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ENDOCRINE MEDIATION IN SOCIAL ADJUSTMENTS OF VERTEBRATES AND INVERTEBRATES

Sponsored by the Division of Comparative Endocrinology
of the American Society of Zoologists

A symposium organized for the
XVI International Congress of Zoology

by

Aubrey Gorbman

Department of Zoology
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Seattle, Washington

ENDOCRINE-EXOCRINE MEDIATED BEHAVIOR IN INSECTS

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ABSTRACT

In recent years we have seen an extraordinarily rapid increase in our knowledge of the roles of endocrine systems in the social adjustments of animals. This discussion will be at least in part, a review of the literature, but will confine itself to insects and to behavior therein which has been shown to be influenced in some way by endocrine and/or neuroendocrine systems. As a further restriction, it may be stated that I am particularly interested in situations in which a product of an exocrine gland (pheromone) plays an intermediary role in the endocrine influenced behavior.

A few minutes would be well spent in discussing some of the general properties of these remarkable exocrine substances. The term pheromone, proposed several years ago by Karlson, et al,[1] is restricted to situations involving intra-specific communication. Thus a pheromone may be defined as a substance secreted to the exterior of an animal which upon perception by an individual of the same species, leads to a behavioral and/or developmental response in the latter. Although the role of pheromones in communication has been more thoroughly investigated in insects than in other groups, communication systems employing chemical substances in this way are likely to be found in all groups. In the lower phyla chemical communication is almost surely the predominant form of communication.

On the basis of their physiological action, pheromones may be divided into two major categories, those with a primer effect and those with a releaser effect (Wilson & Bossert, 1963).[2] Pheromones with a primer effect alter the physiology of the organism, often acting to prepare it for some new biological activity. In general, it is the endocrine system of the organism that plays the central role in this physiological alteration. Behavior resulting from the action of primer pheromones may be of two types. Occasionally there is an immediate behavioral response to the perception of the pheromone, as in releaser pheromones; more commonly, the behavior is delayed until the appearance of some secondary stimulus, the pheromone merely having primed the animal for the performance of the behavior in question. Examples of primer pheromones will be discussed below. The second category, that of pheromones with a releaser effect, includes pheromones

the perception of which results in an immediate and specific behavioral response. Here the term releaser is used in the classical ethological sense. The mediation of the response is quite clearly entirely through the C.N.S. The majority of pheromones known at present are of this releaser type. They are frequently placed in a number of functional categories such as aggregation substances, territory and home range marking substances, alarm substances, sex attractants, and trail laying substances. Examples of some of these categories will be discussed briefly. Attention will be directed to some of the theoretical conclusions drawn by Wilson & Bossert[2] regarding the relationship between the molecular size and structure of pheromones, and factors influencing their effectiveness for communication such as diffusion rate, threshold of perception, etc.

In the chain of events leading from the production and excretion of a pheromone by one individual through the perception of the pheromone by another individual to the behavioral and/or developmental response of that individual, the endocrine system may be inserted at either of two points: a) in the producer individual, the production of the pheromone may be under endocrine control; b) in the recipient organism, the physiological effect of the pheromone may be mediated by the endocrine system. This latter situation is doubtless true of the primer pheromones of the social insects, such as the queen substance of honeybees, and the pheromones inhibiting the production of reproductive caste members in termites, though the physiological mechanisms by means of which the effects of these pheromones are brought about are in no case fully understood. It is frequently presumed that as these substances are ingested by members of the colony, taste receptors are stimulated, thus paving the way for the postulation of a neuro-endocrine pathway for mediation of the response, but the hypothesis that they are absorbed without alteration from the gut into the blood and can thus act directly on endocrine organs has yet to be ruled out.

Perhaps the most elegant example of an endocrine-exocrine interaction of the sort we are discussing is that of the maturation pheromone of the desert locust, Schistocerca, as elucidated by Loher.[3] In this case the endocrine system is involved both in the production of the pheromone and also in mediating the effect of the pheromone in recipient individuals. This case will be discussed in some detail.

As the example of endocrine-exocrine mediated behavior in insects to be discussed in the most detail, I have chosen that with which I am most familiar, the endocrine control of mating behavior in cockroaches. Females of a number of species of cockroaches produce sex pheromones which are of

prime importance in the attraction of males, and in the subsequent release of their mating behavior. Here we are dealing with pheromones of the typical releaser type. However, their production by the producer individual is under endocrine control. Published results[4] on the endocrine control of sex pheromone production in females of the cockroach, Byrsotria fumigata, may be summarized as follows: a) adult females ordinarily fail to produce sex pheromone if their corpora allata have been removed shortly after the imaginal molt; b) there is a high correlation between the production of sex pheromone and successful mating; c) implantation of corpora allata into previously allatectomized females can induce pheromone production. Subsequently, extensive mating tests in which allatectomized females, artificially coated with sex pheromone to enhance their attractiveness, were exposed to males, have shown that pheromone production is the only aspect of the female's sexual behavior affected by removal of this endocrine organ. In addition, pheromone production has been induced in a small number of cases by treatment of allatectomized females with juvenile hormone extracts. These results correlate well with what is known of the role of the corpora allata in the female reproductive cycle in cockroaches. Recent experiments on the role of the brain in the control of the reproductive cycle, suggest that the corpus allatum is an intermediate link in the control of pheromone production and other phenomena associated with the reproductive cycle and that the real control center lies in brain, possible in the pars intercerebralis neurosecretory cells, a situation not unlike that known to exist in certain other insect species.

1) Karlson, P. & A. Butenandt (1959), Ann. Rev. Ent. 4:39.
Karlson, P. & M. Lüscher (1959), Nature 183:55.

2) Wilson, E.O. & W.H. Bossert (1963), Recent Prog. Hormone Res., in press.

3) Loher, W. (1961), Proc. Roy. Soc. (B) 153:380.

4) Barth, R.H. Jr. (1962), Gen. Comp. Endocrin. 2:53.

ENDOCRINE EFFECTS OF CROWDING AND ISOLATION IN INSECTS. Kenneth C. Highnam, Dept. of Zoology, The University, Sheffield, 10, England.

Differences in reproductive performance are among the most characteristic features which separate solitaria from gregaria phases in Locusts. In the gregaria phase of Locusta, Schistocerca and Nomadacris, a smaller proportion of the ovarioles produce mature oocytes when compared with solitaria; in addition, the actual number of ovarioles may be less in the gregaria ovary, and there may be fewer ovarian cycles in the gregaria females. Further, the maturation time is shortened in gregarious Schistocerca and Nomadacris, although curiously, solitary Locusta have a shorter maturation time.

In many insects, reproductive performance in the female is known to be controlled by hormones. It is probable, therefore, that the differences in the reproduction of solitary and crowded female locusts is a reflection of underlying differences in the endocrine balance, which may additionally affect many other aspects of phase differences.

The endocrine control of reproduction in the female Schistocerca has been examined in detail. Both the pars-intercerebralis-corpora cardiaca neurosecretory system and the corpora allata are involved: the former controls protein synthesis by the fatbody and the latter the passage of protein into the oocytes. The number of fully developed eggs produced by a female is controlled by the availability of protein (determined by the neurosecretory system) and by the ability of the oocytes to take up the protein (determined by the corpora allata). The activity of the neurosecretory system controls the rate of maturation of the ovaries.

The endocrine system can therefore affect reproductive performance in a way that mimics phase differences in reproduction in Schistocerca. Can differences in the endocrine systems of solitary and crowded locusts explain these reproductive differences?

The male pheromone in Schistocerca affects the activity of the neurosecretory system of the female. Solitary females would therefore be expected to possess relatively inactive neurosecretory systems, and this is found, in fact, to be the case. Induced activity of solitary females activates the neurosecretory system and the reproductive performance is correspondingly shifted in the gregaria direction. Similar effects are found in Locusta females, but in reversed phase, and it is assumed that the neurosecretory systems of gregarious Locusta are inactivated by the presence of an inhibitory pheromone.

Other endocrine effects may control behavioural differences in solitary and gregarious locusts (Carlisle & Ellis, 1959; Haskell & Moorehouse, 1963) and these may be linked with the endocrine differences in the adult females.

INTERRELATED SOCIAL AND ENDOCRINE FACTORS IN POPULATIONS OF RODENTS. John J. Christian, Div. of Endocrinology, Albert Einstein Med. Ctr., Phila., Pa.

Endocrine responses to increased social competition, resulting from increased numbers, aggressiveness, or both, serve as feedback regulators of population growth. Adrenocortical function and sequelae increase and reproductive function decreases with increased "social pressure", defining the latter as the sum of 2 variables: numbers and behavioral aggressiveness, which together determine the total number of social interactions in a population. Inhibition of reproduction seems to be most important in regulating populations of rodents. Limited food and cover, adverse climatic conditions, and predation (including disease), may limit populations, but perhaps mainly by increasing competition. Increased disease and mortality may result from reduced resistance with increased social pressure.

Most evidence for endocrine responses to changes in population size comes from laboratories. Few studies of relationships between adrenal cortex and numbers of rodents in natural populations have provided interpretable data, mainly due to uncritical use of adrenal weight to assess cortical function. There may be weight changes associated with maturation or sex, but these do not invalidate the tenet that increased social pressure leads to increased adrenocortical function in terms of glucocorticoid secretion.

Adrenocortical function increased and reproductive function decreased with increasing numbers of mice in social contact in static or in freely growing confined populations. Adrenal weight and morphology, thymus weight, body weight, and similar criteria were used to assess cortical function in most of these studies. Recently, these conclusions have been confirmed with measurements of adrenal ascorbic acid, cholesterol, and corticosterone(B), as well as by plasma B and adrenal weight (2,3). Wild Norway rats responded to changes in relative population density with parallel changes in adrenal weight. Reduction of rat and deer populations resulted in proportional reductions in adrenal weight, although maximum adrenal weight is reached in the initial phase of natural population decline. Eosinophile counts have ben used to assay endogenous adrenocortical responses to changes in population, but some of the rapid changes suggest increased medullary rather than cortical activity. Increased numbers alone do not account for the endocrine responses. Behavioral interactions with or without physical contact are equally important. Adrenocortical mass in Mus is related to social rank (low rank - large adrenals, except possibly for those so submissive that they are ignored by more dominant mice). Aggressiveness and increased numbers are needed to evoke increased adrenocortical activity with increased population size. Aggressive mice react to increased numbers while non-aggressive species fail to react.(2) Different strains of the same species react differently. Adrenocortical and reproductive responses to increased density are much less in docile

albino than in aggressive mice. Adrenocortical responsiveness to ACTH varies with species and circumstances. An amount of ACTH producing a 35% increase in adrenal weight in Mus has little effect in Peromyscus leucopus, although the reproductive systems were affected equally. Grouped mice respond to ACTH more than isolated mice. Docile albino rats show striking increases in B secretion with increased density without a significant rise in adrenal weight (4) in contrast to the marked weight changes in wild rats. These data emphasize the need to use adrenal weight with caution and the inapplicability of comparisons between strains and species in terms of adrenocortical response. A few exposures to 20 secs. of fighting daily leads to greater increase in adrenocortical activity in male mice than continuous caging in groups of 8. These results show a spectrum of responses to density with different levels of aggressive behavior and demonstrate that increased numbers alone are insufficient to elicit increased endocrine responses. This is illustrated by different sizes of population reached by Microtus and Mus in the same cage. Microtus reached an asymptote of 36 with a 38% increase in adrenal weight (fully mature voles) whereas Mus has attained populations of 130, or 230 when given reserpine. Also, within broad limits adrenal and reproductive responses to changes in population size are independent of space. Thus, with behavioral factors playing so important a role, comparisons of densities reached in the laboratory and in the field are unjustified and have little meaning. It appears that with increased aggressive behavior, often an accompaniment of breeding season, only a few contacts between animals may produce marked adrenocortical responses and that increased numbers will increase the rate of contact, thus the response.

Somatic growth is suppressed with increased density and in a populatin the degree of suppression is related to social rank. Effects of growth have been seen in natural and confined populations of voles and mice. Outbreaks of disease often occur in natural populations of high density. This is expected, as increased glucocorticoid secretion suppresses defense mechanisms against infection and parasitism. These effects have been confirmed experimentally. Also, the prevalence of glomerulonephritis in populations of deer and woodchucks is relative to density, although the mechanisms are unknown.

Adrenal medullary function changes and increases with changing density,(5). Possibly mass deaths from hypoglycemic shock are due to exhaustion of glucose reserves with increased medullary activity.

Reproductive function in females is affected adversely by increased density, although adrenocortical mass does not increase as much as in males. There is cortical fasciculata-reticularis hyperplasia, accelerated X-zone involution, and conspicuous suppression of reproductive function. Besides inhibited maturation, there is increased intrauterine mortality, depressed fertility, diminished lactation, intrauterine effects on young affecting later behavior, and effects on nursing young persisting for sev-

eral generations. Suppression of maturation appears to be a more important factor in curtailing reproduction than effects on mature animals in populations of voles and mice. Sexual maturation apparently is suppressed with much greater ease than sexual function in adults. There are 2 possible explanations for this difference: (a) younger animals in a population are subordinate and, therefore more affected, and (b) reproductive function is intrinsically more susceptible to suppression in immature animals than in adults. The second is probably the more important. Secretion of gonadotrophins is inhibited by much less sex steroid in immature than in adult rats (6,7). Therefore, it was hypothesized that increased adrenocortical activity would entail an increase in adrenal androgens sufficient to inhibit gonadotrophin secretion in immature animals, but only partially, if at all, in adults. Physiologic doses of adrenal androgens inhibit gonadotrophin secretion in immature mice and delay maturation (8,9). ACTH in intact Peromyscus leucopus, P. maniculatus, and Mus will prevent normal ovarian development for prolonged periods with evidence of increased androgen production (10). For up to 3 weeks of treatment there is no follicular maturation, ovulation, or formation of corpora lutea. This action of ACTH is non-adrenal mediated and cortisol (F), to a lesser extent B, supports normal maturation in adrenalectomized mice in doses which suppress growth (11). Thus, possibly 3 mechanisms act to suppress maturation in populations of mice: (a) possible direct effects of corticoids, (b) suppression of gonadotrophin secretion by adrenal androgens, and (c) the non-adrenal mediated action of ACTH. A related discovery was that immature mice secrete appreciable amounts of 17-hydroxycorticosteroids (17-OHCS), especially F(12). 17-OHCS disappear with maturity and involution of adrenal X-zone. 17-OHCS and F/B ratios increase with crowding and 17-OHCS persist with suppression of maturation and X-zone persistence (13). Primarily B is secreted after maturity. These facts raise a question of the need for F for normal maturation of female mice. Increased F may account in part for suppression of somatic growth, especially of young and subordinate animals, with increased numbers. X-zone involution is accelerated in female and delayed in male mice with increased density. Adrenal androgens involute the X-zone, but are not as potent in this as testosterone. Therefore, adrenal androgens may account in part for failure of normal maturation if F is needed. Testicular position and vaginal perforation have been shown to be poor indices of reproductive condition, and it is notable that adrenal androgens will induce vaginal opening in immature mice.

Growth of confined populations of mice is limited by one of two reproductive mechanisms, or both, in addition to delayed maturation. In one type a decline in birthrate accounts for slowing and cessation of population growth, and with this mechanism there are marked effects on adrenals and testes of males and little effect on females. The second mechanism is declining infant survival. In these populations, females are most affected and males relatively little. Reasons for these differences are not known, but

they pinpoint two different effects of density with a common result. Most populations show some combination of the two mechanisms. Complete inhibition of maturation and adult reproduction adds a third type of mechanism. Most populations probably reflect all three mechanisms.

Studies to relate endocrine function to changes in numbers of natural populations have been disappointing. They have suffered from the assumption that adrenal weight is always an index of corticoid secretion. Adrenal weight cannot be equated with function in the usual way for many species, such as Microtus, Clethrionomys and to a lesser extent Peromyscus due to complex morphological zonal changes associated with sex, age, and immaturity. Mus, as an example, has an X-zone which is involuted at maturity in males and first pregnancy in females. Microtines have an X-zone that behaves somewhat differently and may have another zone that changes at puberty. These morphologically complex adrenal cortices and their weight changes are difficult to interpret. All native small cricetids so far examined seem to have adrenal zones of unknown function that change with puberty or later. Immature zones in male Microtus are involuted and replaced by adult cortex when maturation is complete and seminal vesicle weight has reached 1.4 g. All stages of transition from immature to adult cortex makes finding a constant relationship between body size and adrenal weight almost impossible for male Microtus. The wide range of adrenal weights reported for male Microtus probably reflects these morphological factors (14). Suppression of maturation delays involution of these zones accordingly. Unfortunately, samples from natural populations usually contain mature and immature adrenals and all stages in between. Seasonal increases in adrenal weight reported by Chitty (15) may reflect increasing numbers of immature animals, increased cortical mass with increased aggressiveness during the breeding season, increasing numbers, or all three, but the data probably are confounded by the above factors. These variables invalidate the use of adrenal weights, especially for microtines, to assess cortical functional changes with changes in population unless only fully mature animals are used. Uncritical use of weights, especially with suppression of maturation as a factor, has led to false conclusions concerning adrenocortical function and population changes. However, when they can be used reliably, adrenal weight appears to be the best way to assess the effects of long-term chronic stimuli, as chemical or comparable methods reflect the immediate situation.

Adrenals of female Microtus gain weight with maturation to a greater extent than usually is associated with estrogens in other species (16). They also may have an X-zone, although this has not been critically studied. The increase in weight at maturity seems to be due to tremendous hyperplasia of the reticularis. Reasonable relationships between body size and adrenal weight are possible, especially if only fully mature animals are used.

Progressively increased inhibition of maturation occurs annually in both sexes of Microtus, Clethrionomys, Peromyscus, Mus and probably other species as numbers increase. It also occurs in

confined populations of rodents and in yearling woodchucks. Therefore, most voles or Peromyscus in summer or fall may be sexually immature and appear young, especially at higher densities. Growth may or may not be inhibited. If growth and maturation are both inhibited, an animal may be several months old and called "young" if its age is not known. Most Microtus begin winter in an immature condition. However, animals that have fully matured earlier usually show no evidence of sexual regression at any time in our experience, and only those animals that have not fully matured show evidence of sexual regression. The baculum is used to establish whether or not a male was ever mature or partially so. VanWijngaarden agrees that there is density-dependent inhibition of growth and maturation in the spring and summer, but states that regression is unassociated with density in the fall. There may be seasonal differences in threshhold for regression which would reconcile these divergent results. In voles and mice there is a change in sex ratio at birth in favor of females with increasing density. Fully mature animals (voles, mice) rarely show evidence of sexual regression in fall or winter, although these animals become a small fraction of the population by winter and are difficult to collect. Adrenal weight reflects the immature condition of winter animals. However, Peromyscus all underwent sexual regression in fall, regardless of prior state of maturity. Immature adrenocortical zones in Microtus eventually will regenerate after castration; so regeneration may be a potential complication of sexual inactivity.

The problems discussed emphasize the need to use adrenal weight critically if it is to be an indicator of andrenocortical function in relation to population changes. Without proper validation weight data are meaningless and uninterpretable. Many studies of adrenal weight in relation to changes in natural populations fall into this category. Animals of known age have not been used, although they are necessary for properly interpreting data when inhibition of maturation is involved.

Another problem relating to endocrine-density relationships has been the lack of consideration of non-random distributions of animals in natural populations. For example, Microtus and Rattus may have high local densities with an area-wide low density. Such local colonies of rats appear to behave as discrete populations and adrenal weights respond accordingly. Finally, physiologic responses evidently relate more closely to the logarithm of population size rather than to absolute numbers. Therefore it seems proper to use logarithmic instead of arithmetic functions of population size. Even so, numbers alone are of limited value unless it is known whether they represent relatively "high" or "low" populations. In many instances this will be determined by behavioral factors. Adrenocortical function reflects relative densities unless all other factors remain constant.

The above factors are emphasized in the hope that they will be considered in future field studies so that meaningful data will be collected and valid conclusions drawn regarding relationships between adrenal weight and density. Also, if the points discussed

are considered, it seems evident that the criticisms of Negus(17) and others are invalid and are based on incorrect assumptions and inadequate data.

Heavy dependence on laboratory studies of populations for models will be necessary until data are available from critical field studies with animals of known age. Nevertheless, our experience with laboratory and field studies leads us to conclude that the former are valid and useful models from which we have been able to project and design appropriate experiments in the field or laboratory. In summary, based on evidence from the laboratory and limited field studies, we conclude that endocrine feed-back mechanisms, responding to a combination of numbers of animals and behavioral factors, controls population growth and limits population size even though external factors may sometimes truncate the growth curve.

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THE ENDOCRINE REGULATION OF MIGRATORY BEHAVIOR IN ANADROMOUS TELEOSTS. William S. Hoar, Department of Zoology, University of British Columbia, Vancouver 8, Canada.

The word anadromous, in its etymology, focusses on the most spectacular event in the life cycle of the fish - the up-river migration prior to spawning. In contrast, the downstream movement of juveniles is often relatively inconspicuous. Smelt (Osmerus mordax), alewives (Pomolobus pseudoharengus), sticklebacks (Gasterosteus aculeatus), sockeye salmon fry (Oncorhynchus nerka), pink salmon (O. gorbuscha) and many others leave the rivers soon after hatching, in what appears to be largely a planktonic displacement of small active fish. They show little or no relation to the bottom and are carried by the current or actively swim along with it. This feature of anadromy was emphasized by Huntsman (1948 and earlier) a quarter of a century ago and many of his arguments are just as valid today as they were when he first advanced them. It would be pointless to argue that this downstream movement of the very young fry is entirely or always a passive displacement. Migrant pink salmon fry, for example, show a highly specialized, precisely timed, nocturnal swimming in the surface waters and this may bring them into particularly favorable situations for seaward transport. Other species of salmon lack this intense swimming activity at the surface during the night. There seems to have been an evolutionary specialization in seagoing behavior and the most likely path of phylogeny has been from fortuitous downstream displacements to precisely timed nocturnal swimming in swiftly flowing streams where seaward movement becomes rapid and inevitable (Hoar, 1958).

The juveniles of some anadromous teleosts spend a prolonged period in freshwater before embarking on the journey to the ocean. The Atlantic salmon (Salmo salar) and the coho salmon (O. kisutch) are two familiar examples. As fry, they are cryptically colored for life near the river bottom and relate themselves to particular territories in the streams where they live. In contrast to migrating fry, they settle on the bottom at night and are less active; sometimes they move into shallower waters. Downstream displacement of resident fry is not uncommon when the rivers are in flood or when territories are too few for the population (Huntsman, 1945; Chapman, 1962) but these species do not normally leave the rivers. In fact, they lack the physiological capacity to deal with salt water at this stage and possess behavioral responses which enable them to avoid it (Huntsman and Hoar, 1939; McInerney, 1963). Profound morphological, physiological and behavioral changes - collectively referred to as the smolt transformation - precede the seaward migration and transform the gaily colored, bottom-dwelling fish into a silvery pelagic animal fitted for life in sea water. In some species and under certain conditions, the actual exodus from the rivers may be a downstream displacement of fish which have lost their territorial behavior; they swim up from the bottom and are active at night. However, in at least a few species, it is probably more specialized. The sockeye salmon (O. nerka) for example, is a lake-dweller during its freshwater juvenile stages. Its

precisely timed mass migrations from geographically intricate lake and river systems may require more complex orientation machinery. Precise mechanisms have not been demonstrated.

Several zoologists have postulated a causative role for specific hormones in the migration of anadromous fishes (Fontaine, 1954; Baggerman, 1960). The evidence, however, is still largely speculative. It has been amply demonstrated that fishes possess endocrine glands comparable to those of the higher vertebrates and that the hormones which they produce are intimately concerned with the regulation of metabolism. Seasonal changes in the endocrinology of fishes living in our latitudes are also well documented and known to be profound during spring and autumn - the seasons of anadromous migrations. These facts are accepted but the causal relationships between the hormones and migration are not clear.

In theory, one might envisage a general, permissive action for the hormones or, alternatively, one might postulate specific roles with respect to different components of the migratory sequence. In the first instance, the seasonal activation of the neuroendocrine system would elevate the level of metabolism, alter the excitability of the animal and increase its alertness to stimuli. The fish might then be susceptible to different stimuli or come within the sphere of other influences which would determine migration. The hormonal action in this case is a general one, acting on the appetitive behavior rather than the consummatory acts (Hoar, 1955). In contrast, the second type of hormonal control would be expected to activate or potentiate specific behavior patterns in a rather precise manner. There are few convincing demonstrations of such hormonal regulations (Hoar, 1963). The spawning reflex, which Pickford (1952) elicited in Fundulus by injecting neurohypophysial hormones, seems to fulfil the criteria.

The present discussion is an attempt to examine the evidence for a hormonal regulation of anadromous migrations and to evaluate the probable nature of these endocrine controls. From the foregoing interpretation of anadromy, a special hormonal control of the downstream migrations of fry would scarcely be anticipated. There would not seem to be any biological reason for it if their movement is largely a downstream displacement and, in fact, all attempts to show endocrine activation in migrating salmon fry have given negative results (Hoar and Bell, 1950; Eales, 1963a). On the other hand, the species which show a smolt transformation after a period of freshwater residence and the pre-spawning adult migrants may be expected to migrate in response to some altered endocrinology. The spawning migration will be considered first.

The Spawning Migration

Evidence for hypophysial control of reproduction is consistent in all groups of vertebrates. The activation of neural centers stimulates pituitary secretions which directly or indirectly alter the morphology, physiology and biochemistry in preparation for sexual activities. Thus,

it seems logical to look first to the pituitary for causative hormones of migration (Hoar, 1963). There is now one well-established example of direct pituitary control of pre-breeding migratory behavior. Although this example comes from the amphibia, it will be described as the kind of experimental evidence required for a satisfactory demonstration of rather specific endocrine regulation. The eastern spotted newt, Diemictylus (Triturus) viridescens, migrates from its terrestrial habitat to water before spawning. This "water-drive" is independent of the gonad but requires the pituitary hormone prolactin (Grant, 1961). The classical methods of endocrinology, involving gland removal and replacement therapy, have shown that prolactin is so specific for this reaction that it can be used as a delicate assay technique for the hormone. There are no comparable experiments linking specific endocrines with migratory behavior of fishes. Recent work on the endocrinology of reproductive behavior in the stickleback (Gasterosteus aculeatus) is the most pertinent of the fish studies (Baggerman, 1962; Hoar, 1962a).

Threespine sticklebacks as juveniles are schooling fish. In some places they show an anadromous migration from coastal regions to the shallow freshwaters of streams and ponds. Sometimes there is no such migration and the fish are landlocked. Whether or not there is a migration, the onset of breeding is marked by a cessation of schooling and the establishment of territories. Marked agonistic behavior, involving both males and females, breaks up the schools and scatters the fish throughout the areas available for nest-building. This might be considered the terminal phase of the migratory behavior. Gonadectomy does not interfere with the development of high level aggressive behavior at this time but low pituitary activity does (Wai and Hoar, 1963). Gonadectomized fish maintained under long photoperiods become almost as aggressive as the normal fish in nature; under short photoperiods the level of aggressive behavior is minimal. There is good evidence that the long photoperiod regime produces a high level of pituitary gonadotropins and that the reverse is true with the short photoperiod (Baggerman, 1957; Hoar, 1962b). Indications are that the luteinizing hormone, LH, is the active pituitary factor (Hoar, 1962a). After this agonistic behavior has been established by the pituitary, the gonadal steroids secreted by the interstitial cells (also under control of LH) come into play to regulate the later phases of breeding behavior.

The pituitary is also implicated in some of the earlier phases of the anadromous migration of the stickleback. Baggerman (1957, 1962) showed that the pre-breeding physiology was associated with a change in salinity preference. In this test, fish are given an opportunity to choose between fresh and salt water. During the winter, they show a consistent preference for the seawater at a time when they are normally resident in this environment. The preference is reversed with the onset of the breeding season when they normally migrate into the rivers. Removal of the gonads did not alter the preference but changing levels of thyroid hormone did. When fish with salt water preference were treated with thyroxine or TSH they developed a freshwater preference; anti-thyroid drugs induced a salt water preference in fish which initially preferred freshwater. Baggerman argues that this test is a measure of migration

disposition and believes that the thyroid hormone performs a causative role in stickleback migration. This point of view was also advanced earlier on the basis of biochemical and physiological studies, which indicated that high levels of thyroid hormone disturbed the electrolyte balance of the marine stickleback so greatly that the fish were forced to move into fresh water in order to survive (Fontaine and Koch, 1950). This work has not been confirmed. Studies of Gasterosteus aculeatus in our laboratory have indeed shown an altered electrolyte metabolism and an increased activity of the thyroid, as measured radiochemically, but there is no evidence that these changes are sufficiently marked to compel the migration.

Both gonadal steroids and thyroxine have been shown experimentally to increase locomotor activity of fishes. Hoar et al (1955) found that hormone-treated goldfish swam faster and were more responsive to electrical stimulation while similarly treated juvenile salmon made a more persistent response to water currents. In comparable experiments, Baggerman (1962) found that sticklebacks swam more rapidly and showed increased "fluttering behavior" - thought to be associated with migration. It has been suggested that a similar intensification of locomotor activity may occur during sexual maturation under natural conditions and increase the appetitive behavior of pre-breeding fish (Hoar, 1955). A contributory rather than an initiating role is indicated for the gonadal hormones since the anadromous migrations frequently take place prior to the development of secondary sex characters; the appearance of sex characters is a good indication of strong activation of the hormonal tissues of the gonads and rising levels of sex steroids.

It should be emphasized that there is absolutely no evidence of an altered and specialized endocrinology prior to or during the upstream migration of some anadromous fishes at certain stages in their life cycle. Juvenile rainbow trout (Salmo gairdneri) and sockeye salmon (O. nerka) sometimes migrate upstream into lakes (Northcote, 1962). The latter species is more pertinent to the present discussion but the controlling factors have been more carefully analyzed in the former. In both cases, adults sometimes spawn in the rivers flowing out of lakes and the juveniles, in due course, migrate up into the lakes. This migration is evidently a function of increasing size and elevated water temperatures (Northcote, 1962). It occurs in juveniles at a time when thyroid activity is particularly low. There is no reason for implicating either gonadal steroids or the thyroid hormone.

In summary, there is ample evidence of intensified activity in several of the endocrine glands (pituitary, thyroid, gonads and adrenal cortex) during the reproductive period (Fontaine, 1954; Baggerman, 1960). At present, however, there is little valid evidence for a specific hormonal triggering of the anadromous migration. The endocrine effects may be very general metabolic ones; the change from a feeding resident fish to a roaming pre-breeding animal could be a part of the general maturation

process, quite distinct from the altered endocrinology even though the two occur simultaneously. The significance of the work on the stickleback is still not clear: it is obvious that the salinity preference reaction cannot be an element of behavior when fish commence a migration from fully saline waters or when landlocked species migrate from a lake into the rivers. Moreover, the aggressive behavior which seems to be pituitary controlled is a terminal activity. Fundamental correlations between specific hormones and the anadromous migration - of the sort found in Diemictylus - remain to be demonstrated.

The Smolt Transformation

Histological studies of the thyroid gland of the Atlantic salmon (Salmo salar), carried out 25 years ago, showed a highly active gland in the smolt; an intimate relation between the thyroid hormone and the smolt transformation was suggested (Hoar, 1939). This finding was subsequently confirmed by several investigators; an altered histology of the thyroid was also noted in other "smolting" salmonids. The literature is well known (Fontaine, 1954; Baggerman, 1960). Fontaine, in particular, extended this work to other anadromous fishes and also examined several different endocrine glands. A strong activation of the endocrine system during the smolt transformation is well established. The regulatory action of specific hormones in the anadromous behavior is much less certain. Again, as in the previous section, the attempt here is to evaluate the findings in terms of general metabolic functions in contrast to specific triggering roles in behavior.

The thyroid gland has received most attention and, yet, after 25 years of investigation there is scanty evidence of a specialized role for it in the behavior of the smolt. G.J. Eales, working in my laboratory, has just completed a careful study of the iodine metabolism of the steelhead trout (Salmo gairdneri). The populations of trout from which his samples came have reasonably strong migratory tendencies and characteristically show a smolt transformation at the end of the second year. Both radiochemical and histological techniques show an active gland in the smolt stage. However, this does not necessarily mean that the smolt is experiencing an elevated level of thyroid hormone or even that its tissues have higher hormonal demands. Studies with radioiodine show sharply increased excretion of iodides during the smolt stage; this seems to be correlated with a general elevation of metabolism and an elevated capacity to deal with electrolytes in preparation for the marine life. In this species, there is no evidence that the increased thyroid activity is more than a response to an iodine deficiency.

Eales (1936b) investigated several factors which might be related to thyroid activity in the steelhead trout. He found thyroid activity to be greater in small fish (logarithmic relationship to size), in more saline waters (when fish were compared in sea water and in fresh water with an equal iodine content), at higher temperatures, when fish were more active (induced to swim in strong currents), during sexual maturation

and under long photoperiods if the fish were of migrant age. All of these conditions are known to increase the metabolic load or to be associated with elevated metabolism or increased activity. The data argue strongly for a generalized function of the thyroid in metabolism rather than a specific role associated with migration. This is in agreement with an earlier suggestion based on observations of several species (Hoar, 1959).

Fontaine (1953) has reported an elevation in the protein-bound iodides of the smolt and the sexually maturing stages of several anadromous fish and considered this evidence of increased thyroid hormone demands and utilization during the migrations characteristic of these stages. However, changes in the nature of the plasma proteins - known to occur during these periods - may actually bind more of the iodine to the plasma protein and remove it from the sphere of thyroid action. In this way the iodine deficiency may actually be aggravated and there may be less, rather than more, hormone circulating in the blood (Leloup and Fontaine, 1960; Eales, 1963b).

The most convincing demonstration of the role of the thyroid in the smolt transformation and migration might be expected from experiments of thyroid removal and replacement therapy. This is difficult with a gland as diffuse as the teleost thyroid but Harry Kalleberg (personal communication) reports that destruction of the Atlantic salmon thyroid with radioiodine during the parr stages did not interfere with the activities of the smolts which develop normally. These data have not yet been published. It also seems significant that Eales (1963b) failed to show any inhibition in the silvering of steelhead trout at migrant age when held under short photoperiods and 5°C, even though thyroid activity was at its very lowest under these conditions. By such experiments, the extended speculation concerning the action of the thyroid at the time of the smolt transformation will eventually be settled.

The most tangible evidence for an endocrine effect on the behavior of juvenile salmon during migration comes from the salinity preference work. McInerney (1963) has recently found that the various species of Pacific salmon show a seasonally changing pattern of salinity preference throughout that part of their life when they normally move from fresh water to full ocean salinity. He has correlated the temporal preference changes for progressively more saline waters with estuarial patterns of salinity and presents strong arguments for an orientation mechanism based on this reaction at the time when young salmon move into the ocean. An endocrinological basis for the changing preference is suggested by Baggerman (1960, 1963) who found that she could alter the salinity preference by a manipulation of the thyroid (TSH injections or antithyroid drug therapy). Some preliminary experiments reported by McInerney (1961) indicate that somatotropin can also alter this preference reaction. This work cannot be properly evaluated until the physiological basis of the salinity preference reaction is understood in relation to the action of these hormones at the cellular level. In itself, salinity preference cannot be important in the initiation of the smolt migration and may

not be involved in orientation once the fish has entered water of ocean salinity. However, it may - as Baggerman has argued - be a good measure of migration motivation and an indication that the endocrine system is basically responsible for this motivation.

In summary, evidence of a special endocrinology during the smolt transformation is adequate. Changes in the pituitary, the thyroid and the adrenal are well documented with resultant alterations in growth, general metabolism and the metabolism of electrolytes. At present, however, there is no evidence that the hormones do more than change a resident feeding fish, into an active roaming and frequently more pelagic animal which then encounters a different set of environmental stimuli. Their actions may not even be this specific; the smolt transformation may depend on general growth and maturation with associated but not regulating hormonal changes. This is a similar conclusion to that reached in connection with the spawning migration. In both cases, the interaction of the animal with its environment depends on the endocrinology but there is little evidence that any specific hormone controls any particular reaction. At present these conclusions are very tentative. In evolution, hormones have sometimes become related to patterns of behavior in specific ways but until the classical methods of endocrinology have been more rigorously applied to the problem and until more examples are carefully examined further generalizations are unwarranted.

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THE EVOLUTION OF ENDOCRINE SYSTEMS

Sponsored by the Division of Comparative Endocrinology
of the American Society of Zoologists

A symposium organized for the
XVI International Congress of Zoology

by

Howard A. Bern
Department of Zoology
University of California
Berkeley, California

This symposium is dedicated to the memory of

Professor Gregory J. van Oordt, of Utrecht,

who was to have been the Honorary Chairman

OPENING REMARKS. Howard A. Bern, Department of Zoology, University of California, Berkeley, California, U.S.A.

The present symposium can hardly attempt to cover all that can be said about the evolution of endocrine systems. The four endocrine areas selected for discussion are ones where the abundance of new data justifies an evolutionary survey. Reproductive adaptations and their hormonal control, the structural evolution of the adenohypophysis and the "molecular evolution" of its hormones, the evolution of the endocrine pancreas and the hormonal control of carbohydrate metabolism, the evolution of neuroendocrine mechanisms among arthropods, represent some other topics whose consideration would also have been readily justified by the information presently available, if the time allotted had been sufficient to permit it. However, it is hoped that symposia such as the present one - with evolution as the pervading theme - will continue to occur frequently and will involve authorities with various points of view, to the end that zoologists in general will be regularly apprised of the contributions of comparative endocrinology to evolutionary biology.

INTRODUCTORY STATEMENT. G.J.van Oordt*, Dept. of Zoology, University of Utrecht, The Netherlands.

Though there is evidence that in some cases vertebrate endocrine glands have originated from homologous invertebrate organs — the best example being the vertebrate thyroid gland and its precursor, the endostyle of the lower chordates — we are still wholly ignorant of the evolutionary origin and history of most other endocrine glands.

In addition, many investigations during the last two or three decades have revealed several common endocrine phenomena in Vertebrates as well as in Invertebrates. E.g. in both sections of the animal kingdom neurosecretory processes, as well as organs, secreting true sex hormones, may be found, and in some cases organs with a gonadotropic action and even a functional control of the latter by the nervous system have been detected in widely separated invertebrate phyla.

Though our knowledge about these endocrine phenomena has recently increased enormously, it must be emphasized, on the other hand, that the evidence for a common evolutionary origin of these phenomena is still very meagre; consequently, I am of the opinion that at present the concept of an independent evolution of the endocrine phenomena, having arisen in such different groups, must be preferred.

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* Deceased April 22, 1963.

EVOLUTION OF FORM AND FUNCTION OF ENDOCRINE ORGANS WITH SPECIAL REFERENCE TO THE ADRENAL GLAND. M.Fontaine, Laboratory of physiology, Muséum National d'Histoire Naturelle, 7 rue Cuvier, Paris.

Among the most highly organised Vertebrates, the adrenal gland are constituted of two different parts, by their structure, their embryological origin, the nature of the hormones secreted and their function. Study of the lower Vertebrates shows some of the stages of evolution which have led to this state, i.e. the gathering, on the one hand, of the elements of the medullary and, on the other hand, of the cortical tissue, which were at first scattered, and then to their union in a single organ.

For a long time, the homologous formations of the adrenal cortex of Mammals were called "interrenal", in the case of fish, because the first to be recognised as such were those of the dogfish, which are in fact situated between the kidneys. Here, we shall speak of the adrenocortical cells of tissues.

As regards the homologous formations of the medullo-adrenal, we shall call them chromaffins, although the medullo-adrenal cells are not alone in giving this reaction. It was generally admitted that the adrenocortical tissues came from the coelomic mesoderm, the chromaffins from the neural crests, but according to WITCHI, BRUNER and SEGAL (53), the adrenocortical in Amphibia comes from the intermediary mesoderm. CERTAIN (61) concluded that the three histogenetic lines have a common origin : interrenal tissue, mesonephros, medulla of the gonad.

The chromaffin tissues have been described in different Invertebrates, but it is first in Cyclostomes that the two adrenal tissues are co-existing and, in certain points into contact. The chromaffin tissue and the adrenocortical tissue are widely dispersed, from the cardiac region to the cloaca, bu they are found especially along the walls of the blood vessels and in the mesonephros.

The Selachians give us some examples, on the one hand, of the agglomeration of these cellular masses in several widely scattered organs, on the other hand of a associated location of the chromaffin and adrenocortical tissues, which might be considered as prefiguring that observed in Mammals. It is thus that certain islets of chromaffin tissue, enveloped in the cortical tissue have been discovered in Raia clavata.

In the Teleosts, the chromaffin and interrenal tissues are again widely scattered; in some species (e.g.the Salmo salar), above all in the cephalic kidney, in others (e.g.the Eel)around the cardinal veins, or on the vein walls themselves. In certain species, the two cell types are entirely separated (NANDI,62), but in others, they are often side by side (OVERBECKE,60,NANDI 62) Among Perch and Cottus, one finds medullo-adrenal tissue surrounded by adrenocortical.

It is possible that beside these tissues, whose significance has been well demonstrated by different workers, we ought to place the Stannius corpuscules (S.C.) present in numerous Teleosts, absent in others, and which show a great variety of form and positions. They appear as endocrine glands with holocrine secretion

and they have long been connected, under the name of posterior interrenal with the anterior interrenal; they were thus included in adrenocortical formations. Nevertheless, it has been objected that their embryologic origin is different from that of the adrenal gland because they seem to derive from Müller or Wolff ducts, but CALLAMAND (43) has advanced arguments in favour of the presence of adrenocortical hormones in the corpuscules of the european Eel. BOBIN (48) has emphasized certain homologies between the adrenocortical cells and those of the S.C..RASQUIN (56) has shown that, like the cells of the zona glomerulosa of the adrenal cortex, those of the S.C. contain granules of phospholipids, whose density varies according to the hydromineral balance of the fish (Astyanax mexicanus).

Finally, the presence of hydrocortisone and cortisone in the S.C. of the Salmo salar has been demonstrated (FONTAINE and LELOUP 59)(sample of 10g). It is true that FORD (59) did not find any corticosteroids in the S.C. of Oncorhynchus nerka, but we don't know what weight of this organ he used. PHILLIPS and MULROW (59) were unable to isolate any C 14 corticosteroids from a medium in which the S.C. of Pseudopleuronectes americanus incubated in the presence of 4 C14 progesterone, but it is possible that the quantity they used (174mg 5) was insufficient. On the other hand, it is not impossible that specific differences exist, regarding the quantities of steroid produced, differences which can be related to the conditions of the medium (particularly regarding the salinity of the water where the animal lived) or with the stage of the life-cycle at the moment of sampling.

Thus, the participation of S.C. in the adrenocortical function of various fish is not excluded, and one may suppose that they play a physiological role analogous to that of the zona glomerulosa in the higher Vertebrates. The fact that they do not seem to react to hypophysectomy by any histologic or cytologic modifications authorises this comparison. One may object that the most abundant corticosteroid found in the S.C. is the hydrocortisone, i.e. a glucocorticoid. Nevertheless, we must not forget that the distinction between mineralo and glucocorticoids was established from results obtained on the kidney, but that, on the nasal gland of birds or the gill of fish, certain glucocorticoids appeared very active in the hydromineral regulation.

Furthermore, former studies having suggested a connection between the activity of the S.C. and genital activity (FONTAINE and HATEY 55, OLIVEREAU 61),we have found out and showed the presence of relatively important quantities of sexual steroids (androgen and oestrogen) in the S.C.of Salmo salar (CEDARD and FONTAINE,unpublished). Thus, we feel inclined to put forward the hypothesis that the S.C.are primitive formations bearing witness to a common origin of the adrenal cortex, the mesonephros and the medulla of the gonad, an origin recalled above. These primitive formations (1)range themselves among those which WILMER(60)

(1)The number of S.C.diminishes from the representatives of the least evolved groups to those of the most evolved.In certain species, there is a considerable reduction in this number between the young and the adult stages (BAUCHOT 52).

named as coelomoducts (serially arranged tubes connecting the coelom with the outside) and, in the S.C., the specialisation of biogenesis, be it towards the constitution of sexual steroids or towards that of corticosteroids, is perhaps still not accomplished. It may also be remembered that the adrenal cortex in Mammals themselves synthetisizes several sexual steroids, and HILL's results (44) already suggested that certain grafted ovaries might secrete steroids which assure an adrenal function. It doesn't therefore seem impossible that certain formations, having a common origin with the genital and adrenocortical sphere might synthetize corticosteroids and sexual steroids. If it is thus with the S.C., these should include the most complicated enzymatic equipment, and evolution would be accomplished, yet again, through a loss of enzymes. However, it is quite obvious that this is only a hypothesis and we plan to find out, by cultures in vitro, if the S.C. of the Salmo salar, are really capable, not only of stocking, but of synthetizing these steroids.

In Amphibia, the adrenocortical and chromaffin tissues are more or less connected by vast blood sinuses in cords, on the ventral surface of the kidney into which they penetrate.

From Amphibia to Reptiles, and from Reptiles to Birds, the interpenetration of the cortical and medullary tissues is accentuated and the adrenal becomes more and more compact. CHESTER JONES and PHILLIPS (60) have emphasized that this concentration of the elements of the adrenal cortex corresponds embryologically with the appearance of the metanephros. One should note that among Lizard the chromaffin tissue surrounds the adrenocortical tissue which is exactly the reverse of what we see among Mammals. The adrenal gland of birds is formed of a network of cords of cortical tissue, whose spaces are occupied by strings of medullary tissue. Among Mammals, the adrenal gland is isolated by a conjunctive capsule, the medullary tissue gathers itself in the center surrounded by cortical tissue, which is divided into several zones (zona glomerulosa, z.fasciculata, z.reticularis, and in certain Mammals a zone "X" which is juxta-medullary). However, one must emphasize that accessory or aberrant adrenocortical tissues are frequently observed and that certain (brown fat) are considered by some authors to be capable of synthetizing corticosteroids, facts which witness that the issue of individualisation into two dual organs has not yet been perfected. On the other hand, in a child, the Zuckerhandl organs which are completely independant of the adrenal cortex contain both, adrenaline and noradrenaline, the percentage of methylated hormone increasing with age. Among young dogs, rabbits and guinea pigs, and cats, we find, in the retroperitoneal tissue, rates of noradrenaline sometimes superior to those of the adrenal glands. This tendancy towards a drawing near of tissues as different as the medullo and adrenocortical which manifests itself in the course of the phylogenetic evolution, is easily seen by the embryologists (RAPOLA, 62)

Does this close association present any physiological interest? SHEPHERD and WEST (51) observed that the proportion of adrenaline (in % of catecholamines) present in the adrenal gland of a Mammal or Bird is found to be greater in proportion as the cortical tissue itself is greater. For COUPLAND (53), the immediate proximity of the cortical tissue determine the degree of

methylation of the noradrenaline which leads to the adrenaline. Certain facts seem to support this theory, especially the histochemical data of WRIGHT and CHESTER JONES (55) on the Lizard, where only little islets and streaks of chromaffin tissue scattered among the cortical cells synthetize adrenaline. But a wider investigation does not allow us to confirm this hypothesis to day. Thus the chromaffin tissue which is perfectly distinct from the cortical tissue, e.g. in Selachians may contain, bedides noradrenaline, significant quantities of adrenaline.(SHEPHERD and WEST,52, SHEPHERD, WEST and ERSPAMER,53, von EULER,53, OLIVEREAU,59).HARRISON and HOE,60 have developed an interesting theory based on the effects of adrenaline on the conveying of blood to the cortical tissue. HARRISON (57) showed that among Mammals, the adrenaline constricts the arteriae medullae and thereby diverts blood flowing through the gland into the cortical capillary sinusoids. The contiguity of the two formations assures maximum efficiency for this response mechanism to agression, by assuring a better irrigation of the adrenal cortex,and by faciliting an increased production of corticosteroids in the event of any stress. According to HARRISON and HOE (60), the generalisation of this kind of anatomical disposition among land Vertebrates corresponds to the fact that the non-aquatic medium is more unstable than the aquatic and generates more frequent and intense stress.

As regards the 3 essential cellulary types of the adrenal cortex of Mammals which are arranged in zones, it seems that they are to be seen already in fish where, nevertheless they have not been observed co-existing, but following each other in time. According to ROBERTSON and WEXLER (59), the cortical cells of immature Oncorhynchus are relatively small, very much granulated, and might be compared with the glomerular cells. With genital maturation, the growth of the cells and their arrangement in cords suggests an analogy with cells of the fasciculated type. Finally, authors suppose that cells of the final or degenerating stage correspond with those of the zona reticularis . One should note that this idea supports the theory, at present, however, out of favour, according to which the 3 cellular types co-existing among Mammals represent evolutionary stages of the same cell.

The evolution which has just appeared in the study of phylogenesis is also found in the Mammal ontogenesis. The 2 anlages of adrenal cortex and medullary gland develop separately for 20 days, then the chromaffine anlage penetrates the cortical tissue and, by the 3rd month the histological differentiation of the adrenal gland is accomplished. Some specific variations have been described (ADAMS,58). The studies in vitro (BLOCK and BENIRSCHKE,59,60,62) seem to show that human foetal adrenals differs from the adult in producing 3 β-hydroxy Δ6 steroids such as pregnenolone and dehydroepiandrosterone in preference to Δ4-3-oxo steroids such as hormones produced by the adult (cortisol, corticosterone, aldosterone). This could well come from that the foetal cortex has a low 3 β-hydroxy-steroid dehydrogenase activity. NANDI and BERN's experiments in vitro (60) on the adrenocortical tissue of the Teleosts do not show any parallelism between biochemical ontogenesis and phylogenesis.

5

Comparative Biochemistry of adrenal hormones

Since the chemical proof was given of the presence of large quantities of 17-hydroxycorticosteroids in the blood of Salmo salar (FONTAINE and HATEY,54), many works have shown the existence and specified the nature of corticosteroids present in the blood or the interrenal of the lower Vertebrates, others have described the production in vitro of cortical hormones from the adrenocortical tissue from Selachians to Mammals (bibliography in CHESTER JONES, PHILLIPS and BELLAMY,62 and paper of BERN, de ROOS and BIGLIERI,62). The predominant corticosteroids are aldosterone, cortisol and corticosterone, but great differences exist according to the species and actual physiol. conditions of the subjects, in the respective rates of corticosteroids produced. Thus it seems impossible at present to get a precise idea of the biochemical evolution from the data we possess. Let us indicate simply that no corticosteroid has been found in 142g of Amphioxus (CHESTER JONES and PHILLIPS,60) and that aldosterone has not been found up to now among Myxinoidea, while cortisol and corticosterone have been (CHESTER JONES, PHILLIPS and BELLAMY,62a). This peculiarity, if it is confirmed, is probably connected with the poikilosmotic character of this group's representatives, which, however, manifest a certain ionic regulation (FONTAINE,58, ~~CHESTER JONES~~,BELLAMY and CHESTER JONES,61,CHESTER JONES,PHILLIPS and BELLAMY,62a). From the PHILLIPS's 59 data,it is probable that the amount of corticoids of plasma is lower in Elasmobranchs than in Cyclostoms and Teleosts. It is also possible that certain species and groups are characterised by the presence of some not yet identified corticosteroids (CHESTER JONES, VINSON, JARRETT, CHARMAN, quoted in CHESTER JONES and al. 62b)(observations on the australian brush tailed possum Trichosurus Vulpecula, and in PHILLIPS, CHESTER JONES and BELLAMY,62: observations on Lacerta viridis L. and Natrix natrix L.).

As for the catecholamine hormones (adrenaline and noradrenaline), they have already been shown to exist besides chromaffin tissue in certain Invertebrates (OSTLUND,54,OSTLUND and FANGE,62) although adrenocortical tissue has not yet been found there. Noradrenaline and adrenaline have been found in the entire scale of Vertebrates.

Evolution of the function

Just as there is great unity of adrenal hormones present or susceptible of beeing synthetized in certain physiological conditions in diverses groups of Vertebrates, so there seems to be also a great generality in their intervention in diverse metabolisms (hydromineral and glucidic), but with some actions which may be different according to the targets as far as the hydromineral metabolism is concerned.

Among Myxinoidea, in diluted sea water, the injection of aldosterone or D.C.A. is accompanied by a diminution of the amount of Na in the muscle, but it is the only significant action observed so far (CHESTER JONES, PHILLIPS and BELLAMY,62a). Among the Petromyzontia (Lampetra fluviatilis) aldosterone diminishes the loss of Na (BENTLEY and FOLLETT,62).

6

Among Elasmobranchs, the interrenalectomy seems to raise the level of plasma potassium (data from HARTMAN,LEWIS,BROWNEL,ANGERER and SHELDEN,44, analysed by CHESTER JONES,57). It would be very interesting to know the respective action of the diverse corticosteroids on the kidney, the gills and the rectal gland (BURGER and HESS,60).

Among Teleosts, the data from several authors, be it on fresh water fish (SEXTON,55, SPALDING quoted by CHESTER JONES 57, CHESTER JONES and al.59,62a, REID and EGO,62, HOLMES and BUTLER, 63), or on saline loaded fresh water fish (HOLMES,59,FAVRE,60) don't always agree, and require further research. If the action of corticosteroids on the fish kidney (CHESTER JONES, PHILLIPS and HOLMES,59) seems comparable to that exercised on the Mammals kidney, it is possible that the action is different on the gills; some authors have shown a diminution in the input of Na to the levels of the gills under the action of corticosteroids.EDELMAN, YOUNG and HARRIS(60) have not remarked any action of the corticosteroids studied (hydrocortisone, 9 α-fluorohydrocortisone and aldosterone) on the kinetics of loss in electrolytes in some stenohaline or eurhyhaline sea water fish, transported into fresh water. Among Amphibia, the aldosterone seems to act positively on the active transport of Na to diverse targets (skin, bladder, kidney)(MAETZ,JARD and MOREL 58, CRABBE 61).Aldosterone acts also on the active transport of Na to the bladder of Testudo graeca (BENTLEY 62).

Among birds, one must above all emphasize that the action of corticosteroids on the nasal gland produces an increase in the excretion of ClNa (HOLMES,PHILLIPS and BUTLER 61,HOLMES and BUTLER 61), the glucocorticoids beeing particularly efficient (PHILLIPS and BELLAMY 62).

Among Mammals, and in various degrees, the corticosteroids stimulate the reabsorption of Na and the excretion of K. They act thus on the cells of renal tubes, but also on the cells of other emonctories, sweat and salivary glands. It seems that, in Mammals, the kidney have become the most efficient target of corticosteroids from the point of view of hydromineral regulation, and that all the targets react in the same way, while it is not the same in other Vertebrates.

As regards the glucidic metabolism, the corticosteroids certainly play a part in its regulation, from fish to mammals, for it has been shown that injections of corticosteroids in representatives of diverse groups brings about a rise of glycemia and the stocking of glycogen in the liver (HOUSSAY,MAZZOCO and RIETTI 25, DI BENEDETTO and MAZZOCO 33, MAES 37, HARTMAN,LEWIS, BROWNER,ANGERER and SHELDEN 44, COULSON and HERNANDEZ 53,NACH 55-56,CHESTER JONES 57, PICKFORD and ATZ 57, HUNTER and JONSON 60, GRENMAN and ZAROW 61).

Various results show also that in fish as in mammals, stress and muscular activity induce an increased secretion of corticosteroids (FONTAINE and HATEY 54, HANE and ROBERTSON 59,FONTAINE and LELOUP-HATEY 60, LELOUP-HATEY 60-61, ROBERTSON,KRUPP,THOMAS, CUTTING, HANE and WEXLER 61).

It is probable that the study of the mode of action of the adrenal hormones at cellular level and in particular on certain enzymatic mechanisms, will reveal an evolution to this level. Already, SHUNG KAI CHAN (62) has shown that the corticosteroids have no enhancing effects upon the three transaminase systems in tadpole livers, contrary to that observed in Mammals, horned toads and painted turtles.

The action of the corticosteroids on the transaminases probably has appeared among Amphibia and Reptiles during evolution.

The existence, from Myxinoidea to Mammals of a pituitary hormone controlling the activity, at least in part, of the adrenocortical elements, is shown by numerous works (bibliography in PICKFORD and ATZ 57, CHESTER JONES, PHILLIPS and BELLAMY 62b). The mammalian ACTH acting on the adrenocortical elements of fish under certain temperature conditions (FONTAINE and HATEY 53) and the pituitary extracts of Myxine on the adrenal cortex of Mammals (discussion in the paper by CHESTER JONES, PHILLIPS and BELLAMY 62a and the same authors 62b) it seems that, if a specificity of action exists (the chemical specificity beeing probable to judge by the results obtained among Mammals), this only concerns certain modalities of the hormone's action, such as its variations in activity in relation to the temperature (a hypothesis which suggests a similarity with the results obtained on the T.S.H. by FONTAINE M. and FONTAINE Y.A. 57-62).

As for the medullo-adrenal secretion (adrenaline-noradrenaline), we must above all emphasize that heart of Myxine contains some significant quantities of adrenaline and noradrenaline and that it hardly reacts at all to these hormones, except after pretreatment by reserpine or dihydroergotamine (BLOOM, OSLUND + von EULER, LISHAJKO, RITZEN and ADAMS-RAY 61). From fish to Mammals, adrenaline and noradrenaline act on various targets, though sometimes in different ways. Thus they stimulate the intestinal contractions of Elasmobranchs, while inhibiting the same in Mammals. Adrenaline dilates pupil of Mammals and contracts that of the Carp. The catecholamines don't exercise any precise action on the gills of Elasmobranchs, while a very important dilatatory action is observed on the gills of Teleosts, as on the coronary vessels of the Mammal's heart (OSLUND and FANGE 62). The ratio of adrenaline and noradrenaline activity varies greatly according to the species. We have no space to develop these various aspects, whose evolutionary significance is, in any case, doubtful.

In conclusion, the morphological evolution of adrenal glands continues long after the end of the biochemical evolution. Adrenaline and noradrenaline are already found in certain Invertebrates and exist in all Vertebrates. The most important corticosteroids are already present in lower Vertebrates . It is probably the same for the majority of endocrin glands synthetizing non proteic hormones, and in particular the thyroid gland. On the contrary, as regards the hypophyseal hormones or the neurohormones, the biochemical evolution frequently continues after the acquisition of an anatomical structure which is fixed in its essential features. As for the evolution of functions, it has been continued, in relation to the medium by an evolution of equipment: targets, of their sensitivity and probably of their enzymatic

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A REAPPRAISAL OF THEORIES OF THYROID EVOLUTION. Aubrey Gorbman, Dept. of Zoology, University of Washington, Seattle, Washington.

On the basis of available information biochemical and morphological theories of evolution of thyroid function and structure have been devised. In at least some instances the original data on which these theories were based is no longer as valid as before; in other instances new data have appeared which bear on the question. Accordingly, the time now is ripe for a reevaluation of the theories of thyroid evolution, and for decisions concerning the retention or rejection of various aspects of these theories.

Facts, or supposed facts, upon which the theories are based are the following:

1. Iodoproteins are of general occurrence in nature, and in some invertebrate species these iodoproteins contain thyroid hormones, even in the absence of a thyroid gland.

2. Halide transport (and, in particular, iodide transport) also is a generally distributed property, and significantly it is a property of the foregut mucosal epithelium.

3. In protochordates, which have no thyroid gland, certain specific cell groups in the endostylar epithelium selectively metabolize radioiodine.

4. In embryonic development, which might be taken at least to reflect the evolutionary development of the thyroid gland, iodide accumulation is an earlier property than protein binding of iodine.

5. The cyclostome vertebrates also display features which may be taken as primitive: a) an extensive embryonic origin of thyroid from the pharyngeal floor in contrast with the restricted one of higher vertebrates, b) an exocrine mode of secretion of thyroprotein in ammocoetes, c) a "prethyroid" development of thyroid catheptase.

As usually expressed, thyroid evolution conceptualized from the preceding takes the following pattern. Thyroid hormones (thyroxine, triiodothyronine) were available as accidental protein iodination products long before they were eventually incorporated into metabolic schemes in a regulatory role. Once they were of metabolic significance, then there was adaptive advantage in creating an organ (thyroid gland) to provide a more intense and dependable source of the hormones. The

foregut was a logical place to develop the thyroid because active iodide transport in various parts of the foregut provides localized areas of high concentration of iodine. The easiest way to achieve such high concentrations of iodine is to transport it into a closed, or almost-closed space (lamprey subpharyngeal lumen, or thyroid follicular lumen) and to permit it to react with protein in the transporting cell or in its adjacent lumen. Such reaction produces iodotyrosines easily, and under proper circumstances, the iodothyronines or thyroid hormones themselves. The protein-bound hormone could be freed readily if the parent protein were allowed to move posteriorly where intestinal digestion frees the hormone (as in ammocoetes). However, in the much more efficient iodination-storage organ, the completely closed thyroid follicle, there evolved a localized intrafollicular thyroidal protease, or catheptase, for hormone-liberation. Superimposed upon this basic mechanism for hormonogenesis was the evolution of adenohypophysial regulation of rates of hormonal production, storage, and release, and the ultimate linkage of this regulation to external environmental events through the hypothalamus.

(1) With respect to the first of the above numbered "facts" some doubt has been raised recently by Taurog et al. (1960). These workers found that when a reducing agent is added to the various fluid media during the isolation and analysis of thyroid hormone-containing tissues, much less hormone is found than when conventional techniques are used. They suggested, therefore, that thyroxine often may be produced as an artifact during analytic procedures, and they question on this technical basis the claims of others who found thyroxine in invertebrate non-thyroid tissues (Gorbman et al, 1954; Antheunisse and Lever, 1956; Limpel and Casida, 1957; Roche et al, 1959, etc.). The warning of Taurog and his colleagues may indeed have merit, and in Taurog's laboratory (Tong and Chaikoff, 1961) colleagues were unable to find I^{131} labeled iodothyronines in a snail and in the cockroach. However, it must be pointed out that in a series of experiments in which they found iodothyronines in a fresh water mollusc Gorbman et al, (1954) failed to find it in an annelid. It seems unlikely that the labeled thyroxine found under these circumstances was artifact in nature. One may even wonder whether in the Taurog procedure the presence of reducing agents may not, in fact, even be destroying or removing some thyroxine. Satisfactory assurance of this seems to be lacking.

Accordingly, at the moment it seems permissible to retain the premise that thyroxine, as an "accidentally" produced substance of general occurrence may have preceded the thyroid gland itself in evolution.

(2) Although there is less assurance nowadays in the thought

that a specific type of cell ("chloride cell") is concerned with halide transport by the gills (Burden, 1956), there is still ample evidence that the branchial epithelium is able to engage in this activity (Krogh, 1939). In addition, accumulating evidence has substantiated the ability of the salivary glands, gastric, and intestinal mucosa to transport halides, illustrating that this is a fairly common property of foregut and midgut epithelia (Brown, 1956; Brown-Grant, 1961).

(3) There has been a lingering difficulty in deciding whether iodination of protein in the protochordate endostyle is intracellular on extracellular. Although radioiodinated protein does occur in close association with the "iodination centers" of Barrington (Barrington, 1959), it remains possible that these are points where materials iodinated elsewhere are concentrated by ciliary action of the endostylar epithelium. However, regardless of this it is clear that Amphioxus and some of the tunicates (Roche et al., 1950; Covelli et al., 1960; Tong, Kerkoff, Chaikoff, 1962) may form I^{131}-labeled iodothyronines. In some experiments it has proven difficult to demonstrate thyroid hormones in tunicates. Enteropneusts have not yet been found to have an ability to form iodothyronines, though monoiodotyrosine is produced (Barrington and Thorpe, 1963).

(4) Evidence taken principally from chick and rabbit embryos still indicates that there is a progressive biochemical differentiation in the thyroid, with iodide accumulation the first property developed; this is followed by the appearance of iodotyrosine, and iodothyronines, and finally hypophysial regulation of these phenomena is expressed.

(5) There has been no additional information with respect to the development of the cyclostome thyroid, nor with respect to its catheptic properties. It is interesting, however, that proof is still lacking that the endostylar epithelium is subject to the regulatory action of TSH (Barrington and Sage, 1963). Similarly, in adult hagfish goitrogens and TSH have little effect in thyroidal I^{131}-uptake (Gorbman et al., unpublished). These findings suggest that the pituitary-thyroid regulatory interrelation found in higher vertebrates is not yet fully evolved in cyclostomes. This situation deserves further study. Furthermore, the hagfish thyroid differs from other vertebrates in its virtual lack of follicular colloid.

THE EVOLUTION OF NEUROSECRETORY CELLS AND SYSTEMS. Ragnar Olsson, Dept. of Zoology, University of Stockholm, Sweden.

E. Scharrer (46) has, in several papers, drawn attention to the enormous importance of the concept of analogies for comparative studies in the field of neuroendocrinology. The intense work in this field during the last decade has given us an increasing knowledge of how neuroendocrine mechanisms can exhibit striking similarities although they must have evolved in totally unrelated animal forms. In other cases, forms with an undoubted kinship may display astonishing divergencies in structure and function of equivalent organs. Because of this fact the relationship between the various kinds of neuroendocrine units which have been described has often been neglected, with the result that our knowledge of the evolution of these structures is very meager.

Before trying to consider the very scattered facts available which may throw some light on the evolution of endocrine cells within the nervous system, it seems to me necessary to make a brief review of the morphological characteristics of the cells we call neurosecretory (n.s.) cells. This will be done with special regard to similar structures in the n.s. cell relatives in the nervous tissue, especially the ependymal cells, astrocytes and neurons.

Cytoplasmic network. A number of investigations of various n.s. cells have shown that they generally contain a very well developed ergastoplasm in the perikaryon. This is only what could be expected in cells which are synthesizing protein secretory materials. It is also quite in accordance with the condition in several other endocrine cells (31) where it seems to be established that the secretory products are synthesized in relation to the ergastoplasm, and are eventually conveyed to the agranular endoplasmic reticulum to be enclosed in Golgi vesicles and form membrane-bound secretory granules. Some exceptions to this general rule of secretory formation have been reported, but nevertheless there seems to be no question about the similarities between n.s. cells and ordinary gland cells in this respect (47). In neurons we find the Nissl substance with the same fine structure and distribution, at least in primitive neurons where no clumps of Nissl substance seem to occur (24).

We may conclude that the secretory synthesis, at least from the morphological point of view, gives us no reason to distinguish between n.s. cells and several other cells with secretory properties.

Secretory granules are a most consistent feature in the cytoplasm of different types of n.s. cells. Enwrapped secretory products of identical ultrastructural appearance are, however, a common feature of several endocrine organs (31). It is also clear that granules of the same appearance may be seen in ordinary neurons (22) where they may represent the catechol-amine bodies of motoneurons (6). A current investigation on the brain of the Tunicate Oikopleura has showed that the gill nerve of this animal contains but three fibers. One of them is easily distinguished from the others because it abounds in granules about 100 mμ in size which give the axon an appearance identical to n.s. cell processes of other animals. It is probable that these granules contain a neurotransmitter of non-cholinergic nature. As a conclusion it is obvious that the n.s. granules (Elementargranula) are not peculiar to these cells and they do not allow us to make any conclusions on morphological grounds about the nature of cells which carry them (22).

N.s. cell processes. The n.s. cell typically consists of a cell body, perikaryon, which gives off one or more cell processes. The secretory material is discharged at the termination of this process, which, in analogy with the conditions in nerve cells, usually is called the n.s. "axon". For the same

reason other n.s. cell processes, which usually retain the contact with the matrix layer of the nervous system, have often been called "dendrites".

The so-called n.s. dendrites have repeatedly been described in vertebrate preoptic cells, in lampreys (43) and in higher animals (11). In those cases, however, when the processes penetrate the ependyma layer and release secretory material into the ventricle they seem definitely not to deserve the name dendrites. They have a proximo-distal flow of secretory material which is terminally discharged at a surface. A dendrite would be expected to have postsynaptic nerve terminals or free endings and conduct a disto-proximal nerve impulse. In my opinion the apical n.s. cell processes could be compared to neuronal dendrites only when they do not discharge secretory material (33), or when a receptor function (12) has been suggested for them.

As regards the so-called axonal processes of n.s. cells, a wide variety of appearances and modes of secretion have been described. The processes may terminate close to blood vessels or their sheath of surrounding cells (29), at the connective tissue surface bordering the nervous system (13, 39, 49), or in close association with other secretory cells (18, 26, 28, 35, 45, 48). The apical release of material was considered above but here must also be considered those secretory processes which penetrate hypo- or epidermis and possibly discharge their secretory product to the exterior (5, 10). Finally, n.s. cells have been described which completely lack processes (2) and release their material to vessels close to the perikaryon.

It is quite clear that neither the appearance nor the distribution of n.s. cell processes can be used as characteristics of this special cell type.

Microvesicles. A very rich literature exists about these structures in n.s. cells and their resemblance to the synaptic vesicles which usually cluster near a thickened membrane in neuronal terminal and lateral synapses. In many cases this seems to be a neuronal character of the n.s. cells, but it must be remembered that microvesicles have also been described in non-synaptic regions of neurons (51), Pacinian corpuscles (41), and in astrocytic processes (34). The synaptic vesicles are probably the carriers of acetylcholin or some other transmitter substance in neurons, and some n.s. cell microvesicles may have a similar function (30). There are, however, various types of vesicles in primitive neurons (24) as well as in n.s. cells (23, 28) which may not be identified with synaptic transmission, but may have evolved a function specially for the cell carrying them.

Cytoplasmic filaments of various dimensions have repeatedly been reported from n.s. cell processes. Depending on size and structure they have been called neurofibrils, neurofilaments, and neurotubules (canaliculi, neuroprotofibrils), in analogy with similar structures in neurons. Cytoplasmic filaments are, however, found also in astrocytes (34) and they seem to be a general feature of the different kinds of ependymal cells (14). Bundles of fine filaments are also seen in the coronet cells of saccus vasculosus (3). The most primitive neurons so far investigated (from Coelenterates) totally lack neurofilaments but contain neurotubules (24). If we turn our attention to chordate epidermal cells we will consistently find cytoplasmic filaments in lower vertebrates (25, 50) and even in the very primitive integument of the lancelet (40).

It is apparent that the occurrence of different kinds of tonofilament, glia filament, and neurofilament tells us nothing about the relationship of the different cell types, at least not so long as their functional significance is unknown to us. They are just structures which are common in epidermal cells and their descendants.

To sum up: There are apparently no morphological characteristics which

definitely establish certain secretory cells of neural origin as neurosecretory cells. One reason for our fragmentary knowledge on the evolution of these cells is probably the difficulties of establishing which cells are n.s. ones. This is especially the case in the several primitive conditions which may be most useful for evolutional studies. This fact forces me to consider briefly the whole complex of secretory phenomena in nervous tissue, with special regard to what can be considered as primitive conditions.

For this purpose it may be instructive to bring together, in a diagrammatic form, some cell types to which have been ascribed neurosecretory properties and to compare them with other cells of neuroectodermal origin. The accompanying, extremely simplified, drawing is an attempt to make such an comparison easier and it will serve as the basis for the following discussion.

A. This could be the symbol for common epidermal gland cells or ectoneural gland cells in the superficial nervous system of Asteroids (49) which all release their secretory products (arrow) to the surroundings of the animal. If the diagram represents the hollow nervous system of a vertebrate this symbol might as well represent a primitive ependymal, subcommissural, flexural or infundibular cell, which now releases its material into the ventricle (37).

B. Ependymal cell which transports material from the ventricle to the vessels of the basal surface (32, 48).

C. With apical release to the ventricle as well as basal release to the pia surface this cell illustrates an advanced subcommissural cell according to several authors (16, 21, 36), who ascribe to these cells the properties of discharging active principles which are usually supposed to be regulators of water balance. With the basal release of material only, as it is drawn here, it illustrates a "Stützzelle" from the ectoneural radial nerve of Asteris (49). This cell delivers its secretory product to the connective tissue surface of the nervous system from where it presumably reaches the hemal canal fluid. Changes in the sea water salinity give marked responses in secretory content in these cells.

D. A primitive preoptic cell with secretory release apically to the ventricle as well as basally to internal capillaries. Also certain tuberis lateralis cells seem to belong to this type (17).

E. A n.s. cell which sends a process to the epidermis in certain polychaetes (5) and fresh water oligochaetes (10). It is possible that the secretory material is released to the exterior (9).

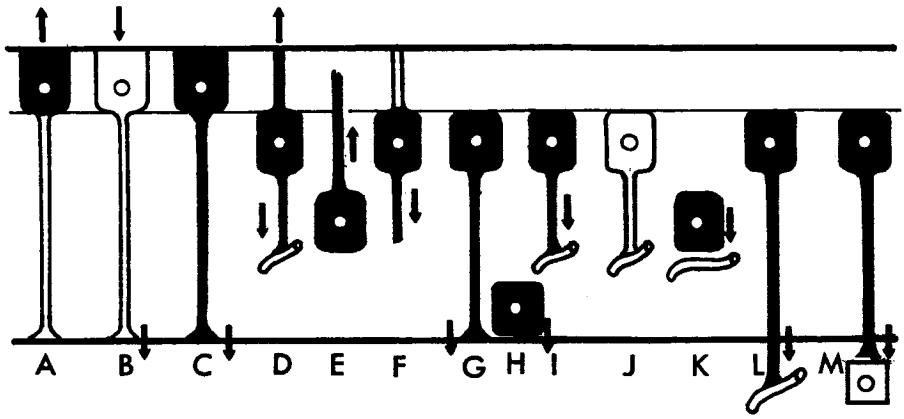
F. A n.s. sense cell as described from the ectoneural nerve system of Asterias (49) or the hypodermis of Tubifex and Nais (10). They may send processes (receptors?) to the surface and basal secretory processes which come in close contact with other n.s. cells. Vertebrate preoptic n.s. cells with ventricular receptors (12) would belong here.

G. A preoptic cell in hypothalamus of lower vertebrates or a Dahlgren cell in the caudal spinal cord of elasmobranchs (19) releasing secretory material at the pial surface of the nervous system.

H. A secretory cell closely apposed to the connective tissue sheath of the nervous system, without or with only short processes. The so-called dorsolateral n.s. cells of Asterias are described in this manner (49).

I. A very common type of n.s. cells, e.g. paraventricular, supraoptic, and urophysial cells. This is much the same as G but the release is now to internal capillaries. Also the specialized subcommissural cells (38) belong to this type.

J. Astrocyte. Several transitional forms seem to exist between ependymal



cells (B) and astrocytes (15) as well as between different kinds of macroglia (42).

K. A secretory cell without processes, directly releasing its secretory product to internal vessels. This condition has been described for some n.s. cells of Lumbricus (2) and it may also illustrate a chromaffin cell.

L. A n.s. cell which leaves the brain to reach external vessels.

M. The cell terminates in intimate relationship to the target cell.

The production of secretory substances is a most fundamental function of the primitive epidermis as well as of the neuroectodermal cells (7, 8, 37). The diagram gives some idea about the great variability which has been developed by secretory elements and there are also borderline cases between most of the types illustrated here. Furthermore, we may add to it the neurons as a group of specialized "short-distance" secretory cells.

It would, of course, be premature to try to arrange these cells, as they have been pictured here, in some evolutional series without even discussing the problem of nervous conduction.

A second outstanding feature of epidermal tissue is its ability to produce cells which may conduct nerve impulses. This is not a typical feature of neurons alone for non-neuronal conduction is found in several other cases, e.g. in ciliated epithelia, myoepithelial cells, and striated muscle cells. Its occurrence has also been suggested in glia cells (20). Different n.s. cells have been investigated in this respect several times (4) and it is likely that they may liberate their secretory products under neurohumoral control (30). We need much more experimental study regarding impulse conduction in n.s. cells. At present it seems, however, reasonable to believe that this property may exist, at least in specialized types of n.s. cells, although this may not necessarily mean that we have to consider these cells as good neurons.

It is usually not very difficult to make some wild speculation about the evolution in a field like this one where we are not hindered by too many facts. But even being aware of these dangers I think that we may trace some important stages in the evolution of the n.s. systems.

The original mode of secretory release is discharge at the apical surface as is seen in a number of epidermal gland cells. It is possible that already some cells of this type may have evolved the capacity of producing biologically active substances, telergones (27), probably as a means of controlling

sexual phenomena in aquatic populations (9). The deuterostome central nervous system is unique in usually being hollow, a fact which enables the gland cells which develop here to retain the ancient mode of apical secretion, although the secretory products are now delivered for internal use. This is the condition we find in ependymal cells, primitive preoptic cells and tuberis lateralis cells.

Superficial cells which originally release their secretory material to an apical surface or which develop in an area of such cells, may specialize for an internal release in two other ways. In the first case they may liberate their material by means of basal processes to an area of body fluids for eventual transport to the target cells. This condition is perhaps found in its most simple form in Asterias ("Stützzellen"). The superficial cells may also withdraw from the apical surface and reach the body fluid area by means of processes or the perikaryon itself. Such a transformation has been reported several times e.g. in nemerteans (44), sipunculids (1), echinoderms (49), and vertebrate ependymal cells (38). In some of these cases the transformed cells are considered as good n.s. cells.

The next step in the evolution of n.s. systems seems to be the accumulation of scattered n.s. cells into "organs". This is nothing unique. The phylogeny of some other endocrines in the vertebrates gives us the same picture of originally scattered functional units which in higher forms associate into organs. The n.s. organs are, however, never very well-defined which is a result of their peculiar mode of secretory release. Polarity in relation to capillaries is a common feature of endocrine cells, and the n.s. cells are extremes in this respect. By means of long processes and a restricted secretory release at the terminal end they may form neurohemal organs far away from the cell bodies. Such neurohemal areas, which probably permit a better control of secretory storage and release, must have been developed independantly several times during animal phylogenesis.

The last step in the development of n.s. systems is a close relationship to other endocrines. It has repeatedly been reported that n.s. processes in such systems may terminate in close proximity to cells which must be believed to be target cells of the n.s. substance. It is possible that this direct contact is a valuable specialization which was made possible by the concentration of target cells and thus made the roundabout way of the hormones through body fluids unnecessary.

According to this point of view the most specialized n.s. cells have acquired properties which make them very like secretomotor neurons. The secremotor control of the n.s. cells themselves is still very obscure. In several cases n.s. cells have been described which are believed to combine sensory and secretory properties. It is possible that such a cell may itself pick up the appropriate environmental stimuli and carry out the secretory release. In other cases n.s. cells develop in close association with a receptor organ which may indicate a functional connection between the sensory and secretory elements. It is possible that the centralization of n.s. units to "organs" ("nuclei") and their incorporation into nervous systems of increasing complexity has put them under intimate neuronal control. But still they differ from neurons in the very important respect that they cannot form synapses with other members of the same kind; they may only form the end-link in neuronal series, just as some other innervated gland cells do.

It may seem astonishing that I have tried here to discuss the evolution of n.s. phenomena in various animal forms without being able to arrive at a conclusion regarding the definition of neurosecretion. If anything, this paper must have been mainly confusing as regards the concept of neurosecretion. I feel, however, that neurosecretory cells could not been considered as neurons, nor as neuroglia; they are just neurosecretory cells. It is no longer

possible to arrive at a clear-cut definition of n.s. cells because this term includes a variety of neuroectodermal cells with the only property in common - let us hope so - that they produce biologically active substances other than neurohumors. If we give up using the term "neuron" indiscriminately for all cells which are believed to be n.s., we will also regain the possibility of defining the neuron.

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EVOLUTION OF NEUROHYPOPHYSIAL FACTORS AND FUNCTIONS.

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Information about the distribution of the family of short-chain peptides secreted by the vertebrate neurohypophysis has more rapidly accumulated than about that of the other protein hormones. Some general conclusions may perhaps already be tentatively drawn.

(1) All the neurohypophysial hormones so far discovered conform to the same chemical pattern. They are all octapeptides containing an S-S linked ring of five amino acids and a tripeptide side chain. Five of the active principles have been identified, namely oxytocin, 8-arginine vasopressin, 8-lysine vasopressin, 8-arginine oxytocin (vasotocin) and 4-serine, 8-isoleucine oxytocin (ichthyotocin or isotocin). The occurrence of at least one further related peptide can be suspected on pharmacological grounds. (2) Neurohypophysial extracts from the various vertebrate groups can usually be shown to contain two active peptides. There are exceptions: only one hormone (vasotocin) has so far been found in certain groups of fishes, and mammalian posterior pituitaries may contain three (Example: lysine vasopressin, arginine vasopressin and oxytocin in the neural lobe of peccaries and warthogs, (Ferguson, Heller, Lederis & Pickford, 1962). This statement must be made with the reservation that admixtures of small amounts of a known or unknown hormonal peptide may not have been identified when other neurohypophysial principles preponderate. (3) Vasotocin (8-arginine oxytocin) seems to be most widely distributed hormone in the phylum. It has been found in cyclostomes, bony fishes, lung-fishes, amphibians (both urodeles and anurans), reptiles and birds, and is probably phylogenetically the oldest of the neurohypophysial hormones. In mammals it is replaced by one of the vasopressins. Arginine vasopressin appears to be the more commonly occurring variety. It has been identified in many eutherians including man, but has also been found in Tachyglossus (= Echidna) (Sawyer, Munsick & van Dyke, 1960) and in several species of the Marsupialia. Lysine vasopressin seems to be characteristic for the Suiformes. Chromatographic and bio-assay results suggest that it occurs in the domestic pig, the European wild pig, the wart-hog, the peccary and the hippopotamus. All the other Artiodactyla so far investigated seem to carry arginine vasopressin and this applies apparently not only to several Families of the Ruminantia (Cervidae, Giraffidae, Bovidae) but also to the Tylopoda (both camels and lamas have been investigated).

Vasotocin or the vasopressins are usually accompanied by a second active peptide which in assays on mammals has little pressor-antidiuretic, but pronounced oxytocic activity. The

second peptide in the Actinopterygii seems to be 4-serine, 8-isoleucine oxytocin (ichthyotocin) (Heller et al., 1961; Acher et al., 1962). Interestingly enough the oxytocic principle in lung-fish pituitaries is not ichthyotocin but has the pharmacological properties of the oxytocic hormone of amphibians (Follett & Heller, 1962). The second hormone in mammalian and avian glands has been identified as oxytocin. Oxytocin may also be present in reptile neurohypophyses. The situation in amphibians is still obscure, there are hints (Morel et al., 1961; Acher, 1963) that they synthesize an as yet unidentified oxytocin-like principle but this problem, like that of the identity of the elasmobranch hormones, has to be further investigated.

The close chemical relationship of the various neurohypophysial hormones explains why they display the same qualitative effects when tested by conventional assay methods. However, quantitatively their pharmacological spectrum may vary greatly. It may therefore be inquired whether the differences in the phyletic distribution of the neurohypophysial hormones have also a functional significance. Useful information of the effects of homologeous neurohypophysial hormones has recently become available. It may be tentatively summarized as follows: Vasotocin injected into lampreys (Lampetra fluviatilis) has apparently no effect on their water metabolism, it does not produce water retention (Heller, 1963) and does not alter water uptake or modify urine volume (Bentley & Follett, 1962, 1963) but increases renal sodium excretion significantly (Bentley & Follett, 1963). Teleosts show again no antidiuretic effect, but actions of neurohypophysial peptides on renal sodium excretion (Maetz, 1963) and on the extrarenal handling of sodium (Meier & Fleming, 1962; Maetz, 1963) have been reported. Relatively small doses of vasotocin produce a reduction in urine flow in amphibians. An effect on tubular water reabsorption is clearly demonstrable in anurans, but reduction in the glomerular filtration rate is probably the main effect in urodeles (Heller & Bentley, 1963). The two main Subclasses of the Amphibia differ further in that neurohypophysial hormones increase water transfer through skin and urinary bladder in frogs and toads but not in urodeles like Triturus, Ambystoma and Necturus (Heller & Bentley, 1963). In so far as the effect of neurohypophysial hormones on their metabolism of water appears to be a purely renal one, these urodeles therefore resemble the higher vertebrates. Sodium absorption by the skin is increased by neurohypophysial hormones in both urodeles and anurans. But while urodeles kept in tap water and injected with vasotocin show a net sodium gain owing to active uptake through the skin and a decrease in renal sodium excretion, we (Heller & Bentley, 1963) failed to find a change in the overall sodium balance in frogs kept in tap water after the injection of large doses of oxytocin. Neurohypophysial hormones injected into free swimming

African lungfish (Protopterus) do not alter their water balance (Heller, 1957; Heller & Bentley, 1963), but in contrast to their terrestrial relatives the urodeles, increases sodium loss.

In reptiles small doses of vasopressin produce marked antidiuresis caused both by a decrease in the glomerular filtration rate and an increase in tubular water reabsorption (Burgess, Harvey & Marshall, 1933; Le Brie & Sutherland, 1962). Sodium reabsorption in water snakes is somewhat increased at low urine flows. Vasotocin injected into chickens produces antidiuresis (Munsick, Sawyer & van Dyke, 1960) but the mechanism of this effect has not been investigated. Since birds, unlike reptiles, are able to produce a hypertonic urine the tubular effect in this vertebrate class may be expected to be more marked.

The fragmentary evidence so far assembled suggests that throughout the vertebrate phylum the neurohypophysis is concerned with the regulation of the water and salt metabolism. But while the emphasis in the more primitive vertebrates appears to be mainly on the handling of sodium, effects on the economy of water became more prominent in animals who lead a terrestrial life. It would also seem - and much further work is needed to verify this impression - that in those animals in which urine volume is modified by neurohypophysial hormones, there is an evolutionary trend from a glomerular to a tubular mechanism of action. To what degree the emergence and disappearance of the renal portal system is linked with this development is an interesting subject for speculation.

In mammals the neurohypophysis influences not only the metabolism of water by the action of vasopressin but, by means of oxytocin, also milk ejection and perhaps parturition. It seems therefore possible that the 'oxytocic' hormones of lower vertebrates affect also reproductive functions. This problem is clearly an important subject for further comparative work.

BEHAVIORAL ADAPTATIONS OF MAMMALS TO ENVIRONMENTS

Sponsored by the American Society of Mammalogists

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by

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BEHAVIORAL ADAPTATIONS OF MAMMALS TO THE DESERT ENVIRONMENT.

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The physical environment in low-latitude deserts poses an ecological challenge of such difficulty that few kinds of mammals have been able to meet it by physiological regulation. In a desert where ambient temperatures routinely exceed the tolerable levels of body temperature, mammals and birds face a dual problem. Not only must they avoid acquiring excessive quantities of heat from the environment, they must contrive to lose to the environment the heat produced by their own metabolism. The problem is compounded by the fact that the most readily available mechanism for heat loss when ambient temperature equals body temperature is the evaporation of water, and water, by definition, is in extremely short supply in deserts.

Two primary classes of solutions to the desert-imposed problems of heavy heat load and local water scarcity are available: (1) relaxation of the limits for physiological homeostasis with regard to either thermoregulation or osmoregulation (or both), and (2) avoidance of the problems by means of behavioral adjustments. When examining adjustment to environment by an intact and normally functioning mammal, it is obviously unprofitable to maintain rigidly the man-made distinction between physiology and behavior (Bartholomew, 1958). The two, together with morphology, obviously act as a functional unit under natural conditions. It is, however, instructive to examine some of the ways in which desert mammals by behavioral adjustments can maintain themselves in situations where environmental conditions do not exceed their capacities for physiological regulation.

Despite the almost overwhelming physiological demands of a severe low-latitude desert, a mammal can escape the dilemma of too much heat and not enough water if it can wait til the sun goes down, or move a few centimeters underground. Thus, the most obvious way for a small mammal to live in the desert is to be nocturnal and fossorial. The parameters of this mode of desert life have been delineated by Bodil and Knut-Schmidt Nielsen (1952 and preceding); the effectiveness of this pattern of heat avoidance and minimal water loss in excretion, defecation and respiration, is witnessed by the large populations of rodents, and their dependent population of carnivores, which occupy all but the most severe deserts of the holarctic.

A very large mammal obviously cannot be primarily fossorial; therefore, if it lives in a desert region it cannot avoid thermal problems. The type-case of the large desert mammal is of course the dromedary camel. Knut and Bodil Schmidt-Nielsen and associates (1957 and preceding) have shown that toleration of dehydration and hyperthermia allows this species to adjust to extremely demanding conditions. By storing heat during the day and unloading heat at night it can avoid excessive dependence on evaporative cooling. Its mobility is such that it can seek out surface

water when the limits of its tolerance of dehydration are approached. This solution of heat storage during the daytime and periodic drinking is of course possible only for a large mammal.

The scarcity of small diurnal desert mammals may be considered as an indication of the difficulty of the problems they face. The rapidity of their metabolic turn-over, their inability to seek out surface water, and their small size and consequent limited capacity for heat storage have forced them to a precarious, behaviorally maintained balance between physiological capacity and environmental stress.

The antelope ground squirrel, Citellus (Ammospermophilus) leucurus is a case in point. Hudson's (1962) detailed study has shown that the success of this small (85-100 gram) diurnal rodent cannot be attributed to any single striking specialization to desert life, but rather to a complex of physiological and behavioral adaptations which together allow it to cope with its difficult physical environment. Its key capacities appear to be (1) physiological and behavioral acceptance of hyperthermia, which contributes to the existence of (2) a broad zone of thermal neutrality with no sharp upper critical temperature, (3) dependence on salivation instead of panting under conditions of severe heat stress, which minimizes the metabolic increment to its heat load, (4) omnivorous eating habits which allow extensive carnivory, with an attendant acquisition of water, and (5) a pattern of activity characterized by frequent retreats underground in the daytime which allows it periodically to unload heat acquired during its surface activities.

The mohave ground squirrel, Citellus (Xerospermophilus) mohavensis shares a number of attributes for desert life with C. leucurus with which it is sympatric in the Mohave desert of California, but its primary adjustment to heat and aridity is one of complete withdrawal. It both aestivates and hibernates and thus faces the desert environment only during the least demanding parts of the year (Bartholomew and Hudson, 1960,1961).

A striking feature of the behavior of rodents of the family Heteromyidae, members of which are pre-eminently successful in the desert regions of North America, is their capacity to undergo brief periods of dormancy during which body temperature falls to within 1 or 2°C. of that of their surroundings. These periods of torpor can occur over a range of ambient temperatures from 5°C. to 30°C. and at any time of year. (Bartholomew and Cade, 1957; Bartholomew and MacMillen, 1961; Tucker, 1962). Such brief, and often daily, periods of torpor afford an obvious saving of metabolic energy. Tucker has shown that the California pocket mouse, Perognathus californicus, will maintain a regular cycle of daily torpor indefinitely if its food ration is reduced slightly from its normal ad libitum consumption. The duration of daily torpor is inversely related to daily food ration. The mouse adjusts its periods of torpor to the food available; the smaller the food

ration the longer the period of torpor. Such a behavioral response allows a granivorous desert mammal to make nice adjustment between its food supply and its energy expenditure. R. E. Carpenter (unpublished) has found a similar pattern of response in a kangaroo rat, Dipodomys merriami. He points out that in deserts because of erratic rainfall and the consequent irregular seed production, seed-eating rodents are less apt to be faced with complete seasonal absence of food than with chronic shortages of food which may last for months or even several years. Daily torpor, the occurrence and duration of which is determined by availability of food, would allow a desert rodent to maintain itself for prolonged periods on a reduced food supply.

Relatively few small and middle-sized diurnal mammals other than rodents have successfully met the challenge of the intense heat of the desert. Among this small company, the jackrabbits, Lepus californicus and Lepus alleni, are conspicuous. Although the mechanism which allows these animals to remain above ground throughout the long desert day has not yet been determined in quantitative terms. Knut Schmidt-Nielsen (unpublished) has proposed an intriguing theory. He suggests that the large, highly vascular ears of jackrabbits radiate body heat to the sky which on a clear dry day may be as much as 25°C. below the animal's body temperature. If a jackrabbit crouches in a depression in the shade of a shrub or a rock, it is screened from solar radiation and radiation from the hot surface of the ground. Even if air and body temperature were both at 40° the animal could get rid of its metabolic heat by radiative loss from its ears to the sky and thus avoid dependence on evaporative cooling--only a large mammal can afford to depend to an important extent on evaporative cooling.

A mammal that can accept hyperthermia instead of mobilizing its resources for heat dissipation, not only avoids using its limited water resources for evaporative cooling, but also avoids increasing the metabolic component of its total heat load. The hyperthermia can also be self-limited, for an elevated body temperature places an animal in a more favorable condition to lost heat by radiation and conduction. Thus, behavioral acceptance of hyperthermia is an important asset for any mammal in the desert and appears to be one of the attributes found in a number of taxa which occupy desert regions. A striking example is offered by a pair of Australian flying foxes Pteropus scapulatus and Pteropus poliocephalus, both of which spend their days roosting in exposed positions in the tops of trees. The former occurs widely in the arid interior where daytime temperatures above 40°C. are commonplace, while the latter occurs only in the more moderate coastal regions. Pt. scapulatus is better adjusted behaviorally to hot conditions than is Pt. poliocephalus. Its upper critical temperature is no higher than that of the latter species, but it remains undisturbed by several degrees C. of hyperthermia and does not fully mobilize its mechanisms for heat loss (wing-flapping, covering of vasodilated wings with saliva, and panting) until body temperature approaches 41°C. In contrast, Pt. polio-

cephalus initiates these same responses when its body temperature approaches 39°C. (Bartholomew, Leitner and Nelson, 1963).

It is apparent, even from the limited number of examples mentioned here, that knowledge of behavior offers an indispensible key to understanding the ways in which diverse types of mammals have adjusted themselves to the unique difficulties presented by the desert environment.

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АДАПТИВНЫЕ ЧЕРТЫ ПОВЕДЕНИЯ МЛЕКОПИТАЮЩИХ СТЕПНОЙ ЗОНЫ ЕВРАЗИИ. А.Н. Формозов, Институт географии Академии наук, Москва, СССР.

В этой "Евразиатской степной области" обитает 31 вид типичных степных млекопитающих (эндемов зоны), 14 видов, водящихся в степях и пустынях, 14 видов, населяющих только горные степи и горные тундрово-степные местообитания и 30 видов из числа убиквистов и видов, проникающих в степи из лесной зоны (всего около 60 видов).

Степи благоприятны лишь для немногих "жизненных форм" млекопитающих. Из свойственных аридным и семиаридным областям Евразии 10 биологических групп (Formosov , 1956) 4 представлены в степях малым числом видов, распространенных спарадично и по существу не характерных для зоны.

Группа "Травоядные одиночные и колониальные, оседлые и полуоседлые обитатели нор, деятельные в течение всего года" виды (Microtus , Lagurus , Ochotona и др.) и близкие к ним по характеру питания "Травоядные одиночные и колониальные норники, впадающие в длительную спячку" - резко преобладают по численности населения в степях. Характерны также для зоны "Подземные роющие активные весь год потребители корней"(Spalax , Myospalax , Ellobius) и "Стадные крупные травоядные=номады, не имеющие постоянных убежищ" (Saiga , Gazella , Equus).

При относительно большом сходстве погоды летнего периода на всем пространстве степей Евразии очень резки различия зимнего режима западной, средней и восточной части зоны. Они обусловливают резкие отличия поведения представителей одной биологической группы или даже близких видов, обитающих в разных районах степей.

BEHAVIORAL ADAPTATIONS OF MAMMALS TO MOUNTAIN ENVIRONMENTS.

Richard D. Taber and Robert S. Hoffmann, Montana State University, Missoula.

Mountains occur at all latitudes. They are peculiar in consisting of steep, broken topography, but may (especially in Central Asian Highlands and Andes) include large upland alpine areas of gentle contour. Characteristic elements of the mountain environment are instability due to lightning fires, land-slips, avalanches, wind-throw and erosion; meterological extremes such as strong insolation, radiation, and wind---great diurnal temperature extremes and great extremes between sun and shade; seasonal regularity; well-landmarked for orientation and learning; and close juxtaposition of strong contrasts due to changes in altitude, aspect and shading, and differences due to air-drainage, soil and snow accumulation, etc.

Winter extremes are critical for mountain mammals in arctic and temperate areas, and elicit pronounced behavioral responses. Small mammals do not have the capacity to move long distances and so winter where they summer--they are permanent residents. Their adaptations for wintering, mostly instinctive, involve taking shelter in a nest or under snow or soil, and living on stored food or fat, or else foraging beneath the snow. A few, such as the hare and porcupine, do not take permanent shelter, but are protected by fur and/or fat; their feeding behavior not much modified.

Because of the sharp environmental contrasts within a short compass to be found in the mountains, many other mammals seek optimum environmental conditions through movement. Seasonal movements (migrations) are characteristic of the larger mountain herbivores and their predators. Migratory patterns are apparently learned by the young during the first cycle of movement; an element in this learning is directional, as shown by transplant experiments.

A striking aspect of the behavioral responses of mammals in mountains is their wide range of adaptability to various habitat conditions over a large geographic range. This is coupled with a pronounced restriction of some species to specific habitats in portions of their ranges in response to the presence of closely related species. This is exhibited by a number of sympatric species groups, such as *Eutamias amoenus*, *E. ruficaudus* and *E. minimus*. Mountain mammals are thus both adaptable to a wide range of environmental conditions (broad niches), and highly adapted specifically to limited habitats (restricted niches), depending upon their behavioral responses to closely-related competitors. This results in clear-cut interspecific behavioral hierarchies in certain montane species. Absence, or removal, of a dominant species in a montane fauna may then result in a change in the behavior and niche ecology of other species in the fauna.

BEHAVIORAL ADAPTATIONS OF THE COATI (NASUA NARICA) IN THE PANAMA RAIN FOREST. John H. Kaufmann, Museum of Vertebrate Zoology, University of California, Berkeley, California, U.S.A.

Coatis belong to the family Procyonidae, and Nasua narica ranges from Arizona to the Isthmus of Panama. Within this range it inhabits successfully almost every terrestrial habitat, though it seems to be most abundant in the tropical rain forest. There, females and young males live in bands of 5-20 members, while adult males are solitary. My two year study of the coati was done at the Smithsonian Institution's research station on Barro Colorado Island in the Panama Canal Zone. Direct observations were made of wild coatis, and most of the data were obtained from five bands and several adult males which were followed intensively for periods of up to nine months.

Barro Colorado lies at 9° 10' north latitude, where there are approximately twelve hours of daylight throughout the year. It is a tropical rain forest locality, with an average of 107 inches of rain per year, and extremes of 75 to 145 inches. There is usually a well marked dry season from January through April, with maximum rainfall in October and November. There is much variation in rainfall from year to year, with wetter periods of about four years alternating with drier ones of about two years. The temperature varies from about 75°F. at night to 85°F. during the day, with extremes of 70°F. to 90°F.; there is no annual variation in temperature.

Barro Colorado is almost completely forested with trees up to 150 feet tall, lianas, and large herbaceous plants in the understory--over 1000 species of spermatophytes on an island of six square miles. There are several fairly distinct strata from the ground level to the canopy of the tallest trees, providing a great abundance of ecological niches. A succession of fruits is available throughout the year, but fruit production is concentrated in the dry season.

Barro Colorado has naturally established, self-maintaining populations of most of the animals native to the region. A wide variety of invertebrates and small vertebrates is available to the coatis as prey, while many species of birds and mammals compete with them for food. Potential predators include large birds of prey, snakes, and several species of carnivorous mammals. Of the numerous external parasites, ticks and bot flies are most conspicuous.

The coatis' day began about ½ hour before sunrise, when they descended from one of the roost trees scattered throughout their home range. They foraged throughout the day, with perhaps one or more brief periods of resting and grooming. About ½ hour after sunset they ascended into a roost tree for the night.

The coatis' chief year round source of food was the invertebrate fauna of the forest floor, including worms, millipedes, arachnids, insects, land crabs, and snails. Vertebrates were only incidental items in the diet. Of those taken, lizards were the most common; a few caecilians, frogs and mice were also eaten. The coatis ate fruit whenever it was available. Fruit

was especially important during the dry season, when single species temporarily became the most important item in the diet. Water is never lacking in the rain forest, and the coatis paused frequently to drink from streams and pools of rain water. They located most of their food with their excellent sense of smell, and could pinpoint small insects two feet beneath the ground or several feet above their heads. They sniffed along, pausing frequently to dig small pits in search of invertebrates, and occasionally to dig holes up to three feet deep in search of taran tulas and lizards. They also used their strongly clawed front feet to rip apart rotten logs and stumps, wild pineapple plants, and small palms. A band of foraging coatis is rather noisy and unalert, which probably accounts for their lack of success in catching many vertebrates. They have not evolved the efficient hunting techniques of more specialized carnivores such as the cats. Coatis are skillful climbers and get much of their fruit directly from the trees. They are aided in climbing by their sharp, strongly curved hind claws, and their long tails which serve as excellent balancing rods.

With their varied diet, the coatis competed for food with many birds and other mammals. The most conspicuous of these competitors were the monkeys. Howlers eat mostly leaves, but also much fruit, and were frequently seen feeding in the same trees with coatis. The two species usually ignored each other. White-faced monkeys, however, eat more fruit, and this was the only species that was seen to go out of its way to make contact with coatis, usually chasing them from the fruit trees. Agoutis and brockets eat fruit from the ground; peccaries eat fruit and also root in the litter; armadillos dig small pits in search of invertebrates; tayras and squirrels eat fruit both from the ground and from trees. Encounters were seen between all of these species and coatis, and in almost every case they ignored each other.

Just as the coatis were not very important predators on other vertebrates, they did not seem to suffer much from predation. Ocelots, pumas, large boas, hawks, and owls probably concentrate on more easily available prey such as rodents. No attempts at predation on coatis were observed, but coatis do have a number of typical alarm reactions. They have a distinctive alarm grunt, and also respond to the alarm calls of other species such as agoutis and squirrels. An alarmed coati usually dashes a short way up a tree, freezes until the source of alarm is identified, then jumps down and runs off. A very effective reaction is the scatter-and-freeze pattern. A band of coatis suddenly alarmed explodes in all directions. An instant later, every coati seems to have disappeared. They may be clinging to tree trunks or standing on the ground only a few feet away, but so neatly is the maneuver performed by the entire band that they seem to have vanished.

The Barro Colorado coatis had a definite annual cycle of social relations, in which the nonbreeding season occupied approximately the second half of the calendar year. The bands of females and young males were loosely organized, having no leader and no dominance hierarchy, and frequently breaking up into subgroups which remained apart for several hours or even a day or two. There are

some advantages, however, to living in even such loosely organized groups. Mutual grooming serves to reduce the infestations of external parasites, to promote the healing of wounds, and to help preserve the band structure. All members of a band benefit from the alarm signals, and young coatis have an increased chance for survival when their mothers are absent or dead, since they receive care from other members of the band. Also, more prey such as lizards is caught when forced to run the gantlet of an entire band rather than having only to escape from a single coati. Coatis have a full repertoire of signals for communicating with each other, most of them serving to achieve the adaptive ends of hostility without the physical risk involved in actual fighting. Vocal and olfactory signals seem to be more important than visual ones.

Bands were usually slightly hostile to other bands when they met, and considerably more hostile to solitary adult males. Adult males were also hostile to each other. All of this hostility lessened after repeated contact, and large aggregations composed of several bands and several adult males were often seen feeding peacefully together in and under large trees in fruit.

Each band studied had a definite home range of about 100 acres. Within each home range was a smaller core area which received heavy, consistent use throughout the year. The peripheral areas of the range were visited only infrequently except for short periods while certain fruits were available there. Almost all of the roost trees were located in the core areas, and each band spent about 80% of its time there. The home ranges of different bands overlapped, but the core areas did not. Encounters in the core area of one band were no more hostile than encounters in the overlapping peripheral areas.

The adult males also had home ranges, but they tended both to wander and to settle down at especially good food sources more than did the bands. The home ranges of the males overlapped those of other males, and also those of the bands.

The home ranges of the bands and males were not territories in the strict sense, since they were not defended. But the pattern of home range use, especially the use of core areas, achieved effective spacing of the population in a relatively homogeneous habitat.

The breeding season began with a mating period of about one month early in the dry season. During this period, one adult male joined each band. He traveled, groomed, fed, and slept with the band, and mated with the females; however, he remained completely subordinate to the females. Other males that tried to approach the band were driven off by the male with the band, or by the females. After the mating period the male left the band.

About one week before the end of the ten week gestation period, the pregnant females became hostile to the nonbreeders in the band and left to become temporarily solitary. During the following week they foraged in a restricted area of the home range, and built a series of tree nests. Most were small and flimsy, but toward the end of the week a larger, stronger nest was built, and it was here that the young were born.

For about six weeks the young stayed in the tree nests. During this time the mothers spent about ½ the day foraging, and ½ in the nests caring for the young. These females were not hostile to other mothers when they met, but were hostile to the nonbreeders in the band, and to adult males. When the young were able to climb well and more or less keep up with the band, they were brought down from the nests and the band reunited. Some initial hostility was shown to the nonbreeders in the band, but this disappeared in a few days. The males which had just reached their second birthday were becoming more and more independent, and within a month or two they left and became solitary adults. For the first few weeks after the young left the nests, the band spent about ½ the day foraging and ½ in trees resting, grooming, and nursing the young. Females refused to groom or nurse their young on the ground, and were receptive only in the comparative safety of the treetops. As the young grew stronger and better able to keep up with the band, the time spent in the trees decreased and the routine of the nonbreeding season was established.

The coatis on Barro Colorado took full advantage of the food and safety available at all levels of the forest, and maintained a unique social system that offered the advantages of group living and effective spacing, yet was flexible enough to allow them to take advantage of especially fine concentrations of food. Probably the principal reason for the coatis' success, not only in the tropical rain forest, but in other habitats as well, is the very flexible and opportunistic nature of their behavior.

TERRITORIALITY AS A BEHAVIORAL ADAPTATION TO ENVIRONMENT IN UGANDA KOB. Helmut K. Buechner, Department of Zoology, Washington State University, Pullman, Washington, U. S. A.

Territorial behavior in Uganda kob, Adenota kob thomasi (P.L. Sclater), an African antelope, may function as a mechanism to space out the herds and prevent overuse of the forage supply, A physiographically confined population of about 12,000 kob (estimate based on 2 aerial counts) in the Toro Game Reserve, western Uganda, appears to be orientated around 15 territorial breeding grounds distributed throughout 400 km^2 of habitat. A territorial ground consists of a group of small, more or less fixed territories defended by males. Females enter the ground to breed.

The environment is limited eastward by the Albert Escarpment (800 m), westward by cattle grazing along the Semliki River and ultimately by an escarpment in the Congo Republic, southward by broken country toward the Ruwenzori Mountains (5,588 m), and northward by marshes along Lake Albert.

The Reserve is located about 1° N of the equator where the days are of equal length throughout the year. Night temperatures average about 21° C and mid-day temperatures about 31° C. Rainfall is about 90 cm annually and falls mostly in thunderstorms during the "long rains" from April through June a d the "short rains" in September and October. Sufficient rainfall occurs during the dry season from January through March to produce excellent growths of grasses.

Vegetation consists of an open, sparsely wooded grassland. Most of the grasses are perennials achieving a height of 1 to 1.5 m. The vegetation is burned over in a patchwork pattern during the long dry season, and the driest areas are burned also in July. Regrowth is so rapid, even without rain, that the burned areas become green within a week. The pattern of burning and rainfall results in a year-long supply of fresh, growing grasses.

Under the favorable conditions of climate and vegetation, the kob breed throughout the year.

In response to good visibility and an abundance of short, fresh grass, the kob spread out widely during the dry season. With the onset of the long rains, the animals concentrate around the territorial grounds where they are able to keep the grasses grazed down for some time. Ultimately, growth of the grasses is so rapid that close cropping cannot be maintained. Reburning in July produces new grass on which the kob immediately concentrate.

The numerical preponderancy of Uganda kob compared with the six other ungulates in the Reserve is striking, and the high density may be essential for the well-developed system of territorial grounds. In the Queen Elizabeth National Park, Uganda, 130 km

south and in the Albert National Park, Congo Republic, 200 km south, where the densities of the populations are about one-fourth as great, territorial behavior is not as well defined as in the Reserve.

Territorial Grounds

The requisites of short grass, good visibility in most directions, and proximity to permanent water and soil licks, together with social convention, seem to limit the number of territorial grounds. Usually the grounds are located on a ridge, knoll, or slightly raised area, but three of them are situated on a relatively low plain about 8 km from the lake. Seven of the 15 grounds have existed within 300 m of their present location for at least 30 years, according to an African Game Guard who has lived in or near the area all his life.

Small, temporary territorial grounds occasionally form, indicating constant pressure to space out the population.

The average ground is occupied by about 15 males in a central area of concentrated activity and up to twice as many on peripheral territories. Within the central area, which is about 200m in diameter, individual more or less circular territories vary from 10 to 50 m in diameter. Usually the territories can be recognized by a central area of closely cropped grass on heavily trampled ground and boundaries clearly demarcated by longer, less grazed grass. The boundaries are less distinct on nearly bare ground where most of the mating occurs. Peripheral territories and many of those in the central area of activity remain more or less permanent over a period of months, whereas territories in places most frequented by females may change in number, size, shape, and position, depending upon the number of males involved.

Territorial Behavior

Territorial behavior in Uganda kob has been described and documented by film (Buechner, 1961). Briefly, a male in possession of a territory defends his boundary against neighboring males through ritualized display. If a neighbor approaches his boundary, the male walks threateningly toward him with lowered, posteriorly directed ears. Often this behavior is sufficient to reaffirm the boundary. If not, the males turn their heads back and forth slowly prior to abruptly lowering the heads to fighting position. Usually they feign without clashing horns, but occasionally brief contacts ensue.

Interchanges of males occur through highly ritualized fighting when a male dashes in to contest a territory. Ears are laid back, phalluses are erected, and tails wag rapidly. Heads are lowered and the kob spar with their horns in vertical position, using

very quick movements, following which the heads are jammed together with the rostrum on the ground and the horns interlocked in horizontal position. Tails are tightly appressed when the heads are rammed together. The males push and twist in the locked position, frequently exchanging position by turning 180° and then resuming their pushing. Often the horns are locked over the neck and twisted, placing the males nearly in a side-by-side position. When a contestant eventually tires, he breaks away, turns, and runs with the victor in close pursuit for 50 to 300 m. A third male may run in to break up a fight by butting his horns in the side or hind quarters of one of the combatants, sometimes inflicting severe wounds. Heavy fighting lasts from 1 to 20 minutes.

Females enter the territorial ground for the purpose of mating. They move freely between territories on a given ground, and often concentrate in favorite territories. If a female leaves a territory and enters an adjacent one, the first male stops at his boundary and permits the neighboring male to take over the female without attempting to fight.

Orientation to Territorial Grounds

The extent to which individuals are attached to a particular territorial ground can be seen from a tabulation of distances at which reobservations were made. Using succinylcholine chloride as an immobilizer, 147 adults (104♂; 43♀) were marked between July 1962 and April 1963. In addition, 34 fawns were caught by hand. The study was centered primarily around three territorial grounds (TGs 3, 6, 7) each separated by 3 to 5 km. About 90% (133) of the adults and 71% (22) of the fawns were reobserved. The observations are tabulated in distances from the territorial ground or its vicinity where the animal was caught originally. Of the 2,173 observations, 97% were within 1 km of the original ground. This percentage is somewhat biased by the fact that many of the males were captured and reobserved on or near the territorial grounds. However, leaving out observations at the territorial grounds, 95% of the kob were still seen within 1 km of their original ground.

Number of kob	TG	Distance (km) from original TG 0.5	1	2	3	4	5	Total
97 ♂	802	862	163	19	6	3	7	1,862
38 ♀	43	129	56	9	7	1	7	252
20 fawns	0	15	31	9	1	3	0	59
155	845	1,006	250	37	14	7	14	2,173

Shifts from one territorial ground to another were noted in 5 males and 5 females. In three cases the male was territorial. Two of the territorial males were on central territories, each for two days, and one occupied various peripheral territories on and off for a period of three months. Male Number 6 from TG 7, for example, was captured out of a large herd 1 km from TG 7 on 10 September and was seen within $\frac{1}{2}$ km of his original territorial ground on 26 occasions and within 2 km on two occasions through 23 January. On 8 March he appeared on a central territory of TG 6 where he remained for two days, after which he returned to the vicinity of TG 7 where he was observed twice.

The greatest distance between points of observation for a male involved Male Number 25 from TG 6. He was captured $4\frac{1}{2}$ km NNE of TG 6 on 21 January and was seen on 21 February 2 km SSW of TG 3, a distance of 10 km. A week later he was in the peripheral zone at TG 6 where he remained for one day.

In 1959, only one of 50 tagged territorial males was observed at another territorial ground. He was not territorial.

Males not on territorial grounds usually join bachelor herds that move in the proximity of a territorial ground. Some serve as herd males for groups of females and young; others lead independent lives. The locations of 12 independent males could be predicted within 200 m or less over periods of up to six months. Three of these same males often took charge of 20 to 30 females, but they were more attached to the ground than the passing herd of females.

Although 90% of the reobservations of females were within 1 km of the original territorial ground, it seems that females may be somewhat less restricted to the vicinity of a particular ground. Of the five females that shifted from one territorial ground to another, one appeared in estrus at a ground 5 km from the original one at which she was captured. The longest movement was recorded on Female Number 2 of TG 3. From the immediate vicinity of TG 3 she moved to 1 km NE of TG 6 on 1 February and returned sometime within the following week. A month later she was observed 4 km SSW of TG 3, making a total distance of 9 km between the most widely separated points at which she was seen.

Discussion

Territorial behavior undoubtedly has several functions in the life of the Uganda kob, including: (1) stimulation to breed, (2) maintenance of high natality to compensate for high mortality imposed by predation, (3) ensurance of the best genetic combinations, and (4) prevention of overuse of the forage supply by spacing out the herds. Only the function of spacing is considered here.

Social organization of the population in orientation to territorial grounds is evident from: (1) close attachment of individuals to a given territorial ground and its vicinity; (2) self-restriction of individual males to a selected area away from a territorial ground; (3) movements of herds in patterns related to a territorial ground; (4) temporary formation of small territorial grounds; and (5) convention in maintaining large, permanent territorial grounds in the same locations over a period of many years.

Apparently one consequence of the well-defined social order is subdivision of the population into more or less concrete units associated with the territorial grounds. Social and genetic continuity is maintained in the whole population through a low percentage of individual shifts from one territorial ground to another. Distribution of the territorial grounds throughout the environment results in spacing out the population. As a spacing mechanism, territorial behavior may represent a behavioral adaptation of a high-density population to a confined environment. The survival advantage achieved seems to be prevention of overuse of the forage supply, as suggested by Koford (1957) for territorialism in the vicuña.

During the period from 1957 to 1963 the population of kob remained relatively stable, according to aerial counts made in May 1958 and October 1961; and the vegetation showed no evidence of significant deterioration. At some territorial grounds there is evidence in the composition of the vegetation that cattle overgrazed local areas prior to 1924 when the area was closed to grazing and habitation because of rinderpest and sleeping sickness. However, it is evident at the present time that fresh, growing forage of a variety of excellent grasses is available to the kob throughout the year. The interrelated patterns of burning, rainfall, and distribution of the animals through territorial behavior provide an ideal adjustment between the population of kob and the vegetation in the Reserve.

Acknowledgements

Field research was supported by grants from the National Science Foundation. I appreciate the cooperation of the Uganda Department of Game. Aerial counts were made by W.M. Longhurst, University of California. Walter Leuthold, University of Zürich, assisted greatly in the field. My wife, Jimmie, helped with the study from its inception.

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BIPEDAL ARMED FIGHTING IN CHIMPANZEES. A. Kortlandt, Zoological Laboratory, Plantage Doklaan 44, Amsterdam-C, The Netherlands.

(1) Data on the influence of habitat on the occurrence of "humanoid" behaviour in non-human Primates were collected by means of questionnaires, interviews and experimentation. They include reports from 145 respondents on (incipient) weapon-use and (incipient) predatory behaviour by non-human Primates in captivity, reports from 90 respondents on similar behaviour and on tool-use in the wild, and film analysis of certain experiments conducted by the author.

(2) The natural varieties of weapon-use observed in the great apes are: (a) aiming and throwing of objects, (b) clubbing with large sticks, (c) dropping of objects from trees. Stabbing is outside the normal behaviour inventory. According to zoo and field data, the development of effective agonistic throwing and clubbing, in phylogeny as well as in individual life, appears to depend on a semi-terrestrial life in a partially open habitat, i.e. in wooded savannas and similar types of landscape.

(3) In chimpanzees, agonistic throwing and clubbing seem to be primarily a semi-instinctive defence reaction against non-conspecific natural enemies. Savanna-dwelling chimpanzees that have been captured as sub-adults use clubs to attack a leopard when one is shown to them. It seems logical that such forms of armed fighting would develop only in habitats where arboreal avenues of escape are often unavailable, and where there is sufficient room for such weapons to be used effectively, i.e. in savannas and park-like environments.

(4) When agonistic throwing and clubbing are performed with full intensity, by adult and sub-adult chimpanzees (*including those of forest origin*), and probably also by adult gorillas, these activities are always carried out while standing, walking or running bipedally; i.e. in a posture that is very appropriate to a savanna habitat, but very impractical in a forest habitat.

(5) Thus we have to assume that the chimpanzee and the gorilla, which are by now almost exclusively semi-terrestrial forest-dwellers, have descended from more human, eurytopic and partly savanna-inhabiting ancestors, which were forced to retreat almost entirely into the rain forests when the early hominids started to use stabbing weapons, particularly the spear. This explanation and its implications may be designated as the "dehumanization hypothesis" of ape evolution.

(6) This hypothesis is supported: (a) by ecological parallelism of the evolution of agonistic true throwing in lower Primates, particularly baboons, (b) by ecological parallelism in the development of predatory behaviour

by baboons, chimpanzees and australopithecines in Africa east of the 29° meridian, (c) by the fact that a forest habitat seems to effect a retardation of the general behavioural development of infant chimpanzees, to suppress their vocal communication and to curb the tendency towards the development of social organisation in mammals in general.

(7) Finally, the dehumanization hypothesis on ape evolution may explain why the great apes did not create a more humanoid type of culture; in spite of the fact that their brain capacity is almost equal to that of the australopithecines, and in spite of the fact that, in captivity, they often spontaneously invent or imitate human technological and cultural achievements.

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INVERTEBRATE CHEMORECEPTION

A symposium organized for the
XVI International Congress of Zoology

by

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ON THE MECHANISM OF THE GLUTATHIONE-RECEPTOR OF HYDRA LITTORALIS.

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The feeding response of some coelenterates is known to be stimulated by juices from their prey. Thus far trace amounts of two biologically ubiquitous small molecules have been shown to activate a feeding response in some coelenterates: Loomis has shown reduced glutathione to activate feeding in Hydra littoralis and Fulton has shown proline to act similarly on Cordylophora. This report concerns the activation of the feeding reflex in Hydra littoralis by reduced glutathione. The goal of this study is to understand the mechanism by which a simple peptide can activate its complementary receptor to set off a coordinated biological response.

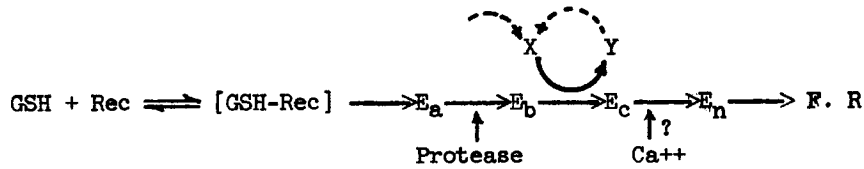
The hydra-glutathione system offers many experimental advantages for the study of a chemoreceptor: 1) The action of the excitatory substance can be studied on an intact live organism. 2) Hydra can be grown in a relatively simple, rigidly controlled, fluid environment. 3) The response is initiated by a specific compound, the activity of the glutathione residing in the specific structural characteristics of its peptide backbone; the response is not dependent upon the generally known reducing properties of this molecule. 4) Glutathione is readily available in the pure form, and has many analogues. 5) The response to glutathione can be determined simply and accurately by visually measuring the duration of the mechanical process of mouth opening.

The hydra used in these experiments are genetically alike, are in the same stage of development, come from logarithmically growing cultures, and have been grown in a rigorously controlled and defined environment. Accordingly, we always had available large numbers of hydra responding to glutathione nearly in synchrony. A quantitative and reliable assay of the feeding reflex was thus made possible.

The following information has already been gathered about the glutathione-activated chemoreceptor system: The receptor-effector systems are probably localized in the area immediately around the mouth and on the tentacles. The activity of the glutathione resides in the size and configuration of the γ-glutamylalanylglycine backbone of the tripeptide. At pH 6.2 concentrations of glutathione greater than 5×10^{-5} M activate all of the receptor-effector systems. The concentration of glutathione eliciting a half maximum response at this pH is 10^{-6} M. In order for a response to take place, the glutathione must be constantly present at the receptor site. The association of glutathione with the receptor is rapidly attained; the affinity of the receptor for glutathione is high. After glutathione combines with the receptor, o.5 minutes are needed for all the events necessary for mouth opening to occur. Once the reflex begins, it continues for 25 to

35 minutes. The response does not stop because of any alteration in the glutathione molecule, but rather because of some inherent property of Hydra. The duration of the response is probably directly related to the conversion of some limiting substance X (arginine phosphate?) to its product Y. Lowered temperatures increase the duration of the feeding reflex, probably by decreasing the rate at which the supply of X is exhausted. It takes about 24 hours for X to be resynthesized either from Y or anew. In the absence of glutathione the response can be stimulated by certain proteases. The protease probably acts before the step involving the consumption of X. Furthermore, the presence of small amounts of calcium ion in the medium surrounding the Hydra are required in order that a response may occur. The calcium appears to be involved in steps activated by either glutathione or the protease.

The following figure gives a schematic outline of the glutathione receptor-effector system as summarized above:



Rec represents the receptor; E_a, E_b, E_c and E_n, enzymes; X, the limiting substance; Y, its metabolic product; and F. R., the feeding reflex.

The extent of the feeding reflex could be expressed as either the average time during which the mouths of the experimental hydra remained open in response to glutathione, or as the reciprocal of the interval between the moment the hydra were placed in the glutathione solution and the time the mouths opened. A plot of either of the measurements of the feeding reflex against molarity of reduced glutathione gives a curve nearly identical to an enzyme saturation curve except that (1) a true saturation is reached, and (2) the curve does not intersect the origin, deviating from a true saturation curve at glutathione concentrations presumed to be "below threshold."

In order to get a more reliable index of the feeding reflex, and in order to determine unequivocally the constants associated with the curve, it is possible to derive a series of equations from the assumption that the feeding reflex is initiated by the combination of glutathione with the receptor.

In more general terms, given an activator, A, and a receptor, R, the measurable effect $\mathcal{E}$ (in this case duration of mouth opening, or the reciprocal of the time it takes for the mouth to open) is a reliable measure of the combination [AR]. The dissociation constant of the activator with receptor is called K_A.

$$\text{i.e.} \quad A + R \rightleftharpoons AR \longrightarrow (?) \longrightarrow \mathcal{E}$$

By then assuming that the maximal effect $\mathcal{E}_M$, is proportional to the total number of receptor sites, R_T, available, and that $\mathcal{E}$ is proportional to the number of receptor sites in combination with the activator, i.e. AR, equations similar to the Michaelis-Menton equation can be derived. However, since the feeding responses "below threshold" were not proportional to the glutathione concentration, a different equation was derived to express the data which "placed the greater emphasis" on results obtained above threshold concentrations of glutathione. This was done by taking the reciprocal of the Michaelis-Menton type equation, and multiplying through by the concentration of A. The equation derived is:

$$\frac{(A)}{\mathcal{E}} = \frac{(A)}{\mathcal{E}_M} + \frac{K_A}{M}$$

This equation is analogous to the second form of the Lineweaver-Burke equation and to an equation developed by L. Beidler for a mammalian taste chemoreceptor. When $(A)/\mathcal{E}$ is plotted against (A), a straight line is obtained at all concentrations of glutathione above threshold. The slope of the line is $1/\mathcal{E}_M$, and the intersect is $K_A/\mathcal{E}_M$. Thus, the K_A can be accurately determined.

Unlike data published in the Lineweaver-Burke and Beidler plots, at low (sub-threshold) concentrations of glutathione the curve swings asymptotically upwards. This deviation is repeatable, and gives indication of being a quantitative index of threshold.

Using this method of expressing data, it is possible now to study changes of the K_A under different experimental conditions, and to determine K_A's of analogues, K_i's of inhibitors, and K_A's and K_i's for essential and inhibitory metallic ions respectively.

A detailed description of these methods, and results concerning changes in K_A, K_i, and $\mathcal{E}_M$ with pH for glutathione, some of its analogues, and an essential cofactor (Ca^{++}) will be presented. The results will be discussed in relation to ionizable groups on the receptor-effector involved in the binding of these substances, and in relation to the possible role of these groups in the mechanism of action of the receptor.

ASPECTS OF CHEMORECEPTION IN CRUSTACEA. Michael S. Laverack, Gatty Marine Laboratory and Department of Natural History, St. Andrews, Fife, Scotland.

The majority of published work on chemosensory functions of crustacea has been concerned with behavioural aspects of the subject. It has been suggested that at least some of the behavioural responses described may be ascribed to stimulation of highly sensitive mechanoreceptors due to the method of substance presentation, rather than stimulation of chemoreceptors. The work of Hodgson (1958) and Case & Gwilliam (1961), however, has shown that chemoreceptors are present on the appendages of several crustaceans and that these can be stimulated by application of appropriate chemical materials.

The present paper is concerned with an electrophysiological investigation of the chemoreceptive properties of sense organs situated on the dactylopodite of the first walking leg of Homarus vulgaris, Portunus puber and Carcinus maenas. The innervation of the dactylopodite of the first walking leg is solely sensory. Removal of the exoskeleton from the segments close to the protopodite - dactylopodite joint exposes the nervous supply. In this situation there is considerable anatomical sub-division of the nerve into many fine bundles any of which is readily separated from the surrounding dermal structures. Electrophysiological recording utilising straightforward amplifying and oscilloscope apparatus is relatively simple in this situation.

Responses from chemosensory elements are intimately associated with responses from mechanically sensitive units. In unteased bundles of fibres activity associated with tactile hairs is always seen and is very difficult to obviate even by separation of small groups of fibres. The chemical solutions used to stimulate the chemoreceptors were run from a pipette on to the side of the dactylopodite and in only a few cases was the chemical response divorced from a primary mechanical response. The tactile responses comprised a rapid burst of impulses that occurred when sea water drops deflected the hairs of the dactylopodite. Usually this activity started after only a short latency and ceased within 300 - 400 msecs. But the response sometimes lasted for up to 1½ - 2 seconds, showing gradual adaptation after a single drop had been applied. These observations are important when considering the responses to chemical solutions made up in sea water and applied in the same manner as control drops.

The application of solutions (0.001 - 0.1 M) of quinine, various sugars, cumarin and vanillin have all been unsuccessful in evoking recordable activity in the sensory nerves. Case & Gwilliam (1961) have indicated that certain amino substances, in particular glutamic acid, may be stimulatory on the end organs of several marine crustaceans. In the present work

aspartic acids tryptamine, glutathione, urea, tyrosine, γ-amine butyric acid, glycogen, glycine, cysteine, methionine, serine, leucine and histamine have all proved non-stimulatory. Glutamine (0.1M) had effect on only 2 out of 30 trials, whilst glutamic acid apparently had no effect, at any concentration up to 0.1M, and gave rise to small indeterminate responses when applied in saturated solution.

Only two substances have been found to invoke activity consistently. These are trimethylamine oxide (TMO) and betaine (used as solutions of their hydrochlorides, buffered to pH 7.2). These substances are known to occur widely in invertebrate and vertebrate tissues.

Extracts of fish tissues which contain TMO also stimulate the chemoreceptors of crustaceans, but extracts of mussels (Mytilus) were non-stimulatory. This species does not contain betaine.

Three types of response have been noted. In type 1 no mechanical response occurred, and the latency of the chemoreceptor unit was very long, several seconds elapsing before the unit fixed. The pattern of discharge showed a gradual mounting frequency, lasting some 20 - 30 seconds, followed by slow adaptation and a quick cessation of activity only when the organ was washed with clean sea water. The second type of receptor showed a latent period of 0.5 to 1 second. These units were always associated with phasic mechanoreceptors that obscured the early part of the record. When discharge began the initial frequency of these chemoreceptor units was high, adapting back to zero in 10 - 30 seconds. A third type of receptor exhibited tonic long-lasting activity that was modified by addition of chemical solutions. The frequency of these units was maintained at about 20 - 30 impulses/sec., rising for a short period as the stimulating drop impinged on the limb, falling below the basal frequency immediately after this, rising to about double the initial frequency for about 10 seconds only to fall back to the initial level again later.

Some chemical specificity of the receptor mechanism is indicated by these results. Betaine and TMO possess the radical $(CH_3)_3N$ in common in the molecule. The amino acids listed above do not have this grouping but choline does, and has proved to be stimulating. Quaternary ammonium compounds (tetra-methyl ammonium $(CH_3)_4N$, dimethyl glycine $(CH_3)_2N$, sarcosine (CH_3N) and glycine $(NH_2.CH_2)$, are all non-stimulating. It is thus suggested that the $(CH_3)_3N$ moiety may be of importance in determining the stimulatory activity of chemical compounds on crustacean chemoreceptors.

The end organs involved in these chemoreceptor responses remain unidentified. There seem to be three possibilities. The setal-tufts (hair-fan organs) shown by Hodgson in fresh water Cambarus to be involved in chemoreception, and which

also are involved in monitoring tactile stimuli (Laverack, 1962). The stout unbranched hairs that project from the surfaces of the walking legs, and in the crabs the pore canals at the distal end of the dactylopodite. These are thought to be innervated but there may be some confusion with the tegumentary glands also present in the same situation.

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AMINO ACID DETECTION BY MARINE INVERTEBRATES. James Case and G. F. Gwilliam. University of California, Santa Barbara, California and Reed College, Portland, Oregon.

A survey employing behavioral and, where feasable, electrophysiological means has been made of the ability of a variety of marine invertebrates to detect amino acids. All forms which we have investigated and found suitable for quantitative study can detect some amino acids at concentrations sufficiently low to suggest they serve prominently as guides to food sources. At this writing unmistakable amino acid sensitivity has been shown in several crustaceans, including Carcinides, Cancer, Callinectes, Pugettia, Libinia, Pachygrapsus, and Pagurus; in the polychaetes Nereis and Chaetopterus; and in the ctenophore Mnemiopsis.

Of these, only the crustaceans have been examined electrophysiologically, by recording from extensively subdivided bundles from the dactyl innervation while stimulating the dactyl with test solutions. Since our earlier report, which was based largely on Carcinides (Case and Gwilliam, Biological Bulletin 121:449-455), Pugettia, a kelp eating crab, and Cancer have been shown to have unmistakable dactyl chemoreceptors, as revealed by both behavioral and electrophysiological methods.

The two polychaetes, despite their different feeding habits, both detect amino acids. Nereis, probably best described as an omnivore, and which has already been shown to have clearly defined acceptance and rejection responses (Case, Comp. Biochem. Physiol. 6:47-56), has been found to react similarly to amino acids. Chaetopterus, a highly specialized filter feeder, expresses sharp rejection thresholds to certain amino acids by reversing the flow of water through its tube. While acceptance responses may occur in the form of increased water pumping in the normal direction it is possible that this also represents rejection.

Mnemiopsis, which preys upon zooplankton, evidences unmistakable feeding responses which may be elicited by pipetting small amounts of sea water extracts of animal tissues or of pure chemicals on the lips, tentacle pocket area or arms. While by far the greatest sensitivity is to certain amino acids, sufficiently high concentrations of substances which could not remotely be considered appropriate gustatory stimuli appear to cause exactly similar feeding responses, creating the impression that this animal lacks a rejection response mechanism to chemical stimuli confined to the mouth region.

PROPERTIES OF LIMULUS CHEMORECEPTORS.[1] Saul B. Barber and Wilbur F. Hayes, Biology Department, Lehigh University, Bethlehem, Pennsylvania.

Contact chemoreceptors are concentrated in Limulus on the spines of the coxal gnathobases of the walking legs except for the last pair. They are also found on the chelae of the prosomal appendages, including the chelicerae, and on the spines of the chilaria. The latter is a greatly reduced appendage of a rudimentary pregenital somite. The distribution and detailed structure of these sense organs were first described by Patten (1894, Quart. Jour. Micr. Sci. 35: 1-96). His description of the gnathobase spine organs indicates that each consists of a canal that extends from the inner to the outer surface of the cuticle. Each canal contains a cuticular "tubule" and each tubule contains the distal process of a sensory cell. The cells are arranged in spindle-shaped clusters in the lumen of the spines and there are many clusters in each of the spines. The cuticular tubule with its sensory filament is said to end flush with the outer surface of the cuticle. The proximal process from each sensory cell contributes to the gnathobase nerve before the latter joins with the large leg nerve.

We have examined some of the properties of the gnathobase receptors using electrophysiological techniques. Fine nerve bundles were teased from the nerve of excised, but otherwise intact, gnathobases and action potential responses of these bundles were recorded while stimulating the spines mechanically and chemically. Many nerve bundles yielded responses to both tactile and chemical stimulation and, in most cases, the units responding to tactile stimulation were clearly different from those responding to chemical stimulation. Many of the bundles yielded responses from several units and were thus too complex to be analyzed in terms of the responses of individual receptor units. In some bundles, however, there were few enough active units so that such analysis was possible.

A typical response of a chemoreceptor unit consisted of an initial repetitive burst of action potentials followed by similar bursts at irregular intervals for many seconds. The number of spikes per burst varied from 1 to 30 or slightly more. Intraburst spike frequency, although not carefully analyzed, varied in one unit from about 30 to 60/sec. and there seemed to be no correlation between this variable and number of spikes per burst. Intervals between bursts varied from a few hundredths of a second to several seconds. Once again there was no obvious correlation between this variable and number of spikes per burst. Similarly there was no obvious correlation between time after initiation of stimulation and length of burst nor between the former and intraburst frequency. Nevertheless, the responses clearly adapted with time and it does seem that the average interburst interval became longer with time, but in a highly irregular sequence. Further, the possibility of an average, but irregular, decrease in burst length and intraburst frequency with adaptation is not ruled out without more careful analysis. (cf. Barber 1961, Amer. Zool. 1: 435).

Crude aqueous extracts of marine clams have consistently elicited strong responses from the gnathobase chemoreceptors. Similar responses are also obtained by stimulation with other crude preparations such as yeast and beef extracts (10% in sea water). Solutions of some individual amino acids in sea water also often elicited unequivocal chemoreceptor responses. A 0.5M. solution of glycine was usually the strongest of these and this was generally followed by taurine, proline, ala-

nine and sodium glutamate. In no case, however, did a single amino acid elicit a response as strong as the clam extract.

One solution that was used, however, did come close to mimicking the response to clam extract. This solution, prepared by mixing equal parts of 0.5M. glycine, taurine, proline, alanine and sodium glutamate, was 0.1M. with respect to each of the amino acids and 0.5M. with respect to total amino acids. In the great majority of responses analyzed this mixture elicited responses that were clearly greater than those of any of the amino acids, although never quite as large as the clam extract responses. In a few cases the response to glycine was greater than that to the mixture. If, as Patten's structural studies indicate, the unit responses were being recorded from the axons of primary sensory cells, then the results obtained with the amino acid mixtures may be interpreted to indicate multiple, peripheral stimulation sites for each unit. However, the irregular nature of the unit responses are such as to suggest that synaptic influences on the recorded responses may not be ruled out.

Although many of the units responding to tactile stimulation were distinguishable from chemoreceptor units there is evidence to indicate that some tactile units also responded to chemical stimulation. One unit has been so identified on the basis of spike height and waveform, and several others, examined in retrospect, strongly suggest similar properties. Two other units are believed to have responded to the osmotic properties of the solutions and these units are also suspected of sensitivity to tactile stimulation. In all of these units it is, once again, impossible to decide, without further observation, on the precise sites of stimulation which are influencing the recorded responses.

Some attempts were also made to determine if distilled water solutions of the amino acids had effects on the chemoreceptors different from those of the sea water solutions. These results were very variable and may have been influenced by the probability that prolonged or repeated exposure to distilled water solutions was harmful to the receptors. Nevertheless enough instances of responses to distilled water solutions comparable to those of sea water solutions were obtained to indicate that the effect, if any, on the responses of the chemoreceptors was not a striking one. Similarly the responses to equal concentrations of DL, L and D alanine were compared and no striking differences were observed.

Some preliminary experiments similar to the above have also been performed on receptors of the chilarial spines. These receptors yielded electrophysiological responses to clam extract, the mixture of amino acids and glycine which were similar to those recorded from the gnathobase spines. Responses to the other amino acids, however, were not as consistent as those displayed by the gnathobase receptors. One major difference of the chilarial receptors was a type of "off response" elicited from many units. This response appeared when stimulating solutions were siphoned from the receptor chamber and the responsible units were identified as identical to some of those responding to application of the chemicals. Although mechanical effects apparently contributed to this response there was evidence to indicate that chemical effects were also operating. The off response was typically a single burst of up to 50/sec. which usually adapted to zero in a very few seconds. When the same units were stimulated by solution

application, complete adaptation occurred over a much longer interval. At least one unit displayed responses to tactile stimulation as well as to solution application and removal. Units suspected to be entirely osmotic in stimulating effect were also observed among the chilaria receptors.

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FINE STRUCTURE OF INSECT OLFACTORY RECEPTORS. Eleanor H. Slifer, Dept. of Zoology, State University of Iowa, Iowa City, Iowa.

The sense organs on the antennal flagella of one or more species of insects from each of several different orders (Orthoptera, Isoptera, Hemiptera, Homoptera, Coleoptera, Hymenoptera, Diptera) have been examined with both the light and electron microscopes. Interest has been concentrated on those which are penetrated by a solution of dye (crystal violet) applied to the external surface of the intact insect since experimental evidence obtained earlier indicates that they have an olfactory function.

The cuticular surface of these permeable structures - which are usually, but not always, hair-like or peg-like in shape - is perforated by a large number of minute openings with a diameter of 0.1 μ or less. The distal tips of the olfactory dendrites end within or pass close below these pores and are there exposed directly to the surrounding air. No cuticle is present over them. The number of sensory neurones in the group which innervates such a structure varies both with the species and with the type of olfactory receptor and may range from a few to as many as sixty. A short distance above the cell body each dendrite in the group suddenly narrows and assumes the structure of a cilium with nine pairs of peripheral fibrils. A central pair is not present. A ciliary collar is usually found at the base of the cilium. One or two basal bodies lie below the collar and are connected to it by fine fibrils. Rootlets with a conspicuous periodic structure extend proximally from the basal bodies and end not far above the nucleus. As the slender ciliary portion of the dendrite passes distally and approaches the inner surface of the antennal cuticle the nine pairs of peripheral fibrils disappear. Beyond this the dendrites contain only neurofilaments. Upon entering the lumen of the peg each dendrite divides once or several times so that the peg is filled with a large number of fine branches. These have a diameter of 0.1 μ or less. At close intervals groups of excessively fine filaments leave the surface of these dendrites and enter the pores in the cuticular wall. The diameter of the filaments is within the same size range as is that of the neurofilaments within the dendrite. It is not certain that these filaments comprise the ultimate receptor surface although it is tempting to suppose that they may be.

No other cellular elements besides the dendrites and the fine filaments which are associated with them are present in the peg lumen. The dendrites and filaments are bathed in a fluid which is continuous with that in the vacuoles of the trichogen and tormogen cells through which the dendrites run below the antennal cuticle. In some types of olfactory receptors the ciliary portions of the dendrites are enclosed within a cuticular sheath which is invaginated from the peg cuticle; in others the sheath is absent.

The general description just given applies, for the most part, to those sense organs which have been demonstrated to be permeable to dyes in all of the species examined but, as would be expected,

variations in both cuticular and cellular details occur. These can not be included here. Finally, it is highly probable that certain of the other structures on the surface of the antennal flagellum which have not yet been shown to be permeable may, eventually, be found to have an olfactory function.

THE FINE STRUCTURE OF THE LABELLAR AND ANTENNAL CHEMORECEPTORS OF THE BLOWFLY Phormia regina.

Joseph R. Larsen, Dept. of Entomology University of Wyoming, Laramie, Wyoming and V.G. Dethier Division of Zoology, University of Pennsylvania, Philadelphia, Pennsylvania

The present study was a morphological one at the level of the electron microscope to ascertain the number of nerve fibers in the various chemoreceptors of the labellum and the antennae. A study was also made of the epidermal structures associated with the sensilla and an attempt was made to determine the relationship of the nerve fibers to the tip of the hairs. All tissues were fixed in a chilled 1% S-collidine buffered osmium tetroxide and embedded in a mixture of 90 parts butyl and 10 parts methyl methacrylate using 2% Luperco CDB to initiate polymerization.

The chemosensory hairs of the labellum observed in cross section are similar to those previously described by Dethier (Quart. Rev. Biol. 1955) being characterized by the presence of two lumina, one thin-walled, the other thick-walled which contains the distal processes of the nerve fibers. In all of the cross sections of hairs in Phormia at least three nerve fibers were observed. Many hairs contain four nerve fibers and in some hairs five nerve fibers are present in the small cavity. The nerve fibers extend from the tip of the hair proximally to the epidermal cell complex where the nerve cell bodies are located. The nerve fibers then pass out of the epidermal complex from the nerve cell bodies to the CNS.

In the region of the hair socket the nerve fibers enter into a cuticular scolopoid structure. An apparent reduction in the size of the nerve fibers from the base of the hair to the shaft of the hair is not in fact a reduction in the size of the nerve fibers themselves but merely the passing of the nerve fibers through the scolopoid body. There is no evidence of the scolopoid body in the small lumen of the hair shaft. The nerve fibers in the shaft of the hair are not enclosed in any type of sheath other than the small lumen of the hair itself. The scolopoid body appears to be a continuation of the cuticle from the base of the small lumen of the hair down through the region of the hair socket. The cuticular scolopoid body completely encloses the nerve fibers isolating them from the surrounding tissue in the region of the socket. In addition the entire scolopoid structure is surrounded by a membrane which separates the scolopoid body from the cytoplasm of the trichogen cell.

The nerve fibers are not bathed in the cytoplasm of the trichogen cell. They are encased in a membrane which surrounds each nerve and each nerve cell body. This membrane is not around the nerve fibers while they are in the scolopoid body. The nerve fibers going proximally from the nerve cell bodies leave the epidermal cell complex and pass on to the CNS. At the point where the nerve fibers leave the trichogen cell there is a membrane which surrounds the nerve fibers and encloses the entire epidermal cell complex associated with each hair. There is good evidence that one of the nerve fibers terminates on the base of the hair where

it undoubtedly acts as a mechanoreceptor.

An additional membrane was observed in the socket region. This membrane appears to arise from the inside wall of the socket and pass across the lumen of the hair and attach to the scolopoid body at the base of the hair. This membrane appears to extend as a partial diaphragm across the vacuole of the trichogen cell. The membrane may serve to transmit movement of the hair to the mechanoreceptor which is attached at the same point where the membrane attaches to the scolopoid body.

Near the base of the hair the trichogen cell is completely enclosed by the tormogen cell. At the proximal end of the epidermal cell complex the trichogen and tormogen cell are adjacent to each other. The entire epidermal layer in the proboscis is underlayed by a syncytial layer of tissue and not tracheae as suggested by many earlier workers. There are many nuclei present in the tissue but cell membranes between the nuclei have never been observed.

The interpseudotracheal papillae are minute sensilla basiconica which project through the syndesmoses between the pseudotracheal and interpseudotracheal plates on the oral surface of the labellum. Dethier (Quart. Rev. Biol. 1955) employing methylene blue and silver preparations described the presence of two distinct nerve fibers and suggested the possible existence of a third nerve fiber. The cuticular portion of the sensillum consists of a peg surmounting a broader base which consists of a sclerotized annular area. The lumen of the peg is an extension of the vacuole of the trichogen cell. The nerve fibers extend up through this vacuole. The nerve fibers, however, are completely isolated from the cytoplasm of the trichogen cell by a cuticular scolopoid body. In the papillae the scolopoid body extends well up into the lumen of the sensillum as opposed to the scolopoid body of the labellar hairs which seems to terminate at the base of the hair shaft. Also in the papillae the scolopoid body completely encloses each individual nerve fiber, while in the labellar hairs the scolopoid body is seen as an invagination between the individual nerve fibers. The scolopoid body is probably what earlier workers described as the inner sclerotized annular area lying within the sensillum.

In all of the papillae observed there were always four nerve fibers present.

The principal site of olfactory receptors in the blowfly as in most insects is the antennae. Nevertheless the identity of the olfactory receptors is made difficult by the great numerical density of receptors, the variety of forms and the minute size of all. By studying all types of antennal receptors with the electron microscope it has been possible to gain some idea as to the nature of the olfactory receptors. The sensilla of the antenna are located on the fleshy apical segment and within large pits which are a conspicuous part of the antenna.

Antennal pits are confined to the dorsal, ventral and inner lateral distal half of the segment. There is some variation in the number, size and exact location of pits from one individual to the next, but in general the males possess from 9 to 11 and

the females from 11-16. Some pits are simple pocket-like cavities others are multichambered. Their orifices are ringed with stout, short, spinous projections of the cuticle. Within the pits are found three types of sensilla: thin-walled pegs, thick-walled pegs, and coronal pegs, so called because of their crown-like appearance in transverse section. Only one type of sensillum occurs in a pit. The surface of the antenna is thickly clothed with non-innervated spines with which the numerous sensilla are interspersed. The sensilla are of three general types: pointed, tapering thick-walled pegs; short, rounded, thin-walled pegs, and minute stellate pegs.

All of these pegs possess many features in common. They are a hollow cuticular peg all or part of whose wall is extremely thin and very possibly perforated; a cuticular sheath or tube, the scolopoid body, extending from the basal region of the peg a variable distance down into the underlying tissue; one to many deep - lying bipolar neurons whose dendrites pass into the scolopoid sheath thence into the lumen of the peg.

Since there are many hundred-fold more cell bodies associated with the pegs than there are axons in the antennal nerve, and since synapses do not occur in the antenna, it follows that there is extensive fusion of axons. Axonal fusion is a common feature of the insect sensory system. There is some density in the appearance of the neural material filling the lumina of the pegs, but thus far it has not been possible to relegate a particular internal appearance to a particular type of peg. The interiors of the dendrites are markedly reticulate. In longitudinal section there appears contorted tubules suggesting that the dendrite has subdivided into many parallel branches.

A conspicuous feature of the cuticular walls of the thin-walled pegs is the indentations in their surface. Each peg possesses from 180 to 360 which constitute from 7-14% of their surface. The electron dense outer layer of the cuticle does not enter the openings of the pits. At the bottom of each opening is a material that is not part of the cuticle and seems to be continuous with the sheets and nets of nerve tissue filling the lumen. At the base of the openings, and only there the material occurs in the form of fine parallel strands. In cross-section these appear as round tubules. It is difficult to ascertain whether or not these pits are indeed perforations into which the nerve fibers come, although this is the most likely interpretation.

The thick-walled surface pegs are the most common sensilla on the antennae. Some are straight and acutely pointed while others, are gracefully curved. Each peg is innervated by one to four neurons whose cell bodies lie at a considerable distance beneath the surface. The scolopoid sheath is scalloped to accomodate the various number of dendrites. The walls of the peg also contain the pits described in the thin-walled pegs.

The coronal pegs in the pits and the stellate surface pegs are few in number and are unlikely candidates for the function of olfaction. The thick and thin-walled sensilla on the surface and in the pits are the most numerous sensilla on the antenna.

FUNCTION OF INSECT OLFACTORY SENSILLA. Dietrich Schneider, Deutsche Forschungsanstalt für Psychiatrie, Max-Planck-Institut, Abt.f.vergleichende Neurophysiologie, München 23, Kraepelinstrasse 2, Germany

Investigations of the olfactory sense in insects aim to answer the following main questions: 1) Which is the sensillum type involved in this function; 2) What is the qualitative range of the individual sense cells (the receptive units); 3) What is the quantitative range of the whole sense organ and its individual cells; 4) What is the course of the intensity-reaction curve; 5) What is the functional connection between the stimulus and the receptor (generator) potential; 6) What is the functional connection between the receptor potential and the spike discharge; 7) What is the primary effect of the odorous molecule on the receptive membrane.

Classical methods using the motor reaction of animals under the influence of a stimulus have in some cases successfully been used to answer the above questions. In a few fortunate cases, sensilla basiconica have been identified as olfactory sensilla (see 2, 3) while the function of many other so called "olfactory" organs is not yet clear. While it is principally impossible to determine the qualitative range of individual sense cells with these methods, it is in some cases very well possible to determine the threshold. If the whole animal "answers" the stimulus, the observer will very likely see the reaction induced by the most sensitive of the receptor cells (7). The course of the reaction curvé beyond the saturation of the reactive behavior as well as details of the cellular function are out of reach for the behavior-physiologist.

Electrophysiological methods have successfully been used to answer some of the above questions.

I. DC-recordings from insect antennae demonstrate summated receptor potentials of many units. These so called electroantennograms (EAG) give information on the quantitative range and the intensity-reaction function of a large number of sensory units. In the case of an antenna with only one type of highly specialized receptors the EAG gives some clue of the specificity. Furthermore, the EAG is a useful tool for bioassay work in isolating biologically active substances (4, 5, 6, 7, 9).

II. Single unit recordings (DC and RC) with a microelectrode attached to the sensillum supplies additional information. In moths, beetles and cockroaches, the olfactory function of sensilla basiconica has been shown (1, 7, 8). The units are of the phasic-tonic type. Immediately after the onset of the odor current, the spike reaction shows a frequency peak of up-to 250 impulses/sec. This initial burst slows down to a plateau. The amplitudes of the peaks and plateaus depend upon the stimulus strength.

Contrary to the highly specialized units, the units of the general olfactory sense react to different stimuli and with comparatively high threshold. These units proved to be the receptor cell endings of sensilla basiconica. Qualitative as

well as quantitative differences of responses from single olfactory units might be sufficient for the central-nervous-system to discriminate a rather large number of different odors. Specialized units responding to a pheromone (like a sexual attracting substance) also respond to "inadequate" stimuli with much higher threshold (1, 8).

Unit receptor potentials are typical and well recognized: the periphery of the sensory ending is depolarized. These slow potentials elicit (generate) the nerve impulses (1, 7, 8). Some substances also induce a reversed slow potential (hyperpolarization) which blocks spontaneous and evoked nerve impulses (1).

So far, no method has been able to answer the principal question concerning the nature of the primary effect of the odor molecule upon the acceptor molecule. Since pheromones and also some food and prey odors are effective in very low concentrations, the amount of energy transfered to the receptor membrane is very low indeed. The architecture of the odor molecule is of decisive importance, since only the correct geometrical isomer of a sexual attracting substance will work with the low threshold. Inadequate isomers need a very high concentration to stimulate the receptor cell (7).

Electrophysiological methods also answer the question of pheromone specificity. In the subfamily Saturniinae (Saturniidae, Lepidoptera) lure glands of females from different genera have electrophysiologically been shown to be equally effective upon the males of all tested species of this subfamily. While this is a lack of specificity, lure substances of different families certainly differ. The *Bombyx* (fam. Bombycidae) attracting substance and the *Lymantria* (fam. Lymantriidae) attracting substance are without effect upon Saturniinae and vice versa. In *Bombyx* and *Lymantria* these substances are not only physiologically different in cross stimulation experiments but differ also in their chemical composition (6, 7).

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SOME POSSIBLE MODES OF ACTION OF HUMIDITY AND WATER RECEPTORS IN INSECTS. L. Barton Browne, C.S.I.R.O., Division of Entomology, Canberra, A.C.T., Australia.

Insects, as the only truly terrestrial invertebrates, are faced with considerable problems in maintaining a sufficiently stable state of water balance, and behavioural responses play an important part in the solution of these problems. The existence of sensilla which respond to atmospheric humidity and water is well established but little definite information is available about their modes of action. Some possible ways in which the receptors could function are suggested in this discussion.

1. Responses to atmospheric humidity.- There are at least three broad principles on which hygroreceptors could operate; viz. as hygrometers, as evaporimeters or as chemoreceptors sensitive to water vapour. Insects with each of these types of receptor would have, if their responses were not affected by temperature *per se*, characteristic reactions in humidity gradients at different temperatures. Insects with hygrometers would select atmospheres of the same relative humidity irrespective of the ambient temperature. Those with evaporimeters would select atmospheres in which the evaporation rate from their receptors is similar. The humidity responses of these insects would, therefore, be roughly related to the saturation deficit of the air. Forms with chemoreceptors would show a preference for atmospheres with equal absolute water content. Behavioural evidence has been obtained which is consistent with the existence of hygrometers (Hafez 1950, 53, Thomson 1938, Pielou and Gunn 1940) and of evaporimeters (Lees 1953, Wellington 1949). Some of the possible modes of action of receptors of these two general types are discussed.

Two general characteristics of the humidity responses of insects are relevant to the ensuing discussion. Many insects show a wet reaction when desiccated and a dry reaction when hydrated. There is evidence that different sets of receptors are involved in the two types of response (Bentley 1944, Begg and Hogben 1946, Roth and Willis 1951). It is of interest to consider whether it is conceivable that changes in the concentrations of the body fluids could directly 'switch' receptors on or off, or whether it is necessary to postulate that the switch-over occurs centrally. It also seems to be a common occurrence that insects are more sensitive to differences in humidity when at least one of the choices offering is near saturation (Lees 1953, Pielou 1940, Willis and Roth 1950). This characteristic, if assumed to be a property of the receptors themselves, may give an important clue to their mode of action.

Hygrometers.- A sensillum functioning on the principle of a hygrometer would necessarily consist of a hygroscopic material and a transducer. The endocuticle is known to absorb moisture and its thickness is influenced markedly by its water content (Fraenkel and Rudall 1947). It is not difficult to envisage systems wherein mechanical receptors could be stimulated by stresses caused by these changes in thickness. It seems feasible, therefore, that a hygrometer type of receptor would consist of a suitable mechanoreceptor associated with an area of cuticle without a lipid layer. The fluids on the inside of the cuticle consist of dilute

aqueous solutions and the water content of the endocuticle would vary with distance from the hypodermis. The innermost part of the endocuticle would be in equilibrium with the internal fluid and the outermost part with the moisture of the air at its surface. This would tend to reduce the sensitivity of the system.

Pielou (1940) has demonstrated that the amount of water taken up by the cuticle of Tenebrio molitor is from 20 to 40 times more sensitive to changes in humidity above 90 per cent than at those below 80 per cent. This suggests that the general high sensitivity of insects to high humidities may be a characteristic of their hygrometer-type sensilla. This high water uptake in high humidity ranges also may provide a mechanism for reversal at a peripheral level. The small available amount of information suggests that the body fluids of hydrated insects would have a vapour pressure equivalent to a relative humidity of virtually 100 per cent whereas those of desiccated individuals would be in equilibrium with relative humidities as low as 97 per cent. Considerable variations of the water content and hence possibly of the thickness of the cuticle could be expected in this range. Such changes could conceivably have the effect of putting receptors into and out of the range of thicknesses in which changes would produce variations in sensory input.

(b) Evaporimeters.- Evaporation from or condensation on a receptor could produce at least three major effects. The water content of the receptor cell and of extra-cellular fluids, the volume of the receptor or other cells, and the temperature of the receptor could be influenced. A hygroreceptor operating on the principle of an evaporimeter could be, in effect, either a receptor responding to intra- and extra-cellular concentrations, a mechanical receptor responding to cell volume, or a temperature receptor. A simple temperature receptor would, however, appear to be of limited use because its input would be influenced by ambient temperature. Such a system would be of value only if the insect were capable of making temperature comparisons in time. Even in this event input from simple temperature receptors could lead an insect in a gradient of humidity only to the highest or lowest humidity available. A wet and dry bulb type of arrangement in which the insect could compare the temperature of receptor cells from which evaporation is occurring with others which are protected from water loss would appear to be of much more use.

It is of interest to consider the possibility that there may be an optimal rate of evaporation for maximal sensitivity in an evaporimeter-type receptor. It seems that the transpiration rate should not be so great that localised areas of high humidity are caused to form at the outer surface of the receptor. The rate of transpiration from the sensillum should be limited by its membrane rather than by the rate of removal of water from the air layer at its surface.

It is unlikely that changes in concentration of the general body fluids would influence the evaporation rate sufficiently to enable reversal to be explained in terms of changes in receptor function.

Behavioural experiments can do no more than tell us whether the humidity responses of an insect are better correlated with relative humidity or evaporation rate. It seems that detailed information on receptor function can be obtained only if it proves possible to record electrically from the sensilla. It is

difficult to see how direct recording can be made from the receptor cells without upsetting its water relations. It is possible, however, that microelectrode recordings from within the antennal nerves of some insects may prove useful. Information about the fine structure of suspected hygroreceptors would also be of great value.

2. Water taste.- A specific water taste receptor cell has been demonstrated to be associated with the labellar chemoreceptors of the blowfly (Evans and Mellon 1962). The activity of this cell is inhibited by solutes, electrolytes being more effective than non-electrolytes. It seems that a water taste receptor could respond either to water as a chemical and be specifically inhibited by solutes, or to the osmotic pressure of the stimulating solution. Present evidence does not allow a definite decision between the two possibilities. The data relating to inhibition by non-electrolytes is not entirely in consistent with the osmometer theory. Although in the individual hairs tested by Evans and Mellon the detailed relationship between osmotic activity and inhibition vary to some extent, the general degree of inhibition by solutions of equal osmotic activity is of the same order. It should be realised that the non-electrolyte solutes tested possess terminal hydroxyl groups and may therefore be suitable compounds for competitive inhibition at water receptors. The greater effectiveness of electrolytes would, according to either theory, seem to be best explained in terms of some specific effect of charges on the membrane of the receptor cell. It seems that the mode of action of these receptors will be elucidated by further experiments along the lines of those carried out by Evans and Mellon.

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MORPHOLOGICAL INVESTIGATIONS ON THE SENSE ORGANS OF THE LABELLA OF THE BLOWFLY, CALLIPHORA ERYTHROCEPHALA MG. Werner Peters and Senta Richter, I. Zoologisches Institut, Freie Universitat Berlin, Berlin-Dahlem, Germany.

One might think that a hundred years of morphological work on the labellar sense organs would have been sufficient to yield a picture without any contradictions. However, we still have a variety of opinions. The main questions concern the number of sense cells and the total number of cells belonging to one hair, the ending of the distal processes of the sense cells, the origin of the Binnenkanal, and the occurence of different types of labellar hairs. Moreover, information on the development of these hairs is urgently needed. I have tried to solve some of these questions as far as possible with the light miscroscope, and my colleague Dr. Senta Richter has done some work with the electron microscope (e.m.).

The labella bear 4 different types of hairs: 2 types of mechanosensory hairs which are scattered on the upper parts of the labella, the interpseudotracheal papillae, and the marginal bristles which are situated on the lateral parts of the labella. Only the latter are usually called "labellar hairs," but it seems to be more correct to call them, in contrast to the other 3 types of hairs, marginal bristles.

The interpseudotracheal papillae are reduced hairs. The hair itself has been shortened and the tip probably "retracted" by the subsidence of the cuticle surrounding the tip; moreover, the whole organ has sunk into the surrounding cuticle. The tormogen and trichogen cells as well as the sense cells lie deep in the interior of the labellum. They are connected with hair and socket by a tube, up to 40 μ long, formed by the distal parts of these cells. There are 4 sense cells of similar size and staining behavior. Their distal processes extend into a tube which is equivalent to the Binnenkanal of the marginal bristles. In Methylene blue preparations there are dark blue granules on the surface of the sense cells as well as on their distal and proximal processes which are absent after the distal processes have entered the Binnenkanal. These granules seem to be related to some sort of nerve sheath. The Binnenkanal ends at the tip of the hair but the ending has not yet been observed with the electron microscope. Graham-Smith (1930) thought that the interpseudotracheal papillae might act not only as chemosensory but also as mechanosensory organs. Yet the obvious stiffness of the membrane between hair and socket, the shortness of the hair (barely extending beyond the surrounding cuticle), and the similar staining properties of the 4 sense cells seem to indicate that these hairs act only as chemosensory organs.

The marginal bristles, standing on a ridge on the lateral parts of the labella, are of different sizes. Their length ranges from 50-500 μ and the diameter at the base from 4-12 μ. The question arises whether there are groups of hairs of equal size

arranged in a more or less fixed pattern. To solve this question all hairs of a labellum were carefully measured and mapped. The result was, that there are no clear groupings of hair sizes and no pattern in the arrangement of hairs of different size.

Since the members of this symposium are familiar with the structure of the marginal bristles, we may at once begin with the development of these hairs. The proboscis of Calliphora develops as a flat evagination of the lower part of the head. Some of the continuously dividing cells of the epidermis in the lateral parts of the future labella differ from their neighbors by their largeness and their large mitotic spindle which lies parallel to the surface of the epidermis. These large cells may be called stem cells of the marginal bristles. They divide. One of the resulting cells produces in a second division the future tormogen and trichogen cells, hence it may be called stem cell of the hair forming cells. The other yields successively 5 sense cells, hence it may be called stem cell of the sense cells. All the cell-bodies subside into the interior of the labellum, but remain in connection with the surface of the epidermis by a more and more narrowing strand of protoplasm. As soon as the trichogen cell is formed, its distal part begins to surround the distal parts of the sense cells. Afterwards this part of the trichogen cell is surrounded by the tormogen cell; and thus about 48 hours after the beginning of pupation, the basic configuration of the hair is achieved. This configuration is obtained by 2 simple processes: the subsidence of the proximal parts of cells and the envelopment of their distal parts by the hair-forming cells. Until about 60 hours after the beginning of pupation there seems to be no change in this state. Then a second phase of hair formation begins. The wall of the trichogen cell, as far as it envelops the distal processes of the sense cells, secretes a material which forms a tube, open on both ends - the anlage of the Binnenkanal. During the second phase of hair formation the distal parts of the trichogen cell and of the sense cells grow out. The Binnenkanal is at first in a central position, ending distally at the tip of the growing "hair" and proximally where the distal processes of the nerve cells enter the trichogen cell. About 5 days after the beginning of pupation it is in an excentric position. About 6 days after the beginning of pupation the trichogen cell begins to secrete cuticular material on the outer wall to form the hair wall, and on the inner wall to form the definite Binnenkanal. At the same time the tormogen cell forms the socket.

Two processes remain to be mentioned. During the first as well as the second phase of hair formation, the nuclei and protoplasm of the tormogen and trichogen cells grow many times faster than those of the surrounding epidermal cells,which means an increase in the degree of polyploidy. The trichogen cell is always a few steps ahead. Presumably the final degree of polyploidy is a question of time, and the first anlagen of hairs become eventually the largest hairs. The vacuoles of the tormogen and trichogen cells appear about 60 hours after the beginning of

pupation and show a tendency to extend more and more into the distal parts of these cells. Finally the lumen of the hair proper and of the socket are occupied by the large vacuoles.

The development of the marginal bristles is divisible into 2 phases, as we have seen already. In the first phase those parts are formed which lie beneath the surface of the cuticle. It seems to be the more important phase, as it includes the principle processes of hair formation. In the second phase the parts which extend beyond the surface of the cuticle are formed. The Binnenkanal is composed of 2 parts whose extension indicates that their different structure is closely related to the 2 phases in the development of the marginal bristle. Yet both parts are formed by the trichogen cell, and hence must have a common name. Stürckow (1960, 1962) has called the proximal part "scolops," and Larsen (1962) has called it "scolopoid body"; but no one has applied these terms to the distal part. As long as we do not know enough about the homologies in this field I would prefer the neutral name Binnenkanal for both parts.

In cross sections of the hair the distal processes of 4 sense cells can be seen in the lumen of the sclerotized distal part of the Binnenkanal, while the proximal part is characterized by 4 folds of its wall which separate the distal processes of the four sense cells (e.m.). In its lower part an additional compartment is to be found, containing the distal process of the fifth sense cell (e.m.). All marginal bristles possess 5 sense cells. Four of these show similar staining properties while the fifth sense cell stains more like the hair-forming cells. The fact that the strong distal process of this sense cell ends in the proximal part of the Binnenkanal while the distal processes of the other 4 extend to the tip of the hair (which alone responds to chemical stimulation), strongly suggests that the fifth sense cell is the mechanosensory cell postulated by Wolbarsht and Dethier (1958) from their electro-physiological studies.

The sense cell bodies and their processes are enveloped by a sheath (e.m.), ending as soon as the distal processes of the nerve cells enter the Binnenkanal; the sheath of the sense cells of the interpseudotracheal organs may have the same structure.

At the junctions of the larger nerve bundles as well as in the labial nerve itself one can find giant cells, singly and in groups. In each labellum there are about 30 of these cells. Their staining behavior and the fact that they are stained during the same short period like the sense cells indicates that they are nerve cells too. Usually they are tripolar. One of the processes extends into the direction of the subesophageal ganglion, while the other two extend into different bundles of nerve strands. Their ramifications may be followed for considerable distances, but finally they disappear among the proximal processes of the sense cells. Synapses were not found. So one can only speculate on the function of these giant nerve cells: 1. They may integrate information coming from the periphery. 2. They may act as

inhibitors and thus increase contrast.

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THE FINE STRUCTURE OF INSECT CONTACT CHEMORECEPTORS

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Two-toned, thin-walled setae on the tarsi and labella of *Stomoxys calcitrans* L. were identified as contact chemoreceptors by stimulation of individual setae and groups of setae and by silver nerve stain.

Location maps of tarsal contact chemoreceptors prepared for ten flies of each sex indicated that prothoracic legs bore the most chemoreceptors while metathoracic legs bore the least. Contact chemoreceptors were concentrated on tarsi; a few were identified on tibiae and femora. Total numbers on opposite leg pairs were equal or nearly equal. A classification of four types of contact chemoreceptors was proposed on the basis of external morphology and occurrence. Type 1 (short, blunt-tipped) occurred in a ventral position in the ventro-lateral areas. Type 2 (with one flexure, acutely-tipped) was found in ventro-lateral areas while type 2 (with two flexures, acutely-tipped) occurred in dorsal and lateral areas. Type 3 (long, acutely-tipped) was found only in the distal ventro-lateral portions of prothoracic tarsomeres one through four. Type 4 (very long, acutely-tipped) occurred on all legs in the distal lateral portions of fifth tarsomeres. A comparison between the sexes in numbers of tarsal contact chemoreceptors revealed slight differences in dorsal and lateral areas except on the metathoracic legs in lateral anterior positions where the number on males was nearly 2.3 times greater. The greatest differences between sexes, however, occurred in the ventro-lateral areas, which contained over 75 percent of the receptors on the leg, and consisted of greater numbers of type 2 contact chemoreceptor setae on males.

The numbers of labellar contact chemoreceptors on *Stomoxys* were identical for both sexes.

Electronmicroscopic investigations of tarsal and labellar tissues revealed that as the distal nerve fibers proceeded from the neurons, they were enclosed in a cuticular sheath. At the base of the seta the sheath appeared to coalesce with the exocuticle of the receptor seta. The distal nerve fiber serving a tactile function terminated at the base in the vicinity of a membrane and the exocuticle of the setal wall, as shown in Fig. 1, while the remaining distal fibers proceeded up the lumen to the site of action and terminated unmodified directly beneath a pore in the papilla (Fig. 2).

The numbers of neurons associated with ventro-lateral types 1 and 2 were usually five; three to five neurons seemed to be associated with labellar contact chemoreceptors of Stomoxys.

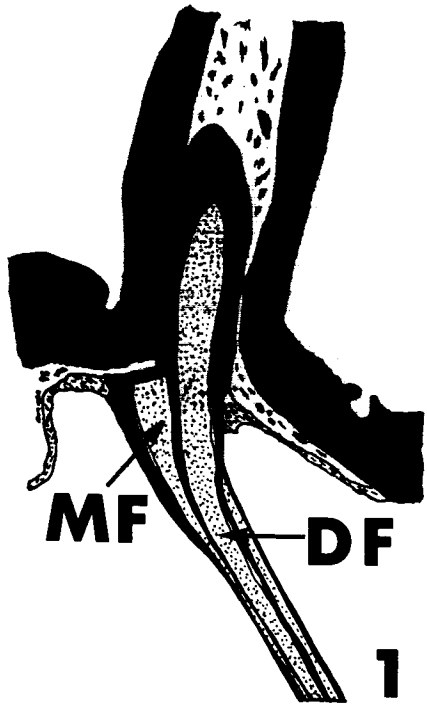
Sections of contact chemoreceptors of Stomoxys calcitrans L.

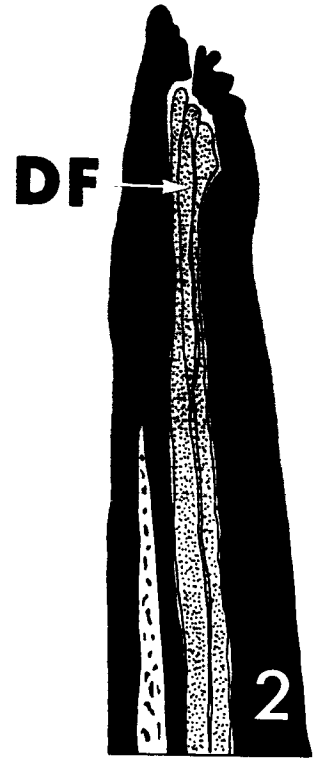
Fig. 1. Longitudinal section through the base of a labellar contact chemoreceptor. The distal fibers lie within a cuticular sheath in the subhypodermal areas. A distal fiber (MF) presumably serving a mechanical function terminates at the base while the remaining distal fibers (DF) proceed into the thick-walled lumen of the seta.

Fig. 2. A type 2 tarsal contact chemoreceptor in longitudinal section. The distal fibers (DF) terminate unmodified beneath a pore in the papilla.

Fig. 3. Cross section through the base of a labellar contact chemoreceptor showing mechano-fiber (MF) and distal fibers (DF) enclosed within a cuticular sheath.

Fig. 4. Cross section of a type 2 tarsal contact chemoreceptor. Four distal fibers lie within the thick-walled lumen of the seta.


MF
DF
1


DF
2

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3

4

TARSAL CHEMORECEPTION IN THE BUTTERFLY, VANESSA INDICA.

Masutaro Kuwabara, Department of Biology, Faculty of Science, Kyushu Univ., Fukuoka, Japan.

Using the proboscis extension response on tarsal contact with sucrose solution, the rejection threshold concentrations of cations mixed with sucrose of threshold concentration were determined in the butterfly, Vanessa indica, and following order of effectiveness was obtained:

Na^+ K^+ Li^+ Rb^+ Cs^+ Mg^{++} Ca^{++} Ba^{++} = Sr^{++}

Bivalent cations were far more effective than monovalent ones. Investigation was also made on anions, but any differences in effectiveness were not found among several kinds of anions.

Sometimes, presumably due to a certain physiological condition of the butterfly, Na^+, K^+ and Li^+ released the proboscis extension by itself. This reversal of the effect was most often seen in Na^+ and the insect which showed the positive proboseis response to K^+ or Li^+ responded also to Na^+ positively without exception.

In the condition, in which these cations released the proboscis extension response they worked additively with mixed sucrose. The mixed solution containing sucrose and NaCl in 1/2 of threshold concentration respectively always released the proboscis extension response, and pure solution of NaCl showed summation with sucrose applied to another leg.

Whenever Na^+, K^+ or Li^+ could not release the proboscis extension by themselves, they depressed the response to sucrose even contralaterally. It was remarkable that organic and also inorganic acids never released nor depressed the proboscis extension response in any concentration. Also in the electrophysiological study of single chemosensory hair the response to acids has never been obtained.

Every agent showing the depressing effect when they were mixed with stimulating sucrose showed depression effect also contralaterally. At least there must be assumed the existence of the receptors which respond to agents depressing the proboscis extension response and discharge impulses on application of such agents.

Although generally the rejection threshold was lower when depressing agent was applied mixed with sucrose than in the case when it was applied contralaterally, Na^+ showed exceptionally lower rejection threshold when it was applied contralaterally.

Bivalent cations depressed effect of mixed sucrose regardless of the concentration of the latter, but when the former were applied contralaterally they could not depress the sucrose response at all if the concentration of stimulating sucrose solution was higher than certain concentration.

To explain the results wer must assume at least two kinds of receptors: 1) One is excited by sucrose Na, K etc., and

sends impulses, to a nurvous center releasing the proboscis extension. 2) The other is excited by Na, K etc. and sends impulses to another nervous center inhibiting the proboscis extension. Changes in responsiveness of the effect of Na^+ and others on the proboscis extension.

The responses of single chemosensory hair were examined electrophysiologically. Two kinds of hairs were found by Takeda (1961) in my laboratory. One of which was sugar receptor and its response to sucrose was depressed by Na^+. Another one was sugar-NaCl receptor which respond to sugar and also to NaCl. Existence of water-receptor was confirmed thereaftr. The existence of NaCl receptor which seems to be necessary for the explanation of the reversal of proboscis extension response to Na^+ is possible, but it is not yet confirmed.

Electronmicroscopical investigations were made, and it was shown that the single tarsal chemosensory hair of Vanessa contains 4 chemoreceptors. Farther analysis of the characteristics of single receptor seems to be necessary.

TASTE HAIR STIMULATION AND PROBOSCIS RESPONSE IN CALLIPHORA ERYTHROCEPHALA. L. de Ruiter, Dept. of Zoology, Groningen University, Haren (Gr.) and C.J. den Otter, Dept. of Zoology, the University, Leiden, the Netherlands.

Electrophysiological records of the messages sent to the central nervous system by the chemoreceptors of taste hairs in intact flies (Calliphora erythrocephala Mg.) in response to stimulation with NaCl or glucose show great variation. Intensity and time course of the train of action potentials set up by successive identical stimulations of the same hair in the same individual differ widely.
Taste hair stimulation results in proboscis movements, as shown by the overt behaviour of the fly, and more sensitively by electromyograms taken from the proboscis. Here, too, marked diversity of responses to identical stimulations is found. Obviously part of the latter variation may be due to information from other sources than the taste hairs reaching the motor centres, but in any case the problem arises how accurately the fly is informed as to its chemical environment by its taste receptors, and with what degree of precision this information is translated into suitable, adaptive commands to the effectors.
To study this problem, we are making simultaneous records of chemoreceptor messages and muscle activity in the proboscis. With myogram electrodes in three strategically chosen sites in the proboscis, it appears possible to detect all activity of the various muscles responsible for the movements of this organ, and perhaps also to identify the muscles contributing to a given movement on the basis of the recorded spike forms.

ELECTROPHYSIOLOGICAL ANALYSIS OF INHIBITION AND SPECIFICITY IN LABELLAR CHEMORECEPTORS OF THE BLOWFLY. R. Steinhardt, H. Morita, and E. S. Hodgson, Dept. of Zoology, Columbia University, New York.

The accessibility of few-fiber chemoreceptor preparations in the labellar hairs of the blowfly, *Phormia regina*, and the extensive studies on feeding behavior of these flies, have led to rapid advances in correlating the functions of receptor cells with the behavioral patterns which they mediate. Following the introduction of electrophysiological techniques capable of detecting the activities of single labellar chemoreceptor cells, the search for electrophysiological-behavioral correlates was based upon the assumption that feeding behavior was elicited by chemicals stimulating the sugar (S) or water (W) receptors, while those chemicals which inhibited feeding upon sugars (salts, alcohols, etc.) stimulated another receptor cell, generally called a salt receptor and designated, because of the larger potentials usually recorded from it, the L receptor. From a biochemical point of view, however, it is difficult to imagine a common receptor mechanism acted upon by such different compounds as hydrocarbons and electrolytes. Therefore, we investigated the possibility that entirely different receptor mechanisms might be producing seemingly identical behavioral responses, such as the "rejection" of sugar solutions.

When the activities of the chemoreceptors in a labellar hair are recorded electrophysiologically, it can be seen that alcohols and other hydrocarbon chain compounds produce two types of effects, depending upon the concentrations applied. The threshold effect of the hydrocarbon chain is a reversible inhibition of all receptor types. The excitation which sometimes results from higher concentrations is a symptom of irreversible injury. Ethanol in 40-50% solution inhibits completely both the salt fiber response to 0.125 M NaCl and the sugar fiber response to 0.250 M sucrose. N-butanol gives similar results at concentrations of 0.3-0.4 M. N-octanol emulsions in water first inhibit the water response completely; then they cause an injury discharge from all modalities. Further quantitative analysis of alcohol inhibition is difficult because of the low solubility of the higher alcohols and the high rate of evaporation from the stimulus capillary tube, making it necessary to push the labellar hair further into the tube with successive tests in order to get comparable degrees of inhibition.

Hydrocarbon amines proved to be more convenient compounds for further quantitative study of the hydrocarbon inhibition. Effective concentrations are easily soluble in weakly acid (pH 6.5) solutions, and the rates of evaporation, as measured by shifts to lower pH values, are negligible. N-hexyl, n-octyl and n-dodecyl amine (as ammonium ions) were tested in 0.125 M

NaCl. The concentrations of the amines for 50% inhibition of the salt response, when recording from the hair tip, are 1.6-2.3 mM hexylamine, 0.3-0.375 mM octylamine, 1.0-2.0 mM dodecylamine. Concentrations which produced injury after-discharges within a 15 second stimulus duration are 25 mM hexylamine, 10 mM octylamine, and 0.125-1.0 mM dodecylamine.

Octylamine was selected for quantitative studies on the relationship between inhibitor concentration and impulse frequency because of its high effectiveness and the large gap between inhibition and injury concentrations. For these experiments, the sidewall recording technique was used exclusively, since it gives reproducible responses to all modalities with a constant stimulus duration under 500 msec., provided the temperature is controlled within 0.5° C. and the initial impulse frequency (first 100 msec.) is disregarded. The concentrations of octylamine for 50% inhibition of the three modalities are 0.02-0.04 mM for 0.25 M sucrose, 0.110-0.175 mM for 0.125 M NaCl and 0.75-1.2 mM for distilled water. The inhibitions of the salt and water responses are roughly linear with varying octylamine concentrations. Sugar responses, however, are sharply inhibited at very low concentrations, and are completely blocked by 0.01 mM octylamine. From these data, it appears that the behavioral effects of brief stimulation by threshold concentrations of hydrocarbon-chain compounds could be explained by the differential inhibition of the S receptor. In higher concentrations of the hydrocarbons, the effects upon behavior might be explained by irreversible effects upon all receptor types.

In addition to the differing thresholds of susceptibility to inhibition by hydrocarbon compounds, certain differences in the characteristics of inhibition of L and S receptors are also apparent. When different concentrations of NaCl are tested, the degree of inhibition at a given concentration of octylamine is directly related to the magnitude of the control response to salt alone, indicating that the mode of inhibition remains the same. Tests with two concentrations of sucrose show the same dependence upon the magnitude of the control response, but the slope of the inhibition curve is much steeper at the low sugar concentrations. The characteristics of the salt and water inhibition suggest a non-competitive interference with receptor depolarization. The sugar inhibition appears to reflect two parameters and suggests that hydrocarbon inhibitors attack enzyme-like complexing sites in addition to blocking of some strictly nervous component of the response. To explore this possibility, freshly prepared solutions of alpha- and beta- glucose (less than 15% muta-rotation) were tested for evidence of enzymatic specificity in the sugar response. Alpha-glucose stimulates the S receptor at a concentration of 0.07 M. Beta-glucose, however, gave no response at 0.14 M. These results suggest complete specificity between

the alpha and beta forms and, together with other data, lend support to the hypothesis of enzyme involvement in the processes bringing about depolorization of the sugar receptor. Further studies are now underway to determine the exact nature of the enzymatic complex in the sugar receptor response.

(This investigation was aided by U. S. Public Health Service Grant No. AI 02271, and one of us--R.S.--holds a Traineeship under U.S.P. H.S. Training Grant GM570-03.)

ELECTROPHYSIOLOGICAL STUDIES OF A SINGLE TASTE HAIR OF THE FLY DURING STIMULATION BY A FLOWING SYSTEM. Brunhild Stürckow, I. Physiologisches Institut der Universität des Saarlandes, (665) Homburg/Saar, Germany.

If the fly is feeding or drinking, the otherwise-folded labella of the proboscis are opened to a disk. In this way the taste hairs around the disk are retracted from the food. Their sensitive tips are in contact with taste substances - of low as well as high concentrations and viscosities - only for a few seconds. However, we have done experiments with prolonged stimulation of a hair in order to get more knowledge of the taste hair. Electrophysiological studies were carried out.

It is known from the papers of Hodgson et al. (1955), Hodgson and Roeder (1956), Wolbarsht (1957), Wolbarsht and Dethier (1958), and Evans and Mellon (1962) that the taste hairs on the labellum are equipped with special receptor cells for sugar, salt, water and mechanical stimulation. It is not known if the combination of the sense cells - usually five are associated with each hair - is the same in each hair or not. We examined stimulation by bending of the hair, water, salt and sugar. Only hairs which did not respond to the mechanical stimulus were examined further.

A flowing stimulus was developed at the suggestion of Prof. R. Stämpfli which permits stimulation with exactly the same concentration for several minutes and allows a quick change from one solution to another. The system used for the flowing stimulus consists of a glass-probe with several individual channels that taper uniformly. The wide ends of the channels are connected via special taps to bottles containing different solutions for stimulation. By means of the taps any desired solution can be run through the probe. The tip of the probe consists of a wider capillary with a tip diameter of about 80 μ glued over the tapering ends of the channels. The tip capillary provides the mixing space for the test solutions. The velocity of the test solution is limited by the flexibility of the hairs to an average of 0.25 ml/hour. At this velocity the content of the mixing space (70-100 nl = 70-100 x 10^{-9} liter) is changed completely within 1 to 1.5 sec. The hairs are so flexible that they can only be put into the stimulating solution when it is not flowing. Mechanical stimulation can also be carried out with the probe by bending the hair during the absence of flow. The fluid ejected from the probe flows off along the outside of the tip capillary and down along a filter paper. Analysis of expelled fluid shows that only traces of the non-flowing fluids diffuse into the flowing solution. If, for example, the test solution is changed from 1 M NaCl to distilled water, the final water contains NaCl equivalent to 1mM. This slight error applies more or less to all the solutions that were tested. Flowing systems were used with 2, 3, 5 and 7 channels.

Each channel contained an earthed platinum-electrode. Another platinum-electrode was put into the base of the excised proboscis

and connected to the high impedance input of a preamplifier (across a capacitor in order to avoid currents due to diffusion and electrode potentials which may amount to as much as 40 mV depending on the flowing solution). The amplified signals were recorded by an oscilloscope and a tape recorder, the second track of which was used for comments recorded from a microphone. The moment of switching the solutions was marked by a square pulse activated by a foot control. Later on the patterns of excitation of a single hair were photographed from the tape recorder by a Grass camera at a speed of 20 msec/cm.

So far, a complete change of solutions requires at least 1 sec., but the response to the new solution is observed sooner. The first impulse to salt, for example, in 10 successive tests of 1 M NaCl following water varies from 390 to 1110 msec. after the time of switching. The time between the moment of switching and the first impulse due to the new solution includes the inertia of the flowing solution (during switching the flow stops for a very short time) and the latency of the nervous structures (which includes the latency of the dendrite and cell body and the diffusion of the solute through the hole - or holes - in the cuticle of the hair tip if the dendrite does not reach to the hole - or holes). Since the inertia of the flowing solution alters only as a function of the viscosity, the latency of the nervous structures must differ in the example mentioned at least between 390 and 1110 msec.

For demonstrating the reliability of the flowing system and the reproducible excitability of the chemoreceptor by salt and sugar, hairs were not used which gave an observable response to water. They are useful for studying the response of the salt and sugar receptors by changing the stimuli between water as a conditioning environment and salt or sugar as the test stimuli.

The response of the salt receptor is very regularly reproducible with 2-8 tests/min lasting 4 or 8 sec. In one case 90 tests with 1 M NaCl were applied to a hair in three quarters of an hour. The time between the moment of changing from water to salt and the first impulse due to salt varied in 45 tests scattered over the whole experiment from 360 to 1110 msec. (mean and standard error 625 $\pm$ 95 msec.). This time did not change significantly between tests at the beginning of the experiment (mean and standard error of 10 tests: 770 $\pm$ 260 msec.) and at the end of the experiment (mean and standard error of 10 tests: 570 $\pm$ 200 msec., $P < 0.6$). This result indicates that the latency of the salt receptor (in the above-mentioned sense) does not markedly change during repeated short tests and conditioning stimulation with water. However, the frequency of the spikes of the salt receptor declines slowly. Within 20 min during which 44 tests were given a remarkable decline was seen.

Since the rise time of the stimulus (1 to 1.5 sec) is long,the frequency of the spikes of the taste hair reaches no sharp peak. The slight fluctuations of the spike frequency seem to be a regular property of the salt receptor. Impulses from one or more

cells other than the salt receptor cell occur, but only rarely during stimulation by 1 M NaCl. In our recording situation, they can only be identified by their disturbance of the regular pattern of response from the salt receptor. Since these spikes are very rare, the total spike frequency does not differ significantly from the frequency of the salt receptor impulse.

If a hair with an obviously identifiable impulse from the salt receptor cell is tested first with a given concentration of NaCl, then with 0.5 M sucrose, and again with the original salt concentration, the response is unaffected. However, some hairs respond to NaCl solutions with an impulse that is neither the usual salt spike nor the water spike. With repeated tests of 1 M NaCl, these hairs exhibit wide variations in spike frequency. In such records, two spike types are clearly discernable: one with a regular and low frequency (probably the salt receptor spike), and another with an irregular and high frequency. In prolonged experiments, water alone begins to evoke a few impulses when earlier there was no response to water. After several tests of 0.5 M sucrose, the response to salt recovers only after two or three tests with salt. This inhibition by sucrose along with the above observations indicates that salt solutions activate two receptor cells in these hairs; one of these cells may be the usual salt receptor, but the other (or both cells) is not specifically responsive to salt.

These observations, using a flowing system to ensure that the stimulus quality and intensity were defined and constant, indicate how many varities of patterns of response are possible even with simple stimuli. Furthermore, there is the complexity that each hair usually has 5 sensory cells, while the adequate stimulus is known for only some of them (four: water, salt, sugar and mechanical stimulation; or less than four). The kind of sensory supply varies among the hairs. Our results indicate that there are hairs with a mechanoreceptor and a water receptor as well as hairs without one or both of them. Hairs with a water receptor have a complicated pattern of response. The excitation of the water receptor depends in a complex way upon the nature and quantity of solutes present (see also Evans and Mellon, 1962). One should consider furthermore that a prior test often influences the results of a succeeding one.

I am indebted to Prof. R. Stämpfli for his suggestions concerning the flowing stimulus.

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GENERATOR POTENTIAL OF INSECT CHEMORECEPTORS. Hiromichi Morita, Dept. Biol., Fac. Sci., Kyushu Univ., Fukuoka, Japan.

The special structure of chemosensory organs of insects enables us to study their responses more directly than vertebrate chemoreceptors. From a side wall of a chemosensory hair, which was penetrated by the tip of a micro-needle, we can record the electrical activities of chemosensory cells before, during and after stimulations. These types of records, with other experiments, lead us to postulate that the initial electrophysiological steps of chemoreception occur as follows: chemical stimulants depolarize the receptor membranes at the tip of the chemosensory hair; the depolarization initiates impulses in the cells near the hair base; the impulses are conducted toward the hair tip as well as to the C.N.S. This scheme was obtained with labellar chemoreceptor of the blow-fly (Morita, 1959; Morita & Yamashita, (1959) and was applied to olfactory receptors of silk-worm larvae (Morita & Yamashita, 1961). In the present paper, records from other insect chemoreceptors will be shown. All these records are explained by the same scheme as mentioned above. The materials used are: contact chemoreceptors--- labellar chemosensory hairs of the blow-flies, Calliphora vomitoria & Lucilia caesar, and the house-fly, Musca domestica; tarsal chemosensory hairs of the butterfly, Vanessa indica. Olfactory receptors--- sensilla basiconica of silk-worm larvae, Bombyx mori; olfactory sensilla of cockroach, Periplaneta sp. (Tateda et. al.)

Another advantage of studying insect chemoreceptors with this method is the ease with which the duration of stimulus is controlled. Thus, we can observe the rapid restoration of receptors from excited to resting states. To do so, contact chemosensory hairs were stimulated by a solution contained in a glass capillary mounted on a glass arm (5mm wide, 2mm thick) with vaseline. The arm was fixed to the moving iron piece of an electric relay, which was driven by a Grass stimulater model-S4C. It is very difficult to record generator potential without any artifact. There are two main sources of artifact: one is an electrical leakage between the solution and the ground (this can be neglected in the above mentioned situation); another is a type of frictional electricity. The latter can be minimized by making no contact with leucite or plastic material at any stage of handling the capillary.

Generally any solution induces water-spikes below a certain concentration, and water receptors also show small but clearly detectable generator potentials. Since we have no chemosensory organ including only one receptor, it is difficult to study the relation between the amplitude of generator potential and the frequency of impulses. However, this was done with the sugar receptor in a hair where salt and water receptors had lost their

function. The correlation coefficient between the two variables was found to be 0.85, based upon 108 comparisons.

Salts mixed into a sugar solution depress the frequency of impulses from the sugar receptor, and reciprocally, sugars depress the salt spikes in the analogous situation. This was pointed out by Hodgson (1957), and was confirmed by several authors. Such an interference of mixed solutions can also be observed in generator potentials, which indicates that the interference occurs at or before the stage of depolarization of receptor membranes. Restoration of depolarized membrane to the resting state is very rapid. In normal receptors stimulated during fractions of a second, the restoration is almost complete within 20-30 msec. Only in the case of stimulation by a high concentration of sucrose, does a residual depolarization seem to last as long as half a second or more. We have no complete explanation of this amazingly fast restoration. However, we have observed that when a sugar receptor was partially damaged by a long application of acetic acid, the depolarization lasted more than one second. A similar prolongation of depolarization is sometimes observed in old experimental preparations. These observations suggest that the receptor membrane, in its normal condition, may be capable of inactivating stimulus molecules.

There are many substances which, when mixed with sugar solution, are not accepted by the fly. These substances are generally considered to initiate impulses in salt receptors. However, many substances rejected by the fly do not stimulate salt receptors, but inhibit responses of both sugar and salt receptors to adequate stimuli. Among them, $CaCl_2$ and quinine were studied, and were shown to hyperpolarize the receptor membrane during their applications. After withdrawal of the solutions, $CaCl_2$ depolarizes the membrane accompanied by trains of salt spikes (or, in some cases, water spikes) as an after-effect, but quinine leaves a long lasting hyperpolarization. The effect of $CaCl_2$ is somewhat variable. Sometimes it depolarizes the membrane and initiates the impulses in the salt receptor during its application. In some preparations we have observed very large (up to 5-7 mV) positive- or negative-going potentials. These last as long as several seconds in the case of stimulation by salts, but never in the case of sucrose or water application. Such potentials are always observed in the tarsal chemosensory hairs of Vanessa when stimulated by salts. The origins of these potentials are not known.

ELECTRICAL SIGNS OF CHEMORECEPTION. Myron L. Wolbarsht, Naval Medical Research Institute, Bethesda, Maryland, U.S.A.

The electrical responses of external chemoreceptors will be considered primarily here, although similar problems are certainly encountered in such internal receptors as the carotid sinus and synapses with chemical transmitters. Both taste receptors, in which stimulus is presented in the liquid phase, and olfactory receptors where the stimulus is primarily in a gas phase, share the same problems.

The electrical responses of a chemoreceptor possesses two components, the receptor potential and the impulse potential. The receptor potential arises as a result of the transduction of the chemical stimulus into some effect on the polarization of the receptor membrane. The term "receptor potential" is applied to those non-propagated potentials recorded near a receptor. It may refer to an intra- or extra- cellular potential and may be hyper or depolarizing in nature. The term "generator potential" is reserved for those potentials existing intracellularly at the site of impulse initiation. Since the term receptor potential is usually applied to all the potentials recorded in the vicinity of a receptor or a group of receptors, it may represent, in fact, the summed responses of the many dissimilar types of cells of a whole organ. At some point, the receptor potential results in the generator potential, either in the same cell where the chemical stimulus acts or in a different cell.

The way in which a chemical stimulus affects the membrane of the receptor involves many processes. It is in this step that the specificity of a given receptor for a particular chemical or group of chemicals is determined. However the stimulus acts, by activation of an enzyme, by acting as the substrate for some chemical process (although the amounts of the stimulating chemical involved seems to preclude this hypothesis), or by binding to specific loci on the membrane and thus opening pores, there is one end result. The conductance of the membrane is changed (usually lowered). This may be an ion specific change (sodium is the most likely) or a generalized resistance change. It is likely that each receptor contains many receptor sites and that changes take place only in the patches of membrane surrounding each active site. Some receptors (the mammalian taste bud cells) may contain several dissimilar types of sites, each sensitive to a different group of chemicals. (Beidler, 1960). The receptor specificity is compromised somewhat by such an arrangement, but higher sensitivity and response to a broader array of stimuli may be made possible.

We usually think of the receptor or generator potentials as being depolarizing in nature so that application of the stimulus produces an immediate impulse response. It is also possible that

the action of the chemical stimulus would be to hyperpolarize the receptor membrane (by making it less conducting) so that when the stimulus is removed, impulse activity will be generated by a post -inhibitory rebound of the cell membrane. This might give rise to the phenomena of contrasting after tastes, for example.

Almost all insect chemoreceptors are bipolar in nature. The action of the chemical stimulus is upon the dendrite and impulses apparently are generated at the cell body or axon hillock. Often the dendrite is quite long and thin. It is difficult to understand how minute changes in the polarization of the end of such a dendrite can have very much influence by decremental induction upon the impulse activity of the cell, but apparently, such is the case. (Morita, et al, 1959). Capacitance, which is usually invoked to explain anomalous decremental conduction, can not be used in the present case as the effects may have enormous time constants.

In the vertebrates the taste receptors have dendrites, or hairs but do not possess axons. Impulses are apparently initiated in the second order neurons which are in intimate contact with the receptors. The olfactory receptors have a short axon which conducts impulses. It is not at present known whether these impulses are initiated in the dendrite or the axon. (Beidler and Tucker, 1955).

The site where the chemical stimulus is transduced into the original slow potential may or may not be capable of electrical excitability, so that the impulse may or may not invade the region of the original chemical stimulus. Many problems are associated with the detection of this occurrence. Modifications of the impulse activity may be expected if the impulse invades the receptor site.

The recordings of the receptor potentials have been most commonly used as a research tool in olfactory systems such as the antenna of the moth, (Schneider, 1957) or the olfactory mucosa of some vertebrates. (Ottoson 1959, Beidler, 1960). In these cases, the recordings are from many cells, presumably of different types, and the potential seen is a summed one. Although many correlations have been made between certain characteristics of this potential and the various chemical stimuli, certain difficulties are immediately evident. The olfactory mucosa is a complex structure with many of its characteristics unrelated to the chemoreceptors. The application of the chemical stimulus can give rise to potentials which are seen by the recording system but apparently are not effective on or produced by the chemoreceptive system. For instance, many of the activities of the olfactory mucosa of the frog can be recorded from long dead frogs or can be mimicked, under the appropriate conditions, by electrodes placed on absorbent cotten soaked with Ringer's solution (Mozell, 1962). Potentials are set up when various odors

presented to this system. These potentials are abolished by the application of cocaine, usually considered to be an agent specifically effective on the nervous system. Such a potential would have little, if any, relation to the membrane potentials of the chemoreceptor and might, in fact, mask it. A similar artifact may exist in the olfactory system of insects.

The insect as a whole is insulated from its environment by the cuticle. Where the insect is in contact with the environment through its chemoreceptors, some break in the cuticle must be made. If a map of the external resistance of the insect cuticle is made, those points of low resistance may represent chemoreceptors, however, joints, spiricles, and the eyes may have a low resistance without being chemoreceptors. On the other hand certain of the chemoreceptors, for example, the olfactory receptors, may be adapted for reception of chemicals in non-aqueous phases. In this case, the aqueous electrolyte electrode may not measure a resistance lower than the rest of the insect.

In some fashion all chemoreceptors give rise to impulses to carry the information about the chemical stimulus into the central nervous system. This is true even in insects. The information is often coded elaborately. Frequency modulation, total number of impulses of a given receptor, and cross correlations between the responses of the various types of receptors are all used in order to allow the animal such discrimination as he may possess between the diverse stimuli which are presented to him. This may be done in several stages so that higher and higher centers perform more and more computation upon the original and the altered signals. The experimenter can distinguish the electrical impulses from only a few receptors simultaneously. From this he must determine the specificity of each type of receptor and how the information is encoded. This is a formidable problem for even so simple a receptor as the contact chemoreceptor on the labellum of the dipteran, Phormia. Here a single hair contains three, four, or even five bipolar neurons. (Larsen, 1962; Stürckow, 1962). All differ in their sensitivity to various chemicals. A particular stimulus is applied to the tip of the hair and acts upon the dendrites of the various neurons producing some changes in the polarization of each. Some neurons will respond with impulses. The comparison between these responses enables the fly to decide what stimulus is applied to the tip and take the action demanded by it: to eat it if it is food; to do nothing if it is not; or avoid it if it appears to be harmful. The fly can decode this information. The experimenter is not yet able to, but much progress has been made. Perhaps by the next congress, we shall be considerably more successful in solving this particular problem at least, if not the larger one of olfactory discrimination in man.

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THE PHYSIOLOGY OF ECHINODERMATA

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THE FUNCTIONS AND PROPERTIES OF THE ECHINODERM NERVOUS SYSTEM.

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The nervous system of echinoderms consists of (1) bipolar sensory cells scattered throughout the integument and parts of the gut, (2) intermediate (or association) pathways, threaded through the spaces above the basement membrane of the epithelium and between the tapered inner ends of the epithelial cells, and (3) motor neurons which transmit excitation from the association pathways either directly to the musculature or through intercalated relay neurons.

In the asteroids and echinoids, the association pathways are represented peripherally by a plexus underlying the skin; in the remaining classes of the Ophiuroida, Holothuroida, and Crinoida dermal ossicles invade the epithelium and obliterate the plexus. The sensory cells accordingly transmit excitation into nerve tracts which plunge through the body wall into the interior of the body. Both arrangements connect the afferent pathways with the radial nerves and, through them to the nerve ring.

This paper deals with the conditions of nervous elicitation of the responses of the movable organ systems of the asteroids and echinoids. The movements of the spines, pedicellariae and tube feet are considered in relation to the form and properties of the excitation circuits through which they are innervated. Experiments are cited which (1) throw light on the geometry of the pathways of the skin of the asteroid arm and echinoid test through which the responses of pedicellariae, spines and of other action systems mounted on the body wall are mediated, and (2) relate to the role of the central pathways of the radial cords and nerve ring in creating co-ordinated systems of movement, for example, the tube feet and (in echinoids) the spines. There is anatomical evidence that in both groups the peripheral plexus consists of (a) a more superficial layer of fibres of indeterminate weave, and of deeper fibres which are oriented into linear pathways directed transversely to the radial cords and connected to them. The geometry and conduction properties of the pathways are discussed in the light of their experimental analysis, and reference is made to inhibition phenomena, decremental conduction and the chemical mediation of synaptic transmission. The role of the central pathways is considered in respect of the integration of complex movements (e.g. the steps of the tube feet), pacemaker control and the co-ordination of unidirectional patterns of movement of the spines and tube feet. Reference is made to the varying degrees of interaction of the peripheral and central control of movement of the different action systems, and to the variations of interaction found in different classes of echinoderms.

PHOTOSENSITIVITY IN ECHINOIDS. M. Yoshida, Tamano Marine Laboratory, Shibukawa, Tamano, Okayama, Japan.

Echinoids are known to be eyeless and yet many of them respond to light. No electrophysiological work being available, only overt responses can be used as a sign of the existence and the degree of photosensitivity. Such responses may be classified into three types; (1) direct response, (2) reflex responses of component parts of the animal and (3) behavioural responses as a whole.

(1) Direct response; In some urchins (Arbacia lixula, Centrostephanus longispinus, Diadema antillarum and D. setosum), melanin granules in chromatophores distributed over the skin and tube feet disperse under light and concentrate in darkness. The response has been known to be independent of the presence or the absence of the central nervous system. Further work with an improved optical system has proved that chromatophores respond directly to light, suggesting that the photosensitivity resides within the chromatophore itself (Yoshida, 1956). It is also shown that chromatophores distorted by centrifugation still retain their reactivity to light (Yoshida, 1960). Though it is attractive to assume that these responses play a role in dermal photosensitivity, similar to that played by photomechanical movements in more elaborate photoreceptors, conclusive evidence for it has not yet been obtained.

(2) Reflex responses; In many urchins it has been known that responses of spines, podia and pedicellariae to a sudden increase ("on") or decrease ("off") in illumination require the presence of the radial nerve. Among those, the spine response of Diadema is not only striking but also suited for experimental purposes for various reasons. (1) Operational units bearing a part of radial nerve and spines can be isolated in the form of sectors and these spines show active movements in response to both "on" and "off" for several hours, though most of the succeeding account is based on the "off" response, for it is more sensitive, vigorous and prolonged than the "on" (Millott & Yoshida, 1960; Yoshida, 1962). (2) The spines are so long that their tips can be illuminated to help visual observation or for photographical recordings of their movements without affecting the underlying skin which is shown to be photosensitive (Millott, 1954). (3) The fact that the radial nerve itself which is more or less uniform along its whole length can be stimulated by obliterating a minute light spot of only about 10 μ in diameter shone onto it (Yoshida & Millott, 1959), is also advantageous, for a fairly stable photic condition can be secured by shining light beams onto the radial nerve and not onto the irregular surface of the skin where the stimulating light beams are often interfered with by a number of small spines and pedicellariae which are continually moving.

Our first attempt was to discover what effects varying the intensity and duration of both light and shade exert upon the character of the spine response (Millott & Yoshida, 1960). The criteria used were the reaction time (the interval between the moment of "off" and the first sign of a response), the number of beats during a standard period, the amplitude of contraction and

the duration of reaction (the period from "off" to the end of all contractions). It was shown that variations in the intensity and the duration of the preceding illumination exerted similar effects on all the above features. On the other hand, variations in the intensity and the duration of shading were found to differ in their effects. The intensity affected all the features of the whole reaction but the duration did not affect the initial part of the reaction. The differing effects of light and shade led us to suggest that the "off" response may be a rebound from inhibition due to light which, when re-admitted, diminishes any "off" reaction that may be in progress.

Inhibition: The above suggestion was substantiated by experiments with two light spots, the intensity, the position and the time of "on" and "off" of which were all made controllable independently (Millott & Yoshida, 1960). At first the two were superimposed. When one (L_2) of the two was admitted after "off" of the other (L_1) whose intensity was kept constant, the reaction was inhibited to a degree roughly proportional to the intensity of L_2. When the two were separated the inhibitory effect of L_2 still appeared and it was exerted over distances as great as 6 mm. It was thus possible to make the effect of light at one point inhibit the response to a shadow cast elsewhere. The effect was here again roughly proportional to the intensity and the size of L_2. Similar inhibitory effects of light were also shown on the outside surface of the skin.

Analysis of initial process: In order to reveal more of the mechanism involved in eliciting the "off" response, attention was paid to the events occurring between the moment of "off" and the first sign of the reaction, the period which must play a decisive role on the events occurring later. Since a process leading to a final result, the spine jerk, is set in train at "off" of a light (L_1), the time course of the process must be identical under identical conditions of L_1. When a second light (L_2) is admitted after "off" of L_1, any change in effectiveness of L_2 (as expressed in terms of the threshold intensity to inhibit the response) with time at which it is admitted, may be considered as an expression of a change in the process released at "off" of L_1. At all intensities of L_1, the inhibitory threshold of L_2 stayed constant at first and then increased suddenly at a critical point. The interval between "off" of L_1 and the critical point became shorter at higher intensities of L_1 and at higher temperatures (Yoshida, 1962). The abrupt change in effectiveness of L_2 hints at an additional mechanism existing between the receptive system and the effector. The mechanism may be activated as a result of primary interaction between excitation and inhibition and the activity thus ensuing may be inhibited through the receptive system. The period during which the inhibitory threshold stays constant may be an expression of the latent period of the primary interacting system. It is pertinent to recall our earlier work on the inhibitory pathways (Millott & Yoshida, 1960), in which it was suggested that interaction between excitation and inhibition may occur in the radial nerve and at the periphery. It seems plausible that the

latent period concerns the central inhibition and the additional mechanism concerns the peripheral inhibition. Applicability of some of the photosensory laws was then examined.

Ricco's law: The reciprocity of area (A) and intensity (I) of light was examined for both the preceding and the inhibitory light by keeping one of them constant while the other was subjected to test. It was found that, regardless of whether the size of light to be tested was larger or smaller than that used as reference, the total intensity (I x A) to produce a constant response stayed constant over a wide range, suggesting that the extent of the receptive field in the radial nerve may be fairly large.

Weber-Fechner law: Since it is difficult to envisage how a negative entity (the Weber's fraction will be negative for "off" responses) can possibly affect any biological system, the whole event was analysed in terms of the efficacy of light (L_2) remaining to inhibit a response after "off" of the preceding light (L_1). It was shown that the inhibitory threshold of L_2 was a constant fraction of L_1 when the latter was fairly bright. It is obvious that this is another way of expressing the Weber-Fechner law.

Light and dark adaptation: Threshold determinations with test flashes after a period of light or dark adaptation as have been done customarily for "on" responses, are not adequate in the case of "off" responses, because in order to follow the changing level of sensitivity the intensity of light to test the threshold level should also be altered accordingly, which will in turn disturb the state of sensory adaptation. The method used here was as follows (Yoshida, unpublished). After a period of general illumination of the whole preparation, a minute area was illuminated by a light (L_1) of constant intensity. Changes in sensitivity level was followed at 5 min. intervals by determining the liminal intensity of light (L_2) necessary to inhibit the response which should have appeared at "off" of L_1 if L_2 was not admitted. It was found that the sensitivity level fell after the general illumination and rose during darkness, the time course of which depended on both the adapting and the testing light conditions. Whether the two spots (L_1 and L_2) were spatially coincided or separated did not affect qualitatively the course of dark adaptation. This rules out a possible change in sensitivity during illumination with L_1, because the two being separated, the site at which the sensitivity level was to be tested had been in darkness all the time after general illumination was cut off. Again, the fall in sensitivity occurred when L_1 and L_2 were shone onto an area which had been shaded during general illumination. All this suggests that the decrease in sensitivity is caused by persisting inhibitory effect of the general illumination on the whole receptive system.

When the two testing spots were placed at an outside ampullar pore, the sensitivity level as expressed by liminal intensity of L_2 changed but little after light adaptation. It is possible that here the two beams have stimulated through the pore an organisation existing outside the test, where the effect exerted by general illumination must be far less. A search in this vicinity has revealed near the oral side of the outside ampullar pore a

structure which has a close connexion with nerve fibres which have just emerged through the test (Yoshida & Yoshida, unpublished). It is premature to attribute photoreceptive functions to this structure, but attention may be drawn to the fact that amburacral margins are shown to be most sensitive and also that Millott (1960) has called this structure "podial ganglion".

(3) Behavioural responses as a whole;

Covering reaction: It has been known that many urchins and sand dollars often cover themselves with opaque objects. This has been studied recently by Millott (1956) who demonstrated in Lytechinus variegatus a beautifully coordinated action of spines and tube feet which carry covering objects over various routes in response to movement of the light source. It was also shown that urchins can be photosensitized by injections of fluorescent dyes so that they cover in dim light. Though removal of lantern with the nerve ring attached did not abolish the action of urchins to take up such objects, he admitted the possibility of the nerve ring to be involved in such highly coordinated action seen in intact animals.

Taxis: Phototactic responses which have been known in many adult urchins resemble superficially the tropotaxis which is seen in animals with well-developed photoreceptors. Being an eyeless form, echinoids must appreciate the distribution of light and dark through their dermal sensitivity by detecting unbalanced illumination over the skin. Apart from the work of Holmes, the role of the nerve ring on these responses has been studied only little. Our recent study (Yoshida & Kobayashi, unpublished) on Temnopleurus reumaticus which reacts positively to light has shown that if urchins with one radial nerve transceted near the oral nerve ring were placed between two light sources faced each other, they never moved toward the source to which the operated side was facing, even though the light intensity at the unoperated side was weaker than the other. In a single horizontal beam, they often turned round where they were placed before starting locomotion toward the light source if the operated side happened to face it. Confirming the need of the nerve ring for tactic movements, these experiments further suggest that the light source may be located by the surface facing to it and that the information may be sent through the radial nerve to the nerve ring which ultimately governs the coordinated locomotory reaction.

Larval phototaxis of echinoids is known among embryologists by experience, but no work has ever been done specifically on it. We (Yoshida & Neya, unpublished) have recently found that larvae from early gastrulae to plutei of Hemicentrotus pulcherrimus react positively to a light below 1 lux in luminous flux. The effect of a horizontal parallel beam, e.g., 0° of divergence, was but little but if it was diverged by 5°, they were found aggregated near the wall of the brighter side. They also gathered in a parallel beam when applied vertically, forming en mass an appearance like a waterspout. It is informative that the presence of an air-water interphase was needed to induce such aggregations, though the ways with which each larva swims up the photic gradient are yet to be determined.

SPINE MOVEMENTS IN RESPONSE TO PHOTIC STIMULI AND THEIR IMPLICATIONS, IN THE ECHINOID DIADEMA. Norman Millott, Dept. of Zoology, Bedford College, University of London, Regent's Park, London, N.W.1, England.

The aboral primary spines of Diadema antillarum react to localised shadows by an orientated reflex movement, followed by continued rapid oscillation which distinguishes them from responses to non-photic stimuli.

The direction of the first movement is determined by the position, relative to the reacting spine, of the light spot on the skin (surface factor), of the oral pole (oral directing factor) and of the radial nerve through which the reflex is consummated (ambulacral directing factor). These factors appear to be manifestations of the routes by which nervous excitation reaches the spines: in the case of the surface factor, through the superficial nerve layer; in the instance of the oral and ambulacral factors, from the radial nerve. Nervous effects following the two routes interact at the spine base. The oral directing factor appears to be the outcome of the preponderantly oral disposition of the nerves entering the spine base from the nearest radial nerve.

Effective excitation can be transmitted through the superficial nerve layer more than half way round the ambitus, and latency studies indicate that transmission over the surface can occur at significantly different speeds in afferent and efferent pathways, so that this layer has properties inappropriate to an anastomosing network.

Each of the categories of spines, ambulacral and interambulacral, shows responses to a given stimulus that are similar in amplitude and duration, which, together with the effect of cooling selectively a radial nerve and its associated structures, suggest that members of each category are responding to a common drive from this nerve.

Clamping selected spines afforded no indication that proprioceptive relays at the bases of primary spines are involved in the spread and co-ordination of spine movements. Such relays, furthermore, would not explain the direction of the first movement of some spines in response to photic stimuli, but their possible participation in surface transmission is not precluded.

The gradients observed in latency and in the frequency of oscillation of interambulacral spines situated at increasing distances from a radial nerve, change differently with temperature. They suggest that spine oscillation is governed by discharges from the radial nerves rather than by self-exciting circuits, either localised at the spine base or extending between this and a radial nerve. This emphasises the rôle of the radial nerves as centres for co-ordination and integration, so that current notions of echinoid nervous

organisation in terms of a "republic of reflexes" (von Uexküll) are inadequate and need considerable revision.

The study was made in collaboration with Dr. K. Takahashi.

PIGMENTATION OF THE ECHINODERMS. Gwynne Vevers, The Zoological Society of London and Dept. of Zoology, Bedford College, University of London.

Coloration of echinoderms. The integument has "melanin," carotenoids, porphyrins and naphthaquinones, varying in distribution according to the class. In starfishes the principal pigmentation is due to carotenoid, although porphyrin has been found in three species. Brittlestars have carotenoids, melanin, and riboflavin; there is a suggestion that they also have pteridines. Sea-urchins, and apparently also crinoids, have naphthaquinone in the test and spines and carotenoid in the gonads; there are also unidentified iron-containing pigments of nuclear origin. Some holothurians have a pigment of unknown composition, and what is presumably a "melanin."

Origin and function of the pigments.

Carotenoid, which is perhaps the most widespread, is presumably derived directly from plant food in those species which are algal browsers or suspension feeders. Carnivorous starfishes must obtain their carotenoid from the bivalve molluscs and other invertebrates on which they feed. Starfish integument has yielded β-carotene, cryptoxanthin, astaxanthin, one or more keto-carotenoids and certain unidentified carotenoids. In at least one species (*Marthasterias glacialis*) the terminal eye spots have been shown to contain β-carotene and astaxanthin. There is some evidence that eye spots are sensitive to light, and this is likely in view of the wide occurrence of carotenoproteins in light sensitive organs. In addition Rockstein has extracted a light sensitive pigment from the dorsal skin and eye spot of *Asterias forbesi*. Many have failed to find Vitamin A in starfishes, but precursors, such as β-carotene and keto-carotenoid are abundant. In many starfishes carotenoid is linked to protein to form red, brown, green or violet carotenoproteins.

Ophiuroids also contain abundant carotenoid, principally β-carotene, an acidic carotenoid and a number of xanthophylls. Both asteroids and ophiuroids tend to store more xanthophylls and acidic carotenoid than hydrocarbon carotenes; it has been suggested that these animals may metabolize the latter more rapidly than the former. Pigment spots, containing β-carotene and esters of acidic carotenoid, have recently been described on the aboral surfaces of ophiuroid arm ossicles. As with asteroid eye spots their function is unknown, but they are sensitive to light and/or aerial oxidation.

In echinoids carotenoid is principally, if not exclusively, restricted to the gonads, although the marked light sensitivity of these forms suggests that it may be present in the skin.

Melanin is particularly abundant in ophiuroids and echinoids. It probably occurs in holothurians, where phenolases have been found in the coelomic fluid. In ophiuroids recent work on the polychromic Ophiocomina nigra show that melanin occurs in the integument in several different oxidation states, giving black, browns and fawn coloration. The melanic forms of this species contain large amounts of fluorescent pigments, particularly riboflavin, and possibly also pteridines, whereas in non-melanic forms these pigments are in low concentration. Fontaine has suggested that the fluorescent pigments are concerned in melanogenesis and that their absence inhibits this process. There is still no explanation to account for the differential distribution of the various types of melanin and of the fluorescent pigments within a population or even in a single individual, although it has been noted that the proportion of light-coloured Ophiocomina in a population increases with depth.

In echinoids melanin is abundant in the skin and amoebocytes, and notably so in the axial organ of Diadema.

Melanin may function in echinoderms generally as a screen against injurious radiation, and possibly also as part of a light adaptation mechanism. The detailed work of Millott and others on light sensitivity in Diadema is being described by Yoshida in a paper in this symposium.

Porphyrins have been found in the integument of three species of asteroids, but in no other echinoderms. Astropecten irregularis and Luidia ciliaris have protoporphyrin and chlorocruoroporphyrin. Asterias rubens has only the former, whilst the related Marthasterias lacks porphyrin. This sporadic occurrence of porphyrin is puzzling. These pigments do not contribute to the general coloration of the animals, although in Asterias rubens pale individuals have less protoporphyrin than violet-brown individuals. In asteroids porphyrin is probably an excretory by-product formed from chlorophyll, or it may be concerned in integumentary photoreception, since porphyrins are known to render living tissues more sensitive to light.

Lipofuscin has recently been found in the axial organ of Diadema, but is absent from that of Paracentrotus and Arbacia.

Naphthaquinones have been known in echinoids since the pioneer work of MacMunn who discovered echinochrome in 1883. They occur in no other animals, with two possible exceptions. These are the purplish colour of the bones of the Pacific sea-otter, Latax lutris, which feeds largely on Strongylocentrotus droebachiensis, and the red pigment of the crinoid, Antedon bifida, which appears to be similar to the naphthaquinone of Diadema.

Polyhydroxyquinones have been found in a Jurassic crinoid.

Naphthaquinones are widespread in the test and spines of echinoids and several different pigments have been described. They also colour the eggs of some species, and also the axial organ and certain of the amoebocytes. Their reduction is easily reversible and MacMunn thought that echinochrome might be a respiratory pigment, but later work has never shown them to absorb oxygen. It has also been suggested that they may be involved in cellular oxidative processes but this seems unlikely in view of their presence in such large amounts, most of which is concentrated in calcareous tissue. Their supposed effect on egg respiration and sperm activation has not been confirmed.

The following suggestion is here put forward for the first time. Certain naphthaquinones are known to be algistats and have been used to control the growth of Cyanophyceae in fresh waters. In 1934 Mortensen and Rosenvinge described a blue-green alga parasitic on the test of Echinus esculentus. This alga has been repeatedly found on pale individuals of this echinoid in the English Channel. It is therefore suggested that naphthaquinone may act as an algistat to protect the test and spines of echinoids, and that when it is absent or in low concentration these structures become more prone to attack by blue-green algae.

RESPIRATION IN ECHINODERMS. A. Farmanfarmaian, Pahlavi University, Shiraz, Iran.

The literature on the physiology of respiration in echinoderms is briefly reviewed from the historical point of view. The relative merit of the various methods such as the Winkler method and manometric techniques employed in investigations on echinoderm respiration are considered.

The mechanics of respiration including renewal of ambient oxygen; respiratory surfaces; transport of oxygen into the coelom and to the various internal organs; and respiratory control are discussed in connection with representatives of the five living classes of echinoderms. The functional morphology of the respiratory organs of these organisms is analyzed and special adaptations such as cloacal pump and respiratory tree in holothuroids; nidamental chamber pump in the asteroid Pteraster tesselatus; and external gills, petaloids, and Stewart's organs in echinoids are described.

Evidence from recent experiments are presented to show that the water vascular system which characterizes this phylum is intimately involved in the respiration of these organisms. It is argued that the water vascular system may have evolved to function primarily as a respiratory surface. This may have been necessitated by the appearance of the hard calcareous body wall found in most members of the phylum.

In connection with oxygen transport and storage, the role of the three fluid systems, namely the water vascular fluid, the hemal sinuses fluid, and the perivisceral fluid are considered.

The results of recent investigations are presented to show that the cellular corpuscles and the various pigments such as echinochrome and erythrocruorin found in these body fluids do not have a significant role in the transport or storage of oxygen in the species studied thus far.

Finally, the various levels of oxygen consumption in representative members of the phylum are compared and the effect of ecological variants such as temperature and osmotic pressure on respiration are discussed.

ASPECTS OF DIGESTIVE PHYSIOLOGY AMONG ECHINODERMS. John M. Anderson, Dept. of Zoology, Cornell University, Ithaca, N.Y.

As in many other features, echinoderms show considerable diversity in mechanisms and adaptations related to feeding and digestion. Significant aspects of digestive physiology include not only details of the digestive process itself, occurring within the lumen of the gut, but also the related structural features of the digestive system, gross and microscopic; sources and characteristics of digestive enzymes and accessory secretions; mechanisms of absorption and transport of nutrients; and adaptations for storage and mobilization of nutritional reserves. This survey is an attempt to draw together the results of a representative sampling of the experiments and observations that have contributed significantly to our present understanding of digestive physiology in echinoderms, to interpret these results in a comparative manner wherever possible, and to point out aspects of the subject which might repay further study. Meaningful comparative generalizations are difficult to draw in echinoderms because in many cases detailed information is lacking, and in many others the information that is available is somewhat uncertain, based as it is upon old studies involving questionable methods. In every group a great deal of work remains to be done.

The modern crinoids are particle-feeders. The meager available details of the histology of the coiled digestive tract show only what appear to be mucous gland cells in the digestive epithelium, and nothing seems to be known of the physiology of digestion in this class (1,2). In ophiuroids, the extremely varied components of the diet are passed with the aid of mucous secretions into the simple sac-like gut, which is without an anus. The epithelium liningthis cavity, like that in crinoids, shows no cells that might be interpreted as specialized for enzyme secretion (3). Extracts of the gut wall of one species have been shown to contain a protease and an amylase, with indications that a lipase may be present (4). Further details appear to be lacking.

Function in the coiled gut of holothuroids has been considerably studied. Digestive enzymes have been extracted from the wall of the gut as well as from tissues of the *rete mirabile*, a mass of hemal tufts adjacent to the wall of the intestine (5). These extracts show hydrolytic activity against starch, fibrin, peptone, and various disaccharides (5,6). In the absence of histological evidence showing secretory cells of a zymogenic type in the lining of the gut, it has been proposed that the enzymes active in the gut lumen are released there by amoebocytes, present in large numbers. The amoebocytes are believed to transport zymogen granules from secretory cells in the peritoneum of the *rete* tubules into the intestine (7). The wall of the holothuroid intestine has been repeatedly found to be impermeable to nutrients in solution. This has led to the suggestion, with some supporting evidence, that amoebocytes are also responsible for absorption and transport of products of digestion. They supposedly move through the subepithelial lacunae in the gut wall and are distributed via the

hemal system (5,8). There is evidence, however, that the fluid of the perivisceral coelom plays a significant role in nutrient transport. Appreciable quantities of glucose and NPN are found in this fluid in Stichopus, particularly studied. While during starvation the glucose level falls, the level of NPN is apparently regulated within certain limits, losses being replenished from some unidentified source. Very shortly after refeeding the glucose level rises to normal, but the presence of food in the gut has little effect on NPN levels in the coelomic fluid (9). Further studies on the possibility of glucose transport across the gut wall (10), and especially upon the relationship between the hemal system and the perivisceral coelom in nutrient transport, should yield interesting and significant results.

Like that of holothuroids, the gut of echinoids is long and coiled. It tends to be longest and best developed in the regular urchins, which utilize a varied diet of plant and animal materials bitten off and ingested by means of the elaborate lantern mechanism. The flattened sand-dollar types, adapted to a diet of detritus and particulate matter, have reduced lanterns and a shorter gut. The irregular urchins, also detritus feeders but handling their food by means of special flagellary tracts and mop-like tube feet, have lost the lantern but retain the long digestive tract. In all, the tract consists of esophagus, stomach, intestine, and rectum. A narrow siphon, of problematic function, parallels the stomach and provides a by-pass from esophagus to intestine. The hemal system is well developed in association with the gut (2).

Mucous glands and granular, basophilic secretory cells occur in the lining the the esophagus; the products of these cells are perhaps responsible for the relatively low pH (6.0) reported for the contents of this region (11). The epithelium of the stomach is very tall and contains an abundance of granular gland cells thought to be the source of digestive enzymes (11,12). Intestine and rectum are lined by undifferentiated cells without evident secretory activity. The gut contents here are less acid (pH 6.8) (11). Amoebocytes are numerous in the gut wall at all levels. A varietyof digestive enzymes has been demonstrated in extracts of the gut wall from several different species. These are usually active in neutral or slightly acid media and include amylases, proteases, saccharases, and an alginase; the presence of a lipase is questionable (13,14). Tested separately, extracts of esophagus and stomach show somewhat greater activity than those of intestine. Experiments with C-14-labeled food show that most of the activity is removed from the gut contents in the esophagus and stomach, before food has even reached the intestine, confirming the relative significance of the fore part of the gut in digestion and absorption (15). Absorption and transport may at least to some extent involve amoebocytes, which ingest particulate matter from the gut and move into the adjacent hemal vessels (11,12). Experimental evidence clearly shows, however, that the fluid of the perivisceral coelom is a more significant vehicle for the transport of soluble nutrients. In addition to coelomocytes and the proteins involved in clotting, this fluid contains reducing sugar and non-

protein nitrogenous compounds. The sugar concentration is highly variable (0-13 mg percent), falling during starvation and increasing again upon refeeding; glucose injected into the coelomic fluid of a fasting animal is almost completely removed by the tissues within a few hours. As in holothuroids, NPN levels in the coelomic fluid are maintained with little fluctuation (at about 5 mg percent), even during starvation; upon refeeding there is a slight surge in NPN concentration, indicating that the regulation is rather loose (14,16). Sea-urchins fed C-14-labeled algal disks show within a short time a marked rise in radioactivity in the coelomic fluid, chiefly associated with galactose. This compound must have been released from a more complex galactoside during digestion of the algal food. As the early peak subsides, activity increases in the coelomocyte fraction of the fluid and in the tissues of the body in general, including the gonads. The activity remaining in the fluid is now distributed among amino acids and other unidentified compounds (15). The site of nutrient storage in sea-urchins, and particularly the role of the gonads in storage, remain problematic. As the spawning season approaches, the gonads naturally increase in mass many times, and their content of such substances as glycogen, lipid, and proteins also vastly increases. These changes are clearly related to gamete production, but it has been shown that the gonad can furnish nutritional reserves for a starving sea-urchin. In Echinus, and perhaps in other forms, there is a second growth phase in the gonads during the summer feeding period, after spawning is completed; nutrients stored in the gonads at this time sustain the animal through the ensuing winter (17). Nutrient levels in the coelomic fluid show no seasonal peaks reflecting intensified translocation of materials to the gonads; the "continual dribble" of nutrients absorbed from the gut must suffice at all seasons (18). Allocentrotus, a deep-sea echinoid, stores lipid (up to 30 percent dry weight) in the gut wall (19), and glycogen and lipids have been histochemically demonstrated in the intestinal lining of other urchins (12). It seems reasonable to conclude that nutrient storage in different types of echinoids involves the gonads and the gut wall in varying degrees.

The typical asteriod is a predatory carnivore, but many species are known to exploit particulate food gathered with the aid of mucus and flagellary currents (20,21,22). The digestive tract is not coiled but passes in the oral-aboral axis directly from mouth to anus; it consists of a divided stomach followed by a short intestine or rectum. Paired diverticula, the pyloric caeca, extend into each ray from the pyloric stomach, and these may bear elaborate appendages, Tiedemann's pouches, interpreted as flagellary pumping organs. These are especially well developed in species known to utilize particulate food (22,23). The rectum bears a group of caeca of unknown function, small in "typical" asteroids but remarkably large in some others. The lining of the digestive tract in general is a tall, columnar, flagellated epithelium provided with a brush border in many regions. Mucous gland cells are abundant throughout the system; in the stomach and intestine

these are the only identifiable secretory elements (some exceptions). In "typical" forms zymogen cells are localized in the epithelium lining the sac-like pockets of the pyloric caeca; these cells do not occur, or at least not in significant numbers, in either portion of the stomach (24,25). In specialized types, zymogen cells may occupy certain tracts in Tiedemann's pouches and occur in large numbers in the central ducts of the pyloric caeca and the radial parts of the pyloric stomach, as well as in the glandular pockets of the caeca (22). Typical zymogen cells contain rows of basophilic granules, almost always in association with a small clear vacuole of unknown significance (22,26). The flagellated digestive epithelium maintains an active circulation in the gut contents, regular currents moving between the central regions and the radial parts. The flagellated peritoneum covering the digestive organs maintains a corresponding circulation in the coelomic fluid bathing their external surfaces (27). It is generally to be understood that food is digested as it lies within, or enfolded by, the cardiac stomach, but under the influence of enzymes produced in the pyloric caeca. Patiria, operatively deprived of its pyloric caeca, ingests food which then remains undigested in the stomach (25). Digestive enzymes of asteroids include proteases, active against a variety of experimental substrates (gelatin, fibrin, egg-white, casein, peptone, peptides) under pH conditions ranging in different experiments between 6.6 and 8.4 (28, 29). Although the pH of the gut contents is somewhat lower than that of sea-water, the only proteolytic enzyme found in extracts of pyloric caecum having a pH optimum in the acid range (pH 4.8) appears to be a cathepsin, activated by HCN (29). Some investigators have detected amylases in extracts of stomach and caeca, or caeca only; some report lipase activity in caecum extracts (28). Absorption of nutrients and storage of reserves are largely localized in the pyloric caeca. C-14-labeled glucose, glycine, and palmitic acid administered with food become concentrated in the caeca, which store some glycogen and large amounts of lipid (24,30). The glandular pockets of the caeca are the usual sites of storage, but Odontaster deposits lipid in the walls of Tiedemann's pouches (31). Nutrients must obviously be translocated from the stores to the tissues; for example, reserves in the caeca are progressively depleted as gonads enlarge in anticipation of spawning (32). The circulating coelomic fluid seems to be the most appropriate vehicle for this transfer, but all investigators agree that this fluid contains very small amounts of reducing sugar (about 7 micrograms/ml) and of nitrogenous compounds (total N about 63 micrograms/ml, of which about 13 micrograms represent excretory ammonia N). Only small and transient increases in these values can be detected during or after feeding, perhaps reflecting leakage across the cardiac stomach wall. Pyloric caeca and other tissues remove from the coelomic fluid C-14-labeled nutrients injected into it, and isolated caeca maintained in vitro in sea-water or cell-free coelomic fluid establish an equilibrium with these fluids in terms of labeled nutrients. Under these conditions the uptake of nutrients is inhibited by metabolic inhibi-

tors. In coelomic fluid but not in sea-water, added glycine is turned over by isolated organs less rapidly than glucose, suggesting competitive relations with other amino acids in the natural body fluid. To explain the low nutrient concentrations in the coelomic fluid, it has been proposed that soluble nutrients flowing from stores in the caeca into the coelomic fluid are constantly taken up under equilibrium conditions by the caeca themselves as well as by other metabolizing tissues bathed by the fluid. The rate of uptake by any tissue will be set by its own metabolic demand at the moment. Except after extreme starvation the caeca will always contain a large surplus of reserves, continually replenished by absorption from the gut contents. It has been suggested that the net movement of material will be out of the caeca, into the fluid, into other tissues; although the amount exchanged may be large, rapid untake by the tissues will prevent the establishment of appreciable concentrations in the circulating medium. Such an arrangement enables the asteroid to utilize as its body fluid a liquid practically identical with sea-water, containing vanishingly small levels of organic substances and thus minimizing problems of osmotic balance with the external medium (30,33,34). It is to be noted that nutrient concentrations measured in the body fluids of echinoids and holothuroids are much greater, and that starvation and feeding bring about changes in nutrient levels not observed in asteroids. These facts suggest the existence of fundamental differences in nutritional physiology between these groups which call for further study.

In preparing such an abstract, it is obviously impracticable to cite an authority for each fact gleaned from the voluminous literature on the subject. Support for the statements given will however be found in the following sources, as indicated by the parenthetical numbers:

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REPRODUCTIVE PHYSIOLOGY OF ECHINODERMS. Richard A. Boolootian, Department of Zoology, University of California, Los Angeles.

The extent to which echinoderms have shown annual reproductive cycles and the variation of these cycles with latitude, the factors which induce the reproductive cycle and the biochemical changes accompanying it, the specific conditions which induce spawning, and the nervous and hormonal mechanisms which are involved in reproduction are all significant parameters of considerable interest to echinoderm biologists. A review of the literature leaves one with the impression that echinoderms do not generally reproduce throughout the year, but rather, do so in a restrictive period. This is true for the polar, temperate, and tropical regions. A distinctive pattern is found for each region, the most limited breeding period is found in the polar region and the most extended, in the tropics. In arctic and antarctic species, spawning and breeding occurs for a limited period of time in spring and summer when the plankton blooms occur and other conditions are favorable for development and settling of the larvae. Although spawning occurs for only a brief time in the polar regions, it is likely that the period of gonad build-up and the formation of gametes covers a much wider span of the year.

In temperate seas, spawning may occur over a brief or extended period of time. In a few cases, it may last the greater part of the year, suggesting that the animal is continuously breeding. Echinoderms of brief, prolonged, or almost continuous spawning periodicities may occur side by side in the same environment. An extended breeding season may mean that the individuals of a species are producing several successive broods during the year, or that they are breeding asynchronously, i.e., some are in early stages of maturation, some are getting ready to spawn, some are spawning, and still others are already spent.

If a large proportion of a population is successively spawning, several broods are being produced. However, even if only a small proportion of animals spawn at any one time, several broods may still be produced by a given individual and not synchronously with other individuals, as appears to be the case in the purple sea urchin, Strongylocentrotus purpuratus. Koehler (17) noted at Naples, Italy that it takes six to eight weeks for a new set of gametes to develop. Fox (10) found new eggs in nine days at Roscoff, France. Therefore, there is no doubt that several broods of eggs could be produced by these urchins. In his study on Centrechinus setosa of the Red Sea, Fox showed that during one week a population was completely devoid of eggs, following a well synchronized spawn. And within one week, a new mass of eggs was detectable (11). It is worthy to note that brooding of the young is a common habit of arctic and antarctic echinoderms, pelagic existence of larvae being uncommon. Echinoderms from temperate seas which have planktatrophic larvae presumably have short breeding seasons since the larvae depend upon the appropriate plankton for survival. Spawning of such organisms is likely to be synchronized at a favorable time in some manner rather than

occurring sporadically over a longer period of time. Those species of echinoderms in temperate waters produce yolky eggs and are more likely to show a prolonged brooding season since the larvae are not likely to be dependent for as long a time on the plankton which is not always abundant.

Echinoderms of tropical seas develop a great proponderance of pelagic planktatrophic larvae; 80 - 85% of the species of echinoderms having larvae of this type, as compared to 55 - 65% in temperate seas, and only 5% in the arctic and antarctic. Echinoderms from great depths of the sea presumably do not have such larvae; what little is known about such forms indicates that they produce large eggs, and that many brood their young. The above considerations indicate that polar, temperate, and tropical seas echinoderms each have a distinctive pattern for breeding season and type of larvae. Some species in each area deviate from the typical pattern.

When the breeding season of a temperate species is studied at various latitudes, it is apparent that the season is extended as one goes south. Species which have been studied with respect to latitude are: Arbacia punctulata (14), Strongylocentrotus purpuratus (2), and Paracentrotus lividius (19). The possibility that the breeding of echinoderms might be regulated by a natural cycle of growth and development seems to have received little attention, and no experimental data are available. The length of the breeding cycle, and its placement could be looked upon as a function of the rate of growth, development, and maturation of the tissues of the gonads, ending in spawning, and followed by a quiet period when the gonad once again begins to gather reserves and gametogenesis is again initiated for the next reproductive season. Therefore, the reproductive cycle is regulated endogenously. This cycle should operate at the same time, no matter where the animal is placed geographically. The very fact that the breeding season in temperate forms is prolonged when in decreased latitude suggests that exogenous conditions have something to do with the timing of events of the breeding cycle. Also, the fact that more broods than one may be found in warmer climates, for species of echinoderms which produce only one brood in the north, again suggests the importance of exogenous factors in the reproductive cycle.

Most of the experimental work done on the role of exogenous factors on the entrainment of the reproductive cycle emphasizes temperature. Other factors such as light, salinity, and food have held little interest. Evidence of photoperiodic control of reproduction in echinoderms has been documented in only one species, Strongylocentrotus purpuratus (4). In this species, long day length stimulates the production of spermatogonia, whereas short day length stimulates the production of mature spermatozoa. Other studies note that a spring breeding season is well correlated with increasing day length. Whereas in autumn and winter breeders, it is correlated with shortening days. The longer breeding season of echinoderms at lower latitudes might be photoperiodically controlled. Correlations alone are not

convincing. The importance of food to reproductive cycles of echinoderms is extremely important and perhaps very difficult to assess. The information available on the relationship of food to breeding is obscure. It is well known that an animal, when starved for a long time, may not have the reserves to develop gonads. In the starfish, Pisaster ochraceus, the hepatic cecae store food during the rest period, and they are depleted when the gonad grows, the size relationship between the two being reciprocal (8). If no reserves are available, the gonads cannot mature. Similarly, in the sea urchin, Strongylocentrotus purpuratus, there is a failure to breed when starved for a sufficiently long time; before the breeding season the gonad tissue being used to maintain the animal (8). The importance of food for larvae also must be taken into account as a factor of some importance and one which might account for timing of some reproductive periods. The causative factors and relationships are obscure.

Heavy runoff due to spring rains may have profound affect on reproductive cycles of many echinoderms. No study specifically directed to this problem has been published. There is indication that temperature has some importance in regulating the annual reproductive cycle of echinoderms, though no experimental work has been done.

Two urchin populations which occupy the same latitude yet live in waters of different temperatures have been studied (3). Due to oceanographic conditions at the study site, temperature differences of 15°C exist in adjacent waters. The data obtained to date suggest that a significant difference in rate of gonadal growth and gametogenic activity exists between the two populations. The cold water population had a relatively short period of gonadal growth, whereas the warm water population had a more extended period of gonadal growth. Since light conditions are the same, and an abundant supply of food was in both areas, it appeared most probable that temperature played a major role in influencing the rate of gonadal growth and gametogenesis.

Some work has been done to determine the variation of chemical composition of echinoderm tissues in correlation with the reproductive cycle. In the sea urchin, Strongylocentrotus purpuratus, during the growth of the gonad from its inactive to the gravid state, the deoxyribonucleic acid increases thirty-two fold in the male, and six fold in the female. But ribonucleic acid, which is stored in considerable amount in the egg but not in the spermatozoa, increases about seven fold in the male, and twenty-nine fold in the female. Protein increases are about twenty fold in the male and about thirty fold in the female. Lipid increases ten fold in the male, and twenty-six fold in the female. Cyclic variations of some of these constituents have been studied monthly in Strongylocentrotus purpuratus, in Strongylocentrotus franciscanus (13), Allocentrotus fragilis (5), Pisaster ochraceus, Pisaster giganteus, and Patiria miniata (13). In a study of Asterias forbesi (1) the fat reserves seen of the sections of cecae disappear after six to eight weeks of starvation. Whereas the zymogen and the mucous secreting cells still remain function-

al. In other studies (15), fractionations of the lipids showed the presence of phosphatized steroids, aldehyde lipids, and neutral fats. Large amounts of glycogen is stored in the Echinus (22) and Strongylocentrotus purpuratus (12). The variation of nitrogen and amino acid content of the gonads of the urchin, Strongylocentrotus lividus have been followed (20, 21). Lipids are of importance to the economy of many echinoderms, and there is some likelihood of stored lipids early in the season as reserves for the reproductive process. Where pyloric cecae are present, these serve as depositories, but in urchins without such organs, most of the storage occurs in gonads or in other tissues, for example, the intestinal epithelium and coelomocytes.

Several factors have been considered as playing a major part in spawning: temperature change, calm waters after a period of disturbed waters, disturbance after a period of calmness, the changes of pressure, physiology, nutrition, illumination, and chemical influences. It is likely that several factors working simultaneously or in succession may be of importance, especially some physical factor, and a chemical factor following it. Spawning in echinoids is thought to be synchronized by chemical or hormonal agents. Echinus females respond to sperm water, and males to egg water (11). Some information on the variation of spawning activity of Strongylocentrotus purpuratus over its entire geographical range is well-worth noting. Regardless of geographical origin of Strongylocentrotus purpuratus populations, spawning is synchronized (2). Therefore, it is likely that the time of spawning may be gentically determined, whereas the other processes associated with reproduction may be under environmental control.

Studies on nervous and hormonal interrelationships of the breeding process of echinoderms are scarce. The presence of steroid substances in echinoids is well known (6), though their function is unknown. Recent studies on the metabolism of estradiol by tissues of Strongylocentrotus franciscanus (7) are interesting. The fact that neurosecretory cells have been found in ophiuroids (9) and asteroids (23) may eventually link the relationship between neurosecretion and reproductive activity. The existence of behavioral responses bearing upon reproduction, such as the shoreward migration and aggregation of some sea urchins (22, 16) and ophiuroids and crinoids (18) is worthy of mention. In these cases, the mature gonads secrete substances which alter the behavior of the animals in response to stimuli of the environment causing them to migrate and aggregate. This kind of behavior has not been analyzed. Prospects in the field of reproductive physiology of echinoderms are excellent. Interesting work has indeed been done, but there is a tremendous amount still undone.

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COMPARATIVE HISTOLOGY OF THE ECHINODERM WATER-VASCULAR SYSTEM.

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The water-vascular system is part of the echinoderm coelom that has become specialized mainly to act as a hydraulic mechanism. It consists of a 'central' ring vessel round the oesophagus, from which arise an axial tube leading to a madreporic complex, five radial canals and various accessory structures, such as polian vesicles, probably concerned with maintenance of turgor in the system. The madreporite is a pore or series of pores connecting the system with the external medium; it is possibly also concerned with pressure maintenance, though at present this is by no means certain. Recent work indicates that, while certain inorganic substances are maintained within the system at a level higher than that of surrounding fluids, the system does not appear to have a vascular function at all: transport of essential substances is relegated mainly to the perivisceral coelomic fluid, leaving the water-vascular system to operate special protrusions from the body wall, the tube-feet. These structures are modified in the various classes for many different functions, and their histological structure, as well as that of those parts of the system concerned with their protraction, reflects these functions.

The protractive force in crinoids is developed in the water-vascular canal, in ophiuroids in a special part of the tube-foot canal itself, and in asteroids, echinoids and holothuroids in ampullae accessory to the tube-feet.

The crinoid tube-foot system is mainly concerned with feeding; in the comatulids at least, and probably in most stalked forms as well, the animal uses a mucous net method for catching its food, the net being produced, spread and collected by the tube-feet. The tube-feet of both arms and pinnules are in groups of three, and those of the pinnules, by far the most numerous and the most important in feeding, are of different lengths, lie at different angles to the horizontal and have different functions related to the net. The longest tube-feet are the main food-trapping organs; they lie almost horizontally. The medium-sized tube-feet project upwards and outwards at an angle of about 45°, while the shortest project almost vertically upwards, forming an avenue to the food-groove. To take up these positions the two groups of feet opposite one another are protracted together by hydraulic pressure built up in the adjacent part of the water-vascular canal itself. Muscle fibres stretch across the cavity of the canal, except at its centre, and the canal is of such a shape that when these fibres contract they first constrict the canal, forming a chamber, then reduce the volume of the chamber so that water-vascular fluid is forced out at each side to the two groups of tube-feet.

The ophiuroid tube-foot system is concerned with feeding and, in some, with burrowing. While in general their powers of protraction are somewhat limited, in a few species they can extend to some length. In many forms there is a similarity with the crinoid protractive mechanism, in that the radial canal is constricted at intervals, though the methods by which they do it, in this case by muscular sphincters, are very different. Another difference is that there are no muscle fibres traversing the canal to build up hydrostatic pressure: the inherent elasticity of its walls forces fluid back down the canals towards the tube-feet. But in addition

part of the canal of each foot is swollen into an ampulla-like structure with a muscular wall the contraction of which forces fluid into the lumen of the tube-foot. A valve cuts off the ampulla and its tube-foot from the rest of the system. In those ophiuroids with only weak powers of protraction the ampulla alone is present as a source of hydraulic pressure, while in a few species with considerable powers an additional median non-muscular vesicle may be present accessory to the radial canal and lying in a special cavity in the vertebral ossicle. In those cases in which parts of the radial canal and accessory structures are used to take excess fluid when the tube-foot retracts, the valve isolating a tube-foot and its ampulla from the rest of the system is provided with opening muscles.

In the other three living classes, asteroids, echinoids and holothuroids, great powers of extensibility of the tube-feet are almost universal, together with considerable strength. In some at least of all three groups the feet are used in locomotion, and therefore powerful postural bending is required. This is brought about mainly by the action of the retractor muscles of one side of the foot working against the pressure of the fluid in its lumen. So for normal protraction and for bending a powerful hydraulic pump is demanded, and this is present in the form of the typical bag-like ampulla, internal to the integument. As in the ophiuroids, the tube-foot / ampulla system can be isolated from the rest of the system by a valve, though, unlike them, no valve so far described is provided with muscles for opening.

The histology of the ampullae of these three groups is basically very similar. A connective tissue basketwork and a muscle layer are sandwiched between two layers of coelomic epithelium, that continuous with the perivisceral coelomic epithelium externally and that continuous with the lining of the rest of the water-vascular system internally.

Besides its mechanical functions in the life of the animal, the tube-foot / ampulla system has another: respiration. When the tube-foot is extended its wall is extremely thin, and gaseous exchange takes place between the sea-water and the water-vascular fluid in the lumen; likewise, when the foot is retracted and the ampulla is expanded exchange takes place between the fluid in the ampulla and that in the perivisceral coelom. So a circulation of fluid within the tube-foot / ampulla system will be advantageous, and in many the coelomic epithelium bears cilia in two bands, one beating in a distal and one in a proximal direction. In most echinoids, in addition, the pore through the integument is divided into two, to assist the circulation of fluid, and in the special tube-feet on the dorsal side of most irregular echinoids the pores of each pair are widely separated, and the tube-feet and their ampullae are stretched between them.

The basic histology of the tube-feet of all five classes is very similar. The outer epithelium is continuous with that of the rest of the body, there is an underlying nerve plexus, a basketwork of connective tissue to prevent bursting, a retractor muscle sheath and a lining of coelomic epithelium.

In the pinnular tube-feet of crinoids, with their division of labour related to the handling of the mucous net, there are important histological differences in the retractor musculature.

The longest tube-feet have muscles restricted to their oral sides, so that they can actively bend inwards towards the food-groove only; the medium-sized tube-feet have retractor muscle columns on their oral and aboral sides, so that they can bend outwards towards the longest tube-feet to pick up the mucous strands as well as inwards towards the groove; the shortest have a complete cylinder of muscle, so that they can move in all directions, and their main function is to push the food-laden mucus into the food-groove.

The external epithelium of the crinoid tube-foot is drawn out at intervals into finger-like papillae. Each papilla is solid, but has a single muscle fibre running its length. Also present in each papilla are five or so single-celled mucous glands, opening at the tip, and a group of sensory nerve cells with processes protruding from the tip of the papilla as a tuft. Though the feeding action is too rapid to observe directly, it is highly probable that as the tube-feet flail about violently the papillary muscle fibres contract, forcing strings of mucus from the glands to form the food-collecting net.

The epithelium of the ophiuroid tube-foot, too, is raised at intervals down its length and at its tip into papillae which contain mucous glands and sensory elements, but, apparently, no squeezing muscles. Quite possibly the voidance of the secretions is brought about by a flattening of the epithelium resulting from normal protraction of the tube-foot.

The epithelium of the strong tube-feet of asteroids, echinoids and holothuroids is more specialized. Terminal suckers are present on many for efficient adhesion during locomotion and feeding. These structures have apparently evolved several times, and the problems of their operation have been solved in different ways.

In asteroid suckered tube-feet the connective tissue of the foot is expanded distally to form the main framework of the sucker, and the non-elastic fibres are so arranged that the pull of the stem retractor muscles is conveyed to the most peripheral fibres of the framework, so that the diameter of the sucker is maintained. Mucous glands embedded in the sucker disk supply extra adhesion and facilitate the efficient working of the sucker. The sucker is operated by special levator muscles in the tube-foot lumen which originate among the stem retractors and insert at the distal end of the lumen. When they contract they pull up the centre of the disk, thus creating a suction between its surface and that of the substratum. Detachment is brought about by another series of muscles, the radials, also at the distal end of the lumen; when they contract, they pull the disk downwards to release the suction.

In echinoids with suckered tube-feet the main framework of the disk is a complicated calcite skeleton, functionally equivalent to the connective tissue framework of the asteroid disk. The main part of this skeleton is a rosette of five or more ossicles arranged round the distal part of the tube-foot lumen and at which the stem retractors insert. The rosette occupies most of the disk. Proximal to the rosette is a series of much smaller ossicles, the frame, to which are attached the levator muscles.

There are no radial muscles: detachment is brought about by relaxing the levators, when the pull of the stem retractors, conveyed by the skeletal rosette, lifts the edge of the disk and breaks the adhesion of the sucker.

The ultrastructure of the sucker cuticle has recently been investigated. The external wall of the epithelial cells is thrown into perpendicular microvilli, protruding through a double layer of cuticle, the outer a thin, dense layer and the inner a thicker, less dense layer. Between the epithelial cells are minute granules of very electron-dense material, probably pigment, and also between them are various kinds of amoebocyte and their disintegration products, some separated from the external medium by only the walls of the microvilli. The presence of microvilli is generally associated with a surface across which the passage of materials occurs, and this conforms with the respiratory function of the tube-feet.

The holothuroid sucker also has a calcite skeleton supporting the disk, but it is generally made up of a single ossicle. The function of this seems to be merely to maintain the full diameter of the disk, and adhesion is obtained solely by the stickiness of the mucous secretion from glands at the disk surface.

Most of the asteroid, echinoid and holothuroid tube-feet without suckers are found on burrowing members, and they show a variety of form and function aimed mainly at producing mucus for lining the burrow walls. For instance, among the starfish the phanerozones have mainly pointed tube-feet, heavily charged with mucous glands, which can reach up the sides and over the top of the arms to plaster mucus on to the burrow wall so that a feeding and respiratory current can be maintained between it and the animal's body. Similarly, among the echinoids some spatangoids have mucus-producing tube-feet which make and maintain a respiratory and feeding connexion from the burrow to the surface of the substratum, others which produce a sanitary soak-away from the posterior end of the burrow, and others which pick up particles of the substratum and pass them to the mouth for feeding. The ones which 'plaster' the burrow walls have special muscle-operated mucous glands reminiscent of the muscular papillae of crinoids. The tube-feet of spatangoids show the greatest specialization and division of labour of these organs seen in echinoderms, and, further, must surely take pride of place as the most remarkable hydrostatic organs in the Animal Kingdom.

THE BIOLOGY OF GNOTOBIOTIC ANIMALS

A symposium organized for the
XVI International Congress of Zoology

by

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GNOTOBIOTIC TECHNOLOGY. P. C. Trexler, Dept. Microbiology and Immunology and Dept. Surgery, Albert Einstein College of Medicine, Bronx, N. Y.

Interest in animals reared in the absence of all microorganisms originated with the pioneers of microbiology and is an obvious extension of the pure culture methods used in the study of microorganisms. The rearing of the first vertebrates free of microbes was reported by Nuttall and Thierfelder in 1895. However the technical difficulties involved in maintaining a sterile environment without interferring with the rearing and study of the animal are so great that comparatively few investigators engaged in this effort before the fifties. The number of investigators and laboratories involved have increased rapidly with the solution of the technical problems and the availability of animals from institutional and commercial breeders. In contrast the study of invertebrates and plants in a sterile environment has not suffered from these technical handicaps.

The maintenance of the sterile environment required for the prolonged rearing of laboratory animals has been accomplished by the use of mechanical barriers to limit the migration of contaminating organisms rather than the usual sterile and aseptic technic. The first such apparatus termed "isolator" was described by Küster in 1915. The sterile area within the isolator can be observed, manipulations performed and materials introduced and removed without viable contamination (Trexler, 1961). Observations are made through glass viewing ports or walls constructed of transparent plastic. Manipulations are performed through rubber gloves attached to isolator walls or even the flexible walls of the chamber itself. The area in which manipulations are performed can be extended by incorporating a protective garment in the wall of the chamber. The garment, with an external personnel airsupply, permits the investigator to enter the chamber for the length of the garment without penetrating the protective mechanical barrier. A waist-length garment or jacket can be entered as readily as a pair of gloves attached to the isolator wall. Personnel in impervious clothing have entered sterile rooms. The protective garment was ventilated by a double airhose, a single hose with a protective filter or a blower carried by the operator. The blower used air from the sterile area and maintained sterility by bacteria-tight filters on the inlet and outlet ports. The impervious clothing is used to prevent contamination of the sterile area by the investigator and as protection against the peracetic acid shower used to decontaminate the garment before entering the room. Supplies are sterilized in a double-doored pass-through lock that forms part of the wall of the sterile chamber. The sterile lock may be operated with steam under pressure so that food, bedding, water and other supplies can be sterilized and directly introduced or removed. Supplies that are ordinarily steam sterilized can be placed in a variety of bacteria-tight packages, sterilized in an autoclave and then introduced through a germicidal bath or lock to avoid the use of expensive and heavy sterile locks.

A variety of isolators can be purchased including expensive tanks that are essentially modified steam sterilizers and simple kits containing all of the parts necessary to assembly a flexible film plastic isolator (Trexler, 1961). It is also possible to rent an isolator from an animal breeder,stocked with the animals and the sterile supplies required for an experiment. A series of isolators or dryboxes are offered for the control of environment conditions in industry and the laboratory. The variety of equipment that is available or that can be readily assembled makes it possible to perform practically any laboratory task in a sterile environment.

Initially the animal is introduced into the sterile environment from a stage in the lifecycle in which it is usually free of contamination. Chickens, turkeys and Japanese quail have been obtained free of contamination by passing embryonated eggs through a germicidal bath or lock just before hatching. The gravid uterus of mammals can be extirpated and passed through a similar bath into an isolator where the young are removed aseptically. To reduce the danger of contamination from maternal tissue the young are transferred to another isolator via the sterile locks that are fastened together to provide a sterile passageway. The young may also be introduced into a sterile isolator by means of a caesarean-type operation. The surgically prepared abdomen of a gravid animal at term is placed against a thin plastic membrane that forms a section of the floor of an isolator. With the larger species, such as swine, a commercially available plastic surgical drape with appropriate adhesive is used to assure a tight connection. Guinea pigs, dogs, cats, monkeys, swine, sheep and goats have been obtained by the above procedures and weaned on a sterile milk formula. Canned sterile infant formulas have been used for much of this work. Mice, rats and rabbits have also been obtained by these methods but they are more difficult to raise since the young must be hand fed a special formula for several weeks (Wostmann, 1959). New strains of rats and mice are usually foster suckled on dams that have been reared in a sterile environment. Colonies of rats and mice have been maintained free of contamination for over twenty generations. Guinea pigs, rabbits, quail and chickens have also reproduced in a sterile environment though they are usually obtained from contaminated stock. Thousands of gnotobiotic animals have been shipped by private vehicle and public transportation. Local as well as intercontinental shipments have been made in isolators in which the animals can be maintained in the laboratory and in specialized isolators for more efficient transportation. The same degree of contamination control can be maintained while enroute as in the laboratory.

The term "germfree" and its equivalent in other languages is probably most frequently used to designate the above animals. In recent years many laboratory and farm animals have been produced free of pathogens. The term "germfree" occasionally is used for these animals since the term "germ" refers primarily to a disease producing microorganism. A group of scientific terms (Dougherty, 1959) has been used based upon "axenic" (without strangers) and "gnotobiote" (known biota). The former term and derivations are frequently used to designate the number of species intimately associated with the subject organism. Categories based upon the number of species involved are significant in the culture of invertebrates since many of them can be grown when associated with another species (monoxenic) but not in isolation. Apparently the common laboratory and farm animals are not dependent upon the presence of another species for growth. Their response to the presence of microorganisms depends more upon the particular species involved than upon the number of species. Since laboratory animals are frequently studied in association with pure cultures of microorganisms as well as in isolation, a single term is needed to designate both groups because the same methods are used to prevent viable contamination from the environment. The term "gnotobiote" referring to organisms grown with a specifically defined biota or in the absence of other species, meets the requirements. The development of this field of investigation depends to a great extent upon the development of contamination control methods, the availability of apparatus, animal supply and special problems in the design of experiments in addition to the requirements for similar studies in ordinary animals. A term such as "gnotobiotics" can serve a useful purpose in designating this field of investigation.

Many experiments involving gnotobiotes must be designed so as to distinguish between the effect of the absence of microbiota and other environ-

mental factors. In general gnotobiotic animals are compared with another group as identical as possible (frequently termed "conventional") but reared in the open animal room or laboratory. A third group must be used consisting of a comparable group having the microbiota of the animals in the open room but maintained within an isolator. At least in some of the early studies the "isolator controls" were unsatisfactory due possibly to inadequate control of humidity and temperature. Various environmental conditions can effect the animals directly or through secondary effects via the microbiota. Microbic growth in the water, food and bedding depends to a great extent upon the frequency with which they are changed and the humidity. The suitability of the isolator environment and husbandry practice can be determined by a careful comparison of the isolator and open-room reared conventional animals. The microbiota itself should be kept comparable by sterilizing the environment and supplies for both groups and using aseptic technic in servicing the open room animals. Forming these groups from split-litters is not entirely satisfactory because resistance factors as well as microbiota are transmitted from the dam to the offspring. The three groups can be formed from animals reared in separate colonies under the respective conditions but tied together genetically by the proper exchange of breeding stock. Gnotobiotic technique makes it possible to control the microbic component of the laboratory animal more precisely than heretofore. How best to use this in the design of experiments will be determined as the field matures.

The purpose of this symposium is to demonstrate the usefulness of the gnotobiote as a research tool. This has been attempted by reviews of various aspects of the problem and reports of current studies. No attempt has been made to provide a complete survey. It is rather obvious that the gnotobiote by itself is essentially an in vivo culture medium as well as a source of sterile products. The gnotobiote can be used to determine the effect of the microbiota upon the host in both health and disease. These studies determine the effect of the microbiota in toto or of the various components. Extrapolation of observations made with microbiota derived from pure cultures to conventional animals must be made with caution because of the complex interactions within the microbiota and the effects of the very complex microbic population on the host. Most of our knowledge of the relationship between microorganisms and higher animals is concerned with parasitism. The gnotobiote provides the opportunity to explore other relationships and to determine if a sound basis can be established for some of the speculations of Metchnikoff.

The control of the microbic component of the laboratory animal as far as species are concerned makes it possible to obtain more uniform specimens for study. The laboratory animal can be considered both as a model for investigating phenomena and a tool or reagent for biological testing. Uniformity and reproducibility appears to be of prime importance to permit verification of observation by the original investigator and by others. The history of experimental biology is replete with examples where such verification could not be made. The quality of laboratory animals have been increased by the elimination of pathogens - the Specific Pathogen Free Animal. However it has been impossible to devise practical tests to demonstrate the absence of the great variety of parasites and pathogens that cause important diseases in laboratory animals and thus have effective quality control. Many forms are harmless components of the normal flora until the animal is subjected to some stress which then results in overt disease. The entire problem of intercurrent infection in the laboratory animal can be by-passed through the use of gnotobiotes. The investigator can use various microbiota associated with different groups of animals just as inbred strains are now used to reduce genetic variability. Practical quality control tests can be used that are based upon tests for the presence of any contaminant and not just active or

potential pathogens. The elimination of the indeterminable microbiota associated with the conventional animal greatly simplifies microbiological examination. The practicality of such an endeavour depends upon the development of production methods that are economically feasible and the determination of combinations of host and microbe that will provide a useful animal for both experimentation and biological testing. At the present time these problems are under investigation and appear soluble.

In general terms the development of gnotobiotics adds another dimension to the laboratory animal and should be of assistance in developing the organismal aspects of biology. The use of these animals has added to knowledge of the role of microorganisms in both health and disease. The precise control of viable contamination required by the pursuit of gnotobiotics has been applied to studies of the treatment and care of patients, industrial processing and the sterilization of space craft.

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THE SEARCH FOR MICROBIAL CONTAMINANTS IN GNOTOBIOTIC RATS AND MICE.* Morris Pollard, Lobund Laboratory, University of Notre Dame, Notre Dame, Indiana, U. S. A.

It is important to have base-line information on the microbiological flora of experimental animals in order to plan their use in specific biological problems. This too is a prerequisite to the designation that animals are gnotobiotes (also referred to as germfree or axenic). Germfree methodology has been perfected (1) so that mice and rats can be produced in significant numbers and varieties; however, the designation "germfree" has been valid only in relation to bacteria and parasites. Procedures for detection of parasites and bacteria have been developed, and used to demonstrate that germfree animals are free of such organisms (2). The occurrence of pleuropneumonia-like organisms (PPLO or Mycoplasma spp.) in germfree animals has been investigated; and techniques are being developed whereby the animals can be searched thoroughly for viral flora. The animal placenta is thought to be permeable to viral agents such as lymphocytic choriomeningitis, cytomegalic virus, and murine leukemia virus (3, 4, 5). Under such a circumstance, it may be anticipated that germfree animals should have an indigenous viral flora which may be disseminated within the population by congenital routes. The experiments on which such information is based are equivocal; and may be more definitive when experimental animals of known microbial content (germfree) are utilized.

At present, the following genetic lines of animals are being maintained and propagated at Lobund Laboratory:

Mice - Swiss-Webster, CFW, Balb C, ICR, C3H and C57 Bl
Rats - Wistar, Fischer, and Sprague-Dawley
Guinea pigs
Rabbits

The original germfree mice and rats in the Lobund colonies were Swiss-Webster and Wistar respectively. They were delivered by caesarian section and transferred to sterile isolators where each animal was hand-fed until weaned. All subsequently added genetic lines of mice and rats were foster-nursed on germfree Swiss-Webster or Wistar animals. Therefore emphasis in the search for PPLO and for viruses has been directed at Swiss-Webster and at Wistar animals, from which all other lines were derived by foster-nursing procedures. Rats and mice from the germfree colonies have been examined by the following procedures:

1. Examinations for PPLO (Mycoplasma spp.). One hundred and thirty-eight germfree rats and mice were killed by cervical fracture, and organs from each animal were collected, pooled, and processed; in all instances with fresh sterile instruments. The autopsies were performed in a sterile isolator so as to avoid possible contamination of the specimens from the operator or the environment. The tissue pool was homogenized in PPLO medium by means of a glass tissue grinder, and the specimens were then transferred to the transfer booth of the Bacteriology Laboratory. Each tissue homogenate was inoculated into standard PPLO medium (liquid and semisolid) and incubated at 35°C. in an atmosphere of 95% nitrogen and 5%

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carbon dioxide. The cultures were passaged three times at six day intervals and examined carefully before being recorded as positive or negative for PPLO. Animals examined by the procedure outlined above were selected at random from colonies of germfree rats and mice of the Lobund Laboratory. In addition, thirty mice were subjected to 700 r whole body x-rays, and 15 days later they were examined individually for PPLO. In order to assure ourselves that the examination procedures described above were accurate and sensitive, germfree animals were infected with standard strains of PPLO (T-1, Campo, and the Eaton agent); and the organisms were recovered by culture procedures for as long as four weeks after inoculation. By the same culture procedures, PPLO was isolated from tissue cultures which were naturally contaminated with the organism. Thus far, PPLO has not been detected in the germfree rats and mice of Lobund Laboratory.

2. Examinations for viral flora. Four hundred germfree rats and mice were individually examined for viral agents by the following procedures:

a. Serum specimens from individual animals were examined for antibodies to polyoma, adenovirus, "K" virus, mouse hepatitis viruses.

b. Tissue sections of submaxillary and of lacrimal glands were examined for histological evidence of cytopathology.

c. Mice were tested for ectromelia infection by skin test with vaccinia virus. All mice developed a vaccinia reaction, indicating that they lacked immunity to related viruses.

d. Tissue cultures were prepared from trypsin-dispersed germfree fetuses and kidneys of newborn rats and mice. Nutrient fluids were changed at four day intervals. Cell monolayers on coverslips were stained and examined at weekly intervals for evidence of cytopathology. Tissues which showed degenerative changes were passaged to fresh tissue cells prepared from germfree animals.

e. Fecal material from the animals in individual isolators was pooled, homogenized and extracted in physiological salt solution and inoculated into tissue cultures which had been prepared from whole fetuses or from kidneys of germfree rats and mice, by the technique outlined above. Nutrient fluids were changed at four day intervals. Tissue cultures were examined at intervals during the next month for evidence of cytopathogenic effect.

f. Visceral organs were collected from individual animals. They were homogenized and extracted in physiological salt solution and inoculated onto tissue cultures prepared from homologous germfree fetuses and kidneys. Nutrient fluids were changed every four days for one month, in the course of which tissue preparations on coverslips were stained and examined for evidence of cytopathology.

g. Mice and rats were inoculated subcutaneously with methylcholanthrene and oil. Sarcomas developed at the point of inoculation. One mouse tumor has been transplanted through seven passages in homozygous germfree mice. Tumor fragments were placed on germfree fetal mouse fibroblast cultures; and the cultures were examined periodically for evidence of cytopathology. Fragments of tumors, from various transplant passages were embedded in epon, sectioned, and examined by electron microscopy for evidence of virus-like inclusions in the tumor cells.

h. Some germfree rats of the Wistar strain developed spontaneous neoplasms of the mammary glands. They were examined histologically, in tissue cultures, and by electron microscopy for evidence of viral inclusion bodies.

i. Germfree mice of C3H and C57 Bl strains have been exposed to four doses of 150 r x-rays at weekly intervals. They are now being observed for evidence of leukemia.

j. Germfree mice of the C3H strain are being treated with lactogenic and mammotrophic hormones in order to elicit tumorigenesis in the mammary glands. They are being examined for evidence of preneoplastic mammary nodules.

Thus far, the only evidence of viral contamination has been an intranuclear eosinophilic inclusion body in the lacrimal glands of germfree male rats, aged six months or more. This lesion has been noted in the glands of Wistar, Fischer and Sprague-Dawley rats. Virus has not yet been isolated from such tissue. Ultra thin sections of the tissues are now being examined by electron microscopy for evidence of virus-like structures in the cells. All other examinations for viral flora in mice and rats have been negative.

Although viruses may be inapparent (latent, dormant) by conventional procedures, they may be activated from the latent or "eclipse" stage of replication by several procedures; for example, by (a) administration of small doses of x-rays which induced clinically apparent viral leukemia in otherwise normal C57 Bl mice (6); (b) a rapid passage-sequence of lung tissue extracts of mice which activated a pneumonitis agent (7), or a hepatitis agent (8) with blood passages; (c) preparation of tissue cultures from "normal" kidneys of monkeys in which Simian viruses appeared (9); (d) repeated transplantation of chemically-induced tumors, in the course of which viral agents were detected in the cells by electron microscopy (10). The search for viral agents in germfree animals continues. If viral agents are found in germfree animals it will create new problems in determining how they traverse the placental barrier, how they can be eliminated, and whether they alter the physiological status of the animal.

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GERMFREE RESEARCH: METABOLIC AND NUTRITIONAL STUDIES.

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INTRODUCTION. There is no doubt that mammalian metabolic and nutritional processes are modified in important, and at times, critical ways by the "normal" microflora present in ordinary healthy animals. It is difficult to study these complex and varied interactions with ordinary laboratory animals and ordinary laboratory techniques because of the inability of the investigator to rigidly control the varied, and varying, microflora present in such experimental animals. The techniques of the germfree laboratory, however, allow not only the rearing and maintaining of animals free of microorganisms (by present tests), but also allow the investigator to maintain rigid control over microbial flora which he may introduce at will, and which may be a single species or multiple species, in order to determine the role specific microorganisms play in specific situations. We will not attempt to review in any comprehensive way the studies so far reported in these areas; for this, the reader is referred elsewhere (1)(2)(3)(4)(5)(6). Our discussion will center around some studies carried out during the last several years at the Department of Germfree Research of the Walter Reed Army Institute of Research and the Department of Surgery of the Albert Einstein College of Medicine. A number of other investigators collaborated in some of these studies, particularly Dr. Floyd Daft of the National Institute of Arthritis and Metabolic Diseases of the National Institutes of Health, and Drs. Erving Geever and Hyman Rosen.

II. GENERAL DESIGN OF EXPERIMENTS, TERMINOLOGY. In our experiments, conventional animals are born and raised in the same sort of cages and isolators and eat the same sterilized diet as the germfree animals, though, of course, the isolators for the conventional animals are contaminated. This is done in an attempt to control environmental factors (other than microbial), diet, handling, etc., to which the germfree and conventional animals are exposed. In some experiments, animals germfree until weaning, are purposefully contaminated with a mixed microflora from open-animal room animals and maintained in isolators. Such animals are called "conventionalized". When a single microbial species is introduced into previously germfree animals, the animals are called monocontaminated. Occasionally, multi-contaminated animals are also studied simultaneously in the usual laboratory open-animal room. These are termed open-animal room animals. The problems of the microbiologic testing of these animals are described elsewhere in this symposium.

III. METABOLISM OF UREA AND CHOLINE. (These studies were carried out with Leo V. Crowley, Lt.Col. M.S.C., R.E. Horowitz, O.J. Malm, P. Prentiss, and N. Brown (7)(8). There is evidence which suggests that some of the specific metabolic processes occurring in the animal organism may be dependent upon enzyme systems of commensal bacteria rather than on endogenous enzymes of the animal. The germfree animal lends itself superbly to the study of these problems. It is possible through a few well designed experiments to obtain definite answers to problems which require a great number of complicated experiments when undertaken with conventional animals. For example, studies with antibiotics, such as that reported by Dinzis and Hastings (9) suggested that much of the hydrolysis of urea was bacterial dependent, but whether all the hydrolysis was dependent on bacteria remained equivocal and inferential.

Working with germfree and conventional rats, my colleagues and I have shown conclusively that (a) the enzymic hydrolysis of urea by the conventional rat is effected only by the urease of its bacteria (7) and (b) the enzymatic conversion of choline to trimethylamine by the conventional rat is similarly dependent wholly on its microbes (8).

IV. SCURVY. For a number of years, my colleagues and I have been studying the metabolic and nutritional effects of severe injury. We had noted sharp and prolonged drops in plasma ascorbic acid concentrations, urinary ascorbic acid excretion, and in "tissue saturation" as judged by load tests (10)(11). Later, we showed that burned guinea pigs not only behaved biochemically as scorbutics but also physiologically, as judged by the healing of test laparotomy wounds (12)(13). We are anxious to know what role, if any, the "normal" microflora of ordinary animals plays in the metabolism of ascorbic acid. To this end, the responses of germfree and conventionalized guinea pigs to a scorbutigenic diet were followed. These studies were carried out with Bud Tennant, Erving Geever, Roger Laundy and Floyd Daft (14). The behavior of the germfree guinea pigs was significantly different from that of their conventionalized counterparts; in each of several experiments, the onset of scurvy was remarkably slower in the germfree guinea pigs. The conventionalized guinea pigs gained weight for 8 - 12 days after the removal of vitamin C from their diet, after which their food intakes and body weights decreased rapidly. All but one of the conventionalized guinea pigs died in 19 - 29 days. This behavior is identical to that of open-animal room guinea pigs receiving this sort of diet. The germfree guinea pigs continued to gain weight for 24 - 36 days after the start of the scorbutigenic regimen. Thereafter, their food intakes and weights decreased, but not as rapidly as those of the conventionalized guinea pigs. All but one of the germfree animals died in 35 - 57 days; one was sacrificed at 60 days. At autopsy, all the guinea pigs showed evidence of weight loss, and the periarticular hemorrhages, particularly of the stifle joints, characteristic of scorbutic animals.

The guinea pigs in another experiment were sacrificed after 21 days of ascorbic acid deprivation for a comparative histopathologic study of their tissues. By this time, the conventionalized animals had begun to lose weight, although slowly, while the germfree animals were still gaining weight. We had anticipated that morphologic signs of scurvy would be present in the conventionalized animals but absent (or very minor) in the germfree, and this turned out to be so. At necropsy, all the conventionalized animals showed the stifle joint hemorrhagic stigmata of scurvy while none of the germfree animals had these gross lesions. Microscopic examination showed impressive pathologic changes typical of scurvy in the conventionalized animals only.

The germfree animals developed scurvy, therefore, on the scorbutigenic diet, but much more slowly than the conventionalized animals. It is likely that there is a continuing movement of ascorbic acid from the tissues and blood into the gut and back again. Some of the ascorbic acid is probably metabolized by the enteric bacteria (15) and, therefore, no longer available to the host. How much of the body's ascorbic acid may be "lost" by this means is not known, but our experiments suggest that this amount is large enough to increase dramatically the rate of onset of scurvy in ordinary guinea pigs as compared with germfree animals in which this sort of "loss" would not occur. It is also possible that the "endogenous" metabolism of ascorbic acid is different in animals with and without microflora. Studies are underway to delineate these factors. To date, we have found that the rates of decline of the tissue and blood contents of ascorbic acid (ascorbic acid + dehydroascorbic acid) are faster in conventionalized guinea pigs

on the scorbutigenic diet than in germfree guinea pigs. This work was done with the technical assistance of Benjamin Floyd and John Diggs.

Four groups of guinea pigs were used in the preliminary study; (a) conventionalized, (b) conventionalized deprived of vitamin C for 7 days, (c) germfree, and (d) germfree deprived of vitamin C for 7 days. Both the total liver and adrenal ascorbic acid levels and their concentrations declined sharply in the conventionalized guinea pigs after 7 days deprivation of vitamin C; remarkably, the germfree animals deprived of vitamin C did not experience any loss of ascorbic acid in their livers or adrenals. The blood ascorbic acid levels were consistent with the foregoing; the germfree guinea pigs experienced only a minimal decrease in total ascorbic acid concentration after 7 days vitamin C deprivation, while the decrease was marked in the conventionalized. These observations will be extended by the use of C^{14} labeled ascorbic acid.

V. DIETARY LIVER CIRRHOSIS. These studies were started several years ago, because of the observations of György (16) and Rutenburg et al. (17) that certain antibiotics delay or prevent the development of experimental dietary liver cirrhosis of conventional rats. These observations led Rutenburg and his associates to conclude.. "cirrhosis in rats on a choline-deficient diet is caused by intestinal bacteria and not by the choline deficiency." This view was also expressed recently by Salmon (18) as a result of work with antibiotics and chemotherapeutic drugs. He stated "..enteric microbial agents or their noxious products are essential to the production of the syndrome.. it seems that the effect of the antimicrobials is not a choline-sparing action but a direct protective action against a harmful agent." However, our experiments with germfree, conventionalized, conventional and open-animal room rats on a 4% casein, choline-free diet, lend no support to this view. These studies were carried out with Nesbitt Brown, Floyd Daft, and Llewellyn Ashburn. Erving Geever has participated in the more recent studies.

In our studies with Lobund rats (1960-61), not only did the germfree rats on the choline deficient diet develop hepatic cirrhosis, but they developed it faster than their conventional, conventionalized, or open-anim al room counterparts. We had not anticipated this in view of the antibiotic experiments mentioned above and other experiments of ours which showed that while about half of the choline fed to conventional rats is degraded to trimethylamine (apparently not effective as a lipotropic agent) very little, if any, of the choline fed to germfree rats is so degraded (7). Thus, if small amounts of choline were available in the diet of the germfree and conventional rats, the effective lipotropic action of this choline might be greater in the germfree, because of the absence of bacterial enzymic activity to degrade the choline to TMA.

We also had found that feeding Neomycin daily to germfree rats does not delay the development of liver cirrhosis due to choline deficiency as it does when fed to open-animal room rats. Supplementation of the experimental cirrhogenic diet with choline, however, did prevent cirrhosis in the germfree rats.

During the past year and a half, several cirrhosis studies were conducted with rats of the Fischer strain. This was necessitated by the temporary unavailability of germfree rats of the Lobund strain. We found again, that the germfree rats on the choline-deficient diet develop liver cirrhosis, but in these experiments, slightly slower than their conventional counterparts. Fischer rats purposefully monocontaminated with E. coli were also placed on the cirrhogenic diet. This organism was chosen because it was shown to produce vitamin B_{12} in vitro. Since vitamin B_{12} given to conventional rats on a choline-deficient diet will prevent the development of liver cirrhosis, it was thought

that the rats monocontaminated with E. coli might be "protected" while on the choline-deficient diet. This did not turn out to be the case. The E. coli rats, in fact, developed cirrhosis slightly faster than the germfree, in two concurrent, but separate trials. This difference was observed regularly through varying periods of ingestion of the diet, i.e., as early as fifteen weeks, but was more impressive at twenty-two weeks, when the experiment was terminated. The difference in findings between this and our previous work may be related to strain differences in the rats. All three groups (germfree, conventional, and E. coli monocontaminants) showed generalized deposits of lipoids in the lungs, lymph nodes, spleen and bone marrow. This material was usually intracellular within histiocytes. Critical comparison revealed somewhat less severe involvement of the germfree group as in the case of the liver findings. This is in keeping with Ashburn's finding in our earlier cirrhosis experiments (19). Another constant observation in our recent experiments was the finding of patchy renal tubular necrosis of varying severity, but less severe in the germfree group than in the other two. The changes were often associated with renal concretions. Sporadic findings were scattered among the three groups and were without consistent localizations to any one. These included pneumonitis and atelectasis, focal basophilia (H and E stain) and focal increased affinity for colloidal iron of the specialized fat in the hibernating gland and in the supporting stroma of the salivary glands and a cushion of cartilage about the root of the aorta.

Currently, three experiments are underway. The first is to determine whether vitamin B_{12} given orally will prevent the development of liver cirrhosis by germfree Fischer rats on the choline-free diet. We assume that it will, just as it does in the conventional rats. This demonstration is necessary before we proceed further with our purposeful bacterial monocontamination experiments to test our hypothesis that under certain conditions conventional rats develop this form of dietary liver cirrhosis more slowly than germfree rats because certain gastrointestinal bacteria produce a protective nutrient, e. g., vitamin B_{12}. This same hypothesis is the basis for the second experiment which involves the prevention of coprophagy by conventional rats and conventional rats treated with antibiotics, eating the choline-free diet. It is our feeling that the protective effect of antibiotics observed by Gyorgy, Rutenburg and Salmon is due to alteration of the gastrointestinal flora by the antibiotics so that organisms which make an "anticirrhotic" protective nutrient predominate, and that this nutrient becomes available to the rat by coprophagy. The third experiment, underway at the National Institutes of Health (Floyd Daft) is with Lobund rats to confirm our original observation that germfree rats of this strain develop cirrhosis faster than their conventional counterparts.

VI. DEVELOPMENT OF CHEMICALLY DEFINED DIETS. We have previously pointed out that while germfree animals are free of viable microbes, at present, their immunologic reactions remain complicated by the presence of dead microorganisms and certain other protein and polymer antigens in the presently used diets. Presumably, these complications can be largely avoided by using chemically defined diets of the type described by Greenstein and his colleagues (20) for open-animal room rats. In several preliminary studies, carried out several years ago, we worked out the technical details of preparation, sterilization (by filtration) and entry of this type of diet. At that time, we had carried out several experiments to test the nutritional adequacy of these diets for mice. The chemically defined diet was started at weaning. The filtered diet seemed adequate for one group of conventionalized mice, but it was not adequate for germfree mice. The germfree mice failed to gain weight, developed diarrhea, and died in 4 to 7 weeks. The conventionalized mice, on the other hand, grew well and a few reproduced. As far as we know, this is the first report of mice reproducing on a chemically defined diet; Greenstein and his

colleagues had shown that rats are able to reproduce on such a diet.

In view of the apparent better growth of germfree rats than germfree mice on the chemically defined diet, we have decided to concentrate for the time being on the development of such a diet for the rat. During the past year, a number of experiments with several diet formulas varying principally in amino acids were carried out with germfree, conventionalized and conventional Fischer rats. The rats were started on the chemically defined diets at weaning. They grew and looked healthy, but none grew as rapidly as their counterparts on a standard rat chow. The germfree rats on the chemically defined diets, though, gained weight as well as their conventionalized litter mates reared on the same diets. The early mortality of the germfree rats was, however, greater than that of the conventionalized rats. Complete necropsies were performed on some rats sacrificed after 12-15 weeks on one of these diets. Microscopic examination of all tissues was made using H. and E., Rinehart and Wilder stains. There were no unusual findings except for the smaller size of the conventionalized and germfree rats and their organs as compared with their counterparts fed our stock "semi-synthetic" diet L-356. Benjamin Floyd, R. E. Horowitz, Bud Tennant, Phoebe Prentiss, F. Daft and E. Geever have collaborated in these studies.

We are continuing our efforts to establish appropriate chemically defined and eventually synthetic diets for germfree rats, mice, guinea pigs, and other species. Such diets will allow for the first time the precise investigation of specific nutrient needs and the effects of specific microbes on these needs. Germfree animals reared with such diets will also offer unrivaled opportunity for the study of natural resistance and fundamental immunologic interreactions among animals, microorganisms, and allergens.

VII. BODY COMPOSITION. Little is known about the body composition of germfree animals except for the well-documented fact that the gastrointestinal tract, particularly the cecum and large intestine, is remarkably enlarged (principally because of an increase in its contents) in germfree rats, mice, and guinea pigs. Accordingly, as part of our overall metabolic and nutritional studies, we have recently begun measurements of the body composition of rats and mice which are germfree, monocontaminated with a single organism, or multicontaminated. Nitrogen, fat, water and calorie concentrations and contents were measured in the livers, gastrointestinal tracts, carcasses and total bodies of these animals (5). The results of the various studies were not entirely consistent; this is not surprising, since the factors governing body composition are complex, and by no means completely understood. It is such an understanding, in fact, which we hope to gain.

In general, there were profound differences among these groups of mice and rats. While male germfree, conventional and monocontaminated (*E. coli*) mice had, in general, the same tissue protein contents, the *E. coli* mice usually weighed less than the conventional or germfree, had remarkably less body fat and calories, and a significantly greater proportion of water than did the mice of the other two groups. Peculiarly, these differences were not observed among similar groups of female mice. The intestinal tract of the *E. coli* monocontaminants was similar in size to that of the germfree.

In contrast to the mice, the multi-contaminated rats (in this case "conventionalized") had nearly twice the body fat and calories of the germfree and less body water, although their body weights and body protein contents were the same.

The livers of the germfree mice and rats were generally somewhat smaller and contained less protein than those of their conventional or conventionalized counterparts. Leo V. Crowley and Bud Tennant have collaborated in these studies.

VIII. NITROGEN METABOLISM. Our studies of some classical parameters of metabolism and nutrition in germfree animals which we had begun at the Walter Reed Army Institute of Research are just getting underway at the Albert Einstein College of Medicina (1). We had found in preliminary studies that germfree rats of the Lobund strain excreted more fecal nitrogen than conventional rats on isocaloric food intakes with protein levels which varied from 5% of the diet by weight to 40%. Fecal nitrogen excretion of both conventional and germfree rats decreased during starvation, but, again, the germfree rats excreted about twice as much fecal nitrogen as the conventionals. At all but the highest level of protein intake, we found no difference in urinary nitrogen excretion by the germfree and conventional rats. At the highest level of protein intake, the conventional rats excreted somewhat more nitrogen in the urine. These results were confirmed and extended in a series of studies with Fischer rats. There was a modest increase in fecal nitrogen excretion by both conventional and germfree rats as the daily nitrogen intake was increased; caloric intake was constant at 60 cal/day. The increase in fecal nitrogen from the low nitrogen intake to the high for the conventional rats was about 28 mg/day, as contrasted with an increase in daily nitrogen intake of 864 mg. For the germfree rats, the increase in fecal nitrogen from the low nitrogen intake to the high was 92 mg/day, as contrasted again with an increase in the daily nitrogen intake of 864 mg. Urinary nitrogen excretion in these experiments was about the same for the germfree and conventional rats at the three levels of nitrogen intake (14%, 20% and 40% of the diet by weight). Bud Tennant, Benjamin Floyd, Nesbitt Brown and Hyman Rosen have collaborated in these studies.

Generally, about half the total fecal nitrogen is postulated to be bacterial nitrogen. If this is so, then the germfree rat excretes considerably more "non-bacterial nitrogen" in the feces than the conventional rat. In a preliminary study of the partition of fecal nitrogen, we have found that about 70% of the total nitrogen excreted by the germfree rats on various protein intakes is filterable when the aqueous fecal suspension is filtered through ordinary laboratory filter paper, while only about 50% of the total nitrogen excreted by the conventional rats on these same diets is filterable, the difference being statistically significant. In no case is any of the filterable nitrogen precipitable by trichloracetic acid. The fecal nitrogen does not represent (except perhaps to a minor degree) undigested, unabsorbed nitrogen containing food, either in the germfree or the conventionals. What are the differences in the rates of turnover of the intestinal epithelial cells of germfree and conventional rats? Are there differences in the secretion or transfer of plasma proteins and other nitrogenous materials into the gut? Experiments are underway to answer these questions.

IX. Studies of the effects of microorganisms on the response of mice to food deprivation and on wound healing of guinea pigs have been summarized elsewhere (1).

CONCLUSION. These experiments buttress the growing evidence that the normal microflora present in ordinary healthy animals modify their metabolism and nutrition in a variety of ways. Methods are now at hand for studying in a controlled way the effects of specific microorganisms (singly and in combination) on specific metabolic and nutritional processes. We can expect such studies to unravel and clarify some of the complex and varied metabolic interrelationships between the animal host and his microflora.

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THE CONTRIBUTION OF GERMFREE RESEARCH TO OUR UNDERSTANDING OF THE CELLULAR KINETICS AND RESPONSES OF THE INTESTINAL MUCOSA.

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Life in a germfree environment is the most artificial and refined state in which a whole animal can be studied (1). It permits the precise definition of the interrelationship of a whole organism with its environment, and to this extent can be considered an extension of cell and organ culture research. The germfree state entails the disruption of the association between higher and lower forms of life which is so ubiquitous and axiomatic that it is called "conventional" in the English literature and "classic" in French. A prime example of such a symbiotic association is the intestinal flora which established itself in the beginning of life on earth. The importance of intestinal commensals in various species of invertebrates and vertebrates is demonstrated by the consequences of their removal (2). I will illustrate in certain insects and mammals the complications arising from their suppression by antibiotics and shall contrast these with the effect of antibiotics in the germfree guinea pig. I will also show the profound effect of the germfree state on reproduction growth and nutrition, and will illustrate this in insects by examples from the work of Koch (3) and Frank (4).

The association of hosts with their symbionts has also a morphologic counterpart and affects organ development (5). Organs which conventionally are in direct contact with a bacterial flora are in the germfree state significantly reduced in weight as is the lymphatic tissue draining these organs. The prime example is the mammalian intestinal tract. In the absence of an intestinal flora there is also a striking reduction of lymphocytes and particularly of plasma and preplasma cells and of reactive lymphoid follicles. This parallels the depressed immune globulin fraction in the serum of germfree animals. In contrast, the macrophage system which is most abundantly developed in relation to the gastro-intestinal tract, remains unaffected by the microbial status of the host, as Bauer recently pointed out (6). The number, distribution and apparent activity of macrophages throughout the lymphatic tissue are equal in germfree and conventional animals. There exists, then, in the lymphatic tissue of germfree mammals a dichotomy between the dormant component of cells capable of antigenic stimulation with a corresponding scarcity of immunologically competent cells and a fully functioning macrophage system showing evidence of active phagocytosis. This is probably related to the phylogenetic evolution of the lympho-reticular system as immune globulins are not formed by lower forms of life which display distinct phagocytosis. Upon appropriate stimulation the dormant component of the lympho-reticular system is capable of ready response which depends on the nature of the antigenic stimulus and the mode of its administration. A sequence of lymph node tissue changes following oral bacterial challenge of germfree guinea pigs will be demonstrated and the evolution of secondary

follicles will be traced in several experimental models. Major histologic changes also occur in the intestinal mucosa in response to oral bacterial challenge. For such a study the germfree animal represents an important experimental model because it eliminates the complex of anti-microbial host factors induced since birth by exposure to the conventional environment (7). The impact of such an exposure will be illustrated in the cecum of the newborn guinea pig which displays a burst of mitotic activity and hyperchromatic staining of crypt gland epithelium, empty goblet cells and increase of nuclear debris in the tunica propria. This will be compared with the cecum of an adult germfree guinea pig which resembles that of a prenatal conventional animal in that it shows a uniform epithelial lining, well distended goblet cells and sparse cellularity of the tunica propria. The intestinal mucosa, like the blood or the epidermis, is in a state of dynamic equilibrium; cells are constantly lost and replaced. The feedback mechanism which controls this renewal system is not entirely understood. However, the rate of cell renewal is profoundly influenced by the presence or absence of an intestinal flora. Abrams (8), using appropriate quantitative techniques, recently determined the extent of this influence on cell population dynamics in the ileum of mice. He found the life span of intestinal epithelial cells in germfree animals increased by about 50 per cent over that of their conventional controls. In conventional animals the accelerated loss of epithelial cells is compensated for by an increased rate of migration along the villi and by increased cell proliferation in the crypts. His count of crypt cells in mitosis was 1/3 higher in conventional animals than in germfree controls. He noted an increase in all mucosal dimensions of the conventional animal, particularly in the length of the crypts, the size of the non-villous component, and in the development of the lamina propria. His data are in agreement with Gordon's observation of a 30 per cent reduction of surface area of the intestinal mucosa in germfree rats (9). The differences in life span, rates of migration along the villus and corresponding physiologic maturation and in the total mass of the epithelial cells in germfree and conventional animals may influence intestinal absorption. However, this has not as yet been established.

In contrast, the morphologic manifestations which correspond to the different rates of tissue movement in the two types of animals are well recognized in mammals. They have not been sufficiently studied in invertebrates. However, I noted a much taller intestinal epithelium in germfree than in conventional snails. In guinea pig ileum the germfree state is characterized by a narrow non-villous portion of the mucosa, shallow crypt glands with well distended goblet cells, slender villi with a strikingly uniform epithelial lining, and a delicate lamina propria with sparse mesenchymal cells. In contrast, the conventional animal shows an altered ratio of villous to non-villous portion of the mucosa, increased height and altered shape of the crypt glands. The villi are more blunt and there is an increased

cellularity of the lamina propria. This highly reactive reticular tissue is attuned in its response to the stimuli it receives from its intestinal environment. The natural stimuli of conventional life elicit what Roessle has called a physiologic inflammation. What we consider "normal" is but a special form of adaptation of the host, a mere segment on a scale of possible responses, having its starting point in the germfree gut (10).

The initial stimulus to raise germfree mammals and birds came from Pasteur, as an extension of his discovery of the microbial nature of the processes of fermentation and putrefaction. Trained originally as a chemist he brought to experimental biology the appreciation of the significance of purity or homogeneity of material. He wanted to raise experimental animals with "pure" nutrients devoid of the commensal intestinal bacterial flora and after a norm of such germfree animals had been achieved he wanted to determine the metabolic effect of the contamination of the "pure" nutrients with single and multiple species of microbes. The actual experiment testing the effect of such contamination was first performed at his institute in 1922 by Cohendy and Wollman (11). They noted that germfree guinea pigs succumbed to oral challenge by Vibrio cholerae while their conventionally raised controls fed the same sterilized diet survived the infection without ill effect. Formal and I recently confirmed the pathogenicity of V. cholerae for germfree guinea pigs. A significant number succumbed although more mature animals were used and present-day techniques have eliminated gross undernutrition which plagued the earlier workers. In contrast, all controls survived. The natural intestinal flora of the conventional animal appears to exert a protective action. Vibrios were eliminated from the gut within 24 hours leaving no histologic sequelae. In contrast, a series of histologic alterations accompanied the ingestion of V. cholerae in the germfree group (12). Already 3 hours following challenge the mucosa of the cecum showed emptying of goblet cells, altered shape of crypt glands and increased cellularity of the tunica propria. Corresponding changes were present in the small intestines and colon. The tissue changes progressed slightly within the first 24 hours. Depending on the ability of the individual animal to withstand the physiologic effect of the infection--loss of fluid and electrolytes into the intestinal lumen--the guinea pigs succumbed or survived. Vibrios persisted in the intestinal tract of survivors and their presence was associated with a continued inflammatory reaction leading to a "conventionalization" of the intestinal mucosa and its lymphatic apparatus. Therefore, the establishment of vibrios in the intestinal tract (vibrios do not penetrate the mucosa) evokes an infection of the mucosa rather than a mere multiplication of the organisms in the intestinal contents.

The seeding of the intestinal tract following contamination of a germfree animal occurs very rapidly. Wagner reported that as few as 9 cells of Shigella flexneri, type 2 and 3 will establish a bacterial cell population of 10 to the 9th power per gram of cecal content within 18 hours (13). Such rates of multiplication

are not necessarily associated with overt clinical disease but do elicit an inflammatory response of the intestinal mucosa. This is further illustrated by the tissue reaction of the germfree guinea pig to E. coli, a natural commensal of the conventional animal. While this organism is nearly non-pathogenic to the germfree guinea pig it acts similarly to V. cholerae in that it colonizes the entire intestinal tract without demonstrable invasion of host tissues. Its presence in the gut lumen elicits in the intestinal mucosa an inflammatory reaction which leads to the "conventionalization" of the gut mucosa and its lymphatic apparatus. In contrast, E. coli does not multiply in the small intestinal content of the conventional animal but settles in the cecum and colon.

In this context Foeckler's experiments on reinfection of axenic larvae of the meal bug Stegobium (Sitodrepa) paniceum L. with various yeast symbionts are of great interest (14). Host-foreign yeast cells distributed themselves differently in individual intestinal epithelial cells than host-specific symbionts; they colonized midgut epithelium which is never involved by host-specific symbionts, and they were shed during molting while host-specific yeast cells are retained.

No differences in the histologic patterns of response to oral bacterial challenge have been noted in germfree animals and their conventional controls because the anatomical organization of the intestinal mucosa which delimits the type of tissue reaction is basically alike in both instances. However, the intensity of tissue reaction is greatly influenced by the germfree state. B. subtilis, a harmless intestinal commensal of the conventional guinea pig, causes fatal illness in the germfree. Depending on the pathogenicity of the agent for a particular strain and species of germfree animal, a whole scale of responses have been observed which range in the guinea pig from commensalism with minimal histologic changes in the case of monocontamination with B. megatherium to fulminating acute shigellosis with a corresponding acute ulcerative enterocolitis in the germfree raised and then Shigella flexneri infected guinea pig. Dysentery bacteria of the same serotype proved non-pathogenic to germfree rats and mice in Wagner's experiments. The fatal outcome of the dysentery infection in the germfree guinea pig could be prevented by prior oral challenge with E. coli.

These results of germfree research have given us new perspectives of the biologic role of the intestinal flora. I have stressed the impact of oral bacterial challenge on cellular kinetics and responses of the intestinal mucosa. However, gnotobiotics can be expected to contribute greatly to a solution of the problem, originally posed by Metchnikoff: To what extent is the natural flora of conventional life beneficial, and how could it be improved for the better health not only of animals but man?

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THE RESPONSE OF THE GERMFREE ANIMAL TO INFECTION. Walter L. Newton, Lab. of Germfree Animal Research, National Institute of Allergy and Infectious Diseases, N.I.H., Bethesda, Maryland.

Germfree animals can be particularly useful in furthering our knowledge of the relationships and reactions between the animal host and the organisms it normally harbors or is invaded by. It is possible to study host responses to individual, or to particular combinations of, parasitic or commensal organisms without the complicating presence of other organisms. A more precise evaluation and measurement of basic defensive mechanisms such as tissue response, antibody production, and various immunologic phenomena would seem to be possible with animals that have had no contact with viable organisms prior to experimental challenge.

Also, with such a tool, it is possible to study the course of infection, and the behavior, of individual microbial species *in vivo* in the absence of any extraneous multiflora, some of which might be closely related to the organism under study. Not only can single infections be followed, but possible synergistic relationships between two micro-organisms can be explored. Finally, some assessment of the effect of the so-called "normal" flora on host responses can result from comparative studies of the germfree and conventionally-reared animal.

At present, the amount of published information on the behavior of various infections in a previously germfree animal, and the response of the latter, is rather limited. Furthermore, some species of animals currently reared germfree are not as sound as their conventional counterparts. For example, guinea pigs reared without a flora seem to experience some unknown nutritional deficiencies, inasmuch as they do not grow as well as the conventional animals. On the other hand, germfree rats and mice appear to be quite normal specimens. Because the nutritional state of a germfree animal may influence, to some extent, its serologic response to infection, for example, comparisons with the conventional animal must be made carefully. Nevertheless, several interesting studies have been made and are underway, in various laboratories throughout the world. This paper will be an attempt to present and discuss some of the information gained, thus far, from induced infections in germfree animals.

Interesting relationships have been established from studies of the behavior of certain parasites in a host without other organisms. *Endamoeba histolytica*, a protozoan parasite which can cause amoebic dysentery in man, and ulcerative lesions and death in conventional guinea pigs, apparently will not survive

in the lumen of the germfree guinea pig. If, however, the latter is infected with bacteria, even a single species, prior to challenge with the amoebae, ulcerative lesions are obtained. A synergistic relationship between the amoeba and a bacterium in the pathogenesis of the disease is indicated. Certain other parasitic protozoa survive as well or better in the germfree host as in the conventional.

Several species of intestinal worm parasites have been shown to grow to maturity apparently normally in a host without a bacterial flora. Of particular interest from the broad biological point of view is the finding that the host specificity demonstrated by many parasites under conventional conditions does not necessarily hold in germfree animals. Certain parasitic helminths of rats and of mice, for example the tapeworm, Hymenolepis nana, will not develop to maturity in conventional guinea pigs. However, they will develop to maturity in germfree guinea pigs, and the latter will discharge viable tapeworm eggs in the feces.

The response of germfree animals when exposed to infection has seemed to vary with the challenge organism, with the animal species and even among strains of the latter. Germfree guinea pigs seem to withstand oral infection with a single species of a variety of common bacteria such as Escherichia coli, Aerobacter aerogenes, Staphylococcus aureus, Bacillus subtilis. On the other hand, Shigella flexneri administered orally has been reported as lethal for these animals, while not for conventional guinea pigs. In fact, germfree guinea pigs, as currently reared, appear to have about the least resistance of any germfree animal. It has been the experience of most investigators that guinea pigs will not survive for more than a day or two when brought to the outside environment from a sterile environment. In contrast, most germfree rats, chickens, and white Swiss mice, though often visibly affected, will survive "conventionalization". There is evidence, however, that the kind of environment into which the germfree animal is brought, e.g., a crowded animal room versus a relatively clean office-laboratory, can affect survival results.

Germfree rats have been shown to be more susceptible as measured by LD_{50} dosages, to B. anthracis spores than conventional controls; with other bacterial species no difference was observed. A similar variation has been observed with germfree mice. Additional examples of individual responses and relationships will be presented in the paper. However, the available evidence suggests that the germfree animal, at least initially, is more susceptible to infection, in general, than the animal which is reared under continuous exposure to a varied flora.

Studies by several investigators have suggested possible reasons for this. Germfree animals tend to have a reduction in lymphoid tissue, plasma cells and other histologic elements generally associated with the defensive mechanisms of an animal. Also, while the values reported have varied with the species and the methods employed, most investigators have found that the levels of gamma globulins in the serum of germfree animals are well below those in animals reared under conventional conditions. Since antibodies are known to occur primarily among these globulins, the germfree animal appears to be deficient in whatever protection may be afforded by antibody. However, they are not entirely devoid of gamma globulins. Also, when they are inoculated with a flora, or brought out from a germfree isolator into a room with animals harboring a varied flora, the levels eventually increase to values similar to those of the conventional animals. This change is gradual and several weeks appear to be necessary to attain "normal" levels. There is also evidence that the type of organism with which the germfree animal is inoculated can influence the levels attained. Less information is available on the effect of infection upon the cellular elements, but it would appear that the histologic picture returns to normal; there is an increase in plasma cells and in lymphoid tissue.

The available data suggest that the various defense mechanisms normally occurring in the conventionally-reared animal also occur in an animal reared in a germfree state. Inoculation with bacteria or with non-viable antigenic materials produce the appropriate agglutinating or precipitating antibody. In some instances, the rate of development or the titres seemed to differ between the germfree and conventional animal, whereas in other types of response no difference was obtained. Also, from the histopathologic standpoint, tissue response to invading organisms such as parasite larvae has been similar in the germfree animal to what occurs in the conventional, with any difference being more of a quantitative than qualitative nature. Thus, the absence of stimulation by a viable flora seems to keep certain components of the animal's normal defense mechanisms at a comparatively lower level of concentration or activity. However, that some stimulation does occur is suggested by the presence of bacterial agglutinins in germfree rat serum and antibody-like reactions in the sera of germfree mice to bacteria such as *E. coli* or *Staphylococcus aureus*. One possible explanation for the presence of this reactivity is that the food of the germfree animals contains antigens which are stable under steam sterilization. These may be from dead bacteria or from antigenically-related substances in the diet.

A final point in connection with the response of the germfree animal to infection touches upon the most celebrated characteristic of the animal -- it's enlarged cecum. This organ which is unusually large and thin-walled, sometimes to the point of causing the death of the animal, shrinks down to a more normal size once an infection has been established in the intestine. Apparently, even a single bacterial species, Clostridium difficile can bring about this change, although all bacteria are not equally effective.

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THE GERM-FREE ANIMAL IN A CLINICAL RESEARCH LABORATORY. Dwain N. Walcher, M.D. and James J. Schaffer, M.D., Dept. of Pediatrics, Parker R. Beamer, Ph.D., M.D., Dept. of Pathology, Ind. University Medical Center, Indpls., Ind; Philip C. Trexler, Albert Einstein College of Medicine, New York, N.Y.

Use of the germ-free animal in this communicable disease diagnostic and research laboratory was first contemplated in 1956 when it became apparent that the use of tissue culture alone could not provide an explanation for the etiology of the non-bacterial diarrhea in many patients.

Although enteropathogenic Escherichia coli had become accepted as a frequent cause of epidemics of diarrhea in new-born nurseries, there were many infants and small children with infectious diarrhea from whom neither bacterial pathogens nor viruses could be recovered.

The concept of a germ-free mouse serving as an unique culture medium was introduced into our laboratories in 1959.

To gain experience in the response of the gnotobiotic mouse to viral monocontamination, a standard, mouse-adapted strain of Coxsackie B_1, Connecticut 5, was inoculated into suckling mice. Germ-free animals made conventional and returned to an isolator, germ-free animals made conventional and kept out of an isolator, and Indianapolis conventional animals served as controls. Five-hundredth ml of a viurs suspension diluted $10^{-6.8}$, $10^{-7.8}$, and $10^{-8.8}$ was injected intraperitoneally into 24 hour old suckling mice. When possible, as many as four animals were sacrificed at 3, 7, 10, and 14 day intervals. Tissue culture titrations of the brain and the remainder of the body indicated that the virus was present in greatest quantity in the brain. Virus persisted longer in the germ-free animals, still being present at 14 days in both brain and body suspensions, but was not detected on the 10th or 14th day in the conventional animals.

Histopathologic studies demonstrated various degrees of alterative inflammation (i.e., exudation of fluid, cytoplasmic and nuclear degeneration, and necrosis of individual cells) in the brain, interscapular adipose tissue, liver, myocardium, and certain other sites. In several instances, especially in the germ-free animals, irregular and indistinctly outlined small accumulations of a few lymphocytes and occasional macrophages were associated with degenerative changes. Recognizable anatomic changes occurred in approximately 60% of the conventional animals and in slightly more than 80% of the virus-infected germ-free mice. Although difficult to evaluate in quantitatively precise terms, the histopathologic changes in the latter groups seemed to be more advanced during a comparable period of infection, with a slightly greater tendency to the development of foci of

cellular exudation, especially in the brain, and to a lesser degree, in the interscapular aditpose tissue, liver, and myocardium.

These observations suggested that the germ-free animal quantitatively tolerated more virus short of death, but fewer virus particles were necessary to initiate infection. The study was repeated during the following year, and confirmatory data were obtained.

The inadvertent contamination of an isolator with a coagulase negative staphylococcus produced results at variance with those previously obtained. Preliminary observations indicated an increased degree of resistance in germ-free mice challenged with Coxsackie B virus of the same titer. A similar effect was obtained when this cell-free filtrate was used in conventional suckling mice.

Anal swab cultures or material obtained by rectal catherization from infants and children with the clinical syndrome of infectious diarrhea were prepared by conventional techniques and inoculated into tissue culture monolayers and into germ-free suckling mice. During one such study, equivocal changes in the tissue culture cells were observed; but convincing evidence of an agent, even though serially passaged, was not demonstrated. Direct inoculation into germ-free animals did not demonstrate the presence of an unusual agent. However, introduction of the tissue culture fluid into the gnotobiotic suckling mouse produced focal necrosis in the liver and staining of the mouse peritoneum with bile. Back passage of the mouse specimens into tissue culture cells produced distinct cellular damage. Attempts are currently being made to characterize and identify the viral agent thus recovered.

In another phase of our work, gnotobiotic pigs were being used to study the effect of recently isolated respiratory viruses. Shope's work on the relationship of swine to influenza virus prompted consideration of this avenue of study.

Due to an inadequate number of control animals, the symptomatic response of gnotobiotic pigs which had been inoculated with virus was confused at first with an underlying metabolic problem. It was soon recognized that germ-free piglets were hypoglycemic and remained in this state for several weeks. Early glucose supplementation of the milk diet prevented a high mortality. Studies of the circumstances surrounding the deficit in lactase activity in the germ-free pig have suggested that the intestinal flora may significantly influence this activity.

These preliminary studies emphasize the fact that the germ-free animal provides us with an excellent means of (1) studying

significant histopathological changes induced by a single microorganism, (2) the alterative changes in these effects when more than one agent is introduced, (3) the modulating effect of intestinal flora on certain enzyme activities, and (4) thus, a far reaching effect on body chemistries.

SPECIFIC INHIBITION DURING DEVELOPMENT

A symposium organized for the
XVI International Congress of Zoology

by

S. Meryl Rose

Department of Anatomy
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New Orleans, Louisiana

ACTION OF ADULT TISSUE EXTRACTS AND THEIR FRACTIONS ON THE EARLY DEVELOPMENT OF THE CHICK EMBRYO. Pierre Lenicque, The Wenner-Gren Institute, University of Stockholm, Sweden.

Cell multiplication and differenciation under the influence of neighbouring tissues may include two different mechanisms, acting in complementary ways, i.e. inductors creating new tools for further differentiation of the cells, and inhibitors restricting the number of tools already existing in the cells. The object of the present study of the action of adult tissues and some of their fractions on the early development of the chick embryo was to test the validity of this theory by means of large series of experiments followed by qualitative anatomical analysis and quantitative chemical analysis. We tried also to detect the nature of these complementary factors. The experiments were done mostly on the development of the brain. But preliminary works were done with mesodermic tissues; blood and heart. The tissue extracts were prepared with either an isotonic saline buffer (Tyrode) or an appropriate phosphate buffer for extracts to be fractionated before use. A temperature of 0-4°C was maintained while the tissue was dissected free from foreign tissue, washed, homogenized, and ultra centrifuged at 15,000 R.P.M. In some cases, the fractionations of the supernatants of brain or heart extracts were performed either by flow electrophoresis in a 0.03 M phosphate buffer pH 8.2 at 200 volts, or by chromatography on a DEAE-Sephadex column in a 0.02 M phosphate buffer pH 6.8 in which an increasing gradient of salinity was established. The blood extracts were fractionated by salting out. The final concentration of the extracts was found to range from 600 γ to 1000 γ per ml. The analysis by spectrophotometry of the brain extracts fractions showed the presence of nucleotides in the most electro-negative ones and traces of hemoglobin in the slightly positive one. Four main fractions were obtained also by chromatography. The very first ones (0.02 M buffer) contained proteins (among them traces of oxyhemoglobin), the following ones were rich in nucleotides. The antigenical properties of these fractions were tested against an antibrain serum. Only the fraction eluated by the 0.02 M phosphate buffer contained antigens specific for brain. Three fractions of heart extract could be prepared by chromatography. The first one contained traces of oxyhemoglobin; the two other ones were very rich in nucleotides. The fractions of extracts were adjusted to pH 7.5 and made isotonic before us.

The extracts to be test IN OVO were either injected under the blastoderm or released directly on it. Preliminary experiments with tissues extracts and with saline showed that the treatment must be performed at the moment of regression of the primitive streak (18-20 hours old eggs).

The diagnosis was made by anatomical and histological analysis of the different organs. In the case of brain and blood extract experiments, chemical analysis could also be done

An exact picture of the quantity of proteins contained in the tissues of the head in comparison with the quantity of proteins synthetized by the whole embryo could be done by measuring the total nitrogen content of the head as well as the total nitrogen of the body. In our experiments, the border line head-body was a tangent to the auditory vesicle drawn from the superior maxilla to the spinal cord. The action of the extracts on the brain and head could be detected and measured by analysis of the variations in the ratio $\frac{\text{Total N (head)}}{\text{Total N (whole embryo)}}$ as a function of development in normal and in brain extract treated populations. In order to determine precisely the development of blood in blood extracts treated populations and in controls, the embryo with the complete circulatory system, containing all the blood was removed from the egg and homogenized. The hemoglobin content of the homogenate was determined by measuring its benzidin peroxydase activity.

Results.

It was found that the eggs treated with brain extracts almost invariably produced living embryos with regularly pulsating hearts and blood with high hemoglobin content, but the heads developed abnormally in about 37% of the cases. The malformations of the brain could be grouped into two main types.(fig.1) 1) The folds of the neural plate had failed to fuse completely, and the neural plate was spread around the ectomesoderm 2) The cerebral vesicles were closed, but remained small and poorly differentiated. We could see also some cases of omphalocephaly. When tested by analysis of the ratio $\frac{\text{nitrogen content of head}}{\text{nitrogen content of embryo}}$, the action of brain extract on the development of populations of embryos resulted as an inhibition of the normal synthesis of the proteins of the head. The regression lines of the variations of these ratio were calculated as a function of age in brain extract treated populations and in controls. An analysis of variance was made, and the difference between the level of the line of regressions was highly significant ($P < 0.1\%$). The influence of the concentration was studied: An extract of brain containing 700 γ nitrogen per ml. and which provoked such head abnormalities in 37% of the treated eggs, was found to loose its activity when diluted to contain only 140 γ/ml. The quantitative analysis of growth of the head showed clearly that the brain extracts had an inhibitive effect on the development of the head. But what about the mechanism? The histological analysis showed two types of defects. In some cases, the neural plate could not fuse; it spread over the ectomesoderm and even in some places folds in many folds. Some sections showed an hypertrophy of the neural tissue where the rate of mitosis was found to be higher than in the controls. The other kind of defect was the dedifferentiation of the neural tissue. The study of the nature of the active agents of the brain extract performed first in our laboratory in Sweden, and pursued this

performed first in our laboratory in Sweden, and pursued this year with D. McCallion and J. Trott in Canada showed that these two types of abnormalities can be attributed to two different kind of substances: 1) Some of the fractions rich in nucleotides, (either prepared by electrophoresis or by chromatography) provoked an increase of the rate of mitosis and an abnormal development of the neural plate followed by failure of closure of the neural tube and mechanical retardation of the total growth of the head. 2) An other fraction rich in both proteins and nucleotides provoked a characteristic dedifferentiation of the neural tissues in 10% of the case. This is a rather low percentage, but this phenomenon cannot be attributed to random because it never happens in the controls injected with Tyrode. In an experiment run during a week we could observe this dedifferentiation in 16 living embryos in a group of 67 treated eggs. The chemical analysis of this brain extract fraction showed that it contained proteins one of which was antigenically specific for brain, some nucleotides, and some traces of hemoglobin. Table I shows that 1) the whole brain extracts contain substances acting against the normal development of the head and a substance which retards the whole growth, but not the differentiation, of the embryo; 2) the brain supernatants (total saline soluble brain proteins) cause head abnormalities in 37% of the cases; 3) these supernatants contain one or a group of proteins, eluated by a 0.02 M phosphate on a DEAE-sephadex column, which works against the formation of neural tissues, and several groups of nucleoproteins promoting the growth of the neural plate in an abnormal way. The action of heart extracts was also tested. The whole heart extracts were very toxic, but the biological action of some of its saline soluble proteins could be studied IN OVO. These fractions were obtained by chromatography on DEAE-sephadex. The first one was eluated by a 0.02 M phosphate buffer pH 6.8. It was analyzed by spectrophotometry and had the spectrum of proteins. It contained some traces of oxyhemoglobin. The two others, eluated by more concentrated salines were very rich in nucleotides. The first fraction when injected IN OVO caused malformation both on mesodermic and neural tissues and the development of blood as measured by the benzidine peroxydase activity was much poorer than normal. The third fraction had also an action against the development of the embryos. The most characteristic defects of the heart extracts treated embryos were heart and omphalomesenteric veins abnormalities. The action of blood extracts were also studied. The serum was found to be harmless. The curves of the average development of benzidine peroxydase activity of blood in function of age were studied in large populations of blood extract treated embryos and in controls treated either by Tyrode solution or by brain extracts. The blood extract partially inhibited blood development. Similar experiments have been done on the development of the chick

embryo IN OVO by Braverman (1958, 1961), by Clarke and McCallion (1959), and IN VITRO by Katoh (1961). When working with brain extracts these authors could observe brain defects and subsequently inhibition of brain development. When using extracts of mesodermic nature (heart, muscle plus cartilage, kidney), they could observe defects in the development of mesoderm. However, these malformations were accompanied by some brain defects. Braverman (1961)was also able to demonstrate a regional specificity of inhibition within the brain i.e. extracts of optic and cerebral lobes affected the development of brain in a manner entirely different from extracts of brain stem. Katoh (1961) concluded from his graft experiments that polarity governs the spread of inhibitory information in differentiation. IN VITRO experiments performed by Emmanuelson showed that an increased mitotic activity was provoked in chick embryos in regions homologous to the source of RNA they were treated with. When working IN OVO with brain extracts, Braverman, Clarke, McCallion and Lenicque could observe always the two same types of abnormalities as we have described here: 1) multiple folds of the neural plate and abnormal development of the head, 2) dedifferentiation of the cells of the neural tissues. These two kind of defects can now be attributed to two kind of brain extracts fractions. The "multiple folds" seem to be caused by a fraction rich in nucleotide, the "dedifferentiation" by a fraction containing proteins, but poor in nucleotides. It can be concluded that it is highly probable that the differentiated tissues contain several groups of antagonistic agents, the balance of which ensures a harmonious embryonical development.

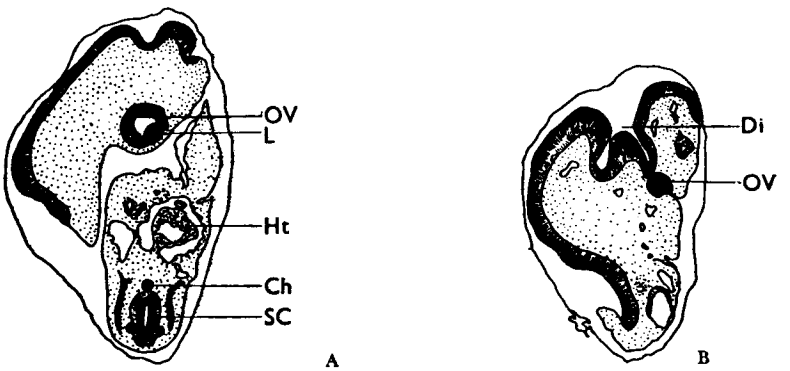

Fig. 1. Action of brain extract on development of the head. Examples of anomalies denoted as type 1. In A and B, the sections pass through the 2nd cerebral vesicle. In A, the section also passes through the body at the level of the heart.—The head is small, the folds of the neural plate have not fused at the level of the 1st and 2nd cerebral vesicles, and the eyes are atrophied. Di: 2nd cerebral vesicle. OV: optic vesicle. L: lens. SC: spinal cord. Ch: chord. Ht: heart.

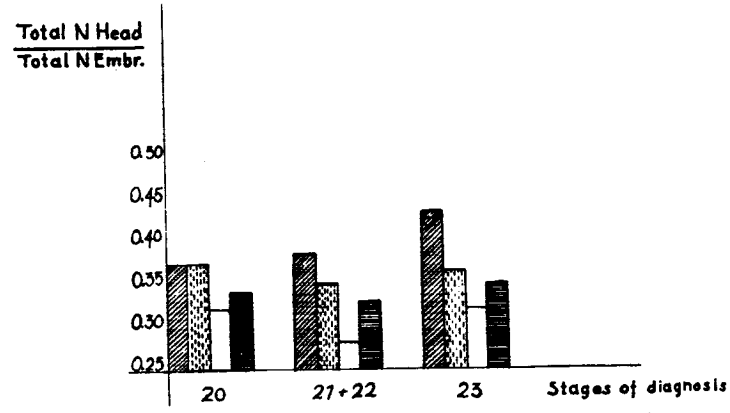

Fig. 2. Action of brain extract on development of the head: variations in mean ratio $\frac{\text{Total N head}}{\text{Total N embryo}}$ as a function of stages of development in control populations (////) and in populations treated with brain extract (▬▬). The treated populations are divided into those of normal appearance (▬▬) and those in which the normal morphogenesis of the head has been disturbed (□). Statistical analysis shows a highly significant difference between the height of the columns representing treated embryos (▬▬) and controls (////).

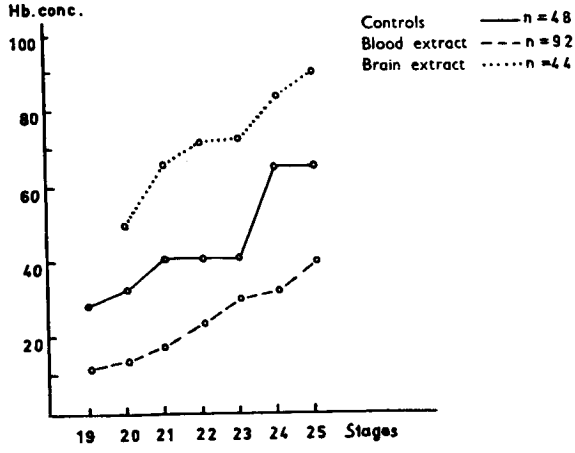

Fig. 3. Curves of the average development of benzidine-peroxidase activity of blood from stage 19 to stage 25: normal embryos (controls), embryos treated with blood extract and with brain extract.

TABLE I

ACTION OF BRAIN EXTRACT AND FRACTIONS OF IT (%)

Treatment		n	N	Head ≠ N	Dead	Remarks
Total Brain		54	22%	37%	41%	Growth retardation
Saline Soluble proteins		357	37	40	23	(3% of Mesoderm ≠ N)
Fractions obtained by chromatography						dedifferentiation of neural tissues
	A.proteins	79	43	10	47	
Buffer 0.02M	B.+ nucleotides	223	27	38	35	
0.20M	A.+nucleotides	38	32	21	47	
	B.+nucleotides	34	20	27	53	
Electrophoresis						
	A.proteins	109	43	11	46	Most electropositive fraction
	B.proteins	40	66	5	29	
	C.nucleotides	38	25	20	55	
	D.nucleotides	89	48	33	19	Most electronegative rraction
Controls						
Heated brain extract t > 55°C		118	80	5	15	
Saline (Tyrode)		100	73	8	19	No dedifferentiation of neural tissues

TABLE II

ACTION OF FRACTIONS OF HEART EXTRACT

Fractions	n	N	Only head ≠ N	Only Mes. ≠ N	Brain + Mes. ≠ N	Dead
I A.proteins	19	32	11	21	10	26
I B.proteins	77	47	17	17	0	19
II + nucleotides	28	75	0	0	4	21
III + "	72	67	0	8	12	13

(N = normal; ≠ = abnormal; n= number of embryos)

SELF INHIBITORY FACTORS IN THE REGENERATION OF FRESH WATER PLANARIANS. Etienne Wolff, Laboratoire d'Embryologie expérimentale, Collège de France, Paris, France.

The study of fresh water planarians endowed with the power of total regeneration (Dugesia lugubris, Polycelis nigra) has elucidated certain factors which play a role in morphogenesis during regeneration. The morphogenesis of regenerating tissues can be explained by a succession of inductions and inhibitions. Whereas inducing actions are responsible for the reconstitution of the lacking organs, inhibition mechanisms prevent, at all levels of the body, repetitive regeneration of an organ. Morphogenetic experiments have demonstrated the reality of these inhibitions.

1. Inhibiting effect of the brain

Several authors have shown that the brain of a planarian exerts a powerful inhibition against the differentiation of a head which has been grafted to the anterior region. A less inhibitory action is exerted against the head by areas posterior to the head, and no inhibition occurs when the head is grafted behind the mouth (Chevtchenko 1937). In general each zone of the planarian inhibits strongly the differentiation of a homologous region grafted at this level. The strength of inhibition decreases with distances.

Lender (1955-1958) has pushed further the explanation of these phenomena. Planarians of the species Dugesia lugubris and Polycelis nigra, deprived from their heads, were reared in water containing head homogenates. The individuals thus treated did not regenerate brains. However the whole head was not inhibited, since the eyes were reconstituted. This result can be explained easily, since other experiments have shown that the crude homogenate contains both the substance which induces the eyes and that which inhibits the brain. An accumulation of neoblasts is found in place of the brain, but these cells do not differentiate.

If the same experiment is carried out with a tail homogenate, no inhibition of head regeneration is found, and the brain differentiates normally.

The homogenate of the head does not lose its inhibiting activity when it is heated to 60°C. Following centrifugation at 10 000 g, the inhibiting activity is found in the supernatant. The inhibiting substance is not species-specific, since the homogenate of the brain of Dugesia lugubris or Dugesia gonocephala inhibits the differentiation of the brain in Polycelis nigra.

.../...

Thus it seems to be demonstrated that there are brain inhibiting substances in the brain of the planarian. These substances are diffusible. The strength of their action apparently decreases with distance. The anterior posterior gradient of brain inhibition can be interpreted as a diffusion gradient of an inhibiting substance.

2. Inhibiting effects of other organs

The question arises as to whether other organs, and possibly all organs of the planarian, emit inhibiting substances for the development of homologous organs. Recent experiments by C.Sengel give strong evidence that homogenates of the pharynx have an inhibitory effect on regeneration of the extirpated pharynx of Dugesia lugubris. Regeneration of this organ is significantly delayed in comparison to untreated planarians. 12 - 15 days after the beginning of the treatment, 34 percent of the experimental planarians are regenerating their pharynx, as against more than 80 % in the controls. Homogenates of other regions of the planarians have no such inhibiting effect on the regenerating pharynx.

It will be noted that the pharyngeal zone, deprived from its pharynx, has no inhibitory effect, whereas this same zone, including this organ, has a strong inhibitory effect. The same action is exerted by homogenates of pharynx isolated from the surrounding tissues. These results can be related to the inducing action of the so-called pharyngeal zone. Only the pharynx as a differentiated organ has an inhibiting action.

Inhibiting substances elaborated by other organs were not investigated up to now. If such substances were demonstrated in each case, the theory of the physiological gradients would find a satisfactory explanation and a positive basis. The results summarized here lead to an explanation, in which physiological gradients can be interpreted in terms of diffusing substances, the effect of which is more or less intense according to the distance of the source and to the concentration of the inhibiting substances.

THE DIRECT CURRENT FIELD: A PRIMITIVE CONTROL AND COMMUNICATION SYSTEM RELATED TO GROWTH PROCESSES. Robert O. Becker, M.D.*

All living organisms possess to some degree the property of self-repair. A major characteristic of this process is its "relatedness" to the total organism. In regenerative growth processes, for example, the part formed under normal circumstances is quite appropriate to replace the missing part, and, in fact, in those animals most highly endowed with this ability it is an exact duplicate. It would appear that the regenerating part is furnished with information from the remainder of the organism indicating the desired physical parameters to be fulfilled, and that some error-sensing process determines the overall organizational pattern of the regenerate to best fit the furnished instructions. This concept implies the existence of some communication system capable of passing appropriate and meaningful signals in both directions between the regenerating cell mass and the remainder of the organism. Certain characteristics of this control or communication system may be predicted. Since the process of self-repair may be considered to be a basic attribute of living things, the control system governing the process should be equally primitive. It should utilize physiological mechanisms of a nature that could have been operating in the earliest living organisms. Secondly, since the function of the system in regenerative growth is to relate the growth to the entire organism, the system must in some way be present throughout the entire organism. However, the parameters at any point in the organism must somehow be representative of that specific point. Knowledge of the system values (? coordinates) at any point should be an expression of the anatomical configuration of the organism at that point. Thirdly, in view of its primitive nature, one would further predict that the system possessed other control and communication functions of a basic nature in addition to control over regenerative growth. During the past several years we have made certain observations on the direct current electrical activity of the central nervous system indicating that this activity constitutes an organized control and communication system of a very primitive type possessing the previously mentioned characteristics. It appears to be related, among other things, to the sensing of trauma and to the control of repair processes following trauma. Presently available data indicates that this phenomena is based upon some solid state or semiconduction property of the neurone, and that the current flow is electronic rather than ionic in nature. The system parameters, as presently known, would seem to furnish a logical basis for study of the role of electrical and neural factors in many types of growth processes.

METHODS: Details of technique have been reported in previous publications, and the interested reader is referred thereto (1-5).

OBSERVATIONS: A. Surface Patterns of Direct Current Potentials. In 1932 Burr (8) published the first of a long series of papers dealing with the direct current potentials found on the intact surfaces of a variety of organisms. He related changes in these potentials to certain physiological functions in-

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cluding growth & development (10), tumor formation (12), sleep (13), and ovulation (11). At the same time, Burge (7), working with the surface cephalo-caudal d.c. potentials, demonstrated a reversal of this potential with anesthesia and predicted a causal relationship between the two. We have examined these cephalo-caudal potentials in a phylogenetic series, and observed a sequence of magnitude that appears to be related to either body size or complexity. Man appeared to occupy an anomolous position in this regard, showing the only reversal of the head-negative polarity. According to the standard concepts, these potentials represented a relatively simple dipole field generated in some fashion by the entire organism and unrelated to any specific tissue (9,14). If this is true, then moving the recording electrode along the dorsal midline from the cephalad portion toward the reference electrode at the caudal end should result in a relatively smooth decrease in field magnitude to zero. However, the potentials measured in this fashion showed a surprising complexity which was related to the general organizational pattern of the central nervous system in each organism. Wherever major masses of neurone cell bodies aggregated, there were evident relative "positive sinks" in the field pattern. These areas were single in planaria, annelids, and fish (Fig. 1), all of which have single cephalic, neuronal masses despite other neural differences of considerable magnitude. In the higher vertebrates the development of additional cord enlargements concommitant with the development of specialized limbs was associated with two more "positive sinks" located approximately over the cellular accumulations (Fig. 2). In man, with the relatively enormous development of the central nervous system compared to body size, particularly in the cranial area, the entire recording was positive, but again the three cellular accumulations were more positive than the intervening areas. Also in these vertebrates the limbs had well developed d.c. gradients of their own with the cord enlargements positive, and with an increasing negative gradient distally. This spatial relationship between the surface d.c. potentials and the general organization of the central nervous system in such a wide variety of life forms appeared to be of some significance, and the thesis was formulated that a causal relationship existed between the two.

B. Direct Current Potentials of the Central Nervous System and Their Relationship to the Surface D.C. Pattern. It was initially observed that the surface d.c. gradient along an amphibian limb decreased to zero if the nerves entering that extremity were completely sectioned. More importantly, however, the gradient on the contralateral limb decreased by approximately 25%. Similar phenomena were noted in humans in chemical or pressure nerve block (6). In addition, section of the spinal cord (in amphibians) at the level of the foramen magnum produced a depression in all limb d.c. gradients during the phase of spinal shock. As spinal shock was replaced by reflex hyperirritability, the limb gradients returned to normal or above-normal levels. The conclusion was reached that the surface d.c. potentials were a second-order phenomenon related to some activity of the central nervous system. On theoretical grounds, it seemed highly unlikely that summated action potential activity could be the generating source for the steady state potentials. It was also theoretically unlikely that polarization or electrostatic potentials could be long maintained within a conducting mass without some actual current flow taking

place. The electrical activity that could best fulfill the requirements was a longitudinal direct current potential along the long axis of the neurone accompanied by some small level of actual direct current flow. In 1940 Gerard & Libet (16) observed that a steady d.c. potential existed across the amphibian cerebral cortex in an axonodendritic direction. They noted changes in the magnitude and polarity of this potential with changes in cerebral irritability, and suggested that this axonodendritic polarization controlled, in part, the level of neural irritability. Subsequently, Goldring (17,18) extended and confirmed these observations both in animals and in humans. Our observations on the central nervous system proper have been confined to the spinal nerves. In this area several lines of endeavor have been explored to determine whether or not actual d.c. flow could be detected in the nerve fibers. The galvano-magnetic effect (Hall effect) was investigated in intact amphibian limbs, and some evidence was obtained for the existence of a neural-dependent, axial current flow (3). The observations indicated a current that was solid state or semiconduction in nature, rather than ionic. This was further evaluated by a study on the injury currents in isolated nerve sections. It was observed that longitudinal steady current flow and potentials were maintained even though a segment of the nerve was frozen with liquid nitrogen at 77° K (4). The existence of a steady current flow requires the presence of a complete circuit loop, and a search was instituted for the in- and out-flow pathways. Our initial concepts were for a unidirectional flow in the peripheral nerves with a circuit completion pathway via some other tissue. Observations, however, indicated that the entire circuit path lay within the central nervous system (except perhaps at the peripheral terminals, where a short, higher resistance pathway exists through other tissues). We found that the axial d.c. potential gradients along spinal motor nerves were distally-negative, while those along spinal sensory nerves were distally-positive (5). The direction of the polarization, therefore, is related to the normal direction of message transmission, or, perhaps, more fundamentally, to the axonodendritic axis (6). (This requires that the peripheral fibers of the sensory nerves be viewed as dendritic in nature, despite their histological appearance.) We may therefore summarize our findings as follows: the surface d.c. potentials are a second-order phenomenon produced by the flow of small steady electrical currents within the underlying neural elements. The currents are polarized in an axonodendritic fashion, and are conducted by some element of the neurone possessing a solid state molecular lattice permitting electronic conduction. This property of the central nervous system is organized, therefore, into a complex electrical field which is related to the entire organism. Since electrical fields with associated currents have not only scalar (magnitude) but vector (directional) parameters, then any area of the body may be represented by a "set" of electrical coordinates. It is tentatively proposed, therefore, that one of the environmental parameters of each cell or group of cells may be a certain set of values of this electrical field in an organism.

C. Functions of the D.C. System. 1. Neural Irritability Level: The work of Gerard, Libet and Goldring on the relationship of the axonodendritic potential to the irritability of the cerebral neurones had been done on isolated cerebral tissue. In our measurements of the surface d.c. potential pattern, we had noted that a midline fronto-occipital vector of d.c. potential was

present on the dorsal surface of the head, with the frontal area negative with respect to the occipital. All other measurement vectors across the head were lower in magnitude and, in fact, the transverse vector was generally zero. We noted further that the magnitude of the fronto-occipital vector was an expression of the state of consciousness of the animal. Anesthesia produced by any one of a variety of agents was associated with a fall in the magnitude of the potential to zero, and occasionally to a moderate reversal of the normal polarity. We postulated that this organization of the d.c. pattern was the resultant of d.c. activity within some midline structure of the brain (? reticular formation). In accord with the concept that the irritability level of the neural tissue was controlled by the d.c. potential, we postulated that the action of the anesthetic agents was to produce a decrease or reversal in this electrical vector by interfering with the electronic transfer. If this is the case, then variation in the level of consciousness should be produced by "overriding" the internal currents with externally administered electrical currents. Experimentation has proven the validity of this hypothesis: administration of low amplitude currents in a reversed direction (frontal-positive) produced EEG patterns (delta waves) and behavior typical of deep anesthesia in amphibia. The converse was also noted: animals deeply anesthetized with chemical agents could be made to demonstrate "awake" EEG patterns with the administration of "normally" oriented direct current (frontally-negative). Since we had previously determined that the internal currents were most probably semiconduction in nature, it was then postulated that a magnetic field of sufficient strength applied in a transverse direction to the normal midline current vector should produce a deviation of significant numbers of charge carriers from the vector resulting in a decrease in the total current flow, and a corresponding decrease in cerebral irritability level. It was subsequently found that a 3.8 kilo gauss field applied at exactly 90° to the midline of the amphibian head produced a drop in the fronto-occipital d.c. potential, and the appearance of delta wave patterns in the EEG (Fig. 3). The evidence would indicate that, in the cerebrum at least, the organized d.c. field determined the level of neural irritability.

2. Sensing of Trauma: The perception of pain is a complex phenomena; the phenomenon of delayed pain (20) is still somewhat difficult to explain on physiological grounds. In amphibians multiple-point recordings of changes in the d.c. field attendant to hindlimb trauma have shown that the time delay before cerebral d.c. changes approximated that of delayed pain. It would seem possible that the d.c. field transmits information of a basic nature related to body damage, i.e., that it informs the organism of the event and possibly of the extent of the injury via a relationship to currents of injury.

3. Control over Regenerative Growth Processes: Preliminary experiments have been done on the d.c. field changes occurring during regenerative and non-regenerative healing processes following limb amputation in two species of amphibians (2). Regenerative growth was noted to be accompanied by a complex series of changes in the d.c. field along the axis of the amputated extremity. The sequence of changes in the field appeared to be related to the two phases of the regenerative healing process. The non-regenerative healing process, on the other hand, demonstrated an initial injury current which was gradually reduced to a value appropriate for the normal d.c. field at the point of amputation.

The administration of very small amounts of electrical energy polarized to augment the naturally-occurring potentials appeared to accelerate the two phases of the regeneration process individually. However, for technical reasons we failed to induce regeneration in the non-regenerating form by duplicating "regeneration sequence" of d.c. field changes. Other authors have reported the production of major alterations in growth patterns by the administration of low values of direct current (15,21,19). These observations would seem to be in keeping with Singer's observation on the essential role of the central nervous system in limb regeneration. We would postulate that the responsible activity of the central nervous system in this regard is the direct current system.

SUMMARY AND CONCLUSION: The central nervous system of a variety or organisms has been shown to possess the ability to generate and transmit small, steady electrical currents. This direct current activity is organized into a complex field pattern dependent on the organization of the central nervous system itself. Some evidence has been accumulated which indicates that this direct current system functions as a primitive analog-type control and communication system. It is sensitive to the presence of electromagnetic force fields in the organism's environment, and appears to respond to traumatic stimuli administered to the organism. The system in turn appears to have a controlling influence over the basic level of neural irritability and self-reparative processes following trauma. Perhaps most importantly it appears to be a representation of the entire organism as a functional unit. In this respect it is an objective, measurable expression of the field concept and furnishes a logical basis for further theorization and experimentation.

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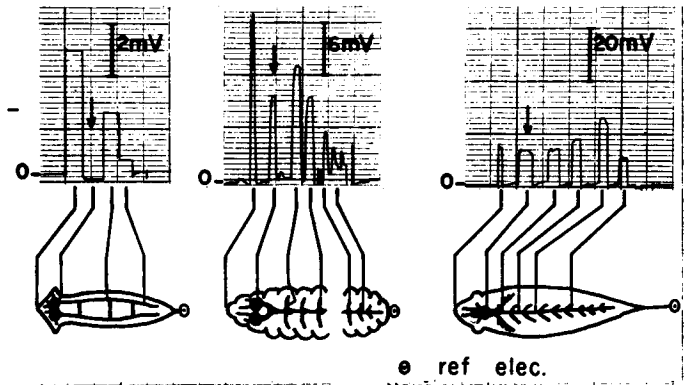

Fig. 1. Midline cephalo-caudal d.c. potentials in planaria, earth worm and fish. Arrows indicate potentials over the single main nerve cell mass. Reference electrode at tail in all cases.

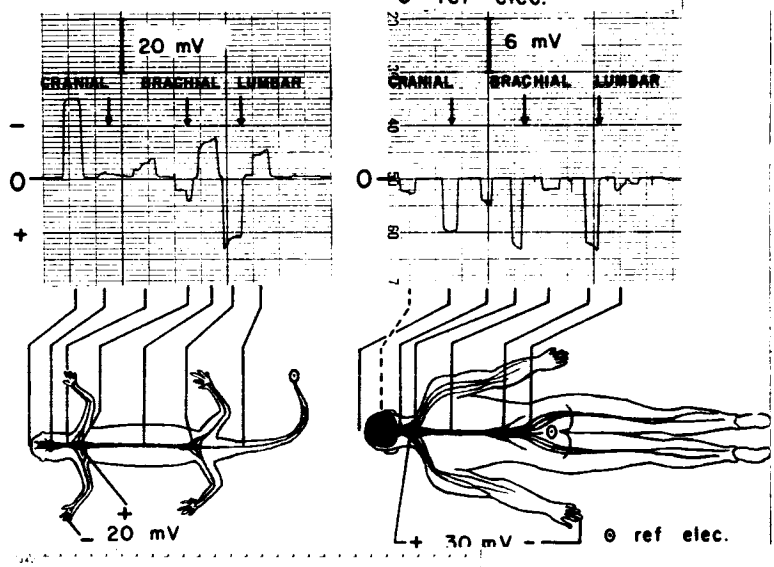

Fig. 2. Same as Fig. 1 except in salamander A. tigrinum and man. Arrows indicate potentials over each of two major nerve cell masses.

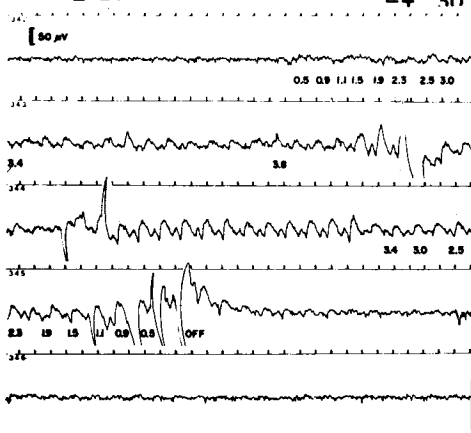

Fig. 3. Fore-to-hind brain EEG recording in salamander (lightly tranquilized) before, during, and after application of transverse, uniform magnetic field. The recordings are sequential from top to bottom; the numbers beneath the tracing indicate field strength in kilogauss. From 3.8 on the second record to 3.4 on the third, a 3.8 Kg. field was steadily applied. The time markers on each record are 1/second.

INHIBITION IN POLARIZED SYSTEMS

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Different rather specific inhibitory products can be found in different parts of an organism as indicated by Drs. Lenicque and Wolff. If these really function as controlling agents there must be some way of removing them from their cells of origin to the cells which they control. For example, in Lineus different levels of the body contain different inhibitors. Those from a mid body region prevent head regeneration in isolates from more posterior regions. There are also inhibitors from the mid region that prevent tail regeneration in anterior isolates (Tucker, 1959). These inhibitors seem to be qualitatively different at different body levels. Dr. Tucker's suggestion is that one set of inhibitors prevents tail regeneration as they move from tail toward head and that another set inhibits head formation as they move in the opposite direction. It would be rather difficult to study some aspects of polarized control in these nemertean worms because they so successfully resist being held in one position. We have turned to Tubularia. The stems of this hydroid regenerate hydranths rapidly and since they are nonmotile can be held in any position.

The stems of Tubularia are totipotent. An isolate from any position can become a hydranth, or, if it is very short, it may become just the anterior part of a hydranth. All stem regions are potentially hydranth and all are potentially the anterior part of a hydranth. When one combines small regenerating isolates, the more anterior partner produces anterior structures. The partner lying behind is prevented by this union from producing what is being produced anterior to it. It is relegated to the production of those structures not forming anterior to it (Rose, 1957).

That this control which operates in an antero-posterior direction is polarized can be shown in another way. When two pieces of regenerating stem are grafted together by their anterior ends, both form all of the anterior structures. When neither piece lies posterior to the other, even though the pieces are intimately fused, neither controls the other.

What could the nature of this highly polarized control be ? It was already known from the work of Lund and Barth (rev. by Lukiewicz, 1962) that the polarity of regenerating stems of hydroids can be reversed by applied electric currents. Ordinarily, as indicated above, control spreads from anterior to posterior. When an electric current reverses the polarity, control must travel in the opposite direction. Since it was already known that rather specific inhibitors exist, the question arose as to whether these were agents of control moving in a

bioelectric field.

The first test of part of this hypothesis was designed to test whether the effect of partners in graft combinations could be changed by applied currents. Pieces of stem were isolated and allowed to regenerate until the critical stage just before tentacle formation. These are already structurally polarized at this time. When two anterior parts are combined and made to face the positive pole in a current of 104 $\mu a/mm^2$ and 12-30 volts the anterior partner controls the piece behind it and a complete hydranth develops. When the combination is made to face the negative pole the control is blocked and both develop as anterior parts (Rose, 1963). The combinations are kept in the current only 4 hours during the critical period when anterior partners are known to control more posterior partners. Although the currents used can change polarity if used for much longer times (Levin, 1961), they do not do so in 4 hours. Instead, in this experiment they are blocking the passage of control along an already polarized transmission system. When the current is reversed it does not change the direction of control. The posterior piece does not then control its more anterior partner. The observations are consistent with the hypothesis that the controlling agents are positively charged and move in the polarized system from anterior to posterior. At this point it also seems possible that an effect such as Ling (1962) visualizes in his association-induction hypothesis could be at work. However, the fact that there are specific inhibitors makes one consider the possibility that these are chemical agents of control whose passage can be blocked by an electric current.

Homogenates of regenerating primordia were mixed with cooling agar about to set. After the mixture had set, capillary punches with inside diameters the same as those of stems of Tubularia were used to obtain cores. The cores were tightly inserted into the coelenteron in the mid-region of stems. There was room at both ends beyond the core for hydranths to regenerate. These cores were inserted in freshly cut stems. Without any other treatment the stems containing cores regenerated at the anterior end. When the anterior end of core containing stems faced the negative pole in currents having the same characteristics that blocked control by grafts, no regeneration occurred. When they faced in the other direction regeneration proceeded normally. Neither cores alone nor current alone blocked regeneration. Neither did agar plugs without homogenate in the current. These findings indicate that the inhibitors from the homogenates are positively charged with respect to the sea water-agar. Only when the positively charged substances were carried by the current into the region where regeneration was expected did regeneration fail.

The electrophoretic tests were refined in an attempt to learn whether there are regionally specific inhibitors. In these tests homogenates were obtained from either anterior or posterior parts of primordia of hydranths. They were put into starch wells at pH 8.6 and materials from them migrated either toward the positive or negative pole. The regionally specific inhibition is obtained only when the starch cores are inserted in stems in a current just before the time when tentacle ridges are expected. These regionally specific inhibitors behave as though some are positively charged and others negatively charged at pH 8.6.

Several facts taken together indicated that at least some of the inhibitors might be histones or other basic proteins. Very small amounts of trypsin removed the inhibition from homogenates and some of the inhibition was carried by positively charged particles at pHs 8.1 and 8.6. Actually very little histone is soluble in this pH range. This fits with the fact that not all of the areas of inhibition on the starch contained sufficient protein to stain with amidoschwarz. It still seemed possible that very small amounts of histone might be effective.

Homogenates were then made in 0.2 N HCl. The supernatant contains the histones. These very basic proteins extracted in this way are positively charged when spread in starch gels at pH 4.1. They move in the same direction as does the inhibition and are visible after amidoschwarz staining. The 0.2 N HCl extract when run on pH 7 starch is almost insoluble and not enough appears in the starch to give a stain. There is, however, sufficient material that moves in both directions a short distance from the wells at pH 7 to effect inhibition.

If the 0.2 N HCl extract and trypsin are mixed in a starch well, subsequent electrophoresis does not yield an inhibitory agent. This suggests that the inhibitory agents in the 0.2 N HCl extracts are proteins. These extracts contain only the basic proteins.

The basic proteins which are spread in starch from the acid extracts have never in more than 150 cases yielded regionally specific inhibition when inserted in regenerates. The inhibition is total and reversible. It seems possible that extraction with acid removes more basic proteins and different basic proteins than does homogenization. This is being tested.

This work, although done without prior knowledge of the hypothesis that histones may control gene action (Stedman and Stedman, 1950; Huang and Bonner, 1962) seems to be in line with that general hypothesis. This work suggests further that basic proteins of one region moving in the bioelectric field may limit

the action of the same genes that were operating in the cells that produced them.

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THE DNA HISTONE ASSOCIATION AS A BASIS FOR ALTERNATIVE HEREDITARY GENE STATES

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Histone synthesis

Spermiogenesis in the grasshopper, Chortophaga viridifasciata, is fairly typical. Cell elongation is accompanied by a sloughing of the cytoplasm, and a transformation occurs in the type of histone contained in the nucleus. The elongation process provides a useful parameter for the study of the changes in metabolism leading to the transformation. Elongation occurs in two phases. The first involves the cytoplasm and terminates with a spermatid characterized by a large spherical nucleus and a long axial filament, both covered by a thin layer of cytoplasm. Some residual cytoplasm is still retained in the form of small beads intermittently strung along the tail, but most of the cytoplasm contained in the earliest stages is sloughed. During the second phase, the nucleus changes from a spherical to a highly elongate form, the nucleus of the mature sperm having longitudinal and cross sectional dimensions similar to those of the tail. The progress during the second phase can be described in terms of the increasing axial ratio of the nucleus.

The transformation of the nuclear protein occurs during the second phase of elongation and is very similar to the process which occurs in many mammals. It involves a replacement of a histone complement which is fairly typical (similar to that found in adult somatic cells), by a protein which is very basic and contains an increased ratio of arginine to lysine. Such protein has been called "intermediate" or "protamine-like" histone. While this designation is unwieldy, it conveys the proper impression that the protein shares properties common to both typical histones and protamines.

The change in the nuclear protein is easily demonstrated by the changes in the staining properties of the nucleus. These reflect increasing basicity and amounts of protein bound arginine. The staining alterations are most apparent as the nuclear length to width ratio changes from around 5 to 10. The rate of synthesis as determined by incorporation of labelled arginine is also maximal at this stage of development. The grasshopper appears to be somewhat unique in this respect, for in other organisms studied, the initial transformation begins before sloughing of the cytoplasm.

The postmeiotic spermatid is very rich in RNA. This substance is contained in both the nucleus and the cytoplasm. Incorpo-

ration of cytidine, which is appreciable in the early stages, is observed first and primarily within the nucleus. By the time the first phase of elongation has terminated, RNA synthesis has practically ceased, all detectable RNA has been lost from the nucleus, and most of the RNA of the cell has been sloughed as part of the residual cytoplasm. By the time the nuclear transformation begins, the only detectable remnant of RNA exists in the form of a few scattered granules in the cortical cytoplasmic layer surrounding the nucleus and along the axial filament. These granules are most easily detected using acridine orange. As might be expected, autoradiography of thin sections through these cells obtained from animals 5 to 15 minutes after injection of labeled arginine indicated incorporation primarily in the thin layer of cytoplasm surrounding the nucleus. At later times, most of the label is in the nucleus. It is concluded that the histone is synthesized in the cytoplasm by ribosomal machinery which had itself been manufactured approximately a week earlier. The histone then migrates into the nucleus and combines with the DNA.

There are at least two orders of histone synthesis. Synthesis occurs as an essential part of the process of chromosome replication (Bloch and Godman, '55), and also, as above, during an alteration of the chromatin which is independent of the replication process. Whether all histone synthesis occurs in the cytoplasm is doubtful. However, it does seem probable that the synthesis of a histone molecule will in general be both spatially and temporally removed from that of the DNA with which the histone will ultimately associate. This view is at variance with the previously held interpretation of parallel increases in Feulgen and fast green staining as indicating simultaneous syntheses of DNA and histone (Bloch and Godman). This old data is now reinterpreted as indicating complexing of DNA with histone as the DNA is replicated, the process rendering the histone stainable. The revised view is based upon the consideration of two types of relationships between DNA and histone, association and specification, and is supported by the observations described above. These relationships have been discussed at length elsewhere (Bloch, '62) and are briefly reiterated below.

Histone variation

The histones of a cell consist of a heterogeneous spectrum of basic proteins (Luck, et al, '58) which yield a complex with DNA containing roughly equal proportions by weight of both substances, (Zubay and Doty, '59), the histone covering most of the DNA (Davison and Butler, 1956). There are between 3 and 4 amino acids per nucleotide in the complex. According to current coding concepts, which require three nucleotides for the specification of a single amino acid, there is insufficient information in a stretch of DNA to specify the synthesis of the histone with which it associates. Therefore the histone associated with a

given stretch of DNA must have its origin elsewhere. At the level of the nucleus, there is insufficient information in the entire DNA complement to specify the synthesis of a unique histone for each DNA. Hence histone heterogeneity must be relatively limited as compared with DNA heterogeneity. In brief, the genome contains a relatively few genes whose function it is to synthesize a relatively few varieties of histone which combine with the entire complement of DNA.

The question whether the DNA-histone association is a constant among all cells can be answered unequivocally. Replacement of histones is a fairly general feature of spermiogenesis. In some organisms, for example, the squid Loligo opalescens, a succession of replacements occurs each of which appears to encompass the entire genome (Bloch, '62b). In the snail, Helix aspersa, the protamine is lost during fertilization and is replaced by a weakly basic histone which is characteristic of all the nuclei up to a point in development, approximating gastrulation, when they are in turn replaced by typical histones (Bloch and Hew, '60). Thus the transitions seen in sperm can be considered a part of a broader program of changes which extend into early development and perhaps throughout all of development.

Histone variation is then, two dimensional; occuring along the genome in an individual cell, and among homologous DNA's of different cells.

Genetic implications

The observation that a given DNA associates with alternative histones, and the inference that different DNA's associate with the same histone, indicates that the affinity between DNA and histone is inherently non-specific, and suggests that a given segment of DNA in a cell is potentially capable of combining with a number of histones found in that cell. Chromosome replication involves the replication of DNA in an environment containing a variety of histones. Unless the association is random (meaningless) one would expect maintenance of the association during replication, or recovery of the association after replication. In either event, the association would be the replicating unit in somatic division, and such variation in the association as might arise during development would provide a basis for alternative states of the genome which are mitotically transmissible.

The biological consequences of this variation would depend upon the role histone plays in the cell. If histones control gene expression, as was proposed by the Stedmans in 1950 and more recently by Huang and Bonner, these alternative states would be manifested as changes in phenotypic expression.

The idea that genes can exist in physiologically meaningful alternative states has a precedent. In 1960 Brink postulated the existence of an ancillary genetic substance, "parachromatin", to explain such quasi-genetic phenomena as paramutation, in which the genes undergo temporary changes of state whose maintenance is attributable to inherent changes in the gene rather than to stable changes in a controlling cytoplasm. Paramutation was viewed as an abberation of a process of gene change which occurs during, and is restricted to development.

Control of gene activity was postulated to reside in the association between the parachromatin and the orthochromatin or gene proper. The role of the parachromatin is to mediate between the gene and its environment, affording a means of stabilizing changes in function, while at the same time permitting a controlled variability essential for development. A formal set of requirements were postulated which were deemed essential for the parachromatin to fulfill its assigned role. These are as follows: The orthochromatin and parachromatin are coextensive throughout the genome. The association is variable. The association is self-replicating in the sense that it is reproduced during chromosome replication. The parachromatin has its origin in a limited fraction of the orthochromatin, and is hence relatively homogeneous as compared with the latter, and also ambivalent in its ability for association. The parachromatin reaches a ground state during gametogenesis.

The existence of parachromatin has still to be shown, although there is little doubt about the reality of the phenomena for which it is called upon to account. Histones on the other hand, are real substances. Unfortunately, assumptions about their function remain without firm basis in fact. An equation of histones and parachromatin is suggested on two counts. The first is that the behavior of histone as inferred from limited data on its composition and current ideas about the transcription of information from DNA to protein parallels the behavior of parachromatin as predicted by Brink on the basis of its effects. The second is that, granted the inhibitory role which has been proposed for histones, the consequences of their inferred behavior would be the "quasi-genetic" phenomena of the type observed by Brink, for whose explanation parachromatin was invoked.

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NUCLEO-CYTOPLASMIC INTERRELATIONS

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BEARING OF PROTOZOAN STUDIES ON CURRENT THEORY OF GENIC AND CYTOPLASMIC ACTIONS. T. M. Sonneborn, Dept. of Zoology, Indiana University, Bloomington, Indiana.

This paper takes up two questions. To what general picture of nucleo-cytoplasmic relations does the current DNA-RNA-Protein theory lead? If there are results of Protozoan studies which are not obviously explicable in this way, what additional or different relations do they show? The intention is not to criticize current theory, but to point out problems that present opportunities for broadening its scope.

Genes replicate by serving as templates for the formation of complementary DNA; they act by serving as templates for the formation of complementary RNA. That is their sole known indirect genic action. The corresponding primary cytoplasmic action is polypeptide production, which may occur also to a limited extent in nuclei. Wherever it occurs, the production machinery and the composition of the product depend upon genically produced RNA. Primary cytoplasmic action is secondary genic action. All further cytoplasmic actions are currently held to be further, more direct genic actions. The specific folding of a polypeptide follows from its gene-dictated amino acid sequence. Assembly of the units of a multi-polypeptide protein follows from the properties of the component gene-specified polypeptides and of the indirectly gene-controlled local intracellular milieu. The gene-determined proteins function as building blocks of cellular structure and as enzymes for the reactions which yield energy and all of the remaining molecular species of the cell except those taken in as food in the broadest sense. These later cytoplasmic constructions and reactions also follow from the properties of the molecules and of the local milieu, both of which are indirectly gene-determined. The cell and its parts are self-assembled.

Current theory thus portrays a primarily unidirectional nucleo-cytoplasmic relation. Gene-produced RNA is the foundation upon which all cytoplasmic action is directly and indirectly built. Some gene-produced RNA is also believed to be an essential part of the mechanism controlling the timing and amount of genic action by specifically repressing the formation by particular genes of messenger-RNA. Reciprocally, however, the rest of the mechanism, derepression and feed-back inhibition, involves molecules formed or taken in by the cytoplasm. Also in the cytoplasm are formed materials essential for DNA and RNA production by the genes: their triphosphodeoxynucleotide and triphosphoribonucleotide building blocks; the enzymes for synthesizing these nucleotides, for activating amino acids and binding them to t-RNA, for polymerizing amino acids and both kinds of nucleotides; and other protein machinery (e.g. ribosomal protein).

Nucleus and cytoplasm are thus interdependent like the chicken and the egg. The cytoplasm supplies the building blocks, the tools, and the machinery for nuclear and cytoplasmic syntheses; but the specific composition of the materials employed by the cytoplasm in making these supplies is determined by the gene, while

the specific composition of the DNA and RNA products of the genes is determined not by the cytoplasmic contributions to nuclear action, but by specific composition of the initial DNA itself. If this specificity at the basic levels is necessary and sufficient to account for all higher levels of compositional specificity, as the hypothesis of self-assembly holds, then the compositions and actions of all parts of the cell and organism are unidirectionally determined by genic action, all else being derivative.

The preceding formulation of current knowledge and thought on nuclear and cytoplasmic actions and interactions is based mainly on studies of viruses and bacteria. They have genes and gene-strings, but do not have chromosomes or nuclei like those of higher organisms. Evidence for the association of protein with the gene-strings of bacteria is lacking, but about 90% of the material of chromosomes is protein. Some viruses lack DNA; their genes are RNA. No membrane separates the gene-strings of viruses and bacteria from the surrounding material; no nucleoli and probably no centromeres or mitotic apparatus are present. The universal occurrence of these features in higher organisms, including the Protozoa, calls for explanation. Bacteria are largely "bags of enzymes" and enzymic products, with only rudiments of the structural complexity of higher cells. The differences between higher cells and microbes raise the question of whether higher cells have some additional or different features of nuclear and cytoplasmic action and interaction.

This is indeed indicated by the following Protozoan studies. The first to be discussed is the remarkable recent discovery by Gibson and Beale of a particle, the metagon, which acts as a cytoplasmic intermediary between the cytoplasmic killer particles in killer paramecia and the nuclear gene on which the maintenance of these self-reproducing, mutable killer particles depends. Metagons are formed in paramecia containing the killer gene. Each cell has about 10^3. They are not self-multiplying, but are long-lived. When the killer gene is replaced by its non-killer allele, the metagons formed earlier are randomly transmitted without increase to the daughter cells. On the average, their number is therefore halved at each fission until only one is present. When a cell with one metagon divides, the metagon passes to one daughter cell along with half of the killer particles which persist and multiply, maintaining the full normal complement of 10^2 to 10^3. The other daughter cell of course get no metagon; its killer particles disappear completely and irreversibly before the next fission. How long the metagon can persist and function is not yet known; it has been maintained by one product of each fission as long as followed, i.e. about 20 fissions, like a Lederberg "trail". Exposure to RNAase (but not DNAase) inactivates them.

What is the metagon and what are its implication for nucleo-cytoplasmic relations? Do not its properties suggest that it is a programmed ribosome (or ribosomal complex) carrying messenger RNA from the killer gene? Whatever it may be, its infectivity and long life (or immortality!) are features to be added to current

theory, as is the unknown mechanism which control its activity, for one can do the job that 10^3 ordinarily do. Clearly further study of the metagon system is one of the most promising areas for further study of nucleo-cytoplasmic relations.

Two other systems, long under study in Paramecium and Tetrahymena, are among those most closely related to current theory because they are concerned with protein production. The proteins are on the cell surface and are detected as mating type substances and ciliary antigens. Their compositions are determined by genes. In P. bursaria and in certain syngens of T. pyriformis, each mating type has a different determinative genotype. There is always a different gene for each serotypic antigen. Most P. aurelia and some T. pyriformis have genotypes for two or more alternative mating types and all strains of both genera have genotypes for several alternative antigens. However, each cell manifests only one of its potential mating types and as a rule forms only one of its potential serotypic antigens. Genic repressions and derepressions thus appear to be involved. These actions are known in some of the materials to be brought about by cytoplasmic agents which are themselves determined by prior nuclear action. However, the nucleo-cytoplasmic systems show at least two features additional to those covered by current theory.

First, both systems show new aspects of the coordination of genic actions. The dozen or more genic loci for serotypic antigens in P. aurelia are not linked. Each cell always possesses one, only one, but any one, of these antigens, indicating that neither more nor less than one locus of the set is depressed at any one time. Further, any cell can be induced to cease forming its antigen and form another one, indicating that turning off one gene turns on another gene of the set, and that turning on one gene turns all the other off. Current theory accounts for two-loci systems in which one or the other locus is always expressed and for multiple-loci systems in which all of the loci in the system are simultaneously repressed or derepressed. But systems in which all loci, but one, anyone, are simultaneously repressed are not readily explained.

A comparable situation exists with regard to the multiple mating types determined by one locus in certain tetrahymenas. Regardless of which allele (or alleles) is at this locus, it can be regulated so that the cell exhibits any one - but only one - of several mating types. The basic mechanism may be the same as in the multiple serotype system - whatever that mechanism is - but with the different cistrons of the set gathered into one complex locus instead of being unlinked. However, the mechanism of operation need not be the same and in any case the type of coordination manifested by both systems, which are much like aspects of developmental differentiation in multicellular organisms, appears to call for one or more additional elements lacking from current theory.

A second additional feature shown by both the mating type and the serotype systems is the restriction of changes in genic repression or derepression to a brief period early in the clonal history and the hereditary maintenance of the genic state

thereafter through the subsequent hundreds of fissions of clonal life. Strains of P. aurelia carrying the genic potential for two mating types and those of T. pyriformis carrying at one locus the potential for several mating types are fixed shortly after fertilization so that a given line of descent will manifest only one mating type. Experiments show clearly that fixation is due to differentiation of the macronuclei when they develop from products of the syncaryon after fertilization. Thereafter, these macronuclei and their descendants cannot again be altered. All but one of their mating type potentialities have been permanently repressed. Likewise, Nanney has found in Tetrahymena that of two alleles for one pair of antigens, one or the other is permanently repressed in heterozygous macronuclei at the same stage. He has suggested that this phenomenon may in general be due to the greater permeability of the young nuclear membrane to cytoplasmic differentiators of the nuclei, presumably genic derepressors. If so, this feature of nucleo-cytoplasmic relations would not be found in organisms like microbes that lack such a membrane. It has not. Whatever the explanation of this restriction of the responsiveness of nuclei to their intracellular milieu, there is nothing in the current theory of genic repression and derepression that obviously accounts for it.

The examples just cited are extreme. The mating type and serotype relations in different strains and clones show many gradations from extreme rigidity and stage restriction to extreme lability with no stage restriction. The latter is shown, for example, by mating types in certain strains of P. multimicronucleatum in which a single cell, in the absence of fission, changes from one mating type to the other twice a day on a diurnal rhythm. Barnett found a single gene that abolishes the rhythm and sets up the rigid stage-limited nuclear differentiation described above. These facts indicate that the basic difference between the two extremes may be relatively slight and that genic repressions and derepressions are their common denominator, with some unknown additional mechanisms for restricting the decisive action to a single brief stage in clonal history and for perpetuating it.

The next series of examples illustrate nucleo-cytoplasmic relations in the control of gross nuclear behavior. The multichambered agamont of the Foraminiferan Rotaliella possesses four nuclei derived mitotically from one. They are thus genically identical. But they are differentiated: three are small and stay in the oldest, smallest chamber; one is large and lies far away in a young large chamber. The large one disintegrates when the small ones undergo meiosis. Czihak and Grell destroyed the large one by irradiation with a fine beam of ultraviolet. Thereupon one of the remote small nuclei enlarged, moved into a young chamber, lost the capacity to undergo meiosis, and later disintegrated when the remaining two nuclei underwent meiosis. The large nucleus apparently releases into the cytoplasm an agent which inhibits differentiation, enlargement and migration of the small nuclei; but when the large nucleus is destroyed and the source of cytoplasmic inhibitor is cut off, one nucleus ceases to be inhibited and begins

at once to form the inhibitor. This differential action and differential responsiveness among genically identical nuclei, and their coordinated "communication" via the cytoplasm, are difficult to visualize as simple genic derepression or mere provision of precursors, tools, or machinery for DNA or RNA production by the genes. Some additional type or types of nucleo-cytoplasmic interaction, perhaps again involving in part changing membrane properties, seem to be required to account fully for this control of nuclear behavior.

Comparable differentiation of genically identical nuclei into macronuclei and micronuclei and comparable inhibitory action of one macronucleus on another via the cytoplasm (e.g. inhibition of regeneration of macronuclear fragments in the presence of a whole macronucleus) have long been known in the Ciliates Tetrahymena (Nanney) and Paramecium (Sonneborn). They show two features not shown by Rotaliella. First, a single recessive gene in P. aurelia brings about unequal macronuclear division at fission; sometimes the whole macronucleus passes to one of the two daughter cells. The gene clearly acts through the cytoplasm because the action persists for a few fissions after a fertilization at which the syncaryon and new macronucleus acquire the wild type allele: wild type and mutant macronuclei both misdivide in cytoplasm bearing products of a prior mutant nucleus.

Second, different cytoplasmic areas differ in their actions on nuclei and these localizations change with time in correlation with sequential steps in nuclear behavior. For example, at conjugation, surface contact between mates at a definite region initiates meiosis and macronuclear breakdown; a small localized area near the mouth prevents the meiotic product which gets into it from disintegrating, while all those outside of it do; the products of division of the syncaryon which briefly lie in one cytoplasmic region differentiate into micronuclei, those that lie briefly in another cytoplasmic region differentiate into macronuclei. All of these nuclear events are clearly dependent upon both nucleus and cytoplasm because they are modifiable by centrifugation and other treatments at critical stages and by mutation. How is the regular sequence of localized cytoplasmic differentiations to be explained by mere self-assembly?

The solution of this problem comes from our current study of the role of preexisting cytoplasmic structure in the assembling of genic products. The rigid cortex of Paramecium is highly structured with marked differences between the anterior and posterior halves; yet transverse fission yields two identically structured cells. This is accomplished by forming before fission a second set of structures or their primordia in positions that permit equal segregation by fission. The formation and localization of the various parts have long been held, on the basis of results of cutting and grafting operations (e.g. in Stentor, Tartar), to be determined by preexisting cytoplasmic differentiations. We have confirmed this by genetic analyses of several hereditary variations in the number and arrangement of cortical structures in P. aurelia. These variations were inherited in the absence of differences in genes, genic repressions and

derepressions, nuclear size and endoplasm. Only cortical differences were involved. They perpetuated themselves. Even a piece of cortex, stolen from a mate and incorporated in an abnormal position, gave rise to parts that were inherited. Recent studies on the cortex of the egg of the amphibian Xenopus (Curtis) and on the mitochondria of Neurospora (Luck) agree in pointing to the essential role of preexisting cytoplasmic structures in determining where the products of gene action go and what they form. Of course, the nature of the gene products also affects the result; for example, Preer found a gene for a difference in the form of the trichocyst in P. aurelia. Different building blocks, different structures. But also different preexisting organization, different hereditary organization, - even with the same genes. For this sorting out and ordering role of cytoplasmic structure I propose the term "cytotaxis".

Without cytotaxis an isolated nucleus could not make a cell even if it had all the precursors, tools and machinery for making DNA and RNA and the cytoplasmic machinery for making polypeptides. Self-assembly of genic products can go only so far; to go the whole way, cytotaxis must be added on. Strong direct evidence now confirms the old dictum that only a cell can make a cell.

The various examples from Protozoan work reviewed here indicate that current views of nucleo-cytoplasmic relations, based upon studies of DNA-RNA-Protein mainly in microbes, need to be modified, supplemented and extended in several important ways. Doubtless as explorations in Protozoa and higher organisms are carried further, molecular theory will grow and encompass them. The comprehensive molecularization of nucleo-cytoplasmic relations is an enticing and exciting prospect. A brilliant beginning has been made. But it is only the beginning of a long road ahead.

THE NUCLEAR CONTROL OF SYNTHETIC ACTIVITIES IN ACETABULARIA.

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The unicellular marine alga Acetabularia offers a unique system for the study of nucleo-cytoplasmic interactions. The nucleus of this giant cell is located in the basal end (rhizoid) and can easily be removed by amputation. The resulting anucleate cell survives for many months. Moreover, complex cellular systems can be constructed and rearranged microsurgically by grafting together nuclei and cytoplasms in various combinations. Components from different species as well as from previous grafts are equally suitable as grafting units. The transmission of genetic information from any one nucleus to the cytoplasm can be interrupted and reestablished at will.

Enucleation experiments with Acetabularia have revealed the surprising fact that anucleate cells as well as certain cell segments are endowed with great morphogenetic and synthetic capacities which are expressed over periods of several weeks after the enucleation. In this organism the nuclear control of cytoplasmic activities thus appears to be a very remote one, perhaps due to the existence of autonomous cytoplasmic systems which control a number of crucial metabolic reactions, and/or due to the build-up of a large pool of nuclear products in the cytoplasm.

Growth and differentiation of anucleate cells of Acetabularia are accompanied by net synthesis of proteins (1,2). The rate of protein synthesis in anucleate cells, which initially parallels that of nucleate controls, gradually declines during the subsequent two weeks and approaches zero soon after the third week. During this period the protein content of anucleate cells can increase up to 300%. Other metabolic processes, however, such as photosynthesis or respiration, are not affected by enucleation for several months (3,4). Low molecular weight organic compounds, such as sugars or amino acids, begin to accumulate soon after enucleation (5,6). These and other results suggest that the relatively early cessation of protein synthesis in anucleate cells is not caused by a lack of precursor molecules or chemically utilizable energy. This view is supported by the finding that various proteins are affected at different moments after the removal of the cell nucleus. Various enzymes have been studied with respect to their response to enucleation. The synthesis of phosphorylase, for instance, continues for almost three weeks (7), whereas acid phosphatase synthesis declines after one week (8).

The general principle emerged from studies with various enzymes that the synthesis of individual proteins continues after enucleation at almost normal rates for a certain period of time, the length of which is characteristic for each protein. At the end of this period a rather abrupt drop in the rate is found. These observations can best be interpreted by postulating the existence of protein-specific, and therefore probably gene controlled, cytoplasmic pools of nuclear products which become depleted at various times after enucleation. In the intact cell these pools are continously replenished by the cell nucleus. Differences in the pool size at the time of enucleation, or in the rate of breakdown during protein synthesis, could account for the specific response of individual proteins to enucleation. The relative stability of the hypothetical pool substances *per se* can be concluded from the observation that the normally limited synthetic capacity of an anucleate cytoplasm is retained over long periods of time under experimental conditions which do not permit protein synthesis, *e.g.*, during dark-starvation of enucleated cells. On the other hand, an increase in the size of the cytoplasmic pools, as concluded from enhanced synthetic capacities of such cells (9), can be brought about experimentally by suppressing protein synthesis for an extended period of time prior to enucleation. The size of a cytoplasmic pool can be thought to reflect an equilibrium between the rate of utilization and replenishment. Thus, during dark-starvation nuclear synthesis, or at least discharge into the cytoplasm, of pool substances seems to continue. In the absence of protein synthesis the breakdown of the pool is retarded and therefore the produced pool substances can accumulate in the cytoplasm.

It is tempting to speculate that the postulated pools of nuclear products are composed of an RNA which has transferred genetic information from nuclear genes to synthetic sites in the cytoplasm. The finding that under normal growth conditions there is only little, if any, increase in the amounts of cytoplasmic RNA after enucleation (10), would be consistent with this idea. One cannot generalize from these data, however, that the nucleus is the sole source of cellular RNA, especially since under certain conditions, *i.e.*, after dark-starvation of cells prior to enucleation, significant amounts of RNA can indeed be synthesized by the anucleate cytoplasm (11). It is therefore justified to assume that also in the normal, nucleate cell at least part of the cytoplasmic RNA is not directly derived from the nucleus. However, a close nuclear dependence of the synthesis of this RNA fraction is evident from control experiments, in which RNA synthesis could not be provoked upon reillumination of anucleate cells that had been enucleated before dark-starvation (11).

As with proteins, the synthetic capacities are built up during the period of starvation only when a cell nucleus is present. These results tend to rule out the possibility that in this alga a significant portion of cytoplasmic RNA is synthesized by autonomous cytoplasmic systems. The finding that a high percentage of the cytoplasmic RNA is localized in a "chloroplast" fraction (12), does not necessarily contradict this assumption. It cannot be decided at the present time whether RNA exclusively from the cell nucleus, or RNA which has been synthesized in the cytoplasm, or a combination of these, perform indeed the function of a cytoplasmic pool of nuclear information.

In addition to stored information from the cell nucleus, the cytoplasm could also carry autonomous systems which might supplement or modify nuclear-controlled cellular activities. A study of species-specific proteins in Acetabularia could provide a tool for the separation of nuclear and cytoplasmic systems which control protein structures in interspecific graft combinations. So far only a single enzyme, acid phosphatase, was found to occur in species-specific molecular forms as defined by starch gel electrophoresis. Three forms of this enzyme, which occur in Acetabularia mediterranea (med), Acetabularia crenulata (cren) and Acicularia Schenckii (acic), have been investigated.(13, 14).

Approximately two to three days after the transplantation of a med nucleus to an anucleate acic cell, a change in the electrophoretic mobility of the acic host phosphatase is observed. A new form, which is electrophoretically intermediate between acic and med phosphatase, appears for a short period of time. Then a final change to med phosphatase takes place. The complete disappearance of the host phosphatase is not caused by the removal of the host nucleus, since in acic control cells the acic phosphatase is still detectable many weeks after the enucleation. The existence in the transplant of a stage in which all phosphatase activity is represented by the intermediate form. suggests that a conversion on the molecular level has taken place. In the reciprocal graft, an acic nucleus transplanted to an anucleate med cell, a conversion of phosphatase types does not occur and the med enzyme persists. In binucleate hybrid cells, which are prepared by grafting together one med and one acic rhizoid and allowing the graft to regenerate a new stalk, only the med phosphatase is detectable. It can be concluded from these experiments that the process of conversion proceeds preferentially in one direction and that the responsible factors are present in the med cytoplasm or are synthesized in a host cytoplasm under the control of a transplanted med nucleus. Once present in the cytoplasm, the converting factors remain active even after great dilution of such a cytoplasm with factor-negative cytoplasm. Thus a nucleus-containing

acic rhizoid, which has been removed from an acic-med binucleate cell, can regenerate to a full-grown acic cell that still contains med enzyme as the sole component. After a second cycle of regeneration, however, the original acic type phosphatase reappears. Although the factors behave in these interspecific grafts as if they were autonomous cytoplasmic units, they are ultimately controlled by nuclear genes.

Changes in the electrophoretic mobility of acic phosphatase can also be brought about under in vitro conditions by combining homogenates prepared from cells of both species. Complex metabolic systems are not required, since the conversion reaction(s) occur also in mixtures of supernatant fractions which are devoid of chloroplasts and mitochondria. Partially successful attempts to separate the converting factors from med phosphatase make it unlikely that the exchange of protein subunits is responsible for the observed effects. Experimental data suggest that both enzyme forms have in common a basic structure which is modified through enzymatic reactions of still unknown nature.

The third phosphatase type, found in Acetabularia crenulata (cren), does not interact with either one of the other two phosphatase types. In nuclear transplantation experiments between cren on one hand, and acic or med cells on the other hand, the phosphatase type of the nuclear donor species always appears as a new component in addition to the persisting type in the cytoplasm of the host. Likewise, in binucleate hybrid cells, cren-med or cren-acic, both enzymes are synthesized simultaneously.

In summary, the experiments with species-specific molecular forms of phosphatase have failed to provide evidence for autonomous cytoplasmic systems which control or modify the expression of the species-specific protein structures. Future experiments with a variety of proteins are needed before these findings can be generalized.

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THE ROLE OF THE NUCLEUS IN EMBRYONIC DIFFERENTIATION, AS REVEALED BY NUCLEAR TRANSPLANTATION STUDIES IN AMPHIBIA.

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The techniques of nuclear transplantation in Amphibia have been used principally to investigate the question of whether or not nuclei of somatic cells undergo stabilized changes in the course of embryonic differentiation. In *Rana* (Briggs and King, 1952), *Xenopus* (Elsdale *et al.*, 1960), *Siredon mexicanum* (Signoret, Briggs, and Humphrey, 1962), and *Pleurodeles waltlii* (Signoret and Picheral, 1962), successful transplantation of blastula nuclei promotes unrestricted normal development of the recipient eggs. However, transplantation tests of nuclei from late gastrulae and older embryos have shown that many of these nuclei undergo changes which restrict their capacity to promote the normal development of test eggs.

In *Rana pipiens*, an extensive series of tests carried out with endoderm nuclei of late gastrula, mid-neurula, and post-neurula stages (all from the anterior mid-gut region) indicated that a progressive nuclear change occurs during embryonic differentiation (Briggs and King, 1957). These changes were best expressed in nuclear-transplant embryos that gastrulated normally, but then showed deficiencies, especially in their ectodermal derivatives, at later stages. Serial transplantation of late gastrula endoderm nuclei demonstrated that the nuclear condition responsible for these developmental deficiencies is highly stabilized and heritable (King and Briggs, 1956), Briggs, King, and Di Berardino, 1960.

Endoderm nuclei taken from progressively older embryos of *Xenopus* also show a decrease in their ability to promote normal development when transferred into unfertilized eggs (Fischberg *et al.*, 1958; Gurdon, 1960). There is, however, a difference between the results in *Rana* and *Xenopus*, in that the nuclear change in *Xenopus* is not evident until the neural stage. Fischberg and his collaborators have interpreted this difference between the two species on the assumption that nuclear differentiation proceeds less rapidly in *Xenopus* than in *Rana*. The fact that a limited number of macroscopically normal transplant-embryos, obtained from donor nuclei of differentiated intestinal epithelial cells of *Xenopus* tadpoles, has led Gurdon (1963) to question whether stable nuclear changes really do occur.

The reasons for these differences in evaluating the results of nuclear transplantation studies will be examined and the questions that remain to be answered will be discussed.

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STUDIES OF NUCLEO-CYTOPLASMIC INTERRELATIONS IN GIANT CHROMOSOMES OF DIPTERA. Ulrich Clever and Wolfgang Beermann, Max-Planck-Institut für Biologie, Tübingen, Germany.

Any discussion of nucleo-cytoplasmic interrelations must center around two main questions: 1. How do the chromosomal genes exert their control of metabolic processes in the cytoplasm? 2. Does the cytoplasm, in its turn, influence the activity of the genes and, if so, what is the mechanism of this action? The giant chromosomes of the Diptera lend themselves particularly well for a study of these problems in higher organisms. As is now generally accepted, the local modifications in the structure of these chromosomes called "puffs" represent synthetically active genes (see BEERMANN, 1962, for review). The distribution of active sites within the genome as well as the relationship of their activity to other physiological processes within the cell can therefore be studied with relative ease.

The structure, the chemical characteristics, and the synthetic capacities of puffs are well known, at least in a general way. Histochemical procedures show that two groups of substances are present in the puffs which are absent, at least in large amounts, in normal bands: RNA and a higher protein (BEERMANN, 1952, PAVAN and BREUER, 1955, PELLING, 1959, SWIFT, 1962). The content of DNA and histone, on the other hand, seems to remain unchanged during puff formation. The concentration of these substances in puffs is, of necessity, lower because of the blown-up structure of the puffs (RUDKIN, 1955, SWIFT, 1962).

PELLING (1959) injected tritiated uridine as a precursor of RNA into larvae of Chironomus tentans. Short pulses resulted in an almost exclusive labelling of the puffs and the nucleoli in the autoradiographs. The results indicated that nucleoli and puffs are the main sites of RNA synthesis within the cell and that RNA migrates from here into the cytoplasm. Comparable results were obtained in studies of RUDKIN and WOODS (1959) in Drosophila and of SIRLIN (196o) in Smittia.

EDSTRÖM and BEERMANN (1962), using a microelectrophoretic procedure, determined the base composition of RNA from individually isolated giant chromosomes, puffed chromosome segments and some other cell components. Their main result concerning our problem of the meaning and the composition of puff-RNA may be summarized as follows: (1) The chromosomal RNA differs significantly from that of any other source (nucleoli, cytoplasm) (2) The RNA extracted from chromosome I differs from the RNA's extracted from different puffed segments of chromosome IV, and the latter differ significantly from each other. (3) The chromosomal RNA does not show a base symmetry in any of the investigated cases, nor is the content of guanine + cytidine exactly the same as that for DNA. The differences of the RNA's of different puffs which these investigations indicate, are in agreement with the assumption that it is messenger RNA which is synthesized in the puffs. The results summarized under (3) exclude the possibility that the RNA is a complete copy of both strands of the chromosomal DNA. The RNA may, however, still partly or completely be a single strand copy of this DNA.

The protein in the puffs is not stainable with fast green after the method of ALFERT and GESCHWIND, (1953), it is stainable, however, with fast green at pH 2 (SWIFT, 1962) or light green at pH 5 (CLEVER, 1961). It cannot, therefore, be histone. The precise nature of the puff proteins is not known and we cannot decide whether or not the proteins of different puffs are identical. In order to study the site of synthesis of these proteins PELLING (1959 and unpubl.) injected radioactive amino acids into Chironomus larvae. In pulse experiments the chromosomes were not labelled at all in autoradiographs. The cytoplasm, however, was heavily labelled. Label appeared in the chromosomes only after incubation times of 5 or 6 hours. In new experiments we injected tritiated leucine simultaneously with ecdysone. Ecdysone induces some new puffs within less than two hours. 2 hours after injection the newly induced puffs were still found to be absolutely unlabelled although rather large amounts of the protein had accumulated (CLEVER, unpubl.). In pulse experiments in vitro BIRNSTIEL et al. (1961) found label only in the nucleolar protein fraction of the nuclei. So far we have, therefore, no indication that protein in larger amounts is synthesized in the chromosomes, with the possible exception of the nucleoli.

According to the results reported here puffs may be considered as the chromosomal sites of messenger RNA synthesis. The actual genetic message carried by these messengers, in other words the genetic meaning of the chromosomal sites involved, is as yet almost unknown. In one case an approach to this problem has been possible (BEERMANN, 1961). In Chironomus pallidivttatus some of the gland cells produce a secretion which contains small mucoprotein granules. In the gland of the closely related species C. tentans the granules are absent in the secretion of the homologous cells. The species difference is inherited in a simple Mendelian fashion and is due to a mutation which has been found to belocated in a small region of chromosome IV. The same region shows a small Balbiani ring in the granule producing cells of C. pallidivittatus. It is, therefore, very probable that this locus synthesizes RNA which is directly or indirectly involved in the production of the secretory granules. The precise relationship between this RNA and the protein of the granules is now under investigation.

In microorganisms the rate of messenger synthesis of each individual locus seems to be under the permanent control of external factors. These factors, end products or substrates of the enzymes to be synthesized, seem to act via a system of regulatory genes according to a model proposed by JACOB and MONOD (1961). It is likely, that the controlling system in higher organisms is more complicated than in bacteria and that additional mechanisms might have evolved. This problem may be attacked by studies on the control of puffing in giant chromosomes. In almost all of the loci which from puffs in the salivary gland cells of Chironomus tentans, phases of puffing alternate with phases of non-puffing. The puffing phases must be controlled by factors from the cytoplasm. This is indicated by results of several authors (BECKER, 1959, CLEVER und KARLSON, 1960, KROEGER, 1960, PANITZ, 1960, RITOSSA, 1962, and others). Most of the activities of the related genes must be concerned

with the basal or functional metabolism of the gland cells. As yet, we do not know anything about the regulation of the activity phases of these loci. Some other loci are, however, only active during molting phases or during metamorphosis. In these cases we do know something on factors involved in the regulation of at least two of the loci.

Molting in insects is induced by the prothoracic gland hormone, ecdysone. Target organs of its action are the individual cells of the different tissues. After injection of ecdysone into young last instar larvae puffs appear within less than one hour in sections I-18-C and IV-2-B. In both regions the size of the puff depends on the hormone dosage injected: large puffs are induced if more hormone is injected. Likewise, the duration of puffing depends on the hormone dosage: at both sites the puffs stays longer if more ecdysone is injected. These results have been interpreted to mean that puffing at both loci is controlled by ecdysone in essentially the same way (CLEVER und KARLSON, 1960, CLEVER, 1961).

In normal development the size of puffs I-18-C and IV-2-B changes in relation to larval development. Both puffs appear during the larval molt from the third to the fourth larval instar and disappear after molting is completed. At the end of the last (4th) larval instar they reappear as soon as the pupal molt has begun (Fig. 1). The results demonstrate that in normal development, as in the experiments puffing of loci I-18-C and IV-2-B depends on the presence of ecdysone. In contrast to the similarity of their behaviour after experimental induction of molting there are, however, some remarkable differences in normal development: 1. Puff IV-2-B forms about 1-2 days later than puff I-18-C in the normal pupal molt (and in the normal larval molt), whereas after injection of ecdysone the time lapse is about 30 minutes (periods I and III in Fig. 1). 2. At the end of the larval molt puff IV-2-B regresses much earlier than puff I-18-C (period II). 3. During the pupal molt puff I-18-C does not disappear at all and reaches its maximal size in older prepupae. Puff IV-2-B, however, attains maximal size in the middle of the prepupal period, then becomes smaller and finally disappears (period IV; CLEVER, 1962, 1963b).

In a series of experiments we injected various amounts of ecdysone and measured the size of the induced puffs (CLEVER, 1963a). We found that the reactivities of the two loci against various hormone concentrations differ from one another. The titer of ecdysone rises gradually during metamorphosis and the different behaviour of puffs I-18-C and IV-2-B in periods I and III is a consequence of the different reactivities.

Next we tried to answer the question whether the behaviour of puff I-18-C and / or that of puff IV-2-B at the end of the molts is also due to the actual hormone concentration (periods II and IV). In a first series of experiments we injected ecdysone into larvae which were expected not to have puff IV-2-B, but only puff I-18-C (E_1-E_3 in Fig. 1). Puff IV-2-B could be induced by injections E_1 and E_2, but not by injection E_3. This led to the working hypothesis that high titers of ecdysone are present in larvae during period IV, but not

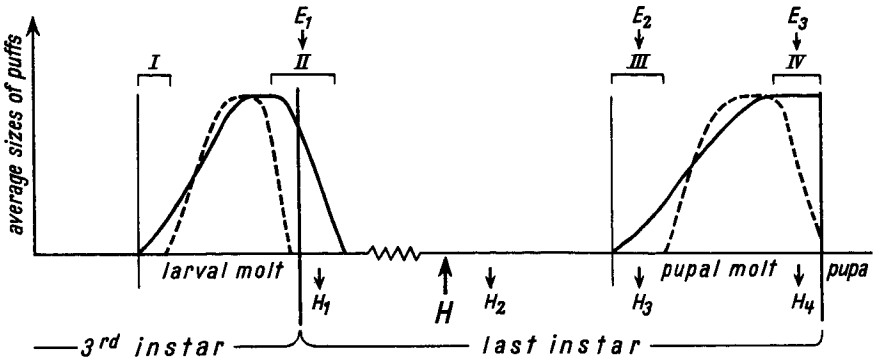

Fig. 1: Puffing of loci I-18-C (———) and IV-2-B (- - - -) during the course of the third and fourth larval instar. I - IV indicate periods of different behaviour of the two puffs. E_1 - E_3 injections of ecdysone. H injection of haemolymph from donors H_1 - H_4.

during period II. Our dosage experiments indicated that very small amounts of ecdysone are sufficient to induce puffs I-18-C and IV-2-B and that the actual hormone concentration in developing larvae is some orders of magnitude higher than necessary for the induction. We tested the ecdysone titer of the haemolymph using larvae in different stages of development as donors (H_1 - H_4 in Fig. 1). The following results are of interest in this connection: (1) Haemolymph from old prepupae (H_4) induced large puffs at both sites, even if puff IV-2-B was not present in the donors. (2) Haemolymph from very young last instar larvae (H_1) never induced puff IV-2-B and only very seldom induced puff I-18-C. The results demonstrate that in period II the titer of ecdysone is very low. The absence of puff IV-2-B in contrast to puff I-18-C in this period is, therefore, interpreted as being due to the different reaction thresholds of the two loci. The fact that puff IV-2-B begins to regress earlier than puff I-18-C during the larval molt may have a different reason. It seems probable that the regression of puff IV-2-B is initiated by the same factor which is effective in the pupal molt (period IV). In this period puff IV-2-B loses its activity although high amounts of ecdysone are still present. It follows that in older prepupae some other process is at work which, with regard to this gene locus, is antagonistic to that of ecdysone. The alterations of puff I-18-C are, by contrast to puff IV-2-B, due only to corresponding changes in the hormone concentration (CLEVER, unpubl.).

In order to obtain further information concerning the mechanism of hormonal control of the two loci, I-18-C and IV-2-B, we tested the effect of some antibiotics known to interfere with cell metabolism at different levels (CLEVER, unpubl.). We injected, simultaneously or in various temporal

combinations with ecdysone, puromycin, neomycin, actinomycin C, and mitomycin. Only actinomycin and mitomycin were found to inhibit puff induction. These results indicate that only those antibiotics which interfere with RNA metabolism inhibit the induction of puffing, while those which interfere with the synthesis of proteins are not effective. It seems, therefore, that the induction of gene activities does not involve the synthesis of new proteins.

Our results are consistent with the hypothesis that the hormone acts directly on the genic level. The action of the hormone might be similar to that of an effector in the regulator-operator systems in bacteria proposed by JACOB and MONOD (1961). Some of our earlier results indicate that loci I-18-C and IV-2-B are independent of each other in the reaction to ecdysone. It would follow, then, that activity of locus IV-2-B is simultaneously controlled by two antagonistic substances, comparable to the "inducer" and the "corepressor" in bacteria. This type of regulation of gene activity by antagonistic effectors has been discussed in the case of catabolite repression in bacteria (MAGASANIK, 1961, McFALL and MANDELSTAMM, 1963). In higher organisms control mechanisms of this kind would a priori seem to be more adequate to the requirements of coordination of intracellular events than those acting only in one direction.

The induction of puffs I-18-C and IV-2-B by ecdysone is followed by a sequence of other changes of genic and cytoplasmic metabolism. Certainly the influence of ecdysone on these processes is not a direct one. Probably it is mediated by the action of loci I-18-C and IV-2-B. A sequence of mutual interactions of cytoplasm and genes seems to be set in motion by the ecdysone. Studies on this system promise to give further information concerning nucleo-cytoplasmic interrelations as well as the mechanism of genic control of animal development.

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THE GENETICS OF SOMATIC CELLS. Jack Schultz, Chairman, Division of Biology, The Institute for Cancer Research, Philadelphia, Pa.

The problems of somatic cell genetics first appeared in Weismann's hypotheses, which are at the basis of evolution theory of the present day, and whose concepts explained the nature of development as well. Arguing from the fact of replication (omnis cellula e cellula), Weismann made one fundamental assumption: that the characteristics of any cell type are due to its genetic determinants. From this assumption, the separate qualities of germ plasm and soma follow; and by a logical continuation the separate qualities of the different cell types of the individual. Making a concrete analysis, Weismann constructed models for the genetic system; the determinants, the ids assembled into chromosomes; the mode of reduction in the germ cells; the segregation of the determinants in the critical divisions of development. These models turned out to be incorrect, in the event: the nature of the meiotic process does not correspond to Weismann's diagrams; but the fact of meiosis and the reduction divisions is of course established. Similarly, it is now agreed that there is a genetics of somatic cells; but just what the rules are is still something to be discovered. We shall consider first the available methods at our disposal for such analysis; some of the model systems that have been proposed on a biochemical and genetic basis; and attempts to arrive at a view of the present status of the problem.

The aim of a genetic analysis is the identification of the units of heredity in a system. This is accomplished when a difference between two types is maintained in the progeny from a mating between them, in other words persists through a cycle of replications in which the character must be determined anew. The degree to which multiple differences in such a mating reassort, giving recombinants, allows the resolution of the differences into minimal units. For the germ cells, these methods are the classical techniques of Mendelian genetics, and permitted the building of chromosome maps. Are the somatic cells susceptible to such analytical methods? What are the limitations of the methods available for transfer of genetic material from one somatic cell to another, and observation of the behavior of differences between the cell types in the progeny from such a mating?

A basis for answers to these questions has been laid by the understanding of the chemical processes underlying the genetic control of protein synthesis. A genetic mutation, which would presumably be responsible for the stable change in the properties of a somatic cell, would be the consequence of a change in the sequence of nucleotides in the DNA molecules of the chromosomes. This primary code determines the sequence of nucleotides on the RNA "messenger", which in turn provides a template for the assembly of the amino acid residues into a polypeptide chain,

this assembly also mediated by information on a transfer RNA. Thus arise the specific proteins which characterize the cell types, and which as enzymes control the synthesis of substances more remote from the primary code.

In such terms, the recombination analysis of the genetic experiment tells us about differences in the primary code. But it only does so when one can exclude the possible modification of the more proximate steps in the sequence of reactions by which the protein is formed, that is, changes involving production or utilization of the secondary RNA template. Such changes might very well have the same effect as the change in the code. These are recurrent questions in genetic analysis, going back to the original debate whether "loss" mutations were physical or physiological. As a result of the *in vitro* analysis particularly, it is possible to be precise about the possibilities. In terms of the somatic cell, the requirement is an understanding of the nature of the differences between the cell types, in terms of their use as genetic markers.

The concepts being developed in microbial genetics are invaluable for the models they offer in guiding such considerations. The variety of methods for genetic transfer discovered in the bacterial and virus work particularly--incorporation of sections of DNA into the genonema, whether introduced as the purified preparation, or associated with a virus carrier, or by mating between cells--this variety has a liberating effect in the search for mechanisms whereby somatic cells may change their genotypes. Similarly, the mechanisms discovered for the control of enzyme synthesis--the interaction of adjacent loci to form a working unit, the "operon", whose components respectively determine the amino acid sequences ("structure"), the inducibility of the enzymes, the repression of its synthesis by way of an effect on the "operator" locus--these, while similar in principle to the much analyzed position effects in Drosophila and maize, have added an element of biochemical precision to the analysis that makes it usable for more definite models, of changes which can occur during the embryonic differentiation of cell types. The problem, however, is not so much an inability to construct useful models in these contexts: it is rather that of an embarrassment of riches, the difficulties arising at the methodological level, of obtaining proof that the elements of the model really are applicable. In point of fact, however, it should be recalled that this correlation between the *in vitro* biochemistry and the *in vivo* genetic analysis has to a considerable degree been accomplished on mammalian systems concomitantly with the microbial studies--an encouragement for the general applicability of these principles.

Two methods are presently available for conventional genetic analysis, by transfer of material from one cell to another. Nuclear transplantation tests the capabilities of a nucleus of one type in cytoplasm of another; and cell hybridization, fusion of cells, allows tracing the consequences of mixture of both nuclear and cytoplasmic determinants. Thus both methods test for the existence of stable differences between the nuclei of cell types, differences that will persist through replication in a new cytoplasmic environment, as well as in the case of the cell hybrids, in a nucleus heterozygous for the chromosomes from the two parental types.

The nuclear transplantation experiments so far have been most successful in the Amphibia. They test the responses of nuclei of various cell types to the opportunities for developmental control offered by the cytoplasm of the egg, and can be compared with the normal development when the nuclei from sperm and egg are in control. A deviation from normal development would be evidence of some irreversible change that has occurred in the differentiation of the transplanted nucleus in the cell type of its origin.

Two questions occur immediately on consideration of such a result: first the relationship of the abnormality in development to the cell type of origin of the nucleus; and second, assuming a relationship to be specific for each cell type, the nature of the nuclear change responsible for the difference, in terms of the biochemical sequence of information transfer. The evidence at present, from the experiments on *Rana* and *Xenopus*, is sufficient to outline the problems. As development proceeds, the nuclei from the more highly differentiated cells tend to give a higher frequency of abnormal development, embryos from the blastula being mostly normal. Among the abnormal embryos, particularly those arrested at early stages, there is a high frequency of chromosome aberrations (DiBerardino). These would therefore be interpreted as a consequence of some type of nuclear differentiation, not specifically part of the differentiation of the specific cell type, but a concomitant feedback process involving perhaps the replication of the chromosomes. It should be emphasized that such changes are of great potential importance, since they offer a source of somatic mutation. They could, for example, be influential in the initiation of the neoplastic process, assuming chromosomes from differentiated nuclei to be especially susceptible to derangement.

But more important for the present discussion are the possibilities of specific restrictions of development according to the cell type. The endoderm nuclei in *Rana* have been shown by King and Briggs to be incapable of supporting normal development, even with a standard karyotype. The results are those to be

predicted on a neo-Weismannian hypothesis: fully normal differentiation in the endoderm, more or less suppression among derivatives of the other germ layers. It should be emphasized that there is at present no clear basis for prediction of what the residual information should be, in the differentiated nuclei, were there to be a progressive restriction. Loci important in the function of the differentiated endoderm cell, may also be influential in the differentiation of the neural crest, given its cytoplasmic environment. The responses of the "endoderm genome" to the egg cytoplasm are evaluated by the transplantation experiment; they tell us that there are no specific genomes for the one germ layer or the other (an improbable hypothesis); that the process in its working out if it is concerned with primary coding is, not unexpectedly, a complicated one.

The degree of complication is indicated by the results with *Xenopus*, obtained by Fischberg and particularly by Gurdon. Here, from nuclei out of visibly differentiated intestinal epithelium, in a very low frequency, normal development may occur. Thus the changes, if nuclear, may be reversible, hence occurring at the proximate stages of information transfer. Such possibilities are not at all remote; witness the discussions of the controlling effects of histones and of RNA. What is called for is a recombination analysis.

Such an analysis would be feasible following the fusion of the differentiated nucleus with a germinal nucleus. This has been accomplished by Subtelny and Bradt. With the continuation of such experiments, it should become possible both to explore the results of aneuploidy following chromosome rearrangement; but more important, to evaluate the possible specific abnormalities associated with nuclei from the different cell types. Will they be dominant over the genotype of the germinal nucleus, or recessive to it? These questions are new ones, and could have different answers for the different cell types.

The direct examination of the chromosomes of somatic cells goes back to Boveri and chromosome diminution in Ascaris. The more recent successes have been encountered in the giant chromosomes of Diptera where two kinds of phenomena have already been uncovered. First there are the reversible changes in the structure, hormone initiated (see Clever, this symposium). In terms of information transfer these apply to the functioning of the cell in the proximate portions of the information transfer sequence: those concerned with the synthesis of new messenger RNA and its transport to the cytoplasm. A second class of phenomena uncovered is of considerable significance in the present context: namely the disproportionate synthesis of DNA at specific loci in the Sciarids (Breuer and Pavan, Rudkin and

Corlette, Swift). This offers the possibility of change in the primary code.

Whether in fact such a change occurs is not yet clear. But in the case of the heterochromatic regions of Drosophila the evidence is definite (Rudkin and Schultz) that in the giant chromosomes, the proportion of the DNA due to the heterochromatic regions is much lower than that in the metaphase chromosomes. If added to the length of DNA in this region, the new DNA might even occasion a change in the reading of the code in special cells.

The heterochromatic state of chromosomes is widespread; strictly speaking, the bands of the giant chromosome are in such a state, from which they are released for activity when the puffs appear for RNA synthesis. The specialized heterochromatic segments maintain this densely coiled state when the euchromatic regions are active, and seem to replicate at a different stage of the mitotic cycle. Such differential timing with respect to replication or function, of the activity of different loci in different tissues in effect gives another mechanism for Weismann's postulate that each cell behaves according to its nuclear constitution.

Somatic changes (variegation) have been shown to depend on proximity to heterochromatic regions. These provided early evidence of the activity of loci on their neighbors (position effects). But specifically in relation to the heterochromatic regions where we now have evidence of differential replication (Lima-de-Faria, Taylor) and disproportionate DNA synthesis, these effects have a reinforced interest. This interdependency of the expression of genes is interpretable in terms of antithetical relations between DNA replication and RNA synthesis. It seems to be present in the sex chromosomes, evident in the behavior of sex chromatin in mammals, may be related to the types of effect revealed by the activators and dissociators of McClintock and possibly related to the paramutation effects discussed by Brink. The effects of variegational rearrangements in Drosophila have been shown actually to be concerned with the DNA synthesis by the use of antimetabolites specifically effecting this process. The interaction of the control of replication and the control of the release of new messenger may very well both be involved in the processes of differentiation.

Thus the analysis of the genetics of somatic cells bids fair to depend on our comprehension of the structure of chromosomes and the mechanism of recombination. The essential problems remain the use of genetic techniques for the resolution of the fine structure of loci in somatic cells. If there are indeed

irreversible changes, consequent for example on disproportionate DNA synthesis, each type of cell may turn out to display its own peculiarities on recombination analysis, and the genetic dissection of cell hybrids to be as difficult as the results are fascinating.

RECENT ADVANCES IN NEUROANATOMY

Sponsored by the Section of Vertebrate Morphology
of the American Society of Zoologists

A symposium organized for the
XVI International Congress of Zoology

by

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MEANINGS OF MIDBRAIN EXPOSURE, PAST AND PRESENT. Tilly Edinger, Museum of Comparative Zoology, Harvard University, Cambridge, Mass., U.S.A.

Paleoneurology has now extended comparative brain anatomy of the Mammalia back to the origins of the class some 180 million years ago. "Fossil brains" have shown this or that detail, and phylogenetic lines have revealed this or that development in the Past which had not been observed in mammals of the Present. It is a major divergence from the concepts established in more than a century of comparative brain anatomy that cerebrum and cerebellum were in broad contact in mammals of the Mesozoic era; the midbrain was likewise not dorsally exposed in some forms that lived in the Paleocene, the oldest epoch of the Neozoic era, in which the eutherian orders were becoming differentiated.

In the majority of fishes, amphibians and extant reptiles, the brain parts lie behind each other, while in extant birds and mammals they overlap each other. In progressive mammals, the midbrain centers tend to recede in importance and relative size as most of their functions become transferred to the enlarging neocortex. The theory is therefore held that a midbrain extending between forebrain and hindbrain occurs only in those mammals whose cerebrum is poorly developed. A tectum dorsally exposed is regarded not only as the mark of a primitive brain but also as a character that assigns a species a low place on the evolutionary scale of mammals.

Whereas the paleontological data that strikingly contradict this concept are few and far between, the recent revival of macroscopic brain research has made known neontological conditions which also conflict with the theory. Different degrees of midbrain exposure and overlapping apparently occur as variations, or are characteristics of closely related, small-bodied species of the lower orders of mammals. Taken together, those large-scale investigations suggest that an exposed tectum can not in every kind of mammal have that single meaning, primitiveness manifested by lack of cerebral progress. In some burrowing forms from several orders, it may well be a secondary development. That also in neontology conditions are recognized which call for a revision of the doctrine was first hinted in a mainly osteological paper; wide midbrain exposure in certain Microchiroptera was explained as not part of a primitive design but as due to a specialization divergent from those of other Eutheria (Starck 1952).

A small fossil, a stone endocast dorsally laid open in an undeterminable cranium from the Upper Paleocene, at first sight seemed to reproduce an extremely primitive brain. Cerebrum and

cerebellum are in contact only laterally, and the corpora quadrigemina occupy an area half the median length of the cerebrum. But the bulging posterior colliculi identified the specimen as the brain of a bat in which that divergent, acoustic specialization had already been achieved -- by an enlargement trend of the tectum itself. Chiroptera had evolved from Insectivora. The endocranial cast of an insectivore, found at the same locality, has only the transverse venous sinuses in the dorsal furrow between cerebrum and cerebellum. On the other hand, there was also collected at the bat locality a skull from an order of higher mammals, Carnivora; and the brain of this oldest fissiped displays almost diagrammatically the orocaudal sequence postulated for primitive mammals. The quadrigeminal bodies lie in a wide transverse area sunken between the cerebral and cerebellar vaults. This configuration is known also in other Paleocene and in early Eocene Ferungulata. In those ferungulate lines that survived into later epochs, brain evolution took the supposedly general course: relative reduction in size and lessened importance of the tectal centers while cerebral development advanced. Thus in an early Eocene Hyracotherium, the depressed midbrain area is approximately half as long as is the tectum in Equus brains, which lies below a high cerebrum that has expanded to almost five times the length it had in the earliest of horses. But a contemporary of the eohippus can be cited to further demonstrate that as little as in tempo was there a standard mode of advance in the evolution of an organ so intimately related with many kinds of progress, including behavioral adaptations. In a macrophthalmic representative of one branch of the Primates, the midbrain was overlapped by the occipital, visual lobe of its voluminous cerebrum already in the early Eocene. In the later Eocene, the cerebrum was still diminutive in Cetacea but nevertheless the tectum was hidden -- in that case by the already enlarged, forward-bulging cerebellum.

On endocranial casts of early Neozoic Multituberculata, the vermis cerebelli is inserted posteriorly between the cerebral hemispheres. These have a smooth surface and are not much longer than the olfactory bulbs. In this mainly Mesozoic, aberrant group whose other characters remotely parallel only the most primitive levels of the other subclasses, absence of the midbrain from the dorsal aspect is obviously not due to the factors which develop this situation in eutherians. What is visible in these brains rather suggests that olfaction was perfected to a mammalian degree whereas the midbrain, invisible, may well not have expanded to marsupial and placental status.

If a large, exposed midbrain were the primitive condition of the mammals, it could be expected in those Mesozoic animals

which in decisive characters reached the grade Mammalia 100 million years before the beginning of the Neozoic Age of (eutherian) Mammals. The area in question can be studied in only three specimens. The conformations revealed are not the same, but in none of these Mesozoic mammals was the midbrain interposed between forebrain and hindbrain as extensively as it had been in their reptilian ancestors and as it was in at least some of the earliest Eutheria. In an Amblotherium, at the bottom of a small median pit between cerebrum and cerebellum, a medially divided structure can be distinguished, that is: colliculi. The forebrain of Triconodon reflects macrosmatism beyond reptilian level with olfactory bulbs half as long as the cerebrum. It is separated on the dorsal surface from the cerebellum only by the transverse sinuses. A partial skull roof of Sinoconodon allows one to deduce some features of the brain in the very oldest of mammals. A long cerebral cavity is set off from the cerebellar chamber by a massive transverse crest descending from the parietal bones. This structure is comparable to the extensive downward thickening between the two chambers in e.g. eohippus, hence its neurological meaning is a low-lying tectum dorsally exposed -- but, in contrast to the eohippus condition, it appears as a short strip across a long brain. Such closeness of cerebrum and cerebellum has been called an anomaly in view of the age and primitive mammalian status of the triconodonts. Actually, comparable to the external configuration in advanced eutherian brains, it is a situation that can not have resulted from comparable neencephalic or cerebellar developments. The possibility has to be considered that it was due to characters also of the midbrain.

The midbrain itself is practically unknown in mesozoic mammals (and multituberculates). In their hindbrain, we know the palaeocerebellar vermis and flocculi; the neocerebellar lobes had not yet developed. The forebrain we know as a rhinencephalon; the cerebral surface was so smooth that, quite possibly, non-olfactory cortex had not been acquired beyond the reptilian primordium neopallii.

We paleoneurologists see most clearly cerebral progress during the Neozoic. We see a small cap rising above a distinctly marked rhinal fissure, and its later expansion into fissured neocortex. We tend to lose sight of the fact that the midbrain, too, had become considerably different from that of reptiles. It is from the microscopic anatomy of real brains -- brains of living mammals -- that we students of only endocranial casts learn about the pyramidal cells in the motor cortex and their cortico-spinal fibers in the cerebral peduncles of the mesencephalon. These are neencephalic parts of the brain stem. They can not have existed before motor cortex was differenti-

ated. Further, one can hardly imagine inside those brains which had just made smell the dominant sense an acoustic system such as that of Neozoic mammals. It is unlikely that the small reptilian nucleus lateralis mesencephali had developed into an important tectal center of acoustic correlation, that the ancient lamina bigemina had been built up into corpora quadrigemina. Presumably, evolutionary drives involving the structures which in the Neozoic are characteristics of the mammalian midbrain set in later than had expansion of the rhinencephalon. Under this view of the long history of the midbrain in mammals, wide separation of forebrain and hindbrain in early Eutheria appears secondary rather than primitive. The later trends that developed neocortical sensory centers did relegate the midbrain to a subordinate role, but whether the tectum remained dorsally exposed or became overlapped does not reflect grades in the mammalian hierarchy. As could be expected of so complex an organ in so varied a class, the meaning of either situation is different in the various branches, and different at different times of their evolution.

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CORRELATION OF HABIT AND STRUCTURE IN THE FISH BRAIN

This report is intended to point out some of the relations between the morphological structure and functions of parts of the nervous system, their exaggeration and abeyance in different fishes and their representation in related forms.

The olfactory nerves, unmyelinated as in other vertebrates, vary greatly in extent. The length of the nerve is determined by at least two factors: the distance of the nasal mucosa from the base of the brain and/or the length of the olfactory stalks that connect the bulb with the brain. Elongation of the skull as the fish grows may be accommodated to by an increase in length of the olfactory fila or by an increase in length of the olfactory stalk.

The considerable differences in the shape and size of the olfactory bulbs in fishes, may be related to the shape of the front end of the head and to the development of the olfactory system. The characteristic lamination in the vertebrate olfactory bulb has a varying but recognizable representation in fishes, being in general more typical in ganoids and less typical in teleosts. In many teleosts, with the failure of an olfactory ventricle to develop, instead of a concentric fiber and cellular arrangement, there is a single pattern of lamination, medial to lateral or lateral to medial depending on the place of entrance of the olfactory fila. Mitral cells of the olfactory bulb in the fish, in the amphibian (frog), where they are less well developed, and in mammals are comparable.

A comparison of the fish telencephalon with that of other vertebrates presents many difficulties and its relations have been the subject of much controversy. The differences stem largely from the lack thus far of a demonstrable lateral ventricle, and the presence of a very extensive and very thin laterally attached roof plate. The major subdivisions of the telencephalon in a frog, where the relations are simple, and in a mammal without the corpus callosum, where they are still somewhat primitive in character, and in the fish, have been compared according to the interpretation which we have adopted and which agrees with that of Dr. Fortuyn. According to this interpretation the dorsomedial segment is termed primordial hippocampus; the lateral part is the primordial pyriform area and amygdala. The dorsal area between the primordial hippocampus and the primordial pyriform area is the primordial general pallium as in the tailed amphibian (Herrick) and as in the tailless amphibians (Hoffman). Such interpretations have been made on the basis of nuclear configuration, specific cell types and connections.

The anterior commissure, present in almost all vertebrates (though not recognizable in cyclostomes), is a major interhemispheric connection of the basolateral part of the amygdaloid complex and pyriform lobe area throughout the vertebrate series. The similarities between the bowfin, goldfish, frog, and opossum are striking and to our mind convincing of the homologizing of the lateral forebrain areas with the amygdala and pyriform lobe. The hippocampal commissure in teleosts has the typical relations dorsal to the anterior commissure and apparently also interconnects the region regarded as the primordial hippocampi. Both the primordial amygdaloid area and the primordial hippocampi are connected with the habenula in a fashion typical for vertebrates.

Centrally located are the striatal areas. The base of the hemisphere is occupied by a region which is variously developed, being very large in the paddlefish and small and poorly developed in the goldfish. Medially and ventromedially is the septal area which probably rises or falls with the relative development of the olfactory and gustatory systems.

There is a correlation between the degree of development of the dorsal thalamus and the nonolfactory cortical and the striatal areas or their primordia. There is also a relation between the manner of life of the animal and the development of these areas. Terrestrial animals need to respond to a wide enviormental range of experience. This is reflected in the gradual appearance (from amphibians to mammals) of specialized cutaneous and proprioceptive terminations and, of course, in the disappearance of the lateral line system. With the differentiation of special terminations, new nuclei and long conduction pathways (spinothalamic and the medial lemniscus), with endings in the dorsal thalamus, make their appearance. As is to be expected then, the piscian dorsal thalamus is possibly the least outstanding portion of the diencephalon even in the highly specialized fishes. It should be emphasized that the optic connections from the retina in fishes project to the ventral nucleus of the lateral geniculate (which is ventral thalamus), to the optic tectum and the pretectal gray. The ventral thalamus of fishes is relatively larger than the dorsal thalamus in the available material.

The hypothalamus is the largest part of the diencephalon in fish and proportionately larger than in any other form. Here certain nuclei and tracts are not common to the main phylogenetic line but are peculiar to the fish or, in some instances, much better developed in the fish than in other forms.

Like other vertebrates, the fish hypothalamus receives olfactory impulses through the medial forebrain bundle and fascicles from the hippocampus and amygdala. The amount of olfactory fibers will be regulated by peripheral olfactory development. Gustatory impulses are relayed to the hypothalamus of fishes by special paths--the size of which depends on the development of the sense of taste in the fish under consideration. Where the entering gustatory fibers are numerous, their regions of termination are enlarged to form gustatory lobes. These lobes may completely distort the usual configuration of the brain stem for vertebrates. The specialized character of such a gustatory lobe is indicated not only by its size but by its specialized histologically laminated character which frequently indicates high sensory specialization and correlation.

From the gustatory lobes associated with visceral afferent terminations of the facial, glossopharyngeal, and vagus nerves in forms such as the goldfish, a large ascending uncrossed secondary gustatory tract goes forward either to relay in the secondary gustatory nucleus for discharge to the hypothalamus or to pass to the hypothalamus directly.

The marked interconnections which exist between the optic tectum and the hypothalamus, at least in many fishes, suggest the modification of visceral and visual-cutaneous correlation in both areas by this interplay. Consequently, there is not a direct relation between the size and the differentiation of the hypothalamus and the relative development of any one of the modalities here discussed, since the hypothalamus represents the correlative activity of many modalities.

The amount of optic tract projection to the tectum in fishes varies from essentially none in the blind fish to a sufficient number to form a macroscopic system in pickerel. It is of interest that although the superficial layer of the optic tectum where optic fibers enter is greatly reduced in blind fish, the deeper portions of the tectum are still well represented. This representation is correlated with the projections to the tectum of the somatic impulses from the brain stem and the visceral impulses from the hypothalamus. Therefore, the optic tectum is essentially a somatic-visceral-optic correlation center in fishes in which the optic projection plays a leading role. Both the hypothalamus and the optic tectum discharge to the lower centers through relays in the tegmentum or reticular formation and to the cerebellum by lobocerebellar or tectocerebellar paths.

Swiftly moving fishes have a large spinocerebellar system and usually at least a large corpus cerebelli. The auricular lobes of the cerebellum, where present, are related to the vestibular system. Impulses relayed over lateral line nerves (V, or VII, IX, and X) pass not only to the copus cerebelli but these impulses indirectly reach a peculiar rostral extension known as the valvula cerebelli. Whether proportionately large or small, the cerebellum of a fish probably has no region homologous to the cerebellar hemispheres of mammals other than the auricular lobes which are demonstrable in some fishes and comparable to the mammalian flocculi. Grossly, and to some extent microscopically the cerebellum in fishes shows then variations in development as do other brain regions in these forms. Such variations reflect, to a great extent, differences in size of the diverse pathways which project upon it.

SOME COMPARATIVE MEASUREMENTS OF THE OPTIC LOBE IN BIRDS.

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The avian optic lobe is remarkable for its size and for the laminar arrangement of the nerve cells of its tectum. This midbrain tectum opticum more closely resembles the cerebral cortex of mammals than any part of the avian forebrain, because in birds, although superficial corticoid areas are found in the cerebrum, there is no neocortex. In the optic lobe there is also a large auditory nucleus, the torus (n. mesencephalicus lateralis, pars dorsalis). From measurements of serial sections of 27 different species of birds the volume of the torus and of the whole lobe have been computed. The volumes of the torus (auditory) and of the lobe (optic) have been compared in each species and the result expressed in per cent. The torus varies from being 2.0% of the size of the optic lobe in a loon (Gavia immer), to being 8.7% in an oilbird (Steatornis caripensis). Taken by orders, the Strigiformes have the largest ratio of torus to optic (7.4%), next come the Capriculgiformes and Apodiformes with an average of 6.5%. These are the groups of birds in which hearing is very highly developed either for echolocation (oilbirds and swifts) or for locating sounds made by their prey (owls). The large relative size of the torus thus seems to have a functional significance. The smallest ratio is found in Sphenisci-, Podicipodi-, Gavii-, Galli-, and Sphenisciformes (average 2.4%). Between these extremes fall birds in all the 13 other orders studied, averaging 4.0%.

Variation in the position and shape of the optic lobes in different birds is shown to be a conspicuous feature of avian cerebral morphology. These lobes of the midbrain are more rounded and dorsolateral in those species in which the cerebral hemispheres do not come far back to more or less cover the midbrain (falcon and emu). The optic lobes are more flattened and ventrolateral in species in which the cerebral hemispheres do come far back over the midbrain (e.g. jay). The size and position of the cerebellum is also important in determining the shape and position of the optic lobes.

Quantitative methods, in the study of the anatomy of the avian brain, can give significant information. The significance appears when the anatomical data are considered in relation to the bird's behavior.

NEUROSECRETION IN TELEOST FISHES: THE CAUDAL NEUROSECRETORY SYSTEM. Uno Holmgren, Zool. Inst. Uppsala University, Sweden.

Different types of neurosecretory systems are known. Among vertebrates it is well established that hormones are synthesized in the hypothalamus of the brain. In amphibians and fishes only one nucleus, the nucleus preopticus is present. The latter nucleus is divided into two parts, the Pars interna and Pars externa, containing neurosecretory cells. The produced neurosecretory material is later transported along neurosecretory pathways down to the neurohypophysis of the pituitary gland, where the hormones are released. The neurohypophysis (Pars nervosa), thus mainly serves as a storage depot for hormones produced elsewhere.

Among invertebrates the intercerebralis-corpus cardiaca-allatum system in insects is well known. In all cases we find characteristic synthesizing- transporting and storage- release elements. A neurosecretory system with similar organization is present in the terminal portion of the spinal cord of various systematic groups of fishes. Studies on the caudal neurosecretory system have shown that great similarities do exist between the general organization of all known neurosecretory systems and this also includes the fine structure of the individual components. It should be noted, however, that the caudal neurosecretory system is so called Gomori-negative. This indicates that the staining response is of minor importance in the discussion of the concept of neurosecretion. However, since 'neurohumors' such as acetylcholin and noradrenalin and similar products also are synthesized by nerve cells, it seems important to establish a definition of neurosecretion. The latter must be based on morphological characters, the cytology and histochemical reactions of the cells. Most important is, however, the information obtained from studies of the fine structure of the individual components, especially the secretory material.

As far as the caudal neurosecretory system is concerned, it has been established that spinal secretory cells in teleosts are homologous to the so called Dahlgren's cells of skates. The neuronal character of the caudal neurosecretory cells is attested by the presence of elements such as dendrites, neurofibrils and Nissl bodies. They seem at the same time to be glandular, elaborating microscopically visible 'granules or 'droplets' which pass along the processes or axons, towards the nerve terminals, ending in an organ where the material is stored and released. The morphology of this hypophysis-like organ may vary considerably and may even be situated dorsal or lateral to the spine. In the great majority of cases the organ, the urophysis spinalis is situated ventral to the spine, above the articulation between the last vertebra and the hypural bone.

Sectioning of the tracts between the spinal neurosecretory

cells and the urophysis spinalis leads to an accumulation of material in front of the lesion. Characteristic neurosecretory 'vesicles', size 1000-2000 Å have been described from the cell bodies and in large numbers from the nerve terminals ending in the urophysis spinalis. The neurosecretory tracts have been described to divide and end in the vicinity of blood vessels. In the case of the neurohypophysis of the pituitary gland, Palay pointed out the presence in the capillaries of a "fenestrated endothelium". Very similar conditions are found in the urophysis spinalis, where the basal membrane of the blood vessels in certain areas is thin and delicate and would seem to allow even granular material to reach the immediate vicinity of the endothelium, which contained endothelial pores, similar to those described from the neurohypophysis of the pituitary gland. This suggests that the neurosecretory material may enter the capillaries, probably in highly dispersed form, since no neurosecretory granules were observed within the blood vessels.

A final strong indication of a neurosecretory system is possible physiological effects of the secretion. There are thus findings which suggest that the system is associated with the functioning of the air bladder and that the secretion is affected by osmotic conditions. However these indications are not supported by morphological data. The caudal neurosecretory system does not show any seasonal variation. It is developed in fishes which are lacking airbladder. The caudal neurosecretory system, which develops later during the ontogeny than the hypothalimic system becomes secretory active considerably after the appearance of the air bladder in certain species.

Experiments carried out on the euryhaline teleost, Fundulus heteroclitus L. strongly suggest some effects of the caudal neurosecretory system on the relative hypertonicity of the fish against hypotonic media. Destruction of the urophysis spinalis and the caudal neurosecretory system in this fish significantly affected the survival of the fish when returned from salt water of 3,4 % to fresh water. Similar effects have not been obtained in other species. Repeated experiments on the possible effects of the caudal neurosecretory system on the buoyancy of fishes have been inconclusive. The finding of some effects on the relative hypertonicity of the fish against hypotonic media is not supported by morphological or ecological data. The caudal neurosecretory system is well developed in both fresh water and salt water species.

The inconclusive data of the studies on the function of the system may indicate a very complex mechanism, the details of which still remain obscure.

Cytological and histochemical studies on the spinal neurosecretory cells have revealed that the Gomori-negative cells show affinity for acid fuchsin, azocarmine, that they are PAS- negative and also Alcian blue negative but respond

positively for histochemical methods for proteins and end-groups of proteins such as ninhydrin-Schiff. Methods for demonstration of RNA have indicated considerable amouts present in the pericaryon of the cells. This is in accordande with the concept of the important role of RNA in protein synthesis. A very intimate relationship between the capillaries and the spinal neurosecretory cells suggests a strong rate of exchange between the neurosecretory cells and the blood.

Studies of the fine structure of ordinary neurons have revealed the existance of so called "synaptic vesicles" accumulated in the synaptic region of the neurons. These "synaptic vesicles" have generally been related to the presence and release of ´neurohumors´ such as acetylcholin and noradrenalin, functioning as transmitting substances. These vesicles, size 100-200 Å are found in some neurosecretory tracts of the caudal neurosecretory system together with neurosecretory vesicles. The significanse of this observation is further discussed below. The´synaptic vesicles´ and neurosecretory granules (vesicles) have been demonstrated both with osmium-and permanganate fixation. The neuronal character of the urophyseal neurosecretory cells is strongly indicated by the fact that considerable amuonts of RNA, neurofibrils and dendrite-like processes are present. The neurosecretory granules are below the resolving power of the light microscope. The neurosecretory material has nevertheless been described as aggregations, clusters or ´colloid droplets´. The formation of such structures is apparently influenced by the fixation and staining procedures. It is also of interest to note that secretory material of the caudal neurosecretory system is preserved in such fixatives as Bouin and Zenker´s solution whereas poor results have been obtained with fixatives such as alcohol and chloroform. These results are similar to those obtained for the hypothalamic system and have been interpreted as an indication of a lipid constituent in the secretion. This interpretation is in line with the observation from material fixed in osmiumtetroxid both for light and electronmicroscopy. Material of colloid character was observed inside neurosecretory tracts of the urophyseal system of the gold fish in tissues fixed for electronmicroscopy in buffered osmiumtetroxid and embedded in methacrylate. Thicker sections were then cut and studied in the dark field microscope. Osiumtetroxide produces insoluble and strongly stained polymerization products with unsaturated lipids but does not prevent large amounts of nucleic acids and proteins to leak out. We are thus likely to find certain differences when comparing the results from fixatives as Bouin, Zenker to osmium. In the former cases the unisotonicity may cause great damage to the tissues.

According to the studies indicated above, material of lipid character is present in the neurosecretory material. Since the secretion also responds to histochemical stains and methods for proteins and endgroups of proteins, it seems likely to

conclude that the stainable material contains a lipoprotein. The latter finding would be in accordance with discriptions of the hypothalamic secretion. However, the staining response of a given neurosecretory substance can be influenced either by the hormonal component or by the "carrier substance".

In the hypothalamic system four stages in the transformation of ordinary nerve cells into neurosecretory ones have been described: 1. Ordinary nerve cells. 2. Ordinary nerve cells with stainable granules and vacuoles in the cell plasma. 3. True neurosecretory cells with traces of secretion both in the cell body and axon, which divides and terminates in a place of storage. The cells have dendrites. 4. Resembles type 3 but is lacking dendrites. When trying to classify the neurosecretory cells of the caudal neurosecretory system in teleosts using the terminology above, it can be noted that the urophyseal neurosecretory system seems to contain: 1. Cells resembling ordinary nerve cells. 2. True neurosecretory cells which in many respects have retained some of the characters of the ordinary nerve cell. Thus the cells have dendrites, neurofibrils etc. but nevertheless show many signs of neurosecretory activity e.g. a large irregular nucleus with indications of material emitted from the nucleus into the cytoplasm, an accumulation of colloid material in the cell body and Herring body like structures along the axon. 3. The studies of the fine structure of the urophysis spinalis have shown that there are apparently neurosecretory cells, which have retained their production of ´neurohumors´, judging from the presence of ´synaptic vesicles´ together with neurosecretory vesicles. This would represent a transitional stage between 2 and 3 according to the terminology above. The most common neurosecretory cell type is lacking dendrites and would thus represent type 4.

RECENT ADVANCES IN OUR KNOWLEDGE OF THE STRUCTURE AND FUNCTION OF THE PINEAL ORGAN. Johannes Ariëns Kappers, Netherlands Central Institute for Brain Research. Amsterdam, The Netherlands.

By morphological as well as by neurophysiological investigations it has recently been established that, in lower vertebrates, the pineal system is predominantly a photosensitive apparatus. This, at least, holds for the parapineal organ of the lamprey, the frontal organ of anuran amphibians and the parietal eye of lizards. Probably, this is also true for the pineal sac or epiphysis sensu strictiori. It has been shown that the ultramicroscopic structure of the sensory cells in these organs is quite similar to that of the sensory cells in the retina of the lateral eyes, especially to that of the rods. Electrophysiologically, inhibitory and excitatory response patterns can be evoked from the amphibian frontal organ and the lacertilian parietal eye by light of different wavelengths and by darkness respectivily. Altered behavior and changes in pigment pattern of the skin were observed after removal or shielding of these organs. There are also indications that the pineal system may play a role in thermoregulation.

In many lower vertebrates, the axons of nerve cells, present in the pineal system, form a pineal or parietal nerve which runs to the epithalamus. For a better understanding of their function a renewed investigation of the distribution and the sites of ending of these fibers seems very necessary. It has been argued that, in aphibians, they may stimulate the secretory function of another circumventricular structure, the subcommissural organ, which shows a very suggestive close topographical relationship with the pineal sac.

So far, the study of the pineal organ in snakes and birds has been rather neglected. In view of their structure they may be the forerunners of the more compact and extensively vascularized pineal bodies present in mammals. In the pineals of birds sensory cells and nerve cells are lacking. Some investigations are suggestive of a secretory function of the bird pineal.

In the mammalian pineal organ sensory cells likewise do not occur. Here, the main type of cell element is the "parenchymal cell" or pinealocyte which show more or less long and slender processes having little bulb-like endings. These protrusions may enter perivascular spaces. By means of electron microscopy it has been conclusively shown that the old hypothesis following which nuclear secretion would occur in the pinealocyte does not hold. The nuclear membrane is deeply infolded in many places. This results in a considerable enlargement of the surface of the nuclear membrane, the significance of which is not known. Furthermore, the pineal organ of mammals contains different numbers of glial elements, especially astroglia, mastcells, and some connective tissue,

accompanying the larger vessels. Some species show pineal pigment cells. Vascularization is rich.

As its innervation is concerned, the mammalian pineal body most certainly does not send any nerve fibers to the epithalamus nor does it receive fibers from the brain proper, at least not such which end in the organ. However, some fibers belonging to the habenular and posterior commissural systems may run within the epiphysis, entering the organ and leaving it again. Neurosecretory nerve fibers are absent. It appears that there is no question of an epithalamo-epiphyseal functional relationship similar to the wellknown hypothalamo--hypophyseal one.

The mammalian organ, on the other hand, is profusely innervated by autonomic nerve fibers, orthosympathetic as well as probably parasympathetic. These fibers reach the organ by way of two nervi conarii, bilaterally present, which may fuse before or after having entered the structure at its caudal side. Especially in Primates, a few autonomic nerve cells can be rather constantly observed within the epiphysis. Autonomic innervation is by far more extensive than the innervation of the pineal vascular system would account for. Moreover, nerve terminals on pinealocytes have been demonstrated. Most probably, this autonomic innervation acts upon the function of the pinealocytes. This is also suggested by changes in the pineal lipid contents after bilateral superior ganglionectomy.

In recent years, biochemistry and histochemistry of the mammalian pineal have been studied by many authors. Both radioiodine and radiophosphorus are taken up at a faster rate in the organ than in any other part of the brain. Moreover, amino acid formation is high. Probably, phosphorus is incorporated in phospholipids, present in large quantities in the pinealocytes of many mammals. Phospholipids being known to represent a source of energy are indicative of the occurrence of many important biochemical reactions taking place in the epiphysis. In comparison to other parts of the brain, relatively high amounts of norepinephrine, histamine and serotonin have been found in the structure.

Besides serotonin, other indoles like melatonin or N-acetyl-methoxytryptamine, 5-methoxy-indole acetic acid and 5-hydroxyindole acetic acid are present and most probably produced in the mammalian pineal organ. It has been shown that melatonin is formed by first N-acetylation and then O-methylation from serotonin. The compound is practically exclusively present in the epiphysis. Per pineal, its quantity is extremely small being for instance only 1/2000 μg in the rat. The activity of this small quantity, however, is amazingly strong. Melatonin, as is the case with some other compounds, causes aggregation of pigment granules in the melanophores of amphibian skin. Whether the substance is produced in the amphibian pineal system seems questionable although pinealectomy in tadpoles prevents the normal

dark-induced blanching of their melanocytes.

The function of melatonin in mammals is not well known. Experiments with pure melatonin on supravital ileum and uterus of rats and guinea-pigs have conclusively demonstrated that this compound does not act as a neurotransmitter substance. However, given in high doses, melatonin gives statistically sound retardation of growth in bodyweight in young rats, and, most probably, suppression of development of the seminal vesicles in males.

By electron microscopy, vesicles containing biogenic amines have been demonstrated in the plurivesicular processes of the pinealocytes. Injections of reserpine cause a release of these vesicles. It is, however, somewhat doubtful whether these club-shaped endings of the pinealocyte processes can be sufficient clearly distinguished from the terminal endings of the autonomic nerve fibers.

In the rat pineal organ, pinealocytes are extremely rich in lipids, especially phospholipids. The pineal lipid contents, possibly functioning as a source of energy in the production of specific compounds, changes under a number of physiological as well as experimental conditions. Quantitative determinations have shown that, in the rat, the lipid contents is lowest just prior to ovulation and highest during diestrous. In old rats this contents is somewhat lower than in young ones.

Ovariectomy causes a general rise in the rat pineal lipid level which could be restored to normal by injections of estron. On the other hand, it has been shown that after hypophysectomy a decrease of the pineal lipid level occurs which can be restored by injections of gestyl. Probably, the increase of the pineal lipid contents after ovariectomy is due to an overproduction of hypophyseal gonadotropic hormone.

These as well as other experimental investigations seem to corroborate the old hypothesis following which the mammalian pineal organ would act upon the development and function of the gonads. There are reasons to suggest that the pineal and the adenohypophysis show a mutual functional relationship, the pineal at least secreting one or more compounds having an antigonadotropic effect. It seems probable that this regulation happens via the hypothalamus but the exact ways and means remain to be investigated exactly neuroendocrinologically as well as on the cytological level.

Experimental modification of the pineal lipid contents in the rat can be accomplished by still other means. Bilateral superior ganglionectomy depriving the organ of most of its autonomic innervation seems to cause an increase of that contents. Reduction of pineal lipids, on the other hand, has been produced by a sodium deficient diet, after adrenalectomy, maintenance of the animals on propylthiouracyl, and by injections of angiotensine. No changes, however, were observed after administration of ACTH, hydrocortisone and

thyroxine. DOCA administration results in a slight increase of rat pineal lipid contents. Osmoregulation has been one of the many functions ascribed to the mammalian pineal organ. Pineal cytological changes after stress have also been observed.

Very remarkably, alterations in the contents of lipids and other compounds were obtained under conditions of continuous light. Mediation seems to be accomplished by way of the lateral eyes but the nervous or neuro-hormonal pathways along which these regulations happen are not known as yet.

Recently it has been surmised that the mammalian pineal organ would also contain a hormone, adrenoglomerulotropin, that stimulates the release of aldosterone by the adrenal cortex. The compound has been tentatively identified as a melatonin derivative. On the other hand, it has been thought that this hormone would be produced by the neighboring subcommissural organ.

From the above survey it is evident that, during recent years, our knowledge concerning the pineal organ has indeed advanced in many scientific fields. The interpretation of a number of results, obtained by experimental investigations, remains, however, rather difficult. A wide knowledge of all facts known at present about the structure, histochemistry, biochemistry, and neuroendocrinology of the pineal body may lead to a better understanding and integration of these results. It is, at least, now very certain that the mammalian pineal body, far from being a mere phylogenetic non-functional relic, is a veritable factory working at a high metabolic rate and producing a number of compounds of high functional importance.

Most probably, the pineal system in lower vertebrates, which is primarily a photosensitive structure, has changed into a secretory organ in mammals, this change being foreshadowed in snakes and birds. Change of function has been accompanied by a very remarkable change of nervous apparatus. Investigations of the functional role of the pineal system in lower vertebrates, especially of its possible action on the function of the gonads, are still wanting. Until now, most research has rather exclusively been concentrated on changes in pigment pattern and general behavior.

QUANTITATIVE COMPARISONS ON THE BRAINS OF INSECTIVORES AND PRIMATES.

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The aim of our investigation is twofold: (1) the understanding of the function of certain structural units of the brain, and (2) the study of the phylogenetic development of the brain and its parts. As to the problems of function we compare brains of animals which are characterized by marked quantitative differences in certain sensory functions, as, for instance, macrosmatic and microsmatic animals. As for the questions concerning phylogenetic development we compare different stages of a phylogenetic scale, or at least a series of forms that comes close to such a phylogenetic scale. Since the direct ancestors of our recent animals are extinct a true phylogenetic series may be reconstructed only from fossil material. Although the brains of such fossil specimen are not preserved, a surprising amount of information on the development of the brain can be gathered from endocranial casts. However, this method, which is known as Palaeoneurology and of which Tilly EDINGER is one of the most outstanding representatives, does not yield reliable data concerning the internal structure of the brain and its finer architectonic and functional units. For this purpose additional data must be furnished by studies on the brains of recent species. The use of recent material is possible because a number of primitive forms have persisted almost unchanged. This is true particularly of the insectivores. Therefore it is obvious that they should be well suited for the reconstruction of early phylogenetic stages of placental mammals. Many - perhaps even all - orders of placental mammals can be traced back to insectivorelike ancestors. The most interesting of the strains derived from the insectivores is the one that follows a line of evolution leading to the prosimians, monkeys, apes, and eventually to man. Thus, the approach should be as follows: the study of different stages in the ascending scale of primates and their comparison with a well established basis of data from insectivores.

Since 1953 we have collected and studied all insectivore species that could be made available, in order to determine whether they have a uniformly primitive type of brain, or if there are species amongst them which show characteristics of a higher evolutionary stage. It was found that the insectivores which can be regarded as basal forms and as such can be used in comparative phylogenetic studies, exist only in the families of Tenrecidae, Erinacidae, and Soricidae, in the form of their terrestric species. These forms have the smallest cortex and the most primitive cortical pattern. In contrast, the semiaquatile representatives of these families, as, for instance, Potamogale and Neomys or the Talpidae and Chrysochloridae, which are specialized for burrowing, but especially the macroptic Macroscelididae, show definite signs of an evolutionary progress. The evolutionary level is judged by the degree of neocorticalization, i.e. the development of the highest cortical centres. Because, so far, it appears that in mammals the development and differentiation of the neocortex gives reliable and most impressive clues to the evolutionary level.

Our previous investigations were comparative studies on the extension of the different components of the cortex on the

cerebral surface (cf. the summarizing account by STEPHAN, 1961). Then in order to be able to include also internal cerebral structures we proceeded with comparative studies of volumes. These investigations are the subject of the present communication.

In order to determine the values for the volumes of finer structural units we have employed the "serial section method", i.e. the measurement of the dimensions of these units in serial sections. This is the only possible way to get a sufficiently accurate delimitation of such units. These quantitative analyses were carried out in the following 21 species: 7 basal insectivores (Tenrec ecaudatus, Setifer setosus, Erinaceus europaeus, Crocidura occidentalis, Crocidura russula, Sorex araneus and Sorex minutus), 7 higher insectivores (Talpa europaea, Chlorotalpa stuhlmanni, Potamogale velox, Neomys fodiens, Galemys pyrenaicus, Elephantulus fuscipes and Rhynchocyon stuhlmanni), 4 prosimians (Tupaia glis, Perodicticus potto, Loris gracilis and Galago demidovii) as well as 3 monkeys (Callithrix jacchus, Leontocebus oedipus and Aotes trivirgatus. Whenever possible measurements were taken from at least two brains of each species. The following 5 subdivisions of the brain were measured: medulla oblongata, cerebellum, mesencephalon, diencephalon and telencephalon. Of the individual parts of the telencephalon we have measured the neocortex, septum, palaeocortex - including the amygdala - the olfactory bulb, and the striate body. In each of the 21 species studied the comparison of the values for the individual cerebral subdivisions revealed a large amount of interesting facts, the discussions of which, however, would exceed the scope of this communication. In the present report we will restrict ourselves to outlining roughly the structural composition of the brain in basic forms of insectivores in general, and to reviewing the changes found in the primates we have studied.

On the average the brain of a basic insectivore is composed of 13% medulla, 12% cerebellum, 6% mesencephalon, 8% diencephalon and 61% telencephalon. In the monkeys studied the average value for the telencephalon is 74% (an unexpectedly low increase) whereas the other subdivisions show a relative reduction in volume. This relative reduction is most conspicuous in the medulla (from 13% down to 4%); it is lowest in the diencephalon (from 8% to 7%) and in the cerebellum (from 12% to 11%). The relatively low increase in volume of the telencephalon in relation to the whole brain at such markedly different levels of differentiation as represented by basal insectivores and monkeys is contrasted by very distinct relative architectonic changes in the telencephalon: from the basal insectivores to primates the proportion of the neocortex grows from 22% to 83%. All other parts undergo a relative reduction in volume which is most striking in the olfactory bulb (from 17% to 0,4%) and the palaeocortex (from 31% to 4%); the striate body undergoes a far less pronounced reduction in volume (from 8% to 6%).

These few data may suffice to demonstrate the relative composition of the brain. But - although useful for a rough orientation - they do not supply information about the true changes, for instance as to whether, in correspondence with its relative decrease in volume from 14% to 4%, the ammonshorn - in the course of the phylogenetic development - does undergo a true reduction, or remains unchanged, or even grows larger. In the latter case the reduction would be only a relative one, due to the enormous growth of the neocortex. This would, in principle, apply also to

the alterations occurring in the other subdivisions of the brain which undergo a relative reduction in volume. Therefore, we soon decided that it would be necessary to introduce a reference system which is independent from brain measurements and to compare directly the absolute values.

A satisfactory reference quantity for the comparison of the absolute values proved to be the body weight, following a method developed by SNELL (1892) and by DUBOIS (1897) which is known as the now generally accepted and widely used allometric method. The typical relations between body weight and brain volume (or the volume of a certain structural unit of the brain) within a group of closely related species are characterized by the allometric formula $h \sim k^r$, i.e. the brain volume is proportional to the body weight raised by the index r (<1). In the system of double logarithmic coordinates, which we have to employ in order to save space, this parabola becomes a straight line ($\log h \sim r. \log k$), the slope of which corresponds to the tangent of the exponent r.

This method can be utilized for our phylogenetic studies by determining in basal insectivores the ratio of body weight to brain volume, or to volume of a given structural unit, and correlating the higher developed forms - i.e. higher insectivores, prosimians and monkeys - with this basal straight line. (A similar method has been employed by WIRZ (1950) after a rough macromorphological differentiation of the brain). We have projected the distances from the basal line on a graded line perpendicular to the former, the scale of which allows at the same time to read by how many percent the values for individual structural units deviate from those in basal insectivores of equal body weight. This method has already been employed by us in a quantitative comparative study of the septum (STEPHAN and ANDY, 1962).

Of the 5 major subdivisions of the brain the telencephalon - as expected - shows the most extensive evolutionary growth (in prosimians 3 1/2 to 5 times the size of the corresponding part in basal insectivores = basal size = BS; in monkeys 6 1/2 to 7 1/2 BS). Also the diencephalon (3 1/2 to 4 1/2 and 5 1/2 to 6 BS respectively) and the cerebellum (3 to 5 and 4 1/2 to 5 1/2 BS) grow larger as compared to basal insectivores. The evolutionary tendency of the mesencephalon is less pronounced (2 to 3 1/2 and 3 1/2 to 4 BS), and is lowest in the medulla. However, the medulla is not actually reduced in volume, which might perhaps be expected in view of the marked reduction of its relative percentage of the total brain. Also the medulla grows larger towards primates; in the prosimians it is twice the size (BS), in monkeys 2 1/2 the size of the medulla in basal insectivores of equal body weight. This is apparently due to the fact that in addition to the elementary structures of the vital vegetative centres new ascending and descending fibre paths from the developing higher cerebral centres and new integrating neuronal systems have appeared. However, also in higher developed insectivores the medulla may reach a size almost equal to that in primates, although in these cases this cannot be explained to the same extent by the progressive development of higher cerebral centres. Maximum values are found in Potamogale, and in this case the very large medulla is clearly the result of the unusually strong development of the trigeminal system. The latter innervates the vibrissae of the upper lip which have probably developed into vibration receptors, which is obviously an adaptation

for catching crustaceans under water. It shows that specializations of this kind can greatly influence the growth of a given cerebral structure. Another example is the extreme development of the anterior corpora quadrigemina in the Macroscelididae. In these animals the value for the midbrain volume reach or are even greater than those for the midbrain in primates. The Macroscelididae have a very powerful, although primitive, optic system which has its highest representations not, or at least not primarily, in the telencephalon, but predominantly in the anterior colliculi of the corpora quadrigemina.

The phylogenetic development of the telencephalon in primates is characterized by an enormous growth of the neocortex (in the monkeys studied: 21 to 24 BS), and also by an unexpectedly powerful development of the striate body (in monkeys 5 1/2 to 7 1/2 BS). In this respect the striate body outranks the remainder of the - essentially - cortical telencephalic regions. Amongst the latter the schizocortex (regio entorhinalis plus regio praesubicularis), hippocampus and septum (i.e. all regions that belong to the limbic system in the strict sense) have a rather low but definite evolutionary tendency. Up to the prosimians these regions double their size and in monkeys they usually show another slight enlargement; however, also in higher developed insectivores they may reach approximately the same size. The hippocampus in particular is extraordinarily powerful in Macroscelididae, more so than in any of the other forms studied (3 1/2 BS). This is a speciality the cause of which is as yet unknown.

The majority of the remaining telencephalic regions belong to the olfactory system. The olfactory bulb (=primary centre of smell) is considerably reduced in all monkeys. In some prosimians, however, (as e.g.in Galago) it is still of an insectivore-like size. These prosimians demonstrate that the evolution of the neocortex and the reduction of the olfactory system are not necessarily correlated. A marked reduction of the olfactory bulb occurs also in semiaquatile insectivores (Potamogale). The amygdaloid nucleus could not be distinctly separated from the secondary olfactory centres of the palaeocortex with their periamygdaloid cortex and were therefore included in the measurements of the latter. This may be responsible for the fact, that in contrast to previously established data on surface measurements, a reduction exactly parallel to that of the olfactory bulb is not recognizable. We believe that a reduction of the secondary olfactory centres does occur, which, however, is compensated by an enlargement of the non-olfactory components of the amygdaloid nucleus. We expect to obtain information on this point by a quantitative analysis of the individual nuclei of the corpus amygdaloideum,

In conclusion, the evolutionary changes in the brain from basal insectivores to lower monkeys can be characterized as follows: (1) Enlargement of all divisions of the brain, - although to a varying degree - in the following order from the lowest to the highest increase in volume: medulla, mesencephalon, cerebellum, diencephalon, telencephalon. (2) Within the telencephalon: enormous growth of the neocortex, marked growth of the striate body, slight enlargement of the schizocortex, hippocampus and septum, reduction of the olfactory centres.

DUBOIS,E.: Arch.Anthrop. 25 (1897) 1-28; SNELL, O.: Arch. Psychiat.23 (1892) 436-446; STEPHAN,H.: Acta anat.44 (1961) 12-59;--and ANDY,O.J.: J.Hirnforsch. 5 (1962) 229-244; WIRZ,K.:Acta anat. 9 (1950) 134-196.

SOME MORPHOLOGICAL AND PHYSIOLOGICAL CHARACTERISTICS OF THE SOMATIC SENSORY SYSTEM IN THE RACCOON.

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Introduction

Differential development of specific portions of the nervous system appears to have been a common phenomenon in mammalian evolution. For example, the brain of the raccoon, when compared with that of a number of other carnivores, exhibits a relatively large development of the forepaw representation within its somatic sensory system. Such a differential neural development may be associated with an unusual development of specific activities within the animal's behavioral repertoire. Thus, the raccoon uses its forepaws to an unusual degree in its manipulation and palpation of objects and surfaces in its environment.

In order to advance toward an understanding of the role that differential neural enlargements might play in adaptive behavior, and more specifically, in the processing of sensory information, studies were undertaken of the electrophysiological and morphological characteristics of the somatic sensory system of the raccoon (*Procyon lotor*).

So far we have traced the somatotopic organization at each of the successive neural levels (dorsal root, bulbar, thalamic, & cerebral neocortical) within this system. This was made possible by the combined use of a variety of electrophysiological and neuroanatomical techniques.

Methods

A. *Electrophysiological mapping experiments*. *Micro*electrode recording techniques were used in sampling evoked activity from single neural units within dorsal roots (10 animals), cuneate and gracile nuclei of the medulla (16 animals), and ventrobasal nuclear complex of the thalamus (12 animals). *Macro*electrode recording techniques were used to sample evoked activity at the surface of the somatic sensory cerebral cortex (20 animals). Sampling of neural activity at each level was carried out by mapping procedures. Mapping involved the systematic and thorough exploration of a volume of tissue by the recording electrode in a search for neural units activated by concomitant mechanical stimulation of the skin, muscles, and joints. The electrophysiological mapping technique is essential for any complete study of projections between two nuclear regions. The location of the exploring electrode tip was marked directly on photographs of the dorsal roots and neocortex, and on drawings of stained sections of the bulbar and thalamic regions containing the electrode tracks. Identification of the responding loci in medulla and thalamus was achieved by either interpolation, or by Prussian Blue or microcoagulation marking methods.

B. Degeneration experiments. Thalamocortical somatic sensory connections were studied by retrograde degeneration methods. The representations of specific body subdivisions within the raccoon somatic sensory neocortex (SmI; Woolsey, Biological and Biochemical Bases of Behavior, 1958) are demarcated by cortical sulci, spurs, and dimples (Welker & Seidenstein, J. Comp. Neur., 1959, vol. 111). Seventeen of these small discrete SmI subdivisions were aspirated unilaterally in 13 animals. Total unilateral SmI ablations were performed in three animals. Thalamic regions showing retrograde degenerative changes in these cases were identified histologically on alternate serial sections stained for cell bodies (thionin) and myelinated fibers (hematoxylin). Extent of neocortical lesions and thalamic degeneration was reconstructed from these sections and depicted on drawings. Myelinated fiber tracts connecting dorsal root, bulbar, thalamic, and neocortical somatic sensory regions were traced by the Marchi method in six animals.

C. Quantitative and descriptive neuroanatomical studies. In order to more accurately assess the relative degree of enlargement of each of the nuclear regions within the somatic sensory system, quantitative measurements are being made of the nuclear volume (Dornfeld, Slater, & Scheffe, Anat. Rec. 1942, vol. 82) and cell density (Agduhr, Anat. Rec., 1941, vol. 80) of functionally homologous regions at each of the cellular levels. These measurements were made on serial sections of these regions stained for cell bodies. The location of the forepaw representation at each level was ascertained previously by the electrophysiological recording and degeneration experiments described above. Description of certain cyto- and myeloarchitectonic characteristics, and of the organization of fiber tracts within the somatic sensory system are being prepared from study of serial sections of normal and experimental material stained by various methods (Bodian, Golgi, Marchi, Nissl, Weil).

Results

Projection of somatotopic pattern within the medial lemniscal system. Information from somatic sensory receptors passes into several pathways within the central nervous system. The intraspinal reflex, spinocerebellar, spinothalamic, and lemniscal pathways and their associated nuclei constitute four major channels for such information. The present investigations are concerned with the last of these, the medial lemniscal system, as described by Rose & Mountcastle (Handbook of Physiology, Neurophysiology, 1959, vol. 1). Within this system, somatic sensory receptors from one side of the body whose cell bodies are situated in homolateral spinal ganglia, send their impulses through the fibers of the dorsal roots. The topographic organization of the somatic sensory receptors from the forelimb is disrupted to a considerable degree within the dorsal roots (C6-T2), and, therefore, also within the dorsal

root ganglia. Thus, there is considerable overlap in the receptor fields of adjacent dorsal roots such that receptors from forepaw digit 3, for example, send their input through at least three adjacent roots (C7, C8, T1). This overlapping of input from adjacent peripheral body regions is also evident among the rootlets within any one of the five dorsal roots studied. The dorsal roots and their ganglia are not organized, therefore, in terms of projections from specific peripheral body parts as are synaptic regions at other nuclear levels of the lemniscal system.

The dorsal root fibers pass up the ipsilateral dorsal columns to terminate within the cuneate and gracile nuclei of the medulla. The relative lack of specificity of organization of the pattern of representation found among fibers of the dorsal roots also persists in the dorsal columns, but within the gracile and cuneate nuclei of the medulla a resorting evidently takes place since the somatotopic pattern there is less diffuse. Thus, for example, the representations of the palmar surfaces of adjacent digits of the forepaw do not overlap with one another as they do in the dorsal roots. The bulbar representation of any small subdivision of the body surface exhibits an organized somatotopic pattern which is similar to that at the periphery, but the overall organization of the representations of all body subdivisions considered together is considerably distorted in relative size, shape, and position. As a general tendency, in the dorsoventral (DV) dimension the more distal body regions within a particular subdivision are represented dorsally within the cuneate and gracile nuclei, whereas the more proximal regions are represented more ventrally. However, due to differential enlargements and distortions of the several subdivisions, there are numerous exceptions to this rule. In the mediolateral (ML) dimension, the projections from the tail and hindlimb are located near the midline, whereas the forelimb is represented laterally. This general organization in the ML and DV dimensions is probably predisposed by the mediodorsal displacement of dorsal root fibers from any given spinal level by the entering fibers from the successively more cephalic dorsal roots.

A large proportion of the cuneate nucleus contains the representations of the palmar surfaces of the forepaw digits, that for each digit projecting to its own specific, anatomically discrete, subnucleus. These digital subnuclei are separated from one another by fibrous laminae. The electrophysiological recording studies of the medulla show that the ML and DV organization of projections from the mosaic of body receptors are generally similar at several anteroposterior (AP) levels within the main body of the dorsal column nuclei. This may indicate a reiteration of input from a particular body locus upon cells in successively more rostral portions of the cuneate and gracile nuclei.

Second-order axons which originate from cell bodies in the bulbar nuclei swing ventrally and medially as internal arcuate fibers, cross the midline and become arranged (just above the pyramidal tract) alongside fibers from the trigeminal second order nuclei. This composite fiber tract constitutes the medial lemniscus and passes forward through the brain stem, reaching the ventroposterolateral aspect of the dorsal thalamus, and, turning dorsally, terminates within the ventrobasal nuclear complex. The mediolateral organization found in the bulbar nuclear groups is maintained among the fibers of the medial lemniscus after their arcuate decussation. The shift of these fibers across the midline results in the hindlimb and tail representations projecting to the more lateral aspect of the thalamus while the forelimb projects to more medial thalamic regions. A general DV inversion appears to take place in the pattern of representation between the medulla and the thalamus since the representations of distal portions of the body subdivisions, which are situated more dorsally in the bulbar nuclei, come to lie more ventrally within the ventrobasal complex. It is not clear how or where this DV inversion of the pattern takes place between the bulbar and thalamic levels. As within portions of the cuneate and gracile nuclei, the thalamic representations of various adjacent, but discrete, peripheral body regions are also separate and distinct, and show little or no overlap in projection. The forepaw region of the ventrobasal complex is also relatively large and is composed of several subnuclei, each of which contains the representation for the palmar surfaces of one of the five forepaw digits. In contrast with the topographic organization of the projections within the cuneate and gracile nuclei, those within the ventrobasal complex do not show marked serial replication in any one dimension. Thus, the thalamic representation of contralateral somatic sensory receptors more clearly resembles their actual somatotopic organization at the body surface.

Efferent axons from the third order cell bodies within the ventrobasal complex pass dorsolaterally to enter into the internal capsule. These radiations terminate at the fourth level of the medial lemniscal system within the SmI cerebral cortex. The fidelity of the somatotopic organization existing within the thalamic ventrobasal complex is preserved in SmI, except that the three-dimensional organization of the thalamic pattern is transformed into a two-dimensional cortical pattern. The distal portions of the body are represented more anteriorly at SmI, whereas they were situated more ventrally within the thalamus. The more proximal body regions, on the other hand, are located caudally in SmI, whereas they projected dorsally within the ventrobasal complex. The pattern of organization reverses itself in the ML dimension between thalamus and neocortex so that the hindlimb

and tail come to lie near the midline and the head more laterally. How and where this ML reversal takes place is not clear.

Within the medial lemniscal system, the spinal ganglia and dorsal column nuclei are ipsilateral, and the thalamic and neocortical regions are contralateral, to the peripheral somatic sensory receptors located on one side of the body. Zollman & Winkelmann (J. Comp. Neur., 1962, vol. 119) have shown that the density of the receptors in the glabrous skin of the raccoon forepaw is relatively high. The present investigations have shown that these receptor regions of the forepaw have absolutely and relatively large representations at dorsal root, bulbar, thalamic, and neocortical levels of the medial lemniscal system. These representations are also characterized by relatively small size of single unit receptive fields and, at all but the dorsal root levels, by the non-overlapping somatotopically organized segregation of individual digits within specific nuclear subdivisions. At bulbar and thalamic levels, these relatively discrete nuclear subdivisions are separated from one another in part by afferent fibers arriving from the next lower level. Homologous, discrete cellular aggregations also exist at the SmI neocortex, but they are organized morphologically in a different manner. Thus, adjacent cortical gyri, with distinctly different projections from the body periphery via medulla and thalamus, are separated from one another by relatively inactive "athalamic" sulcal cortical zones rather than by fiber laminae. Moreover, the neocortical organization is a vertical columnar one with its afferents arriving in parallel arrays. Each cell in a particular column apparently is activated by the same peripheral receptive field (Mountcastle, J. Neurophysiol., 1957, vol. 20). Such an organization does not seem to exist within the thalamic ventrobasal complex, but several cells within the AP cell columns of the cuneate and gracile nuclei appear to be activated by similar peripheral receptive fields.

Reports regarding the topographic organization of peripheral projections at each of these neural levels have been prepared for dorsal roots (Pubols & Welker, Anat. Rec., 1963, vol. 145), medulla (Johnson, Anat. Rec., 1963, vol. 145), thalamus (Johnson & Welker, Physiologist, 1961, vol. 4), and cerebral neocortex (Welker & Seidenstein, J. Comp. Neur., 1959, vol. 111).

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PRINCIPLES AND PROBLEMS OF FUNCTIONAL MORPHOLOGY

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by

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PHOTOELASTIC STUDIES ON THE FUNCTIONAL STRUCTURE OF BONE.

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The locomotor system of most terrestrial vertebrates reminds the engineer of a relatively slowly moving machine. Inertial forces generally do not play a significant role. The individual stages of slow movements may then be considered as a series of static situations. It thus appears justified to analyze the skeleton primarily as a static structure.

The loading pattern of most skeletal elements reflects the important fact that loads are imposed via joints. The forces may then be calculated from the equilibrium conditions at the joints. The loading pattern implies the imposition of bending stresses in the bones.

The degree of adaptation of bone, or the extent to which bone form and structure reflects this, forms an interesting problem in comparative anatomy.

Adaptation to static function implies that the mechanical strength is attained by minimal building material. The strength may include a certain "safety factor", as long as this is equally large in all parts of the element. This means that a structural component formed in this manner must be a body of uniform strength.

A body of uniform strength represents a minimal construction. Two such are known:

(1) Absolute minimal construction. This implies that for a given loading the stress is maintained at a minimum value, in part by a structural redesign of the unit.

(2) Relative minimal construction. This represents an intermediate condition showing some adaptation, though not a perfect fit.

It seems useful to explain this further. The stress in a supporting element such as a column may vary widely depending upon the loading pattern. If the load is axially arranged, i.e. coincides with the geometrical axis of the column, the resultant stresses will be even across the cross-sectional surface (stress equals total load divided by cross-sectional surface). If the load vector does not coincide with the column axis, the column will be subjected to bending stresses, involving compression on the loaded side and tension on the opposite side. These are additive to the major compressive stress and thus result in an unequal stress profile, higher on the pressure than on the "tension side". For this reason pressure stresses induced by excentric loads will always be greater than those due to axial loads. The tensile stresses may acquire extremely high values

with deviation of the effective line of the force from the geometrical axis.

The strength of a structural element is also determined by the properties of the material. It must be balanced in such a manner that the stresses do not achieve fracture values. The greater the loading of an area, the more material of a given strength is required.

Distribution of material in skeletal elements may be determined by the examination of cross sections, or more effectively, by x-ray pictures of bones. The distribution of the material may be measured exactly by means of a densitometric survey.

The stress values can be determined by means of photoelastic studies. These studies utilize models of plexiglas or other plastic. Such materials become birefringent as the material is strained. Stress deforms the spherical elementary particles into ellipsoids, with elliptical cross sections. The short and long elliptical axes lie in the direction of two normal strains σ_1 and σ_2 in the section plane. Transmission of polarized light brings out glowing bands in the spectral colors. Within the region of a monochrome band the difference of long and short axes of the deformation ellipsoids is equal. Such isochrome bands connect all sites of equal normal strain difference $\sigma_1 - \sigma_2$. These isochromes are repeated periodically in different orders of magnitude. Order of magnitude is proportional to the degree of elastic deformation of the material. The lines thus reflect the level of normal strain.

In general the stress level of the material increases in proportion to the increase in normal strain. It is thus possible to generalize that the order-value of an isochrome reflects the material loading at a given site.

Past examinations of plastic models of skeletal elements by this method have correlated well with the results of x-ray - densitometer studies.

These tests are only applicable to the relative minimum construction of a skeletal element; the absolute minimum construction must be evaluated in terms of two parameters. The first is the over-all shape of the bone, while the second reflect its internal structure.

The minimal construction of the bone may again be tested by means isochrome patterns. A bone will be formed with an absolute minimum of material if the loaded bone model shows a minimum number of isochrome orders upon examination. Examinations confirm that minimal construction will be achieved if the bone may be loaded axially. Yet the initially stated reasons suggest that

at least the long bones generally are exposed to a, possibly minor, bending stress.

The structure of bone may be evaluated on several different levels. I will here take up only the architecture of the substantia spongiosa. This involves the following problem: External reasons imply that a loaded body must have a particular dimension and shape. It need not be solid, and its interior may be formed of a framework of plates, bars or tubes. What must be the arrangement of elements in order to obtain maximal strength for minimal density?

The answer remains the same. In order to achieve minimal stress for a given load, each element must be loaded axially. The direction in which the structural elements must be placed in order to achieve this axial loading may again be determined from a photoelastic investigation. The normal strain trajectories of a loaded body indicate the direction of maximum and minimum strains σ_1 and σ_2. The trajectories are represented by two groups of curves that cross each other at right angles. Often one group represents the pressure and the other the tension strains, though all of them may be either pressure or tension strains.

A stressed plastic model shows brightly shining lines in transmitted polarized light. Areas in which the directions of σ_1 and σ_2 are oriented in parallel to the oscillation plane of the polarized light stayed dark. Rotation of the polarizing planes will brighten some previously dark areas, while other (light) areas darken. These dark bands, the isoclines, wander all over the model when the polars are turned from 0° to 90°, thus indicating the directions of the normal strains at each point. The sum of all directions of normal strains, drawn in a contour sketch of the model, represents the trajectorial pattern. Photographical methods can facilitate the drawing work.

Each type of stress involves a specific trajectorial pattern. Axial loading yields trajectories parallel to each other and parallel, or rectangular, to the axis of the stressed body. In bending stress trajectories form characteristic arched systems bending around specific points.

There are two orders of such singular points: (1) Attractive singular points that are surrounded by the (concave) arches of the strain trajectories, and (2) Repulsive singular points to which the convex portions of the trajectorial bends are directed.

All singular points are hydrostatic points in that the strains will be equal in all directions of the observed sectional plane. Here there are no defined directions of the normal strains σ_1 and σ_2. These points and their immediate vicinity then have lower stresses than do adjacent sites.

Bending loads induce an attractive singular point at the site of maximal bending stress, and a repulsive singular point at a site of minimal bending stress.

Spongy bone will have a functional structure when the structural elements of the substantia spongiosa are arranged like the normal trajectories in a correspondingly loaded homogenous model.

Indeed all previously investigated spongiosa structures actually do confirm the theoretically expected and experimentally demonstrated spongiosa formation.

The mammalian vertebral body, stressed axially, shows rectangularly intersecting spongiosa systems arranged parallel to the borders. The long bones, stressed by bending forces, show the typical, arched, cancellous bundles near their epiphyseal ends. These suggest that there is an attractive singular point somewhere near the middle of the diaphysis. Very similar arches are found in the avian bill, often stressed in bending.

The remarkable thing about both examples is there is no bony substance in the proximity of the singular points. These lie relatively in the marrow cavity of the large bones and in the central cavity of the avian bill.

I know of only one example of a repulsive singular point reflected in a spongiosa: the ankylotic knee joint in man. The repulsive singular point here provides the reason why the cross section of the bone increases disproportionately to the bending moment, giving a decrease of strain. A very delicate spongiosa substance then forms at the singular point.

It may be stated in conclusion that photoelastic experimentation has in a whole series of cases permitted demonstration of the adaptation of vertebral bone to mechanical loads.

FUNCTIONAL ANALYSIS OF CONTRACTIVE TISSUES. E.J.Slijper, Zoological Laboratory, University of Amsterdam, Netherlands.

The subject covers such a large field of research, that I shall have to confine myself to one single aspect of it: the microscopic anatomy of the striated musculature of Vertebrates, and especially the question of the staying-power.

It has been known for a long time that in this musculature red and white muscle fibres can be distinguished, depending on the presence or absence of myohemoglobin. There may be either separate red and white muscles or the two types may be found as separate fibres or fibre bundles in the same muscle. Several authors, as for example BOSIGER (1950), KRUGER (1952), GRAY (1958), BREAKKAN (1956), GEORGE c.s. (1958, 1961), RODAHL c.s. (1962) and BODDEKE c.s. (1959, 1963) have pointed to a great number of other characteristics connected either with the red or with the white colour.

In the most typical cases the white fibres have a large diameter, low sarcomeres, a small amount of sarcoplasm, granula and mitochondria, a comparatively high content of water and "Fibrillenstruktur" in the sense of KRUGER with thick innervating nervous fibres and "terminaisons en plaque". They are characterised by the presence of glycogen, by a low content of lipase, vitamin B, succinic dehydrogenase and cytochrom oxydase, and a high content of niacin. The metabolism is supposed to be principally anoxidative (combustion of glycogen). There is only a small amount of connective tissue with a comparatively small number of capillaries. The red fibres have a small diameter, high sarcomeres, a large amount of sarcoplasm, granula and mitochondria, "Felderstruktur" in the sense of KRUGER with thin innervating nervous fibres and "terminaisons en grappe" and a comparatively low content of water. They are characterised by the presence of fat and by a large amount of lipase, vitamin B, succinic dehydrogenase and cytochrom oxydase, as well as by a low content of niacin. The oxydative metabolism is supposed to depend on the combustion of fat. There is a comparatively large amount of connective tissue, which is highly vascularised.

About fifteen years ago it was almost generally accepted that the white fibres showed short and quick twitches, tetanic contractions, governed by the all or none principle. The red fibres were supposed to show prolonged tonic contractions, which could be produced by a summation of nervous stimuli. More recent researches, however, have shown that the matter is much more complicated. It has been demonstrated that in muscles of rats a great number of fibres can be found, which are in many respects intermediate between the two types (miss ROEDE; unpublished). Further it was shown that the difference red-white does not always correlate with the difference narrow-broad (BODDEKE c.s.), nor with the difference "Fibrillenstruktur-Felderstruktur" and the difference in the termination of the innervating nervous fibres or with the speed of the contraction (RODAHL c.s.). According to BULLER (1960), in the cat the speed of contraction is determined principally by the nature of the innervating nerve.

These and other facts lead to the supposition that only some of the above mentioned anatomical characteristics might be connected with the speed of contraction, whereas others might be connected with its duration or with the staying-power of the

muscle involved. These three functional characteristics may correlate in such a way that, for example, a muscle with a low staying-power shows quick and short tetanic contractions, but it must not be supposed a priori that this will always be the case. It is highly probable that there are also quick contracting muscles with a high staying-power.

Some authors suggested that at least the staying-power might depend on the presence or absence of myohemoglobin (i.e. on the colour of the muscle fibre), as well as on the presence or absence of fat, lipase, some other enzymes and on the vascularization of the muscle.

The staying-power of the muscles is supposed to be connected in the first place with the mode of life of the animals involved. Among human sportsmen two different types may easily be distinguished: the sprinters and the stayers. The same thing is known with regard to horses and dogs. The sprinters are built and trained for a maximal development of energy and speed on a short distance and during a short time, whereas the stayers are built and trained for the development of a moderate amount of energy and a moderate speed on a long distance and during a long time.

The difference between sprinters and stayers can also be applied to different species of animals. If we do this, we must, however, be aware of the fact that the inverse correlation between distance (or time) and speed, may be only applied to different types of animals within the limits of the species. The absolute speed developed by different species depends not only on their qualities with regard to the staying-power, but also on other factors as, for example, on the general structure of the animal and especially on its absolute size.

According to our present knowledge, in mammals, birds and fishes the speed of the animals apparently increases with their absolute size, although it does not increase proportionally to their linear dimensions. Moreover, in mammals and birds the speed decreases above a certain size, because the development of the musculature and the resistance of the skeleton do not allow the animals to use a type of locomotion in which a very high speed can be attained. In mammals this is the gallop which, for example, is not observed in elephants. The average maximum speed of small mammals (rats, mice, squirrels) is 10 km.p.h.; in wolves and jackals it is 50 km.p.h., in deer and antelopes 65 km.p.h., in horses and their relatives 65 km.p.h., but in giraffes, camels and hippopotamuses 45, in rhinoceroses 35 and in elephants generally below 35 km.p.h. Hares, however, can attain a speed of 65 and cheetahs a speed of 100 km.p.h.

A.V.HILL has shown that the power developed by the musculature of animals mainly depends on its bloodsupply, which means that it varies with the square of the linear dimensions. The fuel reserves of an animal, however, vary with the cube. This means that generally a big animal will show a certain predisposition to be a stayer, whereas a small animal has the better qualities to be a sprinter, especially because a small animal will also better overcome inertia than a large one. Consequently the small animal will be better adapted to a quick start. Nevertheless the speed of a large animal with the qualities of a stayer may be higher than the speed of a small animal that is a

sprinter.

Typical sprinters are cats, tigers or leopards who may remain almost motionless for a long time and then suddenly pounce upon their prey with a maximal outburst of energy. In this respect they differ in a marked way from wolves or jackals who are typical stayers, just as their prey (antelopes, deer). Rabbits, who fly into their holes mostly over short distances with a sprint of 45 km.p.h. behave in a quite different way from hares who fly over very long distances with a speed of 65 km.p.h. Stayers par excellence among the mammals are the horses and their relatives. The Przewalski horse can develop a speed of 60 km.p.h. over a distance of 12 km and the kulan a speed of 70 km.p.h. over a distance of 10 km. At a speed of 50 km.p.h. the distance is practically unlimited. Typical stayers are also camels and especially dromedaries.

Birds, and especially migrating birds, are in principle stayers; the record is kept by the American Golden Plover, Charadrius dominicus, with a distance of 3600 km without a landing. Fishes, on the contrary, are in principle sprinters. GRAY (1953) demonstrated that from the resting condition a trout may attain its maximum speed in 1/20 of a second. Very pronounced sprinters are some diurnal carnivorous fishes living in stagnant waters or in waters with a weak current, such as the pike, the perch and the three- and ten-spined stickleback. They seize their prey with sudden sprints and, in case of danger fly into the nearest hiding place. Among marine fishes plaice, flounder, halibut and other flat fishes are typical sprinters. Pronounced stayers are, for example some pelagic carnivorous marine fishes such as the mackerel, the tarpon and the albacore, as well as migrating fishes like the salmon (largest distance 3840 km.). Other stayers live in streams like the rainbow trout or they are nearly continuously engaged in feeding like the carp (during 22 of the 24 hours of the day).

As the result of very elaborate researches on the organ weight of 3734 wild animals (chiefly mammals), CRILE (1941) and his collaborator QUIRING found that the difference between sprinters and stayers can also be shown by the development of some interval organs. In some cases this was demonstrated by the development of the heart, the heart weight of a wolf being 314 g and that of a jaguar with the same body weight only 185 g. In most cases, however, the development of the heart and lungs is so much influenced bythe basal metabolic rate of the animals, depending on the climate in which they live, that this overshadows the differences in their qualities as sprinters or stayers.

In this respect the development of the thyroid gland and the adrenal glands and especially the mutual weight relation between these two endocrine organs may be much more important. Generally the thyroid regulates the long term development of energy, whereas the adremals are responsible for the sudden outbursts of energy as we know them, for example, from fright and fear. The weight of the thyroid, however, may also be influenced by the climate in which the animals live. The largest development of the adrenals and of the sympathetic ganglia and nerves attached to these organs, was found in lions, leopards, tigers and other cats, who are typical sprinters. The adrenals are much less developed in wolves and antelopes, who have the characteristic qualities of

the stayer.

However, not in every respect does the adrenal weight give a good indication about the sprinting qualities of an animal, because it depends not only on the size of the medulla but also on the development of the cortex. The thickness of the cortex varies in a marked way with the circumstances in which the animals live with regard to stress. Consequently in rodents, who will fight a lot among themselves, the adrenal weight is supposed to be comparatively high, because of a very well developed cortex.

With regard to the musculature itself there are a number of very significant indications that the colour of the muscle fibres and some other characteristics (the presence or absence of fat and some enzymes), are connected with the staying-power of the animals. In race-horses the amount of white musculature is appreciably larger than in draught-horses; VON BUDDENBROCK (1961) pointed to the fact that the rabbit (a sprinter) has a great percentage of white muscles, whereas in hares (stayers) the amount of red muscles is comparatively high and BOSIGER (1950) showed that the great pectoral muscle of the starling (a migrating bird with very well developed flying abilities) consists of red muscle fibres, whereas in the hen (a heavy short distance flyer) the great pectoral is a white muscle. The quail appeared to be intermediate with respect to the percentage of red and white fibres as well as with respect to its mode of life.

Fishes make a very good subject for researches in this field because they are a much more homogeneous group with regard to their body structure and to their environment, than mammals and birds. This is the reason why BODDEKE c.s. (1959) made researches into the musculature of 21 different Dutch fresh water fishes. They found two different types of muscle fibres, viz.: a) broad, white fibres with low sarcomeres, a poor vascularization and without myohemoglobin and fat; b) narrow fibres with fat, with high sarcomeres and a great amount of capillaries. These fibres are mostly red because of the presence of myohemoglobin. Only in some special cases and in animals that show only a very small number of these fibres, they may be white.

The relation between the diameter of the fibres and their myohemoglobin content is not yet clear in every respect. Narrow fibres, however, will have a proportionally larger surface area; consequently the number of capillaries and the oxygen supply per unit of content will be larger than in broad fibres. Low sarcomeres may be supposed to be connected with quick contractions because the surface area of their components is larger than in high sarcomeres. Consequently in low sarcomeres the interaction between the components is the more adventageous.

It has already been pointed out that in principle fishes are sprinters. This explains why in all fishes examined by far the majority of the musculature of the body consisted of broad white fibres. Typical sprinters as the pike, the perch and the sticklebacks had practically only broad white fibres. This was also the case in typical marine sprinters such as the flat fishes (BODDEKE, 1963). The so called sneakers, like the eel and the thunderfish, have an intermediate position between sprinters and stayers with regard to their mode of life. They have no laternal line strip, their muscle fibres are white and broad, but some of

them may show rather high sarcomeres, pointing to slower contractions.

A very well developed lateral line strip, consisting of narrow red muscle fibres is found in the so called crawlers, like the rudd, the bream, the dace and the tench. Generally the prey of these animals is small and sluggish. In case of danger they do not fly into a hiding place, like the typical sprinters, but they fly with a zigzag movement in or into the open water. Consequently they show a number of characteristics of the stayer without being pronounced stayers.

The pronounced stayers, like the salmon, the rainbow trout and the carp have a very well developed lateral line strip and a subcutaneous layer of narrow red muscle fibres. Moreover they show a number of narrow fibres between the broad ones (mosaic structure). A red lateral line strip and mosaic structure has also been found in a number of marine stayers, such as the mackerel, the marlin and the tunny.

These and other researches have shown that the colour of the muscle fibres and some other characteristics like the presence or absence of fat and certain enzymes, are connected with the staying-power of the muscles, although the colour will not always be connected with this staying-power. In aquatic mammals like the Cetaceans, for example, the dark red colour of the musculature may be much more influenced by their oxygen storing ability with regard to prolonged diving, than by their staying abilities with regard to ordinary swimming.

The correlation of other anatomical characteristics of the musculature with physiological abilities as, for example, the speed of contraction or the duration of the contraction is still an open question. In future researches special attention should be paid to these questions.

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THE USE OF MOTION PICTURES FOR THE FUNCTIONAL ANALYSIS OF VERTEBRATE LOCOMOTION. Milton Hildebrand, Dept. of Zoology, University of California, Davis, California.

Before the turn of the century, most anatomists concerned themselves with describing representatives of the major systematic groups of animals. Gradually, since World War I, attention has shifted to representatives of the major functional groups, and interpretation of structure is supplementing description. Locomotor and feeding mechanisms have received particular attention. Too often, however, the parts of the body have been studied independently, and function has been inferred from structure or estimated by casual observation. Theoretical aspects of posture and locomotion have been discussed, much to the benefit of our understanding, yet theory has at times gotten ahead of knowledge. For example, loading of the foot of the moving ungulate has been estimated without knowing the angle of impact. Rate of muscle contraction has been related to body size without knowing actual stride rates of cats and dogs, let alone of mice and elephants. Swinging limbs have been likened to pendulums without knowledge of the characteristics of their oscillations.

Present day morphologists are placing ever more emphasis on the study of movements of living animals so that we can modify, refine, and extend our interpretations of structure. The moving picture camera is admirably suited for the analysis of motion, and it is not surprising, therefore, that this instrument has recently been profitably used by many laboratories in the Netherlands, England, America, and elsewhere.

I do not have the knowledge or experience to attempt a survey of the many ways that moving pictures can be used by the morphologist. However, in presenting certain results of my own work it shall not be my purpose to detail the findings of one man's research, but rather to illustrate some of the ways that motion pictures can be used for analyses of vertebrate locomotion that permit broad comparisons, new correlations, and insight into heretofore unstudied problems.

Motion pictures are most often used by morphologists to record the relative positions in space of rapidly moving structures. The naked eye cannot see the tongue of a feeding chameleon or the feet of a scampering mouse. Before the invention of the camera, artists consistently misrepresented the positions of the limbs of running horses.

Motion pictures can also measure events in time. The relative speeds of various actions are readily established. If a scale is introduced into the field of the camera, then actual velocities and acceleration rates can easily be calculated.

Another advantage offered by the camera is less scientific but is nonetheless the scientist's reward. Motion pictures reveal not only the fact, but also the beauty of animal movements. Having examined thousands of feet of film showing moving animals in slow motion, I can conjure into my mind's eye the incredibly

lithe body of the dashing cheetah, the rippling flanks of the leaping impala, and the weightless bounds of the spring haas. I have been charmed by the grace, rhythm, and agility of my subjects.

When a horse, lion, or elephant walks, there is an instant when the body is supported by both hind feet and one forefoot. This is followed by a support pattern of one hind foot and the forefoot on the same side of the body. Then comes support by one hind and both forefeet, and so on. There are eight such support patterns before the cycle repeats. Long ago, students of animal locomotion devised a graphic method for representing the gaits of animals by series of eight stylized diagrams. Such a record was called a "footfall formula" by A. B. Howell. The first comprehensive study of animal gaits was made in the 1880's and 1890's by Eadweard Muybridge, who used a battery of 12 or 24 still cameras, triggered in sequence, to record the movements of about 25 kinds of mammals. He established seven footfall formulas for quadrupeds and named seven gaits on the basis of these formulas. Occasional departures were recognized but were considered to be irregular or abnormal gaits and were not named.

In 1944, Howell published a survey of animal gaits as a chapter in his book, "Speed in Animals." He mentioned few animals omitted by Muybridge and emphasis was again on the horse. Twelve gaits were distinguished by footfall formula and named. He also recognized irregular gaits and variations.

A difficulty with the terminologies of Muybridge and Howell is that two animals, each moving according to the same footfall formula, can in fact be using visibly different gaits because of differences in the relative durations of the various support patters. The audible footfalls might be different and also the speed of travel.

The only other man who has made a comprehensive survey of gaits is Paul Magna de la Croix, an Argentinean who, between

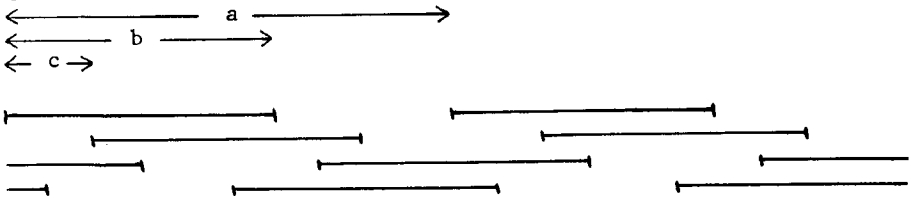

Figure 1

1927 and 1936, published about 17 papers on locomotion. He did not stress the footfall formula and groped toward a better method of analysis. He did not use moving pictures to time locomotor events, however, and although he was perceptive and imaginative his goal was not fully achieved.

My films are studied frame by frame in a viewer equipped with a frame counter. As the film is advanced a record is prepared which

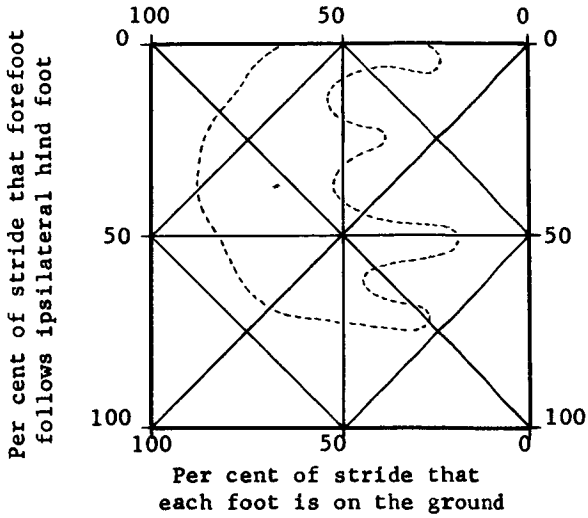

Figure 2

I call a "gait diagram." On graph paper, four adjacent horizontal rows of squares are assigned to the four feet. Vertical rows represent successive moving picture frames and hence equal intervals of time. A square is "lined in" if its respective foot is on the ground. Figure 1 shows the gait diagram for two strides of a walk of a typical ungulate. The grid is deleted. Time advances from left to right. Contacts of the hind feet are shown on the outside, those of front feet on the inside; contacts of the left feet are shown above, those of the right feet below. This record includes all the information contained in a footfall formula and also shows the duration of each pattern of support. It shares with the footfall formula the difficulty that various different gaits cannot be conveniently compared.

At this point in the analysis it is necessary to distinguish between symmetrical and asymmetrical gaits. Let us consider symmetrical gaits first. These have the footfalls of the two feet of a pair (fore or hind) evenly spaced in time, like the footfalls of a walking man. The various walks, trot, and pace are of this nature. Disregarding trivial idiosyncrasies of individual strides, the right and left members of a pair have the same intervals of contact with the ground, and the contacts of the forefeet are usually (but not always) of the same duration as the contacts of the hind feet. Only two variables remain. One can be expressed as the per cent of a complete cycle of motion (or stride) that each foot is on the ground (100 b/a, Figure 1). A slowly moving tortoise may have each foot on the ground 85 per cent of the time. A fast-trotting horse has each foot on the ground about 22 per cent of the time. This variable is not a direct measure of speed, but it relates to speed.

The other variable can be expressed as the per cent of the stride interval that the footfall of a forefoot lags behind the

footfall of the hind foot on the same side of the body (100 c/a, Figure 1). If there is no lag, the limbs on the same side swing together and the animal is said to be pacing. If the lag is 10 to 15 per cent, then, for this gait and the two to follow, the footfall of a hind foot (arbitrarily adopted as a reference) is followed by the footfall of the forefoot on the same side of the body. These are, therefore, described as "lateral sequence" gaits. For gaits having this particular lag of forefoot after hind foot, the footfalls on the same side of the body come as couplets, 1,2--3,4. I call such gaits "lateral couplets gaits." Many ungulates and carnivores walk in this way. If the lag of each forefoot behind the ipsilateral hind foot is 25 per cent of the stride, then the gait is an even four-beat gait, 1-2-3-4. The single-foot of the horse is of this nature. If the lag is 35 to 40 per cent, the lateral sequence is retained, but it is diagonally opposite feet that strike as couplets, 4,1--2,3. These are "diagonal couplets" gaits. When the lag is 50 per cent, diagonally opposite limbs swing together and the animal is trotting. If the lag is more than 50 per cent, the forefoot to strike next after a given hind foot changes from the ipsilateral (lateral sequence family of gaits) to the contralateral (diagonal sequence family of gaits).

It follows that symmetrical gaits can be expressed by two percentage figures. A 35-50 gait is a moderate trot, a 55-22 gait is a fast walk, a 28-5 gait is a running pace, and so on. We can now compare simultaneously as many gaits as we wish, because each can be represented as a spot on a graph.

Such a graph preserves all the information of the old footfall formulas and permits new insight into their relationships. Analysis shows that if the graph is ruled into 16 triangles, as shown in Figure 2, all plots falling within one triangle represent gaits having the same footfall formula, and each triangle has a different formula. Further, there is another and different formula at every intersection of lines and along every line between intersections. Forty-four footfall formulas are theoretically possible. In practice, the 250 plots recorded as this is written (many others will be added in time for the Congress) all fall within the dotted line drawn on Figure 2. It is probable that 16 of the footfall formulas are never used by any vertebrate. The formulas along the diagonal lines and at the intersections include the so-called irregular and abnormal gaits of earlier studies. We see that they are transitional gaits, not abnormal gaits.

The graph also preserves the information about the relative durations of the various support patterns which is present in the gait diagram. To illustrate: the graph is divided into four squares. Consider the square at the upper left. A plot falling at the center of this square represents a gait that exclusively uses support by three feet at a time. Every plot falling on the perimeter of this square represents a gait that never uses simultaneous support by three feet. A plot falling elsewhere in the square represents a gait having three-member support periods

which comprise a percentage of the total stride interval that is proportional to the position of the plot between the perimeter and center of the square. The relative durations of all other possible support patterns can be similarly determined. These variables are quickly established with the use of templates.

Motion pictures also make possible the assessment of variability. If twenty animals of the same kind and size move together over a level, even surface, and their gaits are individually recorded and plotted, the points usually cluster within a diameter of 5 per cent, as measured on the scale of the graph, and rarely does a plot fall as far as 5 per cent distant from the center of the cluster. The eye of a careful observer can detect differences in gaits that vary, when plotted, by about 10 per cent on the abscissa or ordinate.

It is clear from the foregoing discussion that instead of naming gaits according to footfall formulas, which may correspond to a single point on the graph or, at the other extreme, may range over the graph by 50 per cent along the base of a triangle, and by 25 per cent on the other axis, names should be assigned according to a grid system. On the abscissa I propose seven intervals of 10 per cent each, extending from 20 per cent to 90 per cent. These may be called very slow, slow, moderate, and fast walking gaits, and slow, moderate, and fast running gaits. On the ordinate, eight intervals of 12 1/2 per cent are satisfactory. These are the pace, lateral sequence lateral couplets gait, lateral sequence single-foot, lateral sequence diagonal couplets gait, trot, diagonal sequence diagonal couplets gait, and diagonal sequence single-foot. No animal has been found to use the diagonal sequence lateral couplets gait.

Combining the terminologies expressing variation along the two axes of the graph, gait names are established which are descriptive and unambiguous. Thus, a cheetah, but not a beaver, might use a fast, lateral sequence, lateral couplets walk; a charging elephant uses a slow, lateral sequence, single-footing run; monkeys commonly use the moderate, diagonal sequence, diagonal couplets walk.

Asymmetrical gaits have the footfalls of the two feet of a pair unevenly spaced in time, like the footfalls of a man with a limp. The gallops and bounding gaits are of this nature. The two feet of a pair may strike the ground at nearly evenly spaced intervals, or with increasing asymmetry until they actually strike the ground together. Again, disregarding idiosyncrasies of individual strides, the right and left members of a pair usually have equal intervals of contact with the ground. Often, however, the contacts of the forefeet are shorter than the contacts of the hind feet. Clearly there are more variables than for symmetrical gaits and the analysis must be modified accordingly.

A satisfactory method is to plot, on the abscissa of a graph, the per cent of a stride's total duration when one or both hind feet are on the ground. The point in time that is midway between the strike of the trailing foot of a pair (the first to come down

in each couplet of footfalls) and the lift-off of the leading foot of the pair will be called the "midtime." The lag of the midtime of the forefeet behind the midtime of the hind feet is now expressed as a percentage of the duration of the stride and is plotted on the ordinate. Appropriate symbols show the more subtle variables, the most important of which are the lead of the forefeet relative to the lead of the hind feet, and the duration of forefoot contacts relative to hind foot contacts. The result is a graph which is nearly as useful as the graph described for symmetrical gaits.

These methods, made possible by moving pictures, permit the interpretation of gait selection in relation to body size, conformation, maneuverability, ancestry, etc. Some 200 kinds of vertebrates are being compared, including amphibians, reptiles, and representatives of 13 orders of mammals. In some instances, gait selection seems related to phylogenetic affinity. Thus, regardless of size and conformation, the apes and monkeys favor the diagonal sequence gaits which are used by few other animals. Usually, however, gait selection is determined by functional considerations. The trotting walks and lateral sequence, diagonal couplets walks are used by animals with short legs or wide straddle because these gaits effectively maintain balance. Long-legged animals select the lateral sequence, lateral couplets gaits because interference among the limbs is then avoided. All large bulky animals gallop about the same way.

Thus far, my discussion has considered only the durations and relative timing of the contacts of the different feet with the ground. A next step, therefore, should be to determine the vertical and horizontal thrusts of the feet against the ground as the animal moves. This has been done by other investigators for several animals and should be done for many more. The camera can aid in making only part of the necessary observations but serves to indicate the sort of correlations we may expect to find. To illustrate this point, one would suppose that the ponderous elephant could achieve the most continuous support spacing its footfalls evenly in time. In fact, the animal places the feet by couplets. From the relative positions of feet and girdles it is evident that the animal walks so that the instants of maximum loading, not of the footfalls, are evenly spaced in time.

The camera also permits the rate of the stride to be correlated with the speed of the body, and enables the investigator to trace the paths followed by the feet (or other parts) of a moving animal relative to the ground or relative to another part of the body. This kind of analysis has scarcely been tried to date, and in the future surely will contribute new insight into the biomechanics of locomotion. The graceful antelope, dashing rabbit, charging rhinoceros, and scores of others, still hold many secrets which invite our attention.

NON-FUNCTIONAL ANATOMY. D. Dwight Davis, Dept. of Zoology, Chicago Natural History Museum, Chicago, Illinois, U.S.A.

Morphology has been called "frozen function." This is an apt description, but like most generalizations it should not be carried too far. The anatomist must assume, as a working hypothesis, that the structures he is trying to understand were molded by the functions they perform; but to demand a functional explanation for every structural condition is not good biology, and may lead to absurdity. The functional morphologist should always keep in mind the possibility that a specialized condition he is trying to explain may not have a functional explanation.

The difficulty is proving that a departure from the norm is not adaptive. How can we be sure there is not some obscure but important functional relation that has not yet been discovered? This is the familiar difficulty of trying to prove the absence of a correlation when we cannot be sure we know all the possible correlates. In such cases we are forced to fall back on probabilities. If we can find no functional explanation for a factor y, but can demonstrate a positive correlation with a factor x, which is obviously adaptively modified, then there is a strong probability that y is responding to its correlation with x, and not to direct functional demands. Familiar examples of such correlations are the allometric relations between body size and antler size in deer and mandible size in stag beetles, although in these cases it is at least debatable that the allometry of y is not functionally important.

The most obviously non-functional structures are vestiges of organs that have lost their function—the vermiform appendix in man, the splint bones in horses, the peglike tooth vestiges that occur in many mammals. The mechanism involved in such retrogressive changes is fairly simple, at least in principle. But things are not so simple with seemingly progressive changes, changes that seem to require the addition of something rather than subtraction of something already present. Such changes are unintelligible if the resulting condition is non-adaptive, unless they are somehow tied to other changes that are functionally important.

An important but largely unexplored mechanism capable of producing such effects is the growth field, which has been defined as "any influence during the development of an organism that produces a detectable pattern among the elements of any set of structures." Such influences include gradients, pleiotropic effects, the timing of local periods of differentiation and growth—anything that controls local growth rates. If all the elements of a given set of structures are affected, the result of a change may not be adaptive throughout the set—indeed, it is unlikely that it would be. Let us examine some examples of non-adaptive structures that seem to have been modified simply because they are

elements of a set in a growth field.

(1) The bears of the genus Ursus can be arranged in a sequence of increasing size, beginning with the American black bear and ending with the extinct European cave bear (Ursus spelaeus). The cave bear was about ten times as heavy as the black bear. In this size sequence the cheek teeth behave very much like the antlers of deer—there is an allometric relation between the dentition and organism size, which shows up in two ways. The number of premolars decreases steadily as organism size increases. In the black bear all four premolars are present, whereas in the cave bear only the last premolar is left and even it may be absent in the lower jaw. At the same time the crown pattern on the molars becomes increasingly complex as the size of the organism (and the teeth) increases. In the black bear the crowns of the molars are made up of fairly typical cusps, but in the huge cave bear the crowns of the molars are broken up into a great many little bump-like elevations—they are bunodont and rather piglike. Some paleontologists have assumed a functional correlation—that the food of the cave bear must have been piglike because its molars are—but the correlation between tooth size and tooth structure is a far more likely explanation. In another bear—the giant panda, which is no bigger than a black bear—the cheek teeth are enormously enlarged, and here all the premolars are present as in other small bears but the molar crowns are bunodont like those of the cave bear. In both the cave bear and the giant panda the size of the molars is functionally important, but the details of their crown pattern is merely a function of tooth size.

(2) Another example from the bears. In the right ventricle of the mammalian heart there are typically two large papillary muscles, an anterior and a posterior, that are associated with the chordae tendineae of the tricuspid valve. Now, in the bears instead of one anterior papillary muscle there are three, often with indications of even further subdivision. The chordae going to the septal cusp, which lie in the same region of the ventricle, are doubled in number, the ostium between the right atrium and the ventricle is elongated, the apex of the heart tends to be bifid, and even the descending branches of the left coronary artery are increased in number. Other parts of the bear's heart, even other parts of the right ventricle, are unmodified. We get the impression that a certain portion of the right ventricle has for some reason been pulled apart, extending or splitting up all the structures that happen to lie in this region.

This transverse expansion of everything in one localized area of the bear's heart may be functionally important, and it would be difficult to prove that it is not. But another correlation strongly suggests that it has nothing to do with the functioning of the heart. Parsons long ago suggested that the expanded and compressed types of aortic arch pattern in mammals is correlated

with breadth of thorax, and more recently Sawin showed that numerous differences in the thorax among breeds of rabbits can be attributed to growth differences involving whole regions of the thorax rather than isolated structures. In mammals with a compressed thorax, such as the cats and some of the ungulates, the heart is slender, whereas in mammals with a broad thorax, notably the marine mammals, the heart is broad. (The erect posture and altered position of the heart introduces new and different factors in man and the higher primates, where the thorax is also broad). A characteristic feature of bears as compared with more "typical" carnivores is the broadening of the whole body, including the thorax. It is highly probable that the heart is a part of a general growth field, and is broadened in the bears because the general growth field is broadened. Often the time of most rapid growth is not the same for all parts of a structure—the major growth field is subdivided into a hierarchy of smaller, more or less independent, units. If we knew the ontogenetic time-table in enough detail we might be able to explain why only a particular region of the heart, and not the whole heart, is affected in bears.

(3) Many mammals have a sesamoid bone in the abductor tendon at the base of the thumb. In the giant panda this radial sesamoid is enormously enlarged and functionally replaces the thumb; it is a highly adaptive specialization. In the hind foot a tibial sesamoid bone occupies a corresponding position in the tendon of the posterior tibial muscle. In the giant panda the tibial sesamoid is enlarged to almost exactly the same degree as the tibial sesamoid, although it has no special function and if we knew only the hind foot of the panda this enlarged bone would be very puzzling indeed. Obviously the radial and tibial sesamoids represent elements in a common growth field, despite their physical remoteness from each other. The tibial sesamoid is enlarged in the panda simply because the radial sesamoid is, not because its enlargement is in any way adaptive. Incidentally, this example suggests that the serial homology of the limbs, of which the older anatomists spoke, is more than just a typological fancy. The relationships between correspondences in the limbs may go much deeper than mere formal similarity of a pattern.

(4) The normal number of thoracolumbar vertebrae in the Carnivora is 20. The only known carnivore in which this typical number is reduced is the giant panda, which has only 18. This shortening of the vertebral column in the panda cannot be correlated with any functional requirements—it is not itself adaptive. There is considerable evidence that the whole axial region in the mammalian embryo is a single major growth field with an anteroposterior gradient. Minor growth centers appear to develop later within the major field. Any kind of "cephalization"—concentration of the gradient at the anterior end—disturbs the whole axial field, and in extreme cases results in shortening of the field. The most distinctive character of the giant panda is an extreme degree of cephalization, which is highly adaptive. It is evident that re-

duction in the length of the column in the panda has no direct functional explanation, but represents a consequential effect of extreme cephalization. In this animal, in fact, masticatory power has been increased at the expense of locomotor efficiency.

These examples are taken from a recently completed comparative study of the anatomy of the arctoid carnivores, aimed at discovering the morphogenetic mechanisms that lie behind the structural differences characterizing various representatives of this suborder. Over and over again during the course of this study anatomical features that could not be explained as functional adaptations made sense when they were interpreted as elements in a growth field where some other element was highly adaptive. The examples given here are only a sampling of many such relations that were encountered. Once they were looked for with the same diligence as is used in seeking functional relations, these "consequential differences" turned out to be unexpectedly frequent in the Carnivora. I am sure this would be true of any other large group of vertebrates.

The picture in the Carnivora suggests that the growth fields that are altered to produce adaptive changes rarely coincide with the limits of the structure that is undergoing adaptive modification. Unadaptive consequential changes in other parts of the growth field would tend to be buffered out if they reduce the overall efficiency of the organism, or they would limit the extent of the adaptive change, which could proceed only until an equilibrium between favorable and unfavorable effects had been reached. I suspect that far more consequential effects persist than is commonly realized. This suggests caution in interpreting all structural conditions in functional terms, particularly where no clear-cut functional relationship has been demonstrated.

THE FUNCTIONAL APPROACH TO SOME PALEONTOLOGICAL PROBLEMS.

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Fossil skeletal remains often show peculiarities unknown in living forms. As with the morphological diversity of more-or-less closely related groups of fossils, these features permit taxonomic separations and groupings and may be phylogenetically important even if their meaning is obscure. The functional approach permits a more meaningful interpretation, even though it must involve deduction rather than the experimental method. A paleontologist can only make indirect use of physiology, genetics and many aspects of functional anatomy. But he ignores their implications to his cost; and in return he can provide interpretations of time-sequences of millions or hundreds of millions of years in duration. It is proposed here to discuss a very few problems concerning fossil chordates from the speaker's personal fields of interest.

The earliest known (Ordovician-Silurian) chordates were well-provided with dermal bone and denticles (using that term in a broad sense). In many of these early groups no ossified endoskeleton is preserved. In others (e.g. the cephalaspids) the earliest members known are already well-ossified, and among the true vertebrates (except Selachii) heavy ossification in early members, with reduction in later forms, is frequently observed, as stressed by Stensiö. If one could project this apparent trend backwards in time, one would expect a long record of well-preserved ancestors. This is conspicuously not so, which suggests that bone-formation was an innovation. In this case, the functional approach must be physiological. One important possibility is that the calcium phosphate (hydroxy-apatite) of bone was, among other things, a reservoir of phosphate; it is well-known that resorption and new deposition affected many primitive forms. Another possibility is that animals becoming adapted to fresh-waters or brackish waters have to work harder to extract physiologically important cations, and that in these waters calcium is overwhelmingly predominant as compared with seawaters. In these cases heavy ossification may represent deposition of "ballast-constituents" in little-mobile tissues. Later members of many groups seem to have developed control of the amount of bone deposited.

A very good example of a "unique" structure is provided by the paired and median "electric fields" in cephalaspids as described by Stensiö. These are shallow depressions in the dorsal and dorsolateral endocranium, roofed in life by presumably flexible skin with a mosaic of tesserae. The very shallow cavities so contained communicate with the vestibular region of the auditory capsule by relatively wide canals in the endocranium. Stensiö, impressed by the formal resemblance to the pattern of nerves to the electric organs of Torpedo, identified the cephalaspid structures as electric organs. Westoll, and later Watson, interpreted the structures differently; both regard them as receptors

for trains of compression-waves in water.

The auditory capsule and its contained structures and the latero-sensory neuromast system are highly developed in nearly all early chordates, and the endocranial cavities strongly suggest that the acustico-lateralis tracts were very large in comparison with most living forms. Presumably information from water-borne vibrations, and concerning orientation, were of very great significance. In this respect recent work on the function of the semicircular canals, following the fully-mechanistic interpretation of Steinhausen, is most significant. Jones and Spells (Proc. Roy. Soc. B, 157, p.403) have applied the principles of dynamical similarity, and have demonstrated linear log-log. relations between the body-weight and (a) cross-section area of the semicircular canals, and (b) the mean radius of curvature of the semicircular canals respectively, for a considerable range of living tetrapods. A striking feature of their results is that the dimensions for fishes are significantly greater than those for other vertebrates of comparable body-weight. Judging from the cavities in fossils, early fishes may well have shown as great or greater disparity. These differences imply much greater sensitivity in fishes, which Jones and Spells relate to the relative fixation of the head, propulsive mechanism and the fact that fishes move through water.

This discussion thus leads naturally into that of locomotion of early fishes. There is now very clear evidence of the improvement of both the propulsive mechanism and of the control of pitching, rolling and yawing movements in many groups of fishes. This evidence also bears on the validity of the lateral fin-fold theory of the origin of paired limbs. (Incidentally, paleontology has killed Gegenbaur's theory). Paired and median fins in fact are alike in very many respects. We have now considerable experimental evidence that a symmetrical tail can provide direct forward thrust, and that heterocercal and reversed heterocercal tails combine this with, respectively, positive and negative lift, giving "nose-down" and "nose-up" pitching moments about the centre of buoyancy. Both median and paired fins can act as stabilisers against rolling. A degree of oscillatory yawing is almost inseparable from a propulsive caudal fin, but is slight in most modern fishes. Initiation and control of yawing and rolling are largely carried out by pectoral fins, which also play a large part in opposing the pitching-moment caused by an asymmetrical tail. In most fishes the propulsive force is by wave-like antithetic contraction and relaxation of myotomic muscles culminating in tail-flick. In some modern fishes ripple-movements of extensive median fins provide both propulsion and control; this is not generally the case in very early members of any group of fishes.

The cephalaspid ostracoderms provide striking evidence of the nature and evolution of pectoral fins - no separate pelvics develop. A typical early Devonian cephalaspid has a heterocercal tain and flap-like pectorals mesial to the cornua. No internal skeleton has been found in the pectorals, nor are facets for radials developed in the "shoulder-girdle" part of the complex cranio-viscero-zonal endoskeleton of the "head-shield". The

earlier known members are very different. The exoskeleton of the cranio-thoracic region is much more posteriorly extensive, the mobile pectorals are small or absent, and the region of the cornu + fin may be represented by a bone-jacketed keel. In Trematapsis there is only a slight ridge overlain by thickened dermal bone; and the shape (and probably the locomotion) resembles that of an anuran tadpole before the limbs appear. There is very good reason for believing that this represents a primitive condition from which the pectoral "keel" and the mobile fin were progressively developed. Cauna has recently shown that the earliest embryonic rudiment of paired limbs in higher vertebrates is a placode-like thickening; the resemblance to Trematapsis may be highly significant.

The writer recently discussed the apparently poorly-developed pectoral fin-equivalents of Anaspida; Mr. Ritchie has recently found them to be more extensive in some genera, but they show little sign of high mobility. They may also be regarded as products of a ventro-lateral ridge. The pteraspidomorph ostracoderms seem never to have developed paired fins.

The well-known acanthodians have, in early forms, numerous paired "fins", each of which consists largely of a stout bony spine with sometimes no indication of any fin-web; the fin-webs where present were thick and clearly not very flexible. In later members of the group the intermediate paired spines and the opposing anterior dorsal are lost, the spines become slender, and thinner fin-webs with fin-rays develop. Here again there is a well-documented increase in full control of locomotion.

Placoderm fishes show, in many groups, a parallel development. Early forms tend to have keels, developed into long spines, with small and rudimentary pectoral flaps. Later members reduce the spine and develop increasingly mobile pectorals with fin-radials. In some lines (e.g. that leading to Rhenanida) this process seems to have been rapid. Stensiö holds an entirely opposing view, that the keel-like spines are secondarily immobilised fins. The general stratigraphical sequence and the details of various lineages strongly oppose his conclusions.

There now seems little doubt that mobile pectoral fins arose independently in certain ostracoderms and in several groups of placoderms from structures resembling bilge-keels of a ship, the function of which is to prevent excessive rolling. It is of course possible that the development of the strong spine may be adaptive to some secondary function; if so, its need was lost when pectoral fins appeared. Until mobile pectorals allowed control of active swimming, the swimming must have been feeble and tadpole-like; the extension of dermal bone over the anterior part of the body permitted only posterior myotomes to contribute to propulsion. The freeing of more anterior myotomes by posterior reduction in the body armour is correlated with mobility of pectoral fins.

The earliest (Upper Devonian) true sharks had stiff paired fins with long radials, and basals poorly distinguished from the girdles and embedded in the body-wall. By the early Carboniferous the series of basals is partly freed and forms a metapterygial axis.

From this stage onwards the pectorals rapidly developed flexibility. The series of basals of Cladoselache seems to correspond morphologically with the articular ridge of the scapulo-coracoid of many arthrodires, also still embedded in the body-wall.

Holocephali, as is now proved by Ørvig's work on Ctenurella, are descended from ptyctodont arthrodires. The mobile pectorals of Ctenurella seem to be derived from the spinal complex of earlier relatives, and similar changes occur in the dorsal fins.

It would seem therefore that mobile pectoral fins have developed independently in several groups, and that stiff keel-like structures were their fore-runners. These were presumably derived from extensions of myotomes covered with stiff skin which could ossify. There remains the possibility that the whole may have originated in relationship to a placode-like strip.

The early Actinopterygii, the palaeoniscoids, also had rather keel-like (but flexible) paired fins and a strongly heterocercal tail; the pectorals were the main agents in counteracting the pitching due to the tail. The changes towards the homocercal tail of teleosts are well-documented; they are accompanied by the freeing of the pectorals from this main duty. The process seems to be related to changes in the relative density of fish and environment; palaeoniscoids needed lift, teleosts on the whole do not.

The paired fins of the crossopterygian Eusthenopteron are to be described elsewhere in detail. Morphologically they parallel some lower Carboniferous sharks, with the basals partly freed from the body, but apparently retaining a few features of an earlier condition when they were part of the "zonal" skeleton. The radials are few and strong. This fin-type was thus probably derived from a keel-like fin; it gave rise to the tetrapod limb.

The origin of tetrapods from Crossopterygii is now very well documented. It will suffice here to note three points in which functional analysis has interesting applications.

The first is the remarkable change in skull-proportions from Eusthenopteron through Elpistostege and Ichthyostega to typical lower Carboniferous labyrinthodonts. The skull-roof, cranium and adjoining parts of the palate are greatly deformed, while the facial skeleton and jaws are but little modified. This can readily be interpreted as a result of the head needing to be supported out of water, with consequent development of an occipital plate and a neck-region. At the same time the crossopterygian transverse intracranial joint is locked and stiffened by a variety of changes of dermal bones - posterior extension of parietals, suturing of cheek against skull-roof, and fusion of simple parasphenoid with parotic plates to give the tetrapod parasphenoid. Thus many features that have long puzzled anatomists may have a very simple explanation.

Secondly, the axial skeleton and ribs of Eusthenopteron directly foreshadow those of tetrapods. Pleural ribs are absent, and the short dorsal bicipital ribs can be shown to have acted as braces, with the dorsal and ventral muscle-blocks, acting to prevent sagging of the vertebral column under load. Once again an

important tetrapod feature is present in Eusthenopteron and its allies and has a direct functional meaning.

Thirdly, the pectoral fin-skeleton can be shown to have every structural peculiarity of tetrapods in its proximal parts, and was clearly load-bearing; powerful muscle-scars on the under surface of the radius prove this, together with many other features.

Other aspects of the origin of tetrapods have been fully dealt with elsewhere. It is proposed finally to examine one other use to which the writer has applied functional analysis. This concerns the remarkable changes in proportions of the skulls in the origin of mammals and ultimately of mankind. The striking changes in proportion in the palatal region of a mammal as compared with even cynodont and therocephalian mammal-like reptiles were discussed by Parrington and Westoll. They are associated with the reduction of the post-dentary bones of the lower jaw, some of which become ear-ossicles, and the beginning of a dentals-squamosal articulation. This whole process involves the retention of the "facial block" and dental arcade with very little change, while the otico-occipital block is rotated forwards so as to cause considerable compression in the basicranial region. The inner ear structures have to be rotated within the otico-occipital block to maintain approximately the orientation of the semi-circular canals. A very large number of the differences between the skulls of mammals and mammal-like reptiles are in fact interrelated by this process.

In man this cranial flexure has gone very much further; the telescoping of palatal and basicranial structures is intense, and the skull-roof in consequence is dilated; the mandibular condyle is pressed against the auditory meatus, the dental arcade is shortened and so becomes broader (with the wisdom teeth losing full function), and so on.

These changes involve two major cranial blocks - the facial unit and dental arcade, which retain a remarkably similar orientation in the normal pose throughout, and the otico-occipital block. The latter is concerned with support of the head, and maintains throughout a nearly constant angular relationship between the occipital plate and the neck-vertebrae. Hence very many skull-changes are related to posture; mammals had raised the head on the neck and brought the legs under the body, and Homo has become erect. All these processes require greater neuro-muscular coordination, and present to the animal more information about the environment. It is a tempting speculation that the increased intelligence of mammals and of men may be coordinated with just these changes.

FUNCTIONAL ANALYSIS IN A SINGLE ADAPTIVE RADIATION. Carl Gans, State University of New York at Buffalo.

Functional analysis should ideally represent an advanced level in the interpretation of vertebrate structure. It must thus proceed beyond the straight descriptive and the homology-oriented aspects, to evaluate the bases or causes of a particular morphologic scheme.

Different approaches are possible and desirable. I have generally relied upon the review of a single, limited group, at present a suborder, of animals. Comparisons of actual behavior recorded in various ways, and analyses of structural changes from species to species have furnished the raw material for interpretation. Analysis has always started with those animals of the group, most specialized for the particular function. The following *a posteriori* discussion attempts to bring out and illustrate some of the theoretical considerations back of this approach.

Analysis of a morphological system requires the solution of two initial problems: Separation of the several influences that mold structures, and determination of the actual functions carried out by the structures.

SEPARATION OF INFLUENCES

It is axiomatic that animal structures carry out functions; they are obviously adapted for this and reflect this adaptation. It is also well known that other aspects of the organism represent remnants of past circumstances. Natural selection incidentally implies a high probability that the phenotype of an individual will have been influenced by historical as well as functional factors. Three complexly interrelated sets of historical events are of importance here:

1. Changes due to random environmental effects that influence an individual during its development. Such changes are responsible for some of the individual intrapopulational variation. The degree of their expression is obviously under the control of the particular genotype.

2. Reflections of the ontogeny of the individual. These record the ontogenetic shift in mode of life history of the species. Each individual must during its development shift its adaptive pattern with the succeeding changes in mode of life (such as from larva to adult). This makes it likely that any aspect of the adult phenotype will represent a compromise, forced by the need for remodelling a (larval) structure, that is adapted for a different mode of life and more or less non-functional in the next (or the preceding) stage. Even forms that show only relatively slight behavorial and structural modification with growth are usually undergoing allometric changes that provide less than optimum design in the adult, in

the juvenile, or in both.

3. Reflections of phylogeny. The past history of a species contributes not only to the phenotype of individuals, but also to their inherent genetic variability. It thus influences and limits the extent, type, rate and direction of adaptive change. Past history in this sense refers not only to a previous adult condition, but also to embryonic and larval adaptations.

All of these historical factors interact and contribute to any observed phenotype. The historical remnants due to the last two factors tend to be eliminated quite gradually as natural selection is for adequacy rather than perfection, and the overall phenotype rather than each separate structure is being exposed to selection.

It is obviously necessary to attempt such a partitioning of influences, in order to comprehend which aspects of a structure remain as evidence of past adaptation and which represent specific current functions. This is clearly impossible when one deals with only a single species; it may become possible by the use of comparative methods. Comparison should first be of related forms, using species from a single (even if complex) adaptive radiation. It should secondly compare these results to those for other similar groups that have been investigated on the same basis. [The functional levels investigated in this manner may well correspond to the evolutionary concepts, respectively of special and of general adaptation.] The method presupposes understanding not only of the structure, but also of the function of a particular system in each of the animals being compared.

DETERMINATION OF FUNCTION

Classical functional analysis consists of a dissection of the animal into its component parts. The function of each element is then deduced from its structure (cf. the origin-insertion-action approach to myology). This approach has two inherent weaknesses: The assumption that the function of an isolated part corresponds to its action in an intact organism, and the assumption that an element's function may be absolutely derived from its structure.

1. The functional relation of the part to the whole is hardly a simple matter. Such factors as tendon direction, and the position of muscle fibers will change both post-mortem and during animal movements. The difficulty is compounded when the mechanism becomes complex, since it has been shown that particular muscles often act during part of a movement cycle only. Their position when contracting is important, yet it is the resting position that is ordinarily described.

2. The derivation of function from structure has certain implicit assumptions that pose severe problems and may be

mentioned. These are:
a. that the architecture of the structure is mainly or entirely molded by the functional influence. This has already been disproved in the discussion of influences.
b. that a structure will have only a single, overriding function. This one structure-one function relation obviously does not exist. There are bones that must alternately withstand tensile and compressive stresses, and more difficult to analyze situations in which manipulation and power exertion are handled by the same structure. These factors cannot be separated by simple inspection.
c. that the structure either does not incorporate a factor of safety or that this be equal in all directions. The difficulty here is the same as for the second point. A structure may have a routine function that loads it to less than 25% of capacity. The maximum load that may be carried without structural failure may in contrast only be imposed once or twice during the animal's life cycle. Yet the structure may be primarily influenced by this rare load, particularly if the load is imposed during an escape reaction or in a similar situation of high selective importance. Many examples furthermore suggest that retention of a more generalized structural pattern, equivalent to an increased factor of safety, has further selective advantage. It implies a degree of random morphological preadaptation, thus increasing the likelihood that the first [always behavioral] step into a new functional pattern will not exceed structural limits. All of these factors combine to complicate the analysis of any structure. They rarely permit direct extrapolation from it to the functional pattern.

The discussion underlines the conclusion that analysis best proceeds from function to structure rather than vice versa. Only in the case of rare, or fossil, animals may the reverse approach be justified. Then it must be used with exceeding caution. The further one departs from living animals, the more tenuous the decisions become.

PROCEDURE OF ANALYSIS

The determination of function by observation also implies difficulties. It ideally begins with a knowledge of the intact animal's activity pattern, passes to the animal's utilization of its body regions in this, and continues to the physical and physiological loading of their component elements. This requirement for a broad view of the overall biology of the species suggests the desirability of working with a limited group of animals, or alternatively of collaborating with a specialist in a particular group.

The activity pattern should first be determined under nearly natural circumstances. High speed (or slow motion) photography employing flash-tubes synchronized to the motion-picture camera, plus frame-by-frame analyzers and variable speed projectors,

provides an ideal adjunct for this phase. The next approach involves the manipulation of the intact animal in special situations. The animal is induced to move across a strange substratum, to feed on modified foods, and to act within a framework in which it may be both observed and where major external and internal forces may be measured by varied apparatus. Even modified or multiple camera angles may provide useful information, while x-ray motion pictures furnish preliminary indication of the correlation of external and gross internal levels.

The next step of analysis may involve structural alterations and observation of the post-operative animal. Here and in the previous step, it becomes necessary to consider the animal's compensation for the modification. Most animals learn to do this very rapidly as can be deducted from the survival in the wild of animals with major fractures or other damage to elements of the feeding or locomotor apparatus. It may ultimately be most profitable to utilize the implantation of miniaturized recording instruments. These permit monitoring of the forces exerted and the sequence of firing of single muscles, as well as the stresses in single bones even of small, intact animals during their normal activity.

The direct measurement of forces is essential because animals represent dynamic systems. The forces exerted during a jump or a digging push are many times those imposed under static conditions. Part of functional "design" has consisted of devices for building up and utilizing large amounts of kinetic energy with limited contractile tissue, or alternately of handling positive and negative acceleration without exceeding permissible stresses.

The accumulation of a reasonable amount of behavioral information, coupled with descriptive data on gross and histological morphology, permits a first approximation to functional explanation. This is carried out for each behavioral mode that possibly involves the structural system under study. It provides an estimate of the influences most important to an element. It is at this level that the results of synthetic studies on the physical nature of connective and supportive tissues, and on the physiology of muscle fibers are most usefully applied.

Many problems of this nature involve situations similar to those of practical concern to engineers and applied physicists. Standardized (and simplified) approaches exist and their terminology is often applicable to the biological situation. Its use does require consistency (thus stress, strain and load are hardly synonyms). New terms have to be adequately defined. The danger in any numerical approach is that its results are only as reliable as the least dependable datum (or assumption). Elegant mathematical derivations are occasionally based upon multiple

levels of "guessed" assumptions about the biological system concerned. This is acceptable only as long as the possible ranges (or ranges of error) for each parameter are carried through the analysis.

Approximation of the functional pattern in a given species is usefully paralleled by similar investigations in related forms. This permits comparison of minor functional and corresponding structural changes. Their relationship is clearly not on a one-to-one basis because: (1) Some of the inter-species changes in morphology may represent "non-functional" causes (i.e. random variation, linkage with characters subject to selection). (2) Functional analysis cannot simultaneously encompass all systems of the animal. The morphological change may be induced by a system not investigated, or understood. (3) Any change in a structure affects adjacent elements. The shift may thus represent a secondary reflection of character directly selected for.

Comparisons of this sort are usefully carried out within the restricted context of (what appears to be) a single adaptive radiation. This reduces the possibility of multiple solutions to a single major problem. The differential success of several lines may suggest clues regarding the pattern of structural utilization. Thus the initial selective value of a particular process is apt to be based primarily on topography and be independent of the ossification centers that modify to provide it. The "choice" of one of two initially equivalent situations may imply later limitation. The same also holds for seemingly equivalent functional pathways. Thus the clues furnished by the spectacular differences in range and abundance of related species have several times served to alert me to interesting implications.

The analysis of groups most profitably starts with those species showing highest development of some major adaptive system. This facilitates conclusions regarding the development of specific adaptations. Since function may be observed only in the Recent, comparison must be on a single time level. Yet related species often obtain elements of a particular adaptation at different degrees of development (indicating selective differences, i.e. adaptation for diverse habitus, and possibly representing secondary regression and not a stage in the development of the adaptation). Comprehension of the pattern in the most modified form will assist recognition of its elements or precursors, even in those (still generalized) forms in which no selection has occurred for one of the several pathways and where the particular pattern remains in a "primitive" condition.

Such analyses are speculative, but help to establish possible alternatives. The overall process should yield a better understanding of the nature of morphological adaptation.

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EVOLUTION OF HIGHER LEVELS OF ORGANIZATION

A symposium organized for the
XVI International Congress of Zoology

by

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EVOLUTION OF HIGHER LEVELS: THE PROBLEM

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Viewed over long periods of time, evolution reveals a pattern of adaptive pulsations following upon shifts between adaptive zones. The pattern is multiple, being repeated many times in the broad adaptive bands designated as phyla. Evidence of this duality of zonal shift and adaptive radiation is documented in the records of many of the phyla. It seems safe to extrapolate from these to all parts of the organic world for which there is evidence of some part of the total cycle. This includes most of the major stocks that have a fossil record. It is within this framework that our problem is cast.

Evolution itself, however, has evolved and there may be danger in projecting the seemingly stable recorded pattern back over life's almost unrecorded first two billion years. During this earlier time most of the basic subdivisions of the organic world were established. With a few possible exceptions, all animal phyla were differentiated prior to the Cambrian. These represent the primary organizational patterns of the animal world and are in a real sense the major adaptive levels of this kingdom. There is little direct evidence of the nature of this early deployment. The Procaryota, as interpreted by Stebbins (4), seem to be derived unchanged from this remote history. They post a warning flag in their wholly different approach to adaptation.

This symposium will be concerned mostly with the more recent parts of evolution, those for which there is a record against which studies of mechanisms based on contemporary life can be tested. Implications concerning more ancient times are, of course, implicit in what is said.

Thus limited, "the higher level of organization" or the concept of "adaptive levels" becomes clear and definable. Such a level is that attained in the course of an organic sequence by modification such that the organisms so modified could and did undertake an adaptive radiation not possible to their ancestors. Both the potential for new radiation and its realization are necessary and sufficient. Were we able to predict success or failure, which we are not, then the definition could specify the attainment of a particular set of necessary conditions. There seems to be little hope of achieving this capability at the present stage of development of evolutionary theory.

To determine the mechanism of evolution it is necessary to treat modern animals and plants. The available time span is distressingly short. Hopefully it is believed that the essential elements of the mechanism can be derived or inferred from this evidence. Thus we pose problems of genetics, adaptation, integration, morphogenesis, ecology and so forth, separately, to stay within manageable limits and within the contemporary scene. In this way the main problems become amenable to critical testing and analysis. The question is asked for each: is there anything in the broad scope of evolution that the processes discovered in such investigations cannot explain?

If the answers validate the knowledge in each area, then the problems of origins of higher levels might be put to rest, at least within the limits that have been set. But general agreement, even within this symposium, cannot be anticipated. The evidence is too complex and the parts are still too inadequately interrelated. If there is less than full validation, then it may be hoped that areas for additional study will be singled out, so that we may probe where the weaknesses seem to lie.

Evolution is basically directional. This is expressed in the phase of adaptive radiation, expecially in phyletic lines. Adaptive directionalism under changing environmental conditions has been adequately related to known mechanisms. Adaptation provides an ordering that brings direction out of the seemingly random initiation of variation, selection and experimentation. There is also, however, a broader directional aspect, evidenced in the trend toward increasing complexity found in most major evolutionary sequences.

This broader directionalism is critical to our questions about the steps involved in the origin of new levels of organization. The problem is to know under what circumstances such changes have taken place and, knowing this, to evaluate their nature and meaning and the roles of the various mechanisms in their attainment.

At the core of evaluation of the circumstances attending changes of organizational levels lies the problem of the adequacy of the historical record. It is from varying interpretations of this that most controversy has arisen. Are there special circumstances under which the new levels arise? If special, are they particular and specific to each case, with nothing in common except their departure from the norm? Or can they be defined and generalized?

Most of the changes that have occurred have not been documented by the record. We merely know that they must have

taken place by what we see of the course of evolution. The inception of radiation of the placental mammals, of which very little is known, is a good example. There are dozens of others like it. Generally, the record is non-existent. Now we can take this absence of record as evidence, and this has been done. Some highly divergent interpretations have resulted. Most importantly, however, we must ask whether there are any cases at all for which there is documentation.

There are many minor instances where observed shifts might indicate a change in adaptive level. But these are largely problematical under our definition. Throughout the animal world there seems to be but one case for which there is a fairly adequate record of the events of a major shift. This, of course, is the record of the origin of mammals. This case indicates clearly that nothing which is not common to usual adaptive radiation operated to produce mammals from reptiles. The transition was carried out, of course, by particular circumstances, but, in kind, it seems representative, if we may judge from more fragmentary evidence of other cases.

The mammal grade resulted from realization of many trends, variously expressed in developing therapsid stocks. It was not attained "all at once," but gradually. There was improvement of, within and between system integration, which laid a basis for exploitation of environments not open to predecessors. Later this potential was realized. Hence, this case fits our definition of the origin of a new adaptive level in all particulars.

But a curious thing occurred. In large part the potential was not realized for about 100 million years after it seems to have come into being. Then over a comparatively very short time, explosive radiations took place. This same pattern now seems to emerge wherever there is enough evidence for interpretation. The basic features of the new adaptive level tend to arise long before the exploitation.

It is this "secondary" problem that may be most in need of investigation. The operational measure of a higher level of organization is not its existence, but its effectiveness. The circumstances of effective activation are central if we accept the evidence of the origin of mammals as a guide with respect to the mode of production of the basically new level. It is to this problem that the question of adequacy of mechanisms may be directed.

The nature and role of biotic balance appears to be critical during initiation of exploitation. Imbalance, or rapid shift, is characteristic, whereas no marked departure

from the established norm can be identified during origin of a new level. Coincidences of time or major extinctions, implying rapidly shifting biotic balance, and initiation of radiations are commonplace. There is no such coincidence of extinctions and times of origins of higher levels.

Modifications in the integration of body systems are fundamentally important in the development of new adaptive levels. After the fact evidence is conclusive. As far as the fossil record is concerned, we are forced to study largely secondary effects. Integration and correlations as envisaged by Schmalhausen (3) cannot be directly seen. Multivariate studies of skeletal features, Schaeffer (2), Olson and Miller (1), offer a possible but still uncertain approach. We may, in fact, not be able to determine precisely when a new adaptive level was reached. Full integration may have been a long process. Radiation in the new zone could occur only when it was complete in essential aspects. This does not imply a nonadaptive stage, merely one lacking the potential of the fully matured integration.

The suggested pattern (origin-gestation-maturation-activation) for higher levels of organization has implications pertinent to the incompleteness of the record. The second stage, being somewhat specialized with respect to its contemporary biota, would tend to be environmentally limited. Chances of finding it preserved are thus reduced. The deltaic and low lying flood plain regimes in which the mammal-like reptiles arose and flourished are highly suitable for burial and preservation. The relatively late geological time and the presence of exposures of these deposits in several parts of the world have been important in revealing this record. No other group has had such favorable circumstances.

In resumé, several aspects of the problems of this symposium stand out. In extrapolating the mechanisms of evolution from our short span of observations over the whole range of life's history, we are bridging an enormous gulf. To do this it is necessary to study the modern evidence field by field with the possible danger of assuming an unwarrented synthesis. The integration of information comes in some large measure from the fossil record, for without it a theory of evolution would be difficult to conceive. The record is very incomplete, so once more extrapolation from a small part to the whole is necessary.

It is in this light that we ask our questions: What are the higher levels of organization of organisms? Can adaptive levels be explained in terms of known mechanisms? The complexity of factors suggests the use of models for investigation. These necessarily reduce the complexity, introducing another

danger--that of simplicity. From individual studies, we may hope to attain a meaningful series of answers, and to test problems we have raised. The pertinence of our questions may lead to a better understanding of evolution.

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GENETIC IMPLICATIONS OF THE EVOLUTION OF HIGHER LEVELS OF ORGANIZATION

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In its early days genetics placed all its emphasis on the segregation of particulate units producing fixed ratios of dissimilar phenotypes. The notion of blending traits had been given the coup de grace, but the new theory of inheritance gave little comfort to students of speciation, largely paleontologists, who were looking for a mechanism to explain the slow accumulation of small increments of difference. Paleontology, along with geology, had become gradualistic in Lyell's time, but now genetics seemed to be propagandizing for biological catastrophism.

With time gradualism has become more and more widely accepted in genetics. A sound hypothesis for reconciling Mendelian segregation with continuous variations was proposed very early by Nilsson-Ehle (6) and East (1), but it was not until the 1940's, after the elaboration of a mathematical description of natural selection by Fisher, Haldane and Wright, that Mather (3) worked out a theory of polygenic inheritance and gave it experimental support. As a result of selection experiments begun by Mather and subsequently carried out by many workers in many different ways, it is now generally accepted that in any population almost any phenotypic character owes its mean value to the interaction of a number of genes, each one of small effect, scattered at various loci throughout the genome, and that the mean of the character can be changed if, through selection, the relative frequencies of the different alleles in this polygenic system are altered.

Thus in most natural populations the genetic variability is much greater than the phenotypic, for the modal phenotype results from many different genotypes. This genetic variability is always available in the constant interaction between population and environment, and the population uses it to respond to any challenge. It does not mark time while waiting for rare mutations to occur.

The versatility of the genetic system in providing the variability necessary to produce a shift in the mean expression of a given character is beautifully illustrated by the work of Waddington. If pupae of Drosophila melanogaster are subjected to heat shock at a certain stage in their development, a considerable number of the emerging flies will have defective crossveins in their wings. By subjecting pupae to such

treatment and breeding from those flies which showed the crossveinless trait, Waddington not only increased the sensitibity of the line to the heat shock, but after some generations of selection he obtained flies with defective crossveins from pupae which had been subjected to no heat shock at all. Further selection of these flies and their offspring resulted in lines giving practically 100 per cent crossveinless with no environmental stimulus whatever. Genetic analysis of these lines showed that the factor producing crossveinless flies were distributed throughout the genome. This experimental work has been repeated, confirmed and analyzed in futher detail by Milkman (5), and there is not the slightest doubt that a similar line can be derived from any sample of a score of flies. By artificial selection a polygenic system can be built up to produce a change in a morphological character.

By a very similar program of selection for sensitivity in the egg stage to ether, Waddington (7) also succeeded in producing a line of flies in which the majority showed halteres modified into a second pair of wings. In this case one is dealing with a character which differentiates an entire order of insects--the Diptera. The fact that one can in a few generations--29 in this case--monkey with so fundamental an element in the morphology of an organism is evidence that such morphological cornerstones are subject to a bit-by-bit alteration and revision by the ordinary mechanisms of selection. The bithorax phenotype in Drosophila is, of course, a reversal of evolutionary events which must have occurred at the origin of the Diptera. But since a partial reversal can result from the accumulation of small increments, it is much less difficult to imagine the change occurring in a Mecoptera-like insect in the opposite direction--the gradual reduction of the metathorax and the transformation of the wings into halteres.

Any type of directional selection--that is, selection for change rather than for stabilization--must change not merely the mean of the character selected, but also the relation of that character to the entire program of the organism in its genetic, epigenetic, morphological and ecological aspects. For the program of any organism is an integrated whole in which every character is confined by the program as a whole within very definite limits.

The experiments of J. Maynard Smith and K. C. Sondhi (4), who were able to alter the number and pattern of the ocelli in D. subobscura, show that selection can alter the system of integration, but that it is harder to force it into asymmetry than to change it symmetrically.

In selection experiments it frequently happens that two samples of a given population, subjected to the same type of

selection, both respond positively but with a somewhat different pattern, suggesting that the marshaling of the available genetic variability has taken place one way in one sample, and another way in the other. In a project carried out by the author (2) for the study of the genetics of resistance to DDT in D. melanogaster, two lines taken from a single population began showing definite response after twelve generations of selection. From generation 12 to 30 both showed a roughly parallel increase in resistance. Then one line ceased responding while the other continued. At generation 60 the resistance of the one strain was 1.5 times that of the other. A third line taken from the same population--selected in a somewhat different way--ultimately arrived at the highest level of resistance, but most of the change occurred in two abrupt steps, one at about generation 30, the other at generation 50.

These data could be supplemented by similar observations from many selection experiments. They indicate the erratic nature of the details of the response in any given line. The complications are such that no statistical or mathematical model can give us even approximate limits.

Two or more samples from a single population are never genetically identical and hence are very likely to respond somewhat differently to selection--to interact differently with their environment. But conversely, two populations which have become genetically isolated by synchronic speciation may react similarly to a similar environmental challenge. Species of a common origin have similar biotic programs, and the changes which have differentiated the species will have left the fundamental ground plans of the programs unaltered. Such species, as they interact with their environment, will react within the limitations of their ground plans, and even though they are no longer capable of genetic collaboration, their gropings and evolutionary experimentations will have a much greater probability of being similar and parallel than those of species of very different origin. To give an extreme example, two species of hummingbirds are more likely to meet an environmental challange similarly than would one of them and a species of sphingid feeding at the same flowers.

Thus within a group of similar species or genera, several lineages may evolve in a parallel direction as a result of the fundamental similarity of their programs. Some or all of them may in time give give to many more genera and species, and in retrospect the whole complex may be recognized as having moved to a new level of organization. Within the complex, synchronic speciation will have occurred in isolated, often peripheral, populations, but not all cases of aquisition of characters diagnostic of the new group need have come about in such circumstances. Some of the species may have aquired them

through diachronic speciation.

To sum up succinctly, the geneticist can say that mechanisms are certainly available for the evolution of new adaptive levels. Biotic programs can be modified over a range which is about as wide as that of human imagination. Such alteration can be carried out by the accumulation of small increments. How long it will take to produce a given alteration is unpredictable. Many alterations have never occurred. Those that do occur may take place in a surprisingly short time, or, although present potentially at a given time, they may not appear until later, perhaps in two or more genetically isolated lines.

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EVOLUTION IN MORPHOGENESIS

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Present means of investigation do not permit us to delve very deeply into the structure of the egg and to ascertain what will characterize its structural level. How the information contained in the genome is used by the egg during its development remains unknown. Before fertilization, the egg in the different vertebrate classes may show structural differences which, however, appear to have scarcely any significance, as is the case with yolk content.

The first detectable sign of change related to organizational level is found in the young gastrula, where experimental techniques permit us to spot groups of prospective regions. From this stage one, the topography of these regions clearly separates two vertebrate groups, the anamniotes and the amniotes.

The appearance of an extra-embryonic area is quite typical of the amniotes, but in this case it is only an embryonic adaptation whose relation with the final structural plan is not evident.

Induction appears at the gastrula stage. With this induction mechanism, organogenetic characteristics develop which are bound to the formation of different structural levels. These characteristics have to do with the inductors and their associations.

Induction of the medullary plate through the archenteric roof is a process typical of chordate development. The situation in the protochordates is still unknown, although Amphioxus provides some information concerning this matter. From the form of the medullary tube in the adult we can deduce that this induction is uniform and regular since the medullary tube from one extremity to the other has, in general, the same structure. With the appearance of the vertebrate type, segregation occurs in the medullary induction which leads to the differentiation of the prosencephalon, deuterencephalon and the spinal cord. Is this real segregation into specific inductions for these diverse segments or is the arrangement a gradient effect of a single inductor? It is difficult at the present time to answer this question. Some changes in the capacities of the inductor are accompanied, from the first vertebrate onward, by similar variations in the competence of the epiblast.

We know, on the other hand, that the formation of an organ generally involves the cooperation of several inductors as is the case in the spinal cord, the otic vesicle, etc. Thus associations between embryonic organs are temporarily established which may have no apparent anatomical or physiological connection in the mature organism. Certain of these instances of cooperation in the vertebrates have their origin necessarily in the first stages. Once established, do such associations remain unchanging and incapable of evolving? The development of the cranium can provide several clues. Amphioxus possesses only a rudimentary visceral skeleton; the neurocranium will appear only with the differentiation of the brain. There must therefore exist between the neurocranium and the brain (and also the notochord, somites, etc.) morphogenetic bonds which should offer a rich field for analysis. Data on the formation of the otic capsule and on the chicken cranium show the cooperation of several inductive systems. It would be interesting to ascertain if these associations of inductors are always the same for the same cranial portion.

The construction of the bony cranium offers an interesting example of induction involving bones associated with the cephalic part of the lateral line system (the "canal bones") (1). We do not know if the cephalic part of the lateral line system had any connection with the cranium when the vertebrates arose. As a system of sensory perception, the lateral line does not seem, at first sight, to be destined for an important role in the organogenesis of the bony cranium. However, at a stage in evolution which we cannot definitely determine, it is integrated into the pool of cranial inductors.

The lateral line system of the cephalaspid ostracoderms is completely enveloped by the bony cranial shield. We cannot determine if its neuromasts exercised a skeletogenic induction of the embryonic mesenchyme, or if the canals were passively taken in by the dermal bones. The neuromasts of the Chondrichthyes show no induction role. In the Osteichthyes, however, the cephalic part of the lateral line system is incontestably integrated into the morphogenesis of the cranium. At the level of the neuromast in Amia, Salmo, etc., the blastema of the canal bones are organized. In the cases studied, the neuromast induces the entire bone, including the bony tunnel which shelters the canal, or it directs only the formation of the tunnel. In the latter case, the body of the bone seems to have freed itself from any influence of the neuromast which was its primitive inductor.

It is possible that such an evolution of the bony organogenesis has taken place in other classes of fishes. If such is the case, one can understand how, in the crossopterygians and

tetrapods, the dermal bones with canals were able to persist in the descendants while the cephalic part of the lateral line system gradually lost its functional and organogenetic importance. It is probable that in the course of this evolution the old canal bones passed under the influence of other inductors which are as yet unknown.

It would be interesting to determine if other organic systems could furnish proof of such an evolutionary change: namely the integration of a new induction system. This would be followed by progressive emancipation of the fully formed organs after disappearance of the primitive inductor because they would have passed under the control of a secondary inductor.

Organic evolution is certainly conditioned by the evolution of induction systems. Unfortunately we do not possess systematic embryological analysis of a single organ for the entire series of the vertebrates. Thus it is impossible at this time to trace such evolutionary patterns. The transference of induction along with other factors effecting changes in morphogenetic pattern are key problems in the evolution of higher organizational levels.

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THE ROLE OF PREADAPTATION AND ADAPTATION IN THE EVOLUTION OF HIGHER LEVELS OF ORGANIZATION

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A major problem confronting students of evolutionary theory is the origin of new major forms of organization in terms of known mechanisms of adaptive evolutionary change. Mechanisms underlying the origin and perfection of single features are at present well understood (3); the problem arises when the many individual features comprising an organism are put together. The adaptation of the entire organism to a particular environment depends upon the adaptive condition of the many integrated parts. All the individual parts must be present and must have reached a certain threshold of development for the entire organism to be adapted. If any part were missing, or had not reached the threshold level, the organism would not be adapted. All parts must evolve together, a condition difficult to explain by known mechanisms of adaptive evolution, because the individual features do not reach the necessary adaptive level together. Hence the models and mechanisms suggested by the synthetic theory contain dilemnas that are difficult to avoid, such as an inadaptive phase in the origin of new groups.

The relationship of an organism to a specific set of environmental conditions may be termed evolutionary adaptation, designating both the process and the state of being. The degree of evolutionary adaptation of a species may vary and can even be lost, but judgement must be relative to a definite environment. It is meaningless to say that an organism is well adapted, poorly adapted, or not adapted, without specifying the exact environment. Extreme care must be exercised in choosing the correct environment when judging evolutionary adaptation. A form intermediate between two major groups may be poorly adjusted to the adaptive zone of each group, but may be perfectly adapted to a particular environment which lies outside both zones.

An adaptation is the complex involving not only the form of a feature, but the function as well. Adaptation, as the state of being, is defined as the ability of a feature to execute its biological role with the least expenditure of energy. As a process, it may be defined as any evolutionary change reducing this amount of evergy. The mechanism for change is natural selection acting on genetic variations which are primarily generated by recombinations in populations of sexually reproducing organisms. Judgement of adaptation is based upon an estimate of the degree to which the whole organism is fitted to its environment.

Preadaptation is the condition whereby a feature, by virtue of its present form, may acquire a new function and thus become a new adaptation (2). The origin of a new adaptation through preadaptation involves only a change in function: the form is already present, having developed through the operation of previously acting selective forces. Old functions of a preadapted feature are not necessarily lost when the new function is acquired. Those adaptations whose appearance is focal to the exploitation of a new niche may be termed key innovations. They are generally the first features of a complex adaptation to appear and are often the result of preadaptation. Nevertheless, key innovations evolve by the same mechanism as other features.

Before describing a model for the origin of new forms of organization, several premises must be established. The change from an ancestral to a descendent group involves many steps, each representing an intermediate stage between the old and the new adaptive zones. The concept of a "single phase" change is unfounded. In the evolution of flying birds from terrestrial reptiles, the steps would be bipedal locomotion, arboreal leaping, parachuting, gliding, and active flight. Some of these steps could be further subdivided. Different features of the ancestral group would evolve independently at different times and rates in response to the selective forces associated with each step. This pattern of change is called mosaic evolution (1). The avian tibiotarsus and tarsometatarsus evolved in connection with bipedal locomotion; the reversal of the hallux with arboreal life; feathers and warm bloodedness with a later arboreal stage; the wing with gliding; the enlarged sternum and its keel with active flight; and so on. Each step in this series involved preadaptation and key innovations, as well as a period of postadaptational adjustment. Each step would set the stage for the next step. The changes appearing at each level are dependent upon those features that appeared in the previous levels. One very important conclusion to be drawn from mosaic evolution is the fallacy that definitive sets of characters can be ascertained a priori for any group of organisms, and that the group can be recognized only when all these features have evolved. No end point can be ascribed to a period of mosaic evolution, and hence no presupposed set of characters can be called definitive. Among the mammals, the flying squirrels stopped at the gliding stage, while the bats went on to active flight.

The nature of the "regions" between adaptive zones is ambiguous. These in-between areas are often designated as unstable ecological zones (= inadaptive) in which selection is severe--a gauntlet through which the organism must pass to reach a new adaptive zone. Hence the belief has arisen that organisms must pass quickly through an inadaptive period, which acts as a bottleneck or threshold that must be surmounted be-

fore the new zone is reached. No real evidence supports this concept of unstable areas between adaptive zones. The in-between areas are better regarded as unsuitable for organisms in the surrounding adaptive zone. The change from one adpative zone to another occurs in a transitional zone extending from one to the other. Organisms in the transitional zone are fully adapted to the ecological conditions and do not experience selection forces of a greater magnitude than those present in the adaptive zones themselves.

Several to many phyletic lines are involved in the origin of new groups. Although little direct evidence supports this idea, it appears most reasonable and does not conflict with any paleontological data. This is the pattern shown in the evolution of the horses. The small number of known forms and lineages associated with the origin of any group is most likely an artifact of the fossil record.

The starting point for the model is the ancestral group of the same magnitude as the descendent group, e.g., the class Reptilia for the Aves or the Mammalia. The ancestral group evolves through time, but remains within its adaptive zone. New adaptive radiations occur and new forms appear; these refill the zone again and again to replace those forms which have died out, but the basic organization remains the same, just as the zone remains the same. The size of the group expands or shrinks as the zone becomes wider or narrower. If one of the zone expansions tends toward a different set of conditions, e.g., marshes at the edge of water, it could be regarded as a transitional zone. But whether the transitional zone will be a temporary extension of the old adaptive zone, or whether it will in time lead to a new major adaptive zone, cannot be determined at any given point in time.

A key innovation, perhaps preadapted, may appear and allow a step away from the old group and into a transitional zone. This is not a step toward a new adaptive zone or new group, since the existence of a new zone is not yet realized and may never exist. An innovation might permit a small radiation, dependent upon the size of the transitional zone, and a period of postadaptational adjustment. Postadaptation, in turn, may lead to another innovation in one or more members of the radiation, permitting another step away from the ancestral group, and to another period of radiation and postadaptiational adjustment. Such a cycle would repeat itself again and again, leading further and further away from the ancestral group with each step and setting the stage for the next one. The adaptations appearing throughout this sequence of stages would form a pattern of mosaic evolution. Most of the forms produced by each radiation would die out as successive radiations occurred, leaving little fossil evidence. Several different phyletic lines showing similar series of changes might persist. This sequence could last as long as the transitional zone exists. If

it disappears, the evolutionary experiment is over, which is probably the most common situation. If the transitional zone remains until a new major adaptive zone is "reached," then a larger adaptive radiation would be permitted by the size and diversity of the zone--the primary radiation of the new group.

Although the primary radiation marks the initial flowering of a new group, no point can be chosen as its origin. Rarely can one feature be cited as the key innovation leading to the origin of a group, nor can a single point be designated as the threshold that must be surmounted. No real difference exists between the continued evolution of a stem group within its adaptive zone and the evolution of a branch group in the transitional zone. The evolutionary mechanism in both is exactly the same. The only difference is that in the transitional group there is a steady change of niche relationships associated with the changes in the environment. The appearance of a key innovation of preadapted features, and of small adaptive radiations with postadaptational adjustment seems to be exactly the same in both.

This model of successive stages with radiations and periods of postadaptational adjustment at each step is in agreement with the known fossil record and the mechanisms of adaptive evolutionary change advocated in the synthetic theory. If these mechanisms can explain adequately evolutionary change within a form of organization, then they will explain change from one form to another. No new mechanisms need be proposed. The process is adaptive in that all intermediate groups are fully adapted to their environment.

Most of the problems and dilemmas involved in explaining the origin of new organizational levels by mechanisms of adaptive evolutionary change are artificial, having come about by looking backwards from the descendent group to the ancestral stem. Although many unanswered questions remain, understanding the mechanisms of adaptive evolution associated with the origin of new forms of organization involves no real problem if we realize that all evolutionary change should be studied by starting at the ancestral group and working forward through time.

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THE ROLE OF ECOLOGICAL FACTORS IN THE EVOLUTION OF HIGHER LEVELS OF ORGANIZATION

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Evolutionary processes leading to the origin of single characters and lower levels or grades are largely regarded as adaptive, i.e., as being determined by the interactions between the gene-pools of populations and their environments. For the origin of higher categories and their basic features (Baupläne), judgement about the role of ecological factors, and hence adaptation, is less unanimous. However, in recent years some new approaches to this problem have emerged which I should like to outline by presenting and analyzing some case histories.

The higher fishes (Chondrichthyes, Sarcopterygii and Actinopterygii) are members of one adaptive radiation, arising from one common stock. Their key character is the possession of lobed fins stiffened by archipterygia which combine morphological and functional traits exhibited in several ways by the earlier placoderms.

The central line of this radiation is represented by the Sarcopterygii. They have preserved to a greater degree than the other lines, the morphological and ecological features ascribable to the first higher bony fishes. With lobed fins and aerial respiration, they were adapted to a mode of life combining benthonic features with those related to the surface and the atmosphere. They were thus capable of either leaving the water temporarily, or surviving seasonal droughts in the same way as lungfishes. These characteristics represent the preadaptations from which the amphibians originated, and the mode of life of the earliest tetrapods could not have differed much from that of their rhipidistian ancestors.

The general trends of the two other major lines of higher fishes can be clearly analyzed. The Chondrichthyes have given up their respiratory relationship with the surface and the atmosphere by taking oxygen directly from the water through the gills. The major groups of chondrichthyans have preserved ancestral benthonic features, but increased swimming efficiency, resulting from the deposition of fat or the swallowing of air, has enabled some of them to have a rather pelagic life. Spawning has been transferred from the littoral zone by the forms that give birth to living young, thus breaking the ties to the bottom that exist in egg-laying forms. Originally sharks and batoids used the bottom for resting and spawning, and in a good many cases, also for feeding. The bottom also provided cover for the batoids through their burying habit. The mobile and

enlarged pectoral fins of the batoids adapted to this habit have then been put into use for locomotion, permitting reduction of the trunk and tail.

The Actinopterygii initiated their history by using the lungs as a means of buoyancy. The air bladder can be closed by sphincters around the ductus pneumaticus for better control of the gas volume. The efficiency of gill respiration was improved to satisfy the oxygen demands of more active swimming by increasing the mobility of the dermal bones surrounding the gill region in the primitive actinopterygians. These changes, however, interfered with the original jaw action, induced the forward shift of the jaw mechanism and led eventually to the protrusive mouth of the teleosts. The endoskeleton of the fins, released from lifting the body, was reduced and largely replaced by fin rays. The fins became adapted in shape, structure and position to increase speed and maneuverability.

Thus the bony fishes have integrated the water into their mode of life as a supporting element and as the only source of oxygen, substituting in these respects the bottom and the surface respectively. By forming schools, they evolved a way of replacing the protection provided by the rocks and plants and other objects of shallow water, and made their way into the open sea. Schooling became an integral part of the reproductive behavior away from the littoral zone as a nesting and spawing site. The teleosts increased their egg number, abandoned the Mullerian ducts, which were apparently unfitted to handle the large number of eggs, and evolved pelagic larvae without benthonic features. But even then they still retained one ancestral relationship to the surface by swallowing air for the initial filling of their swimbladders. Finally, the Acanthopterygii improved gas secretion from the blood to such a degree as to render possible the initial inflation of that organ. Thus by gradually replacing the functions which had been carried out originally by the bottom and the surface, the bony fishes became adapted to a way of life which satisfied all demands from the surrounding water. This is not to say that no new relations evolved, either to the bottom or to the atmosphere, or even to the world outside of the water. They were, however, new relations, and not the old and original ones.

It is in terms of change in mode and way of life that we must describe the history of both grades and clades. This can be further demonstrated by two more examples. The flatfishes have evolved from serranids, with which they share the habit of sleeping on the bottom. The perch-flounders (Psettodidae) are little more than slightly asymmetrical serranids. One of their eyes, either the right or the left, with equal frequency, is shifted to the margin of the head, thus enabling the fish to

lie on its side and still benefit from binocular vision. This modification has been exploited by the perch-flounders which use the bottom as an ambush site and dart out for their prey. This use of the bottom in relation to feeding behavior has been elaborated by the two other groups of flatfishes. The flounders (Pleuronectoidea) use it as a hunting site; the soles and tongues (Soleoidea), as a grazing site. Some flatfishes have reduced the oil globules in their eggs, which sink to the bottom. This is a first step toward making the bottom, in addition to its other roles, a spawning and development place.

The angler fishes (Pediculati) catch their prey after attracting it with their well-known lures. This trick, however, works only if the carrier of the tackle is hidden. The goose-fishes of the northern seas (lophiids) achieve concealment by lying flat on the bottom. The bat-fishes (ogcocephalids) and sargassum-fishes (antennariids) hide themselves by burying into the sand, by hiding among rocks, corals or sea-weed, or simply by the astonishing degree of similarity between themselves and their environments. Although retaining their original mode of life, these fishes have found a way into the open sea with the drifting sargassum. The deep-sea anglers (ceratids) have become wholly pelagic fishes by adapting themselves, and their way of feeding, to the cover provided by the darkness of deeper waters, fishing there with a luminescent lure.

What generalizations can we draw from such case histories? Obviously, we must further define such comprehensive terms as "bottom fish" or "pelagic fish" by examining the particular relationships and interactions between the individual demands of the organisms and the ecological factors satisfying these demands. The relationships between organisms and their ecological factors evolve in relative independence and show the same principle of mosaic evolution which has been discussed in recent years for morphological features. It is the mode of life, the total of these relations and interactions, on which we must focus our attention. The system of interactions is the ecological niche of a species, the ecological zone of higher groups. If we take into account only the ecological factors themselves, many of the essential features escape investigation. The environment supplies a vast variety of prospective resources, but these become integral parts of the ecological zones, and hence, of the evolutionary situations only according to the use organisms make of them.

The relations between organisms and environments evolve and determine the further course of evolution after they have originated. This dual character holds equally true for morphological, physiological and ethological traits, as well as for faunistic and distributional patterns. The evolution of all

these traits results from and determines the evolution of the ecological zones, which themselves must be regarded as the very units of evolution. From this it follows that analysis of all these traits, and their phyletic sequences, can be utilized for the reconstruction of the history of a group in terms of the origin and radiation of the group and the building up of its ecological zone.

These rather brief statements cannot do justice to the host of objectives and methodological problems involved. So far experience has shown that the origin of evolutionary lines and the ecological zones built up by them can be traced back to the evolution of ethological key characters which opened up a new way of living in an old environment. They are followed by morphological key characters which are adaptive to the new way of life, and which establish and fix the direction of the oncoming evolution.

Explanatory natural history, based upon an ecologically oriented evolutionary biology, bridges the gap between descriptive and causal-analytical branches of biology in much the same way as the New Systematics does in the study of lower categories. Groups should no longer simply be described, but their origin and adaptive radiation should be explained. Every trait shown by their members should be investigated both as to its evolutionary causes and effects. This includes behavior which, evolving by variation and selection, plays a key role in stimulating and directing the further evolution.

The higher fishes have reached their level of organization in an old habitat; their mode of life, however, integrates relations to the water, the bottom and the atmospheric air. The higher bony fishes and the chondrichthyans have relaxed their inherited relations to the atmosphere, and in their evolutionarily most successful lines, most or all of the original benthonic traits. Although some of them have found and established new relations to bottom or surface, others have evolved into truly aquatic animals, satisfying all their demands from the surrounding medium. At the same time, and from the same ancestry, the tetrapods have evolved by relaxing their aquatic ties and strengthening those to the air and ground. Thus, new habits open up new ways of life in old habitats, and new ways of life open up new habitats. Ecological relations have a dual role: they are the results of evolution and the starting points for new evolutionary endeavors.

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THE ROLE OF NATURAL SELECTION AND EVOLUTIONARY RATES IN THE EVOLUTION OF HIGHER LEVELS OF ORGANIZATION

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Natural selection is the mechanism which controls directional changes in the genetic composition of natural populations by its action on the phenotype. The role of selection has been amply demonstrated by experimental studies. To the population geneticist the action of natural selection is merely the differential reproduction of genotypes. To the naturalist, the morphologist, and the paleontologist, the phenotypic changes observed in living and fossil populations are, by implication, the result of the same process. The demonstration of selection within natural populations, employing characters of known functional significance, is more difficult. Evidence of this type has been provided by the study of color patterns in land snails, industrial melanism in moths, mimicry in insects, occlusal patterns in fossil cave bears, and size and toe pads in geckoes.

There are usually two points of view regarding the selective significance of characters in natural populations. One is based on the assumption that such characters are nonadaptive if their functional significance cannot be demonstrated. Some proponents of this view believe that the observed lack of variation is evidence that these characters are nonadaptive. By contrast, others assume that highly variable traits are nonadaptive. Many students explain the evolution of such traits by invoking genetic drift and mutation pressure. The alternative point of view is that all the characters of an integrated organism are basically adaptive. The functional significance of these characters, however, may not be apparent because natural selection is rarely unidirectional; it is the resultant of changing vectors acting on functional complexes of the organism in the population.

The study of fossil lineages enables the student to note changes in the phenotype through time. The effectiveness of the complex forces of natural selection can be measured as rates of evolution. The investigation of rates was mainly initiated by G. G. Simpson (9), who pointed out that two types can be recognized--taxonomic and morphological. Originally he described three basic sorts of taxonomic rates which can be measured in terms of origin, longevity and survivorship in supraspecific and specific categories. All rate studies involve three basic problems which are (a) the inherent difficulties in any taxonomic system, (b) the gaps in the fossil record, and (c) the reality of the phylogenetic scheme. Williams (12) has discussed these difficulties in relation to brachiopod evolution.

According to Simpson (10), the chief difficulty in the morphological approach arises with emphasis on the evolution of parts of the organism, rather than the entire organism. Morphological rates can be measured in terms of change in single characters, in functional complexes of characters, in lineage allomorphosis, or in changes from archetypal ancestors involving incompletely known phylogenies (8), (11). Kurtén (5) has attempted to correlate his morphological studies with the taxonomic rates of Simpson.

Biologically, slow and moderate rates of evolution do not present an interpretative problem in terms of the ordinary microevolutionary processes. On the other hand, a rapid rate of evolution involving the origin of new types or levels posed a new problem for which Simpson proposed the hypothesis of quantum evolution. The limitations of this hypothesis involving the existence of a nonadaptive phase and its relation to genetic drift, were soon recognized by Simpson himself (10). In 1953 he modified his original concept of quantum evolution by emphasizing the importance of adaptation.

Rapid evolution poses two questions: Does it require a special genetic mechanism? Is the action of selection during the rapid phases different from that during the slower phases?

The rapidity of change appears to be most dramatic in those groups whose origin is poorly known. Often this apparent rapidity disappears with the discovery of new fossil material from the proper biofacies and lithofacies. However, the origin of new adaptive types, often expressed within the nomenclatorial system as new taxa, does seem to be more often associated with rapid evolution.

A rate of evolution in a lineage might be expected to vary inversely with the average generation time since all events involving genetic change take place between, rather than during generations. Actually most students (1), (10) have now come to the conclusion that there is no real correlation between generation time and rapidity of evolution. The apparent origin of the Elephantinae in the early Pleistocene has been used as evidence to support the lack of correlation between evolutionary rate and generation time, and it represents an example of rapidly evolving organisms with long generation time. Recent studies on populations of African and Indian elephants (2), (3) imply that the ancestral elephants could compensate for long generation time by means of partially isolated populations of adequate size. The available evidence suggests that the effective size of the breeding population was large enough to provide sufficient variability for selection to be effective. Therefore it may be concluded that rapid evolution in long

generation organisms does not require special genetic or selective mechanisms. However, Kurtén (5) in his studies on species longevity in Pleistocene mammals, concludes that short generation forms have speciated more rapidly than longer generation ones.

In the shift from one adaptational level to another are there any special requirements which cannot be accounted for by the generally accepted microevolutionary process? The relatively few cases in which the shift is represented in the fossil record seem to show little reason for involving special selective or genetic mechanisms. Early in the history of the Perissodactyla, for example, the ceratomorph and hippomorph divergence occurred. Radinsky (7) has observed that Homogalax, the most primitive ceratomorph, and Hyracotherium, the most primitive hippomorph, could easily be placed in a single genus if the classification was a horizontal rather than a vertical one. The difference between Homogalax and Hyracotherium primarily involves the degree of development of the cusps and lophs on the molars. This sort of distinction has been considered as an intraspecific difference in other related forms. In retrospect, this observable difference heralds the development of two adaptive types in the Perrissodactyla.

Although the analysis of change leading to new adaptive levels can usually be done only ex post facto, there are examples in the modern biota that indicate a possible breakthrough to a new adaptive level. The polar bear probably represents a new adaptive level in the ursid phyletic line. Its close genetic relationship to the living brown bears is demonstrated by the ability of the two species to produce viable, fertile hybrids. The recent origin of the polar bear from one of the Pleistocene forms of the brown bear is clearly indicated by the fossil record, and by the relatively minor morphological differences between them. It is obvious that the polar bear represents a departure from the usual ursid way of life, and its exploitation of the new habitat is theoretically unlimited.

Simpson (10), and more explicitly Bader (1), imply that phenotypic variation as studied by the paleontologist is a direct reflection of underlying genetic variability. It is for this reason that Bader compared variation in two lines of oreodont artiodactyls to determine whether there were any differences between the variation in the rapidly evolving merycochoerine and the slowly evolving merychyine lines. In most of the characters studied the rapidly evolving line showed less variation than the slowly evolving one. Bader believes these observations indicate that strong selection pressure pushed a greater number of alleles through the population (1). As a result of this a more homozygous group was produced at

each stage with a consequent lower variability. Underlying these observations is the belief that populations of organisms must await the accumulation of new mutations in order to break through into new levels of organization. Actually, recent studies on canalization of development, genetic homeostasis, and genetic assimilation provide us with new mechanisms to explain the above observations. It is now quite clear that modal phenotypes may actually be determined by many different genotypes in the population. Natural selection operating on hidden variability released by recombination is capable of explaining shifts in the rapidly evolving and phenotypically less variable oreodonts. Moreover, homozygosity may result in an increase in phenotypic variance as discussed by Lerner (6). Therefore phenotypic variation may or may not be correlated with genetic variability.

The significance of comparing evolutionary rates has been questioned. There is no question regarding the utility and validity of comparing rates in closely related lineages. Rate comparisons between unrelated phyla, however, are perhaps less significant. Yet the study of species longevity, biologically defined, particularly when closely related living forms are available for comparison, would afford a valid comparative method for the study of evolutionary rates.

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THE ROLE OF EXPERIMENTATION AND PARALLELISM IN THE EVOLUTION OF HIGHER LEVELS OF ORGANIZATION

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The concept of biological improvement as a fundamental aspect of evolution is as old as the realization that organisms have evolved through time. In the decades since Darwin the concept has been explored from many points of view, including the reproductive mechanism, morphogenesis, phylogenetic differentiation, and centralization of structure and function in both the embryo and adult. The underlying theme in all this deliberation relates to an increased independence of the integrated organism in relation to its environment and to a greater plasticity in the organism-environment relationship.

Improvement thus involves general adaptations that maintain genetic, developmental and physiological constancy within the population or the organism and which are therefore necessary for survival. This is essentially what Cannon (1) and Waddington (7) mean by homeostasis. Lewontin (2) would expand the definition to mean constancy of survival and reproduction, or constancy of fitness. In spite of these conceptual differences, it may be concluded that biological improvement is, in effect, refinement of homeostasis.

An increase in physiological or biomechanical efficiency, as an index of improvement, is relatively easier to evaluate at higher levels of organization than at lower ones, in mobile organisms than in sessile ones, and in living organisms than in extinct ones. The evaluation must therefore involve structures and functions that are understandably adaptive. Since the criteria of improvement are not absolute, the evaluation must also be relative and comparative. Changes in the hard parts of sessile organisms, as in many lineages of mollusks or graptolites, can be followed for millions of years and are presumably adaptive, but in many cases they have no known functional meaning. The best evidence for improvement in a temporal sequence is found in organisms with mobile hard parts that can be preserved as fossils. This is why the vertebrates, in general, provide more examples of improvement than the invertebrates or plants. The sharks, actinopterygians, synapsid reptiles (3), and various mammal groups show well-defined grades of increasing efficiency that can be defined in relation to the feeding and locomotor mechanisms.

The concept that organisms have evolved through a series of successive levels or grades has always been associated with

a mechanistic concept of improvement. A level is usually defined in terms of broad adaptations evolving in more or less parallel fashion in different lineages with a common ancestry. Parallelism is simply the development of similar character complexes in lineages that have a common ancestry, and therefore a similar genetic potential for these complexes. Similar selection pressures must be involved, as in convergence, so the critical point for parallelism is the inherited genotype which is shared to a greater or lesser degree by all the lineages involved.

Experimentation, which is basic to de Beer's mosaic evolution, may be defined as the tendency for organisms in related lineages to develop in parallel new adaptations involving various combinations of primitive (ancestral), intermediate, and advanced (descendant) characters. These combinations reflect different degrees or kinds of interaction between genetic recombination, mutation and prevailing selection pressures. Every new viable combination must be integrated into the total organism which, in turn, must be adapted to some particular environmental situation. Some conbinations, however, are undoubtedly more efficient than others, not only for a particular way of life, but also for meeting the more general problems common to all the environments that a group of organisms is able to occupy. Simpson (6) has pointed out that breadth of adaptation and retention of adaptation are correlated. In retrospect, it is modification of the broader adaptations that has characterized the change from one higher organizational level to another.

Experimentation and parallelism (as well as adaptive radiation) are clearly related phenomena, and in many ways one is the expression of the other. Both are based on common ancestry and both involve subsequent independent modification. The limitations imposed on the experimentation are, in fact, largely responsible for the parallelism. In addition to the restrictions related to the ancestral genotype and to selection, there are also those associated with morphogenesis. The data of experimental embryology consistently indicate that the course of normal individual development is canalized, and that it cannot be altered in a significant manner. As the stabilizing selection of Schmalhausen implies, an adapted norm of development obviously has an advantage over all deviations from this norm. Since, however, the organism-environment relationship is never stable for an extended period, viable deviations will, in time, be favored by the changed selection pressure and experimentation will occur. It is probable that genetic variability, as expressed through the far more limited range of viable epigenetic change, represents the principal basis for experimentation.

Simpson (5) has stressed the continuum between general adaptation, which is advantageous in nearly all environments,

and specific adaptation in a single ecologic niche. General adaptation, however, must begin much lower in the adaptational spectrum. All the organisms in a lineage complex must fit into sufficiently distinct ecologic niches to make selection for divergence or experimentation possible, but at the same time the niches must overlap enough to produce parallelism. It is only because nearly the same "specific" experiments have occurred a number of times under the guidance of selection that we can call the adaptation a broad one. Broad adaptations, involving two or more lineages, thus arise as specific adaptation, and their similarity is a result of many interacting genetic and environmental factors.

Divergence does not necessarily diminish opportunity for parallelism in general adaptation, but it may alter the way in which the parallelism is accomplished. The various versions of the holostean feeding mechanism, of the atlas-axis complex in the higher tetrapods, of the four-chambered heart in reptiles, birds and mammals, and of the cursorial locomotor mechanism in mammals, reflect different degrees of divergence and parallelism.

Ideally, a sharp separation between successive organizational grades should not exist. Even if the origin of a new grade is monophyletic with grade and clade coinciding, it is probable that some experimentation has occurred involving nearly successful approaches to this grade. Lineages enter a new level only in retrospect, and the evolutionary mechanisms involved are no different from those promoting cladogenesis within a level. An adaptation becomes broad because, through coincidence, the genetic changes plus selection point in one direction, that of general functional improvement.

At the time a broad adaptation arises its significance may be very circumscribed. There may be a long lag before diversification involving this adaptation is possible. This is so because other related organisms which are nearly as successful are occupying the available niches. In the history of the actinopterygian fishes the teleost level was probably first attained by the Middle Triassic, but teleost diversification did not really begin before the end of the Jurassic. There is also evidence that the teleosts had a polyphyletic origin with several lineages reaching the teleost level at different times. Hence the same broad adaptation may evolve heterochronically in lineages of common ancestry.

It is becoming increasingly evident that the origin of a higher grade of organization is not always related to a well defined threshold, as this term is usually defined. The evolution of the actinopterygian feeding mechanism passed through perhaps four major grades (4) involving a mosaic of inter-

related modifications which mostly either improve or supplement older functions.

Improvements involving radical change in function and environment are radical in an evolutionary sense mainly because we can consider only the beginning and the end of what must have been a long and complex transition period. The changes from agnathan to gnathostome, from rhipidistian to amphibian, from archosaur to bird, or from insectivore to bat, represent profound modifications in total organization.

On the basis of what we can deduce regarding the origin of general adaptations, a long period of experimentation involving both embryonic and adult structures and functions must have occurred. Every viable experiment throughout the transition phase was functionally integrated and adapted to some particular ecologic niche. We have no knowledge of how many "nearly successful amphibians" or "almost birds" became extinct along the way because their total organization was somehow less efficient for a particular environment than that of some other close relative. We can only say that the trial and error period in the origin of higher levels is rarely represented in the fossil record, mainly because the center of origin was very circumscribed.

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EVOLUTION
AT THE
POPULATION AND INTERPOPULATION LEVELS

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ISOLATING MECHANISMS AND INTERSPECIES INTERACTIONS IN ANURANS*

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The concept of geographical speciation, with roots in Darwin's writings in "The Origin of Species", has come to be a widely accepted one for organisms in general as well as for the one group under particular discussion here. For anuran amphibians there is now a large body of empirical data, particularly from temperate latitudes, where present distributions reflect the effects of Pleistocene climatic shifts, and these data leave little room for doubt that geographic speciation has been the major source of increase in number of species in these latitudes at least. Nevertheless, interesting and difficult questions present themselves when one attempts to understand the course of geographical speciation.

Our whole present day concept of "biological species" is based on the functioning of reproductive isolating mechanisms which prevent or restrict interbreeding of sympatric species populations. The course of speciation then must be the course of acquisition and perfection of attributes that will serve as isolating mechanisms. In well studied cases, it is clearly evident that sympatric species are restricted in their interbreeding by complexes of several factors. At least some of the components of these complexes must have had their origins in the past history of geographic disjunction, and they must have evolved through time both before and after the initiation of sympatry.

The main questions of interest are: (1) how do the changes that may lead ultimately to reproductive isolation of sympatric species populations have their beginnings? (2) how do geographically speciating populations interact if and when they become sympatric? The anuran amphibians have been used extensively in the last decade as material for approaching these problems particularly because of the relative simplicity of their isolating mechanism complexes and because of the relative ease with which important isolating mechanisms may be pin pointed and measured. The postmating mechanism of genetic incompatibility may be measured with comparative ease in those anurans with generalized reproductive habits, because the external fertilization permits involuntary hybridization and permits determination of the embryonic or life history stage at which genetic incompatibility becomes evident. In generalized anurans, the complex of premating isolating mechanisms usually consists of the ecological factors of time and/or place of breeding and the ethological factor of differentiation in mating call of the male with discrimination by the female between this call and the calls of other sympatric species. The usually restricted period of reproductive activity during any given year makes assessment of the significance of the ecological isolating mechanisms simpler than in animals with protracted breeding seasons. The mating call, which appears to be generally the most important isolating mechanism, can be measured by sound spectrography, and the response of the female to differing calls can be determined by simple choice experiments.

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Investigations of disjunct, allopatric populations provide clues to the kinds of divergence that may take place without sympatry of closely related species. Even here, however, one cannot be certain that sympatry has not occurred in the past, with the present allopatry being of secondary origin, and one cannot be certain that observed differentiation has not occurred through interaction with once coexisting but presently extinct populations. Nevertheless, our best estimate of how differentiation proceeds under geographic separation comes from the study of these disjunct allopatric populations. A few examples will illustrate the diverse ways in which differentiation may proceed. As postulated by many authors, differentiation should occur through the agencies of differential mutation rates and of differential selection pressures as the separate populations adjust to and maintain their equilibria in different ecosystems. It would be unexpected to find all measurable attributes being selected equally under different situations, and the following examples are chosen to illustrate a diversity of possibilities.

The significant differentiation insofar as the attainment of the level of "biological species" is concerned is that differentiation which will affect interbreeding, or in other words will serve as a component in the isolating mechanism complex. The disjunct allopatric populations of spadefoots that have been called *Scaphiopus holbrooki* and *S. hurteri* by morphological systematists have differentiated an excellent key character for separating these in a taxonomic key, but there is no obvious (at least) differentiation in any attribute that would serve as an isolating mechanism (W. F. Blair, 1962). By contrast, slightly lower (about 500 cps) dominant frequency in the call of *P. streckeri* provides sufficient difference to permit female *P. streckeri* to discriminate between this and the call of male *P. ornata* (Blair and Littlejohn, 1960, W. F. Blair, 1962). This call difference in turn appears to result from the greater body size of *P. streckeri*, since the dominant frequency in anuran calls is inversely proportional to body size. Therefore we may postulate that the call difference that provides the means of species identification is the indirect result of selection for an adaptive character, body size. It does not detract from this case that there is also the postmating isolating mechanism of genetic incompatibility (Mecham, 1957, 1959). This simple mechanism whereby differentiation in body size provides pleiotropically the differentiation in mating call is rather common, e. g. in *Microhyla* (W. F. Blair, 1955). However, there are numerous cases of relatively complex modification (as in frequency shift, notable in the family Leptodactylidae and in others) that cannot be related to simple morphological characters. Another is that of major modification in duration of the call. A striking case in point is that of the *Bufo cognatus* group (Bogert, 1960) in which two species which are rather differentiated morphologically but have essentially similar calls of about 20 seconds in duration. The third species has a call only about 0.7 second in duration and appears to be widely disjunct from the species it most resembles morphologically, but it does overlap narrowly the geographic range of the other species. The cause for such a major change in call duration is unknown.

In general, therefore there seem to be two major possible origins for differentiation in mating call: (1) the simple pleitropic effect of structural modification (i.e., body size), (2) the selective advantage of species identification in mixed breeding choruses. In addition, there are some cases that are presently unexplainable, unless explained by item 2 above.

Some species populations may, of course, live out their evolutionary life spans without ever becoming sympatric with another species of the same genus. It is probably true, however, that most do encounter other related species sooner or later in their span of existence as a species. The effects of such populations on one another are related, of course, to their degree of preadaptation for coexistence at the time of sympatry. There is, as has been pointed out by many workers, the possibility of fusion of the populations if contact is made before relatively effective isolating mechanisms have been acquired. A case of this in Bufo woodhousei has been analyzed by A. P. Blair (1941) and Meacham (1962). Incipient complexes of isolating mechanisms may be molded and strengthened (reinforced) as has been discussed for various anuran situations by W. F. Blair (1958) and Mecham (1961). Lastly, it is possible but intuitively rather improbable that the differences acquired in isolation would be so effective that the populations would not influence one another under subsequent contact.

The approximately 30 species of toads (Bufo) of North America give some insight into the evolution of isolating mechanisms both prior to and subsequent to the stage of sympatric existence. These 30 species fall into 11 species groups which have been extensively hybridized (W. F. Blair, 1963a). No intergroup cross has yet proved to produce fertile F_1 hybrids; intragroup hybrids have proved fertile as tested in the relatively large B. americanus and B. valliceps groups. Members of the same species group are usually, but not always, allopatric in distribution and presumably represent in large part the results of Pleistocene fragmentation of ranges. The species of Bufo that occur together at a given location in North America then are usually effectively isolated by the postmating mechanism of genetic incompatibility. At Austin, Texas, for example, there are 5 species of Bufo (debilis, punctatus, speciosus, valliceps and woodhousei) none of which appears capable of introducing its hereditary variability into any of the other species even if premating mechanisms should fail. In addition, and apparently antedating this mechanism, there are ecological and ethological isolating mechanisms. One theoretically possible way in which these sympatric populations might affect one another would be through the production of sterile hybrids which might in turn affect the chances of success of the parental populations. Natural hybridization of two of these species has been studied by Thornton (1955) who found among 114 mated pairs, 85 homospecific B. woodhousei, 20 homospecific B. valliceps, 7 ♀ B. woodhousei x ♂ B. valliceps, and 2 reciprocal matings. The latter cross results in embryonic death of the F_1, so the only effect would be wastage of gametes in both parental populations. The reciprocal cross results in heterotic, sterile intersexes with male secondary sex characters and deficient calls. The

effect of this cross is potentially greater than that of the reciprocal, since the heterotic F_1 hybrids might compete effectively for the necessities of existence. Critical evidence remains to be obtained, but we would expect that the deficient call would not allow the hybrids to compete effectively in attracting a mate.

In the relatively few instances in which 2 members of the same species group have become sympatric, maintenance of populational distinctness rests mainly with the complex of premating mechanisms. This seems not entirely true, for both in the B. valliceps group (author's unpublished data) and in the B. americanus group (W. F. Blair, 1963b) there is evidence of some reduction of hybrid viability and fertility. In the B. americanus group, B. woodhousei occurs sympatrically in part, at least, with 4 of the 5 other species. Characters of the mating call suggest that call difference has been reinforced in zones of interspecies contact (W. F. Blair, 1962). The greatest interspecific similarity in call exists between B. woodhousei and B. hemiophrys, which is the only species of the group with which it does not occur sympatrically. In B. americanus and B. terrestris, both of which partly overlap the range of B. woodhousei, the calls are more different from those of B. woodhousei in the overlap populations than in the non-overlap ones. In addition to these factors, there is a highly evolved complex of premating isolating mechanisms, particularly of time and place of breeding, as described by A. P. Blair (1941, 1955) and various others. Sympatric hybridization is known between B. woodhousei and 3 of the 4 other members of the americanus group with which it occurs (various authors). In spite of this, the sympatric populations tend largely to maintain their identity as separate breeding systems. Just how much introgression actually occurs remains to be determined.

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CRYPTIC SPECIES AMONG SOUND-PRODUCING ORTHOPTERA. Thomas J. Walker, Dept. of Entomology, University of Florida, Gainesville, Florida, U.S.A.

In sexually reproducing animals speciation occurs when isolated populations of a single species develop genetic differences which will prevent interpopulation gene exchange or make such exchange detrimental (as in the case of the hybrids having reduced viability). Populations which have passed this "speciation threshold" are sometimes so similar as to escape detection by ordinary techniques, and in such instances they can be termed cryptic species.

If cryptic species are allopatric there is little chance of their being detected. Indeed closely related allopatric populations may be classified as subspecies rather than species even when they are easily distinguishable by ordinary techniques.

If cryptic species are sympatric (have overlapping ranges) and synchronic (sexually responsive adults occurring at the same time), they should be revealed by a study of the mechanisms which insure intraspecific meeting and mating and make unlikely interspecific meeting and mating. Such mechanisms are of primary importance for the continued co-existence of cryptic species.

If cryptic species are sympatric and allochronic, they should be revealed by careful studies of life histories.

In crickets (Gryllidae) and long-horned grasshoppers (Tettigoniidae) the calling song is ordinarily responsible for the sexes coming together prior to mating and is therefore the most effective isolating mechanism among sympatric, synchronic species (Alexander 1962, Walker 1957). Careful studies of calling songs and seasonal life histories of the crickets and long-horned grasshoppers of eastern United States began with the work of B. B. Fulton and a few other field biologists and have been continued in recent years principally by R. D. Alexander and me. These studies have revealed that nearly one third of the species in these groups had gone unrecognized by those relying solely on morphological comparisons of preserved specimens.

The following examples are from the approximately forty cryptic species now known among the crickets and long-horned grasshoppers of eastern United States. They are selected to

illustrate the ways in which cryptic species have been detected and the degrees of morphological distinctness which have been found once the cryptic species were recognized by other means. Numbers in parentheses refer to the categories listed in Table 1.

The katydids known as Amblycorypha uhleri produce the most complicated songs yet described for insects. In the coastal plain region of southeastern United States two species are easily recognized on the basis of striking differences in the song; and once specimens are sorted on the basis of song, morphological distinctions are evident. (3)

The cone-headed grasshoppers presently known as Neoconocephalus triops can be separated into two species on the basis of differences in calling songs and seasonal life histories. Individuals of one species overwinter as adults and mate in early spring while individuals of the other species overwinter as eggs and mate in midsummer. So far individuals of the two species cannot be reliably separated on the basis of morphological characters. (2)

Until recently the weed-inhabiting tree crickets (Oecanthinae) were considered to be a single species--Oecanthus nigricornis. Studies of the calling songs and seasonal life histories revealed five species in the eastern United States (Walker 1963). (1) (3) (4) (5)

R. D. Alexander and R. S. Bigelow (1960) have pointed out that the most frequently collected field crickets in northeastern United States belong to two species which are largely indistinguishable morphologically and completely indistinguishable on the basis of the calling song. The species differ in seasonal life history, one overwintering as a late instar nymph and maturing in the spring and the other overwintering in the egg stage and maturing in July or August. Not only is there no evidence of gene exchange between the two species in the field, but no hybrids were produced in a series of controlled laboratory crossing experiments. (6)

Table 1 shows six possible categories of cryptic species which are sympatric and originally detected by studies of calling songs and/or seasonal life histories. Examples of each category have been described above. Most of the cryptic species so far unrecognized among sound-producing Orthoptera belong to category 3, and it seems likely that categories 2, 5, and 6 exist because of our inability to find clear-cut morphological distinctions rather than because of the lack of such distinctions.

Table 1. Detection of cryptic species among sympatric sound-producing Orthoptera.

Category	Non-overlapping Differences in		
	Calling Song	Seasonal Life History	Morphology (properly evaluated only after species had been initially separated by other means)
(1)	yes	yes	yes
(2)	yes	yes	no
(3)	yes	no	yes
(4)	no	yes	yes
(5)	yes	no	no
(6)	no	yes	no

Since few groups of insects have received more careful attention by competent museum workers than the gryllids and tettigoniids of eastern United States, the occurrence of a high proportion of previously unrecognized species is especially noteworthy. Similarly high proportions of cryptic species probably await discovery in other groups.

The common occurrence of sympatric cryptic species suggests that many allopatric populations classified as subspecies are in reality species. Indeed allopatric populations which differ morphologically too little even to be called subspecies are undoubtedly distinct species in some cases.

Unless the speciation of eastern tettigoniids and gryllids has recently been of an unusual sort, genetic incompatibility between insect populations may characteristically precede conspicuous morphological differences.

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POPULATION STRUCTURE IN ANATOLIAN CYPRINODONT FISHES.

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In Anatolia five valid species of cyprinodontid fishes can be distinguished (VILLWOCK, MS.): Aphanius fasciatus C. et V. (living only near the Mediterranean), A. cypris HECKEL (on some places near the Mediterranean, but also on the Anatolian high-plateau; only in fresh-water), A. chantrei GAILLARD (in the Kizil Irmak basin, descending towards the Black Sea near Samsum and Carsamba), A. anatoliae (morphologically very similar to A. chantrei, practically restricted to the earlier Central Anatolian lake basin, descending towards the Aegaean Sea near Ephesus, a highly polymorphic superspecies), and Kosswigichthys asquamatus SÖZER (practically scaleless, with more than one row of funduline-like, conical teeth; living only in Lake Hazer in Eastern Anatolia). There can be no doubt that, in spite of the great differences in certain characters, the Anatolian cyprinodontids form a natural unit (ÖZARSLAN, 1958) which arose independently of the new-world genus Cyprinodon: an interpretation also proposed by MILLER (1950).

In all Anatolian cyprinodontids the chromosome number is the same (ÖZTAN, 1954, KARBE, 1961). Crosses between different species and genera yield sterile males, the sterility of which is based upon characteristic abnormalities during the reduction division. For some hybrid females are fertile back-crossing with either parent species is possible. In the back-crosses males are sterile and females fertile. By repeated back-crosses fertile males can be obtained. The latter fact suggests that a greater number of genes is responsible for complete male fertility (VILLWOCK, MS.).

Each species, with the exception of K. asquamatus which is restricted to one large lake, is represented by a great number of local populations which are well isolated from one another. This is especially true for A. chantrei and A. anatoliae. Most of the populations of these two species were united by AKSIRAY (1952) under the name of A. chantrei and described as subspecies by the same author. Although there are many similarities between these fishes with transversely striped males, according to sterility barriers between the two population groups they have to be accepted as different allopatric species. In the widely distributed A. anatoliae some populations are sexually isolated from others, thus A. anatoliae can be called a superspecies. It is interesting to note that male sterility occurs between populations living far from one another, whereas those living spatially between yield fertile hybrid males with the two extreme populations. One case is known in which some of the male population hybrids (brothers) are fertile, others sterile. This means that at least one of the panmictic populations bears, in a latent stage, some genes which may be useful in later speciation processes (VILLWOCK, MS.).

The superspecies A. anatoliae is polymorphic in so far as the make-up of the scale-covering is concerned. Some populations are normally scaled, in others all individuals are scale-reduced. At first sight the two types are so

different that the scale-reduced fish were described as representing a new genus (Anatolichthys KOSSWIG and SÖZER). The scale reduction in these fish does not reside in a simple reduction of the scale number in the longitudinal or in the transverse scale rows. Reduction in this case is the result of the division of the anlage of the single scales into two or even more, i.e. a reduction of the morphogenetic field of each anlage. The smaller the anlage the later will be its phenotypical manifestation. Further division of the morphogenetic field finally results in the complete suppression of scale formation. There exist gradients for intensity of splitting of the anlagen from the dorsal and ventral regions of the body in the direction towards the longitudinal line: here the best-developed scales can be seen, and here scales persist even in individuals with very reduced scales. In normally scaled Aphanius as in many other cyprinodonts the scales cover each other nicely. They number in the longitudinal line 26-29, transversely 8-9 (rarely 10-11 in certain Anatolian species). In "Anatolichthys" the number of scales in the longitudinal line may be as high as 55 scales, in the transverse 15-20.

In two localities panmictic populations are extremely polymorphic, viz. Lake Burdur and a salt lake, called Aci göl. As rep-orted earlier (VILIWOCK, 1958) fishes, either scale reduced or normal, from these two lakes produce completely fertile males with some normally scaled populations of the superspecies. This permitted the study of the polygenic basis of scale formation. It is interesting to note that the scalation of F_1-hybrids between normal and reduced fishes differs according to the place of origin of the normal parent fish: in some crosses scale reduction being clearly the dominant character, in others recessive. It was concluded that the typical composition of normal scalation of an Aphanius can result from very different genetic bases. This was shown to be true by large-scale crosses between normal or reduced fishes for the one parent and the practically scaleless Kosswigichthys for the other (AKSIRAY, 1952, VILIWOCK, 1963). In the latter species the reduction process of the scalation has reached its end phase, some females being absolutely naked, whereas in males there is a double row of very small scales: up to 10 pairs in the longitudinal line. An individual scale measuring 250 μ (ERMIN, 1946) . Kosswigichthys can be accepted as bearing the highest number of polygenes for scale reduction. Whereas normally scaled Aphanius species (e.g. A. chantrei) yield more or less scaled progeny with Kosswigichthys, crosses between the latter species and scaled members of the superspecies A. anatoliae produce remarkable and frequent reductions of the scale system, the different populations giving different phenotypes in the F_1 with Kosswigichthys. Hybrids between the normally scaled population Gelif (from Aci göl) with Kosswigichthys are as sparsely scaled as the F_1 between a scale-reduced population (e.g. GÖLCÜK) and Kosswigichthys.

During the glacial period the water level in the basin of Burdur was so high that it was in a direct contact with the present Aci göl. But while Lake Burdur, in the last 15.000 years, was merely transformed into a salt lake which is now

the home of the cyprinodontid hybrid swarm only, the Aci göl underwent much greater changes as the result of its lesser depth. In modern times there is practically no lake at all where the geographical maps show it. During the summer of dry years there is an area of 160 square kilometers, covered with salt; primarily sodium sulphate. In wetter years, some regions of the lake retain their winterly appearance, i.e. contain water during the whole summer. In many places a layer of only some centimeters of salt water covers a deep layer of mud. The Aci göl basin is a broad, shallow, trough having its deepest regions to the south of its longitudinal axis. In the rainy season the salt concentration is highest here, and we have to imagine that, even if covered by a film of water, salt concentration is so high that an impassable boundary separates cyprinodonts living to the north and south of this barrier, which normally dries up completely every summer. Polymorphic populations of the superspecies live in Lake Burdur and in springs around Aci göl. AKSIRAY and VILLWOCK (1962) compared the frequency of the different phenotypes in the polymorphic populations around Aci göl and Lake Burdur in the course of 15 years according to material from repeated collection trips. It is important to state that even in small polymorphic micropopulations of only some hundred individuals in the Aci göl region no important changes occured during 15 years. Mixed populations retained their polymorphism, and no remarkable changes were observed in uniform populations. Only in one case a slight rise of feebly reduced individuals in a primarily scaled population was found. An important condition was described by the authors: whereas in the salty Lake Burdur the population is highly polymorphic, in a small fresh-water river, of only some hundred meters length on the lake border, the high variability in the lake is substituted by a small monomorphic population of scaled fishes. Although fishes from the lake can enter this small river freely it seems that only scaled ones make use of this possibility. AKSIRAY and VILLWOCK draw attention to the fact that the scaled populations around Aci göl, live relatively far from the salty coast-line in fresh-water springs. This situation favours the idea that the reduced types are better adjusted to brackish conditions; the scaled individuals, although able to tolerate salt water for longer periods, seemingly prefer fresh-water. If this view is correct we should have an indication for different selective values for the differently scaled fishes. But it has to be kept in mind that on the Anatolian high plateau there are other salt lakes (e.g. Yarisli) in which scaled populations of A. anatoliae exist. Furthermore, in other species of Aphanius (e.g. dispar, fasciatus) even in hypersaline biotops scalation is completely normal. Finally it has to be added that a very typical scale-reduced population lives in the Gölcük Lake (near Isparta) which is, today at least, completely fresh-water.

In our laboratory Miss Inke SCHMIDT did a series of experiments in which fishes of the normally scaled population of Egridir (fresh-water) were exposed to high concentrations of sodium sulphate the results of which will be published in detail elsewhere. She found that such exposure of embryos and young fish, up to sexual maturity, yields individuals

with reduced and disorganized scales, producing phenocopies to the hereditary scale reduction under natural conditions. At least we can conclude that there are certain forms in the superspecies in which the normal scaled type can easily be changed by changing the environment. It has to be remembered that in the great majority of cases the type of scalation under aquaria conditions remains unchanged. Up to now our knowledge is meagre in so far as the reason for the high polymorphism of the Burdur- and Aci göl populations is concerned. Principally two hypotheses have been proposed: the mutation and the hybridization hypotheses. According to the first the different phenotypes from normal to scale-reduced arose by a series of mutations inside the macropopulation living in a large glacial lake of which the modern lake of Burdur and the Aci göl are remnants. The question remains as to the mutation frequency for scale formation in a system of polygenes can be imagined to be so high that during a period of 20.000 - 15.000 years the total variability of today could arise. The hybridization hypothesis has been offered in different forms. Crosses of valid species in nature, as the basis for the production of a high variability in the F_x-generations, has to be excluded because the sterility barriers are to well established between the Anatolian Aphanius species. On the other hand by hybridization of a (homozygous) scale-reduced fish, e.g. of Lake Gölcük or Lake Salda (earlier described as Anatolichthys splendens), with a representative of a homozygous scaled population (e.g. Egridir) would result in a highly variable F_2, comparable with the "hybrid swarm" in Lake Burdur. If this hypothesis is of any value the question arises as to how the scale reduced Gölcük population arose. This may have occured in a long preglacial period under isolation, mutation and selection pressure. But certainly, at the beginning of the change to the reduced "Anatolichthys"-type, a stage of genic variability has to be imagined.

Another possibility has also been discussed (KOSSWIG, 1947, 1961). It is a well known fact that identic phenotypes can be evoked by different genes. In a simplified example we can imagine that primary condition for the formation of a normal scalation was the presence of the gene A. In scaled AA-fish, by mutation, a new gene B arose which enables normal scale formation. AABB-individuals will be scaled, too. By lost of A and transfer of function from A to B (MULLER, 1942) the combination aaBB (scaled) will come into existence. When an AAbb-type is crossed to aaBB, the F_1 (AaBb) will be scaled as the parents, but in F_2 the combination aabb will arise, which may represent the reduced form. Only under the condition that during this evolutionary change by genic substitution no species barriers evolved, this idea may show another way for the production of variability by hybridization. We have some knowledge that the scaled condition within the superspecies has not the same genic background in all its populations. These differences reside merely in the fact that the number of gene pairs controlling scale formation on an additive polymeric basis is different in different populations; therefore crosses between scaled individuals from different localities consistantly yield scaled progeny in later generations. There are only some exceptional and slightly

reduced individuals in the progeny of scaled fishes from two neighbor populations of the Acı göl; but there we are confronted with the "hybrid-swarm" members of a most polymorphic stock, so that homozygosity of the parents can not be guarantied.

NATURAL POPULATION REGULATION AND INTERSPECIES EVOLUTION* David Pimentel, Dept. of Entomology, Cornell University, Ithaca, N.Y.

Interspecies evolution controls natural population regulation. The numbers of parasites, predators, herbivores, and competitors are regulated by the dominant action of the genetic feed-back mechanism (Pimentel, 1961) which allows the interacting species to evolve toward ecological homeostasis with their community associates. For example, the relative densities of parasites and hosts create strong selective pressures on their interacting populations; selection influences genetic make-up and this ultimately changes the characteristics of the interacting populations. In this way homeostasis and self-regulation evolve among the species populations of the community.

To understand the relation between community homeostasis and population regulation, let us examine briefly the evidence concerning the ecology of natural populations. Stability or constancy is a prime characteristic of populations in nature. In discussing stability in animal populations, Macfadyen (1957) states that: "It is generally agreed that the same species are found in the same habitat, at the same seasons for many years in succession, and that they occur in numbers which are of the same order of magnitude." This summarizes the view of most investigators concerning the constancy of animal numbers in nature.

We know, too, that the majority of animals in nature can be classified as either a herbivore, parasite, or predator. Few species are true saprophytes. Many species living in decaying matter depend directly upon the microorganisms in the medium. The herbivorous animal feeding on plants; predator feeding on herbivore, parasite, or predator; and parasite feeding on herbivore, predator, or another parasite form the major structure of the community. The individuals and the populations which make up the community respond to both interspecies and other selective pressures. Thus, the community consisting of an interacting network of species has the capacity to evolve as a functional unit. Adjustments take place between species, especially in the supply-demand economy which is basic to the survival of the community. Inherent in this living network is the capacity for self-regulation and stability.

Another characteristic of populations which should be considered is the relative abundance of animals in nature. Andrewartha and Birch (1954) aptly summarize this evidence when they say: "The truth is that the vast majority of species are rare, by whatever criterion they are judged". Supporting this fact is the evidence that the majority of animals are either a herbivore, parasite, or predator. These species must be rare, especially relative to their food. Each species depends upon its food host and

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in a balanced economy the eating species feed only on interest (excess individuals of the eaten population). They do not touch the capital (those individuals which are necessary for the maintenance of the population of the eaten species).

When a new species is introduced into a foreign biotic community it increases to outbreak levels shortly after introduction. The following cases amply illustrate this point: the Japanese beetle introduced into the United States; the European rabbit introduced into Australia; the European gypsy moth introduced into eastern United States. Because none of these introduced species were genetically integrated in the community and were not a part of the self-regulatory system, their numbers increased uncontrolled to outbreak levels. In time, however, these species and the community will evolve and once integrated the populations of these new introductions will be effectively controlled.

Natural protective mechanisms inherent in many organisms are important to their survival. To escape attack, both plants and animals have evolved protective mechanisms which include various physical structures, coloration, and toxins. Although these mechanisms require energy and other resources for maintenance, their value is repaid by limiting the feeding pressure of the attackers and guaranteeing the survival of the owner.

The preceding information concerning natural populations supports the fact that genetic change resulting from the action of the feed-back mechanism is important in population regulation for the following reasons: (1) Constancy is characteristic of natural populations because community systems are homeostatic. (2) Most animals are either a herbivore, predator, or parasite and feed only on interest of their host populations and thus must be rare, especially relative to their food source. (3) Animals which feed on living matter are part of a reactive system, one capable of self-regulation. (4) Newly introduced species reach outbreak levels because they are not integrated in the new community and not part of the self-regulated system. (5) Protective mechanisms of the food host help limit the numbers and feeding pressure of the eating species in the community system.

Before demonstrating how genetic change may significantly influence population trends two other explanations for the regulation of natural populations--competition (Nicholson, 1933) and environmental heterogenity (Andrewartha and Birch, 1954)--should be mentioned. Competition is the more popular of the two, perhaps because of its relative simplicity. As a population increases in numbers, the resources available to the competing individuals become in short supply and limit reproduction. Finally births equal deaths and the number of individuals of the population reach a state of equilibrium. Further support of the functioning of this mechanism comes from many excellent laboratory experiments.

Competition, however, apparently is not the dominant mechanism regulating the density of populations in nature because animals seldom destroy their food or other necessity. Also if the majority of animals are rare relative to their food host there is little opportunity for competition to be a dominant regulatory mechanism in nature.

Environmental heterogenity focuses upon the variability and randomness of the environment and the relative scarcity of the necessary resources in space and time. I prefer to call this environmental discontinuity (Pimentel, Nagel and Madden, 1963). For example, changes in weather make the environment favorable one time and unfavorable another. Host food may be present yet a shortage actually exist because of wide distribution of food in space and time. These conditions will effectively limit animal numbers. Environmental discontinuity apparently is not the dominant mechanism regulating the density of populations in nature because of the great instability inherent in such regulation. If, as mentioned, stability and constancy are important characteristics of natural populations, then environmental discontinuity cannot play a dominant role in regulating natural populations.

Based on the information we have about natural populations, the genetic feed-back mechanism may be the dominant controlling factor To illustrate the significant effect that changing the genetic make-up of a host population has upon its eating population, 3 examples follow. To simplify I have used 2 alleles but in nature the feed-back would function through polygenic action. Initially there would be little resistance to the eating population by the food host, but gradually under intense selective pressure resistance would increase until it controlled the eating population.

Assume there is a plant population of 100 individuals and these occur in the ecosystem in the following genotypic proportions: 25 plants are homozygous and carry 2 susceptible alleles; 50 plants are heterozygous and carry 1 susceptible and 1 resistant allele; and 25 plants are homozygous and carry 2 resistant alleles. By "susceptible" is meant a plant on which the animal can feed and its population increases rapidly, whereas on the "resistant" type feeding by the animal is limited by some protective mechanism and the population cannot maintain itself. An insect population is distributed with 10 insects per plant. There are 250 insects on the 25 susceptible plants, 500 on the 50 intermediate plants, 250 on the 25 resistant plants--making a total of 1,000. Because the insects on the susceptible plants can feed easily, they reproduce at a high rate or 1.5 per individual; on the intermediate plants the insects just maintain themselves and reproduce at a rate of 1 per individual; and on the resistant plants where feeding is difficult the insects reproduce at a rate of only 0.5 per individual. The insect progeny produced on the susceptible plants is 375; on intermediate plants, 500; on resistant plants, 125. This results

in a total of 1,000 showing that the animal population just maintained itself on the plant population with the proportions of these 3 genotypes.

Using another experimental ecosystem, assume the plant population of 100 has a higher proportion of the susceptible genotype or 36. The same number (50) of intermediate genotypes are present, and the number of resistant genotypes has decreased to 14. Starting again with 1,000 insects distributed at 10 per plant and employing the same reproductive schedules per plant genotype, we find the insect population increased to 1,100. Thus, by increasing the proportion of susceptible plant genotypes and decreasing the proportion of the resistant plant genotypes, the animal population increased.

This next example demonstrates what happens when the proportion of resistant genotypes is increased. The plant population is still 100 but with 14 susceptible genotypes, 50 intermediate genotypes, and 36 resistant genotypes. Starting with 1,000 insects distributed at 10 per plant and employing the same reproductive schedules per plant genotype, we find the insect population decreased to 890 in the next generation.

In these examples the numbers of host plants were kept constant to demonstrate the effect of genetic change in the host upon the eating population. In nature host numbers vary, but this only slows the evolution of feed-back mechanism slightly. As the density of the eating population increases, it feeds heavily on susceptible host-types. The proportion of this genotype is reduced while that of the resistant genotypes increase. The increased proportion of the resistant genotypes reduces the reproduction of the eating population. As the density of the eating population decreases, selective pressure on all the plants declines. When the numbers of the eating population are relatively few in number, the susceptible host types regain their original advantage. With a sparse eating population the number of susceptibles destroyed by the environment will be smaller than the number of resistant genotypes. The burden of maintaining the protective mechanism places the resistant genotype at a selective disadvantage relative to the susceptibles. Therefore, the proportion of susceptibles will increase and, as a result, the eating population will increase again. This second increase of the eating population should not be as great and the following decline should not be as drastic as before. A dampening of the fluctuations continues and in time an equilibrium is established. Then the resistance in the host population is such that the eating species population uses only "interest" from the host population.

Four mechanisms of population regulation have been proposed: competition; environmental discontinuity; parasitism, predation,

and other forms of interspecific interference; and genetic feed-back. I feel that populations are not controlled by any one single mechanism but that the mechanisms often operate interdependently. As the new species is integrated into the community dominant control changes from one mechanism to another. When a species is introduced into a new community, frequently it will increase to outbreak levels. At this time competition and environmental discontinuity are the more important mechanisms regulating the numbers of the eating species while the numbers of the host are severely controlled by the feeding pressure (parasitism, predation, and grazing) of the eating species. Initially with the eating population at high density, intense competition for food exists between the individuals and limits the increase of this population. Concurrently, the severe feeding pressure of the eating species causes the host population to become sparser until it breaks up into numerous discontinuous colonies. Under these conditions some hosts have time to grow, reproduce, and maintain the population. Additional control of the eating species is also afforded by its enemies and interference from other species. If all of these factors are successful in initially limiting the eating species then the feed-back mechanism can evolve and eventually play its dominant role.

The preceding discussion illustrates how populations may be regulated in nature. These ideas are now being tested in the laboratory in the following described experiments.

To investigate how genetic changes in the housefly host (*Musca domestica*) affect the density and population characteristics of a wasp parasite (*Nasonia vitripennis*), 2 populations are used: (1) a "control", in which the genetics of the host population is held constant, and (2) an "experimental", in which the genetics of the host population may respond to parasite density. Initially 100 parasite adults were introduced into each of the 2 population cages. Daily 750 housefly pupae from an unselected control stock are placed in with the control-parasite population; the same number of pupae from a selected experimental-host stock are placed in with the experimental-parasite population. After the pupae are exposed for 24 hours, they are removed and held in separate vials. In 4 to 5 days those pupae not parasitized produce adult flies. For the experimental line these adult flies are placed in another cage and held for the production of progeny, providing more host pupae for the experimental-parasite population.

The parasitized control and experimental host pupae are held until all the parasites have emerged. The emerging parasites are returned to their respective population cages. Because the genetics of the parasite populations may also change under the experimental design, these changes are carefully followed.

The measurement of genetic change in the housefly and parasite populations is made about every 3 months by comparing the development, reproduction, and longevity of the parasite strains using the 4 possible combinations. To initiate a genetic test a sample of houseflies and parasites is withdrawn from each of the parasite populations and from each of the housefly lines and large numbers of all cultured. For each combination a parasite pair is placed with 10 housefly pupae and each day these pupae are replaced with fresh pupae of the same stock until both parasites die. Each of the parasite-host combinations are replicated 100 times.

Measurable evolution has taken place in both the parasite and host populations during the more than 3 years the experiment has been in operation. The experimental host population has become more resistant with the result that original average reproduction of 138 progeny per experimental-female parasite has dropped to about 45. Parasite longevity on the experimental-host population has decreased from 7 to 4 days. In the population cages, selective pressure on the experimental-host population has declined from 97% to 89%, and the density of the experimental-parasite population has declined from an average of 3,437 to 1,760. In addition the amplitude of the population fluctuations has decreased. Interestingly enough the parasite population has evolved toward some avirulence. Evolution in this system has been towards a state of ecological homeostasis.

The ecology and evolution of this parasite and host is being investigated under conditions which allowed both the genetic make-up and host density to change. A special population cage, consisting of 30 plastic boxes joined together with plastic tubes to make a multicell structure, provided ample space-time structure for unaffected parasite-host interactions. The adjoined cells permitted individual colonies to be established within each cell and the connecting tubes allowed limited dispersal within the cage.

In the multicell cage, the distance between colonies and the establishment of new colonies was determined by the rate at which the parasite found and destroyed colonies and also by the rate at which the host established new colonies. A special agar media held in shell vials served as food and oviposition site for the flies and also as larval food. One vial per day was added until the 7 vials were present in each cell. Thereafter, the oldest vial in each cell was removed and a fresh vial introduced. The vials were held in a screen rack off the bottom of the cage. In this way the parasites were not hindered in their search for hosts. Since the larval stage of the housefly is about 5 days, all the larvae had left the vials and pupated on the bottom of the cage by the time the vial was removed.

A comparison was made of the population characteristics exhibited by a control or newly associated parasite-host system and an experimental one in which some ecological homeostasis had evolved. The control 30-cell population system was started with wild parasites and hosts, and the experimental population system used parasites and hosts removed from the experimental system in which the parasite and host had evolved together longer than 2 years.

The population fluctuations in the control system were severe during a 78 week period. The density of the host averaged 6,071 and ranged from 2 to 12,482, and parasite density averaged 3,590 and ranged from 11 to 13,131. In the experimental system the population fluctuations were damped during a 63 week period. The density of the host averaged 6,809 and ranged from 3,953 to 13,116 and the parasite density averaged 1,436 and ranged from 100 to 4,168. The number of cells occupied in the control system was smaller (averaged 24.3 and ranged from 1 to 30) than the number in the experimental system (averaged 28.7 and ranged from 16 to 30). The parasite-host populations in the experimental system were more stable and occupied a larger number of cells than those populations in the control system. This is providing the experimental populations with an opportunity to make greater use of their environmental resources. Because of the greater chances for survival and persistence of the experimental system than the control, the experimental system clearly has the evolutionary advantage.

Different regulatory mechanisms were apparently dominant in these 2 population systems. The average density of the parasite population in the control system was 3,589. At this equilibrium density, births equaled deaths. With the control parasites producing 138 progeny per female, 136 of these would have to be destroyed each generation to allow a parasite pair to survive and replace the parent pair. This would maintain the state of relative equilibrium. Competition, environmental discontinuity, and other factors were responsible for limiting the numbers of the control parasite population. To illustrate, let us assume that competition was the only limiting factor and it eliminated the 136 progeny each generation. In contrast parasite density of the experimental system averaged 1,436 in this system. While this is a lower average density than in the control system, at equilibrium births have to equal deaths. The average reproduction of the female parasites in the experimental system was 45 progeny. The decline from their original reproduction of 138 progeny per female parasite to only 45 was due to the action of the genetic feed-back mechanism. In the experimental system to have births equal deaths, 43 of the 45 progeny would have to be eliminated each generation. For simplicity again let us assume that competition was the factor which eliminated the 43.

The genetic feed-back mechanism, therefore, accounted for almost twice this number or 83.

The action of the genetic feed-back mechanism reduced the growth rate of the parasite population in the experimental system and contributed to the homeostasis of the parasite-host system. This low growth rate of the parasite population permitted competition to assist in controlling the parasite without much of a time lag response. With the parasite population increasing slowly there was less tendency to overshoot the carrying capacity of the environment. There was also less chance for extreme overcrowding and the resulting severe fluctuations which were typical in the control system. As a result of the genetic feed-back mechanism operating as the dominant control and competition as a secondary control force the population fluctuations in this experimental system were damped and greater homeostasis was characteristic.

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THE REGULATION OF NUMBERS IN NATURAL POPULARIONS

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by

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SOCIAL AND ECOLOGICAL FACTORS IN POPULATION REGULATION OF THE AUSTRALIAN MAGPIE, GYMNORHINA TIBICEN. Robert Carrick, Division of Wildlife Research, C.S.I.R.O., Canberra, Australia.

(This resume is provisional, prepared on tour without access to original data that are not yet fully analysed.)

The population ecology of G. tibicen has been studied during 1955-62 at Canberra. In a 5-square-mile study area 700 birds have been individually colour-banded; 2500 others have been group-banded, and 200 territorial groups have been studied. Three field experiments have been done on the proximate factors that stimulate or inhibit breeding.

The open pasture and savannah woodland of this area are typical of a wide region well within the permanent geographical range of the species. It feeds mainly on invertebrates taken on or near the surface of the ground, and it also takes amphibia and reptiles as well as carrion and plant foods when necessary. It lives in social groups of 2 to 10 birds, average 4, with a maximum of 3 adults of either sex in a group; each group has at least one adult male and female, otherwise any combination of age and sex.

The optimal habitat, consisting of a pasture feeding-ground with adjacent trees for nesting, roosting and shelter, is subdivided into territories each permanently occupied by a social group. These territories vary in size from about one to 20 or more acres, average about 10, and each contains up to 20 or more trees. Sub-optimal habitats of two types are also recognized; one has inadequate tree cover consisting of one or several bushes or low trees, and the other lacks a feeding area adjacent to the occupied trees. Social groups form in the open treeless pasture where they hold feeding territories, and this habitat is also occupied by non-territorial birds that form loose flocks. Thus the population can be classified according to the quality of habitat occupied:

a) Permanent groups vigorously defend the optimal territories from all other magpies, and occupy them by day and night continuously throughout the year. Successful breeding is virtually confined to these groups, whose members do not willingly leave the group or territory.

b) Marginal groups hold territories with feeding-ground but inadequate cover, and roost up to several miles away in woods not otherwise used by magpies. Breeding may occur, but success is low. These birds attempt, as a group or as individuals, to gain entry to the preferred habitat.

c) Mobile groups exist mainly during the breeding season and commute between separate tree and feeding areas. Breeding occasionally takes place but no young are fledged.

d) Open groups live in pasture territories without cover, roost in woods, and do not breed. These territories may be short-term or held for several years while the group, and each individual member, continually seeks to improve their habitat status.

e) Flocks consist largely of immature birds which are slightly nomadic in open pasture areas, though many individuals return daily during successive weeks or months from the woodland roost to the same feeding-place. Adults form new groups, and these flock birds attempt to enter or displace groups in better habitats.

Changes in the status and composition of groups occur in several ways. Death or injury of the dominant male usually leads to replacement of the group; loss of adult females may be followed by entry of new females only; most juveniles leave their natal group during the first year, others in the second or third year, and a few remain permanently.

Testes mature in all habitats and social situations, but are largest in adult territorial males and smallest in one-year-old birds. Some females mature at 2 years of age, but full development of the ovary requires adequate proximate stimuli from group status and habitat quality, especially a suitable nest-site. It is inhibited by socio-sexual factors such as insecure tenure of the territory due to unresolved conflict with neighbouring groups or intruders, or to dominance from other females of the same group. There is considerable variation in the ovarian response of different adult females to similar stimulatory or inhibitory situations. This has been tested in field experiments involving the introduction of artificial nest-sites into open-group territories, and of intruder birds into permanent territories.

The study area supports c. 200 territory (permanent, marginal and mobile group) individuals, with little seasonal or annual variation; these include fully 100 adult females, i.e. potential breeders. The flock on this area consistently numbers c. 540 birds, c. 175 of which are adult females. Thus only c. 38% of the adult females have the opportunity to breed, and non-breeding due to inter-group aggression and intra-group dominance reduces this figure to c. 25%. The overall sex ratio is 57 males : 43 females; in territorial groups it is 43:57, and in flocks 63:37.

Annual productivity is low, and in good seasons only about one juvenile per adult territorial female reaches the free-flying stage in January. Territorialism and the associated socio-sexual interactions have greatly reduced breeding population size and the amplitude of seasonal fluctuations in numbers, and have led to a high degree of numerical and spatial stability. In 1962, 17 of the original 40 permanent groups present in 1955 still occupied the same territories, and 9% of males and 16% of females were in the territories in which they were first banded in 1955. Body weights, fat reserves and stomach contents of birds in each habitat throughout the year provide no evidence that food is a limiting factor. Predation on free-flying

birds, especially those in good territories, is light. Diseases cause important mortality, particularly in the flocks which have been seriously reduced by *Pasteurella pseudotuberculosis* and *Aspergillus* that did not affect nearby territorial groups.

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COMPARATIVE ECOLOGY OF RED-WINGED AND YELLOW-HEADED BLACKBIRDS DURING THE BREEDING SEASON

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At higher trophic levels, because of energy loss, food is more likely to become in short supply before other environmental requisites, such as cover and nest sites. Moreover, the relevant parameter for the regulation of natural populations is rate of food intake and, hence, availability, rather than absolute quantity. For example, if an organism utilizes only a small fraction of the absolute quantity of food present, this may mean either that it is not food limited or that the critical minimum rate of food intake is reached while there is yet much food remaining. For herbivores in general food is easily obtained but may be of poor quality. If quality is low enough it may be impossible for food to be digested at a rate sufficient to permit reproduction or even survival. On the other hand, food availability rather than food quality is often a problem to carnivores. For maintenance and reproduction carnivores must be able to capture individual food items at a rate which may be surprisingly high. For example, British workers have demonstrated that titmice in woodlands in winter must obtain an average-sized insect every few seconds throughout the day in order to survive.

In the present study we have investigated the energetics of food exploitation in two marsh-nesting blackbirds, the Red-winged Blackbird (Agelaius phoeniceus) and the Yellow-headed Blackbird (Xanthocephalus xanthocephalus), during the breeding season. They are graminivorous most of the year, being insectivorous only while breeding, and are particularly suited for detailed time and energy budget studies because they are common and easily observed in the low marsh vegetation. Data presented here were largely obtained during 1961-1963 on the Turnbull National Wildlife Refuge, Cheney, Washington. Additional data are available from other areas in eastern Washington and southern British Columbia where both species of blackbirds occur together, and also from Seattle in western Washington where only the Redwing breeds.

The Redwing, the smaller of the two species, is not a food specialist and utilizes many species of arthropods, some with wholely terrestrial life cycles and others with aquatic larval stages. The breeding season is relatively protracted and two broods may be reared. The larger Yellowhead, however, utilizes a comparatively small number of species of insects and they are for the most part forms that are available to the birds for only a brief period. Utilization of the marsh habitat occurs only during the period of maximum emergence of aquatic insects, only

a single brood is reared, and renestings following failure of the first attempt are rare. Data obtained by general observation and counts of cast skins of naiads reveal a correspondingly restricted period of emergence of damselflies at Turnbull. Moreover, the supply of food is determined largely by factors influencing emergence and not by the intensity of predation following emergence, i.e. the availability of food on day $x+1$ is largely independent of the rate of predation on day x.

Energetics of Exploitation. The food brought to nestling Redwings includes a wide variety of Coleoptera (adults and larvae), lepidopterous larvae, Orthoptera, Odonata, and Arachnida. Odonates, particularly damselflies, are prominent in the diet of those birds nesting in marshes producing large numbers of these insects, but the Redwing is always less dependent upon them than is the Yellowhead and breeds in many areas where odonates are completely unavailable.

Food for the young is gathered on the marshes, their moist sedgy borders, and on adjacent grasslands. Even on marshes where both species breed together, the Redwing feeds more in sedge and less on the marsh than the Yellowhead, and in some areas up to 90% of feeding trips may be to the sedges. Data available do not reveal any consistent diurnal pattern of feeding areas.

By contrast, over 80% of the biomass of insects brought to nestling Yellowheads in eastern Washington after the third day consists of odonates, particularly damselflies. That these are primarily obtained soon after emergence is shown by field observations and by analysis of age stages of samples of food taken from the throats of nestlings. There is also a regular pattern of change of forage area during the day, females foraging significantly more in the grassland from 0600-0700 and from 1700-1900 than at other times of the day.

These data are consistent with the hypothesis that early in the morning the birds feed largely upon previously emerged but inactive adults. Later in the morning these adults become active but emerging naiads become available and the birds turn to them. In the afternoon the tenerals begin to move away from the marshes to the adjacent grasslands and the birds correspondingly shift their foraging areas.

The rate of food delivery to the nests is more highly variable and is less easily determined since it has been impossible to measure the average amount of food brought per trip. However, there is usually a peak in the number of trips per nestling per hour around 1000 and another less well-marked peak in the early evening.

Further evidence of the dependence of the Yellowhead upon emergence of damselflies was obtained in 1962, a year of frequent bad weather during the breeding season. During cold, rainy weather there is little damselfly emergence and in 1962 there was massive starvation of nestlings during spells of bad weather. Redwings, however, being less dependent upon supplies of insects affected by bad weather, survived much better than Yellowheads in 1962. Scattered data from other localities in eastern Washington and southern British Columbia indicate that Yellowheads are dependent upon damselflies throughout the Pacific Northwest. Intensive studies have not been made elsewhere but there are data suggesting that in some areas chironomids or orthopterans may replace odonates as the prime food source.

Conclusions. The exploitation of marshes by moderately large passerines such as blackbirds is possible for only a restricted portion of the year when emerging aquatic insects are common. Smaller species, such as marsh wrens, are able to meet their energy requirements by utilizing a wider variety of smaller arthropods and have long breeding seasons. Redwings breed successfully in areas where large insects are not really abundant but the Yellowhead is restricted to breeding where large insects are available in abundance. If these fail and there are no alternative food sources to which the birds can turn, the nestlings starve. Breeding density and the geographical and ecological ranges of the two species will be considered in the light of these data on the nature of environmental exploitation.

(a) Regulation of breeding density. The factors responsible for controlling breeding density are difficult to determine since in birds a simple relationship between a population and its food supply, as found in *Daphnia* for example, does not exist. Rather, complex patterns of social behavior are usually the proximate regulators of density and the ultimate factors resulting in their evolution may be difficult to determine. Both species of blackbirds are polygamous and territorial during the breeding season. Where they breed on the same marshes they are usually interspecifically territorial, the Yellowheads occupying the deeper water and the Redwings the shallower water near shore. In marshes where only one species is present, it typically occupies the entire marsh from shore to the outer edge of emergent vegetation.

The size of territories of both species is influenced by the presence of individuals of the other species. Redwing territories contract when Yellowheads arrive in spring, and while the reverse cannot be observed, it is likely that Yellowheads would expand shoreward if Redwings were not there. In more open areas where damselfly production is higher and the emerging naiads concentrated on a smaller number of stalks per unit area,

the larger Yellowhead is presumably more efficient. Conversely, in more dense vegetation with lowered productivity of large emergent insects, the smaller Redwing may be more efficient. Territorial behavior and habitat selection have presumably evolved in response to this difference. Whereas the two species differ strikingly in the food delivered to the nestlings, they are apparently similar enough in their requirements to preclude the possibility of their efficiently exploiting the same simple vegetation in different ways.

Territorial behavior also limits population density intraspecifically, but more data are needed before it will be possible to relate feeding rates to territory size and location. Nonetheless, even though the territory supplies only a variable fraction of the food utilized for breeding purposes, the location and size of the territory may be of great importance in influencing the reproductive output of territorial individuals. First, the total number of birds utilizing a given area is limited. Second, the better the territory the more food may be gathered close to the nest and, consequently, the shorter the distance to be traveled per trip and the greater the possible rate of food delivery to the nest. Since rate of delivery appears to be of great importance in these birds, the potential value of the territory in this regard may be great.

(b) Regulation of geographical and ecological ranges. As a breeding bird the Yellowhead is restricted to the arid and semi-arid regions of western North America, ranging north into the arid regions of interior British Columbia and the prairie provinces of Canada and south only as far as the lower Colorado River. Two features of the marshes of humid regions may make them unsuitable for Yellowheads. First, such marshes are usually surrounded by woodlands, useless as foraging areas for this species, so that alternative food sources are probably completely unavailable if food supplies on the marsh fail. Second, such marshes are usually characterized by having dense mats of vegetation and less open water. Hence, less sunlight penetrates and productivity of large aquatic insects should be much lower. Consequently, emerging insects may not be sufficiently abundant for a long enough period to permit successful breeding. Those marshes at the extreme eastern portions of the range occupied by Yellowheads are noteworthy as being the most open marshes of the area.

The Redwing breeds from the Atlantic to the Pacific and from southern Yukon to Central America. It is less dependent upon massive emergence of large aquatic insects and is able to obtain enough food for reproduction in marshes with dense vegetation. This may be why the species is able to breed on almost all marshes in North America. The only marshes at

Turnbull without Redwings are those completely occupied by Yellowheads and it is likely that in the absence of Yellowheads, every bit of marshland would support breeding Redwings.

The distribution of the Yellowhead within its geographical range is remarkably spotty, many apparently suitable marshes being unoccupied. One such marsh at Turnbull, singled out for special study, produced few damselflies. Other marshes with more dense mats of emergent and floating vegetation are also lacking in breeding populations of this species.

POPULATIONS OF HARE AND ROE-DEER IN DENMARK. Johs. Andersen, Game Biology Station, Kalö, Rönde, Denmark.

Roe-deer (Capreolus capreolus) and Hare (Lepus europaeus) are two common and popular game species in Denmark, as in several other European countries. The literature on these species as well as on their exploitation is considerable and can be traced centuries back, although population studies as such must be of very recent date.

About 27,000 roe-deer and 400,000 hares are bagged in Denmark per year. The total area of the country is 43,000 sq. km (16.600 sq. miles), but woodland, the habitat of roe-deer, only contributes 10 per cent while 75 per cent of the area is productive farmland. The chief habitat of the Hare is this open, cultivated land although, to some extent, it also occurs in woodland. The roe-deer is characteristic of the numerous small woods and plantations, but the adjoining farmland is visited extensively during the night, when they come out to forage.

During a number of years the two species have been studied in great detail in Denmark, particularly in well-defined areas. As one would expect, the population dynamics of the two species show characteristic differences, but parallel features can also be pointed out.

The techniques used for obtaining the data on roe-deer have already been published. It is repeated here that the population which originally inhabited the woods belonging to the Game Research Station was eliminated in 1950 and analyzed as to population turnover.

It became clear that 75 per cent of the population had to be killed before the sample could be considered representative. The animals were killed in an order which was determined by chance, but on analyzing the data afterwards it was found that bucks are exposed to a greater risk than are does.

When, in 1956, the district had approached roe-deer saturation a special study, based on capture, marking, and release, was initiated. Also when using this technique a representative sample must be of the order of 75 per cent of the population.

During the last 6 years the population has remained stationary in spite of a high birth rate.

Deaths and casualties are of rare occurrence, but there is a pronounced emigration during the summer, when a certain proportion of each age group leaves the district. At an age

of one year or more the majority of the emigrants are males, hence the remaining population comprises a relatively large number of females.

The autumn density of the population is about one animal per 1.6 ha of woodland, a density which even the most trained observer is likely to underestimate. To us, who live in the district, the figure would be difficult to believe had we not the chance to handle the individual members of the population every winter.

It is not possible to provide an explanation for the constant population level, but apparently roe-deer show no reaction to changes in climate. It seems quite improbable that the supply of available food should be a limiting factor. Psychological pressure (in all its vagueness) seems, after all, the most probable cause. Since emigration coincides with the striking territorial behaviour of the rutting season this may afford an explanation.

During the latest years the population appears to decline slightly, but the age composition and relative production has remained unchanged. Deterioration of cover may be the causative agent: the study area is an isolated wood of 165 ha. In 195o it held 117 deer while 27 per cent of the area was dense thickets. To-day, when the population is about loo deer, only 8 per cent of the area provides day-cover for the roe-deer.

The Hare (Lepus europaeus) is a much more difficult animal to study. After several years' study of hares in open districts, with several unknown factors operating, we acquired the shooting rights on a small island, Illumø, in the southern part of Denmark.

The size of the island is loo ha (247 acres) and well above half the area is intensively farmed. Predators and other factors which obviously would affect the hare population are absent. There is no shooting, and the population is not interfered with in any way. The crucial problem was to census the hare population each year. A direct count is entirely unsatisfactory.

The hare is nocturnal and spends the day in a shallow depression in the ground. It sticks to this hollow unless somebody comes quite close. As a matter of fact one can walk about on the island for several hours without seeing a hare although the population amounts to loo-3oo. Young hares weighing less than about two kg (half the adult weight) are practically never seen during shooting and trapping operations.

Through repeated trappings (in nets) at the most suitable time of the year as many hares as possible are caught. They are all marked, age and sex is determined, and they are released. After about a week another trapping takes place. The trapping operation requires about 6o-7o people and 1-2 miles of netting (specially made). During autumn and winter 5-6 trappings are carried out, and gradually a 1oo per cent trapping efficiency is approached. All hares are tatooed and given an ear-mark. Young hares from the summer of the same year can still be distinguished quite satisfactorily by about 1 Oct. (only about 1 per cent doubtful). At this time the first two trappings are made, and 8o-7o per cent of all hares present are trapped. During subsequent trappings a large proportion of recaptures are obtained, and the proportion not recaptured can be estimated rather accurately. Later in the autumn unmarked hares are obtained, they were all born late and escaped the October trapping because they remained in their hollows, and were not detected unless the drivers happened to spot a few and take them by hand.

The trapping around 1 Dec. would, therefore,disclose a number of young hares which, apparently, did not exist on 1 Oct., but at the same time a large proportion of the hares which were young on 1 Oct. have lost the juvenile features, and they would have been recorded as old hares had they not been marked.

At all times the population suffers a mortality, but it varies between age-groups and years. Samples of a hare population obtained any time during the autumn are entirely misleading, be they obtained through trapping or shooting, and they cannot be used for studies on population dynamics.

Two trappings around 1 March are particularly suitable for population estimates because juvenile hares are absent and the vegetation offers no cover. Under these circumstances 9o-95 per cent of the population is captured in two trappings. The proportion of recaptures enables us to estimate winter mortality (a number of dead hares are actually found during the period October-March), and the mortality among males, females, young, and old hares can be separated.

Thus the March-population is estimated. What happens during the summer is essentially unknown, but the autumn trapping gives an estimate of the survivors. Furthermore, the number of (surviving) young derived from the population of old hares can be estimated.

In contrast with the roe-deer populations the hare population fluctuates widely, and climate (spring temperature and June + July rainfall, two independent factors) seems to

affect the autumn population size decisively.

How this climatic regulation works is unknown, but it is largely responsible for the size of the population. A surprising feature is that the number of surviving young is subject to wide fluctuations. If the number of surviving adults is high, the number of young is relatively low, and vice versa. The relationship shows a correlation coefficient of no less than -o.8.

One must conclude that the climatic conditions determine the upper limit of the total hare population. The size is regulated within the limits set by climate and operating on the number of young admitted to the population during the summer.

We are inclined to believe that the potential fertility of the female, 8-lo young, is realized, but that a varying proportion of the young die through lack of space.

Here again the difficult concept of psychological pressure enters the picture.

However, the alternative is also possible, viz. that the potentially possible maximum of young is not realized in certain years - also because of crowding.

We have a peculiar example of an overcrowded roe-deer population. The average number of fawns born to each female of the Kalø herd is 1.8. This is close to the potential maximum. In the Rye Nørreskov herd - 5o miles from Kalø - the number of fawns born to each female was found to be o.9 in 1954. This forest was completely fenced and, consequently, no regulation through emigration was possible. An attempt at regulation through reduced fertility seems possible.

Marking experiments have shown that whereas roe-deer populations ordinarily regulate the population size through emigration the hares remain stationary from birth to death.

It is a well established fact that even in the absence of obvious enemies an animal population will only reach a certain density. The extensive marking experiments give information on the ways in which different species approach this density dependence limit.

The data presented here are only a few aspects of the large body of data concerning roe-deer and hare populations.

The areas under study still hold the original populations, and thanks to the marking experiments we have a perfectly clear picture of the contribution of 6 generations to the

populations.

This winter's trapping of roe-deer yielded 75 individuals and, as usual, we found appr. 40 per cent of fawns. The majority of adults were recaptures, only six being new.

The share of these 75 animals in the present population is being calculated.

On Illumø 4 trapping operations were made during the autumn 1962. This spring we had to postpone the spring census of hares because the severe winter prevented us from carrying out the two usual trappings around 1 March. This is, however, the first deviation from a rigid procedure.

THE REGULATION OF EUROPEAN SPRUCE SAWFLY NUMBERS IN THE MARITIME PROVINCES OF CANADA FROM 1937 TO 1962. M. M. Neilson and R. F. Morris, Canada, Department of Forestry, Forest Entomology and Pathology Laboratory, Fredericton, N. B.

The European spruce sawfly, Diprion hercyniae (Htg.), was first discovered in Canada in 1930 and by the mid-thirties had attained outbreak proportions in spruce forests over large areas in the Gaspé and New Brunswick. The accidental introduction of a polyhedrosis about 1938 was responsible for the rapid decline of these infestations, and under the influence of both disease and introduced parasites, densities throughout the range of the sawfly have been fluctuating at very low levels for the past 20 years. Population studies have been conducted on this insect in New Brunswick since 1938 and data are now available for 52 consecutive generations over a 26-year period. Morris' key-factor approach, using parasites, disease and weather, as the key factors has been used in the analyses of the data. Although disease largely obscured the effect of other control factors during high sawfly densities, larval parasites have been largely responsible for maintaining the low densities experienced in New Brunswick for the past 20 years. A pronounced change in the relative importance of the different parasite species and relative amounts of cocoon predation attributable to small mammals and wireworms has occurred during the change from high to low host densities.

SOME FACTORS CONTROLLING INSECT POPULATIONS LIVING ON SCOTCH BROOM. O.W. Richards, Dept. of Zoology & Applied Entomology, Imperial College, London, England.

Scotch broom, Sarothamnus scoparius (L.) Wimm., is a common plant in England on disturbed sandy soils. It supports a large fauna of herbivorous insects, many of which are confined to it. At the Imperial College Field Station, Silwood Park (England), 9 Lepidoptera, 5 Diptera, 1 Hymenopteran, 7 Coleoptera and 13 Hemiptera are regularly found feeding on it and with them is associated a very large complex of parasites and predators. Such an assemblage is more tightly held together than the group of species one might find in some more general habitat and the interrelations of the populations of the various species are of considerable interest. The ultimate controlling factor, the destruction of the host-plant, is rarely apparent though occasional small plants are killed by Psyllids.

With Dr. N. Waloff, populations of the leaf-eating beetle, Phytodecta olivacea (Forst.)(Chrysomelidae) were studied for the five seasons (1954-58)(Richards and Waloff, 1961) and since then with Dr. J.P. Dempster we have studied the populations of the five Mirid bugs (Heteroptera - Miridae) which live on the plant and it is with the beetle and the bugs I am now going to be concerned.

The life-cycle of Phytodecta runs as follows. Adult beetles spend the winter in the litter beneath the broom and climb up onto the plant in the spring, usually in late April. There they feed, mate and lay eggs until early July when they begin to return to the litter for a second hibernation which only one-third to one-fifth survive. The offspring of the spring beetles become mature larvae towards the end of July; they also enter the litter to pupate. The new generation of adult beetles crawl up onto the broom during August and early September, feed up for about a fortnight, and then without mating re-enter the litter for hibernation. The spring population of adults consists, therefore, partly of "old beetles" and to a large extent of their offspring. Soon after leaving the litter, a rather small proportion of the beetles make a dispersal flight for which we now have fairly substantial evidence.

The principal agencies destroying the beetles are Hymenopterous egg-parasites (rare), larval parasites (mostly a Tachinid fly, Meigenia), a Braconid parasite of the adult (Perilitus), a fungus which attacks the adult and larva (Beauveria) and most important of all predation by various insects, especially Mirid and Anthocorid bugs and earwigs (Forficula). The importance of predation on the eggs and young larvae can be indicated by a "key-factor" analysis by the method of Morris (1956) as modified by Varley and Gradwell (1960). This analysis may underestimate the importance of Beauveria which may have caused a number of unexplained adult deaths but was perhaps not always detected in the early stages of infection or may have developed later in the year after sampling had ceased.

The predation by the Mirids is of a peculiar type. The species are primarily vegetarian but supplement plant-juices with the contents of almost any egg or small insect they come across. They can probably develop satisfactorily if necessary without any animal food. Even the Anthocorids which are obligate predators feed much more on Psyllids and Aphids than they do on the early stages of Phytodecta. They are much more abundant than the beetle. As a result these predators are very important to Phytodecta and

their action on that species is in some sense density dependent though their own numbers are probably unaffected by this particular source of food.

Owing to a combination of the age of most of the plants with exceptionally cold winters, the quantity of broom in the habitat studied was halved in the winter 1955-6 and nearly so in 1957-8. Probably as a result of this predation was much heavier and reproduction of the beetle much less successful in the summers of 1956 and 1958 than in other years. It seems that on any particular quantity of broom there may be a ceiling for the beetle population determined by predation (i.e., approximately a steady state). The reduction in the quantity of broom affects the beetle by increasing predation (since most of the year's hatch of predators manage to get onto the surviving plants) rather than by reducing the beetle's food supply.

Table 1.

Success of reproduction in Phytodecta when the quantity of broom varies. Broom measured in 100 armfuls; adult beetles in hundreds

Year	1954	1955	1956	1957	1958
Quantity of broom	48	46	21	26	14
Adults in spring	180	162	170	64	68
Estimated predation on la. & egss, %	86.1	79.1	98.9	77.8	91.6
Autumn adults produced per spring ad.	1.89	2.34	0.31	1.15	0.79

If one assumes for the moment that the predator population is constant on a particular quantity of broom, then an upper limit is set to the size of the Phytodecta population. If the population increases above this more eggs and larvae will be discovered by random searching and predation will be heavier. This leads to no necessary reaction on the predator population because of the relative unimportance of this particular prey. The process is probably density dependent for the prey but not for the predator.

The assumption that predator density on broom is constant is evidently unrealistic even if it is independent of Phytodecta-density which is really all that is involved in this particular example of a control-mechanism. However, since 1958 we have been making a detailed study of the principal predators concerned, namely five species of Mirid bugs. Their populations seem to provide quite unusually difficult sampling problems. Partly because their habits alter during development from egg to adult, partly (perhaps) because disturbance of any sort also leads to non-random movements on the plant, it is not yet possible to get reliable figures for seasonal population changes. The numbers sometimes seem to rise after all hatching has ceased and before there are any winged adults to immigrate. Nevertheless with four years' data some idea has been obtained of the factors influencing the size of Mirid populations.

There are five species of Mirids which live on broom, three of Orthotylus and one of Heterocordylus in the Orthotylini, and one of Asciodema (Phylini). All are confined to broom except the last which also lives on gorse (Ulex europaeus L.)and rarely, Genista. There is thus an interesting evolutionary problem - how these species have come to live together and how they avoid destructive competition.

All five species have a very similar life-cycle. They hibernate as an egg laid in the bark of some part of the plant. The eggs, according to the species, hatch between March and June. After passing through five instars, they become adult in May, June or July. The adults live for 2-4 weeks and die soon after reproduction is over.

In order to study these insects about two acres of broom were planted in 1957, about 1600 plants in 24 rows. Thus, the area has gradually been colonised by the bugs and their various enemies and we have studied their populations in a period of rapid change. Although there is now a dense area of broom with most of the plants 6-12 ft. high, the situation is still not in equilibrium. If the age of the plant which normally lives for 12-15 years is an important factor from the nutritional point of view, as seems quite possible, no real equilibrium may develop. Although it would not be possible to analyse the situation formally in terms of key factors, it is at least possible to point to two or three processes which could together account for most of the observed facts - namely the coexistence of five species of very similar habits and the relative stability of the populations on areas of well-established broom.

Important factors are (1) parasitism of the nymphs, (2) emigration of the adults, sometimes also (3) destruction of the eggs. Predation on various stages also occurs but is probably less important.

The movements of the adults have been especially studied by Dr. N. Waloff and, for one year, Dr. K. Bakker, especially by using a number of suction traps and also trap plants grown at various distances from the experimental area. I do not wish to anticipate their first paper on the subject which will shortly be published. It may be said, however, that in the early adult period many of the bugs emigrate. Up to 50% of the population may leave and in some species, at least when the population reaches a high density, the movement appears to be density dependent. Immigration is here relatively unimportant because there is no adjacent area of broom nearly as big as our own plot.

The nymphs of all these Mirids are parasitised by Braconids of the genus Euphorus which lay their eggs mainly in the first instar and emerge as a full grown larva from the fifth instar which is killed. The parasites have one generation a year, hibernating, probably as a full-grown larva, in a subterranean cocoon. There appear to be three species of Euphorus involved. One attacks Heterocordylus and rarely Asciodema or Orthotylus adenocarpi. Another attacks Asciodema, the three species of Orthotylus, and apparently also many other Mirids including those which live in the surrounding grass. A third species attacks the three Orthotylus and also the genus Pachylops which lives on gorse. It seems that the hosts of the parasites are to a considerable extent determined by the time of year at which they occur. Thus the parasite of Heterocordylus occurs very early in the spring and has a chance to attack only a few of the very earliest occurring of the nymphs of Asciodema or O.adenocarpi; the other two Orthotylus are too late. In the laboratory, the parasites may be induced to lay in different hosts and there seems to be little real specificity.

Slides have been prepared to show how these parasites have affected two of these species - Heterocordylus and O.virescens - and I have an estimate for the adult emigration for the first of these species in the peak year. I do not want at the moment to

draw any further conclusions from these data except to suggest that the complexity of the relations of these species with one another and with Phytodecta is such as to discourage one from attempting to construct any simple mathematical model.

Table II

Maximum numbers on one day of Heterocordylus in 1000's

Year	Maximum no. of nymphs	% parasitism of nymphs	Maximum no. of adults
1959	50	19.6	19
1960	315	38.9	83
1961	1980	68.0	145*
1962	310	94.2	14

* 187,000 adults emigrated; winter 1961-2 90.8% of eggs destroyed.

Table III

Maximum numbers on one day of Asciodema in 1000's

Year	Maximum no. of nymphs	% parasitism of nymphs	Maximum no. of adults
1959	87	0	65
1960	492	28.3	181
1961	1044	58.9	110*
1962	233	75.2	35

* winter 1961-2 85.9% of eggs destroyed.

Table IV

Maximum numbers on one day of O.adenocarpi in 1000's

Year	Maximum no. of nymphs	% parasitism of nymphs	Maximum no. of adults
1959	11	12.5	15
1960	214	8.8	182
1961	1597	6.6	524*
1962	454	4.1	331

* 443,000 adults emigrated.

Table V

Maximum numbers on one day of O.virescens in 1000's

Year	Maximum no. of nymphs	% parasitism of nymphs	Maximum no. of adults
1959	36	19.7	23
1960	270	10.4	218
1961	1174	25.6	174
1962	1831	24.7	266

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RECENT ADVANCES IN SIMIAN MALARIA

Sponsored by the British Society for Parasitology
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A symposium organized for the
XVI International Congress of Zoology

by

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RECENT ADVANCES IN SIMIAN MALARIA - INTRODUCTION. G. Robert Coatney, Laboratory of Parasite Chemotherapy, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland, U.S.A.

There was considerable interest in simian malarias shortly after the turn of the century and, then, interest wained until the classic work of Sinton and Mulligan, in the thirties, led to a clear understanding of the then known species. Following their work, the malaria of monkeys provided an important tool for research on the parasitology and chemotherapy of human malaria.

The demonstration by Shortt and his colleagues of exo-erythrocytic stages in the liver of a monkey infected with *Plasmodium cynomolgi* pointed the way for finding, shortly thereafter, the same stages of *Plasmodium vivax* in man. Also, the similarity of the response of *P. cynomolgi* and *P. vivax* to drugs, made the finding and development of some of the more effective antimalarials easier and more rapid. Primaquine, the most effective curative drug against relapsing malaria, was selected as the best of the 8-aminoquinolines in trials against *P. cynomolgi* and the results were translated directly to *P. vivax*. Even now, work on the immunology of simian malaria promises to be applicable to that of man, providing valuable information on cross-relationships of the various species.

The discovery that at least two species of malaria from lower primates are transmissible to man has led to a renewed interest in the monkey parasites. Expansion of our knowledge has led to the recognition of six species of malaria in monkeys of the Far East where three years ago only three species were recognized. At the same time, knowledge of the distribution of each species has increased, and those known originally from only the type host have now been found to occur in a number of different host species.

It goes without saying that newer information on taxonomy, morphology, life history, hosts, vectors, and geographical distribution will produce modification and amplification of the information presented during this symposium but, nevertheless, it seems appropriate and necessary to focus our attention on the knowledge now available.

THE SPECIES OF SIMIAN MALARIA: TAXONOMY, MORPHOLOGY, LIFE CYCLE AND GEOGRAPHICAL DISTRIBUTION OF THE MONKEY SPECIES. Don E. Eyles, Far East Research Project, Laboratory of Parasite Chemotherapy, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Kuala Lumpur, Malaya.

Discussion of the species of monkey malaria is best done on a regional basis for, unlike the species of man, none of the monkey malarias, so far as known, has a cosmopolitan distribution. Most of the research has been done in the orient where there are many species of macaques and languars. The malarias of this region will be considered first.

PLASMODIUM INUI.

This species was originally described by Halbestaeder and Prowazek in 1907 and described in detail by Sinton in 1934. It was confused with P. cynomolgi until the description of Sinton. Its periodicity is quartan.

Morphology: Produces no, or only slight, enlargement of the erythrocyte and, if stippling is produced, it is less marked than in P. cynomolgi. The cytoplasm of the trophozoite is not dense and the pigment is moderate in amount. Considerable amoeboidicity is seen in young trophozoites. The mature schizont has 12 to 14 merozoites in the form of a rosette. The preerythrocytic cycle lasts for about 12 days and the mature schizonts are small (about 20 to 24 microns in diameter). They are characterized by the presence of pseudocytomeres.

Life cycle: Mosquito infection is usually difficult. Infections produced in the rhesus monkey and undoubtedly the more usual natural hosts also are moderate but persist for very long periods. This long chronicity may thus explain its high prevalence in nature. It is presumed that the exoerythrocytic stages are persistent.

Geographic distribution: Southwest India, East Pakistan, Thailand, Malaya, Sumatra, to Celebes, the Indochinese region, the Philippines and Formosa.

Natural hosts: Macaca irus (various subspecies), M. mulatta, M. nemestrina, M. cyclopis, M. radiata, and Cynopithecus niger. It, or related species, occurs in the Malayan species of Presbytis.

PLASMODIUM CYNOMOLGI.

This species was also described in 1907 by Mayer. It was not always differentiated from P. inui until the work of Sinton and Mulligan (1933) clarified the relationships, and showed that it is a tertian parasite and P. inui is quartan.

Morphology: The blood stages resemble those of human P. vivax. The trophozoites are amoeboid and the enlarged erythrocytes are distinctly stippled with Schüffner's dots. The number of merozoites is 16 to 20. The preerythrocytic stages were first seen by Shortt and his colleagues and have since been studied in detail by Garnham and colleagues, by Bray and by Eyles. The preerythrocytic cycle is about eighty days. The mature parasites are large, 50 to 60 or more microns in diameter, may be lobed; pseudocytomeres are not present.

Subspecies bastianellii was described by Garnham in 1958 and has recently been accorded specific rank by Bray. The blood stages are indistinguishable from those of typical cynomolgi. It differs from the typical in that the preerythrocytic cycle is shorter (seven rather than eight days), and the non-lobed mature schizonts show pseudocytomeres. This author does not consider the elevation to species rank to be wise at present.

Life cycle: Mosquitoes are usually easy to infect. There have been many studies of sporozoite infections in rhesus monkeys (but not in the usual natural hosts). An acute but usually non-fatal infection is produced which is often self-limiting and not as persistent as P. inui or P. gonderi. Strains differ in this respect and some recently isolated strains studied by this author persist at high levels for many months. Exoerythrocytic forms persist for very long periods, producing relapse in treated monkeys.

Geographic distribution: Southwest India and East Pakistan to Malaya, Java (type locality), Cambodia, probably Formosa, and the Philippines. There seems little point at present in differentiating the species, P. cyclopis, as it has not been shown to differ in any fundamental way from P. cynomolgi. P. c. bastianellii and P. c. cynomolgi are so far known only from Malaya; all of the other records can be ascribed only to species as the exoerythrocytic stages have not been studied.

Natural hosts: Various subspecies of Macaca irus, M. nemestrina, M. mulatta (East Pakistan), M. radiata, M. cyclopis, and Presbytis cristatus.

PLASMODIUM KNOWLESI.

This species was described by Sinton and Mulligan from a strain isolated by Napier and Campbell which was studied in detail by Knowles and Das Gupta prior to its description. The type host was Macaca irus, undoubtedly from Malaya. P. knowlesi is unique among primate malarias, having a 24-hour asexual cycle.

Morphology: The ring forms are frequently very abundant and the high degree of synchronicity gives the aspect of P. falciparum at this stage. The older trophozoites are compact with very dense

cytoplasm and heavy pigment. Schizonts when mature contain about 12 merozoites and in severe infections a distortion of the erythrocyte is produced. Gametocytes are round and heavily pigmented. The preerythrocytic stages were described by Garnham, et al.; the cycle requires about five days. The mature exoerythrocytic parasites are about 40 microns in diameter and are unlobed. Garnham described flocculi of cytoplasm in this species which only became associated with nuclei late in the cycle.

Life cycle: In the rhesus monkey a fulminating infection is produced which is ordinarily fatal within a few days. In the natural hosts the infection is moderate and apparently persists for a long time. Mosquito infection has been difficult and not many studies have been done using sporozoite-induced infections. It is not known if the exoerythrocytic stages are persistent. This species, because of its severe course, has proved valuable in pathological studies.

Geographic distribution: Known only from Malaya, Java and the Philippines. The avirulent subspecies arimai of Formosa is of uncertain status. Pending further study there are insufficient morphological grounds for the differentiation of the subspecies sintoni of Brug.

Natural hosts: Various subspecies of Macaca irus, M. nemestrina, and Presbytis melalophos.

PLASMODIUM OSMANIAE.

This species was described by Shortt, et al., in 1961 and was recently renamed P. shortti by Bray on the basis that P. osmaniae is not available due to conditional publication after 1960. Bray added no new information on the species, so for the present it seems best to retain the name P. osmaniae.

Morphology: P. osmaniae is a quartan parasite with stippling of the Schüffner type but less prominent than in P. cynomolgi. Erythrocyte enlargement is described, but again to a lesser extent than in P. cynomolgi. Sinton in his redescription of P. inui describes erythrocyte enlargement and stippling, and his figures show stippling perhaps similar in degree to P. osmaniae. Mohiuddin also described a strain with prominent stippling, and the author has seen newly isolated strains presumed to be P. inui with fairly prominent stippling in Malaya. Perhaps studies now in progress with the exoerythrocytic stages of this species will throw light on the position of P. osmaniae.

Life cycle: In rhesus monkeys produces moderate to fairly intense infections which persist for long periods. Sprozoite-induced infections have not been described.

Geographic distribution: Southwest India.

Natural host: Macaca radiata.

PLASMODIUM FIELDI.

This species was described (1962) from Macaca nemestrina in Malaya by this author and his colleagues.

Morphology: Periodicity is tertian. Rings are large and the older trophozoites non-amoeboid and compact. Schüffner's stippling is very heavy and occasionally produces unique eosinophilic masses in the cytoplasm of the erythrocyte. There is no enlargement of the erythrocyte. Oval and fimbriate cells are common. The number of merozoites is smaller than in P. cynomolgi, averaging about 12. The schizont is encircled by an intense eosinophilic border apparently representing condensed stippling. The species has superficial similarities to P. ovale of man. Studies of the exoerythrocytic cycle and immunological studies may provide light on the relationships.

Life cycle: In rhesus monkeys produces very mild infection. Exoerythrocytic stages unknown. Mosquito infection is difficult.

Geographical distribution: Known only from Malaya.

Natural hosts: Macaca nemestrina and M. irus.

PLASMODIUM COATNEYI.

This species was also described by this author and his colleagues (1962) from Malaya. It has the distinction of being found first in a vector mosquito, Anopheles hackeri. It has since been found in Macaca irus on a number of occasions.

P. coatneyi has a tertian cycle and produces severe infections in rhesus monkeys. The outcome is occasionally fatal, not as the result of the initial parasitemia, but after long continued severe infection. P. coatneyi shows the phenomenon previously associated only with P. falciparum; that is, the older asexual forms tend to disappear from the peripheral blood so that mostly ring stage parasites are seen. This is so marked in animals with a single brood that the parasite count may alternate by a factor of 1000 times from day to day.

Morphology: Ring and older trophozoite stages are nearly identical to P. falciparum including the marginal forms and tenue forms. Multiple chromatin is frequent. Schizonts are also similar to P. falciparum except that the pigment coalesces at a later stage. Mature schizonts usually contain about 20 merozoites (27 have been seen). The gametocytes are oval but the pigment is of the rice-grain type seen in P. falciparum. If one accepts the

subgenera proposed by Corradetti, et al., it is difficult to make an assignment for this species, as the exoerythrocytic stages are not known.

Life cycle: See introductory notes on cycle above. The infections in rhesus monkeys are intense but in Macaca irus only mild infections are produced. The species is very persistent in both hosts. Mosquito infection is difficult. There are interesting cross-immune relationships with P. knowlesi.

Geographic distribution: Malaya and the Philippines.

Natural hosts: Subspecies of Macaca irus.

There are no species of malaria shared by the oriental monkeys and those of the continent of Africa. This may be due to a discontinuity in the range of the vertebrate host for between Pakistan and Arabia there are no monkeys. There would appear to be fewer species in Africa for only P. gonderi and the lemur parasite, P. girardi, are known. However, investigation has probably not been done as thoroughly as in the oriental region.

PLASMODIUM GONDERI.

P. gonderi was originally described by Gonder and Berenberg-Gossler as P. kochi and was treated as a subspecies of P. inui by Sinton and Mulligan. It was redescribed by Rodhain and van den Berghe in 1936 under the name P. gonderi.

Morphology: P. gonderi is tertian in periodicity and has many points of similarity with P. cynomolgi. It is slightly less amoeboid and produces less erythrocyte enlargement. The merozoite number is smaller and it produces infections of longer chronicity than most strains of P. cynomolgi studied. The young parasites tend to invade reticulocytes, whereas P. cynomolgi prefers normocytes. The preerythrocytic stages were described by Garnham, et al. The cycle requires slightly less than eight days. The parasites approaching maturity are smaller than P. cynomolgi and are not vacuolate. Pseudocytomeres have been seen and the nuclei resemble those of P. knowlesi in showing three or four densely staining granules in a paler matrix.

Life cycle: In the rhesus monkey the early infection is moderate. It is not known if the exoerythrocytic stages persist. It is difficult to infect mosquitoes with some strains.

Geographic range: Africa from the Cameroons and West Africa in Liberia and the Congo.

Natural hosts: P. gonderi has been found in several species of mangabeys including probably Cercocebus atys, C. galeritus agilis and C. aterrimus. In the Cameroons it was isolated from

the drill, Mandrillus leucophaeus. Ionesco-Miahaiesti, et al., described a malaria from East African baboons, but it is not known if this represents P. gonderi.

PLASMODIUM GIRARDI.

This species was described from the Madagascan lemur, Lemur fulvus rufus by Buck, et al., in 1952.

Morphology: The periodicity has not been studied. The infection was studied by Buck, et al., first in an intact animal in which a light infection was present; when splenectomized the infection became heavy. The trophozoites were compact and round or oval with blackish-brown pigment. The erythrocytes were not enlarged and the merozoite number was 8 to 10. The authors compared the parasite to P. inui but Bray calls attention to a resemblance to P. malariae. The exoerythrocytic stages are not known.

Life cycle: Virtually nothing is known.

Geographic range: Known only from Madagascar.

Natural hosts: Known only from Lemur fulvus rufus.

Just as there are no malaria species in common between oriental and African monkeys, the malarias of the American monkeys are still different. Only two species are known.

PLASMODIUM BRASILIANUM.

This species was described from Brazil by Gonder and von Berenberg-Gossler (1908) from Cacajao calvus.

Morphology: Periodicity is quartan. Young trophozoites may by amoeboid and are frequently highly vacuolate. As growth proceeds they round up and become more compact and brown pigment appears. Band forms are seen and the mature trophozoites nearly fill the erythrocyte. The mature schizonts have eight to 12 merozoites. No material erythrocyte enlargement or stippling has been described. The exoerythrocytic stages have been seen recently by Garnham but have not been worked out in detail.

Life cycle: Mosquito infection is difficult but possible in A. freeborni. Blood-induced infections in various hosts have been studied in detail by the Taliaferros. The infection remains chronic for long periods. It is not known if the exoerythrocytic stages persist.

Geographic range: Dunn and Lambrecht have recently published an excellent study. They found this species in a variety of monkeys from Yucatan to Brazil and Peru.

Natural hosts: Members of the Cebidae but not the Hepalidae. Ateles fusciceps, A. geoffreyi (two subspecies), A. paniscus, Cebus capucinus imitator, C. albifrons, C. apella, Alouatta villosa, A. palliata, Lagothrix lagotricha, L. infumata, Samiri sciurea, and S. boliviensis.

PLASMODIUM SIMIUM.

This species is known only from the original description by da Fonseca (1951) who found it in Alouatta fusca in the State of Saõ Paulo, Brazil.

Morphology: The periodicity was not definitely determined. The trophozoites were amoeboid and Schüffner's stippling was clearly present. Hypertrophy of the erythrocytes was produced, and the number of merozoites was higher than in P. brasilianum, the majority presenting 16 or more up to a maximum of 27. Morphologically, it would appear to be related to P. cynomolgi and P. vivax.

Life cycle: Little known.

Geographic range: Known only from type locality, State of Saõ Paulo, Brazil.

Natural host: Known only from Alouatta fusca.

It is hoped that a continuing study of the malarias of monkeys and apes (and man too) will elucidate the interrelationships of these malarias.

THE SPECIES OF SIMIAN MALARIA IN APES. Robert S. Bray, London School of Hygiene and Tropical Medicine, London, W.C.1., U.K.

The morphology, distribution and life-cycle of *Plasmodium reichenowi*, *P. vivax schwetzi*, *P. malariae* of chimpanzees and gorillas, *P. pitheci* of orangutans, and *P. hylobati* of gibbons are described and discussed.

THE VECTORS OF SIMIAN MALARIA: IDENTITY, BIOLOGY AND GEOGRAPHICAL DISTRIBUTION. R. H. Wharton, Institute for Medical Research, Kuala Lumpur, Malaya and McWilson Warren, Far East Research Project, Laboratory of Parasite Chemotherapy, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Kuala Lumpur, Malaya.

Interest in the vectors of simian malaria has been stimulated by the transmission of the Malayan parasite *Plasmodium cynomolgi bastianellii* from man to monkeys by the bite of infected *Anopheles* mosquitoes. Until recently no vectors in nature had been identified, though simian malaria occurs widely in Asia, Africa and in central and southern America. The overwhelming evidence that *Anopheles* mosquitoes are responsible for the transmission of human malaria, and the records of the development of simian parasites in anophelines suggest that these mosquitoes are the most likely vectors in nature. However, insufficient attention has been given to the possibility that culicine mosquitoes may be involved, and recent reports support an earlier finding that *Aedes* mosquitoes may be vectors.

In the African region, no natural vectors have been found for the chimpanzee and gorilla parasites, *P. reichenowi*, *P. vivax schwetzi* and *P. malariae* (=*rodhaini*). *A. gambiae* is a poor vector for *P. v. schwetzi* and failed to support the development of *P. malariae* of chimpanzee origin. The mangabey and drill parasite *P. gonderi* also failed to develop in *A. gambiae* but has been transmitted experimentally with *A. atroparvus* and *A. aztecus*. Several West African anophelines have recently been identified as vectors of the rodent malaria parasites, *P. berghei* and *P. atheruri*, but there are at present no indications of the vectors of simian malaria. Nothing is known of the vectors of the Madagascan lemur parasite, *P. girardi*.

In the central and southern American region no natural vectors have been found for *P. brasilianum* which occurs in several monkey species, but *A. aquasalis* (=*tarsimaculatus*) and *A. albimanus* are recorded as laboratory hosts. The infection has been transmitted experimentally using *A. freeborni*. Bromeliad-breeding anophelines which are known to feed readily in the forest canopy would appear to be the most likely vectors in nature. No observations have been made on the brown howler parasite, *P. simium*.

In Asia, where there is a much wider range of simian malaria parasites, several anopheline species of the *A. leucosphyrus* group have been identified as vectors in nature. *A. hackeri* is a vector of the monkey parasites, *P. coatneyi*, *P. cynomolgi*, *P. fieldi*, *P. inui* and *P. knowlesi*, while *A. balabacensis introlatus* transmits *P. cynomolgi*, and *A. leucosphyrus* is known to carry *P. inui*. These mosquitoes were identified as vectors in Malaya by inoculating sporozoites from wild-caught mosquitoes into uninfected rhesus monkeys, *Macaca mulatta*. Another member of the group, *A. balaba-*

censis balabacensis is an important vector of human malaria in many parts of south-east Asia, and from observations on its behaviour in Cambodia is strongly suspected to be a vector of monkey malaria. A recent report suggests that Aedes pseudomediofasciatus may be a vector of P. cynomolgi in India. No information is available on the natural vectors of monkey malaria in other areas, nor is there information on the vectors of the anthropoid ape parasites P. pitheci, P. hylobati or the new parasite from the Malayan gibbon.

Studies on the development of simian malaria parasites in the laboratory have been of limited assistance in determining the identity of the mosquito hosts in nature. In general the Oriental members of the sub-genus Anopheles have been poor hosts for P. cynomolgi bastianellii while many species of the sub-genus Cellia are satisfactory laboratory hosts. Members of the A. barbirostris group are refractory and commonly show black spores on the gut. In the A. hyrcanus group A. lesteri is moderately susceptible but the remaining species are poor or refractory hosts. A. umbrosus and A. letifer are slightly susceptible but sporozoites fail to develop. On the other hand certain Anopheles (Anopheles) mosquitoes from the Palearctic and Neartic regions are efficient laboratory vectors of P. cynomolgi and P. c. bastianellii. Included among the highly susceptible mosquitoes in the sub-genus Cellia are A. maculatus and A. sundaicus which are vectors of human malaria in Malaya, and A. stephensi, A. culicifacies and A. fluviatilis which are important vectors in India. Observations with mosquitoes of the A. leucosphyrus group have unfortunately been very limited, but indicate that most species are readily infected, including A. riparis whose hosts in nature are not known. Although A. balabacensis introlatus is an efficient laboratory host for the P. cynomolgi strain which it transmits in nature, it is relatively insusceptible to P. c. bastianellii. Differences in susceptibility to different P. cynomolgi strains have also been noted with other anopheline species such as A. maculatus. Partial development of P. cynomolgi occurs in Mansonia uniformis, and experimental infection to the sporozoite stage has been observed in Aedes (Aedes) pseudomediofasciatus in India.

Mosquitoes appear to be less readily infected with P. knowlesi, P. inui and the recently described P. fieldi and P. coatneyi, but observations are limited. P. knowlesi and P. inui have been transmitted using A. aztecus, A. stephensi and A. atroparvus and it has been reported that P. knowlesi will develop in Aedes reginae and Armigeres subalbatus (=obturbans).

Observations on the biology of the vectors is restricted to Malaya where A. hackeri, A. leucosphyrus and A. balabacensis introlatus are proven hosts, and to Cambodia where A. b. balabacensis is the probable vector. Studies have been made in a number of areas chosen to represent the various conditions under which human malaria is transmitted and where monkeys live in

fairly close proximity to man. The Malayan peninsular may be divided into three main zones, the coastal mangrove swamps, the lowlands with fresh-water swamp forest, and the inland hills. Each zone presents further subdivisions depending on the terrain, forest clearance and type of cultivation, and there are transitional regions where the various zones merge. Data regarding the vectors of human, simian and mouse deer malaria are summarised as follows:

MALARIA	COASTAL MANGROVE	LOWLAND SWAMP FOREST	INLAND HILLS
Human	A. sundaicus A. campestris	A. letifer	A. maculatus
Simian	A. hackeri A. pujutensis (?)	A. pujutensis (?)	A. leucosphyrus A. b. introlatus
Mouse deer	A. baezai (?)	A. umbrosus A. roperi A. letifer	
Unknown		A. donaldi	A. riparis

In all areas a comparison was made between the numbers of mosquitoes attracted to human bait on the ground, and to monkey bait at both ground level and on a platform in the forest canopy. The bait was partially enclosed in a net which was searched at intervals throughout the night.

In the coastal mangrove zone A. hackeri and A. pujutensis show a definite preference for monkey bait, and appear to feed almost exclusively on monkeys in the mangrove canopy. Eight malaria isolations have been made from A. hackeri out of twelve inoculations including all the known Malayan parasites. A. hackeri was first found breeding in fallen bamboo in rain forest, but in certain coastal regions the larvae breed freely in the clean water which accumulates in the cut bases of cultivated nipah palms (Nipa fruticans). Adults in all stages of the gonotrophic cycle may also be found at the base of the palms and precipitin tests on blood from freshly fed females confirm that A. hackeri feeds mainly on monkeys. The overall malaria infection rate in A. hackeri is 1.5 per cent (sporozoite rate 0.7 per cent) and 44 per cent of the population is parous. Though long-tailed macaques (Macaca irus) in the mangrove are highly infected (8/8) the A. hackeri population feeding on the monkeys appears to be small. An average of about one A. hackeri was caught per monkey per night, giving a transmission rate of about 2.6 infective bites per monkey per annum. A. pujutensis is also suspected to be a vector of simian malaria in the coastal regions. Neither A. hackeri nor A. pujutensis bite man readily, and though A. hackeri can be raised easily in the laboratory from eggs, and adults can be artificially

mated, all efforts to induce the females to feed in the laboratory have been unsuccessful. The human vectors A. sundaicus and A. campestris are not attracted to monkeys in the canopy and maintain a low level of endemicity in the human population. A. campestris has been suspected to be a vector of simian malaria but does not appear to be involved in transmission.

In the lowland swamp forest the vector has not been determined. A. pujutensis which breeds in casual ground pools in the forest is attracted exclusively to monkeys in the canopy. The infection rate in Macaca irus is 30-40 per cent and a moderate endemicity of 10-30 per cent is maintained in man in some areas by A. letifer. Large numbers of sporozoites from A. umbrosus, A. letifer and A. roperi have been inoculated unsuccessfully into rhesus monkeys. The infections in A. umbrosus, A. roperi, and occasionally A. letifer are now known to be Plasmodium traguli, a parasite of the mouse deer Tragulus javanicus. Suspicions that Mansonia uniformis might be a vector of simian malaria have not been substantiated, but Mansonia mosquitoes feed readily on monkeys in the canopy. The origin of the infections found in A. donaldi is not known, and this mosquito is a poor experimental host for human, monkey and mouse deer malaria parasites.

In the forested areas of the inland hills the anopheline population is never high. A. leucosphyrus shows a clear preference for monkey bait in the canopy, while A. balabacensis introlatus is attracted equally to bait in the canopy or at ground level. Small numbers of A. riparis, another member of the A. leucosphyrus group, have been caught on monkey bait in the canopy. A. maculatus is abundant where the forest is cleared and is an important vector of human malaria but a few are attracted to monkeys in the canopy. The proportions attracted to human bait in the canopy and at ground level were, A. leucosphyrus 145:50, A. b. introlatus 24:20, and A. maculatus 5:37. P. inui has been isolated from A. leucosphyrus caught biting man on the ground and P. cynomolgi from A. b. introlatus caught on monkey bait. Two additional inoculations from A. b. introlatus failed, and also failed to produce infections in man. Sporozoites from one A. riparis and from numerous A. maculatus were inoculated into rhesus monkeys unsuccessfully. Sporozoite rates of 0.4 per cent in A. leucosphyrus and 1.5 per cent in A. b. introlatus have been recorded, but the numbers of mosquitoes caught have been small, and the transmission rate was estimated to be about one infective bite per year. In a small sample of Macaca irus monkeys all were infected (6/6). Malaria in man is transmitted by A. maculatus which maintains a high endemicity of 40-50 per cent in valleys where the forest has been cleared. Four members of the A. leucosphyrus group occur in the forest, A. leucosphyrus breeding in clean shaded seepage pools, A. balabacensis introlatus in animal wallows, A. riparis in pools at the edge of streams and A. hackeri in fallen bamboos or very rarely in ground pools.

Studies have also been made in a monsoon forest area of Cambodia where the human malaria rate was over 50 per cent; monkeys were common and 30 per cent were infected with P. cynomolgi. A. balabacensis balabacensis was the human vector and had a 6.2 per cent infection rate around villages compared with 1.7 per cent in uninhabited forest 1.2-3.5 miles away. However the mosquito population in the forest was much higher than in the village and A. b. balabacensis was strongly attracted to both monkeys in the canopy (295) and to man on the ground (203). The densities of Anopheles in the monsoon forest were much higher than in rain forest, 20 A. b. balabacensis per monkey per night in Cambodia compared with 0.3 A. leucosphyrus per monkey in Malaya. Circumstantial evidence indicates that the village infections were mostly of human origin, whereas it seems most likely that those from the forest were simian. Even so, none of 13 monkeys inoculated with sporozoites developed infections so the source of the deep forest infections remains an enigma.

Data on the culicine mosquitoes attracted to monkey bait have also been collected. Members of the Aedes niveus group feed preferentially in the canopy but no malaria infections have been found. Mansonia mosquitoes which transmit a zoonotic form of Brugia malayi in some parts of Malaya are attracted in large numbers to monkeys in the canopy but do not appear to be involved in simian malaria transmission, though natural infections of an unknown malaria parasite have been found in several species.

The distribution of the Anopheles leucosphyrus group of mosquitoes is closely linked with simian malaria in Asia. Members of the group are found from South-west India and Ceylon, eastwards to the Celebes and north to China, Formosa and the Philippines, covering the known range of simian malaria in this region. All species share a common broad ecological niche, the monsoon and tropical rain forests. They are all "wild" species, not resting by day in houses, though the human vector species are not averse to entering houses at night to bite. Attraction to bait appears to take place early in the evening and the great majority have arrived at their host by midnight, though many do not bite until much later. Despite their forest habitat there is a striking relationship between the various species and primates - they appear almost to be "obligate primate feeders".

Internal relationships within the group indicate that there are three main lines headed by A. riparis, A. hackeri, and A. leucosphyrus which graduate in their linkage with man. The riparis line includes species whose feeding habits are virtually unknown but the only adults ever taken were on monkey bait in the forest canopy. The hackeri line includes A. hackeri, a vector of simian malaria and A. pujutensis, a probable vector, but both species have little or no contact with man; A. hackeri var. sulawesi however is a recognised human malaria vector in the Celebes. The A. leucosphyrus line, which is the most widespread,

also includes vectors of both human and simian malaria; A. leucosphyrus is a vector of simian malaria in Malaya, and human malaria in Sarawak, while A. b. balabacensis transmits human malaria over a wide area from Assam to North Borneo. These species are of particular interest since they provide an intimate link between man and monkeys, and if simian malaria is transmitted to man in nature, it is likely to be in situations where A. leucosphyrus or A. b. balabacensis are common. In the tropical rain forests of Malaya the link is present, but the mosquito population is small. However, in forested areas of Cambodia, Thailand or Burma the possibilities for cross-transmission are not remote.

DISTRIBUTION OF SIMIAN MALARIA PARASITES IN VARIOUS HOSTS.

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The simian malaria parasites are considered to fall into six groups as follows: 1. Quartan parasites, with quartan periodicity in the blood, producing Ziemann's stippling in the erythrocytes and undergoing lengthy sporogony and exoerythrocytic schizogony: P. inui, P. shortii, P. brasilianum and P. hylobati.

2. Ovale group, with tertian periodicity and producing very heavy stippling of the erythrocyte: P. simiae and P. fieldi.

3. Gonderi group, with tertian periodicity and producing light stippling of the erythrocyte: P. gonderi and P. coatneyi.

4. Benign tertian group, with tertian periodicity, and producing Schüffner's dots in the erythrocytes: P. cynomolgi, P. bastianellii, P. cyclopis and P. pitheci.

5. Quotidian group, with daily fever, with rapid sporogony and exoerythrocytic schizogony: P. knowlesi.

6. Hepatocystis spp., including H. kochi and allied African species, and H. semnopitheci and allied Oriental species.

The distribution of these parasites in the different species of monkeys is briefly reviewed, and the range of natural and experimental hosts is discussed.

The evolution of the simian malaria parasites may now be briefly considered, and it is essential here to include lemurs and man, at the bottom and top of the tree respectively, in order to speculate on the relationships. It is easiest to assume the existence of an undifferentiated primate ancestor which persists throughout the geological ages more or less unchanged, and from which branch specialised groups of animals.

In the early Tertiary age, first the lemurs and then the New World groups of pithecoids and ceboids separate; at this time, the quartan parasites and Hepatocystis alone have been evolved - this is the quartan level, and primates which have branched here carry only quartan parasites and Hepatocystis.

The mainstream of primate development continues and off it branches, in the Oligocene period, the Old World monkeys, which have now acquired "ovale", "gonderi" and "benign tertian" groups of parasites, possibly in this order, which approximately represents a gradient in severity of host reaction. Later still in the same age, arose the quotidian group (P. knowlesi). Quartan parasites still persist.

At the Pleistocene level, primate evolution arrives at the higher apes and finally man, and to the previous quartan and benign tertian parasites are added the virulent Laverania, while Hepatocystis disappears.

Just as certain phylogenetic features are reproduced during ontogeny of the host, so do primitive or fundamental stages persist in the life cycle of a parasite which in its natural form is never seen today in the later evolved animals. Thus the New World monkeys are insusceptible to normal infections of the later evolved P. bastianellii, but will support them adequately in the primitive exoerythrocytic cycle in the liver; the chimpanzee shows normal tissue forms of the human parasites, though its erythrocytes are insusceptible to all but the stabilised quartan.

Speculations on the evolution of the malaria parasites are prone to fallacies in three directions at least - concerning the parasite, the host and the vector, and it is as well to regard any theory as highly tentative.

EXPERIMENTAL ADAPTION OF SIMIAN MALARIAS TO ABNORMAL HOSTS.

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A. Adaption to Monkeys and Apes:

Many species of malaria parasites, similar at least morphologically to one of the four human types, have been described from naturally infected simian hosts. A brief review of the experimental studies on the transmissibility of these simian malarias to abnormal simian hosts; i.e., monkeys and apes other than the type hosts from which natural infections were described, is presented.

Vivax-Types:

Plasmodium cynomolgi (Mayer, 1907) Mulligan, 1935, naturally infects the long-tailed (*Macaca irus*) and the pig-tailed (*M. nemestrina*) macaques. Infections with this parasite have been experimentally induced in guenon (*Cercopithecus*) and rhesus (*M. mulatta*) monkeys.

P. cynomolgi bastianellii Garnham, 1959. This subspecies is known only to naturally infect long-tailed (*M. irus*) and pig-tailed (*M. nemestrina*) macaques. Experimentally, this subspecies has been established in rhesus (*M. mulatta*) and capuchin (*Cebus*) monkeys and in baboons (*Papio*).

P. cynomolgi cyclopis Inoki *et al.*, 1942. This subspecies which naturally infects *Macaca cyclopis* has been experimentally established in rhesus (*M. mulatta*) monkeys.

P. gonderi Rodhain and van der Berghe, 1936, naturally infects mangabeys (*Cercocebus*) and drills (*Mandrillus*). It has been experimentally established in rhesus (*M. mulatta*) monkeys and in baboons (*Papio*).

P. schwetzi Brumpt, 1939. Natural infections of this species have been reported only from chimpanzees (*Pan*). This species has not been established experimentally in other simian hosts.

P. simium Fonseca, 1951, has been reported as a natural infection only from the howler (*Alouatta*) monkey.

Malariae-Types:

P. inui Halberstadter and Prowazek, 1907, naturally infects macaque monkeys (*M. irus*, *M. nemestrina*, *M. mulatta*, and *M. lasiotis tcheliensis*). Experimentally, it has been established in guenon (*Cercopithecus*) monkeys, and baboons (*Papio*).

P. brasilianum Gonder and von Berenbert-Gossler, 1908, originally was described in Brachyurus calvus (type host). It also occurs naturally in spider (Ateles), capuchins (Cebus) and howler (Alouatta) monkeys. This parasite has been experimentally established in Aoutus and Callithrix monkeys.

P. rodhaini Brumpt, 1939, the quartan-like parasite of chimpanzees, has not been established in other simian hosts.

P. hylobati Rodhain, 1941, is a quartan-like parasite of the gibbon, Hylobates lensciscus. It has not been established in other simian hosts.

Falciparum-Types:

P. reichenowi Blacklock and Adler, 1924, and P. pitheci Halberstadter and Prowazek, 1907, are falciparum-like simian malarias which are known to infect only anthropoid apes; chimpanzees (Pan) and orangutans (Pongo), respectively.

P. coatneyi Eyles et al., 1962, is the first falciparum-type malaria species to infect lower apes. It also represents the first type strain to have been isolated from the definitive mosquito host (Anopheles hackeri) rather than from the simian host. This species was experimentally established in rhesus (M. mulatta) monkeys. The subsequent isolation of this species from a naturally infected long-tailed macaque (M. irus) would make this simian the type host. Experimental studies to transmit this species to other simians or man have not yet been done.

Ovale-Type:

P. fieldi Eyles et al., 1962, was described from a natural infection in the pig-tailed macaque (M. nemestrina). To date this species has been experimentally established in rhesus (M. mulatta) monkeys only.

Non-Human-Type:

P. knowlesi Sinton and Mulligan, 1932, a quotidian parasite which is completely dissimilar to any of the human malaria species, naturally infects the long-tailed (M. irus) and pig-tailed (M. nemestrina) macaques. This species has been established in a variety of simian hosts representing five genera; namely, Macaca, Cercocebus, Cercopithecus, Papio and Hylobates.

B. Experimental Adaption to Man:

Studies on the transmissibility of the above-mentioned simian malarias to man have been limited. There have been no published reports of attempts to establish infections in man with P. cynomolgi cyclopis of monkeys, P. hylobati of gibbons and P. pitheci of orangutans. Attempts to establish infections in man with P. gonderi of monkeys and P. reichenowi of chimpanzees have failed.

The first simian malaria species to be experimentally transmitted to man was P. knowlesi. This was accomplished in 1932 by the inoculation of parasitized blood. To date, this parasite has not been transmitted from monkey to man by the bites of infected mosquitoes. In 1939 and 1941, respectively, the quartan-like parasites of chimpanzees (P. rodhaini) and of monkeys (P. inui) were experimentally transmitted to man. In 1955, the vivax-like parasite of chimpanzees (P. schwetzi) was transmitted to man.

The successful passages reported above were all accomplished by the inoculation of parasitized blood. In 1960, the first recognized natural (mosquito) transmission of a simian malaria to man obtained with P. cynomolgi bastianellii. During the same year, mosquito transmission from monkey to man likewise obtained with the classical or M strain of P. cynomolgi. In 1961, mosquito transmission from man to man was accomplished in 12 prison volunteers with both of these cynomolgi parasites, as well as from man back to the monkey using the bastianellii parasite. In 1962, it was further demonstrated that a strain of P. cynomolgi recently isolated from the jungles of Malaya could also be transmitted from monkey to man by the bites of infected mosquitoes.

Most recently, P. brasilianum, the quartan-like parasite of spider monkeys, has been transmitted to man by the bites of infected Anopheles freeborni mosquitoes. Six serial passages of this parasite to Caucasian prison volunteers have obtained to date. In contrast to P. cynomolgi which could not be transmitted to Negro volunteers, P. brasilianum has been established in a Negro by the intravenous inoculation of parasitized human blood.

There can no longer be any question as to whether simian malaria should be classified as a true zoonosis. The vivax-like P. cynomolgi (M strain and subspecies bastianellii) can be transmitted with relative ease by mosquitoes not only from monkey to man, but also from man to man as well as from man back to the monkey. The recent mosquito transmission of the quartan-like P. brasilianum from monkey to man by mosquitoes represents a second example of a zoonotic simian malaria. The problems created by such zoonoses are obvious. However, in terms of a program of malaria eradication, the true importance of such zoonoses cannot adequately be evaluated without further intensive studies.

SCIENTIFIC USE OF NATURAL AREAS

A symposium organized for the
XVI International Congress of Zoology

by

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Pacific Science Board
National Academy of Sciences-National Research Council
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SCIENTIFIC NEED OF NATURAL AREAS. Kazimierz Petrusewicz, Inst. Ecology, Pol. Ac. Sci., Warsaw, Poland.

The scientific need of natural habitats may summed up in one simple sentence: natural habitats form a point of reference, a yardstick for an important part of the processes taking place on our Earth. The importance and significance of possessing a point of reference and a knowledge of the yardstick of processes taking place in nature is just as great to biological sciences as a knowledge of the laws and regularities governing the economy of nature is essential to permit of directing the processes taking place in nature and bringing about changes desired.

It would seem that these matters are so obvious that proving them is like breaking down doors already standing wide open, and that nothing further can be said on this subject except truisms, and yet life continually provides examples of the under-estimation, disregard or lack of understanding of the laws and regularities governing economy of natur; itprovides examples in cases in which man by his activities, intended to produce favourable results, produce losses to himself and to humanity in general, since through having disregarded the laws governing natural economy, side or secondary processes are evoked which cause harmful effects far greater than the benefits obtained. Despite the fact that the matters dealt with are so obvious, the importance of this knowledge and the ways in which a knowledge of the laws of natural economy can be obtained isxoften under-estimated or not realised. The causes of this state of affairs lie, in my opinion, amongs others, in the state of development and developmental tendencies of contemporary biology.

Contemporary biology is an extremely many-sided and immensely varied field of human activity. Biology comes into contact along a wide front with non-biological sciences forming scientific disciplines extending beyond the science of life sensu stricto. There would be no difficulty in pointing to a large number of biological disciplines each of which investigates separate processes and possesses methods peculiar to itself, and frequently uses a language incomprehensible to biologists of different specialisations. Nevertheless, despite this variety and many-sidedness it is possible - taking as a standpoint not unconfirmed facts but processes already investigated - to distinguish three basic directions, possessing as their final aim separate tasks, of biological research: 1. a knowledge of the essence /or better perhaps, the nature/ of life, 2. investigation of - to use Darwins words - economy of nature and 3. a knowledge of the history of life - evolutionism.

Contemporary biology is characterised by a particularly strong development of the direction aiming at a knowledge of the nature of life. A knowledge of the morphology and physiology of an organism became insufficient long since. Human thought is penetrating increasingly deeply into the most fundamental and elementary manifestations of life, obtaining an increasingly accurate knowledge of life processes and their substrata - the organisation of living matter, up to and including structure and molecular processes. These are indeed imposing achievements: an increasingly profound and fuller knowledge of the structure of nuclein acids and their role in the synthesis of proteins, the structure of protein and its activity, the specific properties of living membrane, and in particular a closer approach to an understanding of the reciprocal interdependences of structure

and activity, and of those of their manifestations which are common to all living beings. Achievements so far attained give grounds for the hope that we are on the threshold of an understanding of the very essence of life and the mechanism of its creation from parent organisms /heredity/, and possible even of the artificial creation of living matter.

This splendid development of research on the essence of life results - even among biologists - in the under-estimation or in fact failure to realise the simple, and it would seem, obvious fact, that even a complete knowledge of the nature of life does not make it possible to understand the laws and regularities of economy of natur, that the laws and regularities governing natural economy are different, belong to a different category of biological phenomena than the laws and regularities revealed by research on the essence of life - the manifestations and processes of life taking place in organisms. The laws and regularities of natural economy belong to the category of processes, the majority of which take place in groups of individuals. Physiological processes - up to and including processes on the molecular level - concern the individual, take place in the individual. We do not know of any manifestation of life beyond the individual one - life consists of these processes in individuals, but processes taking place in nature are not just the ordinary sum of the manifestations of life taking place in individuals.

Let us illustrate this by a few examples. Disease is an individual process, an epidemic - its formation, course and extinction - form a process subject to different laws; the individual falls ill, while the population is affected by an epidemic, the concept of epidemic is senseless in relation to the individual. Different diseases may produce an identical course of the epidemic; the same disease may produce a different course of the epidemic depending on the ecological situation of the population.

Ecology has amassed an enormous number of facts providing evidence that the population or ecosystem are units really existing in nature. Population or biocenotic processes cannot be explained even if we knew the properties of all the components. Natural economy is not the sum of the manifestations of life of different individuals not the sum of their troubles, successes and failures, but mass processes. To generalize, and possible thereby slightly simplify, it may be said that the laws of natural economy such as the development of the population, gradation, regulation of abundance, dying-out of populations or species, competition etc. - are laws of the probability of the existence of different life processes - the laws of the struggle for life in a widely understood sense, ecological and not physiological laws.

I shall pass over the problem - as not directly related to the subject - of the separateness of the laws of evolution. It seems to me that such processes as the law of the irreversibility of evolution, the laws of adaptative or inadaptative radiation,such as the problem of differences between speciation, philetic and quantum evolution are not simply the sum of physiological and ecological processes. Returning to the subject we may state that life processes and processes of economy of nature are different one. The laws of natural economy may be examined in nature, which is where they are in force. Experimental research in laboratories can only explain certain fragments of the processes of natural

economy, can only reply to certain questions posed by these processes which take place in nature.

In the meantime a knowledge of the laws of natural economy is essential, since exploitation of natural resources is necessary. We are living in a period when one species - Homo sapiens - imprints his stamp on the whole of nature surrounding us, when man has changed and continued to change the face of the earth. These changes are necessary and will be lasting. Man as a heterotrophic being feeds on organic matter formed by other organisms. What is important is that these changes in biotic communities should be changes for the better and not for the worse, that they should not be accompanied by injurious side and secondary effects. In order to ensure this it is essential to know the laws which govern nature, which can best be discovered in their natural habitats. Hence the necessity facing mankind for leaving natural habitats in the form of reserves, nature parks etc.

One further property of natural habitats is worthy of emphasis. Many investigations have shown that natural habitats possesses under given climatic and geographical conditions the highest productivity. It seems that this is somewhat of a general regularity. As far as agrocenoses are concerned this is obvious. The time taken for production is - with few exceptions - far shorter in them than in natural associations, the assimilation period of which lasts for the whole of the growing season. But there are data showing that artificial associations even when producting throughout the whole period of wegetation, are often less productive than natural associations. Paczoski found that in districts in which the steppe is a climax formation, that it is not sufficient to leave a certain area uncultivated. Typical steppe associations with high productivity form and are permanently maintained only when grazed by hoofed animals. Similarly Areher and Bunch found that the plant associations which form on the prairies when bison are present yield the largest crops. Grazing, mowing etc. also exert a beneficial effect on the vegetation of the prairie, but the plant associations formed under these conditions are not among those with the highest crop yield, since such associations are only formed under the influence of herds of bison.

Nunberg 1960 after making a penetrating and comprehensive analysis, divides the forests of Poland from the aspect of healthiness and productivity into three categories, distributed in belts from west to east. The maximum productivity and healthiness is found in forests closest to the natural state /eastly lying/.

The statement that natural ecosystems are on the whole more productive is not equivalent to saying that natural ecosystems should be maintained to a maximum degree. Man changes and must changes natural ecosystems into those of greater benefit to himself. The best method of protection is rational and planned exploitation. Exploitation may even be very intensive, but its extent must be determined scientifically. It has been found, for instance, that 80% of the squirrel population in the Siberian forests may be shot without ill effect to the stock as a whole. Even more - after the large predators have been destroyed - chiefly wolves and lynx - it is essential to shoot the deer game. Investigations made in the Bialowieża Virgin Forest and

Kampinos Forest - extensive nature parks in Poland - showed that unless a certain number are shot, hoofed animals first destroy the forest and then themselves decline. Many more such examples can be given. Nevertheless, despite the fact that the best protection is planned exploitation, reserves of natural habitats must be formed, not only in order to obtain a knowledge of the laws and regularities of natural economy in them. A rational, scientifically understood attitude to natural resources induces man to preserve biological forms which today may not be of interest, but which are potentially useful in the future. This compels us to preserve for posterity forms at all levels of organisation: species, geno- and ecotypes, particularly forms of population, ecosystems formed in a natural way.

It is difficult to describe in detail the way in which Polish biologists have made use of ca 430 nature parks and reserves in Poland. A review of scientific works in reserves and nature parks etc. in Poland shows that these studies cover all branches of "field biology". It is for this reason that I have chosen only certain of the papers, the choice being somewhat subjective.

One of the most important achievements of the Polish Nature Conservancy is the preservation of the European bison /Bison bonasus/. The European bison is a species threatened with complete extinction. The chief cause of this was hunting and even more so the destruction of the natural environment - the wildwood. The first, more specific forms of preservation are to be found as early as the 16th century. Bison bonasus and Bos primigenius were declared "royal animals", and ordinary mortals were forbidden to hunt them unless in possession of the sovereign's consent. This did not, however, save Bos primigenius, the last specimen of which disappeared in 1627. The last natural station of the European bison in Europe was the virgin forest of Bialowieza. In 1914 there were 727 European bison at Bialowieza. The was and poaching destroyed the whole population. The last wild European bison was killed by a poacher in 1921. It became necessary to restore the species from specimens in zoos. On Poland's initiative the International Society for Bison Protection was formed. The European bison was saved, and despite losses during the second World War and the serious epizootic in 1953/54 on January 1963, there were in Poland /not counting the specimens in zoos/ 155 bison in five herds: in the Bialowieza bison reserve and a wild herd of 63 animals living at Bialowieża virgin forest, Pszczyna, Gorce and Niepolomice.

When speaking of Bialowieża it is impossible not to refer to the wonderful research work done by Dehnel, who demonstrated that in the shrew seasonal variations in the size of such permanent parts of the body as the skull take place, and made an outstanding contribution to our knowledge of the ecology and biology of these animals.

In the Tatra National Park Myczkowski has made some very interesting investigations on the ecology of forests, in particular on the interrelationships of the snow covering and plant associations. He showed that up to a certain altitude vegetation decided and formed the snow covering, while above this altitude the snow covering formed the vegetation and excluded woods. He also indicated the ways in which the upper limit of the forest could be raised, thus opening up new production areas.

In the Kampinos National Park a great deal of work has been done by the Institute of Ecology of the Polish Academy of Sciences,

both in the field of population ecology and biocenology. In the field of population research the fundamental task, may be generally defined as the search for interrelations between the population structure and the populational processes, or to put it more precisely: investigation whether, under what conditions and to what extent the ecological structure of the population depends on, and influences the course of population processes /reproduction, mortality, migrational tendencies, transformation of the habitat and in the final effect, dynamic of numbers.

It was found that frequently the ecological structure may influence the lot of the individuals either by changing their life processes, or by causing their even unchanged individual life processes to take place under the conditions determined by the population itself /its structure/, and therefore evoke results determined in a certain way by this structure. It is in way that the ecological structure of the population modifies the effects of habitat and biocenosis on the individuals in the population. One of the expressions of this modification may be the changes in birth rate and mortality, general or in different groups of individuals, which in effect produces the fluctuations of the populations size. Work of this kind is carried out on wild boar, roe--deer, hares, micromammalia and birds. Inter alia it was found that in the case of micromammalia, migrating individuals are infested by fleas to a different extent than settled animals, more often fall victims to vipers /Vipera berus/, that their physiological resistance is less than that of settled animals. The mean duration of residence, rate of disappearance, extent of migration etc. were examined. In the field of the predator-victim relation it was found that hawks in about 70% of the cases attack white pigeons in flocks composed of 1 white to 8 black birds; while in a flock composed of 1 black to 8 white they attack black pigeons also in about 70% of the cases. These interesting results show that not infrequently it is not the personal qualities of the individual which are the cause of victory or defeat in the struggle for life, but its situation, that it is different from the remaining individuals.

Very interesting research on the competition of soil predators was carried out by Kaczmarek, who found that the abundance of Formicidae clearly depended on such habitat factors as: humus contents in the soil, humidity of the habitat, compactness or porosity of the soil etc. The abundance of spiders, Carabidae and predatory larvae of Elateridae did not exhibit correlation with habitat factors, but only with the abundance of ants. He also found that there was no competition between spiders, Carabidae and Elateridae, while each of these groups competes with Formicidae.

Field research over a large number of years made it possible to obtain a good knowledge, including a knowledge of definite individuals, of many populations in the Kampinos Forest, from small mammals to wild boar. Plans for the future include intensification of research on migration and the productivity of different layers or food links.

I mentioned earlier on that not infrequently underestimation or even disregard of field research is encountered among laboratory experimenters intoxicated with their undoubtedly great successes, enchanted with the exactitude and precision of their own research work. In social research no reasonable man would

attempt to explain such economic processes as inflation or deflation, prosperity, recesion, or crisis, by means of the physiology or psychology of human beings, since these are processes of a different category. And yet the opinion is often heard that a knowledge of the essence of life will explain everything, or even better, that research on natural economy should be suspended until a knowledge of the biotic properties of all the components of the ecosystem has been obtained. There are, however, certain manifestations in the field of biology which give godd grounds for optimism. I am here thinking of the plan for the International Biological Programme. It is surely not mere chance that the chief problem put forward by the organisers of IBP - the majority of whom are of course experimentalists - is biological productivity, a question par exellence ecological. This gives hope for an intensification in biology of research on the economy of natur.

LES RESERVES INTEGRALES SONT INDISPENSABLES POUR LA PRESERVATION ET L'ETUDE DES ESPECES ZOOLOGIQUES, DANS LES REGIONS TROPICALES.

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1.- GENERALITES.

Pendant longtemps, les zoologistes se sont contentés de déplorer la raréfaction progressive et bientôt la disparition de nombreuses espèces, comme un phénomène presque inéluctable. Les premières réactions ont consisté en des mesures de protection de quelques individus, parfois à grande distance de leur contrée d'origine. Actuellement, on va beaucoup plus loin et, sous l'influence des écologistes, on s'apercoit que la préservation du biotope naturel de l'espèce est au moins aussi importante. La disparition d'une forme zoologique n'est d'ailleurs pas un phénomène localisé mais a des répercussions lointaines dans les biocénoses.

Or, il est devenu de plus en plus difficile d'effectuer des travaux d'écologie dans des milieux primitifs. La plupart des biotopes naturels ont été détruits ou sont lourdement hypothéqués par des facteurs anthropiques. Une seule solution s'impose, d'est la création - de toute urgence - de réserves intégrales, qui paraissent trouver tout naturellement leur place au sein des grands Parcs Nationaux. Dans la plupart de ceux-ci, cependant, d'intenses mesures de management ont été mises en application et ces régions préservées ne constituent plus nécessairement les endroits idéaux pour les recherches dans des milieux non perturbés. Il s'impose donc que dans certaines portions des Parcs Nationaux, l'on admette que le tourisme ou les intérêts économiques directs cèdent la place aux impératifs de la recherche et de la préservation pure.

2.- ZONES TROPICALES.

On a cru longtemps que le probleme ne se posait pas avec acuité dans les régions tropicales et on s'est imaginé que les milieux primitifs recouvraient encore de vastes superficies dans ces zones géographiques. Au cours des dernières années, les chercheurs ont montré que les biotopes y étaient infiniment plus fragiles que dans les autres régions

et se modifiaient rapidement, avec comme corollaire, la destruction de nombreuses éspeces zoologiques. On apercoit rapidement que la plupart des milieux sont marqués par l'empreinte d'interventions humaines historiques ou proto-historiques. Les seules zones encore plus ou moins intactes se situent dans les mangroves, la végétation alpine et sub-alpine des hauts sommets et de rares portions de la forêt ombrophile de plaine.

Si l'on prend le cas de l'Afrique, qui a la réputation d'être le "continent des Parcs Nationaux", sous les Tropiques, on doit admettre malheureusement que les impératifs à la base de la mise en protection de nombreuses réserves, sont essentiellement d'ordre touristique, mais non scientifique. Le zoologiste a quelque peu l'impression que l'on a songé exclusivement à préserver les Vertébrés supérieurs - et plus spécialement les Ongulés et les grands Carnivores - et que les autres éléments des associations zoologiques ont été totalement négligés.

Les recherches dans les régions tropicales montrent, mieux que partout ailleurs, que les espèces zoologiques sont plus menacées indirectement, par destruction de leur biotope, que par des facteurs directs. Sur les flancs des volcans éteints des Virungas, dans le sanctuaire des gorilles qui s'étend le long des frontières du Congo, du Rwanda et de l'Ouganda, une destruction du sous - bois dans les forêts à Hagenia est bien plus à craindre qu'une chasse directe, fort rare d'ailleurs. Au Kenya, l'antilope de Hunter est surtout menacée par la suppression de son biotope. A Madagascar, c'est bien plus l'abattage inconsidéré des lambeaux de forêt ombrophile qui a déterminé la raréfaction des lémuriens, que leur chasse directe. A Java, le maintien, dans son état actuel de la petite forêt semi-marécageuse de la presqu'île de Oedjoung-Koelong est capitale pour la preservation de l'animal le plus rare de la planète, le rhinocéros de la Sonde. Dans les steppes d'Afrique orientale, la destruction des patures par le bétail pose parfois un problème plus inquiétant que celui du braconnage. Le cratère du Ngorongoro est un excellent exemple, quoique des recherches récentes ont montré que les dommages avaient sans doute été exagérément amplifiés.

Les réserves naturelles intégrales qui s'imposent doivent nécessairement être d'une superficie suffisante pour que la totalité du cycle vital des espèces zoologiques s'effectue à l'interieur des limites de la région protégée, et en particulier, la reproduction.

Nous admettons volontiers que, dans certains cas, il y a tout intérêt à protéger des zones qui sont loin de constituer des "climax" naturels. Une politique absolue de non-intervention pourrait, à la longue, être aussi néfaste pour le naturaliste que pour le touriste. Il apparait, en effet, de plus en plus, que les grandes plaines ouvertes d'Afrique orientale, ces steppes couvertes de "graminées courtes", sont des formations d'origine, au moins partiellement, anthropique. Laisser disparaitre, au nom d'un principe absolu de non-intervention, les migrations d'Ongulés dans la steppe Masai, serait un non-sens.

Il convient également que, dans ces réserves, l'homme continue d'être une valence neutre pour l'animal. Si, en dehors des régions protégées, il constitue systématiquement une valence négative, il n'est que trop souvent, à l'intérieur des Parcs Nationaux, une valence positive pour l'animal, qui le parasite littéralement. Comment peut-on encore envisager de la sorte des recherches de psychologie animale?

Plutôt que de discuter plus longuement "in abstracto", il nous semble plus fructueux d'examiner la situation dans deux réserves naturelles où l'accent a été mis essentiellement sur la protection intégrale ou la recherche scientifique. La pratique a d'ailleurs clairement montré que ces impératifs étaient loin de s'opposer nécessairement au tourisme.

3.- LE PARC NATIONAL ALBERT, AU CONGO.

Le Parc National Albert, dans l'Est de la république du Congo, est situé le long du graben occidental, dans une zone en pleine explosion démographique humaine, région intensément cultivée par l'homme. Il est évident que c'est grâce à une mise en protection à une période où rien ne laissait présager une pareille évolution, qui a sauvé l'ensemble biologique le plus varié de tout le continent africain. Son intérêt essentiel, unique même, réside en une incroyable multiplicité de biotopes, et donc de biocénoses, sur une superficie relativement limitée (8.000 km2).

La réserve naturelle est située à la limite de plusieurs zones biogéographiques; elle contribue donc à la protection d'espèces de forêt ombrophile et de steppe orientale. Située des deux côtés de l'Equateur, elle renferme la plupart des biotopes tropicaux et les facteurs biotiques y présentent donc une extraordinaire vairiété. Pluviosité, température, sols, végétation y sont d'une diversité extrême. L'etage des 2.500 mètres, sur les flancs occidentaux du Ruwenzori, est une

des régions de pluviosité maximale de tout le continent africain et n'est situé qu'à quelques dizaines de kilomètres de la rive Nord du lac Edouard, station la plus sèche de tout le Congo.

Une succession étonnante d'edge-effects s'est développée en limite de ces différents milieux. On peut observer une série remarquable de gradients verticaux et horizontaux. Le plus intéressant est, sans nul doute, la transition complète - et pratiquement disparue partout ailleurs en Afrique - entre la forêt ombrophile de la vallée de la Semliki jusqu'au milieu alpin et glaciaire, sur les sommets du Ruwenzori, en passant par le forêt ombrophile de montagne, les bambous, les bruyères arborescentes, etc. Le Parc National Albert constitue donc ainsi une sorte de microcosme de toute l'Afrique, les changements de biotopes - qui s'étendent généralement sur de vastes espaces - étant réduits ici sur de faibles distances. Cette réserve abrite également des biocénoses dont l'existence est liée à celle de milieux très spéciaux (laves, eaux thermales, gaz toxiques).

C'est le long de certaines rivières de cette réserve naturelle que l'on peut observer certaines des plus importantes concentrations de mammifères sauvages de l'Afrique et même de la planète. Les "troupeaux" d'hippopotames constituent une biomasse considérable, sans équivalent ailleurs dans les tropiques, si ce n'est dans les plaines du Serengeti. Ils sont à la base d'un cycle biologique particulièrement remarquable. Le Parc National Albert, au Congo, est contigu au Parc National Queen Elizabeth, en Ouganda, où des principes de protection diamétralement opposés à la conservation intégrale sont en application. Il ne rentre pas dans l'objet de cet exposer de juger deux méthodes différentes; remarquons uniquement qu'il y a là une occasion unique de comparaison, dans des biotopes similaires.

Rares sont les zoologistes qui ignorent que le Parc Albert constitue le seul endroit où le gorille de montagne peut être étudié dans son biotope naturel, qui est peut-être d'ailleurs une adaptation secondaire à des conditions d'altitude.

Il est essentiel d'insister sur l'ampleur des recherches scientifiques qui ont été effectuées depuis plus de trente ans au Parc National Albert et les autres Parcs Congolais. Il n'existe guère de régions d'Afrique - ou même d'ailleurs - où une prospection biologique plus intense a été réalisée. 288 publications, représentant 468 travaux, sont sortis de presse à la date du 31 décembre 1962 et comptent 30. 881 pages. Au total, 4.056 espèces nouvelles - tant zoologiques que botaniques ont été decrites. Les recherches d'écologie ont fait suite aux

travaux de systématique. Il convient d'y ajouter des études dans la plupart des sciences biologique, ce terme étant pris dans son sens le plus large (météorologie, pédologie, géologie, paléontologie, anthropologie, etc.). Ces travaux ont toujours été caractérisés par leur caractère international et rares sont les pays dont des spécialistes n'ont pas été envoyés en mission par l'Institut des Parcs Nationaux du Congo, ou n'ont pas effectué des recherches sur du matériel provenant du Parc National. Ajoutons incidemment que les mêmes méthodes de prospection intensive ont été utilisées dans les autres Parcs Nationaux du Congo et du Rwanda: le Parc National de la Garamba, qui a pratiquement sauvé la forme septentrionale du rhinocéros blanc, le Parc de l'Upemba et le Parc de la Kagera, au Rwanda, haut-lieu pour l'etude du Balaeniceps et du lycaon.

4.- LE PARC NATIONAL DU SERENGETI, au TANGANYKA.

Contrairement au Parc National Albert, où l'attrait majeur pour le biologiste est constitué par la multiplicité des biotopes, le Parc du Serengeti, au Tanganyka est caractérisé essentiellement par l'abondance de la grande faune. L'existence d'une population de près d'un million d'ongulés, avec leur cortège de prédateurs et de charognards, dans une seule unité écologique, est sans égale. 18 espèces d'antilopes vivent dans cet ensemble et on aperçoit immédiatement combien la sélectivité intervient dans la recherche de la nourriture. Les relations interspécifiques prennent une importance majeure au Serengeti.

Actuellement, les écologistes sont réduits à effectuer leurs "études de populations" chez les Invertébrés ou les Vertébrés inférieurs. Chez les Mammifères, seuls les Rongeurs représentent encore des éléments significatifs des biocénoses. Il semble bien que c'est uniquement au Parc National du Serengeti que des études de population d'ongulés soient encore possible sur une très grande échelle. Mais il importe avant tout de préserver le biotope de ces ongulés généralement inféodés à l'existence de vastes plaines à végétation extrêmement courte. Il y a donc une compétition possible avec les troupeaux d'ongulés domestiques et on pourrait craindre que le milieu est en voie de dégradation, par surpaturage et érosion, dans les zones où se reproduisent les troupeaux d'antilopes et de gnous, situées, pour une grande partie, endehors des limites de la réserve naturelle. Il semble toutefois que le risque est bien moins grand qu'on ne l'a craint un moment et qu'une sorte d'équilibre n'est pas à exclure entre les Masai et le gibier.

Si la préservation des steppes du Serengeti a sauvé ces grandes populations, ette contribue également au

maintien d'un phénomène biologique devenu partout extrêmement rare, si pas complètement disparu: la migration des mammifères sur des distances atteignant annuellemment des centaines de kilomètres, parfois des milliers. Il est capital que les deux zones essentielles en rapport avec ces migrations soient presérvées: les "breeding aréas" et les "dry season holding areas", dont une bonne partie ne jouissent pas de la même protection intensive que dans le Parc National lui-même.

Le Parc National du Serengeti montre clairement l'opposition fondamentale entre les ongulés de milieux ouverts, généralement largement grégaires et mobiles et ceux des milieux fermes, habituellement plus ou moins solitaires et casaniers.

La recherche scientifique - si elle a commencé plus tard qu'au Parc National Albert - y est caractérisée par des objectifs particulièrement ambitieux et des équipes de chercheurs sont sur place pour déterminer si une forme prudente de management - et, même, en dehors des limites de la réserve, d'exploitation - peut aller de pair avec la préservation des populations d'ongulés. Une des premières conclusions - quelque peu paradoxale - a montré que la densité de population en ongulés était, de loin, la plus élevée dans les milieux où la productivité végétale était la plus faible. Des travaux sont actuellement en cours grâce à la collaboration de la FAO, du Michaël Grzimek Memorial Fund et des Parcs Nationaux du Tanganyka. Tout comme d'ailleurs dans les Parcs Nationaux du Congo, des installations et un laboratoire sont à la disposition des chercheurs.

5.- SUGGESTIONS ET CONCLUSIONS.

A/ Il est clair que c'est uniquement dans les réserves naturelles tropicales que l'on pourra établir ces "milieux-types" de référence dont l'importance a été mise en avant par une série de récentes conférences internationales. Ces "milieux-types" sont aussi importants pour l'écologiste que les "types zoologiques" pour celui qui s'occupe de systématique.

Dans les régions tropicales, il s'impose de protéger avant tout un certain nombre de "milieux-types" dans les biotopes fermes. En pratique, la plupart des Parcs Nationaux, à vocation essentiellement touristique, ont été établis dans des milieux ouwerts et les menaces ne sont pas tellement urgentes.

La protection d'echantillons de forêt ombrophile de moyenne altitude (1.500 à 2.200 mètres), terriblement ravagée, et de la mangrove, très menacée par de récents projets économiques s'impose de toute urgence. Dans la forêt ombrophile

de plaine, surtout dans le bloc quinéen occidental d'Afrique et en Afrique du Sud-Est, il convient d'etablir des zones - témoins protégées, pour autant que l'on puisse encore trouver des milieux intacts. Dans le bloc congolais et gabonais oriental d'Afrique et en Amérique du Sud, la situation est moins grave.

Il est suggéré que le Congrès de Zoologie de Washington joigne sa voix à toutes celles qui ont demandé instamment que l'on établisse d'urgence cette "liste des milieux biologiques types", protégés de façon absolue et, bien entendu, suivis par des naturalistes et des photos périodiques.

B/ Il semble clair que d'ici quelques décades le biologiste sera réduit à effectuer ses recherches dans les réserves naturelles intégrales, seules zones où subsisteront des biotopes primitifs. Il convient de les établir suffisamment à temps et ne pas attendre qu'une région soit menacée. Ces réserves intégrales doivent être créées lorsqu'il ne semble pas encore y avoir de menaces directes.

Il ne s'agit pas simplement de maintenir en existence les espèces rares dont la liste est tenue à jour par le Survival Service de l'Union Internationale pour la Conservation de la Nature, Il convient également de faire sorte que d'ici quelques années le naturaliste qui s'occupe des vertébrés superieurs ne soit pas contraint à limiter son étude a des animaux tenus en captivité ou à des specimens morts.

La préservation de ces "laboratoires naturels" est donc essentielle, particulièrement sous les tropiques, pour l'avenir de la zoologie. Il convient d'attirer l'attention de tous ceux qui portent des responsabilités et de veiller à ce que les Parcs Nationaux ne deviennent pas de simples curiosités, pour ne pas dire attractions, touristiques. L'évolution des événements dans des pays neufs comme le Congo et le Tanganyka permet d'être optimiste et montre qu'il y a possibilité de concilier les points de vue économique et scientifique.

Jacques VERSCHUREN,
31 mars 1963.

THE SCIENTIFIC NEED FOR ISLAND RESERVE AREAS. Robert I. Bowman, Dept. of Biology, San Francisco State College, San Francisco, Calif., U.S.A.

Of the various insular areas of the world in which priceless nature reserves have been established, I wish to single out the Galápagos Islands of Ecuador for special emphasis because they are famous out of all proportion to their size for their classical examples of biological evolution. Also, in terms of their physiographic setting, biological anomolies, extant pristine conditions, low incidence of species extinction, and historical significance to biology, the Galápagos are truly "Treasure Islands of Science."

Scientific Importance of Islands

Islands have special scientific significance because they present conditions which, though not entirely unique, are generally simpler than those on continents and therefore easier to analyze.

1. Evolutionary studies.— Traditionally, islands have been the Mecca for students of organic evolution, and in this regard the Galápagos are no exception. Some of the reasons for this are summarized below.

(a) Islands are more limited in size and therefore in space resources than continents. The total land area of Galápagos is approximately 3000 square miles, about half of which is contained in one island, Albemarle; the remainder is distributed among 13 main islands and innumerable islets, some less than one-quarter acre in size and separated by stretches of ocean from a few feet to as much as 70 miles in extent.

(b) Islands are sharply defined by water and have a rather high proportion of coastal conditions. In general, water is a more impenetrable ecological barrier to terrestrial species than are topographical barriers such as deserts and mountains.

(c) As a consequence of "insularity" (two preceding points), populations of terrestrial and littoral organisms are more strictly limited in size and area, with little or no contact with similar populations elsewhere. For example, Galápagos populations of the prickly pear cactus (*Opuntia*), the fiddler crab (*Uca*), the gecko (*Phyllodactylus*), and the yellow warbler (*Dendroica*), are effectively isolated from their nearest continental relatives.

(d) Minor character differences are particularly evident among island populations. This may be explained as follows. Small population size means, biologically, a reduced incidence of mutations. However, such mutations as do occur can spread through the whole population rapidly, if they are favorable, and also have a better chance, if they are neutral, of becoming established in the population by the process of genetic drift. Rapid evolution is favored by many small sub-units of a population, which are partially isolated from each other, but which can interchange genes through occasional migrations between them. Such conditions prevail in oceanic archipelagoes like the Galápagos. For example, the terrestrial tortoises (*Geochelone elephantopus*)

or "galápagos" (after which the archipelago receives its name), have distinctly shaped shells on several islands. Species of geospizine finches have evolved island populations, in some instances within sight of each other, which, nonetheless, differ in the size and shape of the bill and in their vocalizations. This phenomenon of inter-island variability of forms was carefully noted by Charles Darwin when he visited Galápagos in 1835, and it gave rise in his mind to some of his later evolutionary views.

The potentiality for rapid divergent evolution in small populations also explains why we have on islands so many peculiarly structured forms. Examples of these are the Galápagos giants, including 30-foot tall cacti (Opuntia echios), 60-foot tall tree composites (Scalesia pedunculata), and the world's largest and only flightless cormorants (Nannopterum harrisi).

(e) Since most of the major evolutionary events in "higher" land plants and animals have occurred on the large land masses, the progenitors of every living thing on oceanic islands must have come from across the sea. Thus islands have been populated by but a few groups of plants and animals that fortuitously have filtered through the barrier of overseas transport on one or more occasions, reached the islands, and overcome almost overwhelming hazards of survival and colonization. Because of this we find that insular floras and faunas are "unbalanced" with many major taxonomic categories completely absent, and representation by others, sporadic. The smaller and more distant an island is from the nearest mainland, the fewer the species that will be found. Because only a few ancestral immigrants have given rise to the entire endemic biota of islands, it is much easier here than on continents to trace the evolutionary history of the descendent forms. Furthermore, remote oceanic islands typically lack indigenous amphibians, terrestrial snakes, and terrestrial mammals. On the Galápagos no amphibians occur but endemic snakes (Dromicus) and rodents (Oryzomys and Nesoryzomys) are found on several of the islands. Only four families of songbirds, and a scattering of families of seed plants, are represented.

(f) Inasmuch as only fragments of the mainland biota are found on oceanic islands, ecological revolutions termed "adaptive radiations" have taken place, with new and vacant niches being filled by forms whose ancestors behaved rather differently on the continents. This phenomenon is best illustrated by the classical example of Darwin's finches. Among the 13 species composing this homogeneous Galápagos group are ecological and structural equivalents of six families of continental song-birds, including the wood warblers, grosbeaks, tanagers, titmice, parrotbills, and blackbirds. It is noteworthy that two species of Darwin's finches are known to use a small stick as a tool to aid in extricating insects from their entrenchments in wood. Among the plants one of the better known examples of adaptive radiation is found in the composite genus Scalesia, which contains many species varying in stature from shrubs to trees and in ecologic distribution from desert to humid forest (Howell, 1941).

Shifts in structure and behavior resulting from this evolutionary process have created distinctive forms. In those few groups of Galápagos organisms that have been fairly well collected, we

find a high degree of endemism. For example, in the vascular plants, somewhat less than 40 per cent are endemic; in ants, 57 per cent; in reptiles, 86 per cent; in birds, 75 per cent; and in mammals, 100 per cent. Since the number of niches is considerably reduced on oceanic islands, so is the number of species and genera.

(g) Islands play a peculiar rôle in the extinction process. Certain aberrant forms, which would be doomed on the mainland, are permitted to survive on islands. This is particularly true of the giant tortoise which is now extinct everywhere except in the Galápagos and Seychelles, although fossil forms of it have been found in various regions of the world. On small islands, populations tend to become genetically uniform so that they are adapted to a peculiar set of living conditions. The great reduction in heterozygous individuals, with their hidden potentialities for preadaptation to sudden environmental changes, makes island populations extremely vulnerable and predisposed to extinction.

One of the remarkable yet fateful features of Galápagos animals, and originally those of other insular areas, is the almost complete lack of fear of humans. Except in the immediate vicinity of human habitation, mockingbirds will pull at one's shoelaces, flycatchers will attempt to sit on one's shoulder, hawks will permit ruffling of their feathers with a short stick, and fur seals may be stroked without showing alarm. Such a widespread trait makes Galápagos animals ideally suited for behavioral studies. It also means that the vertebrate fauna may suffer severely from irresponsible activities of man. As a direct result of human disturbances, no area in the world has suffered a greater number of extinctions of its bird-life than the islands of the Pacific.

For these reasons islands have been termed "evolutionary traps." Furthermore, the original absence of terrestrial predatory mammals (including man) on remote oceanic islands has also permitted certain evolutionary lines to survive, e.g. Galápagos flightless cormorant and penguin. The loss of flight in these birds and a reduction in size of the wings in several families of insects, tends to increase the isolation of the species, with all its genetic consequences.

2. Distributional studies.— In island groups we have the facts of distribution presented to us in their simplest form, and have our most instructive examples of geographical species formation. We are able to grasp more quickly and clearly the causes of species limitation. On Galápagos, for example, one species of Darwin's finches lives entirely within the large mangrove swamps at the west end of the archipelago, whereas another species resides only on islands that support an abundant growth of prickly pear cactus.

A knowledge of terrestrial biotas of islands may contribute to our understanding of distributional problems on continents, and vice versa. For example, when we know through fossils that giant tortoises once roamed widely on the mainlands of the world, it is less difficult to explain their present disjunct distribution on two widely separated island groups in the Pacific and Indian

oceans.

The fish fauna of the Galápagos-Cocos region is a blend of Panamanian and Indo-Pacific forms, with many of the latter found nowhere else in the Americas. Thus, Galápagos is an important station for eastern Pacific biogeographic studies of both terrestial and marine species.

3. Ecosystem studies.— Every biotic community has a unique complex of energy conversion cycles, the most efficient of which is termed the "climax." When man changes the environment he breaks down the conversion cycles and redirects their course, and particularly those which vitalize the soil, upon which all else depends. Pristine communities have the greatest metabolic complexity that a region can provide. Agricultural intervention destroys a delicate balance by simplifying the natural dynamics. The extreme sensitivity of island biotas to man-made disturbances has been explained above. As evidence of this we need only look to the highlands of Galápagos to see the rapid deterioration of the virgin volcanic soils under intensive and rather primitive cultivation practices. Here cutting, fire, and grazing have proceeded with too much haste and without any established scientific planning. Driven by their precarious economic position, the colonists have taken to growing short-term high revenue crops such as potatoes, which they extend precipitously. Extensive forests, some untouched by man previous to 1960, have been disappearing in a matter of months!

Although some biological studies can be successfully carried on without reference to habitats or other creatures and plants, studies on the bio-economy of the land cannot be so separated and must be related to the environmental conditions, and most desirably to those existing in virgin areas. Thus within designated nature reserves where primeval conditions still prevail (such as on Narborough Island in the Galápagos), research on the dynamics of undisturbed conversion cycles can provide us with a fundamental knowledge of the circulation of energy and the transmutation of raw materials in arid and semi-arid tropical environments.

The natural vegetation is the logical source of plant materials for use in the reclamation of depleted and worn out lands, because these plants are already adapted to the local climate. A little experimentation could provide the practical knowledge necessary for pressing them into service elsewhere. The need for new herbaceous materials to aid in the rejuvination of the vast arid and semi-arid regions of the world is exemplified by the situation in the Argentine. Conservationists there have been searching for drought resistant plants in the agricultural zones of the pampa where, until a few years ago, there occurred one of the most varied grass communities in the world. Since climatic and vegetative conditions on the Galápagos are similar to those occurring on the South American mainland, especially around the Gulf of Guayaquil, it is reasonable to believe that the results of botanical research in Galápagos reserves will have practical application elsewhere.

4. "Living museum" studies.— One of the important functions of island reserves is to prevent the disappearance of rare plants and animals and to maintain them in their natural settings. Thus

national parks and wilderness areas constitute "biological banks" with their enormous "genetic reserves," from which we can withdraw important scientific information about "type" organisms and environments. Basic research on the natural inhabitants of these areas may have far-reaching theoretical as well as practical value. The advancement of scientific knowledge and technology has brought into use a stream of new plants and animals, and there is a constant search by breeders for new materials to improve their domestic stocks. An example of the genetic potential of Galápagos organisms is demonstrated by the recent studies of Professor C.M.Rick who has uncovered a rich source of new hereditary characters in the inconspicuous Galápagos tomtaoes that may serve to improve domestic varieties.

We have scarcely begun to discover the full scientific and economic potential of Galápagos that is hidden in the natural landscape and awaiting discovery. However much of value the botanical and zoological gardens, arboreta, herbaria, display museums, etc., may be, nature reserves conceived as "living museums" provide esthetic, cultural, scientific, and educational values that can never be fully realized in a strictly artificial enclosure.

Contemporary man is the creator and the captive of a technological civilization. Its instruments have related him to the earth in a new way. This new way has reflected back upon man himself, forming and altering his values. We are confronted with a pluralistic society with many value systems, and one cannot make a fully satisfactory defense of nature reserves solely on economic or scientific grounds. From a humanistic point of view, man's need for national parks and nature reserves is based on reverence for nature and knowledge of it.

Nature Reserves in the Galápagos

Under an executive decree signed by the President of the Republic of Ecuador in 1934, all major islands (with the exception of the southern half of Albemarle, Charles, Chatham, and Narborough) were to be set aside as reservations for the flora and fauna. Various rare species such as the albatross, flightless cormorant, tortoise, and others, were to be afforded complete legal protection. It was also declared that a board of directors should serve as guardians of the biota and supervise the establishment of research stations.

During the quarter century following the signing of this historic document, there was virtually no enforcement of the law. With the influx of new colonists followed by a rapid clearing of the arable land, especially on Indefatigable and Chatham islands, and with the rise and fall of the U.S. military bases on Seymour and Hood islands and more accidental introductions of exotic rodents and insects, the native biota suffered irreparable damage. However, it is remarkable that, despite heavy human impact on virgin land, during present and historical times, only two Galápagos species, namely, rats of the genus Nesoryzomys, have become extinct, although several island populations of other species apparently have gone under, namely, the finch Geospiza difficilis on Indefatigable, Charles, and/or Chatham; the tortoise on Barrington, Charles, Hood, Jervis, and James (?); the land iguana, Conolophus, on Seymour and James (?). The loss of so many island populations is most regrettable since it is these units that are

so meaningful in evolutionary studies.

In 1958, during the Darwin Centennial celebrations, and in response to the pleas of the XV International Congress of Zoology (London), UNESCO, IUCN, and various other international scientific groups, the Government of Ecuador ammended the statutes of 1934 and set aside as "national parks" the entire island of Narborough and the western half of Indefatigable Island. The importance of this action cannot be over emphasized in view of the fact that Narborough Island is virtually unscarred by man. Only a handful of explorers have ever penetrated the interior highlands, and almost nothing is known about its southern slopes. Furthermore, Narborough is the third largest island in Galápagos (approx. 20 miles in diameter), it is second highest and exhibits all the typical life-zones of Galápagos, it harbors unknown numbers of endemic plants and animals, and active volcanism is of frequent occurrence. Up till now Narborough Island has retained its pristine primitiveness because of the paucity of safe anchorages and the vastness of the lava fields at its perimeter, which have deterred would-be settlers and all but the most determined explorers. In the absence of wardens or regular patrols it is only a matter of time before the natural defenses of this volcanic fortress are penetrated by man, his domesticates, and his symbionts.

Conditions are much more tenuous in the western sector of Indefatigable Island because of the recent intrusions by settlers into what constitutes the heart of the tortoise habitat and reserve. Here and elsewhere in the Galápagos untold numbers of these reptilian monsters still fall under the machettes of oil hunters, and some are illegally impounded for secret sale to tourists and even to scientists.

The Charles Darwin Foundation for the Galápagos Islands

Cognizant of its tremendous task of implementing conservation practices in the far-flung territory of Galápagos, the Government of Ecuador gave encouragement to a small group of leading scientists, headed by Sir Julian Huxley and Professor Victor Van Straelen, to organize an international scientific trust for the preservation of the natural resources of Galápagos. Under Belgian law and by Royal Decree, the Charles Darwin Foundation for the Galápagos Islands was incorporated on July 23, 1959 (Moniteur Belge, Aug. 1, 1959). The Executive Committee of the Darwin Foundation, which is composed of representatives of various nations interested in this problem, and is supported by the United Nations, national governments, foundations, societies, and individuals, has constructed a research station at Academy Bay, Indefatigable Island. From this central location scientists may base their research operations, and educational information may be disseminated to the local population. It is hoped that the Ecuadorian authorities will utilize the basic findings of scientific studies fostered by the Darwin Foundation in their efforts to safeguard the economic, scientific, and educational resources of the Galápagos.

RESEARCH IN WETLAND RESERVES. Luc Hoffmann, Station Biologique de la Tour du Valat, par Le Sambuc, B.d.Rh., France.

Wetland reserves probably include of all natural areas, those with the most diverse habitats and they harbour a rich animal life. They are generally composed of habitats which are truly aquatic, those which are only moist, and those which are truly terrestrial. Interesting edge effects are displaied on the limits, including the possibilities of amphibious life. Moreover, seasonal cycles often result in the same place being aquatic for one part of the year and terrestrial for the other. Finally aquatic habitats are in a delicate balance and undergo very quick evolution. Changes of water level or quality at once have profound effects on the communities and, as they can often be controlled without great difficulty permit experimental research in natural areas.

It arises from this that wetlands offer a particularly rich field for investigation. The basic problems are much the same as in other natural areas but the multiple aspects under which they appear, and the numerous applications to which their solution may lead, make wetland investigation one of the most profitable for science and mankind. The basic questions which arise for the biologist in front of any natural area are those of the structure of the ecosystem and of the special adaptations which allow the species to integrate themselves in the ecosystem. A reply to this can only be an ultimate goal and a significant approach in its direction can only be made for any particular area by teamwork planed over many years.

Inventory and collection. Certainly the first step is to make an inventory of the plant and animal species occuring in the area. This inventory should if possible contain quantitative indications on the most important species as well as details of their seasonal cycle.The quantitative aspect and seasonal cycles can be followed by regular sampling of plants and animals on stations chosen as representative of their habitat-category. In truly waterlogged stations handgrabs or other special devices may be used for quantitative sampling; in dry or only partially submerged areas one may collect on a measured surface with the aid of the special devices widely used today.For higher animals, especially birds, a census has to be made over a comparatively large surface or along some lines. However, quantitative sampling involves difficulties, which are partly technical but which are chiefly problems of the economic use of biologists and technicians. There is no doubt that it would be very useful in many cases to have accurate quantitative data, but if the results are really to give reliable statistically significant indications of total biomasses for an extensive surface, very many samples have to be taken and worked up in the laboratory. This means a lot of work, which will occupy several people for the whole time and will only be possible in big research-teams. In other cases quantitative sampling will probably have to be reduced to some particular species of plants or animals which have special importance in the research programme.

Cartography is an important part of the inventory. Good topographical maps are needed, and a map giving the distribution of plant commu-

nities has to be drawn on a medium scale. Some fragments containing transitional zones have to be mapped on a large scale as they will be very important to judge evolution of the ecosystem in a relatively short time. It will often be necessary to put reference marks in the field, otherwise the location of limits may no longer be possible after several years. Habitat-photographs taken from well determined points at regular intervals should also be collected as they often show in a very instructive manner the evolution of a habitat.

As specimens will be handled daily by the research workers and as determination problems may arise, the constitution of a collection seems essential. This collection, so far as it is only to prove the occurence of a species or to determine it, needs only one or few specimens per species. For species difficult to conserve such as waterplants or small insects, the collection may be usefully completed by photographs or by drawings.

The constitution of an inventory and a collection can no longer be considered as a scientific goal in itself, it is only the forging of an instrument to be used for ecological research. It shows the constituents which by interaction among themselves and with the environment build up the community. However by comparision with later inventories it permits the evolution of the ecosystem to be detected. Therefore, if it is certainly necessary to begin research by making an inventory, one should not spend too much time at this stage but should rapidly make plans how to study the interrelationship of the different constituents of the ecosystem. There are two complementary ways for an approach to this.

The structure of the ecosystem. As any form of life is based on the particularity of the substratum, this should be studied in order to know the conditions and possibilities of life. This means physical and chemical analyses of water and bottom in aquatic habitat, and of soil and soil-solution in terrestrial habitat. The seasonal cycles of the watertable and of the physical and chemical character of water are also of fundamental importance. The climate is also a factor of primary importance and should be studied.All these factors are the conditions of, and give the limits for,the primary production of living material, which in turn is the source of any animal life.

Measurements of primary productivity are still difficult.Different methods have been established both for aquatic and terrestrial habitat, but they are so far not satisfactory for determination of the total productivity of a habitat under natural conditions and the results can only be considered as very rough approximations. However, the measure of primary productivity would be interesting in any wetland-reserve, as it is in some wetlands, and more precisely in estuaries, where the highest productivity rates of any natural area have so far been found. Primary producers are consumed by other organisms which form together the second trophic level; these are eaten by animals of the third trophic level and so on. Each time the organic material or energy mounts from one level to another there is energy loss and also loss of material taken by decomposers. Therefore the biomass is always reduced from one level to the next and from the last goes entirely to decom-

posers. It would certainly be interesting for any wetland reserve to class its fauna according to the trophic levels and to show in this way the pyramid of biomass or energy. This is the raw structure of the biotic community. The next step is to find out for any one animal the others on which it feeds and by which it is eaten. This gives then the structure of the food web, which with its abiotic basis gives the structure of the ecosystem.

Rich animal life occurs, generally speaking, where high primary pro ductivity occurs but this is not always so.A large part of the primary production may not find the animal consumers on the higher trophic levels and come rapidly again out of the cycle through decomposers.This is the case for instance in many temperate eutrophic wetlands where, after the winter pause an explosive development of phytoplancton takes place, followed immediately by a similar increase of zooplancton.This in turn finds only comparatively few consumers such as insect larvae, fish and vertebrates. Although insect larvae in particular may take much profit from this manna and grow rapidly, they can not follow the plancton-production-rate and most of the zooplancton will die unconsumed and be taken out of the cycles by decomposers. Thus the efficiency of communities in bringing energy to the higher trophic levels is variable. It would be interesting to know this efficiency for different wetland types and we shall come back to this point when we dis cuss the applications. The relation between primary productivity and high biomass depends also on the turnover rate of community metabolism. This is higher in warm wetlands and more primary production is needed in tropical marshes to give the same biomass as in temperate.

In turn the densest populations of higher vertebrates occur where they are direct consummers of primary producers.Among mammals, rodents and ungulates are well known to do so but their occurence is chiefly on dry land. However, the same situation exists for some wetland birds for instance for grazing geese and ducks which may occur in very large concentrations;the most spectacular example is probably the crowds of several millions of Lesser Flamingoes on small East African lakes,feeding chiefly on blue-green algae which are the main primary producers on these lakes.

Other birds, although they feed on primary producers, take only special parts of them such as grains or bulbs. Although this food occurs in smaller quantity it has high energy-content and may therefore in favourable cases sustain bird populations of high density. The remaining birds feed on insects and other invertebrates or on vertebrates and belong to the secondary, tertiary or even higher trophic levels. They therefore occur never or only on some special occasions in high concentrations. Such special occasions may in fact be the "eruptions" of plancton in spring mentioned above which may lead to temporary concentration of waders. Typical representatives of the highest trophic level are birds of prey which, in so far as they feed on living vertebrates, occur always only in low population density.

Some wetland reserves like wildfowl refuges have their particular importance for the high biomass of birds, such as wintering ducks and geese, feeding directly on plants. In these refuges it may be of no in

terest or even undesirable to have much energy going into invertebrate life and higher trophic levels. However, more complete nature reserves or National Parks are those whose energy flow takes a rich diversity of directions, which results in a large number of species at all trophic levels, even if this means a somewhat lower biomass of higher animal life.

We come now to the second approach to the structure of the ecosystem. When we tried to begin at the base and to follow energy flow, this means a long chain until the higher vertebrates, which are often the major objects in research, are reached. Therefore one can also take the opposite method and begin with birds and mammals. In order to find the structure of vertebrate communities the biologist does not need to worry about primary productivity and the basic trophic levels. He will find a quicker way by watching directly the birds and mammals and finding out what is their food. This may bring him to a general hypothesis about the structure of the vertebrate communities of any given wetland reserve and its interrelationship with invertebrate life and environment. Valverde showed recently that this method can give within a relatively short time a good idea about structure of vertebrate communities and that very promising research plans can be built on such a hypothesis.

Thus both approaches lead to knowledge of the food web, the first begining with its basis in inorganic nature, the second giving quicker results for the study of higher animals.

Adaptations of the animals to the environment. However, the food web alone gives an unsatisfactory picture of an animal community. It only gives one species as a point and the trophic interrelationships as a line. Even if this is of outstanding importance it is far from what represents all the reciprocal influence between an animal and its environment. Even the trophic interrelationships can only have a very fragmentary description in the food web. It is not only important to know on what an animal species feeds, but also how and under what conditions it gets its food. Likewise one should not only know by what other animals a species is eaten, but also how and under what conditions it is able to escape predators. These precisions alone, not the rough aspect of the food web, will discover all the special adaptations which make understandable the rich diversity of animal life in a wetland reserve. For the problem of interspecific competition as well the food web can only indicate where it may possibly occur, where as only these further observations will show if it really does.

Outside of the purely trophic relationships many others are very important, chiefly those concerning the biology of reproduction. A reserve may provide excellent living conditions for an animal species, but not allow breeding because it does not offer sufficient protection against predators, such as high trees or dense cover for such bird species as herons, rails and others, or islands which keep mammal predators from places where Gull, Terns and Waders nest in colonies. Like wise the behaviour of an animal species figures among the factors making it better or less well adapted to its environment; particularly the social behaviour, which is important in colonial nesting as a well

known defense against predators. Behaviour in general and social behaviour in particular is also, apart from its ecological aspect, a very interesting field of research in wetland reserves. Indeed it seems that colony breeding of birds is in no other habitat so widespread as in wetlands. One finds here colonies of gulls, Terns, Waders, Flamingoes and others which nest on the ground, mostly on small islands; colonies of some Gulls, Terns, Grebes etc. on floating nests; colonies of some Herons, Spoonbills, Pelicans etc. in thick reed beds or other emerging vegetation and finally colonies of some Herons, Cormorants, Storks etc. on trees. This provides rich material for studies on social behaviour and it is certainly not by chance that N. Tinbergen and his school, one of the most famous for the study of behaviour, do most of their research in wetland reserves.

All the special adaptations which allow an animal species to live within an ecosystem may provide limiting factors which are responsible for their restricted numbers and which may reach a level at which the species vanishes. Therefore monographic studies on the ecology of the most important animal species in reserves should be undertaken with special consideration of limiting factors. In many wetland reserves it may be possible to proof a hypothesis made on mechanisms of the interrelationships of animals and environment by experimental research in the field. Often waterlevel or quality can be modified and this will have as consequence an evolution of the biotic community or of the status of some species which is predictable on the light of the hypothesis. The way the evolution takes will show if the hypothesis is good.

A research programme as sketched previously will always depend on the material possibilities of implementing it. Only few wetland reserves have their own research station where a team works throughout the year. In these privileged places such a comprehensive and extensive programme should be followed, whereas in other places the more limited possibilities should preferably be used for research on some particular aspects. Monographic studies seem to be the most promising on these places but the variety of problems is enormous and covers as well fields as physiology or biometry as ecology sensu stricto.

Applications for reserve management. Ecological research has many applications in the economic use of wetlands. It is of importance for fish-culturists occupied with human food production, but is also concerned with other economic uses of wetlands such as harvest of various plants, particularly reeds, or utilisation as pasture. However, these applications will not be considered here and we would only like to discuss the possibilities of using research for the improvement of the reserve itself.

Indeed any reserve should have a management plan which indicates how it has to be treated in order to be most successful. Such a management plan should be, as far as possible, based on the results of ecological research. A wetland reserve may be a particularly pleasing habitat or harbour particularly interesting plant or animal species and it may have been put into reserve with the idea of conserving it as much as possible in the same state. However, most wetlands

are in a precarious balance and very vulnerable to outside influence. There is generally a water exchange with the surroundings and they may therefore undergo changes in water level or water quality. Both these factors should always be under survey and installation permitting complete control of water exchange is desirable. Where this is not possible there will generally be some oportunities for at least a partial control of water exchange. A change of water level and quality will be soon followed by changes in the biotic community. Monographic studies on the ecology, chiefly food and feeding habits of the most important species to preserve will allow an evaluation of dangers incurred by modifications of the ecosystem. In many cases it may then be possible to prevent severe decrease or disappearance of the endangered species. Even wetlands which undergo no changes from the outside may modify themselves by natural succession. In these cases it should be decided if this succession should be allowed or if measures, based on ecological research, should be taken in order to stop succession at a certain level. Such measures may involve cutting young trees or other plants or burning.

Not all wetland reserves have this purely conservative caracter. In countries with dense human population many wetlands have been drained. To compensate for this those remaining should harbour particularly large numbers of water birds including migratory or wintering wildfowl. In some countries even artificial wetlands have been created for this purpose. In these reserves, which should rather be called wildfowl refuges than nature reserves, it may be interesting to increase the productivity. This can be done by increasing the primary productivity through for instance artificial fertilizers. In the Camargue this is done in many cases by introducing cattle which, by trampling down the emergent vegetation, make it produce more and leave more light in the water for development of under water vegetation. Simoultaneously the droppings fertilize the etangs. The productivity for birds can also be increased by influencing the direction of energy flow. Again in the Camargue the commonest fish in fresh water is carp (Cyprinus carpio), and in brackish water mullet (Mugil cephalus). Both grow rather rapidly and after about two years are out of reach of any predator. As far as birds are concerned they are a blind alley of energy flow and may tie up for many years large amounts of energy otherwise available to birds. A simple remedy is to let the etang dry out every two or three years in order to kill the big fish and make room for smaller ones. It would be a much better solution if the carp could be replaced by a smaller fish species such as Carassius which even after several years growth is still available to herons and other birds. Perhaps the herons are much more numerous in the Marismas of the Guadalquivir than in the Camargue merely because there are Carassius there instead of carp.

From these few examples it can easily be recognised that the field of application of ecological knowledge to management is a very rich one. It is today only very poorly applied but it may have an interesting future with the increasing number of biological research stations in wetland reserves.

THE SCIENTIFIC NEED FOR SHALLOW-WATER MARINE SANCTUARIES. Dr. Carleton Ray, New York Aquarium, New York Zoological Society, 630 Fifth Avenue, New York 22, New York. Illustrated with 35 mm. color lantern slides (including underwater). Bibliography.

SYNOPSIS

Arguments for the establishment of parks and sanctuaries usually fall into one of four categories: 1) economic, such as the protection of a resource for exploitation, 2) recreational, such as the establishment of seashores for bathing and surfing, 3) scientific, such as the protection of a study area for some aspect of the natural environment, 4) esthetic, such as the protection of breeding and feeding refuges for rare or especially interesting species. Hunting preserves might fall under (2) above and wilderness areas under (4). It is true that at this date there are extremely few park-sanctuaries of any sort the world over that include the sea. It is also true that the reasons for this are various and complex but possibly stem from an age-old disregard for the sea, an attitude of laissez-faire toward it, and ignorance of it, all well-illustrated by man's continued use of it as the largest waste receptacle available to him.

Largely through the impact of skin-diving and aqua-lunging, which has vastly increased the awareness of the sea on the layman, and also due to an awakening that the sea has great economic and scientific potential, these attitudes are today in flux. Thus, all the problems of conservation with which we are faced by land now cover the entire globe, sea included for the first time. Man is today at a crossroad in marine conservation.

A resolution of the First World Conference on National Parks, Seattle, Washington, June, 1962, called for the establishment of marine park-sanctuaries and for the extension of existing and proposed seashore parks to include a portion of the sea out to realistic limits. The intent was that these marine parks would be perpetuated and regulated by a philosophy exactly as land parks are, by which is meant complete protection of all living things and the establishment of reservations of restricted human use for scientific purposes. Marine park-sanctuaries now in existence are almost to the last one not so set up, being more in the nature of parks in name only.

Of the four justifications for park-sanctuaries mentioned above, I firmly believe that the scientific need will prove to be the greatest and most real in time. This is simply a need for restricted natural areas set aside as outdoor laboratories where the scientist will establish his "control" without which most experiment has little meaning. For several years, biology has been drifting more and more into the laboratory and even biologists have forgotten that they are studying animals in a vacuum

in their experiments. Living things do not exist without the environment to which they are adapted and a constant referral to nature is a requirement even in such specialities as biochemistry, seemingly so removed from the out-of-doors. In my field, physiological ecology, this referral to nature is most striking and there must be an immediate realization that the laboratory animal under investigation differs importantly from the wild animal - and by "animal" I mean protozoan through mammal.

Unless I misread, we are today witnessing a bit of a return to nature by biologists, even those formerly of the greatest laboratory orientation. Biological experiment has been miniaturized and quickened. Collecting and maintenance of living materials has vastly improved. The one thing that has worsened, however, and drastically, is the provision of places for the biologist to work or to gather his materials. Investigation has increased but materials have decreased. This is a problem of the greatest urgency for the marine realm is being turned to in every way as the land shrinks and is denuded. The sea is, biologically vastly more complex than the terrestrial realm, more difficult in which to work, almost unknown relative to land, and -- in a real sense in its shallow areas inshore -- disappearing. It is disappearing before our eyes, but we do not see it since man still largely looks down only at the surface and dreams that what is below is still more or less the same. Here is the importance of the underwater swimmer, layman or scientist. He knows <u>firsthand</u> that it is not the same. There are few improvements on firsthand knowledge.

The mass of man is difficult to reach with the scientific argument. Particularly in the marine field, it therefore behoves the scientist to make his work known, make the importance of the sea known, make our abyssmal ignorance of the sea and the dangers of this ignorance known. For only by public support can study areas be set up. The public will not look kindly on the "stealing away" of an area for restriction for some group of abtuse and isolated investigators if it is held in ignorance, but once let in on the secret, the public will be behind us and our scientific needs. The setting aside of park-sanctuaries is often not looked on kindly by the layman unless it is for his recreation. If equated with standard "conservation", it may receive mixed support as well. Conservation itself has not been dominated by scientists and if there is a need for scientists to become conservationists and push for natural area preservation, there is an almost greater need for conservation to incorporate science. Conservationists and scientists have not worked together enough. By sea, this could be a disaster. The sea is, as said above, too complex for easy lay judgement.

I would now like to mention some points which I believe are of the greatest importance. First, there are points of attitude, "selling points", if you will:

1) Is the sea a limitless wilderness? The Earth is the name of our entire planet, land and sea. One would think that "limitless" and "wilderness", as applied to the sea's entirety, would have been refuted by this date. Rich as the sea is, comparatively unspoiled as it is in most places, it is neither an unspoiled wilderness nor limitless as so implied by the prophets of panacea, in the face of terrestrial decimation. The sea is big, but not as it was a thousand or even ten years ago. There are endangered species there, some species long extinct, areas of vast depletion and pollution. We should not fall into the error of looking at the sea as we did our western plains a hundred years ago. One has only to look into the records of the early adventurers of the New Worldto gain some insight into the decimation that has already occurred. This is particularly true of the inshore waters where the oyster, soft clam, estuarine fishes and river-running fishes have been so widely depleted and even locally extirpated.

2) One Earth. The land does not stop at the shoreline nor does the sea stop at the beach. We look at the Earth without reference to time, as one frame of a motion picture. In time, water levels may turn sea to land and land to sea in their fluctuations. Land is built and destroyed as well by several methods, siltation, isostatic forces, or the action of the tropical mangroves, current and winds. Environmentally as well the sea and the land are indivisable. The sea is a storehouse of the Earth's heat, mollifying temperatures. It receives, in turn, from the land, the basic minerals necessary for the growth of its organisms, particularly nitrogen and phosphorus salts. Thus the land and sea arenot divided by a static line and are environmentally interdependent in producing the sort of planet and the sort of life with which we are familiar on Earth.

The points of attitude are major hurdles that must be put across before the finer points are reached. They are obvious enough and as applied to land, held as true, but they are major hurdles in the setting aside of marine preserves. At this point, we may leave the similarities and concentrate on the special features of the marine realm which make the sea quite distinct from the terrestrial environment and also more complex than the latter:

1) Sea's atmosphere. The terrestrial atmosphere of air is not inert, but it is elementally simple and it is the only bio-

logically uninhabited realm of this planet. The atmosphere of the sea, water, is neither elemental nor inert nor simple. In sea water are literally thousands of biologically active compounds, the products of living things - wastes, active organic compounds , growth-inhibiting and growth-stimulating compounds, vitamins, antibiotics, and many more. Thousands of living creatures, large and small, adult and young, inhabit the water. The biological complexity of the sea's atmosphere is totally foreign to the land. One can never contemplate a marine creature apart from the "living" quality of its atmosphere of water which exists in a buffered balance, but which is capable of being upset. The integrity of the marine environment is therefore equivalent to the integrity of water, of currents, and of drainage from the land.

2) Sea's continuity. In these complex waters, barriers of the sort familiar to us on land are rare. Barriers act to separate populations of animals. Lands are distinct and one can destroy an island without hurting another at all, generally. Sanctuary thus ispossible by the erection of a fence. In the sea, barriers are vastly more complex and subtle - interactions of food supply, light, temperature, salinity, clarity and others all interacting. The water itself forms a continuum. Lakes are islands in the land, but in the sea there are few islands at all. What, therefore, constitutes an area that can be protected as a study area which will not be affected by changes from without? This is a difficult and complex problem and one where we speak today largely from complete ignorance except in special cases. This is not to say that zonation does not occur which it certain- and strikingly does, but it does say that the reasons for discontinuities of population are obscure. In the deep sea, for instance there appears to be a single barrier the Earth over. This is the Wyville-Thompson Ridge which appears to separate the deep fauna of the Arctic Ocean from the rest of the sea. Otherwise the deep sea fauna is pretty much the same the world over. It is this possibility of dispersion of waters throughout the seas that makes the problem of pollution so serious at sea. Atomic wastes should not be dumped since they have a good chance of being broadcast over the whole sea and this is applicable to any foreign thing dumped into the waters. The largely ignored and serious problem of carbon dioxide production by a mechanized society is one which may effect the sea more than the land since most of the carbon dioxide probably enters water and is removed from the atmosphere. In spite of the buffered water, just how much carbon dioxide can be produced before a limit is reached? No one knows.

3) Three dimensions. Lastly, the sea is not a surface, but covers three dimensions. The volume of activity is thus much

greater than its surface area would indicate. Nevertheless, the surface is the active area of the sea, with areas of somewhat lesser activity at various depths. It is at the surface that light is plentiful and the oxygen is available. It is also at the surface that carbondioxide enters, is dispersed, or is utilixed by plants. The sea is like a layer cake, each layer of which is distinct in taste and texture as well as in richness. The tropical rain forest is similar with this layered ecology, but in the sea, the scope is much vaster. One cannot study the sea at any one depth. It is a three-dimensional problem.

It is our attitude toward the sea as apart from the land and our view that once we use up the land, there will be plenty of sea to turn to that will make the establishment of large park-sanctuaries difficult. It is the recognition of the special properties and problems encountered in the sea that will make such park-sanctuaries successful or not. We are today proceeding in the sea inner space, as it has been called in distinction to outer space, in ignorance. How long can we afford it? How long can science afford it? Marine scientists are now in the position of having to become conservationists. Some may not like it, but they must become so or they will find that they will become cataloguers of dead things as has happened so often by land. Ecology cannot exist without study areas, yet marine ecology is faced now and will be increasingly faced with lack of areas in which to work. And these areas, in order to maintain their integrity, must be very large in most cases. In the cases of inshore study areas, they must involve their water sheds, and that is a difficult problem indeed.

Just how critical this problem is, is exemplified by the recent U. S. Department of the Interior surveys of seashores of the east and west coasts, finding pitifully few areas left as possible park-sanctuary sites. This survey did not include the sea proper, but if inshore water is to be protected, so must be the adjacent land. It appears that the only inshore study areas, with few exceptions, that could be successful in time, will be in conjunction with seashores with park status.

The inshore seas are the richest areas in terms of production of life, both in variety and in amount, in the world. A possible exception is the production of plankton in polar seas in summer, but this is short-lived. Inshore seas are also in the greatest danger. Many can no longer be fished due to decimation of stocks and pollution. One often finds that our forebears had good sense in the location of their cities near rich waters, but now the cities have destroyed that richness. One would think that such areas would be well known, being near centers of population, but this is not often the case. The New York bight was once

rich in fishes and shell fishes. This is no longer so nearly to the extent of former years and surprizingly, in spite of the location in New York of centers of learning, this bight has been rightly called, as a part of the mid-Atlantic bight, "a neglected marine gold mine." With more knowledge, increased productivity, and perhaps even a restoring of some decimated species, might result. But where to study? There is not a large study area in the bight, to my knowledge. There are none that are protected with their water shed and therein lies a sad tale for such establishment would be a difficult thing nowadays. These bights are the nurseries of the sea. We must know them better.

Conservation is supposed to conserve, but conservation must not be passive. It also must teach and needs therefore, to increase along the professional scientific lines. If the greatest argument for setting aside of park-sanctuaries is as study areas, as I believe it should be, then this teaching goal must be realized and projected publicly. Our ignorance of the sea in the face of population pressures, pollution, over-exploitation, and the panacea approach to the seas as limitless solutions to our waste and greed, is dangerous.

If one has a complaint, it is a good idea to take it to the boss. In this case, since the sea is mostly international property, a place of laissez-faire, the "boss" will be an international body with power to act. What this will be I do not know, but the place to start may be at the top. Then again, it may be at the bottom. Local efforts at the establishment of study areas should be increased even though the problem is as all-encompassing as the waters.

In conclusion, I would propose that universities establish marine programs on a vastly increased scale and that conservation organizations include the marine in their programs. The need for scientific study areas is obvious. The five points of attitudes and of the sea's special qualities are equally obvious, but a start must be made. The scientists and conservationists, jointly, must make it. Marine conservation lags dangerously and its complexity and difficulty and unfamiliarity must no longer scare us off.

RESEARCH IN DESERT RESERVES

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1. LE DESERT -

Si chacun croit savoir ce qu'est un "désert", il faut avouer qu'il demeure très difficile de trouver à ce mot une définition adéquate, et précise. Il est d'ailleurs douteux qu'il puisse être possible de parvenir à intégrer dans une seule définition des critères empruntés tour à tour à la climatologie, à la géographie physique ou humaine, à la végétation ou à la vie animale.

Par contre il existe une notion du paysage désertique qui, pour qualitative et descriptive qu'elle soit, rend parfaitement compte des particularités d'une des grandes divisions écologiques du globe, d'un "biome", caractérisé par la dégradation de la couverture végétale avec les incidences de cet appauvrissement sur la vie animale - la désorganisation d'une hydrographie à la fois vestigielle et anarchique - la tendance, fonction d'une localisation très poussée de l'eau ou de l'humidité, à la concentration des habitats (végétations "contractées", vie sédentaire humaine limitée à des oasis punctiformes,etc) l'apparition d'une morphologie sui generis (p. ex. massifs dunaires).

Il est bien entendu que le phénomène désertique relève essentiellement de causes d'ordre climatique, en rapport soit avec les circulations atmosphériques planétaires soit avec divers facteurs géographiques (continentalité, relief).

L'ensemble des critères proposés pour déterminer les limites du Sahara trouveront également leur emploi s'il s'agit, à l'intérieur même du désert, d'y définir des degrés d'aridité. On s'accorde en général, même si les divisions proposées ne coïncident pas dans le détail, à distinguer, à l'intérieur des zones arides, un domaine semiaride (la steppe) et un domaine aride (le désert), lui même divisible en plioaride et hyperaride.

2. LE PHENOMENE SAHARIEN DANS LE TEMPS : LES OSCILLATIONS CLIMATIQUES -

Si le Sahara a ses limites dans l'espace, son peuplement actuel ne saurait s'expliquer sans un recours à une autre dimension, le temps. On sait en effet, et ce n'est pas le lieu d'en discuter ici en détail, que la région a connu au cours du Pléistocène, une série, encore imparfaitement déterminée d'ailleurs d'oscillations climatiques (pluviaux et interpluviaux). La richesse des faunes comme des flores subfossiles d'une part, la persistance jusqu'à l'époque actuelle d'une série de formes relictuelles d'autre part, sans oublier le témoignage des figurations rupestres d'animaux, fournissent à l'histoire du peuplement des éléments très importants. Il est inutile d'insister sur l'extrême intérêt de ces ségrégations géographiques, qui constituent une expérience évolutive naturelle que l'on souhaiterait voir étudiée et suivie par les généticiens. Rappelons ensuite l'essai d'établissement d'une carte des isohyètes du Sahara oriental au Néolithique, à partir des exigences écologiques de 4 Mammifères (Eléphant, Rhinocéros, Girafe et Hippopotame)représentés sur les gravures rupestres (BUTZER, 1958). La comparaison des isohyètes pour env. -5000 à -3000 et des actuels montre que le décalage n'est pas énorme (200-300 Km, parfois beaucoup moins), ce qui signifie qu'un très faible accroissement de la pluviosité suffirait à renvoyer Eléphants et Girafes au Tibesti... Cette notion de la haute efficacité dans un sens comme dans l'autre, d'ailleurs, de variations très légères dans la pluviosité doit être soulignée : quelques millimètres de plus ou de moins font ici une différence.

3. CLIMAX ET PEJORATIONS ANTHROPIQUES -

Les formations végétales du désert sont-elles climaciques, représentent-elles un véritable stade d'équilibre avec les conditions du milieu ? A priori, cela peut paraître probable, vu l'immensité des surfaces et l'extrême dilution du peuplement humain.

S'il n'est pas douteux que de vastes régions du Sahara se trouvent actuellement soustraites à toute activité humaine, au moins depuis le Néolithique, il ne l'est pas moins que dans toutes les zones exploitées par le nomadisme comme, plus encore, autour des lieux habités en permanence, la sévérité et l'efficacité des pressions exercées sur le monde vivant (végétaux ligneux, pâturage, gibier) sont indiscutables. Si la steppe à Aristida pungens de la Majabât al-Koubrâ est certainement un climax, où l'absence de tout ligneux est "congénitale", si l'aspect ultra-désertique de la Tanezrouft, du Ténéré ou des grands sérirs libyques ne saurait être attribué à l'Homme, ce dernier soit directement soit par le truchement de ses animaux demeure certainement responsable de péjorations étendues, en particulier de la raréfaction croissante des boisements : les expériences de mise en défends des parcelles protégées IFAN-UNESCO d'Atar (Mauritanie) l'ont clairement montré. Sans doute peut-on penser, en admettant que le taux des actions anthropiques soit demeuré plus ou moins identique au moins depuis le développement du nomadisme chamelier, qu'une manière d'équilibre s'est établi entre le pouvoir destructeur de l'Homme et le pouvoir de régénération de la plante ; mais si la désertification naturelle, climatique, s'est accentuée depuis le subpluvial néolithique, on comprendra la redoutable efficacité d'une action humaine qui ne peut qu'accélérer le rythme de la dégradation d'une végétation dont les conditions de régénération sont devenues, p. ex. pour les arbres, particulièrement aléatoires. Le climat demeure, et de loin, le facteur dominant de la désertification, mais l'Homme seconde celle-ci de son mieux, et avec l'irruption au désert de la civilisation du fusil et du camion - aux méfaits souvent jumelés d'ailleurs - l'incomparable potentiel de destruction désormais à la disposition de l'Homo dit "sapiens" risque de trouver au désert un bien inquiétant emploi.

4. LES HABITATS -

Le désert, s'il sait être monotone et sur des surfaces d'ampleur parfois océanique(1), offre cependant à la vie animale une gamme d'habitats plus riche qu'on ne le penserait peut-être à première vue. Aussi voit-on les zoologistes tenter de dresser, pour une région déterminée, des listes plus ou moins détaillées d'habitats.

L'établissement de semblables catalogues est plus difficile qu'on ne pourrait le penser.

Je préconiserais volontiers la classification suivante :

Classe A. - Habitats non aquatiques

Groupe I. Habitats contractés

1. Oueds et zones d'épandage

Groupe II. Habitats diffus

2. Substrat meuble : sable (dunes, nappes, etc) - 3. Substrat compact : sols consistants, généralement pierreux (regs, hamadas, surfaces de plateaux, etc) - 4. Substrat rocheux (rochers, falaises, éboulis, canyons, ravins encaissés à lit rocheux, montagnes, etc).

/Groupe III. Habitats artificiels : lieux habités, cultures, cadavres, excréments d'animaux domestiques, etc/ (voir (1) p. 3)

(1) de la p. 2. - 3 -
Il m'est arrivé dans l'Empty Quarter occidental de faire en ligne droite, en 22 jours, près de 900 Km, entièrement dans le sable, sans avoir rencontré, un oued ou un caillou.

Classe B. - Habitats Aquatiques -

5. LES TYPES DE DISTRIBUTIONS -

A la surface d'un désert sensiblement aussi étendu que l'Europe ou les Etats Unis, régionalement différencié, et qui, de plus, vient s'installer à cheval sur la limite de deux "empires" biogéographiques, Holarctis et Palaeotropis, on concevra qu'il y ait place pour des types divers de distributions.

Un premier groupe, de beaucoup le plus important, sinon quant à celui des espèces du moins quant à celui des individus et par conséquent des biomasses, comprendra les représentants de la faune saharienne "banale" des différents habitats planitiaires secs, ceux que l'on peut s'attendre à rencontrer de la steppe nord-africaine au Sahel et de l'Atlantique à la Mer Rouge (et souvent bien au-delà). D'autres groupes n'occupent par rapport au "coeur" du désert que des situations marginales, et que l'on pourrait presque appeler "côtières" ils occupent en effet les "bords" du Sahara, qu'il s'agisse d'une limite maritime (Atlantique ou Mer Rouge) ou d'un "littoral" continental (limite désert / steppe au Nord comme au Sud avec toutes les transitions que, d'ailleurs, elle comporte). Un type plus étroit de distribution est fourni par des situations littéralement insulaires, en relief s'il s'agit du sommet des montagnes, en creux dans le cas des ségrégations aquatiques, mais équivalent chaque fois à une sorte d'enkystement, plus ou moins efficace sans doute ; avec les eaux temporaires viendra s'ajouter à une limitation dans l'espace une autre, dans le temps. Montagnards stricts et aquatiques aptères sont des isolats, enfermés dans les habitats clos.

Si le Sahara possède un peuplement de sédentaires, il joue également un rôle très important dans la circulation des Oiseaux migrateurs, voyageurs en transit et souvent, d'ailleurs, en difficultés.

6. ASPECT HISTORIQUE DU PEUPLEMENT -

UVAROV avait insisté en 1938, à propos des Acridiens de la "zone érémienne" de l'Ancien Monde, sur leur "polyphyletic origin", leur "chequered history" et leur "resulting heterogeneous character". Ce qui est vrai des Sauterelles peut être étendu non seulement au reste de la faune mais à la flore et il faut par conséquent tenter de répartir les taxa sahariens en "éléments" au sens de EIG (1918), témoignant par la diversité de leur origine, et de leur âge, de la complexité de l'histoire du peuplement saharien. Si l'on se souvient que le Sahara constitue le plus vaste désert du monde, que son ancienneté semble grande, que son histoire "récente" (i.e. depuis la fin du Tertiaire) a connu toute une série d'oscillations climatiques, et qu'il a quelques 5000 Km de frontières communes au N avec le monde paléarctique, au S avec l'Afrique intertropicale, on ne sera pas surpris d'y découvrir dans la composition de son peuplement animal et végétal, 3 "éléments géographiques" de base : méditerranéen, saharien -saharo-sindien), tropical (ici : soudano-deccanien).

7. LES REPONSES DE L'ORGANISME AUX AGRESSIONS DU CLIMAT -

a) Eau et chaleur - Le problème majeur de tout être vivant soumis à un climat désertique sera, nécessairement, celui de l'équilibre hydrique de ses tissus. Les petits Mammifères (p.ex. Rongeurs), échappant à l'insolation directe grâce à un terrier dont le microclimat sera moins chaud et plus humide que la surface du sol, peuvent d'une part faire l'économie d'une thermolyse éva-

poratoire au niveau de la peau et de l'autre se contenter de l'eau préformée d'une nourriture sèche et de celle obtenue par l'oxydation des aliments, tout en réduisant leurs pertes d'eau au niveau et de l'excrétion urinaire et de la ventilation pulmonaire. Mais la situation des grands Mammifères exposés sans abri à la chaleur et à la sécheresse, est différente, pour lesquels la régulation thermique exigera une certaine dépense d'eau et, par conséquent, pour équilibrer les pertes par l'évaporation, l'urine et les fèces, la possibilité d'une ingestion correspondante d'eau libre.

La Chameau est un cas exemplaire d'espèce particulièrement outillée pour supporter les contraintes du milieu désertique. L'Antilope Addax (Addax nasomaculatus) qui peut prospérer dans des régions sableuses toujours dépourvues d'eau libre et se contenter, même en été (ce qui n'est pas le cas du Chameau), de l'eau préformée de 3 espèces végétales (2 Aristida et le Cornulaca) est certainement, quant à sa résistance à la sécheresse, plus remarquable encore, mais sa physiologie demeure inconnue. Entre ces types et les organismes sahariens hygrophiles, voire aquatiques, tous les intermédiaires, bien entendu, existent.

Sur le comportement des Mammifères et Oiseaux sahariens vis à vis de la radiation solaire et de la température du sol, on a une étude de HEIM de BALSAC (1936) et, pour les Insectes sabulicoles, un exposé de PIERRE (1958). Il n'est pas d'ailleurs certain que les hautes températures de l'air ou de la surface du sol expérimentalement léthales, aient en fait une très grande importance écologique puisque, par des procédés divers, la plupart des espèces échappent à leur action directe.

b) Radiation solaire et coloration animale. - Le problème de la signification de la "livrée désertique" et du mélanisme ne semble pas élucidé, malgré de nombreuses études.

S'il y a bien, dans le domaine des colorations, une marque désertique, et dont la valeur sélective semble très douteuse, comment imaginer cette action du milieu sur le patrimoine héréditaire ?

c) Vent. - S'il est certainement abusif de considérer la fréquence et la violence du vent comme une caractéristique du désert, il n'est est pas moins certain que les effets du vent et sur le milieu physique et sur les êtres vivants y sont particulièrement marqués. Le sujet, en ce qui touche les animaux, a été relativement peu étudié. Le vent jouera un rôle important dans la distribution des Insectes ailés, dans celle de beaucoup de graines et d'une façon générale dans la constitution d'un wind-drift de débris végétaux (et animaux) pouvant représenter pour les Insectes sabulicoles et plus encore pour les "ultra-psammophiles" (KOCH 1961) des dunes du Namib une source appréciable de nourriture (et sans doute, par oxydation, d'eau).

8. LE PROBLEME DES ADAPTATIONS MORPHOLOGIQUES -

S'il faut renoncer comme l'a montré HEIM de BALSAC (1936) à l'idée ancienne d'une adaptation au milieu saharien pour la dimension des conques auditives des Mammifères et pour les membres des Mammifères et des Oiseaux, on a pu, dans d'autre cas, admettre l'existence de caractères statistiquement liés au milieu désertique. On observe, par exemple, que plus des 3/4 des Mammifères sahariens présentent une hypertrophie des bulles tympaniques, et qui plus est, que celle-ci paraissait même progressive et parallèle à l'accentuation des aridités désertiques. On se demande aujourd'hui si la dimension des bulles est réellement en relation avec le degré de sécheresse du milieu, et si, au lieu de constituer un indice d'aridité, l'hypertrophie bullaire ne présenterait pas plutôt un indice de dispersion, "les espèces à bulles hyper-

trophiées ayant habituellement une faible densité de peuplement et, dans les limites d'une même espèce, les animaux dotés de bulles les plus volumineuses étant ceux que l'on trouve dans les zones de peuplement le moins dense pour cette espèce "(PETTER, 1953).

Autre exemple d'adaptation morphologique : les pattes des Ténébrionidés psammophiles du Namib, avec des "sand-swimming feet", des "gliding feet" et des "sand-shoes", etc (KOCH, 1961).

9. UN ECOSYSTEME EN EQUILIBRE INSTABLE -

Si nous possédons bien, et souvent par habitats, de nombreuses listes faunistiques, la structure même des biocénoses est encore très peu connue.

Dans certaines régions dunaires, l'attention a été attiré sur le rôle que peuvent jouer au point de vue alimentaire, en particulier pour les insectes, les transports de débris par le vent, avec constitution fréquente d'un véritable "drift" éolien se déposant sous le vent des dunes.

KIRMIZ(1962) croit pouvoir conclure d'une étude sur la physiologie d'une Gerboise que le "problème de l'animal dans le désert n'est pas tant la chaleur, qu'il peut éviter en menant une vie souterraine et nocturne, que la pénurie d'eau et de nourriture". L'hostilité du milieu rend la persistance, la reproduction et l'extension de la vie précaire ; "Se traduisant dans le paysage par la raréfaction de la végétation, l'aridité a pour effet de diminuer les ressources alimentaires de la faune. Malgré la frugalité des Rongeurs, leur densité de peuplement est fatalement fonction de la quantité de nourriture dont ils peuvent disposer" (F. PETTER, 1961).

BRINCK(1956) a fait remarquer que l'aggravation des conditions désertiques entrainait pour le monde vivant les conséquences suivantes ; 1° réduction dans la densité des individus d'une espèce-2° réduction dans le nombre des espèces 3° tendance à l'indépendance vis à vis de précipitations régulières-4° forte augmentation de la faculté de recourir, en face de conditions adverses, à un stade quiescent (latence, diapause) de types divers (bulbes, graines, stades larvaires, adultes inactifs, etc)-5° spécialisation alimentaire.

Aussi admettra-t-on aisément non seulement que le milieu saharien ait pu exercer un tri sévère parmi les constituants de sa faune et de sa flore mais aussi que la productivité biologique demeure excessivement faible.

Si l'on a reconnu, et décrit, un cycle biologique annuel, on constatera que ce cycle "normal" et un peu théorique, peut se voir profondément bouleversé par l'irrégularité des pluies (et par conséquent l'évolution du tapis végétal) ou d'autres causes de l'aggravation de la sécheresse et de la chaleur. L'alternance de périodes d'aridité extrême (pouvant localement durer plusieurs années..) et d'épisodes humides porvoque de véritables métamorphoses du paysage, en substituant par exemple un tapis fleuri d'annuelles à un reg complétement nu.

Bien que les causes en soient le plus souvent inconnues, on cite des pullulations acycliques (Criquet pélerin, Rongeurs), des migrations occasionnelles, des mortalités, des inhibitions de la reproduction, de singulières extensions de l'éventail alimentaire des Mammifères.

Les rythmes génitaux sont encore peu connus (BODENHEIMER 1953, 1954). Il se peut, d'autre part, que le recours au sommeil léthargique, obtenu en captivité chez plusieurs Rongeurs sahariens, permette à ceux-ci de durvivre à une péjoration excessive des conditions du milieu et, en les libérant dans une certaine mesure des exigences de celles-ci, d'occuper plus aisément des habitats particulièrement inhospitaliers (PETTER, 1956). Les Hélicidés peuvent, durant de longues périodes, plusieurs années peut-être, être en état de vie ralentie.

10. CONCLUSIONS - - 6 -

Par la sévérité des conditions physique, et essentiellement par le caractère accentué d'une aridité dont les excès sont directement hostiles à la vie, le désert chaud vrai occupera, avec les déserts froids des régions polaires, une situation marginale par rapport aux régions où le développement de la biophère est plus favorisé. Sans atteindre, bien entendu, un abiotisme que connaitront seuls les inlandsis, le désert coïncidera cependant avec une singulière diminution régionale du volume de la biosphère, et non seulement le tapis vivant s'amincit mais il se déchire ; et si les trous s'y multiplient, il se sera transformé en un réseau où la vie n'occupe plus guère que les côtés des mailles.

On ne sera donc pas surpris de l'extrême appauvrissement des faunes et des flores : moins de 10 espèces de Phanérogames sur 200.000 Km2 ce n'est pas beaucoup.

Le désert représente donc, écologiquement, un territoire où la vie, raréfiée, ne reste possible qu'à un certain nombre de conditions auxquelles des groupes entiers, communs pourtant en pays humides, ne sont pas parvenus à satisfaire ou que marginalement : Lichens, Cloportes et Escargots sont ce dernier cas, et il leur faudra l'accroissement d'humidité des bordures, continentales ou maritimes, comme de la haute montagne pour les autoriser à pénétrer au désert.

Tendance générale à la raréfaction de la vie, multiplicité des réponses des organismes au agression du milieu, complexité et, parfois, intensité des fluctuations paléoclimatiques, dont certaines phases ont installé au coeur du Sahara des espèces s'y trouvant désormais "enkystées", caractère souvent précaire de l'équilibre des biocénoses, ces divers ordres de faits contribuent tous à conférer à la biologie des déserts un caractère à bien des égards unique et d'un très haut intérêt.

Il est essentiel que l'étude des écologies sahariennes soit poursuivie avant que l'action humaine, qui s'intensifie ici aussi, n'ait trop aggravé la situation naturelle.

Un voeu du "Colloque sur la Conservation de la Nature et de ses Ressources dans les Etats africains modernes" (Arusha, Tanganyika, 5-12 Sept. 1961), et un second, tout à fait similaire, adopté par la "First World Conference on National Parks" (Seattle, 30 Juin-7 Juillet 1962) ont l'un et l'autre insisté sur l'urgence qui s'attache à la constitution d'une collection de types d'habitats assurés d'une conservation définitive. Le très important projet de l'International Biological Program, de son côté, accorde, on le sait, une large place à un "General Survey of terrestrial biological communities" et à la "Conservation of terrestrial biological communities".

On veut espérer que des milieux aussi originaux que les habitats désertiques ne seront pas oubliés et recevront, le moment venu, toute l'attention qu'indubitablement ils méritent.

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