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CONTRIBUTED PAPERS SESSION

8A. BEHAVIOR (INVERTEBRATES)

DIFFERENT RESPONSES TO THE SAME TRAINING IN SYNGEN 1 AND SYNGEN 4 OF PARAMECIUM AURELIA. Beatrice Gelber, Basic Health Research Institute, Tucson, Arizona, U.S.A.

For the purpose of studying behavior at the cellular level, we have developed a training procedure using the protozoan, Paramecium aurelia. For this training, a platinum wire is lowered vertically into a culture of hungry paramecia. At first, the protozoa ignore or avoid the wire, but after it has been repeatedly raised and lowered again after being baited with a very small amount of food, paramecia cling to the wire they had previously ignored, after the wire is cleaned.

Number of paramecia on the wire or near it varies in significant ways and analysis of the data these numbers constitute has guided the development of a theory of behavior. This theory indicates that acquired characters, of which behavior modification may be a special case, are encoded in cellular loci for which the name, modes, is suggested. Modes may be modifications in the way macromolecules of protein or RNA-protein complexes in the cytoplasm are structured on the DNA template. A prediction based on this theory led to the present experiment.

Geneticists have found that mating type in some syngens of P. aurelia is controlled by factors in the cytoplasm, while in other syngens, mating type depends on the macronucleus. It seemed possible that sites for mating type in the cytoplasm might interfere with the availability of modes for restructure by training but such interference should not occur where the macronucleus controlled mating type. Accordingly, cultures of syngen 4 paramecia (cytoplasmic mating type control) were compared with syngen 1 cultures (macronuclear mating type control), after receiving either massed or spaced training. Training consisted of 15 dips of the baited wire (called training trials), which was left down for one minute and raised for one minute between trials for massed training. For spaced training, there was a lapse of 1/2 hour after 5 and after 10 trials.

Results were as expected. Syngen 1 cultures responded equally after massed and after spaced training and did as well 3 hours after completion of training as they did immediately. Syngen 4 gave markedly less response after massed training, though response was greater 3 hours later than it was immediately.

This research was supported by grant HE 05970 from National Institutes of Health.

RELATION OF DISTANCE TO FORAGING INTENSITY ON NATURAL FOOD SOURCES OF HONEY BEES. William R. Lee, Dept. of Entomology, University of New Hampshire.

Following Karl von Frisch's discovery of communication among bees by means of their dances, investigations by R. Boch have shown that bees relate distance from a food source to recruitment of foragers on artificial food sources. The relation of distance from a food source to foraging intensity under natural conditions was studied following the author's previously published results, which showed a shift in the ratio of foraging bees from different apiaries with a shift in the relative distances to the apiaries. Three apiaries were located in a blueberry field and distinctively marked by a combination of genetic markers and two radionuclides, Iodine-131 and Phosphorus-32. Bees were collected at three different collection stations in this field and at one location in an adjacent field, and after collection they were classified as to phenotype and measured as to their radioactivity. Radioactive bees were further classified as having been fed either I-131 or P-32 by the ratio of the G.M. count to scintillation count, as the ratio for the two radionuclides differed by a factor of 28. The origin of each bee was then determined by a combination of its phenotype and radioactivity on an IBM 1620 computer. A total of 1,478 bees was collected during four days of sampling, of which 778 were identified as being from one of the three experimental apiaries. There were significant differences among collecting stations only 210 feet apart, a circumstance which confirms previous experiments. The ratio of foragers from different apiaries varied linearly with the relative distances from their hives for the three collecting stations within the field in which the apiaries were located, but the ratio differed significantly from linearity when the station 1,050 feet outside the center of the field was included in the results. Flight conditions were good, and there was satisfactory homogeneity among sampling periods within the four-day period.

A second experiment of similar size and methods to the first was conducted in an apple orchard. In contrast to the first experiment, low temperature and cloud cover permitted the bees to fly only intermittently during the collecting periods; and there was a significant variation in the ratio of foragers collected during different sampling periods within all locations sampled.

In both experiments one apiary consisted of colonies headed by queens heterozygous for the cordovan gene and mated to cordovan drones; therefore, the colonies' foragers consisted of equal numbers of cordovan and non-cordovan bees. In neither experiment were there any significant differences between the ratio of cordovan to non-cordovan bees at the various locations sampled, nor were there any differences in the proportion of cordovan bees collecting pollen.

PREY-SELECTION & OVIPOSITING BEHAVIOR OF SPHECID WASPS (HYMENOPTERA: ACULEATA). C. S. Lin, Dept. of Biology, Huston-Tillotson College, Austin, Texas.

North American sphecid wasp fauna is represented by about 1200 known species, grouped into 8 subfamilies. Only about 200 species of these wasps have been studied by a number of entomologists during past several decades. A great variety of insect and spider prey is used by them to feed their young. The prey-selection of these wasps reveals their evolutionary and morphological adaptation patterns: from non-specific groups of prey to highly specific as some wasps which hunt only the females of a species. There are 50 species of wasps which hunt on Diptera, 40 on Homoptera, 35 on Orthoptera, 15 species each on Coleoptera, Hemiptera, Hymenoptera, Lepidoptera, and Araneida. Other orders such as Ephemerida, Odonata, Thysanoptera, and Trichoptera are also used as larval food. A summary of prey-preference which is known to exist in these 8 subfamilies as listed below: Astatinae hunt on Hemiptera; Larrinae on Orthoptera, Hemiptera, and Araneida; Trypoxyloninae on Araneida; Pemphredoninae on Homoptera; Sphecinae on Orthoptera, Lepidoptera caterpillars, and Araneida; Nyssoninae on Diptera, Hemiptera, Homoptera, and Lepidoptera; Philanthinae, composed of 2 tribes: Philanthini and Cercerini-- the former prey on Hymenoptera (bees and ants, one species on queen ants only), the latter hunt Coleoptera; and finally, Crabroninae hunt Diptera, Hemiptera, Homoptera, and Lepidoptera.

The nesting behavior of sphecids can be divided into the following categories: (1) fossorials or digger wasps, about 70% of them belong to this group; (2) dwellers, which utilize various pre-existing burrows or cavities to make their nests constitute 20%; and (3) mason wasps, which build their nests and cells with mud or clay. Prey are brought into the nests by the mother wasps by either mass provisioning or progressive provisioning. Ovipositing behavior of these wasps varies greatly: eggs are laid in the empty cells then the prey are brought into the nests; eggs are glued to the wing-base of the prey; eggs are deposited on the venter of the prey's body either in transverse, vertical or in oblique position; laid in between the neck regions; laid on the proleg of the caterpillars; and attached to the femur of the hind leg. In most cases, the prey are partially paralyzed when they are stung by these wasps, and will remain fresh for a few days as the larvae feed on them one by one until full grown. The life history of the sphecids is rather unique: egg incubation period 2 days, larval feeding period 4-6 days, then spin cocoons and become prepupae. The multivoltine species may have several generations during the summer months, but the pupae of the univoltine species emerge as the adults in the following spring or summer to complete one generation life cycle.

PATROLLING BEHAVIOR OF MALE ANTS. Paul B. Kannowski, Department of Biology, University of North Dakota, Grand Forks, North Dakota, United States.

Some species of ants have colonies which produce only male or only female reproductives. The nuptial flights of certain of these species are modified so that the females wait on or near the nest for the males to fly in from distant nests. These males fly low over the ground until they become aware of the presence of females. This type of flight is herein termed "patrolling" behavior. This behavior, including species-specific variations, will be described and also illustrated with movies. Methods (chemical, visual, and acoustical) whereby the males are attracted to the females have been studied.

INTERRELATIONSHIPS BETWEEN WEB-BUILDING BEHAVIOR AND AMOUNT OF THREAD MATERIAL IN THE SPIDER ARANEUS DIADEMATUS CL.

Peter N. Witt, Dept. of Pharmacology, State University of New York, Upstate Medical Center, Syracuse, N. Y., U.S.A.

The geometric orb web of one individual of Araneus diadematus Cl. is known to vary from day to day in size of catching area, mesh width, and thread thickness. All three variables depend on the amount of thread material available at the start of web-building. Observations have shown that animals neither ran out of thread before the web pattern was completed nor was much material left in the glands directly after orbs were built.

Total nitrogen was determined daily from a digest either of the web or from thread pulled from the spinnerets until exhaustion. Under the assumption that thread protein always contains 16% nitrogen (N), total web protein could be calculated.

1 mg/kg physostigmine given by mouth in sugar water raised daily nitrogen in 18 spiders significantly from an average of 36 gamma to 49 gamma in 36 hours. Webs were larger than controls and contained more spiral turns and radii. 24 hours later N was back to control levels. After the body weight of spiders had been increased by lead weights about 30%, regular, wide meshed webs with shorter (10.8 instead of 16 m) and thicker (22.6 gamma protein/m instead of 11.5) threads were built (less radii and spiral turns). Spiders deprived of food for 6 days with webs eliminated daily built webs of control size with wider meshes and significantly less material (59 gamma N/day instead of 74) than before and afterwards.

These and other experiments indicate that the production of thread material can vary. The amount available together with body weight is closely related to size and mesh width in the web and determines thread thickness. It is probable that spiders have integrated at least these bits of information in the first phase of web construction when frame threads begin to limit web size, and the angles between radii determine mesh width.

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A. Christiansen, R. Baum, and P. N. Witt: Changes in Spider Webs Brought about by Mescaline, Psilocybin and an Increase in Body Weight. J. Pharmacol. 136: 31, 1962.

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FOOD PLANT SELECTION OF ADULT OXACIS SUBFUSCA BEETLES. Ross H. Arnett, Jr., Department of Biology, The Catholic University of America, Washington, D. C., U. S. A.

Several populations of Oxacis subfusca Horn (Coleoptera, Oedemeridae) in southern Arizona have been under study for six years. These beetles feed, as adults, on the pollen grains of a prickly poppy, Argemone pleiacantha subspecies pleiacantha Fedde. This poppy occurs in large stands in overgrazed areas in the Pajarito Mountains of Arizona and adjacent regions. Not all of these poppy fields are infested with Oxacis subfusca, however. A series of experiments has shown that the beetles will not feed on the pollen of certain poppy stands, but will readily accept the pollen from others. In captivity, these beetles will feed on the pollen from blossoms only from those stands of poppies that are infested with these beetles in the wild. Moreover, when individuals are marked and released among the rejected poppy stands, they will rapidly migrate back to the original field, passing from plant to plant from the rejected area to an acceptable area. There is no morphologically discernible difference between the poppies in the rejected area and those of the accepted area. In the cases tested, the distance between the two stands was less than 1500 feet (457 meters). The only detectable habitat difference is the presence of livestock watering tanks in the two acceptable areas.

Previous studies have shown that the pollen is used by the female for the manufacture of eggs. She seems to be highly selective in her choice of pollen, but the factors involved remain obscure.

The research reported here was conducted under National Science Foundation Grant no. 25,136.

THE BEHAVIOR OF THE PYGOSTENINI (COLEOPTERA, STAPHYLINIDAE).

David H. Kistner, Division of Natural Sciences, Chico State College, Chico, California, U. S. A.

The Pygostenini make up about 20-25% of the species and individuals of the insect myrmecophiles associated with Driver Ants (Doryline) in Africa. They exhibit many morphological characteristics which have been known for some time but the use of these structures has heretofore been largely a matter for speculation. The use of such structures as the different types of glandular appendages of the feet and the overlapping body parts has been observed and will be explained. In addition, the Pygosteninis also exhibit other behavioral adaptations which will be shown and explained. These behavioral adaptations are most characteristic of the more primitive Pygostenini and are replaced by morphological adaptations in the more specialized genera.

MOTOR AND SENSORY MECHANISMS OF LIFT CONTROL IN LOCUST FLIGHT.

Donald M. Wilson, Dept. of Zoology, University of California, Berkeley, California.

Changing the body angle in the pitching plane by up to 20° in steadily flying locusts often does not result in a change in lift. Since other wingstroke parameters are constant, changes in angle of attack of the wings must compensate the body angle changes (Weis-Fogh, T. 1956. Phil. Trans. B, 239, 553-584.) Flash photographs triggered by a muscle action potential (with an interpolated delay) were used to measure wing profile angles during mid-upstroke and mid-downstroke of both wing pairs. Wind speed, stroke plane angle, stroke amplitude, stroke frequency, and wing profile were used to calculate angle of attack of the wing with respect to the relative wind.

During upstroke the wing positions follow the body pitch angle changes; that is, there is no compensation of wing twisting to hold angle of attack constant. At mid-downstroke, when the lift is maximal, angle of attack of the forewings does compensate body pitching but there is no measurable compensation by the hind-wings. The results are consistent with electro-physiological measurements of activity in the wing controlling muscles but not with predictions made from earlier behavioral observations.

Lift compensation of pitching movements by only one wing pair results in a changed distribution of lift between the two wing pairs. The changed lift distribution creates a torque which tends to restore the body pitch angle to the normal value. Thus some aspects of both lift and pitch control are accomplished by a single muscular mechanism. The extent of forewing twist, and therefore angle of attack, is normally controlled by a two motor unit muscle, the subalar muscle. At low body pitch angles this muscle is more active than otherwise and it then counteracts the large downstroke pronation of the wing.

Necessary to the constant lift-constant pitch reaction are the campaniform sensilla on the main wing veins. These respond to upward, foreward forces on the wing, or forces in the direction of the maximum aerodynamic pull. Surgical removal of these wing sense organs eliminates the ability to control the angle of attack of the wing without affecting other wingstroke parameters.

(A full description will be prepared by E. Gettrup and D.M. Wilson. The work was supported in part by U.S.P.H.S. grant B3927.)

ÉTUDE EXPÉRIMENTALE ET COMPARÉE DE LA PERCEPTION VISUELLE DU MOUVEMENT ET DE LA PRÉDATION CHEZ UN POISSON (PERCA), UN CÉPHALOPODE (SEPIA) ET UN INSECTE (MANTIS). P. C. Boulet, Laboratoire de Zoologie, Université de Tunis, Tunisie.

La perception visuelle du mouvement chez les animaux est encore peu connue. Pourtant, elle est une fonction psychique primordiale, car elle a précédé, dans l'évolution du psychisme, la perception des formes, et elle domine encore celle-ci dans presque toute l'étendue du Règne animal.

Grâce à un appareillage électro-mécanique permettant une étude quantitative de la perception visuelle du mouvement, nous avons démontré que les réactions de Perca fluviatilis dépendent surtout de la vitesse du mobile: ainsi une sphère blanche de 7 mm. peut être pour le sujet le signe d'une proie (réaction +) ou le signe d'un ennemi (réaction -), si l'on modifie la vitesse de l'objet. La dimension de l'attrape a une assez grande influence sur les réactions; par contre la forme de la trajectoire, la couleur et la forme de l'objet ont peu d'importance.

Des expériences analogues sur Sepia officinalis ont montré que cette espèce est, comme Perca, sensible à la vitesse de l'objet. Mais, en outre, Sepia ne réagit qu'au mouvement d'objets à formes déterminées: les formes anguleuses déclenchent surtout des réactions positives, les formes arrondies surtout des réactions négatives. Si l'on considère les facultés perceptives des sujets, le niveau psychique de Sepia apparaît plus élevé que celui de Perca.

D'autre part, de nombreuses observations de capture des proies, par de jeunes sujets notamment, nous ont montré que le réflexe de capture est inné chez Sepia. Cependant, suivant que la proie est un Crabe ou une Crevette, on peut constater deux modes de capture, appelés respectivement "mode R" et "mode N"; or, la distinction pa r Sepia des deux formes de proies, induisant des modes spécialisés de capture, se perfectionne avec l'âge des sujets et pourrait dépendre d'un apprentissage.

Enfin, chez Mantis religiosa, il est prouvé que le mouvement de la proie ou de l'attrape est indispensable pour déclencher la prédation: une Mante peut rester plusieurs heures immobile devant une attrape fixe, et il suffit d'un déplacement infime de celle-ci pour que la réaction s'amorce. Mantis est peu sensible à la forme des objets: une croix noire en mouvement peut provoquer autant de réa ctions qu'un Criquet. Par ailleurs, Mantis continue ses attaques un nombre élevé de fois, même si elles ne sont pas satisfaites. Au contraire, au cours des expériences, Perca et Sepia cessent précocement leurs attaques.

En conclusion, nous essayons, dans ces recherches, de montrer l'importance du facteur mouvement dans les modes inférieurs de la perception visuelle. Cette notion doit avoir sa place dans une théorie évolutive de la perception.

AIR MASSES ASSOCIATED WITH LONG RANGE MOVEMENT OF THE POTATO LEAFHOPPER INTO WISCONSIN. R. L. Pienkowski and J. T. Medler, Dept. of Entomology, Virginia Polytechnic Institute, Blacksburg, Va. and Dept. of Entomology, University of Wisconsin, Madison, Wis.

The potato leafhopper, Empoasca fabae (Harris), winters only in the extreme southern portions of the United States. Each year in April, May and June a northward movement of leafhoppers results in the infestation of alfalfa, potatoes and many other plants in the North Central and Eastern United States and South Eastern Canada.

This northward dispersal is associated with warm south winds and air masses which move up the Mississippi Valley. Medler (1962) has shown an association between average May surface air-flow patterns and potato leafhopper dispersal.

As a condition for long-distance movements by winds, the leaf-hoppers must take flight and either fly or be carried to a fairly high altitude. The initiation of this movement has been associated with the maturation and harvest of host legumes in the South. Convection currents alone or in combination with turbulence are capable of carrying insects to a considerable height. For long-distance transport to occur, the temperature in the horizontal winds must be high enough that the insects can maintain their altitude. Insects cooled below their minimum flight temperature fold their wings (Wellington, 1945). Leaf-hoppers being transported horizontally could drop to the ground due to the following conditions: exhaustion, termination of flight response behavior, being caught in a downdraft, being precipitated out with rain, or by being cooled and not falling into warmer air in which flight might be resumed. The latter two situations could readily occur if the warm air containing the leafhoppers was forced over a cold air mass at a frontal system and cooled due to expansion.

The air mass movements associated with the initial arrival of the potato leafhopper in Wisconsin were studied for the ten year period, 1951 to 1960 (North Central Regional Project NC-29). These systems were generally made up of a low over the Great Plains, a high over the Atlantic Coast, a north-south cold front moving east, an east-west front over Wisconsin and rain occurring in the fall-out area. The counterclockwise movement of air around a low and the clockwise movement of air around a high tended to force a mass of warm, moist air up the Mississippi Valley. If occurring in conjunction with conditions suitable for the pickup of leafhoppers, the warm air movement could continue to carry leafhoppers until forced over a cooler air mass.

Medler, J. T. 1962. Int. Kong. Ent. Wien 1960. III: 30-35.
Wellington, W. G. 1945. Canadian Ent. 77: 7-15, 21-28.

RECEPTIVITY IN FEMALES OF THE COCKROACH NAUPHOETA CINEREA.

Louis M. Roth, Pioneering Research Division, QM R&E Command, Natick, Massachusetts.

After adult emergence there is a precopulatory period of about 3 days, during which the female is unreceptive and will not mate. The female becomes receptive and mates when the corpora allata become active (indicated by yolk deposition in the oöcytes or secretion in the colleterial glands). A low titer of corpus allatum hormone apparently controls mating by inducing female feeding on the displaying male's tergum. Virgin females remain receptive as the oöcytes grow, or mature and are oviposited.

Females remain receptive if mated to males lacking accessory glands (no spermatophore is transferred). Females also remain receptive if mating is interrupted, with normal males, prior to spermatophore transfer or if the spermatophore is transferred but is not firmly cemented in the bursa. The firm insertion of the spermatophore into the bursa results in loss of receptivity by releasing an inhibitory influence which suppresses feeding on the male's tergum. The seat of the inhibitory mechanism originates in an area posterior to the last abdominal ganglion, because cutting all nerves arising from this ganglion induces hypersexual behavior (multiple matings). Nervous stimuli which suppress mating following copulation, originate from mechanical pressure exerted by the spermatophore in the bursa.

The corpora allata are inactive after oviposition and remain inhibited during the entire or almost the entire gestation period, at which time the female will not mate. Removal of the oötheca from the uterus results in resumption of corpora allata activity and the female again becomes receptive.

Females may or may not mate again after parturition. Those that do are individuals whose corpora allata have been inhibited during gestation. In females that do not mate again, inhibition of the corpora allata has broken down during late gestation and the oöcytes have begun to develop prior to birth of the young.

VARIATIONS IN INDIVIDUAL ATTRACTIVENESS TO JAMAICAN SANDFLIES

Douglas S. Kettle, Dept. of Zoology, Royal College, Nairobi Kenya.

In Jamaica three species of sandflies (Culicoides barbosai, C. Furens and Leptoconops bequaerti) feed on man sufficiently frequently as to produce a biting fly problem. These small ceratopogonids rarely enter houses and therefore their populations have to be assessed in the field. It is obviously desirable to estimate biting fly densities by counts of actual numbers feeding on a man in a given time. Numerous field experiments were conducted in which collections were made from the legs and arms of four individuals for 15 minutes in each of four positions. It was relatively simple to calculate the preference of each species for limbs (leg or arm) and site positions. These remained constant from one experiment to another but considerable variation was encountered between the relative attractiveness of the same individuals at different times of day and night. Five people participated in these experiments. Three (C, D and S) were very dark skinned Jamaicans and two (K and L) were light skinned English. One (D) was comparatively unattractive to sandflies and was dropped from the catching team.

On all occasions S caught less than either C, K or L and was about two-thirds as attractive as the others to C. barbosai and C. furens, while he attracted only about half as many L. bequaerti. The simplest response to the other three baits (C, K and L) was shown by C. barbosai, which at dawn and in the morning was equally attracted to all three. This response changed at sunset when C. barbosai became increasingly attracted to L as the light faded. This preference reached a peak immediately after sunset when L caught three times as many as C. Similar behaviour at sunset was shown by C. furens, which was also much more greatly attracted to L than C. However, the behaviour of C. furens in the morning was quite distinct. It differentiated clearly between all four baits placing them in the following descending order of attractiveness -C, K, L and S. The respective proportional catches were 2.7: 2.0: 1.6: 1.0 with C's catches being consistently and significantly larger than those of L. L. bequaerti is diurnal, becoming active shortly after dawn and disappearing before sunset. During the day it was attracted equally to both K and L, preferring them to C, who caught only about three-quarters as many.

It is clear that each species responds to the presence of particular baits in a characteristic manner and that this response changes with the time of day. The specific nature of these responses is emphasised by the fact that observations were made simultaneously on all three species. Therefore, when C. barbosai was being attracted equally to C, K and L in the morning, L. bequaerti was, at the same time, showing a decided preference for K and L, and C. furens was responding differently to each individual bait by preferring C to K, and K to L.

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CONTRIBUTED PAPERS SESSION

8B. BEHAVIOR (VERTEBRATES)

SPAWNING BEHAVIOR OF THE SHIELD DARTER, PERCINA PELTATA (PISCES-PERCIDAE). John G. New, Science Department, State University College, Oneonta, New York, U.S.A.

The shield darter inhabits large streams and rivers in the eastern United States where it is confined ecologically to areas with a gravel substrate.

Spawning occurs in late April and early May. Males establish territories but these are not rigid. Females enter the territory when ripe and ready to spawn but may move out of the territory during the time when males are fighting or when pursued by males.

Male aggressive display often prevents a fight, but fighting does take place and is vigorous especially within the territorial boundaries. Males sometimes defend the territory against females regardless of their readiness to spawn.

Females solicit spawning by presenting themselves in the territory of the male or directly in front of a male when territorial behavior breaks down in the aquarium. Males mount females from the side and first bring the area of the isthmus into contact with the dorsal region of the female. A series of enlarged caducus scales is present in the male along the mid-line of the belly. Each scale has several long spines projecting from it.These scales apparently serve as contact organs. Rubbing these scales on the female, especially in the region of the nape, is stimulatory and if the female is ready to spawn she will permit the male to mount. Spawning takes place when the male has brought his body parallel to, above and in contact with that of the female. The male's dorsal fins are erect, the female's are depressed. His anal fin is to one side of her body and, as both male and female vibrate, the milt flows down the side of the fin closest to her body. The female's ventral region is at the surface of the gravel but is sometimes below. As spawning occurs both fish move forward through the gravel thus distributing the eggs in a line. Spawning lasts about ten seconds.

When ripe, females will spawn over and over again. One female was noted spawning twelve times within a three hour period.

A 16mm movie will illustrate spawning behavior in this species.

EXPERIMENTAL RESEARCHES ON MONOCULAR VISION OF CHAMAELEO AND MELOPSITTACUS. Mario F. Canella, Inst. of Zoology, University, Ferrara, Italy.

Comparative ophthalmologists, after having established that also the Vertebrates with total chiasma of optic nerves have a more or less ample binocular visual field, admitted that binocular vision is a necessary condition for stereo- and bathyscopic perception (Tschermak, Elliot Smith, Walls, Chavasse, Rochon-Duvigneaud, Linksz, Polyak, etc.).

Our first researches, several years ago, on visual behavior of one-eyed Teleosts, Batrachia, Lizards and Birds, gave the proof that in all these animals (not excepted marine Fishes with one fovea and Birds with double fovea) unilateral blindness does not alter the perception of depth and relief. This result has been accepted, in their papers or books, by some french authors (Rabaud, Verrier, Piéron, etc.), and, for the Birds, experimentally confirmed by a german student (Bonner). As for Rhiptoglossa, notwithstanding the availability of one chameleon, it also, deprived of an eye, maintained its marvellous shoot precision, its exact perception of the third dimension; however, Rochon-Duvigneaud, in his treatise (Les yeux et la vision des Vertébrés), observed that this experience, "intéressante mais unique, appelle une enquête plus approfondie".

As they persist in affirming the necessity, also for the Vertebrates with total optic chiasma, of binocular vision in order to estimate, "by a process of triangulation", the distance of the objects, we have recently performed some other researches, not only on several individuals of three species of Chamaeleo (jacksoni, bitaeniatus, lateralis), but also on Melopsittacus undulatus (knowledge on eye and vision of the Parrots is still scarce). The results, documented by many snapshots, have given clear evidence that visual behavior of one-eyed Chameleons and Parakeets, without any preliminary adjustment after blinding, and without monocular parallax, is not at all defective; they do not behave differently from the controls, except for the obvious restriction of the visual field. If both eyes, which normally move independently, converge and focus simultaneously upon an insect or a seed, this does not involve a binocular rangefinding.

HABITAT SELECTION IN BIRDS. Peter H. Klopfer, Dept. of Zoology, Duke University, Durham, North Carolina.

The purpose of our studies is to compare the effective "niche size", or, "degree of behavioral stereotypy" (cf Klopfer and MacArthur, Amer. Nat. 94 and 95) in tropical and temperate zone passerines. Our hypothesis is that tropical birds show increased stereotypy (smaller niches) due to the greater stability of tropical as opposed to temperate zone habitats. This, in turn, permits an enhancement of the diversity of tropical faunae.

The experiments reported here purport to show that northern chipping sparrows, *Spizella pusilla*, though normally strongly preferring pine foliage to oak, can be fairly easily induced to shift their activities to oaks. This is one of the kinds of plasticity a northern bird requires. Whether comparable tropical species are indeed more rigid has yet to be shown. (These experiments are currently in progress. Support is from The National Institutes of Health).

SUN ORIENTATION BY THE ADELIE PENGUIN (PYGOSCELIS ADELIAE).

R. L. Penney and J. T. Emlen, Department of Zoology, University of Wisconsin, Madison 6, Wisconsin.

Adelie Penguins (Pygoscelis adeliae) were transported from coastal rookeries in the Ross Sea area to release points on featureless snow-ice plateaus in the interior of the Antarctic. The departure routes of individual penguins were mapped for distances of 2 to 4 kilometers. Direction and straightness of course were measured. Birds from Cape Crozier (77°S, 169°E) released on the Ross Ice Shelf (80°S, 179°E) took courses to the north. Birds from both Cape Crozier and Cape Hallett (72°S, 170°E) released at an intermediate point to the west on the Victoria Land Plateau (75°S, 155°E) assumed courses to the northeast. Reseases of Cape Crozier birds at Byrd Station (80°S, 120°W) took routes north northwest. Two additional experiments on the Victoria Land Plateau were carried out using normal birds and two groups of birds subjected for ten days to two different light-dark cycles. The normal birds performed the same as the first Victoria Land release two months earlier, but the birds with the superimposed light-dark cycles showed much greater variation in direction chosen as well as behavior upon release. The results of the experiments with normal birds when plotted on a polar projection map reveal routes essentially parallel to a line passing northward through the home rookery. All performances under varying degrees of cloud cover suggest a navigation mechanism which uses the sun as the primary orientation cue plus a biological clock to compensate for the changes in sun azimuth. The basic northward orientation (in reference to the breeding grounds) is thought to be of survival value in steering lost birds to their off-coast feeding area (pack ice) and may segregate and concentrate birds from the various circumcontinental population upon reaching the pack ice.

SOCIAL ACCELERATION OF FOOD-DISCOVERY IN GULL CHICKS. Jack P. Hailman, Department of Zoology, Duke University, Durham, NC, USA

The newly-hatched gull chick (Larus) pecks preferentially at its parent's red beak. This pecking induces the parent to regurgitate partially-digested food. However, chicks do not recognize food and must find it by trial-and-error pecking. The initial experience with food causes the chick to peck at a greater rate and to restrict pecking to food. Thus initial food-discovery is the key to the chick's learning to feed.

Two social mechanisms which accelerate food-discovery have been found. The chick possesses an inborn preference to peck at stimulus-objects which move, possess contrast or are red-colored. The parent's red, moving bill maximizes these stimulus characteristics. Since the parent not only regurgitates food onto the ground, but may also pick up and hold the food between its mandibles, the chick pecking at the bill may initially discover this food very quickly. A similar social-facilitating effect is created by experienced siblings. Since each chick posses a white-tipped dark bill, this moving, contrasting stimulus elicits pecking from siblings. When naive chicks peck at the bill of an experienced sibling while the latter, in turn, is pecking at food, the naive chick's pecking is directed to the food.

AN EXPERIMENTAL STUDY OF THE DEVELOPMENT OF SONG IN THE SONG SPARROW. Rev. James Mulligan, Dept. of Zoology, University of California, Berkeley, California.

Males of this species, *Melospiza melodia*, possess large repertoires of songs which vary greatly in structure. The purpose of this study, which follows an instrumental analysis of variation in the adult song, was to discover whether this song could be developed in isolation, and whether this species has an early sensitive learning period.

Eleven male nestling Song Sparrows were raised by hand and placed in sound-proof isolation chambers, singly and in groups of two or three. Three of these were hatched from the egg and raised by canary foster parents. A fourth was experimentally deafened to test for the importance of hearing. Seven individuals were exposed to training with adult songs at selected periods during development, to study the existence and time limits of learning by imitation.

The results show that this species is capable of developing in isolation a song which is normal in many respects, although lacking in typical complexity and variety. Accurate imitation of adult songs occurred if training was given during the first few months of life, which was several months prior to the period of song development. Later training was not effective. The song of the deafened bird was completely abnormal.

It is concluded that normal song development is partially dependent on experience of adult song, that there is a sensitive period of learning during the first few months of life, and that auditory feedback in the developmental process is essential.

ASCENDING PATHWAYS FROM THE SPINAL CORD IN THE PIGEON(COLUMBA LIVIA). Harvey J. Karten, Department of Neurophysiology, Walter Reed Army Institute of Research, Washington 12, D.C.

Hemisections of the spinal cord of pigeons were made under direct vision at C12-14. The animals were sacrificed 7-14 days post-op. and the brains studied for ascending pathways using the Nauta technique for degenerating axons. Fibers could be followed in continuity to termination in the following regions (all terminations were ipsilateral with few exceptions):

MEDULLA-PONS- Nuclei cuneatus and gracilis; caudal pole of the inferior olive; commissural nucleus of the vagus-solitary complex (nucleus infima); nucleus of thetractus solitarius; medial and lateral portions of the medial reticular nucleus (Cajal) in the ventro-lateral to medial zones; the raphe and paramedian nuclei; cerebellar cortex via distinct dorsal and ventral spinocerebellar tracts.

TECTUM-MESENCEPHALON- Dense terminations in the lateral reticular nuclei to the level of the interpeduncular nucleus, with a diffuse spray of degenerating axons seen throughout the reticular formation and central grey substance of the mesencephalon. Dorsolaterally arching fibers could be followed to termination in the nucleus mesencephalicus lateralis, pars dorsalis, though completely sparing the central core of it which corresponds to the torus semicircularis. The region of termination is possibly homologous to the stratum profundum of the superior colliculus of mammals. Other fibers could be traced to the lateral optic nucleus of Craigie, the stratum lemnisci of the tectum proper, the pretectal and subpretectal nuclei.

THALAMUS- Degenerating axons could be seen penetrating the tectal and posterior commissures and entering the thalamus to terminate diffusely in the ventral portion of the anterior and posterior dorsolateral nuclei of the thalamus. Occasional fibers could be seen in the dorsomedial nucleus of the thalamus. No degenerating axons could be clearly identified in the "ventral" thalamic nuclei: e.g. nucleus rotundus, ovoidalis, etc.

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GENITAL DENERVATION AND SEXUAL BEHAVIOR IN MALE CATS.

LESTER R. ARONSON AND MADELINE COOPER, DEPT. OF ANIMAL BEHAVIOR, THE AMERICAN MUSEUM OF NATURAL HISTORY, NEW YORK.

ACCORDING TO CURRENT THEORY (BEACH, 1956), REDUCTION OF SEXUAL STIMULATION BY THE ELIMINATION OR BLOCKING OF ONE OR MORE SENSORY MODALITIES SHOULD NOT CHANGE THE PATTERN OF SEXUAL BEHAVIOR BUT SHOULD LEAD TO A REDUCTION IN THE LEVEL AND EASE OF SEXUAL AROUSAL. TO TEST THIS HYPOTHESIS, THE NERVE DORSALIS PENIS OF SEXUALLY EXPERIENCED MALE CATS WAS SECTIONED BILATERALLY ALONG THE SHAFT OF THE PENIS. THIS MINOR OPERATION DESENSITIZES THE GLANS PENIS BUT SHOULD NOT INTERFERE WITH ERECTION WHICH IS UNDER CONTROL OF THE PELVIC NERVE.

IN MATING TESTS STARTED ONE WEEK AFTER OPERATION, COPULATING MALES WERE GROSSLY DISORIENTED IN RELATION TO THE FEMALE'S PERINEAL REGION, TO THE EXTENT THAT IN MOST CASES INTROMISSION WAS NOT POSSIBLE. IN ONE OPERATED MALE, INTROMISSION OCCURRED IRREGULARLY BUT THE BEHAVIOR WAS VERY AWKWARD. IN SOME CASES WHERE NERVE SECTION WAS INCOMPLETE OR WHERE REGENERATION HAD OCCURRED, ORIENTATION GRADUALLY IMPROVED TO THE POINT WHERE INTROMISSION WAS AGAIN POSSIBLE. IN OTHER CASES WHERE NERVE SECTION WAS PRESUMABLY COMPLETE AND WHERE REGENERATION DID NOT TAKE PLACE, DISORIENTATION HAS PERSISTED UP TO 20 MONTHS AFTER OPERATION. SHORTLY AFTER OPERATION, ONE SUCH MALE DEVELOPED A STEREOTYPED PATTERN OF LYING ON HIS SIDE AND THRUSTING VIGOROUSLY WITH ERECT PENIS FAR REMOVED FROM THE FEMALE'S GENITAL REGION. IN NEARLY TWO YEARS OF TESTING THIS ANIMAL, THERE HAS BEEN NO IMPROVEMENT IN ORIENTATION. SEXUAL EXCITABILITY IN THIS ANIMAL IS VARIABLE. AFTER OPERATION, SOME OF THE ANIMALS EXHIBITED A TEMPORARY LOSS OF FULL ERECTION, BUT WITHIN A FEW WEEKS ALL OPERATES WERE CAPABLE OF COMPLETE PENILE PROTRUSION AND ERECTION. COMPLETENESS OF NERVE SECTIONS IS BEING DETERMINED HISTOLOGICALLY. OTHER VARIABLES, SUCH AS THE AMOUNT OF PRIOR EXPERIENCE, ANDROGEN LEVEL AND AGE AT TIME OF NERVE OPERATION (PREPUBERAL VERSUS POSTPUBERAL) ARE ALSO BEING INVESTIGATED AS PART OF A LARGER PROGRAM OF STUDY OF THE ONTOGENY AND PERSISTENCE OF SEXUAL BEHAVIOR.

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THE EFFECT OF TEMPORARY ANOSMIA ON COURTSHIP BEHAVIOR IN THE RAM (OVIS aries). Edwin M. Banks, Dept. of Zoology, University of Toronto, R. Bishop, and H.W. Norton, University of Illinois.

In selecting an estrous ewe from a flock, the ram appears to provide himself with olfactory cues of a high stimulus valence. To determine whether the precopulatory behavior of rams temporarily deprived of olfactory sensitivity would be modified, a series of tests was undertaken on 3 sexually experienced, vasectomized rams. The design provided for 24 day-pairs, one of the days being a control run (saline nasal douche), the other day the rams' nasal sinuses were thoroughly sprayed with a topical anesthetic (Xylocaine). This treatment produced a temporary anosmia which lasted for about 3 hours.

Three stimulus ewes, randomly selected from a flock of 50 Western cross-bred animals were tethered to a fence about 10 ft. apart. Prior to being led into the observation pen, one of the ewes, in which estrus was induced by injections of progesterone and estrogen, was tested with a teaser ram to insure that it was, in fact, in estrus. The other 2 ewes were in the nonreceptive phase of their cycles.

The position of the tethered ewes was randomized; the order in which the experimental rams were introduced into the pens was randomized. During the 5-minute test period, the frequency of display of the components of precopulatory behavior, the latency to successful selection, etc. were recorded.

Data from control and experimental series were analyzed by the method of least squares and revealed (1) while anosmic, the rams' behavior differed insignificantly with regard to all measured parameters, including successful selection of the estrous ewe, with one exception; (2) while anosmic, the frequency of display of nudging, one of the important components of precopulatory behavior was significantly higher than during the control series. The implications of this finding will be discussed, in the framework of stimulus specificity.

EVOLUTION OF SOCIAL STRUCTURE IN AFRICAN BOVIDS. Allen Keast, Queen's University, Kingston, Ontario, Canada.

From a relatively late origin in the Miocene the Bovidae have developed into the most successful of the large herbivores, occupying a wide range of habitats and food niches. As a group, the African bovids are undergoing active speciation and ecological radiation (Keast, unpubl.), and they account for the bulk of the African artiodactyl fauna of 111 species, 82 super species.

One of the most interesting facets of bovid evolution is the wide range of social structures displayed. These include the following: (1) solitary way of life (Steinbok, various duikers); (2) typically living in pairs or threes (Oribi); (3) living in small parties, generally with one adult male (Lelwel - Backhaus, 1959; Kudu, Roan); (4) larger aggregations which, seasonally, may be composed of all categories but that spend part of the year as harem herds (one adult male, many females and young), male herds, and solitary males (Thompson's Gazelle - Brooks, 1959; Impala in southern Africa); (5) the same but with three herd types all year (Impala in east Africa); (6) an "advanced" type of social structure where mixed groups, with some solitary males, are typical (Buffalo). In all species solitary males are seasonally territorial (with stamping grounds), the trend apparently reaching a peak in the Uganda Kob, as described by Buechner (1961). Broadly speaking, the degree of elaboration of group structure varies with habitat, solitary antelopes being scrub and thicket dwellers the more elaborate aggregations occurring in open country species.

Horn structures in antelopes have long been looked upon as characters that gives "quite convincing evidence of lack of directedness in their development" (Rensch, 1960). It is noteworthy, however, that simple, stabbing, lethal horns are characteristic of solitary species and those with little social structure, and in which the primitive "defensive" role persists. Recently, Bruhin (1953) has reviewed horns and shown them to have several roles. Walther's fascinating behavioral studies with zoo animals (especially 1958) have shown that, in their dominant, inter-male combat role some species use them in pushing, others in butting, others as fencing or parrying weapons.

There would seem to be no doubt that, basically, horn elaboration has proceeded as a means of asserting dominance without (usually) killing or wounding. Greatly increased frontal area, annuli, spiralling, and hooked tips, not only give a greater pushing surface but prevent the horns of opponents from sliding past each other to the vulnerable side of the body. Once this basic requirement is fulfilled the direction that horn evolution takes varies with the group. This "dominance without injury" is reminiscent of a territorial song and posturing in birds.

Harem herds, ruled by one male who has attained his position by combat, must function in assuring that the maximum number of impregnations are by the fittest males (note Allee's work on Sage Grouse).

PHYSIOLOGICAL EFFECTS OF FIGHTING IN MICE. F. H. Bronson and B. E. Eleftheriou, The Jackson Laboratory, Bar Harbor, Maine, and Purdue University, Lafayette, Indiana.

Adult male mice of the C57BL/10J strain were exposed to: (1) trained C57BL/10J fighters or (2) handling similar to that received by mice exposed to fighters including being placed in a strange cage. Rates of exposure to these two types of treatments were 0, 1, 2, 4 or 8 times/day (12 mice/cell) for one week. Each exposure lasted one minute. A one-minute exposure to a fighter resulted in an average of 20 seconds of fighting and only small amounts of tail wounding. Adrenal weights increased as the number of exposures/day to fighters increased. Body weights (gain) and seminal vesicle weights decreased as amounts of fighter-exposure increased. The same trends for body and adrenal weights were found in mice given the handling treatments but to a much smaller degree. Plasma corticosterone levels were significantly higher in mice exposed to fighters than in those given handling. Adrenal corticosterone levels showed a similar trend but were not significantly different. A primary conclusion is that relatively small amounts of exposure to trained fighters (2-8 minutes/day for one week) resulted in relatively large physiological changes.

ANIMAL MIGRATION. Archibald G. Huntsman, Dept. of Zoology, University of Toronto, Toronto, Ontario, Canada.

The Aristotelian myth of purposive migration takes account of what is necessary for survival, but fails to agree with actual behaviour. If we eat, not in order to live, but merely because appetite is evoked by food or gastric secretion, we should not without warrant ascribe purpose to animals, rather than mere will to act as observed.

This approach to the problem - study of the animal's response to the environmental situation - has been used for salmon to replace the myth of migration seaward for feeding and back riverward for spawning. In descending rivers, smolts do not direct their courses downstream, but descend through failure to keep near the bottom, where they are stimulated to head and swim upstream. On reaching deep water, they retreat from surface light and thus disappear, but for the most part go no farther than the outflow of the river into the sea.

Salmon return on ceasing to feed when fat and vigorous. They do not direct their courses riverward, but wander as directed by the local situation. As being near the surface, they stop wandering on reaching a shallow strong current that fully engages their activity in heading and swimming against it. Only on reaching the shallow steady outflow at the river mouth do they direct their courses steadily against it and thus riverward. They migrate upstream mainly when a freshet subsides, with the current no longer fully engaging the activity evoked in them by the increasing current of the freshet. Leaping over a fall is merely what they like to do, even in still water of sea or pool, but particularly when stimulated by turbulence at the foot of a fall, and is to be observed in descending smolts as well as in adults.

In detail, migration shows great variety and complexity in accordance with environmental conditions. Salmon respond most variously to light, bottom, current and temperature, as well as to the unknown solutes that are responsible for homing.

NEW METHOD OF ANALYSIS OF ANIMAL BEHAVIOR UNDER CONDITIONS OF NATURAL EXPERIMENT. N. Ladygina-Kohts, Museum Darwinianum, Moscow.

We suggest a new method of analysis of animal behavior under conditions of natural experiment to investigate the structure of the activity and the significance of the stimulus and the character of actions it produces. A continuous record of the behavior of the test animals to a certain stimulus should be subjected to a new form of analytical research. Separate actions must be singled out and chronologically recorded taking into account all the operations of the animal in response to the stimulus...

Quantitative evaluation consists in recording every operation the animal performs with the object...

Qualitative evaluation consists in verifying the aim of the operations and their specific practical result for the animal...For this purpose actions are classed into series according to the types of activity devoted towards a given object. We have registered the following types of activity in the structure of the unrestrained behavior of an adult chimpanzee. 1. acquaintance and inspection; 2. treatment; 3. construction; 4. kinetics play; 5. use of tools; 6. retaining the object; 7. rejection... A difference in the structure of the activities with regard to different objects is revealed by comparing the percentage of the operations related to any particular form of activity with the total number of manipulations with the given objects, which enables us to plot a number of curves... When a definite object is handled, the various form of activity are plotted on the abscisse and their frequency in % on the ordinate... When we record the structure of the activity with regard to different objects, they are plotted on the abscisse and the frequency of the operations of each activity on the ordinate. Such curves may be compared in the study of the structure of unrestrained behavior of animal towards a definite stimulus at different levels of phylogenetic and ontogenetic development or the structure of the behavior of the same species towards different stimuli.

Such comparative analysis reveals the significance of a definite stimulus for the animal and the psychological traits in the structure of their behavior.

ANALYSIS OF A BOUNDED PRIMATE POPULATION. C.R. Carpenter, Professor of Psychology, The Pennsylvania State University, University Park, Pennsylvania, U.S.A.

The howler population of Barro Colorado Island has been analyzed repeatedly since 1932. Population variations, distribution, group organization and structure have been investigated. The Collias and Southwick census of 1951 showed 237 animals organized in 29 groups. Sizes of groups were reduced and the structure changed. The majority of the groups had one male. A census in 1959 showed 814 animals organized into about 44 groups. There were 146 adult males, 402 females, 131 infants and 135 juveniles. The grouping structures shown by data collected in the 1930s were re-established. The distribution of groups on the Island was extended. Inter-group interactions were intensified. Observations were made on group equilibra, composition and the socionomic sex ratio problem. Also data were collected on territoriality, reproductive rate in varied size groups, "stress" and population dynamics. Behavioral variations related to population were observed.

XVI INTERNATIONAL CONGRESS OF ZOOLOGY

CONTRIBUTED PAPERS SESSION

10A. PHYSIOLOGY

STUDIES ON LIPID METABOLISM IN THE CECROPIA SILKMOTH. K. A. Domroese and L. I. Gilbert, Dept. of Biological Sciences, Northwestern University, Evanston, Illinois.

Respiratory quotients, as determined by 6 hours of Warburg readings a day for up to 35 days, indicate that lipids are only metabolized after the 17th day of the 21 day pupal-adult developmental period of the male Cecropia moth. Analysis of total lipid and carbohydrate during adult development revealed a progressive fall in carbohydrate but little lipid alteration until ecdysis. The lipid content dropped precipitously between the second and fifth day of adult life. The lipid content of immobilized 5 day old male adults was 57% greater than that of flying adults. These data suggest that lipid is conserved during adult development for later use as an energy source for flight.

That lipid is the substrate of choice for flight muscle metabolism was shown by the following experiments. Flight muscle homogenates contained 24% more lipid than anthrone positive material. The endogenous RQ of flight muscle homogenates was indicative of lipid catabolism. Exogenous C^{14} labeled fatty acids and Kreb's cycle intermediates were actively oxidized by flight muscle homogenates whereas glucose and pyruvate were not. Lipid breakdown was strongly inhibited by iodoacetic acid, malonic acid, KCN and dinitrophenol. Dialysis of flight muscle homogenates reduced the metabolism of endogenous lipid by 90% as compared to non-dialyzed controls. Activity was restored to normal by the addition of ATP, Mg and citrate. Citrate could be replaced by other Kreb's cycle intermediates but not by glucose or glucose-1-phosphate. Isolated flight muscle sarcosomes also required ATP, Mg and citrate to oxidize C^{14} labeled fatty acids. Fatty acid activating enzymes were detected and studied in flight muscle homogenates using the hydroxylamine trapping technique.

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INTERMEDIARY METABOLISM OF LIPIDS AND AMINO ACIDS IN INVERTEBRATES.

H.J. Vonk and D.I. Zandee, Lab. of Comparative Physiology, University of Utrecht, Utrecht, The Netherlands.

Vertebrates are able to synthesize cholesterol, but nearly all insects are unable to do so. This difference makes a comparative investigation of this point in other groups of invertebrates highly interesting. It can be built up from mevalonic acid in some lamellibranchs (1), but not in Annelida (although synthesis of squalene from acetate seems to be possible) (2). In our laboratory it has been found that decapod crustaceans (Astacus) are unable to synthesize cholesterol or other steroles from injected C^{14}-labeled acetate (3), but that the gastropod Helix pomatia is able to do so (4). In the sea anemone this synthesis is probable (5).

All invertebrates investigated so far are able to build up higher fatty acids from acetate (several insects, Astacus, Helix, Anemonia sulcata). It is generally accepted that in vertebrates the unsaturated fatty acids (oleic acid, palmitoleic acid) are derived from the corresponding fatty acids by means of dehydrogenation. In invertebrates we have met with several cases where the labeling of the unsaturated fatty acids after injection with labeled acetate is far higher than that of the saturated fatty acids (4,6). These animals probably have independent pathways for building up saturated and unsaturated fatty acids.

Injection of C^{14}-labeled acetate or glucose (7) also provides a means for determining which amino acids are essential and non-essential for a certain species, as the tracer is found to be present in the non-essential amino acids and not in the essential acids. This is especially important in such cases where ordinary feeding and deletion experiments are impossible or difficult (aquatic and phytophagous animals). Such experiments have been performed with Calliphora, Astacus and Anemonia with the result that their requirements do not essentially deviate from those of the species investigated so far by the classical methods (3,5,6).

It must be borne in mind that all the results on nutritional requirements of vertebrates as well as of invertebrates have been reached with a very limited number of species so that an extension of these investigations is highly desirable.

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BLOOD-GLUCOSE AND LIVER GLYCOGEN LEVELS IN THIOUREA-TREATED CHICK EMBRYOS. Robert C. Thommes and Sr. M. Etienne Truchan, O.S.F., Dept. of Biological Sciences, De Paul University, Chicago, Illinois, U.S.A.

The effects of thyroid inhibition on blood-glucose and liver glycogen levels in the developing chick embryo has been studied.

1.5 and 3.0 mgs. of thiourea in 0.1 ml. of distilled water were injected into the yolk sac on day 8.0 of incubation. Blood and liver glycogen samples were collected from embryos sacrificed at daily intervals from days 9.0 through 18.0 of incubation. Blood-glucose levels were determined by the glucose oxidase method (Glucostat - Worthington Biochemicals). Liver glycogen levels were determined by the "Anthrone Method".

The addition of thiourea by injection into the yolk sac results in the production of a hyperglycemia beginning on day 10.0, reaching a maximum on day 12.0 and ending on day 13.0. The incubation interval of 15.0 to 18.0 days is characterized by a persistent hypoglycemia.

Liver glycogen levels somewhat correspond to blood-glucose levels. Liver glycogen concentrations in 9.0- and 10.0-day embryos are below normal. In the 10.0- to 11.0-day incubation interval, liver glycogen levels rise markedly above control levels, reaching a peak on day 11.0. The period from day 11.0 to 13.0 is characterized by a precipitous decline in liver glycogen concentration, the low being reached on day 13.0. Recovery from this low occurs on day 15.0 with 1.5 mgs. of thiourea and day 17.0 with 3.0 mgs. of thiourea.

The influence of thyroid inhibition upon skeletal muscle glycogen and yolk sac membrane glycogen is currently being investigated. (Supported by research grant A-4527 from U.S.P.H.S.).

MODE OF UTILISATION OF CARBOHYDRATES IN INSECTS. K.N. Saxena & S.S. Krishna. Department of Zoology, Delhi University, Delhi, India.

The fate of certain oligo- and monosaccharides ingested by and injected into certain dipterous, heteropterous and homopterous insects has been traced *in vivo* during a comparative study of the mode of utlisation of carbohydrates in insects. The first step in the utilisation of oligosaccharides is their digestion into absorbable monosaccharides. The degree of digestion of these substances depends not only on the presence of appropriate enzymes but mainly on the rate and mode of action of the enzymes in relation to the rate of conduction of the nutrients from the digestive to the non-digestive zones of the gut. For instance, in all the insects examined, invertases and other glycosidases show transglycosidic activity on the ingested oligosaccharides causing the production of simpler saccharides as well as the synthesis of certain higher oligosaccharides in the gut. But, differences in the above mentioned physiological characteristics of the gut are responsible for complete digestion of the oligosaccharides into monosaccharides in the dipterans and heteropterans and their incomplete digestion and final excretion in the homopterans.

The second stage in the utilisation of carbohydrates involves their absorption in the form of monosaccharides. But some of the ingested sucrose, when present in high concentration, is absorbed undigested from the mid-gut of *Musca*. The rate of absorption of the carbohydrates depends upon their concentration gradient across the gut wall. The glucose after absorption is metabolised to trehalose to maintain a steep concentration gradient so as to facilitate its complete absorption. The fructose is partly absorbed as such and partly after its conversion into glucose in the lumen of the mid-gut. Whatever fructose is absorbed as such is finally transformed into glucose which, in turn, leads to the synthesis of trehalose. Transformation of fructose into glucose in the mid-gut, prior to absorption, accelerates the synthesis of trehalose from the absorbed glucose and thereby facilitates the absorption of fructose by maintaining a steep concentration gradient.

The ingested arabinose is also fairly readily absorbed in the region of the mid-gut but it can be hardly metabolised into glucose or into any other sugar. As the concentration of the pentose in the blood rises, it begins to pass into the lumen of the hind-gut. This serves to maintain a concentration gradient from the mid-gut lumen to the blood so that all the ingested arabinose can be absorbed from the mid-gut. However, the absorbed pentose passes from the blood into the hind-gut and is finally excreted. One of the factors governing the capacity of the insects to survive better on sucrose, glucose and fructose than on arabinose concerns the absence of a suitable mechanism for metabolising the arabinose to glucose or trehalose for participation in various metabolic processes.

THE PHOSPHATE METABOLISM OF THE FACETED EYE IN LIGHT AND IN DARKNESS.

Helmut Langer, Zoological Institute, University of Munich, Germany.

The metabolism of the faceted eye of an insect, the blow-fly Calliphora erythrocephala Meigen, was investigated in connection with its function as a sensory organ. The experiments were performed on males of the wild-type and of the mutant chalky where ommochrome and pteridine pigments are lacking. In former investigations an increased consumption of carbohydrates was found during illumination, the rate of which depended on the wavelength and intensity of the light and on the pigment content of the eyes (Langer 1960,'62,'63). This paper deals with the metabolism of phosphate compounds during darkness and illumination by a mercury lamp emitting the lines between 254 and 436 nm.

First, analyses were made to determine the changes in the amounts of phosphorus compounds in the eyes. Wild-type flies were kept in darkness or under illumination for 6 hours. Then the compounds of isolated eyes were separated according to the method of Schneider (1945). In no fraction did the phosphorus content vary between dark-adapted and illuminated eyes. – In another series of experiments the quantity of free inorganic phosphate was determined. The amount increased with illumination, while the phosphate liberated from acid-instable compounds during 7 minutes hydrolysis (N HCl, 100^0) decreased correspondingly. This effect was more pronounced in mutant c than in wild-type eyes. Thus one must conclude that illumination leads to an increased consumption of energy-rich phosphorus compounds.Specific enzymatic analyses showed significantly less ATP in irradiated eyes than in those kept in darkness. ATP itself, however, represents only 15 – 20 % of phosphorus in acid-instable compounds.

To check the possible influence of adequate stimulation to the turnover of phosphorus compounds, hungry flies were fed with carrier free P^{32}-orthophosphate in concentrated sucrose solution and the extent of incorporation of the radioactive phosphorus in the eyes measured. In preliminary experiments flies kept in low-intensity, diffuse daylight manifested in their eye substances a rapid rise in phosphorus incorporation up to 4 hours after a single feeding and then gradually drop (measurement up to 16 hours). The same curve was obtained for the fraction of acid-soluble compounds which contains the inorganic phosphate as well. A considerably smaller and slower incorporation occurs in the fractions of nucleic acids, phosphoproteins, and phosphatides, the latter of which had not reached a maximum even after 16 hours.

The effect of light and darkness on the turnover rate was determined during the increase of over-all incorporation. At 1, 2, and 4 hours after feeding the over-all incorporation in the eyes was found about 40 % higher in light than in darkness ($P = 0.01$). That the rise varied among the fractions suggests more than a general rise in total metabolism. Acid-soluble compounds showed the least increase (<40 %); nucleic acid and phosphoprotein fractions were only slightly higher (about 50 %). Phosphatides, on the other hand, showed an increase of more than 100 % over their value in darkness; that is nearly three times the average incorporation ($P = 0.01$).

Conclusions: 1) Both the phosphate turnover in the total metabolism and the consumption of energy-rich phosphate compounds of the faceted eye increase during illumination, as was to be expected from the increased carbohydrate consumption. 2) Although their total amount remains constant the turnover rate of the phosphatides shows a large additional increase. Precisely this additional increase during illumination strongly indicates that the phosphatides play a large role in the primary processes of vision or in the ensuing excitation of the sensory cells.

CATION REGULATION IN IMMERSED AQUATIC AND TERRESTRIAL CRABS.

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Cation regulation in immersed crabs showing different degrees of terrestrialness was studied with special emphasis on the Mg ion. In general, highly terrestrial brachyuran crabs maintain low Mg concentrations in the blood. Thus, the blood Mg concentration of Gecarcinus lateralis, Ocypode quadrata and Pachygrapsus crassipes averaged less than 30 meq/l when the animals were immersed in normal sea water; the more aquatic Hemigrapsus oregonensis averaged 47.0 meq/l and the completely aquatic Cancer antenuarius averaged 59.50 meq/l. Anomuran land crabs do not demonstrate such strong Mg regulation. The antennary glands of Ocypode, Pachygrapsus, Hemigrapsus and Cancer are capable of forming urine which is highly concentrated in Mg. Such is not the case for Gecarcinus. Thus, the antennary glands of this land crab cannot be responsible for strong Mg regulation.

As the concentration of the external medium is increased from 50% to 100% and finally to 150% sea water the value for urine concentration /blood concentration (U/B) with respect to Na decreases with each increased increment in the medium in Ocypode, Pachygrapsus, Hemigrapsus and Cancer. This ratio remains constant in Gecarcinus for the same experimental conditions, suggesting that fundamentally different mechanisms are operating in the antennary glands of this extreme land crab.

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IONIC REGULATION IN THE DEVELOPMENT OF MALES OF A SILKWORM: HYALOPHORA CECROPIA. Jan W. Michejda and Ralph E. Thiers*, Biological Laboratories, Harvard University, and Biophysics Research Laboratory, Peter B. Brigham Hospital, Boston, Mass.

The contents of Na, K, Mg, and Ca in the hemocoele and testicular compartments of H. cecropia were studied, employing the multichannel flame photometer, (Nature 174 1050, 1954). The cations studied were evenly distributed between the two compartments in diapausing and in chilled pupae, at approximate concentrations in mEq/l: Na,1: Mg,30; Ca,5: except for K which was slightly accumulated in the testes, 53 mEq/l compared to 45 mEq/l in the hemolymph. At later stages of development K was concentrated in the testes to twice the concentration of the blood, and in diapausing pupae also in the midgut and hindgut. Na concentrations were extremely low in all compartments studied, including the midgut and hindgut.

Injected Na and K was distributed evenly between blood and testes during diapause, indicating the absence of permeability barriers. The injection of Na, K, and sucrose led to accumulation of K in the testes of developing adults but not in diapausing pupae. Experiments on animals perfused with sucrose suggested that the distribution of ions throughout the body was the same as in hemolymph.

The osmotic value was the same in blood and testes; it dropped from 0,60 - 0,70 osm in diapause to 0,30 - 0,40 osm on the 2nd day of development.

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THE EXCHANGE OF IONS AND MOLECULES BETWEEN THE BLOOD AND CENTRAL NERVOUS SYSTEM - A COMPARATIVE STUDY

By J.E. TREHERNE

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The nature of the physiological processes involved in the transfer of substances between the blood and cerebral tissues has been the subject of some controversy ever since the original observation by Ehrlich that certain aniline dyes stained all the tissues of the mammalian body except those of the central nervous system. In mammals the direct measurement of transfer processes and the identification of rate-limiting processes is rendered extremely difficult by the complicated structure and organization of the brain. The purpose of this communication is to summarize some results which have been obtained with the relatively simple arthropod central nervous system and to consider them in relation to the more complex physiological organization of the vertebrate brain.

THE INFLUENCE OF SALINITY ON THE UPTAKE OF GLYCINE BY EURYHALINE ANNELIDS. Grover C. Stephens, University of Minnesota, Minneapolis, Minnesota, USA.

It has been shown that a number of marine invertebrates are capable of transporting glucose and amino acids inwards across the body wall. Unpublished data indicate that this capacity is lacking in fresh water forms. Hence, an investigation of transport in brackish water species is of interest.

Nereis limnicola and Nereis succinea are brackish water polychaet annelids whose chloride regulation, osmotic regulation, and distribution with respect to salinity have been studied. N. succinea is found in more saline regions of the brackish water spectrum while N. limnicola occurs at lower salinities and occasionally in fresh water. Both forms survive well at low chloride concentrations in the laboratory. They also tolerate extended sojourns in sea water or media of greater salinity.

The animals used in this study were maintained in the laboratory for at least 36 hours in the dilution of sea water in which they were to be tested. In the experimental observations, glycine-2-C^{14} was supplied in the external medium for periods of 15 minutes to one hour. At the end of such a period of exposure, the radioactivity of an 80% ethanol extract of each worm was determined. In a number of cases, a distilled water brie of each worm was prepared after alcohol extraction and its radioactivity measured. Initial and terminal measurements of the radioactivity of the medium were also made. All determinations were made using a thin-window Geiger tube.

Both species appear to take up glycine across the body wall. A double log plot of the rate of uptake against wet weight indicates that uptake is an exponential function of weight with an exponent value of approximately 0.5. The rate of transport in N. succinea exceeds that in N. limnicola by a factor of 20 to 30.

In both forms, a dramatic fall in the rate of uptake occurs as the sea water medium is diluted below concentrations of 200 mM chloride per liter (chlorosity of 7.09 grams per liter or salinity of approximately 12.72 parts per thousand). At 150 mM chloride per liter, uptake has declined by 80% and at 100 mM has virtually ceased. This range corresponds quite well with the onset of internal chloride regulation in the two species. Higher salinities produce a modest increase in the rate of uptake. The ratio of alcohol soluble and alcohol insoluble radioactivity does not vary with concentration of the external medium.

Uptake also occurs if the worms are supplied with glycine in a medium of 400 millimolar galactose or 200 millimolar sodium chloride. However, the rate of uptake is lower in both solutions. Since both solutes are somewhat toxic at these concentrations, this decrease in rate of uptake may or may not reflect specific ion requirements for the process in addition to the influence of osmotic concentration.

BIOCHEMISTRY OF THE COELOMIC FLUID OF THE POLYCHAETE NEPHTYS AND CHANGES DURING STARVATION. Mary E. Clark, Dept. of Zoology, University of Bristol, Bristol 8, England.

The sand-dwelling errant polychaete, Nephtys hombergi, has a large amount of coelomic fluid and also a closed circulation containing a relatively small volume of hemoglobin-rich blood. The coelomic fluid plays a significant role in eversion of the proboscis, essential for burrowing in sand, and bathes most of the tissues of the body. In it occur numerous coelomocytes, which form the major part of the blastema in regenerating worms. The coelom is also the site of breakdown of muscle fibers from the body wall during starvation and sexual maturation. Interest in these phenomena has led to a study of the chemistry of this milieu intérieur.

The pH of Nephtys coelomic fluid equilibrated with air is 6.50-6.60. It has a buffering capacity of 8.7 meq/l within the pH range 6.1-7.1. The protein content lies between 0.5 and 1.0 g %, part of which is a hemoglobin. The non-protein nitrogen has a range of 200-250 mg %, of which 50-60 mg % is due to amino acids. Chromatographic separation of the latter shows measurable amounts of 18 amino acids, with a large preponderance of glycine. Cysteic acid appears to be absent in oxidized samples. Anthrone estimates of the total carbohydrate content give glucose equivalents of 190-270 mg %, all of which is alcohol-soluble. Comparison of the constituents of Nephtys coelomic fluid with body fluids of other invertebrates so far studied indicates that it has higher levels of the common organic substances than do most other animals except insects.

Nephtys can be kept in good condition in the laboratory for up to a month without food providing sterile conditions are maintained. During starvation, massive histolysis of body-wall muscle occurs, many fibers being sloughed into the coelom. Coelomic fluid from worms starved 10 days shows a decreased NPN and a 50 % or greater increase in protein level. Total carbohydrate levels drop to around 100 mg % glucose equivalents, but less than half of this is alcohol-soluble. The remainder appears to be a glycoprotein, possibly derived from specialized cells lying in the coelom. It is likely that despite breakdown of the body wall, starved individuals lack enzyme systems capable of maintaining normal levels of amino acids and of mono- and oligosaccharides in the coelomic fluid.

A NEW HYPOTHESIS OF FLUID MOVEMENT IN MAN AND ANIMAL. Filimon Ukradyha, Div. of Cardiology, University of Utah, Salt Lake City, Utah, U.S.A.

The life of various organisms is conditioned by physiological and biochemical processes in the cells. The great importance is the movement of the fluids which supply nutrients and eliminate the metabolic waste products from the organism.

Many investigators have studied the movement of fluids in the living organism, and in this field many studies have been made by Starling. The theory which he proposed in 1896 has been until now the most widely recognized in physiology and medicine (J. Physiol., 19:312,1896).

According to Starling's theory, the exchange of fluid between the tissues and blood is regulated by the hydrostatic pressure of the blood in the capillaries and the osmotic pressure of the plasma proteins, to which the capillary walls are impermeable. He postulated that the hydrostatic pressure of the blood at the end of arterial capillaries is higher than the osmotic pressure of blood proteins; in consequence, the fluid is filtered to the tissues at the arterial end of the capillaries. At the venous end of the capillaries the osmotic pressure is higher than hydrostatic pressure, and fluid is moved back into the blood, i.e. conditions exist for fluid movement from the arterial end of the capillaries to the venous side.

Investigations of Landis (Am. J. Physiol., 82:217,1927; Physiol. Rev. 14:404,1934) and Pappenheimer (Physiol. Rev., 33:387,1953) are the most important contributions in support of the Starling theory.

This mechanism for the movement of fluid in the organism has been criticitized by many investigators (Hill, Bioch. J., 1:55,1906; Lasarus-Barlow, J. Physiol., 19:418,1895; Drinker and Field, Lymphatics, Lymph and Tissue Fluid, 1933; Krogh, The Anatomy and Physiology of Capillaries, 1936 and others).

Applying the method of concentration ratios of various constituents of serum as well as of protein, hemoglobin and hematocrit in the blood by dialysis in man and animal data were obtained which showed that such ratios are the same before and after dialysis. These data led this author to propose a new hypothesis of fluid movement in the organism:

1. Movement of fluids and many diffusible constituents in the organism is accomplished only as a result of complicated diffusion processes and not by filtration and diffusion as is interpreted in physiology and medicine. The existing hydrostatic pressure serves only for the circulation of blood through the body.
2. Movement of fluids and various substances in the organism is accomplished in a steady state by constantly unbalanced osmotic pressure of protein colloids in the blood and protoplasm.
3. Every disturbance of the relative equilibrium of osmotic pressure of the plasma electrolytes also influences the movement of fluid in the organism. The osmotic pressure of the plasma electrolytes in blood (about 7 atm.in man) is a great potential force; when its equilibrium is violated, this tremendous force operates to bring about osmotic homeostasis of the living organism.
4. Constantly unbalanced osmotic pressure of protein colloids and osmotic pressure of electrolytes are the only available forces which condition the movement of fluids and nutrients and eliminate the metabolic waste products from the organism.

HAIR GROWTH PATTERN IN THE RAT AS INFLUENCED BY DIETS LOW IN POTASSIUM. E. H. Dolnick, C. A. Cabell, J. D. Robbins, R. R. Oltjen, Animal Husbandry Research Division, ARS, U. S. Department of Agriculture, Beltsville, Maryland.

An experiment to study effects of mineral imbalance in weanling male rats was designed as a 2x2x2 factorial. The variables, expressed as percent of the synthetic diet were two levels of calcium, 1.2 and 0.3; two levels of phosphorus, 1.2 and 0.3; and two levels of potassium, 0.9 and 0.1. The experiment thus included 8 treatment combinations fed to 9 rats in each group. The feeding period of 14 weeks included the growing phase of the hair growth cycles of the 1st, 2nd and 3rd pelages.

Body growth was excellent in all of the four high potassium groups, averaging 5 grams per day for the first 6 weeks. Over the same period the 36 rats in the four low potassium groups averaged 2.7 grams body weight gain per day. This retarded body growth was expected since the 0.1 level of potassium is below known requirements of the rat.

The normal molts and subsequent waves of hair growth are gradual and therefore not perceptible to the naked eye unless the coat is sheared or plucked to facilitate the study of hair regeneration as hairs emerge. Superficial examination of the groups on the high potassium diet showed no visible signs of hair regeneration that could be detected macroscopically. However, all of the animals fed the low potassium diets elicited patterns or waves of hair growth on the head, back and occasionally along the upper sides. This has not been previously demonstrated in relation to potassium deficiency. The pronounced patterns, symmetrical in arrangement and visible macroscopically, directed attention to the involvement of potassium in the shedding and regeneration of the hair. Histological findings will be discussed with regard to this hair growth pattern.

EFFECT OF VARIOUS SUBSTANCES ON RED CELL PERMEABILITY. F. R. Hunter, Dept. of Biology, U. of the Andes, Bogota, Colombia

Using a densimeter technique, the effect of mercury, tannic acid and triton X-100 on the permeability of erythrocytes to various non-electrolytes has been studied. These experiments were designed to give information concerning the relationship between the molecular architecture of the cell membrane and the movement of substances across it. The effect of these inhibitors alone and in various combinations has been studied using erythrocytes of different species of animals and several non-electrolytes. By appropriate selection of penetrating substances and erythrocytes, different effects of the inhibitors have been obtained. These can best be explained by assuming that the movement of some substances across the membranes of certain erythrocytes depends on some type of active process while in other cases the mechanism is simple diffusion. In some cases, the potentiating effect of mercury plus triton can best be understood by assuming that these two substances act at different sites in the cell membrane. On the other hand, the competitive effect of triton and tannic acid is consistent with the hypothesis that these two substances act at similar sites in the membrane.

CHANGES IN THE PLASMA LIPIDS IN THE BLACK-TAILED DEER THROUGHOUT A YEAR. Sheila F. Stewart, Department of Zoology, University of British Columbia, Vancouver, Canada; H.A. Nordan, A.J. Wood, Central Animal Depot, University of British Columbia, Vancouver, Canada, and I. McT. Cowan, Department of Zoology, University of British Columbia, Vancouver, Canada.

A yearly growth cycle in male and female deer has been observed in a number of species of deer in the U.B.C. colony.

Six animals (3 males and 3 females) of the species Odocoileus hemionus columbianus (V.I. genotype) were observed for a period of twelve months. Their weight increased from late spring (March-May) until the month of October then a marked decrease in both weight and feed intake occurred.

The weight loss continued until December or March. Sometimes this loss was half or more of the weight previously gained. It was assumed that the fat was the principal component being laid down and mobilized, and that a change in plasma lipids would reflect this.

Consequently, at two monthly intervals the animals were bled; total plasma lipid values were determined as well as total plasma cholesterols and a rough estimate of non-cholesterol "fat" was obtained from their difference. All values are expressed as gm./unit of body wt.

During the early period of weight increase both sexes showed an increased feed intake per unit of body weight and no changes in plasma lipids. In the latter part of this period, toward the point of maximum gain, there was a decreased feed intake per unit of body weight with a concommitant rise in plasma "fat" and little or no change in an already low plasma cholesterol level.

At the point of maximum gain both sexes showed a significant rise in plasma cholesterol, this was more marked in the males. In the males, the feed intake was decreased per unit of body weight with a concommitant fall in plasma "fat". The females showed increased feed intake per unit of body weight and a rise in plasma fat.

A period of weight loss ensues in both sexes; the males losing more weight at a greater rate. The loss is accompanied (male and female) by a decreased feed intake regardless of adequate food supplies. In the female, the plasma "fat" levels rise and then fall when the greatest weight loss ensues. The males showed a reduced plasma level throughout this period.*

When weight loss ceases the feed intake increases but no significant changes occur in the lipid levels.

It would appear that the plasma "fat" augments the caloric requirements of the animal during "compulsary" cessation of feed intake at the expense of the body fat depots.

* The period of high plasma fat levels in the males was believed missed during the two month interval.

INFLUENCE OF THE ACTIVE REACTION OF WATER-pH ON FISHES. Theodor Welykochatko, Dept. Zoology-S.B.P.C. - POBOX 970-S.Paulo

1) The active reaction of water-pH is very important and perhaps the chiefest factor of the medium of fishes; chlorides, sulphates, nitrates, carbonates etc., that were used for neutralization, have had no influence on fishes. The aeration neutralized and created the best medium for fishes in aquariums. 2)The optimum conditions of the medium, for the life of stenioh and eurion fishes, are between pH 7.2-7.6; the vital conditions for fishes, that may be considered as normal are encountered within the limits of pH6.0-8.0; above and below of these limits there appear the signs of depression. At the pH values between 6.0-5.0 and 8.0-9.0 the fishes survive with greater or lesser signs of depression, and at the values between 5.0-4.0 and 9.0-10.0 with marked signs of depression the fishes live during some hours or days, with hyperemied and later necrotic branchiaeiand soon die. The medium with pH 4.0-3.0 and 10.0-11.0 is mortal for fishes, where they die during 8-22 minutes, and at the pH 3.0-0.0 and 11.0-14.0 the fishes suddenly die at any circumstances, with marked signs of paralysis of the respiratory center and heart. The death of fishes by acid or alkaline reactions of water, proceeds with almost of the same signs what means the similarity of influence and action of hydroxyd and hydrogen ions. 3) The typical death proceed at the pH 4.0-3.0 and 10.0-11.0 by such phases: a) the fishes swim normally b) after 3.5 minutes they stop and stand immobile c) they frequently go to bottom,then rise to the surface and snatch air - first signs of asphyxia d) they throw themselves by darts to all sides and beat at the glass walls e) abnormal body position: head up and down,swim sideways and belly upwards f) little convulsive contractions of the whole body g) the respiration rhythm alters: increases or diminishes, the mouth opens too much sometimes h) skin and branchiae anemic, but the latter soon become hyperemic and at the margins of them are seen the signs of necrosis i) the convulsive contractions of the body darts and jumps become more frequent k) expressive signs of agony l) the fishes lie sideways at the bottom, frequently with opened mouth. Sometimes the sequence of referred symptoms changes due to the healthy conditions, nutritive state and size of the searshed fishes. By reactions, far from neutral (pH 7.0) these phases diminish and near the neutral-prolong. 4) The conclusion of these experiments are confirmed: a) by structure of the nervous system and innervation of the sensitive organs of fishes b) by biochemical data: native proteins and body humors, amphoterous electrolytes, that are only stable within the limits of pH 3.0-10.0 above and below of which they denature, acquire hydrophobous properties, what leads to the isolation of the organism from the medium, and diminution of ferment activity, disturbance of the main physiological functions of the organism and sudden death of fishes.

THE END

XVI INTERNATIONAL CONGRESS OF ZOOLOGY

CONTRIBUTED PAPERS SESSION

10B. PHYSIOLOGY

TIME MEASUREMENT IN THE PHOTOPERIODIC CONTROL OF DIAPAUSE IN AN INSECT. Perry L. Adkisson, Dept. of Entomology, A & M College of Texas, College Station, Texas.

The diapause of the pink bollworm, *Pectinophora gossypiella* Saunders is under photoperiodic control. Diapause is induced by daylengths of 13 hours or less and prevented by daylengths of 13.25 hours or greater. During the dark or scotophase the insect exhibits a bimodial rhythmical sensitivity to short periods of light. Under typical daylength situations, there are two periods of maximum sensitivity to light, one of which occurs 14 to 16 hours after subjective dawn and the other 10 hours after subjective dark. These two sensitivity periods serve to synchronize time measurement by the population and apparently are entrained by the light-dark transitions of dawn and dusk. If the insect "sees" light during these sensitivity periods diapause is prevented, if it does not, diapause is induced.

VERNALISATION-EFFECT WITH ANIMALS. Dietrich Teschner, Zoological Institute, Technical University, Braunschweig, Germany.

In experiments to the biology of the ground water mite Lobohalacarus weberi quadriporus (Walter)(Limnohalacaridae) I observed an important influence of low temperature upon the beginning of the oviposition. Mites, which are kept in +4° to +5°C for 6 weeks, start egg laying remarkable sooner than those of equal age but without chilling period. There were to find no eggs in these control samples, when the rising generation in the cultures with cold treatment had already developed past the larva to the chrysalis. Obviously the oviposition of these mites is released by a "cold stimulus". Similar results are published about "Imaginal Diapause" of several insects. So in different groups of animals is established an effect of cold on the physiology of development in grown females. As yet a success of a cold stimulus has been found for juvenile stages only: It breaks the diapause of many insects.

In other words: Now are known in the animal kingdom instances, in which a cold stimulus in grown organisms starts the activity of gonads or in juvenile stages finishes a latent stagnation period. In the whole area of biology we find such cases again in the field of botany. Phenomenons of this kind are called vernalisation in the last 30 years. We can compare the start of gonad activity with the induction of ripeness-to-flower; corresponding is the case with the diapause of arthropods and the stagnation period of buds or of whole plants.

The diapause is a specifical physiological condition. We can not call so each stadium in which the development is stopped. In all cases mentioned above is acting a similar mechanism: activity of hormones. The cold stimulus or other environmental factors activate a certain hormone in the brains of animals or in the vegetation cones of plants. This hormone activates other hormones and so starts a series of reactions.

In new botanical literature is suggested to use the term vernalisation only just for the cold induction of ripeness-to-flower and not in the original wider sense. This means in the zoological parallel that not all manifestations of the diapause can be included. The induction of oviposition of mites, however, still belongs to the effects of vernalisation as well as the already published facts of the so-called imaginal diapause. The original conception of diapause means the juvenile stages with latent stagnation of development. According to the usage of the term diapause nowadays the vernalisation is a special case of it.

Deliberately I did not coin a new expression for the effect of chilling with the mites, furthermore I perceive the use of the term imaginal diapause as caused by verbal difficulties. Because of far-reaching parallels to the botanical phenomenons I propose to use the well-known conception of vernalisation. The keynote of the XVI International Congress of Zoology asks for "REUNION OF ZOOLOGY FROM ITS SEPARATE SPECIALTIES". In the problem discussed here is a possibility to recognize common interests not only within zoology but of the entire biology, that is zoology and botany, and to utilize those for reunion.

IDENTIFICATION OF ACTIVE BRAIN REGIONS INVOLVED IN THE PROCESSES OF HIBERNATION. Felix Strumwasser, Jerrell L. Smith, James J. Gilliam and Floyd R. Schlechte, Dept. of Neurophysiology, Walter Reed Army Institute of Research, Washington, D. C.

In many respects, hibernation is an ideal type of phenomenon to study in order to gain insight into the mechanisms by which the central-nervous-system elaborates a long-term change in behavioral state. The large drop in body temperature and resultant reduced activity of all body cells probably represents the most dramatic change in reversible physiological state among all of the higher animals. It has now been established in three species of Citellus that the processes of hibernation are gated by an endogenous cycle of activity presumably built into certain individual neurons; the onset of this cellular activity is approximately of one year period (Strumwasser et al., Proc. Finn. Acad. Sci., 1963).

This paper describes the techniques which have been successfully used to record brain-temperature, heart-rate and the EEG over continuous periods of time, as long as one year. It has allowed a study of mono- and poly-synaptic transmission during entrance into hibernation. The axons of the tufted cells of each olfactory bulb synapse with granule cells in the contralateral olfactory bulb. When tufted cells are electrically stimulated in one olfactory bulb, an evoked potential in the contralateral bulb due to this mono-synaptic path can be studied. During entrance into hibernation, the electrical threshold of initiating a minimum evoked response does not increase more than 30% although brain temperature drops almost 30°C. The threshold is virtually independent of temperature, having a Q_{10} of 1.1 over this large temperature range. This finding is evidence that both the electrical excitability of the conduction process and chemical excitability of the transmission process are compensated for temperature.

The area under the EEG has been computed by on-line electronic integration during entrance into and arousal from hibernation. An intensive analysis has been performed of the olfactory bulbs (OB) and (stereotactically-determined) amygdala (AMYG) in C. beecheyi. When the EEG integral is studied as a function of entrance and arousal temperature, a particular shape of the relationship exists which is highly specific for each of these two brain regions. This relationship is remarkably constant over many separate hibernations. The OB integral/temperature relationship appears as a long and narrow ellipse and indicates a positive activity-temperature coefficient. The AMYG integral/temperature relationship is triangular in shape with the base representing entrance into and the sides of the triangle representing arousal from hibernation. Reasons are developed for considering the EEG integral/temperature plot as an indicator of active and passive brain regions during the processes of hibernation. The integration technique has allowed discovery of an infra-dian rhythm of discharge normally progressing in the amygdala during the alert and the hibernating state. It is clear in C. beecheyi, arousal from hibernation occurs in synchrony with one of the infra-dian bursts emanating from the (stereotactically-determined) amygdala.

THE CRITICAL BODY TEMPERATURE FOR LEARNING IN HYPOTHERMIC RATS.

J. A. Panuska and V. P. Popovic, Department of Physiology, Emory University School of Medicine, Atlanta 22, Georgia.

Hypothermic rats at a body temperature of 29°-30°C are capable of learning to use a lever-activated heat reinforcement apparatus within 48 minutes (Panuska and Popovic, Fed. Proc., 22, 1963). The high percentage (86%) of rats which learned in those experiments suggested that learning might be achieved at even lower body temperatures. In order to test this hypothesis, inexperienced shaved adult white rats in deeper hypothermia were exposed to a similar high motivational situation with the possibility of learning how to heat themselves externally and regain euthermia. This was achieved by cooling the rats to a colonic temperature of 18.5°C, rewarming them to 22.0°C or 24.0°C, and then placing them in a heat reinforcement chamber at a constant 2.0°C environmental temperature. The only object protruding in this chamber was a sensitive lever which when pressed actuated an overhead 250W infrared heating lamp for two seconds. Frequent pressing of this lever would effect rewarming; accidental pressings did not occur frequently enough to produce an increase in body temperature.

In this situation the body temperatures of the rats rose slightly and then leveled. Three hours was the longest duration of any experiment. Activity and body temperature were constantly recorded, and performance was checked visually. In rats which learned to use the heat reinforcement apparatus, performance began with a dramatic change from an average of one accidental reinforcement per minute to 6.1 reinforcements per minute.

Six experimental groups of five rats each were used. In the first group the body temperatures of the rats remained at 28°-29°C until performance began. These rats started to use heat reinforcement steadily after an average time lapse of 41 minutes. With the lowering of body temperature in other groups, the time necessary to show performance increased to 80 minutes (27°-28°C group), 98 minutes (26°-27°C group; one rat failed to perform), and 113 minutes (25°-26°C; one rat failed to perform). The rats in the last two groups (24°-25°C and 23°-24°C) did not manifest learning. All rats which learned how to obtain external heat warmed themselves rapidly to euthermia and continued to use the lever as long as they remained in the experimental situation. Control rats, denied the use of the external heat source, died in deep hypothermia.

It is concluded that inexperienced rats are able to learn a simple technique necessary for survival and use it successfully even when their body temperature is at a low level of 25°-26°C. This is only 3°-5°C higher than the body temperature at which locomotion ceases. While rats at a body temperature of 28°-29°C learned and began to use the heat reinforcement apparatus after an average of 41 minutes, performance took place after longer periods in deeper hypothermia. Rats at a body temperature below 25°C failed to perform during the three-hour experimental period.

BODY TEMPERATURE REGULATION IN THREE SPECIES OF AUSTRALIAN FLYING FOXES. Philip Leitner, George A. Bartholomew, and John E. Nelson, Biology Dept., St. Mary's College of California, St. Mary's College, California, Dept. of Zoology, University of California, Los Angeles, and Dept. of Zoology, University of Queensland, Brisbane, Australia.

Measurements were made of body temperature and oxygen consumption in three species of Australian Megachiroptera (Mammalia: Chiroptera). The species studied included two of the larger flying foxes, *Pteropus poliocephalus* (550 to 1100 grams) and *Pteropus scapulatus* (330 to 550 grams), and one of the smallest, *Syconycteris australis* (15 to 20 grams).

All three species maintain body temperatures within the normal mammalian range in ambient temperatures as low as 5°C. Neither species of *Pteropus* undergoes daily or seasonal periods of torpor and there is no positive evidence that *S. australis* can become torpid. By wrapping the wings around the body, flying foxes can trap a relatively warm layer of air between wings and body. This behavior contributes importantly to insulation, since the temperature of this layer of air may be more than 10°C. above ambient temperature at 5°C. The two species of *Pteropus* respond to ambient temperatures of 40°C. or more with open-mouth panting and fanning of the partially flexed wings, then with salivation and licking, often covering wings and chest with saliva.

Basal metabolic rates of all three species approximate the theoretical values appropriate for mammals of their size. In both species of *Pteropus* the upper critical temperature for oxygen consumption is about 35°C. The thermal neutral zone extends to 24°C. in *Pt. scapulatus* and to either 19°C. (wings at sides of body) or 15°C. (wings enclosing body) in *Pt. poliocephalus*. *S. australis* has no thermal neutral zone but rather a thermal neutral point at about 33°C. The action of the wings in controlling insulation is shown particularly well by *Pt. poliocephalus*. By enclosing the body with its wings this species can reduce its lower critical temperature by 4°C. and decrease considerably its thermal conductance in low ambient temperatures. In *Pt. scapulatus* the curve of oxygen consumption against ambient temperature extrapolates to the usual range of body temperature for the species, indicating that thermal conductance is constant below the thermal neutral zone. In the two other species thermal conductance continues to change below the lower critical temperature. *S. australis*, with a point rather than a zone of thermal neutrality, has a thermal conductance which changes over the entire range of ambient temperatures in which body temperature can be controlled. The factors affecting thermal conductance in *Pt. poliocephalus* are so complex and variable that we cannot fit our data to a biological expression of Newton's Law of Cooling.
(Supported by Grant C-14972 from the N.S.F.)

ASPECTS OF THE POSTNATAL DEVELOPMENT OF THERMOREGULATION IN SMALL MAMMALS. Kari Lagerspetz, Dept. of Zoology, University of Turku, Turku, Finland.

Through the work of Adolph, Gelineo, Hahn, and others the postnatal development of the homoiothermy in the rat has been well documented. The experiments to be reported here deal with the postnatal development of the thermoregulatory mechanisms in the laboratory mouse, in the golden hamster, and in the arctic lemming (Lemmus lemmus). The comparative data on the latter two species is based on the unpublished results of the experiments performed in this laboratory by Mr. Raimo Hissa, M. Sc.

By measuring the differences between the stabilized body temperature and the external temperature in animals at different age levels and ambient temperatures the time-courses of the development of homoiothermy in the different species can be compared. Four periods can be distinguished in the development of the thermoregulation in mice: (1) the development of the first, behavioural thermoregulatory reactions (0 to 2 days), (2) the period of only behavioural thermoregulatory reactions (orthokinesis) (2 to 7 days), (3) the period of the development of homoiothermy at intermediate temperatures (7 to 13 days), and (4) the period of the development of homoiothermy at lower temperatures, i.e., of the cold shivering (13 to 19 days). The establishment of the adult type of electric activity in the brain coincides with the last period.

ELECTRON MICROSCOPE STUDIES OF THE TEMPERATURE EFFECTS ON THE FROG SKIN. Shigeo Takenaka, Dept. of Physiology, Gifu Medical School, Gifu, Japan.

Temperature effects on the frog skin were studied with the electron microscope and two kinds of thermo-adaptation were ascertained. Ringer solution was applied to the inner and outer surfaces of the excised abdominal skin of Rana nigromaculata, the skin temperature being changed between 9°C and 22°C. The following results were obtained: 1) The intercellular space between the epithelial basal cells is larger at 22°C (e.g. about 1.7 - 2.7 μ) than at 9°C (e.g. about 1.2 - 1.3 μ), though in the superficial epithelial cell layer the intercellular space does not change so markedly. This change may correspond to two different temperature coefficients of the resting potentials of the frog skin. 2) By electron microscopy, between the basement membrane and the principal collagenous fiber layer, white pigment cells are found in the matrix, which contains fibroblasts and collagenous fibrils. The degree of mutual contact of the white pigment cells changes by temperature. At 9°C the matrix layer is rather thin and its inner surface is smooth, while at 22°C the inner surface is not smooth and the intercellular space between white pigment cells becomes larger, their contact surfaces being curved more irregularly. By light microscopy it was very difficult to detect the white pigment cells in this region of the frog skin.

SOME PHYSIOLOGICAL STUDIES ON THYROXINE-RESERPINE TREATED WHITE MICE.

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Thyroxine, reserpine, and a combination of the two drugs in definite sequence were given to white mice over an experimental period. Growth rate, litter size, and muscle weakness in the hind legs of the animals were studied.

Data collected, indicated that a sequence of thyroxine for two days and reserpine for one day, with the sequence repeated over the experimental period proved to have greatest effect on growth rate. This growth rate is compared with that obtained when thyroxine alone, reserpine alone, and a combination of reserpine for two days and thyroxine for one day was given.

Another series of experiments was carried out using the same drugs and combinations as before but this time considering the effect on litter size. The sequence of thyroxine for two days and reserpine for one day, with sequence repeated increased litter size most of all. When thyroxine was given alone it either prevented pregnancy, caused loss of females that did become pregnant or resulted in stillborn offsprings. Prolonged reserpine treatment resulted in a very small increase in litter size, as did the sequence of reserpine for two days and thyroxine for one day with sequence repeated.

Prolonged treatment with thyroxine alone or in the sequence of thyroxine two days and reserpine one day produced no unusual leg muscle weakness. The sequence of reserpine for two days and thyroxine for one day gave slight indications of leg muscle weakness. Reserpine given alone over a prolonged period resulted in marked leg muscle weakness.

Chromatographic analysis for free and bound amino acids in the leg muscles of the mice were made for control mice, reserpine treated mice, thyroxine treated mice and combinations of the two drugs. Two amino acids did not appear in the analysis of leg muscle of mice given reserpine over a long period of time.

PRELIMINARY ANALYSIS OF SHIVERING RESPONSES IN WILD BIRDS.

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Thermogenesis in response to cold is effected chiefly by increased physical activity such as exercise, increased muscle tone and shivering. Non-shivering thermogenesis has not been described for birds. During the daylight hours, birds in the cold produce body heat by exercising, but at times of inactivity, especially during the long winter nights in Arctic and North Temperate regions, shivering becomes the most important mechanism for producing heat.

Shivering was determined by 1. the average peak to peak voltages read from a myogram, and 2. the mean power ($volts^2$) of shivering activity over a period of 3 minutes. Four species of wild North Temperate birds were used to measure shivering responses at different environmental temperatures. In all cases, shivering increased linearly with decreasing temperature. Evening Grosbeaks, Hesperiphona vespertina, were acclimated to a series of temperatures from -15° to +18°C and to natural outdoor winter and summer temperatures. Shivering was measured and it was determined that thermal acclimation did not have any effect on the Grosbeaks' shivering responses to a series of test temperatures.

Since shivering requires energy, shivering and metabolic rate as determined by oxygen consumption were measured simultaneously. In all species, a direct correlation between shivering and metabolism was obtained. However, the slopes are widely divergent, due to the different sizes of birds used. When metabolism is expressed per gram body weight, the slopes converge.

(This research was done while the author held a post doctoral fellowship at the National Research Council of Canada.)

TEMPERATURE REGULATION IN THE GOLDEN HAMSTER (MESOCRICETUS AURATUS) ACCLIMATED TO THERMO-NEUTRAL AND COLD ENVIRONMENTS.

Hermann Pohl and J. Sanford Hart, Division of Applied Biology, National Research Council, Ottawa, Ontario, Canada.

Oxygen consumption, measured in an open circuit system, heart rate and electrical activity of the muscles (shivering), detected with fine copper wires attached to the skin of the back, and body temperatures from the cardiac and subcutaneous areas, were determined at temperatures from 30°C to -30°C in unanaesthetized golden hamsters acclimated to 30°C and 6-9°C.

Over the range from 30°C to -15°C, warm- and cold-acclimated hamsters did not differ significantly with respect to oxygen consumption or body core temperature at any given temperature. At -30°C, oxygen consumption of cold-acclimated hamsters was approximately 5 times that at 30°C (1.5 ml/g/hr) and exceeded that of warm-acclimated hamsters, which became hypothermic at -25°C. In spite of a threefold increase in metabolic rate, heart frequency and shivering did not rise in cold-acclimated animals between 30°C and 0°C. Below 0°C, shivering remained significantly ($P < .001$) less than that in warm-acclimated hamsters. At temperatures from 30°C to -15°C, thoracic subcutaneous temperatures remained higher in warm-acclimated hamsters, which maintained smaller temperature differences between central body and subcutaneous tissues than cold-acclimated hamsters ($P < .05$).

In further experiments on cold-acclimated hamsters, chronically implanted thermocouples indicated that temperatures near the heart remained about 1-2°C higher than those near the liver in animals exposed to cold. Increased heat production (without shivering) was associated with elevated temperatures near heart and liver. After replacing the animals into a thermo-neutral environment there was a sudden rise in subcutaneous temperature (about 3°C) and a temporary rise in temperatures of the body cavity (liver area) above that near the heart.

It is concluded that heat production without shivering is an important element in the temperature regulation of the golden hamster, which is able both to hibernate and to acclimate to cold. It is also apparent that important sources of heat production occur in the body core, especially in the cardiac area.

METABOLISM AND SHIVERING IN EVENING GROSBEAKS (HESPERIPHONA VESPERTINA) DURING ACCLIMATION TO COLD IN THE LABORATORY.

J. Sanford Hart and Hermann Pohl, Division of Applied Biology, National Research Council, Ottawa, Ontario, Canada.

Metabolic acclimatization to winter in evening grosbeaks and other small birds is characterized by increased survival time in extreme cold and enhanced ability to maintain high rates of heat production (Hart, Physiol. Zool. 35:224, 1962). In a laboratory acclimation study 8 grosbeaks were kept at 30°C for 12 weeks during the winter, and 8 were kept at 0°C for 5 weeks and at -10°C for 5 weeks during the summer. After acclimation, the oxygen consumption measured with an open circuit system and the electrical activity of the pectoral muscles detected with fine copper wires, were determined at a series of temperatures from 30°C to -30°C on the same day.

Cold acclimation was associated with an increased body weight and an increased oxygen consumption per bird at any given temperature, but the oxygen consumption per gram was the same in all acclimation groups. Acclimation to cold resulted in an intensification of shivering when related either to exposure temperature or metabolic rate. It is concluded that nonshivering thermogenesis, observed in rats and other mammals, does not play an important role in these birds during acclimation to cold.

EFFECT OF TEMPERATURE ON EGG PRODUCTION IN NEODIPRION SERTIFER (GEOFF.) (HYMENOPTERA: DIPRIONIDAE)

I. M. Campbell and C. R. Sullivan

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Preliminary experiments on the influence of temperature on growth and reproduction in Neodiprion sertifer (Geoff.) show that temperature can have a profound effect on egg production. Whereas females held at 20°C, during the cocoon stage, completely utilized their abdominal fat body content in the production of eggs and produced 'full-sized eggs, females held at 10°C over the same stage of development utilized only 80-85 per cent of their food reserves, had a slightly reduced fecundity and a 15 per cent reduction in egg weight. Females held at 30°C underwent little development. However, females transferred from 30°C to 20 or 10°C developed into apparently normal adults except that their egg production was reduced: those moved from 30 to 20°C produced approximately 60 per cent of their potential egg complement and egg size was but slightly reduced, whereas those transferred to 10°C produced only about 15 per cent of potential egg complement and the eggs were considerably smaller. Indeed, under the latter temperature regime some females failed to produce any eggs, the ovaries remained juvenile and the fat bodies intact.

WINTER REPRODUCTION IN THE ALFALFA WEEVIL. Averett S. Tombes, Department of Entomology and Zoology, Clemson College, Clemson, South Carolina.

The occurrence of true winter hibernation in the alfalfa weevil in South Carolina has been recently questioned because of observed winter reproductive activity. During adult hibernation in arthropods the reduction of reproductive activity and often the atrophy of the reproductive organs are common occurrences. Therefore, the objectives of this study have been to observe changes during the winter months in the reproductive organ structure and the rate of egg deposition.

Following a period of summer estivation, extending from mid-July to late September or early October, the adult weevil begins feeding after which the reproductive organ development is initiated. Ovipositioning first occurs within thirty days and continues at a rather uniform rate throughout the winter months.

The internal reproductive organs of both the male and female were studied in detail, both as to size and weight. The female emerged from estivation with non-developed ovaries and oviducts. As development progressed the oviducts doubled and often tripled in length, became convoluted and showed signs of segmentation proximal to the ovaries. The developing ova, one from each segment, enlarged and passed down the oviducts with the accumulation of four to fifteen fully developed eggs in the uterus. This was followed by ovipositioning. Throughout the winter months and into the spring this sequence of development occurred continuously. The only structural alteration noticed was the gradual reduction in ovary length. The male organs experienced little change during the winter months in either size or weight.

It is thus concluded that the alfalfa weevil does not truly hibernate during the winter in this area of the United States although its field activity is noticeably decreased. Reproductive activity continues in both male and female with no atrophy of tissue, no unusual increase of fat and no sharp decline of egg deposition.

STANDARDIZATION OF THE METHODS OF MEASURING THE RELATION BETWEEN CHEMICAL STRUCTURE AND BIOLOGICAL ACTIVITY. Abd El Khalek Hamed El Sebae, Dept. of Entomology, University of California, Riverside, California, U.S.A.

During all the pharmacological, toxicological, physiological, or biochemical studies there are always attempts to correlate a certain type of chemical structure with a certain phase of biological activity; whether it is a type of toxic or non-toxic change.

All these variables and relations must be expressed quantitatively, so as it can be comparable and help in deducing final conclusions. These quantitative measurements must be standardized so as to be generalized as comparable values.

Between these quantitative measurements now in large scale use are the LD50 and LC50 values, which correspond to the amount of toxicant in grams, for every organism or unit weight of it, that is responsible of 50% kill, or the concentration of the toxicant which is responsible of 50% kill respectively under certain specified experimental conditions.

The LD50 and LC50 values as they are now used, neglect the factor of the variation in the molecular weights of the different chemical compounds tested. As the actual unit at the biological site of action is the molecule itself, therefore it is reasonable that the molecular weight should be considered in determining the relative biological potency. This correction is already applied in the enzymatic toxicity tests where the inhibition (I 50) is calculated in terms of molar concentration. Thus it is suggested to generalize the use of molar LD50 and LC50 values.

It is quite clear in the text that recalculation of these values according to the suggested molar system, will affeect the already known picture of the relative efficiency of insecticides, acaricides, rodenticides, molluscicides, trypanosomicides, plasmodicides, and general anti-protozoal chemicals. Also the relative efficiency of the attractants or repellents, or any such compared activity neglecting the molar ratio, might be affected if revised on the molar basis.

We can just pick one example showing the wide difference in molecular wights in some particular cases, e.g. the comparison between hydrogen cyanide (molecular weight 27), and another nitrile used also as a fumigant which is trichloroacetonitrile (molecular weight I44.5).

Finally, it is clear that applying the molar correction is vital for obtaining more standardized, comparable and accurate efficiency values, especially when the biological activity measured, is attributed to the chemical structure.

XVI INTERNATIONAL CONGRESS OF ZOOLOGY

CONTRIBUTED PAPERS SESSION

10C. PHYSIOLOGY

A STUDY OF THE MECHANISM OF ACTIVATION OF THE PROTHORACIC GLANDS.[1]

Herbert Oberlander[2] and Howard A. Schneiderman, Developmental Biology Center, Western Reserve University, Cleveland 6, Ohio.

It has long been known that the brain of insects activates the prothoracic glands. What is activation in biochemical terms? To answer this question an autoradiographic study of nucleic acid synthesis and protein synthesis in inactive and activated prothoracic glands was undertaken.

Larvae and diapausing pupae of a number of Saturniid moths were studied, particularly Antheraea polyphemus and Samia cynthia. Prothoracic glands were activated in various ways; e.g. by the animals' own brain, by injection of hormone extracts, etc. Activation was measured in terms of the amount of incorporation of tritiated uridine into RNA, of tritiated thymidine into DNA, and of labeled amino acids into protein. Grain counts were made of autoradiographs of sections of whole glands.

Within twelve hours after exposure to brain hormone the prothoracic glands were engaged in active nuclear RNA synthesis, and both nuclear and cytoplasmic regions were heavily labeled. As the secretory cycle progressed the glands grew markedly in size and both amount and concentration of labeled RNA increased. Ultimately, RNA synthesis diminished in the enlarged gland cells. There seemed to be little DNA synthesis in response to brain hormone. The course of protein synthesis in the activated glands is now being investigated by a variety of techniques.

1. Supported by grants from NIH and NSF.
2. Predoctoral Fellow of the National Science Foundation.

THE ORIGIN OF THE NERVOUS SYSTEM. L. M. Passano, Dept. of Biology, Yale University, New Haven, Connecticut, U.S.A.

A neurophysiological study of the nerve-nets of scyphozoans and Hydra, together with initial recordings from two species of planarian flatworms, has suggested an alternative to the generally accepted theory of the origin of the nervous system first proposed by G.H.Parker (The Elementary Nervous System, Philadelphia, 1919).

It is postulated that primitive protoneurons showed endogenous non-propagated depolarizations which served to synchronize local effector activity in the colony. When decremental conduction evolved, interconnected neurons coordinated regional activity (analagous to the tentacle twitching of hydroids). Further interconnections eventually led to a nerve-net with many potential pacemakers capable of integrating activity patterns over the entire organism. This might have marked the individuation of the colony and thus the emergence of the eumetazoa.

Subsequently a hierarchy of pacemakers evolved, with some serving to initiate and coordinate long sequences of complex behavior without direct control of effectors. Rather, they controlled specialized subordinate pacemaker loci. One way in which such indirect control could have been effected would be by a frequency modulation system such as seems to operate between the "rhythmic potential" system and the contraction burst pacemaker in Hydra (Passano and McCullough, 1962).

The emergence of a few discrete, morphologically and physiologically specialized control centers with pacemaker properties coincided with the development of specialized sensory receptors near these loci. An analagous situation occurs at present with the marginal ganglia and their associated rhopalia in scyphozoans. The development of specialized nerve-nets (such as the giant fiber nerve-net of the scyphozoan subumbrella, or the through-conduction tracts of actinian mesenteries) with non-decremental conduction, polarized synapses and faster conduction, marked the appearance of specific nerve pathways with function determined by terminal connections. Consolidation of control centers and longitudinal nerve cords led through stages of cephalization (illustrated within present-day platyhelminths) to the "typical" nervous system of higher animals.

This theory differs from Parker's in several important respects: 1) afferent sensory pathways evolved subsequent to "central" neurons rather than visa versa. In more primitive forms it is suggested that external stimuli affect endogenous activity either indirectly through mechanical deformation of tissues surrounding pacemaker neurons, by passive depolarization and electrotonic spread from surface dendritic endings of such neurons, or by direct reception by the neuron cell body. The latter may persist in the so-called "generalized light sensitivity" of many eumetazoans. 2) endogenous rhythmic nervous activity is primitive and widespread whereas "reflex" activity of the kind postulated by Parker is advanced. 3) long-term phases of activity, sequentially organized, are more characteristic of primitive animals than direct responses to environmental stimuli.

SOME ASPECTS OF THE ELECTRICAL ACTIVITY IN THE CENTRAL NERVOUS SYSTEM OF THE SCORPION, HETEROMETRUS SWAMMERDAMI. Kandula Pampapathi Rao, Dept. of Zoology, Sri Venkateswara University, Tirupati, A.P., India.

Electrical activity in the ventral nerve cord is known to occur in the absence of any apparent external stimuli in several invertebrates. Such activity has been shown to consist of either periodic or aperiodic bursts of impulses or repetitive impulses from discrete units. This "spontaneous" activity has been correlated with certain normal activities of the animals concerned, only in very few cases. In most cases no such correlations could be established.

Such "spontaneous" electrical activity was observed when the ventral nerve cord of the scorpion was lifted on to Ag-AgCl electrodes. The amount of activity and the number of units distinguishable, varied with the position of the electrodes. Activity recorded increased as the position of the electrodes moved from the posterior to the anterior along the length of the nerve cord. A greater number of active larger units were evident anteriorly. The activity of most these units was of a regular frequency.

The degree of the "spontaneous" activity not only varied with the level of the nerve cord from where it was recorded but also with the time of the day. In scorpions kept in continuous darkness very little (or no) activity could be noticed in the forenoon. Comparing any given level in the nerve cord, maximum activity was noticed between 4 p.m. and 12 midnight. After 12 midnight the activity diminished reaching very low or zero level at about 8 a.m., only to increase again in the afternoon. In scorpions kept in continuous light the time of onset of increased activity was later in the evening by about four to six hours.

This "spontaneous" electrical activity in the ventral nerve cord of the scorpion is correlated with the diurnal rhythm in the locomotory activity of the animal, which has a similar time course as that of the electrical activity in the two sets of animals (viz. those kept in continuous darkness and those in continuous light). Thus the locomotory rhythm appears to be a direct consequence of the rhythm in the electrical activity of the central nervous system.

While the locomotory rhythm seemed to flow from the rhythm in the central nervous activity, the mechanism of initiation and control of the latter is not immediately apparent, and therfore the neurosecretory system of the animal was examined. It was found that the secretory activity of certain groups of neurosecretory cells in the central nervous system exhibited similar time relations as above. The data seem to indicate the possibility of the products of neurosecretion playing a role in modulating the "spontaneous" electrical activity of the nervous system and thus controlling the diurnal rhythm of locomotory activity of the scorpion.

SOME ASPECTS OF THE RETINAL PHYSIOLOGY OF SALMON (Salmo salar).

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Results of my investigations during the past five years will be reviewed. The structure of the Atlantic salmon retina corresponds to descriptions by previous workers and is also similar to that of the Pacific salmon retina. It is capable of pronounced photomechanical changes in response to variations in light conditions. Retinal epithelial pigment (REP) light-adapts in 60 min. Cones light-adapt in 45 min. Dark-adaptation of REP and cones occurs in 70 min(Can. J. Zool. 39:123. 1961). Retinae of fish exposed to 900 ft-c are more fully adapted to light than those of fish exposed to 0.1 ft-c. Retinae of the former group take a shorter time to attain the same state of adaptation as that of the latter. However, they take a longer time than the latter to attain their own maximum state of adaptation. Retinae of fish subjected to darkness after exposure to 900 ft-c are not dark-adapted even after 70 min. REP of fishes placed in darkness from light of 0.1 ft-c dark-adapts in 45 min. while the cones do so in 25 min. Hypotheses presented in a previous study are examined in the light of these results (Can. J. Zool. 40:561. 1962). REP dark-adapts in light intensities below 0.001 ft-c. Cones are in a light-adapted state in intensities of 0.01 ft-c and above, semi-adapted state in 0.001 ft-c, and dark-adapted state in 0.0001 ft-c and below. It is suggested that the visible spectrum of the yearling Atlantic salmon ranges from 3640 Å to 6900 Å because the retina is in a light-adapted state in that range. In light, lower and higher temperatures bring about an expansion of REP. Cones are not affected. In dark, REP expands with higher temperatures but temperatures exceeding 18.3°C bring forth contraction. Cones contract with increasing temperatures but beyond 14.8°C they expand. Cones exhibit no rhythm in their positions in continuous light or dark. REP demonstrates no rhythm in light but shows one in dark which persists for a day. Significance of these results will be discussed. Comparisons with Pacific salmon will be made (Can. J. Zool. 39:511. 1961). Enucleated eyes from light- and dark-adapted fish were subjected to light and darkness for one hour and fixed in Bouin's. In all cases one eye of each fish was placed in light and the other in darkness. REP remains in approximately the same state it was in at the time of enucleation regardless of whether it was placed in light or darkness. Regardless of whether they were placed in light or in darkness, cone myoids of dark-adapted fish remain expanded while those from light-adapted eyes become expanded or relaxed. It is suggested that the retina of salmon dies within an hour after enucleation. This renders it difficult to ascertain whether the cones in the enucleated eyes are capable of responding to light and darkness, or not. However, it appears certain that the REP is incapable of responding to changes in light conditions when the blood and/or nerve supply are interrupted (Rev. canad. Biol. 21:7. 1962).
(This work was supported by grants from the National Research Council of Canada)

INTRACELLULAR POTENTIALS FROM SINGLE SENSE CELLS IN THE EYES OF THE LEECH, HIRUDO MEDICINALIS.

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Single cells in the eyes of the medicinal leech were impaled with micropipette electrodes (3 mol KCl; R ≧ 4o MΩ in Ringer's solution), and their responses to light stimuli were recorded.

Before penetration, negative graded generator potentials as high as 3o mV were frequently observed, and kept under the electrode for up to over one hour. Very often they were superimposed by one to rarely more than three positive going spikes (duration 3 to 5 msec) on the rising and early falling phase.

Penetration of a cell was assumed on the sudden appearance of a steady negative potential, accompanied by an increase of electrode-plus-preparation resistance. These resting potentials measured up to 4o, sometimes up to 55 mV, and they declined to zero, gradually or stepwise, within periods from seconds to more than one hour.

On illumination the resting potential depolarised (except in some nonresponsive cells), usually by less than half its value, in some cases by over 4o mV, but never beyond zero potential. Amplitude, and also latencies of these responses depended on intensity and duration of illumination in a graded fashion.With strong stimuli depolarisations showed a smooth, reproducible course: They started after a latency of 4o to 1oo msec, rose to a fast transient peak in 1o to 4o msec, followed by a second slower and lower maximum; on continued illumination they then attained a slowly falling plateau. After stimulation the previous resting potential level was regained within about .5 to 2 seconds. Subsequent test stimuli, however, found the cell less responsive for periods from some to over 6o seconds. With less intense stimuli the fast transient peak disappeared. In the lowest intensity range, comprising 1 to 1.5 log units, depolarisation showed irregularities in amplitude of some millivolts, and also in timing; at the threshold end of this range responses occurred or not, appearently at random. Intensities between threshold and appearance of the fast transient peak differed by 2 to 3 log units.

Accompanying these depolarisations, one or a few positive going spikes, timed like the extracellular ones, were occassionally observed; whether they overshot zero potential was uncertain. At the end of stimuli of up to .6 sec duration spikes were never seen. Volleys of spikes (frequency up to 15/sec) occurred in some cases without correlation to light stimuli immediately after cell penetration, or for some seconds before disappearance of the resting potential.

The cells in the Hirudo eye were thus established as functional light receptors. Their properties will be discussed with respect to the photoreceptors of more regular structure and arrangement found in other taxa, to the responses of the leech to light, and to the mechanisms underlying the potentials.

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FUNCTIONAL CONNECTIONS OF SINGLE NEURONAL UNITS IN THE CENTRAL NERVOUS SYSTEM OF THE CRAYFISH. C.A.G. Wiersma and P.J. Mill, Division of Biology, California Institute of Technology, Pasadena, California, U.S.A.

A further analysis of descending fibers at the commissure level was made, and 41 new entities were added to the previous 31 (Wiersma, 1958), giving 141 altogether at this level. Of the 72 descending entities only 3 are primary fiber bundles, respectively for certain second antennal segment hairs, for antenna flagellum hairs and for hairs on the ridge and anterior carapace. As at other cord levels (Wiersma and Hughes, 1961; Wiersma and Bush, 1963), the majority (82.6%) of the interneurons are modality-specific; 39.1% respond to hairs alone, 34.8% to joints alone, 4.3% to light alone and 4.3% to statocysts alone. Fourteen of the interneurons respond to fields which include the body either alone or with the head. Of these only 6 (42.9%) are modality-specific. These 14 fibers are presumably of a high order and may be low-threshold "command" fibers. They all respond to all parts of their field in the descending lead, but at least 1 may conduct impulses in both directions. Ten respond on all three tagmata, 3 on thorax and abdomen and 1 on thorax alone. Of the 10, 4 respond also to visual stimulation, and 1 is unique in that it is the only established entity in which one tagma (the head) inhibits the excitation caused by the other two. In addition 5 other interneurons are of a high order; 3 of these are pure light fibers, the other 2 responding to head parts and to visual stimulation.

"Visual" interneurons are difficult to establish, since visual sensitivity often disappears before other stimulus modalities. For this reason only 9 out of 16 certainly existing light responding units could be established. Of the 16, 4 are sensitive to the intensity of illumination, two increasing and two decreasing with increased illumination. Ten are sensitive to varying speeds of movement of objects in the visual field. The 2 remaining fibers react respectively to "on" and "on-off."

Of the 19 "high order" interneurons 1 is homolateral, 4 heterolateral and 14 bilateral. Because homolateral interneurons of this type receive their input from homolateral ascending interneurons, the latter being increasingly cut during the experiment, the disproportionality shown may be exaggerated.

Of the 69 descending interneurons 12 respond to more than one modality, whereas only 2 of the 62 ascending interneurons do so. These multimodal units are of a higher order and are probably all low-threshold "command" fibers. The much larger proportion in the descending lead is to be expected if the majority of commands are initiated by integration in the "brain."

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NEUROSECRETORY CONTROL OF RIBONUCLEIC ACID IN CRAYFISH HEPATOPANCREAS. Milton Fingerman, Mitsuharu Miyawaki, Chitaru Oguro, and Tadeusz Dominiczak, Dept. of Zoology, Newcomb College of Tulane University, New Orleans, Louisiana, U.S.A., Dept. of Biology, Kumamoto University, Kumamoto, Japan, Akkeshi Marine Biological Station, Akkeshi, Japan, and Dept. of Biology, Medical Academy, Gdansk, Poland.

Neurosecretory products regulate a wide variety of functions in crustaceans. However, the role of neuroendocrine substances in relation to the functioning of the hepatopancreas of crustaceans has been a relatively neglected subject. Removal of both eyestalks results in degenerative changes in this organ (Yamamoto, Zool. Mag., Tokyo, 69:195-201, 1960). Miyawaki et al. (Kumamoto J. Sci., 5:161-169, 1961) had shown by histochemical means that most of the ribonucleic acid (RNA) of the crayfish hepatopancreas occurs in the cytoplasm of cells rich in iron. The experiments described herein were designed to learn whether or not the degeneration of the hepatopancreas after eyestalk removal involves a change in the RNA content. Two genera of crayfishes were used, *Orconectes ozarkae* from Missouri and *Procambarus blandingi* from Louisiana. Treatment of histological sections from intact specimens with ribonuclease resulted in digestion of the RNA and a negative reaction to pyronin. However, sections of hepatopancreas from crayfish that had been eyestalkless for one week continued to react positively to pyronin after treatment with ribonuclease. This observation suggested that eyestalk removal resulted in a change of the RNA to a substance insensitive to ribonuclease. Biochemical experiments were then performed to verify this supposition. Specimens that had been eyestalkless for one week and intact controls were sacrificed for their hepatopancreases. These organs were then homogenized and an attempt was made to extract RNA from each homogenate by the technique of Reddi (Biochim. Biophys. Acta, 23:208-210, 1957). The ultraviolet spectra of the samples revealed that the hepatopancreases of the crayfishes which had been eyestalkless for one week had no detectable RNA whereas the intact controls had a significant quantity. In still other experiments involving extraction of homogenized organs the observation was made that injections of eyestalk extracts into eyestalkless specimens inhibited the loss of RNA. These findings demonstrate that a neurosecretory substance from the eyestalk is necessary for the maintenance or synthesis of RNA in the hepatopancreas of these two crayfishes.

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BIOLUMINESCENCE AND ELECTRICAL ACTIVITY IN A SINGLE CELL.

Roger Eckert, Department of Zoology, Syracuse University, Syracuse, New York.

Single specimens of the luminescent marine dinoflagellate Noctiluca miliaris were held in sea water to the end of a small horizontal pipette by means of slight hydrostatic pressure, and were positioned over the objective of an inverted-type compound microscope. A selector prism in the base of the microscope was used to divert the image of the cell from the oculars to the photocathode of a multiplier tube. A recording capillary electrode was inserted through the thin peripheral cytoplasmic layer into the large internal vacuole. In one series of experiments, stimulating pulses were passed between the lumen of the suction pipette and the sea water bath, whereas in other experiments stimulating current was passed by means of an internal capillary electrode. Stimulating current, recorded potentials, and emitted light were simultaneously displayed and photographed.

An all-or-none 40-70 mv negative-going spike was recorded in response to sufficient current. It reached its peak in about 7 msec. and subsided in another 4 msec. The emission of light begins at about the peak of the action potential, and is never recorded in its absence. The light flash reaches its maximum intensity in about 20 msec. and decays to 50% in another 20 msec. Repetitive stimuli with 100 msec. intervals cause facilitation of the luminescent flashes, while stimulation at intervals shorter than the duration of each flash brings about summation of flashes as well. Neither phenomenon is accompanied by facilitation or summation of the action potentials. Further evidence will be presented to show that flash intensity is independent of the size of the action potential.

Electrical activity in both luminescent and non-luminescent strains of Noctiluca was compared. Both types show the all-or-none action potential in response to similar stimulus intensities. Present also in the luminescent cells, but not in the nonluminescent variety are slow, spontaneous rhythmic potentials similar in sign and amplitude to the evoked action potentials. These are not all-or-none in nature and are not followed by luminescent flashes. The two kinds of potentials do not add with one another.

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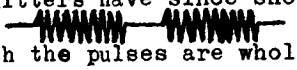
SECOND THOUGHTS ON SOME CLASSICAL MECHANISMS OF SOUND PRODUCTION IN GRASSHOPPERS. W.B.Broughton, Dept. of Zoology, Sir John Cass College, London, England.

The classical view is that the tegmina emit the sound, which is but triggered by the impact of file-tooth on scraper. Thus, in Acridinae, it was assumed that the femur merely evokes tegminal vibration, a view supported by LOHER's (1) tegminal mutilations. Experiments on Chorthippus femora are described.

In Tettigoniidae, the "mirror" was widely credited with true resonance (e.g. PIERCE(2)), but it probably has no special acoustic function, and experiments on Metrioptera roeselii seem to confirm this view.

In both these types of stridulation, and in several others, it has often been assumed that each ultimate individual pulse (simple wave-train) in the oscillogram corresponds to one tooth of the file. This is undoubtedly true of Platycleis spp., Chorthippus parallelus etc., as oscillograms and slowed-up recordings demonstrate; in others it seems far from true, as I first suspected from a chance observation, 5 years ago, of an anomalous oscillogram of Chorthippus biguttulus.

Experiments with minute physical emitters have since shown that an electronic signal of the form  can produce an acoustic signal in which the pulses are wholly replaced by other, ill-defined ones bearing no relation to them, and resembling those produced by sustained mild dissonance. Many grasshopper "pulses" previously regarded as impact-delimited, in fact, resemble these latter; I have suggested elsewhere(3) that they are at least partly harmonic products of reverberation under the tegmina, and, consonant with this, slow playing reveals "sweet", musical vocalization contrasting with the harsh grating of the impact type. Most musical of all are the crickets, unique in showing real resonance (HASKELL, in litt. 1953, PASQUINELLY & BUSNEL(4)). Decticus (Tettigoniidae) is interesting in sounding harsh to us, but only because of quick rise and fall times; it belongs nevertheless to the musical category.

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OLFACTORY RESPONSES IN RABBITS WITH IMPLANTED ELECTRODES.

David G. Moulton, Division of Physiology, Florida State University, Tallahassee, Florida, U.S.A.

Changes in multifiber spike and wave activity following odor stimulation have been recorded from the olfactory bulb and olfactory nerves of unanesthetized rabbits with chronically implanted electrodes[1]. Such responses can be summated on short-time averaging circuits so that activity occurring simultaneously at up to 8 or more points can be quantified and displayed on pen recorders. Thus studies can be made of the spatio-temporal evolution of patterns of activity in the bulb and the relative effectiveness of various odors in stimulating different sites in the olfactory system. The collection and interpretation of such data, however, is likely to be impeded by the existence of fibers originating in rhinencephalic and higher centres and terminating in the bulb, since there is evidence that such fibers may mediate centrifugal influences. The effects on olfactory responses of lesions in the anterior olfactory areas (through which these fibers pass), was therefore investigated. A thermistor bead suspended immediately beneath the nostrils was used to monitor respiration.

In the undisturbed rabbit, habituated to the controlled environment of the test chamber and breathing filtered air, a continuous, high amplitude spike discharge was recorded from bulbar sites. Following the introduction of an odor from an olfactometer into the rabbit's face mask, responses varied depending partly on site and odor. Commonly, there was some increase in activity with each inspiration but no evident change in the level of background discharge. Such responses seldom summated effectively. With some sites and odors, however, and especially following repeated presentations of amyl acetate paired with shock, a marked deactivation of the background activity was seen between each inspiration of an odor. During each inspiration, spikes appeared which were seldom much greater in amplitude than the prestimulus spikes. The result was a decline in the level of summated activity. These effects coincided with increased wave and primary nerve activity and with either an increase or a decline in the amplitude of the respiratory trace.

Following unilateral lesions in the anterior olfactory areas, the decline of activity in response to odor persisted at most relevant sites in the side opposite the lesion. On the same side however, a striking improvement in the signal-noise ratio occurred so that high amplitude summated responses showing little or no initial decline, were recorded. Such sites yielded stimulus -response curves which were indistinguishable from those derived from primary olfactory nerve leads. The consistency of the responses was such that a quantitative comparison of activity evoked by a wide range of odors at different sites in the olfactory became possible. This revealed marked variations in the stimulating efficiency of the compounds at these sites.

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PHOTOSENSITIVITY OF THE FROGS PINEAL BODY.

E. Dodt, W.G. Kerckhoff-Herzforschungsinstitut der Max-Planck-Gesellschaft, Bad Nauheim, Germany.

The sensory function of the frogs pineal body has been studied in adult specimens of R. esculenta and R. temporaria. In a series of experiments performed with Dr.E.Heerd (Physiologisches Institut, Universität Giessen) afferent impulses were recorded from the central end of the cut pineal nerve of the frontal organ (forming the distal part of the pineal body) located beneath the skin between the lateral eyes. Illumination of the frontal organ (Stirnorgan, Stieda's spot) by white light and with stimuli of short wavelengths ($<$ 420 mμ, λ max 355 mμ) caused inhibition, whereas darkness and light of longer wavelengths ($>$ 420 mμ, λ max 515 mμ) produced excitation of pineal nerve fibres. This is the chromatic response of the frontal organ. In addition to the chromatic response in some animals an achromatic response has been found which is inhibitory for light stimuli of all wavelengths between 321 and 727 mμ and is followed by off-discharges. For the achromatic response spectral sensitivity is highest at about 560 to 580 mμ.

In other experiments performed with Dr.M.Jacobson (Physiology Department, University of Edinburgh) photic responses were recorded by means of tungsten microelectrodes from a very localized region of the roof of the diencephalon after removal of the lateral eyes as well as of the frontal organ and / or the pineal nerve. From attempts made to localize the site of the recording electrode it is concluded that the diencephalic responses to light originate from the epiphyseal stalk (proximal part of the pineal body). The photic response of the diencephalon is similar to the achromatic response of the frontal organ, (i) being purely inhibitory to light stimuli between the ultraviolet and the far red and followed by off-discharges, (ii) showing spectral sensitivity curves peaked in the yellow-green region of the spectrum, (iii) exhibiting about equal absolute thresholds (0.0 1 lumen/m^2). After allowance is made for the absorption of light by the overlying tissues (skin, cranium) the threshold of the photic response of the diencephalon is about 100 times that of the frontal organ.

DISTRIBUTION OF CHITIN IN ANIMALS. Charles Jeuniaux, Dept. of Biochemistry, University of Liège, Belgium.

An accurate quantitative method for the detection and estimation of chitin has been devised, using a purified chitinase as highly specific reagent. This method enables the discrimination of the free chitin from the chitin chemically bound to other substances.

A first survey of the distribution of chitin in a number of skeletal and cuticular structures seems to indicate that the ability to synthesize this polysaccharide is a primitive property of the animal cell. Chitin has indeed been found in the shells of Thecamaebaea and Foraminifera, in the walls of the gemmules of Spongillidae, and in the periderm of athecate and thecate Hydroïda. In the other Metazoa, chitin synthesis seems to have been lost in the Deuterostomia. On the contrary, this property has been kept along the evolutionary stem of Protostomia, the absence of chitin being observed in a few phyla only (Platyhelmintes, Nemertina, Sipunculida). In the Protostomia, chitin is a constituent of many different types of anatomical structures, which can be considered as morphological and physiological radiations of chitin biosynthesis.

In addition to the known cases of chitinous structures among Invertebrates, chitin has been clearly identified in the egg shell of Nematoda and Acanthocephala, in the tubes, shells and ectocysts of the Lophophorate phyla, in the hooks of Thalassema (Echiurian), in the different types of chaetae of Annelids, in the peritrophic membrane of Arthropods, as well as in the shells of Mollusks, not only in those of Cephalopoda (including Nautiloïdea) but also in those of different Gastropoda and of the Oyster.

A tendency in the evolution of cuticular and skeletal structures in Protostomia is the increasing quantitative importance of chitin in the organic matrix of these structures. The amount of chitin in lower Invertebrates is indeed rarely more than 20% of the organic matter, but attains 20-37% in the chaetae of Annelids, 20-50% in the cuticle of Insects and 50-85% in that of Crustaceans. In nearly all the groups studied so far, including the most primitive, chitin is principally present in the form of complexes, probably glycoproteic in nature, resistant to the action of pure chitinases. Free chitin generally represents less than 20% of the total chitin. In this respect, Mollusks are opposed to the other groups, their shells containing larger amounts of free chitin (up to 80 p. 100 of total chitin).

THE ORGANIC MATRIX OF THE GASTROPOD SHELL. V.R. Meenakshi, Dept. of Zoology, Andhra University, Waltair, India.

The chemical nature of the organic matrix of the shell and operculum of the apple snail, *Pila globosa* is studied. The histochemical properties of the different layers of the shell are observed and their protein content analysed qualitatively by chemical and chromatographic methods. A comparison has been made between the different layers of the shell on the basis of their chemical and histochemical properties. The mantle and the foot, which secrete the shell and the operculum respectively, are also examined for the nature of the secretions.

The matrix of the periostracum, the so-called chitinous layer, consists mostly of a continuous layer of quinone-tanned protein. Traces of chitin and acidic mucopolysaccharides are also observed in this region.

The organic composition of the inner calcareous layers, the ostracum and the hypostracum differs markedly from the periostracum in their chemical nature. These two layers consist mostly of an acid mucopolysaccharide with traces of protein. This protein is soluble in dilute acids and its tyrosine content is very low. Quinone-tanned proteins are absent in these layers. It is suggested that the acid mucopolysaccharide plays an important role in the calcification of this region of the shell.

The matrix of the operculum consists of a quinone-tanned protein and acid mucopolysaccharide. In this region the tanned protein does not form a continuous layer as in the case of the periostracum.

The histochemical tests performed on the mantle reveal that the secretory cells of the mantle margin supply a phenolic substrate which when oxidised provides a quinone capable of tanning the periostracum. Acid mucopolysaccharide and glycogen are distributed throughout the epithelium. The histochemical reactions of the mantle epithelium are similar to those cells such as osteoblasts, odontoblasts etc., which are involved in calcium metabolism. Hence, in general the mantle epithelium participates in the formation of these calcareous layers of the shell, where as the periostracum is secreted only by the mantle margin.

The modifications shown in the distributions of proteins and acid mucopolysaccharide in the different layers of the shell may be significant in the specialised function of the shell in resisting evaporation during aestivation.

THE DEVELOPMENT OF MUSCLE FUNCTION IN ANTHERAEA POLYPHEMUS (LEP.).

Hans Nüesch, Zoological Institute, University of Basel, Switzerland

During ontogenesis the complex system of tissues develop from undifferentiated cells to the functioning organs. Parallel to the structural differentiation the cells take up their specific functions. One can take for granted that a study of the mutual relation between structure and function during development may tell us much also about function of the adult organ. Muscle is an especially convenient material for this kind of research, as its function (the contraction) may be easily determined quantitatively and may also be elicited by quantitatively graded stimuli.

The flight muscles of Antheraea polyphemus (Lep.) develop within three weeks from small undifferentiated anlagen present in the young pupa. These anlagen consist of a thin sheet of myoblasts which already is innervated and which has the same connections as the muscles of the adult. After a period of mitosis the myoblasts elongate to multinuclear strings. At about eight days after the begin of adult development a few myofibrils appear within these strings. It is at this stage that for the first time the muscles can contract. Only two days later the cross striation appears. Further differentiation concerns multiplication of the myofibrils. In this way the muscle fibre grows in width to the ten- to twentyfold diameter.

The first contractions need a rather strong direct stimulus (15-30 V, 1 millisec. duration at 50 c.p.s.). The very slow shortening only involves the small part of the fibres between the pair of electrodes and is fading away even with the stimulus persisting. Already the following day the muscle also contracts as reaction to stimulation of the ganglion. Later on contraction waves pass slowly over the whole fibre even if stimulated only locally. "Spontaneous" contractions occur four days after the first electrically induced contractions. All different kinds of contraction, as twitch, contraction waves, tetanic contraction of part and at last of the whole muscle need specific stages of differentiation to occur. They all start with a high threshold which during the following days decreases to the value characteristicof the adult, i.e. about one volt.

The significance of these findings will be discussed.

XVI INTERNATIONAL CONGRESS OF ZOOLOGY

CONTRIBUTED PAPERS SESSION

10D. PHYSIOLOGY

RESPONSE OF TRIBOLIUM CONFUSUM TO RADIATIONS AND OTHER STRESSES.

H. S. Ducoff and Gayle C. Bosma, Department of Physiology and Biophysics, University of Illinois, Urbana, Illinois, U.S.A.

A number of workers have examined the acute lethal responses of X-irradiated Tribolium adults; the midlethal dose (MLD) range is 6 - 12 kiloroentgens (kr). Our laboratory's investigations reveal a MLD for larvae of 2.5 - 5 kr; X-irradiation of larvae leads to two other effects, a delay in pupation and, when very late larval stages are irradiated, morphological abnormalities in the resulting adults. First day pupae are about as sensitive as larvae, but by the third day, pupae are as resistant as adults. Eggs are more sensitive than larvae, and the sensitivity of the egg is an inverse function of its age: 2 kr causes 100% lethality in eggs less than 24 hours old. In addition, sublethal irradiation of eggs markedly inhibits the subsequent rate of growth and development.

X-ray effects on eggs and on pupae are mimicked by ultraviolet radiation; larvae, however, appear less sensitive than adults to the stress of high oxygen pressure.

The demonstration will include the dose-response curves of the various stages of the life cycle, examples of the induced developmental abnormalities, and a simple apparatus for exposure to high oxygen pressure.

HISTOLOGIC CHANGES IN THE SPLEEN AND LYMPH NODES OF RABBITS FOLLOWING EXTRACORPOREAL IRRADIATION. Peter Abramoff, Myung Moo Choe, Annamma Cownan, and Geraldine Shea, Dept. of Biology, Marquette University, Milwaukee, Wisconsin.

The effects of X-irradiation of the exteriorized circulating blood of rabbits on their lymphoid tissues and antibody production have been analyzed. In this study extracorporeal irradiation has been accomplished by inserting cannulae into the femoral artery of one leg and the femoral vein of the other and allowing blood to circulate via polyethylene tubing connected to a stainless steel irradiation chamber having a mylar window at the surface of which the irradiation impinges. The irradiation is delivered by a Maximar 100 X-ray unit for one hour, using no filtration.

In this study 278 New Zealand white male rabbits were divided into 6 groups and treated as follows: Grp. I-Normals; Grp. II-injected intravenously with BSA; Grp. III-blood exteriorized for one hour; Grp. IV-extracorporeal irradiation; Grp. V-blood exteriorized for one hour followed by BSA injection; Grp. VI-extracorporeal irradiation followed by BSA injection. Animals were sacrificed in groups of four at various intervals after treatment and spleen and lymph node fragments were removed, sectioned, and stained with methyl green pyronine.

Analysis of the number of pyronine-positive cells shows that the histologic reaction in the rabbit spleen during antibody formation is characterized in this study by a hyperplasia of the red and white pulp regions and an increase in the percentage of pyronine-positive cells in these areas. Following extracorporeal irradiation and antigen injection at a low dosage level there was an over-all decrease in splenic activity as indicated by the depletion of the germinal centers and absence of mitotic activity. The demarcation of lymphoid follicles into the germinal center, mantle layer, and marginal zone was disrupted. However, the fact that the decrease in numbers of pyronine-positive cells was not as great in the experimental group as in the operated-injected group indicates that at low antigen dose levels there is some stimulatory effect of the irradiation. On the other hand, it was found that at higher antigen dosage levels the suppression of cellular proliferation was considerably greater than in the operated-injected control group.

Histologic reaction in the lymph nodes of rabbits following extracorporeal irradiation was similar to that observed in the spleen but the extent of damage was not as severe and was somewhat delayed.

In both studies it was clearly demonstrated from an analysis of the 3 control groups that the operation itself exerts a relatively small effect on the histological picture in the spleen and lymph nodes. (Supported by grant AT(11-1)-820 from the U.S.A.E.C.)

METABOLIC RESPONSES TO 755 CARCINOMA IN C57 MICE.

George H. Fried, Nathaniel Greenberg & William Antopol, Levy Laboratory, Beth Israel Hospital, New York, N.Y.

Alteration of enzyme levels and total oxygen consumption in genetic and experimental obesities and differences in respiratory enzyme levels associated with changes in body size, as reported from our laboratories, indicate a response to homeostatic stress at the level of specific tissue respiratory enzymes. In the present investigation, possible changes in total oxygen consumption and tissue respiratory enzyme levels in C57 mice have been sought in response to the growth of 755 tumors (3-4 weeks) in 16 host animals as compared to 17 controls. Oxygen consumption measurements were carried out manometrically. Lactic and malic dehydrogenases were assayed spectrophotometrically in homogenates of kidney, liver, brain, heart and rectus abdominis muscle. Alpha-glycerophosphate dehydrogenase and alpha-glycerophosphate oxidase determinations were carried out in the same tissues using a monotetrazolium salt, INT (Fried, G.H., W. Antopol, Proc. Soc. Expt. Biol. Med. 107: 523, 1961).

Oxygen consumption levels of the control group averaged 1907 uL/gm/hr, which is similar to the levels for house mice, while the levels in the tumor group averaged 1425 uL/gm/hr, which is significantly lower. Enzymes involved in glycerophosphate oxidation were substantially similar in both groups, thus tending to rule out a qualitative shift of metabolites through the glycerophosphate shunt in the tissues of tumor animals. Glycerophosphate oxidation was absent in the tumor proper. Lactic dehydrogenase levels were significantly higher in livers of tumor animals but showed little variation in the other four tissues. Malic dehydrogenase levels in muscle and heart of tumor animals appeared to be elevated above control levels (not significant).

Thus, while O_2 consumption levels are depressed in the stress to homeostasis of tumor growth, the respiratory enzyme levels appear to reflect little change from the normal, which is in accord with the classical findings of Greenstein and others.

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OBSERVATIONS ON THE HEMATOLOGY AND BLOOD VOLUME OF CAPTIVE WESTERN GULLS. L. Z. McFarland, Dept. of Anatomy, University of California, Davis. -- Western gulls (Larus occidentalis) held in captivity 186-258 days and weighing 857±9 g were studied. Heparinized blood was analysed for venous hematocrit, buffy coat, hemoglobin, erythrocyte count, leukocyte count and differential, refractive index, plasma proteins, and plasma electrolytes. Dried blood smears were stained for glycogen using PAS and for peroxidase activity. Blood volumes were measured by dilution technique after injecting P^{32}labelled RBC from donor gulls. The donor gulls had received 3 intravenous injections of 200 µc $NaP^{32}O_4$ four weeks before their blood was used. P^{32} is incorporated into the developing avian RBC and will not elute. Only average values for the various blood indices will be given below.

The gulls had a hematocrit of 48.7%, a buffy coat of .9%, 3 million RBC/ cu mm, and a hemoglobin content of 17.1 g%. The RBC had a mean corpuscular volume of 160 cu µ, a mean corpuscular hemoglobin of 57.0 µµg, and a mean corpuscular hemoglobin concentration of 35.2. Thus, the RBC of gulls are about twice as large as mammalian RBC, but contain essentially the same percentage of hemoglobin per unit volume. The RBC of the sea gull show a strong peroxidase activity but not the WBC, which is in contrast to mammalian blood where intense peroxidase activity is noted in the neutrophil. Also there is no evidence of either erythrocytes or leukocytes storing glycogen, as does the mammalian neutrophil and eosinophil, since all cellular elements of the gull were negative to the Periodic acid-Schiff reaction.

Direct leukocyte counts indicated 15,300 leukocytes/ cu mm, and the differential count gave a percentage distribution of 37.7% heterophils, 47.7% lymphocytes, 2.3% monocytes, 1.7% eosinophils, 2.7% basophils, and 7.2% others.

The plasma had a refractive index of 1.3421 and a protein concentration of 3.5 grams %. The concentration of plasma electrolytes was 170 meq Na/l, 5.3 meq K/l, and 130 meq Cl/l.

Based on the injection of P^{32} labelled RBC, the average blood volume of these gulls was 71.3 ml, ranging from 69.3 -95.0 ml. This represented 8.34% of their body weight. The intravenous injection of 5 ml of 10% NaCl which stimulated the activity of the nasal salt glands was accompanied by a variable response of 0-80% increase in blood volume. In addition, handling was accompanied by a 0-45% increase in blood volume. In no case was a decrease in blood volume observed.

The relationship between these findings and those reported in mammals and other birds will be discussed.

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EFFECT OF BRADYKININ ON "BLOOD-LUNG BARRIER."

Chryssanthos Chryssanthou & William Antopol, Levy Laboratory, Beth Israel Hospital, New York, N.Y.

Certain compounds introduced into the systemic circulation fail to permeate specific tissues or organs. This selective impermeability has led to the concept of "barriers." We observed that tetrazolium salts, trypan blue, and chlorophyllin do not penetrate lungs of rabbits or mice. Bacterial endotoxin has been shown to alter the "blood-brain barrier." We reported similar effects on rabbit lungs. The present communication deals with bradykinin effects on mouse lung permeability to chlorophyllin.

260 C57 mice of both sexes received 0.5 cc. of 2% copper chlorophyllin intravenously. One group (control) received no additional treatment. Another group received 10 mgm bradykinin intravenously immediately following the chlorophyllin. A third group received 0.3 mg cysteine subcutaneously followed by chlorophyllin and bradykinin. (Cysteine was administered to retard bradykinin destruction). A fourth group was treated with cysteine followed by chlorophyllin.

The mice died or were sacrificed at intervals to 24 hours. Gross examination of the lungs within 1 hour after the injections revealed mostly weak staining in 36% of the controls and generally intense staining in 100% of the bradykinin-treated. The percentage of stained lungs in the controls decreased with time. A parallel drop was noted in the bradykinin-treated animals but the percentage of stained lungs always remained at least double that of corresponding controls. In the group treated with cysteine, bradykinin and chlorophyllin, the percentage of stained lungs dropped slightly within 6 hours and then remained constant.

It is postulated that the bradykinin effect is due to vasodilation and increased vascular permeability. Vasodilation is only temporary. This accounts for the initial high percentage of lung staining and its subsequent drop; increased permeability, on the other hand, could be responsible for the sustained color due to persistence of staining once it involves the lung tissue proper. The lung histology was consistent with this hypothesis. Endotoxin modification of the "blood-brain" or "blood-lung barrier" may be mediated, at least in part, by bradykinin.

Aided by Grant H-2670 U.S.P.H.S. and the Saul Singer Foundation.

THE POSSIBLE ROLE OF BRADYKININ IN DECOMPRESSION ILLNESS.

William Antopol, John Kalberer, Chrys Chryssanthou and Samuel Kooperstein, Levy Laboratory, Beth Israel Hospital and the Medical Department of the Port of New York Authority, New York, N.Y.

Since bradykinin is a bronchoconstrictor and causes bone marrow changes in mice similar to those observed in animals after decompression from high ambient pressure, it was postulated that this polypeptide may play a role in the mechanism of decompression illness (bends).

A total of 38 hereditary obese mice (approx. 45 gm) in two groups were simultaneously exposed to high ambient pressure (6 atmospheres absolute), followed by decompression to sea level within one minute. Immediately after decompression the control group received normal saline subcutaneously and the other group, 5 mgm bradykinin. Within 16 minutes after decompression, 37% of the bradykinin-treated mice died, while only 5% of the controls. The results of these experiments indicate that bradykinin significantly ($P = 0.05$) shortens survival time after decompression; there was no significant effect, however, on the overall mortality (74% died in the bradykinin-treated group and 53% in the controls). These experiments were extended to thin mice which present a very low mortality after decompression. The data obtained indicate that these mice become susceptible to decompression effects when treated with bradykinin. From 32 thin mice exposed to 6 atmospheres absolute pressure for a period of 48 to 72 hours followed by decompression within 1 minute, 12% died in the control group while 56% died in the bradykinin-treated group.

In another series of experiments aminopyrine (a bradykinin antagonist) and 2-(4-phenyl-1-piperazyl methyl)-cyclohexanone HCl (an anti-inflammatory compound) significantly increased survival time and lowered mortality of obese mice exposed to pressure-decompression. The protective effect on survival time of the latter compound was striking; in the experimental group 15 out of 24 mice survived (63%), while 3 out of 24 (12%) in the controls ($P < 0.01$).

Histologic examination of bradykinin-treated mice revealed prominently dilated perivascular spaces similar to those seen in animals exposed to pressure after tracheal obstruction. Bradykinin enhancement of bends could be attributed in part to the bronchoconstrictor effect of this polypeptide. Circulatory changes produced by bradykinin should also be evaluated. The role of endogenous bradykinin in the pathogenesis of bends must be considered. Our findings that bradykinin antagonists prolong survival time and decrease mortality rate in decompression illness are consistent with this hypothesis.

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NITROGEN EXCRETION IN REPTILES. Fouad Khalil and Gaber Haggag, Department of Zoology, Faculty of Science, University of Cairo, Guiza, Egypt (U.A.R.)

The earlier literature about nitrogen excretion in reptiles is shortly enumerated. The non-protein nitrogenous excretory products including total nitrogen, ammonia, urea, uric acid, allantoin, amino acids, hippuric acid, creatine and creatinine are given for Chelone mydas, Testudo leithii, T. sulcata (Chelonia); Scincus officinalis, Chalcides ocellatus, Uromastyx aegyptia (Lacertilia); Zamenis diadema, Psammophis sibilans, Eryx thebaicus (Ophidia) and Crocodylus niloticus (Crocodilia). According to the data obtained, tortoises should be considered as ureo-uricotelic, sea turtles and crocodiles as ammono-uricotelic while lizards and snakes are typically uricotelic. Needhams view concerning nitrogen excretion in animals is discussed on the light of these findings. Excretory products of these reptiles are shed out either as solid concretions or as urine containing an insoluble deposit. A suggested mechanism for the formation of the urinary concretions or deposits is discussed. Soluble urates, dialysing through the Malpighian corpuscles, are supposed to lose their bases in the cells of the uriniferous tubules leading to the precipitation of uric acid. According to this mechanism minimal amounts of water are needed for the excretion of uric acid. Distribution of enzymes concerned with the final formation of the main nitrogenous products in the liver (L) and kidney (K) of representatives of the different orders of reptiles is given in the following table and their bearing on the nitrogen excretion of these animals is discussed.

Species	Xanthine oxidase		Guanase		Adenase		Arginase	
	L	K	L	K	L	K	L	K
Testudo leithii	+	-	+	-	-	-	+	-
Uromastyx aegyptia	-	+	+	+	-	-	-	traces
Chalcides ocellatus	-	+	+	+	-	-	-	traces
Psammophis sibilans	+	+	+	traces	-	-	-	traces
Zamenis diadema	+	+	+	+	-	-	-	traces
Crocodylus niloticus	-	+	-	+	-	-	-	+

THE FORMATION OF PRESUMPTIVE URINE BY THE CRAYFISH ANTENNAL GLAND. Leonard B. Kirschner and Stanley Wagner, Department of Zoology, Washington State University, Pullman, Washington.

Separation of the coelomosac from the rest of the antennal gland enabled us to examine it for the presence of compounds injected into the blood. Both inulin and glucose appear in the isolated coelomosac. The ratio of inulin to glucose is about the same as in blood but the quantities of both are larger than can be accounted for by the blood sinuses alone. This suggests that they appear in another compartment, possibly the tubular lumina. Identity of coelomosac and blood ratios is compatible with the formation of a presumptive urine at this locus.

Small dextran molecules (M. W. 15,000) and I-125 labelled albumin (M. W. 60,000) are excreted in the urine, and urine to blood ratios are about the same as for inulin (2-3). However, larger dextran molecules (M. W. 60,000-90,000) have a urine to blood ratio of about one. The data may indicate that porosity limits the entry of molecules larger than albumin into the presumptive urine.

SUR L'URICOLYSE CHEZ LES INSECTES. Pierre Razet, Section de Zoobiologie, Faculté des Sciences, Rennes, France.

Depuis très longtemps, tous les auteurs affirment, comme un fait indubitable, fermement établi, que l'acide urique doit être considéré comme le catabolite terminal de tout le métabolisme azoté des Insectes.

Une telle affirmation est cependant trop absolue, et par suite non conforme à la réalité. Après avoir recherché sur de nombreux Insectes de tous Ordres (426 espèces), aux divers stades de la vie, les enzymes de la chaîne de l'uricolyse (uricase, allantoïnase, allantoïcase, uréase), après avoir analysé leurs tissus et leurs excreta, l'auteur peut affirmer que l'acide urique n'est le catabolite azoté terminal que chez une partie, importante certes, des Insectes (Thysanoures, Paléoptères, Polynéoptères, Homoptères, Coléoptères (Haplogastra et certains Heterogastra), Diptères pro parte, Lépidoptères pro parte (en particulier Tineoidea, Geometroidea, Sphingoidea). Chez de nombreux autres Insectes par contre, cet acide urique n'est qu'un catabolite intermédiaire, étant lui-même dégradé en composés plus simples grâce à des actions enzymatiques.

La dégradation enzymatique de l'acide urique, l'uricolyse, s'arrête à l'allantoïne, chez les Insectes pourvus seulement de l'enzyme uricase : c'est le cas d'Hétéroptères et de Diptères Brachycères à larves créophages; l'allantoïne est éliminée et peut être caractérisée dans les excreta.

L'uricolyse va jusqu'au stade acide allantoïque chez de nombreux Holométaboles qui possédent simultanément uricase et allantoïnase : Coléoptères Heterogastra (Elatérides et Malacodermes) et Haplogastra (pro parte), Mégaloptères, Plannipennes, Hyménoptères (Symphytes et Pétiolés du groupe des Cynipides), Mécoptères, Trichoptères imagos, Diptères Nématocères Tipulides, Lépidoptères des groupes Bombycoïdea, Papilionoïdea et Noctuoïdea aux 3 stades (larvaire, nymphal et imaginal) : ces Insectes excrétent de l'acide allantoïque.

La dégradation de l'acide urique peut même se prolonger jusqu'à l'acide glyoxylique et l'urée chez les quelques espèces possédant simultanément uricase, allantoïnase et allantoïcase (Collembole Xenylla velchii et Orthoptère Acheta domestica). Toutefois, l'acide glyoxylique n'a pu être caractérisé, car il est détruit par l'action d'une nouvelle enzyme.

Chez aucun des Insectes étudiés jusqu'ici, l'uricolyse ne va jusqu'à l'ammoniaque, car les quelques espèces pourvues d'uréase ne possédent pas d'allantoïcase. Par suite, l'ammoniaque, dans les cas où son excrétion a été observée (larves créophages de Diptères Brachycères, larves aquatiques diverses), ne peut provenir de l'acide urique par la voie classique des enzymes uricolytiques, mais semble d'origine protidique.

L'uricolyse est rarement totale ; le plus souvent, une partie plus ou moins importante de l'acide urique y échappe, et est éliminée sans subir de dégradation.

La répartition des enzymes uricolytiques est généralement très irrégulière dans un Ordre ou une famille donnés : certaines espèces en sont pourvues, tandis que d'autres, parfois très proches sur le plan systématique, peuvent ne pas en posséder.

A PARTIAL PATHWAY FOR THE SYNTHESIS OF URIC ACID IN THE AMERICAN COCKROACH. Donald G. Cochran and Charles F. Bruno, Department of Entomology and Department of Biochemistry and Nutrition, Virginia Polytechnic Institute, Blacksburg, Virginia, U. S. A.

From previous work it is known that the tissues of the American cockroach possessthe enzymic capability of degrading purine nucleoside monophosphates to the uric acid level. The steps in this enzymic process have been indicated, but detailed studies of the various reactions have not been made. It is the purpose of the present report to describe the results of experiments which more clearly define this pathway.

Throughout most of this work thoracic muscles from adult male cockroaches served as enzyme source. The soluble fraction and the mitochondrial fraction yeilded most of the activities which were examined. Usually, the enzymes were partially purified before characterization studies were undertaken.

5'-nucleotidase activity was found in both fractions. Both purine and pyrimidine nucleoside monophosphates were degraded. In unpurified systems several reaction products, including nucleo sides and free bases, were found; but following purification only the corresponding nucleosides were present. This finding lends support to a coupled degradative pathway. Ion requirements, pH optima and several other characteristics of these enzymes have been acertained.

Adenosine deaminases have been identified and partially purified from soluble and mitochondrial preparations. The enzymes appear to be quite specific for adenosine and deoxyadenosine. The soluble enzyme deaminates cytidine to some extent. The two activities also differ in pH optima, but show similar Michaelis-Menten constants (with adenosine) and ion requirements. Adenosine monophosphate was not deaminated by these enzymes, a finding which agrees with previous reports on the absence of adenylic acid deaminase activity in these muscles.

A purine nucleosidase has been observed in the intact mitochondria. This enzyme converts inosine to hypoxanthine at a low rate. The characteristics of this enzyme have been difficult to illucidate, but it appears to be a phosphorylase. Purification experiments have been unsuccessful to this point.

From these results it is now possible to describe a partial pathway for the synthesis of uric acid in the cockroach. The significance of these reactions is discussed in terms of the *de novo* formation of uric acid in insects.

THE CONTROL OF FEMALE FECUNDITY BY IMMUNIZATION AGAINST SPERMATOZOA. Anne McLaren, Agricultural Research Council Unit of Animal Genetics, Institute of Animal Genetics, Edinburgh, U.K.

The fecundity of female mice, as measured by number of young born, is decreased by the repeated injection of spermatozoa from males of the same or a different strain. The extent of the reduction is related to the level of sperm agglutinins induced. A high titre of agglutinins reduces fecundity to zero. The physiological basis of the effect is discussed.

POSTNATAL GROWTH FOLLOWING ASYNCHRONOUS DEVELOPMENT OF THE EGG AND ENDOMETRIUM. Allen H. Gates, Department of Obstetrics and Gynecology, Stanford University School of Medicine, Palo Alto, California, U.S.A.

Mammalian eggs are known to be variable in their rate of Cleavage. In the mouse, for example, eggs recovered at $3\frac{1}{2}$ days post coitus were found to have from 8 to 63 cells (in the inbred strain BALB/c). Such variation may lead to lack of synchrony between development of the egg and of the uterine endometrium, a condition which influences the growth of the resultant fetus (Noyes, et al., Fertil. & Steril. 12:405, 1961).

In the present study, birth weight and postnatal growth in the mouse were investigated following the transplantation of "overdeveloped" eggs ($3\frac{1}{2}$ days) and "synchronously" developing eggs ($2\frac{1}{2}$ days) to the uteri of F_1 hybrid hosts (BALB/c x 129) mated $2\frac{1}{2}$ days previously to vasectomized males. The $3\frac{1}{2}$- and $2\frac{1}{2}$-day p.c. eggs (BALB/c ♀ x 129 ♂) bore genetic markers (presence or absence of eye pigmentation) which were reversed in approximately one half of the hosts. The respective genotypes differed essentially only at one chromosomal locus. The hosts were isolated when pregnant (85 in all) and allowed to litter on $\frac{1}{2}$" wire mesh screens to prevent nursing. Each of the 373 offspring was classified as to sex and genetic tag and was then weighed.

When both developmental stages of eggs were present in the same host, birth weight in offspring from the advanced eggs (mean 1.49 gms.) was significantly greater than in offspring from the "synchronously" developing eggs. This was true whether both were transplanted to the same uterine horn (difference, independent of litter size, sex and genetic tag, = 0.10 ± 0.026 gms., DF = 137, $P<0.01$) or to opposite uterine horns (difference = 0.12 ± 0.028 gms., DF = 90, $P < 0.01$). In litters containing only offspring from $3\frac{1}{2}$-day eggs, birth weight was not altered; however, the length of gestation (mean, 20.45 days) was significantly shorter than that in hosts receiving only $2\frac{1}{2}$-day eggs (difference, independent of litter size = $0.70 \pm .22$ days, DF = 47, $P< 0.01$).

At the sexually mature age of 80 days, offspring from $3\frac{1}{2}$-day eggs (mean ca. 28 gms.) still weighed more than those from $2\frac{1}{2}$-day eggs which were allowed to compete for development in the same uterine horn (difference, independent of litter size, sex and genetic tag = 1.67 ± 0.51 gms., DF = 63, $P < 0.01$). Even when corrected for initial weight at birth, the difference was significant at approximately the 0.05 level of probability.

These findings suggest that in the presence of developmentally advanced eggs, normally developing eggs give rise to offspring which are delivered prematurely and which retain their developmental disadvantage at least until early adulthood.

VENOMOUS ANIMALS AND ZOOTOXICOSES. Afrânio do Amaral, Instituto Butantan, S. Paulo, S. P., Brazil.

Animals causing toxicoses are either veneniferous (snakes, scorpions, spiders, bees, etc.) or poisonous (fish, toads, etc.), snakes outstanding. Ophiotoxicoses may be typically cytotoxic, neurotoxic or mixt. Venomation due to solenotodonts (Viperidae, Crotalidae, the neotropical Cascavel except) is revealed by successive local symptoms, a real enzymic chain-reaction, the starter's role being played by proteinases followed by hyaluronic acid, coagulases, phospholipidases, esterase and other enzymes, or toxins; the split products from the action of these principles on the tissue elements are mostly cholinergic as against such products in animal poisoning which are adrenergic. The Cascavel venenation feature results from crotoxin (crotamin, and/or cleavage byproducts), fibrinogenolysin and hyaluronidase, its late hypo-nephron syndrome being caused by protease and A-lecithinase. Toxicoses by proteroglyphodonts (Elapidae), being due to neurotoxins and hemolysins, consist of serious systemic symptoms, the venom hyaluronidase acting as preventive of local swelling.

In the light of the newer knowledge of venoms, rational medication includes: a) uni- and multi-specific antivenins to counteract unmodified (if any) toxic principles introduced by the bite, their dosage being inversely proportional to the victim's weight and their injection, preferably intravenous in late or grave cases; b) auxiliary, anti-cholinergic, remedies such as analgesics and neuroplegics (e.g., novalgin or phenothiazine compounds; never opium or morphine), complemented by liberal doses of glucose-saline solution, through phleboclysis, with ephedrine or even epinephrine. Against local, hard oedema normally evolving to necrosis: part of antivenin dose, mixed with hyaluronidase (up to 5 mg), may be given in situ, hypodermically, followed by warm, wet dressing of brine or saturated magnesium sulfate solution. Against expected progressive myolysis: metal-chelating agents may be added to specific antivenins so as to inactivate any proteinase involved.

Even specific sera, if given late against zootoxicoses, are likely not to neutralize some toxic principles, apparently non-antigenic, ensuing the venom-tissue interactions, such newly-formed principles probably being different to those appearing in the circulation of the antivenin-producing animals.

MORTALITY OF MICE OF VARYING AGE FOLLOWING INJECTION OF URETHANE. Laurence R. Fitzgerald and Robert Waller*, Department of Anatomy, University of Tennessee Medical Units, Memphis 3, Tennessee.

Mice of both sexes, <1, 3, 5, 7, 30 and approximately 360 days of age, were given injections of urethane (ethyl carbamate) subcutaneously in the interscapular region, and mortality in each age and dose group determined 1, 5 and 24 hours after the injection. Twenty mice were used at each age and dose level. No mice were killed by doses of 1 mg/kg or less, and all mice were killed by doses of 10 mg/kg or more.

When mortality was measured 1 hour after the injection, mice 5 days or older showed an LD_{50} of 5-6 mg/kg. Those mice less than 5 days old had an LD_{50} of 6-7 mg/kg. When mortality was measured 24 hours after injection, the LD_{50} for mice 3 days or older was 2-3 mg/kg while that for mice <1 day old was 4-5 mg/kg.

The mice <1 day old showed no true anesthesia as a result of injection of urethane, performing voluntary movements and responding to stimuli, in marked contrast to the older mice which were in deep anesthesia.

Although it has been shown by others that mice are far more susceptible to the carcinogenic action of urethane, both in types and numbers of tumors, when exposure is begun in infancy (7-8 days), this difference is not reflected in the present studies on toxicity of urethane at various ages.

The marked resistance of very young mice to the toxic action of urethane simulates their resistance to anoxia but must be distinct since resistance to urethane is lost far more rapidly than resistance to anoxia.

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MODELS OF ANAPHYLACTICALLY-INDUCED INFLAMMATION FOR EVALUATION OF DRUGS. Paul S. Prickett, Harry L. Murray, Howard R. Williams, Jr., and Paul M. Lish, with the technical assistance of Russel L. Stratman, Mead Johnson Research Center, Mead Johnson & Company, Evansville 21, Indiana, U.S.A.

Increasing recognition of the significant role of allergy in a variety of seemingly unrelated clinical syndromes, but all involving inflammation, has increased the search for drugs able to influence the immune response or modify the sequelae of the antigen-antibody reaction. For this purpose we have adapted or developed several models of anaphylactically-induced inflammation in this laboratory and have determined their usefulness by testing them with drugs of known biological activities, such as aspirin, phenylbutazone, aminopyrine, and the reportedly anti-allergic agent from egg yolk, N-(2-hydroxyethyl)palitamide.

Four basic models, of which one is *in vitro* and three are *in vivo*, are employed and will be discussed. In each model the guinea pig is the test animal. Drugs are screened initially by the rapid *in vitro* method, which enables the differentiation between antihistaminic and anti-anaphylactic activities. This procedure determines the ability of a drug to inhibit the contractions induced either by histamine or by anaphylaxis in a passively sensitized ileal strip. The second procedure detects alteration of immune response development in active sensitization. Another measures the effects of drugs on the asthma-like reaction following challenge with aerosolized antigen 24 hours after passive sensitization. The fourth test evaluates effects of drugs on a localized anaphylactically-induced inflammation in the ankle joint.

In each of these models, pharmacologic action other than true anti-anaphylactic action can provide prophylaxis or amelioration of the induced pathology. Thus, ancillary tests are needed to differentiate true anti-anaphylactic action from some other unsought action, e.g., antihistaminic. Ancillary tests are necessary only for those drugs showing apparent anti-anaphylaxis in the basic tests. Details of the types of action of the drugs mentioned will be described and compared with previously published information. We will attempt to define the usefulness of this group of tests for the screening and evaluation of compounds that qualify for consideration of utility in inflammatory diseases with an allergy component.

XVI INTERNATIONAL CONGRESS OF ZOOLOGY

CONTRIBUTED PAPERS SESSION

10E. FUNCTIONAL ANATOMY AND PHYSIOLOGY OF MOLLUSCS

Organized for the Division of Comparative Physiology
of the American Society of Zoologists

by

Michael J. Greenberg

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BILATERAL ASYMMETRY IN THE BIVALVIA. C. M. Yonge, Dept. of Zoology, University of Glasgow, Glasgow, Scotland.

Bilaterally asymmetrical bivalves include: (1) those always attached by the same valve, e.g., right in Pectinacea, Anomiacea; (2) those in which attachment differs in different genera, e.g., left in Chama, right in Pseudochama (Chamacea); and (3) those individuals which attach by either valve, e.g., Etheria, Acostaea (Etheriidae: Unionacea).

In Pectinacea and Anomiacea, asymmetry is conditioned by byssal attachment with a notch on the right valve. The notch is very extensive in Anomiacea where byssal attachment resembles cementation. This may lead to cementation (Plicatula: Spondylus: Hinnites) or to freedom (Pecten, Chlamys, Ammussium), the horizontal disposition aiding in swimming. In the Anomiacea, Placuna loses the byssal apparatus and notch and lies free but immobile; Enigmonia retains these, enlarges the foot, and assumes the limpet form and habit.

Asymmetry in Chamacea is associated with a tangential component in shell growth. Here free and attached valves resemble one another, not right and left.

In Etheriidae attachment leads to profound asymmetry; in Acostaea to a twisting from vertical to horizontal disposition during early growth.

EMBRYONIC DEVELOPMENT AND METAMORPHOSIS IN THE BIVALVED GASTROPOD.

Siro Kawaguti and Terufumi Yamasu, Dept. of Biology and Tamano Marine Laboratory, Okayama University, Okayama, Japan.

Tamanovalva limax (1) has a green bivalved shell. Dead shells and fossils of related animals had been classified as Pelecypods for many years (2). Actually, the valves are those of a perfect bivalve with the outlines of the two shells fitting exactly and with a hinge and scars of the adductor; only the larval helical shell of about 250u in breadth at the top of the left valve distinguishes it as a special type. However, the animal shows many definite characteristics of the gastropods in its living part, and it has been described as a Sacchoglossan gastropod.

The animal reaches maturity when it has attained the stage of 3mm body length (3). From that time on, it spawns frequently, once every three or four days at 20-25°C water temperature. It usually finds a mate, but as it is hermaphroditic, it can propagate by self-fertilization when kept in isolation (4). The egg is comparatively large and its embryonic development continues until the snail-form is completed, which takes about 20days. It hatches out as a typical gastropod and soon changes into a bivalved form (5).

When it hatches, it still has some velar cilia and can swim, which is advantageous for its wide distribution through the sea. Usually, however, it creeps on the alga, Caulerpa okamurai, as a snail-form without a free-living stage. Two or three days after hatching the helical shell begins vigorously to put forth marginal portions on both sides, making two lateral extensions. The hinge line begins to appear along the proximal portion of the right extension. Thus the bivalved shell is formed as an extension of the helicoid shell,but the latter also remains functional throughout the whole life.

There are many problems concerning how such a hybrid shell half-way between a helicoid and a bivalved form is organized. The process of metamorphosis will be described in connection with the development of the adductor muscle. The adductor appears near the head retractor starting at the proximal portion of the helicoid shell which is situated at the left side of the animal body. The adductor attaches to the right extension of the shell margin as it begins to form. With the progress of the shell formation, the right attachment of the adductor descends gradually with the right extension, while the left attachment descends to the left extension of the bivalve shell, until the adductor muscle reaches a position at right angles to the valves (6). The tract of the alimentary canal undergoes some modifications during the development.

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PHYLOGENY OF WATER CURRENTS IN PROTOSTOMIAN INVERTEBRATES.

Henning Lemche, Zoological Museum, Copenhagen, Denmark.

In the belief that all functional changes in animals must be gradual, it is attempted to derive the more primitive water currents in protostomians from a single type such as exemplified in anthozoan coelenterates. In polychete embryos, it is seen how the two sides - the somatic plates - meet to form the ventral side of the animal. In an adult, such a fusion would separate a possible inner food-acquiring current through the simple alimentary canal - from an outer one passing along the ventral side as a locomotory or/and respiratory one. Such an inner current would enter between proto- and metatroch where a pair of grooves lateral to the mouth has formed between the rows of cilia, carrying the food towards the mouth. With increasing complexity of the alimentary canal the feeding current cannot directly enter the mouth but, having been searched for food, becomes deflected so as to join the superficial outer current. But the feeding grooves may persist, as is seen in veligers and especially in some adults (Nuculids, Tryblidians,etc.) which molluscs do not pass any veliger stage as larvae but should rather be regarded "adult" veligers. Cephalopods, with their direct development from a kind of coelenterate embryonic stage, will not show these features because of their extensive yolk sac protruding ventrally from the mouth.

Silurian nautiloids such as Gomphoceras with restricted T-shaped shell apertures may have had an organisation intermediate between advanced coelenterates and primitive molluscs, the transverse part of the slit being for water intake and the posterior end of the stem of the T being the place for expulsion of water (and faeces). Apparently, therefore, these water currents are of very high age, as is also indicated by the widespread occurrence in protostomian invertebrates of larval types similar to the veligers of molluscs.

ON THE PHYSIOLOGY OF POSTURAL BEHAVIOUR IN MOLLUSCS. Maurizio Mirolli, The Biological Laboratories, Harvard University, Cambridge, Massachusetts, U. S. A.

In a study of the effects of reserpine, 3 species of Amphineura 18 of Gastropoda, and 1 of Bivalvia have been considered. In all, the administration of the drug deeply affects the postural behaviour. The symptomatology which is induced differs in its details according to the species; however, these differences are related to the particular anatomical organization of the various groups and only represent minor variations within a fundamentally similar pattern of effects. The muscle sheets of the peripheral lacunae are tonically contracted and thus the haemostatic pressure is reduced in the head, foot, and mantle. The tonus of the muscles of the shell is completely abolished. The drug has therefore an opposite effect on the two major components of the mechanical system which regulates the posture of these animals.

At the cellular level reserpine lowers the amount of 5-hydroxytryptamine (5-HT) in the central ganglia. The time course of this effect and of the effect on the posture is the same. The temperature at which the experiment is made influences both effects in the same way.

These results suggest that 5-HT, which is present in high quantity in the central ganglia, plays a physiological role in the control of the posture of molluscs. The following drug interactions were tested: reserpine X 5-HT; reserpine X iproniazid (Marsilid); reserpine X syrosingopine (Syngoserp); and syrosingopine X iproniazid. The results of these experiments support the hypothesized role of 5-HT.

CELLULAR LOCALIZATION OF MONOAMINES IN MOLLUSC NERVOUS SYSTEMS. Erik Dahl, Dept. of Zoology, University of Lund, Lund, Sweden.

The cellular localization of adrenaline, noradrenaline, dopamine, and 5-hydroxytryptamine can now be studied microscopically by means of a highly specific fluorescence method developed by B.Falck. After freeze-drying and treatment with formaldehyde gas the three catecholamines show a green fluorescence and 5-HT a yellow fluorescence.

Ganglia and other tissues of Anodonta piscinalis, Helix pomatia, and Buccinum undatum were studied. Great numbers of yellow and green cells and fibres were found in the cerebral ganglia of A.piscinalis while the visceral ganglia contain both kinds of fibres but only green cells. In the ganglion ring of H.pomatia yellow cells and various types of green cells occur. In both species non-fluorescent cells are more numerous than fluorescent ones.

By spectrophotofluorimetric methods the fluorescent amines of both species were identified as 5-HT and dopamine. A comparison with adrenergic mechanisms of vertebrates makes it highly probable that they act as neurotransmitters.

In the foot of A.piscinalis and the tentacles of B.undatum green subepithelial nerve cells occur which send a fibre towards the epithelium. They appear to be sensory cells. In the tentacles of sea-anemones an apparently sensory-motoric adrenergic system occurs. Neurons of a similar type were also observed in leeches and turbellarians. Thus adrenergic neurons which may have a double sensory and motoric function appear to be wide-spread among invertebrates, including Molluscs.

The present investigation was carried out in collaboration with B.Falck, C.von Mecklenburg, and H.Myhrberg.

SOME ASPECTS OF THE CARDIOREGULATION SYSTEM IN MERCENARIA (VENUS) MERCENARIA. Robert E. Loveland, The Biological Laboratories, Cambridge, Massachusetts.

The heart of Mercenaria mercenaria is symmetrically innervated by two lateral nerves, one entering the auricle on either side. These nerves can be traced ventrally to the left and right cerebro-visceral connectives respectively and presumably have their origin in the visceral ganglion. After passing through the inside of the auricle, branches of the nerve end abruptly on the surface of the auriculo-ventricular valve. By light and fluorescence microscopy it can be seen that the nerve fibers are most concentrated in this region and that the major portion of innervation occurs at the auriculo-ventricular site. Few nerve fibers can be seen in the ventricle.

Two distinct types of neurons are seen in the cardio-regulator nerves by electron microscopy. The first contains agranular vesicles and, in general appearance, resembles a typical unmyelinated motor neuron. The second type is filled with granular vesicles and appears to be a neurosecretory cell. Glial cells are present in association with both types of neurons.

The heart muscle cells are probably syncytial and are imbedded in a rather homogeneous matrix. Two features characterize these cells: (1) the localization of the mito-chondria and (2) cytoplasmic bridges joining the muscle fibers.

The nature of the nerve endings and the relationship of cardioregulator fibers to the heart muscle cells have not been determined. However, it is speculated that innervation occurs at a very localized site and that a wave of excitability spreads out over the heart via the syncytium of ventricular muscle cells.

PHARMACOLOGY OF THE HEART OF STROPHOCHEILUS OBLONGUS. Celso Paulo Jaeger, Instituto de Fisiologia Experimental, Universidade do Rio Grande do Sul, Pôrto Alegre, Brasil.

The effects of neurohumors, drugs, and tissue extracts were studied on the isolated heart of the snail, Strophocheilus oblongus. The heart is excited by acetylcholine, 5-hydroxytryptamine (5-HT), and by tissue extracts made from the nerve ring and the heart of the same snail. An atropinized heart treated with acetylcholine ceases beating. Treatment with 2-bromo-d-lysergic acid diethylamide (Br-LSD) blocks the 5-HT excitor action. On the other hand, the actions of the tissue extracts are antagonized by neither atropine nor Br-LSD.

In situ electrical stimulation of the cardiac nerve produces a cardio-accelerator effect similar to that produced by the action of tissue extracts on isolated hearts. Histological investigation showed that neurohumoral-like material is present both in the nerve ring and in heart tissues. Granules and vesicles of this material appear in greater quantities in the auricle than in the ventricle and seem to be associated with nerve endings. Histological examination of hearts previously submitted to repetitive electrical stimulation revealed that a great part of the stored, neurohormone-like, stainable substance had been released.

The penis retractor muscle of Strophocheilus can be induced to "beat" by the addition of several amines. This effect was used as the basis for an assay of tissue extracts. Auricle tissue extract induces rhythmicity in the penis retractor one hundred times more effectively than does ventricle extract.

The evidence suggests that the Strophocheilus heart is regulated through the release of a neurohormone different from either 5-HT or acetylcholine.

EXCITATION AND INHIBITION OF THE HEART OF APLYSIA. Robert B. Hill, Dept. of Physiology, Dartmouth Medical School, Hanover, New Hampshire.

The heart of specimens of the worldwide species Aplysia dactylomela has been reported not to beat spontaneously when separated from ganglia (Acta Physiol. Latinoam., 2:101). Nevertheless, if the bath is well aerated isolated ventricles will beat vigorously. Ventricles quiescent as a result of lack of aeration may be tetanized by electrical stimulation with repetitive square pulses or be caused to contract by passing direct current through the ventricular wall with either inside or outside made positive. However, a current positive with regard to the inside of the ventricle is more effective and will cause further contraction of a ventricle already contracted by another agent, such as acetylcholine, whereas a current negative with regard to the inside will relax a ventricle already contracted by another agent.

The observation that Aplysia dactylomela ventricles are not accelerated by adrenalin or noradrenalin (ibid) appears to indicate a pharmacological difference from the Mediterranean-centered species Aplysia fasciata (Arch. int. Pharmacodyn. Ther., 28:337) which is the more unexpected since a number of authors have found an inhibitory action of low concentrations of acetylcholine on ventricles of both species. I have confirmed that low concentrations of acetylcholine, from 10^{-12} to 10^{-9} molar, inhibit the ventricle of Aplysia fasciata. Also confirmed is the action of acetylcholine on the ventricle of Aplysia dactylomela. Concentrations of 10^{-7} M and lower will inhibit beating and relax the ventricle. Concentrations of 10^{-5} M and higher can initiate beating and increase tonus. I find that 5×10^{-6} M acetylcholine will stop a beating ventricle in diastole but can initiate beating in a quiescent ventricle. The beating ventricles of Aplysia fasciata and of Aplysia dactylomela respond similarly to adrenalin, noradrenalin, tryptamine, and 5-hydroxytryptamine. At concentrations of 5×10^{-5} M for adrenalin and noradrenalin or of 10^{-8} M for tryptamine and 5-hydroxytryptamine, the immediate effect is positively chronotropic, inotropic, and tonotropic. An inhibitory action follows, which becomes more marked as concentrations are increased. Thus the discrepancy between the pharmacology of the isolated ventricle of Aplysia fasciata and that of the ventricle of Aplysia dactylomela is not confirmed.

THE EFFECT OF CHANGES IN RATE ON THE AMPLITUDE OF BEAT OF BIVALVE HEARTS. Michael J. Greenberg, Dept. of Zoology, University of Illinois, Urbana, Illinois, U. S. A.

Quiescent, isolated ventricles of Mercenaria mercenaria can be induced to beat by electrical stimulation; the changes in amplitude of an isotonic preparation induced by alterations in the rate of stimulation can then be determined. The height of contraction decreases as the interval between beats is decreased from 20 seconds to 0.1 second at 25° C. Tone also rises markedly as the interval becomes smaller than one second. The amplitude-interval relationship is helpful in understanding the following phenomena which are observable in the performance of isotonic bivalve heart preparations:

Acetylcholine usually decreases the amplitude and frequency of normally beating bivalve hearts. However, near threshold, and notably in cockle hearts, only the frequency is depressed while the amplitude of beat increases. This response, in oyster ventricles, occurs even at high acetylcholine doses. Appropriate changes in the ambient potassium concentration of Spisula solidissima hearts produce a similar effect. On the other hand, drugs which primarily increase frequency and tone, such as epinephrine, result in a diminished contraction at near threshold doses. 5-hydroxytryptamine (5-HT) usually increases amplitude. But this effect can be selectively blocked, and then 5-HT also decreases amplitude while increasing rate of beat. It would appear that the effects of agents on interval-dependent processes are important components of bivalve heart drug responses.

As the bath temperature of Mercenaria heart preparations is increased from 4° to 35° C. frequency increases linearly while amplitude peaks at about 10°-15° C. The amplitude-interval relationship suggests that this non-linearity might result, at least in part, from superposition of a frequency-dependent change on a set of temperature-dependent ones.

Two other phenomena indicating an effect of interval on amplitude of contraction are observable in bivalve heart preparations. First is the "rest contraction." Occasional preparations beat in sustained bouts separated by periods of quiescence. The first beat of the bout is large; successive contractions decreasing until a lower equilibrium value is reached. In some instances the bouts are very short (4-5 contractions) and equilibrium is never achieved. The second phenomenon is the appearance of frequent extrasystoles in irregularly beating ventricles (e.g., Pectinidae). These contractions are always smaller than those which preceed them. Post-extrasystoles are potentiated. Phenomena similar to these have been observed for some time in mammalian heart preparations. Probably there are similar inotropic and chronotropic control mechanisms in mammalian and molluscan cardiac muscle.

This study was supported by Research Grant HE-06291 from the National Heart Institute of the U. S. Public Health Service.

SPONTANEOUS ACTIVITY OF SPISULA INTESTINE. Richard A. Nystrom, Department of Biological Sciences, University of Delaware, Newark, Delaware.

The longitudinal smooth muscle of the surf clam, *Spisula solidissima*, shows both phasic and tonic contractions to electrical stimulation of either the gut or the motor nerves. Fast, propagated, spike-like action potentials, recorded with wick electrodes, accompany the phasic contractions and small ganglionic-like discharges, which are localized in certain areas of the gut, are associated with the tonic contractions. Prosser demonstrated that these components can be separated although closely coupled. Greenberg produced two-phase contractions by exciting with acetylcholine.

Isolated segments of intestine frequently, but not always are spontaneously active. Under various conditions, at least four kinds of spontaneous activity can be described. Under normal conditions, the activity of the segment that passes through the heart consists of a series of slow, rhythmic waves -- about one wave per minute. Localized, ganglionic-like electrical discharges can be recorded in regularly occurring bursts. Units of response can be identified; single units occassionally alternate in the basic pattern. These bursts which occur at a frequency of 1.5 cycles per minute are not clearly related to the mechanical waves. Bathing the intestine in 10^{-6}M 5-hydroxytryptamine (5-HT) induces a second type of rhythmic contraction: smaller, isolated contractions are superimposed on the resting waves at a rate of four per minute. At the same time, the 5-HT depresses the rhythmic unit discharge seen in normal activity. Thirdly, 10^{-6}M lysergic-acid diethylamide (LSD), a 5-HT antagonist, greatly enhances the 5-HT response and produces trains of large, rapid contractions, approximately two per second. Frequently, large contractions alternate with smaller ones or trains of smaller ones. Single spikes can be recorded with each contraction. Lastly, 10^{-4}M MS222, a good nerve-blocking agent for *Spisula*, elicits fast, tetanic-like contractions at a frequency of one per second, and, related to these contractions, a rhythmic but continuous electrical discharge.

At the present time, no firm conclusions are obvious. 5-HT must have more than one site of action although the pharmacology remains confusing. A system which involves nervous activation and spontaneous activity of nerve cells is suggested. The relationships between the spontaneously, neurally, electrically, and pharmacologically excited contractions remains for further study.

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XVI INTERNATIONAL CONGRESS OF ZOOLOGY

CONTRIBUTED PAPERS SESSION

11A. ENDOCRINOLOGY

NITROGEN METABOLISM IN METHANDROSTENOLONE[/1] TREATED CHICKS[/2]

H. S. Siegel, Poultry Science Department, Virginia Polytechnic Institute, Blacksburg, Virginia. U.S.A.

Three experiments, involving 330 chicks, were performed. Varying levels of an aqueous suspension of the synthetic anabolic steroid, methandrostenolone, were injected intramuscularly into White Rock males from the fifth to the seventh week of age.

In Experiment I, daily doses ranged to 10 mg/kg/day to give a wide range and to observe possible toxicity. Although no mortality was experienced, weight gains and feed efficiency were significantly depressed. Nitrogen retention was depressed from a control value of 0.52% to 0.28% in the 10 mg/kg groups. Pectoralis muscle weight and nitrogen concentration were significantly depressed, but semimembranosus values were not. Plasma albumin and globulin values were slightly increased which together resulted in a significant increase in total protein concentration.

In Experiment II a dosage range up to 5.0 mg/kg/day was administered to males of three families of paternal half-sibs. Weight gains and feed efficiency were significantly depressed at the 2.5 and 5.0 mg/kg levels. Nitrogen retention was again reduced from 0.68% in the control to 0.61% at 5 mg/kg. Muscle weights, muscle nitrogen and plasma protein levels were not significantly affected at these levels.

The dosages in Experiment III, ranging up to 1.0 mg/kg/day, were administered to males of three sire families. Weight gains were greatest at 0.5 mg/kg and nitrogen retention was highest at 0.25 and 0.50 mg/kg (0.71%) and lowest at the 1.0 mg/kg level (0.65%). Muscle weight and muscle nitrogen values were not significantly influenced.

In none of these experiments were the non-protein nitrogen substances of the plasma significantly changed by the steroid. Family x treatment interactions for testes, comb and hematocrit values indicated that there were familial differences in response to androgenic stimulation.

/1 17α-methyl-17β-hydroxyandrosta-1, 4-dien-3-one, CIBA

/2 Partially supported by Ciba Pharmaceutical Products, Inc.

ETUDE EXPERIMENTALE DU DETERMINISME DE LA MUE CHEZ LES MYRIAPODES CHILOPODES. Robert Joly, Laboratoire de Zoologie, Faculté des Sciences, Lille (France).

Il existe dans la tête des Myriapodes Chilopodes une paire de glandes cérébrales en liaison nerveuse avec le protocérébron. Découvertes par Holmgren (1916), elles furent interprétées comme organes endocrines par Fahlander (1938) et Gabe (1952).

Des études expérimentales réalisées chez Scolopendra cingulata Latr. et surtout chez Lithobius forficatus L. m'ont permis de confirmer la nature endocrine de ces glandes et d'en préciser le rôle dans le phénomène de la mue. Ces études ont été effectuées sur des individus de taille moyenne (maturus junior de Verhoeff), sans distinction de sexe, maintenus en élevage dans des conditions de température (18 - 22° C) et d'hygrométrie (95 - 100 % d'humidité relative) constantes.

Les principaux résultats obtenus sont les suivants :

- 1) L'ablation élective des glandes cérébrales provoque le déclenchement de l'exuviation dans 75 % des cas. Chez les témoins, le pourcentage n'excède pas 28 à 30 %. Lorsque l'ablation est unilatérale, le pourcentage obtenu est intermédiaire entre celui des témoins et celui des opérés bilatéraux.
- 2) L'isolement des glandes par section des nerfs les reliant au protocérébron déclenche l'exuviation.
- 3) L'implantation répétée de glandes cérébrales chez les individus qui en ont été préalablement privés empêche l'exuviation. Celle-ci peut au contraire se produire chez les individus subissant successivement une ablation des glandes et des implantations répétées de fragments de tergites.
- 4) Chez Lithobius forficatus L. l'activité des glandes ne semble se manifester que 10 jours environ après une exuviation naturelle et paraît se maintenir pendant 15 à 20 jours.

Les glandes cérébrales des Myriapodes Chilopodes exercent donc une action inhibitrice sur la mue ; cette fonction endocrine est comparable à celle du complexe organe X - glande du sinus chez les Crustacés.

Il convient d'ajouter que chez les Chilopodes la fréquence des mues est soumise à des variations d'ordre individuel et qu'elle est influencée par un cycle saisonnier où interviennent plusieurs facteurs externes : degré hygrométrique, photopériode, température, alimentation.

VENTRAL NERVE CORD SHORTENING; A METAMORPHIC PROCESS IN GALLERIA MELLONELLA (L.) (LEPIDOPTERA, PYRALLIDAE). Rudolph L. Pipa, Dept. of Entomology and Parasitology, University of California, Berkeley.

Although considerable progress has been made during the past quarter century in defining those endocrine glands which condition postembryonic differentiation in insects, studies of concomitant patterns of tissue and cell reorganization have lagged regrettably. Such reorganization would be anticipated in the nervous system of holometabolous insects, since extensive modifications and deletions of affector and effector organs ensue during metamorphosis.

As part of an extended investigation of neurometamorphosis in Galleria, the course of interganglionic connective shortening was followed by measurements made on more than 200 timed vivisections. All segments do not shorten to the same extent. Variations range from no statistically significant shortening, to partial shortening, to shortening with establishment of contiguity between ganglia, to complete shortening with coalescence of ganglia.

In animals maintained at 30-33°C. shortening commences about 24 hours prior to ecdysis, and is completed 30-45 hours after. Rates at which three of these segments shorten were approximated. These are: 0.27 mm., 0.46 mm., and 0.41 mm. per 24 hours. Much of the shortening which occurs is compensated by subsequent elongation of certain of the connectives. The morphogenic processes of shortening and elongation are not in phase.

The occurrence of axonal coiling during part of the shortening process indicates that an extraneuronal element may be directly involved. Other cytological features have been correlated with stages in the shortening process and shall be discussed.

THE EFFECT OF JUVENILE HORMONE ON TANNING OF THE PUPARIUM IN THE EBONY MUTANT OF DROSOPHILA MELANOGASTER. Henrietta G. Kalicki and Marcello Giordano, Dept. of Biology, Adelphi College, Garden City, L.I., N.Y.

The puparium of the ebony mutant does not undergo tanning--that is, hardening and darkening of the puparium cuticle--as does its counterpart in the wild type. The lack of tanning is correlated with a higher oxidative metabolism and lower tyrosinase activity in the ebony just before pupation takes place. This inverse ratio of high oxidative to low tyrosinase activity is normally existent in the larval stage of ebony and in wild type and is part of a physiological control system involving two enzyme systems which are controlled by the relative concentrations of two hormones. During larval life, oxidative metabolism is high, tyrosinase activity is held in abeyance and the concentration of juvenile hormone is relatively higher than growth and differentiation hormone. This physiological control system is reversed at pupation time; oxidative metabolism is reduced and tyrosinase activity increases and juvenile hormone concentration is relatively lower.

In ebony, juvenile hormone secretion does not decrease at pupation time and larval metabolism is maintained, as is evident in the lack of tanning. To determine whether the excessive concentration of juvenile hormone is responsible for maintaining high oxidative metabolism, third instar larvae were ligated below the brain, in the region of the third to fifth segment. The ebony larvae showed no tanning of the cuticle above the ligature but did tan in the region posterior to the ligature. Wild type larvae treated in the same way tanned on both sides of the ligature. These results lend support to the view that the inhibiting agent which prevents tanning in the larvae is a high concentration of juvenile hormone which provides the proper milieu for the activation of the cytochrome oxidative system thereby inhibiting tyrosinase activity.

ROLE DE LA PARS INTERCEREBRALIS CHEZ LOCUSTA MIGRATORIA L. (ORTHOPTERE)- Adrien Girardie, Laboratoire de Biologie générale Faculté des Sciences, Strasbourg, France.

L'irradiation aux rayons X ou l'électro-coagulation de la région de la Pars intercerebralis pratiquée sur des larves de Locusta migratoria phase gregaria des stades IV ou V permet d'obtenir des effets variés:
1°) Retard ou suppression de la mue, l'animal pouvant mourir à l'état de larve permanente après un long délai (3,5 fois la durée d'un stade larvaire normal). Ces larves permanentes conservent malgré leur âge des ovaires infantiles.
2°) Chez les animaux muant malgré l'intervention on peut obtenir une inhibition plus ou moins complète de la métamorphose, une forte proportion des stades VI obtenus étant des larves surnuméraires, des adultoïdes à ovaires infantiles ou des imagos imparfaits à appareil génital plus normal.
3°) Une modification de la pigmentation: bien que maintenus en élevage dense, une forte proportion de nos opérés prend la pigmentation verte normale chez les solitaires.

Nos interventions sur le protocérébron aboutissent donc dans le premier cas à des résultats identiques à une ablation totale oupartielle de la glande ventrale telle que l'a pratiquée Strich-Halbwachs, ce qui étend au cas de Locusta le mécanisme de contrôle de la mue bien connu ailleurs.

Pars intercerebralis-----Glande ventrale----Tégument

Dans les deux autres cas (2ème et 3ème) elles aboutissent à un résultat identique à une implantation de Corpora allata telle que l'a réalisée L. Joly. Ceci montre que toutes les actions physiologiques des C. allata (contrôle de la métamorphose, de la pigmentation, du développement ovarien) sont sous la dépendance de la Pars intercerebralis comme l'activité de la glande ventrale, mais ce contrôle s'exerce d'une manière tout à fait opposé, un même type d'intervention provoquant le blocage de la glande ventrale et l'hyperactivation des Corpora allata.

RAPPORTS ENTRE MUE ET VITELLOGENESE CHEZ LE CRABE CARCINUS MAENAS L.

Noëlle Démeusy, Laboratoire de Zoologie, Université de Caen, Caen (Calvados), France.

A la suite d'ablations des pédoncules oculaires (P.O) réalisées chez le Crabe Carcinus maenas L., nous avons signalé l'antagonisme qui existe entre la mue et la vitellogénèse (Démeusy N. et Lenel R., C.R. Soc. Biol., 148, pp. 156-158, 1955). Destinés à confirmer cet antagonisme, voici quelques observations relatives à une population naturelle de la Manche (Luc-sur-mer) et quelques résultats expérimentaux obtenus sur ses représentants.

Les femelles parvenues en fin de croissance et qui se trouvent vraisemblablement bloquées au stade d'intermue C^4T, présentent une activité ovarienne accrue. Venant de pondre, elles subissent très rapidement une nouvelle poussée ovarienne.

Des femelles oeuvées, privées de leurs glandes de mue au printemps, offrent dans 100 % des cas, et deux à trois mois après l'opération, des ovaires en vitellogénèse. A la même époque, un certain pourcentage des témoins mue et présente des ovaires transparents (C.R. Ac. Sc., 255, pp. 3062-3064, 1962). Des femelles juvéniles, soumises au même type d'opération, conduisent, dans les mêmes délais, à des résultats identiques (Cah. Biol. marine, 1, pp. 259-278, 1960). Dans les deux cas, la vitellogénèse des opérés se ferait sur un rythme plus rapide que celle des témoins.

Aussi bien chez les animaux indemnes en C^4T, que chez les opérés, l'activité ovarienne constatée semble une conséquence de l'absence de mue. Il se produirait sans doute un transfert vers les ovaires, des réserves destinées à la croissance somatique. Toutefois, ce transfert n'est pas provoqué par le défaut de libération en D° de l'hormone de l'organe Y. En effet, le déclenchement de la vitellogénèse a souvent lieu avant le moment où normalement la glande de mue devrait être activée. Il est peu probable d'autre part que la glande Y sécrète un principe inhibiteur de la vitellogénèse, la réaction de l'effecteur, soustrait à cet éventuel principe, étant très lente.

On peut alors supposer, que dans le cas des opérés, l'ablation des glandes de mue retentit sur le système inhibiteur pédonculaire, entraînant de sa part une surproduction d'hormone inhibitrice de mue (M.I.H). Ce trouble sécrétoire affecterait à son tour un mécanisme particulier agissant sur les ovaires, en l'occurence, la production de la seconde hormone inhibitrice (O.I.H) dont l'existence se trouverait ainsi renforcée.

Chez les Crabes en C^4T, un schéma du même ordre pourrait s'appliquer. On sait en effet que les P.O de tels individus sécrètent une forte quantité de M.I.H (Carlisle D.B., J. mar. biol. Ass. U.K., 36, pp. 291-307, 1957).

A STUDY OF EXO-SKELETAL CALCIUM AND ITS VARIATION IN THE CRUSTACEAN INTERMOLT CYCLE. Cahoon, Sister M. Odile and McWhinnie, M. A., College of St. Scholastica, Duluth, Minnesota and DePaul University, Chicago, Illinois.

Paired portions of branchiostegites of *Orconectes virilis*, injected with Ca^{45} twenty-four hours previously, were removed from intermolt animals before and three hours after injection of one equivalent of premolt eyestalk extract. In pieces removed after injection there was a significant reduction of Ca^{45} ($P < 0.025$) indicating the presence of a molt promoting hormone. This drop was also seen in the measurement of total calcium found in the carapace of intermolt animals injected with premolt eyestalk as compared with the calcium in the carapace of non-treated intermolt animals ($P < 0.005$). At three hours after premolt eyestalk injection, the stomach of intermolt animals did not demonstrate a rise in calcium nor was there a significant difference in Ca^{45} levels in liver, blood filtrate, blood protein or green glands of these animals.

Similar studies using premolt animals injected with intermolt eyestalk extract did not show a significant difference in calcium levels in the carapace before and after injection, indicating that a molt inhibiting hormone was not capable of reversing the molt cycle after it had been initiated.

Environmental studies of early premolt crayfish placed and maintained in water having a Ca^{45} activity of 8 μC/100ml showed that carapace had reached a maximum load of Ca^{45} by twelve days and stomach by nine days. Blood filtrate and blood protein varied inversely while green glands and liver showed little Ca^{45} uptake by this time. The ratio of specific activity of Ca^{45}/mg dry weight to total calcium/mg dry weight of each tissue appeared to vary in six day cycles. Destalked crayfish, under similar conditions, are being studied to determine the relation of eyestalk hormone to uptake of Ca^{45}.

GLANDES ENDOCRINES PROTOCEPHALIQUES ET CYCLE D'INTERMUE CHEZ SQUILLA MANTIS LATREILLE (CRUSTACE STOMATOPODE). P. Drach et M. Gabe, Faculté des Sciences de Paris, France.

Les cellules neurosécrétrices désignées sous le nom de pars ganglionaris X-organi, la glande du sinus et la pars distalis X-organi, bien que de structures entièrement différentes, subissent au cours de l'intermue, des modifications cycliques dont l'étude préalable est nécessaire à toute recherche physiologique.

Les cellules de la partie ganglionnaire de l'organe X ne contiennent au début de l'intermue (A_1) que de rares grains de sécrétion colorables par l'hématoxyline chromique et la fuchsine-paraldéhyde; une augmentation assez rapide conduit à un premier maximum qui correspond à la période de stabilité physiologique (C). Une première diminution survient au début de la période préparatoire à la mue suivante (D_0-D_1'); un deuxième maximum correspond à la mise en place des soies (D_2'); dès lors, la quantité de produit de neurosécrétion diminue progressivement jusqu'à l'exuviation.

La glande du sinus, dont la zonation est moins nette que chez les Décapodes, se montre assez pauvre en produit de sécrétion chez les animaux qui viennent de muer (A_1), puis s'enrichit rapidement au cours du durcissement de l'exosquelette (B_2). L'abondance du produit diminue à la période de stabilité physiologique (C); les fluctuations qui surviennent au cours de la préparation à la nouvelle mue (étape D) sont encore difficiles à analyser.

Le cycle sécrétoire mérocrine des cellules de la partie distale de l'organe X comporte l'élaboration intracellulaire de structures en bulbes d'oignon au centre desquelles apparaissent ensuite des mottes acidophiles; celles-ci quittent la cellule après disparition des bulbes d'oignon et le cycle recommence. Ces différentes cellules fonctionnent de façon asynchrone, suivant un gradient latéro-médial, de sorte que tous les stades du cycle existent à tout moment de l'intermue. L'étude quantitative montre toutefois que l'élaboration des mottes acidophiles est maximale au moment du durcissement du tégument (B_2) et lors de la mise en place des soies (D_1''); elle est naturellement précédée d'une multiplication des structures en bulbes d'oignon.

ADAPTATION CHROMATIQUE CHEZ LE CRUSTACÉ DÉCAPODE CRANGON CRANGON (LINNÉ) PAR EFFET RECIPROQUE. Colette Chassard, Laboratoire de Zoologie, Faculté des Sciences de Paris, France.

Dès que deux ou plusieurs Crangon sont mis en présence, leur adaptation chromatique à un fond blanc est totalement modifiée; l'animal ne réagit plus en fonction du fond mais selon la couleur des individus qui l'entourent. Dix Crevettes sombres (provenant d'un fond noir) sont mises ensemble sur un fond blanc : aucune ne s'éclaircit pendant les trois jours de l'expérience; un animal blanc intégré à ce groupe noir s'assombrit aussitôt. Ainsi, chaque unité du groupe, au lieu de réagir en fonction de la couleur du fond se maintient, ou s'adapte, en accord avec celle de ses congénères. On voit que dans ce cas la réaction résultante de l'ensemble est radicalement opposée à celle de chaque individu qui, pris isolément et mis dans les mêmes conditions s'éclaircit rapidement par adaptation au fond blanc. Dans le cas où deux Crangon seulement, l'un noir, l'autre blanc, sont mis en présence, le sens de l'influence chromatique dépend du stade du cycle d'intermue de chacun d'eux.

Les courbes d'étalement des chromatophores en fonction du temps montrent que ces interactions sont presque immédiates : elles se produisent d'une façon totale généralement en dix minutes; elles sont donc plus rapides que l'adaptation au fond; elles sont d'autre part d'une meilleure efficacité car la migration des pigments est plus complète.

L'ablation des pédoncules oculaires ou l'application sur ceux-ci d'un vernis noir opaque supprime toute réponse; par contre, la réaction se produit entre des animaux séparés par une paroi de verre; c'est donc bien une excitation visuelle qui provoque chez Crangon cette adaptation chromatique à effet réciproque.

Sur fond noir, le phénomène ne semble pas se produire.

Ces faits pourraient expliquer les réactions anormales de Crangon observées par certains auteurs parmi lesquels Brown et Ederstrom (1940) qui constatent : "The behavior of the animals upon backgrounds in the laboratory was moderately irregular. Although the animals were usually light upon a white background and black upon a black one, there were occasional exceptions".

Ces phénomènes d'adaptation chromatique réciproque entre individus sont l'expression d'une sensibilité optique et sélective chez Crangon crangon.

ENDOCRINOLOGIE ET GENETIQUE DU SEXE CHEZ LES CRUSTACES SUPERIEURS. Hélène Charniaux-Cotton, Laboratoire de Génétique évolutive, C.N.R.S., Gif-sur-Yvette, Seine et Oise, France.

Pour rendre compte de l'évolution du sexe, il est nécessaire d'envisager l'existence de deux groupes de gènes :

1) les gènes des caractères primaires et secondaires des deux sexes, tous présents dans chaque individu ;

2) un ensemble de gènes formant la "détermination génétique du sexe" et qui diffère d'un sexe à l'autre ; cet ensemble assure l'expression des gènes des caractères d'un seul sexe.

L'hypothèse de ce double système génétique s'accorde particulièrement bien avec l'endocrinologie sexuelle des Amphipodes Talitridae (*). Le jeune est sexuellement indifférencié et possède une ébauche de glande androgène. Quand cette ébauche se développe, elle secrète l'hormone androgène qui impose la différenciation ♂ (spermatogenèse comprise) et est, de plus, inhibitrice des caractères ♀. Quand l'ébauche ne se développe pas, la gonade s'autodifférencie en ovaire ; l'ovaire (contrairement au testicule) est endocrine et secrète les hormones contrôlant les caractères ♀ externes. La détermination génétique du sexe décide donc de la sécrétion ou de la non-sécrétion de la glande androgène.

Les inversions expérimentales du sexe montrent, d'autre part, que chacun des sexes possède les gènes des caractères sexuels de l'autre sexe. En effet, une ♀, après la greffe d'une glande androgène, acquiert tous les caractères primaires et secondaires ♂. Inversement, le mâle possède les gènes des caractères ♀, comme l'ont montré des expériences récentes : après l'ablation des glandes androgènes, le testicule de certains Talitridae s'inverse en un ovaire et induit l'apparition des caractères ♀ externes.

Il est intéressant de souligner la différence entre le déterminisme génétique de la sécrétion de l'hormone ♂ (qui appartient au groupe 2) et celui de la sécrétion de l'hormone ♀ (groupe 1). Ceci explique que les techniques chirurgicales de l'endocrinologie permettent d'obtenir la sécrétion de l'hormone ♀ chez le ♂ génétique, mais non l'inverse ; en effet, le ♂ possède les gènes pour la sécrétion de l'hormone ovarienne tandis que la ♀ ne possède pas le système génétique responsable de la sécrétion de l'hormone androgène.

(*) Charniaux-Cotton, H. 1962 Gen. Comp. Endocrinol. Suppl. 1, 241-247.

LA NATURE ET LE DETERMINISME DE L'EURYHALINITE CHEZ LEBISTES RETICULATUS (CYPRINODONTIFORMES, POECILIIDAE), T. MOHSEN, Faculté des Sciences, Université de Dakar, Sénégal.

Lebistes reticulatus était considéré jusqu'à récemment comme une espèce d'eau douce stricte. Nous avons démontré expérimentalement, que transférés directement de l'eau douce à l'eau de mer, les individus ne résistent point à une concentration de plus de 50% d'eau de mer. Cependant, si le passage d'eau douce en eau de mer est effectué graduellement pendant au moins 36 heures, tous les individus le subissent sans mortalité. L'euryhalinité ainsi acquise est temporaire, puisque les animaux la perdent au bout d'environ 36 heures en eau douce. Par contre, le passage d'eau de mer en eau douce, se fait d'emblée, à n'importe quel moment, sans besoin d'une adaptation préalable. Il est à noter que devant un choix entre l'eau douce et l'eau de mer, les animaux, même adaptés à l'eau de mer, continuent à préférer l'eau douce, mais qu'ils fuient moins rapidement l'eau salée que les témoins d'eau douce.

Nous avons réalisé, expérimentalement, chez cette espèce, un cycle complet en eau de mer, démontrant ainsi qu'il ne s'agit sûrement pas d'un phénomène de survie en eau de mer mais d'une vraie adaptation, d'autant plus que la croissance des jeunes nés dans ce milieu n'est nullement inhibée, mais au contraire légèrement accélérée.

Cette adaptation à l'eau de mer pose deux problèmes, d'une part celui de son déterminisme, d'autre part celui de la réalisation d'un équilibre hydro-minéral avec le milieu.

Pour ce qui concerne le déterminisme, nous avons envisagé le rôle de la glande thyroïde. Des lots d'animaux ont été traités par la thyroxine et la thiourée, afin de détecter l'action de ces substances sur la possibilité du transfert de l'eau douce en eau de mer. Les résultats obtenus nous paraissent en contradiction avec la règle générale, selon laquelle le passage d'eau de mer en eau douce est accompagné d'une activation de la glande thyroïde et le passage d'eau douce en eau de mer par une inhibition de l'activité de cette glande.

Concernant l'équilibre hydro-minéral, nous pensons que l'hypothèse de poikiloosmose n'est pas à rejeter apriori. Nous espérons pouvoir résoudre ce problème d'une part en mesurant les variations de la teneur globale des ions Na^+ et K^+ dans le corps des individus, maintenus dans des milieux de salinités variées, à l'aide du photomètre à flamme, d'autre part par la mesure directe de la teneur du sang en ces ions à l'aide d'une méthode de microcryoscopie. W

POST - IRRADIATION PROTECTION AND RECOVERY. M. Maqsood, CENTO Institute of Nuclear Science, Tehran (Iran).

Administration of olive oil within an hour after whole-body x-irradiation of male mice at a dose of 625 r (LD_{50} ($_{30}$)) significantly increases the survival rate when compared with the x-irradiated untreated mice. The effects of x-irradiation on bone marrow, spleen, thymus, liver, gonads and fertility rate of x-irradiated and olive oil-treated x-irradiated male mice at different time intervals, following whole-body x-irradiation have been investigated. Olive oil therapy accelerated the rate of cellular regeneration and repopulation in the bone marrow, spleen, thymus and gonads. Whole-body x-irradiation of male mice did not produce any significant pathological changes in the liver as judged from histological examination. Following x-irradiation there was evidence that Kupffer cells were phagocytizing the circulating debris. Administration of esters of certain unsaturated fatty acids, i.e. methyl oleate and methyl linoleate, within an hour following whole-body x-irradiation of male mice at 625 r (LD_{50} ($_{30}$)), accelerated the rate of cellular regeneration in the testes of these animals when compared with the untreated x-irradiated controls, on the 30th day post-irradiation. On the other hand esters of certain other saturated fatty acids, i.e. methyl stearate and methyl palmitate adversely affected the rate of cellular regeneration in the testes of x-irradiated mice. These differences may, in part, be due to differences in the composition of the esters of unsaturated fatty acids used in these experiments.

Whole-body x-irradiation of male mice at a single exposure of 625r decreased the fertility rate and significantly lowered the number of litter size obtained by mating x-irradiated males with non-irradiated females, 1 to 45 days post-irradiation. At this dose level x-irradiated males recovered in their fertility rate as judged from their litter size production 65 to 75 days post-irradiation. Intraperitoneal administration of 1 ml olive oil, improved fertility rate of treated male mice, 30 days post-irradiation. The olive oil-treated mice were twice as fertile as the untreated x-irradiated male mice. There are indications that olive oil therapy by restoring the impaired physiological processes of cells, accelerates the rate of cellular regeneration and repopulation in the gonads of x-irradiated male mice, at different time intervals following x-irradiation. Intraperitoneal administration of 1 ml. of mineral oil or 40 mg of powdered glass (-- 40 microns) in 1 ml of saline within an hour following whole-body x-irradiation at a single exposure of 625r, resulted in marked degenerative changes in the gonads of the x-irradiated mice when compared with those observed in the untreated x-irradiated mice. The harmful effects of mineral oil-powdered glass on the sex organs of the x-irradiated male mice appeared to be, in part, due to certain systematic disturbances which resulted from their intraperitoneal administration following x-irradiation.

Work carried out at the University of California, Berkeley. U.S.A.

SECRETORY ACTIVITY OF THE CORPUS CARDIACUM DURING THE PUPAL DEVELOPMENT OF MUSCA NEBULO.

G.Bhaskaran, and P.J.Deoras, Department of Entomology, Haffkine Institute, Bombay, India.

The corpus cardiacum consists of the deeply stainable chromophile cells and the chromophobe cells. In the early stages of pupal development, the cytoplasm of the chromophile cells are stained red uniformly with Azan. Twenty four hours after pupation, the intensity of the stain in these cells increases considerably. During this period, the ring gland lies behind the brain. Forty eight hours after pupation, the chromophile cells contain fairly detectable colloidal matter. After seventy two hours of pupation these cells contain numerous deeply stained colloidal particles which are gradually released into the lumen of the gland.Just before emergence, the corpus cardiacum is replete with secretory colloids and these are all aggregated into the space adjacent to the aortic wall. A few hours after feeding, in the newly emerged fly, the secreted colloids disappear from the corpus cardiacum. The histological evidence therefore indicates a definite secretory function for the chromophile cells of the corpus cardiacum during pupal development.

HORMONAL CONTROL OF OVARY DEVELOPMENT IN THE HOUSE-FLY, MUSCA NEBULO.

P. J. Deoras, and G. Bhaskaran, Department of Entomology, Haffkine Institute, Bombay, India.

Extirpation of the corpus allatum in a newly emerged females prevents ovary development. The oocytes do not grow beyond the yolk deposition stage. Implantation of corpus allatum from mature females or males, promotes ovary growth in the allatectomised females. Likewise, transplantation of the brain-ring gland complex from 1st instar, 2nd instar or early 3rd instar larvae, promotes ovary growth in these females. On the other hand, the brain-ring gland complex of the late 3rd instar larvae does not promote the growth of the allatectomised females. However, the corpus allatum part alone of the ring gland of the late 3rd instar larvae when transplanted into allatectomised females also initiate yolk deposition in the oocytes. These studies therefore indicate that the Juvenile hormone and the hormone regulating ovary growth are probably one and same substance.

XVI INTERNATIONAL CONGRESS OF ZOOLOGY

CONTRIBUTED PAPERS SESSION

11B. ENDOCRINOLOGY

A MODIFIED INDUCTOR THEORY OF SEX DIFFERENTIATION AS APPLIED TO MAN. John M. Opitz and David W. Smith, Depts. of Pediatrics and Med. Genet, University of Wisconsin School of Medicine.

The discovery of the actively male-sex-determining role of the Y-chromosome in man has facilitated an extension of a modification of the inductor theory of Witschi to the interpretation of normal and abnormal sex determination and differentiation in man. Primordial germ cells, the main inductors of the gonad, migrate from the posterior gut endoderm to the gonadal ridges which consist of a medulla of mesonephric blastema and a cortex of celomic epithelium. They are distributed to medulla and cortex in both sexes and enveloped with follicle cells which are homologous with granulosa and Sertoli cells. The follicle cell, the cortical inductor, responds best to an XX, but also to an XXX constitution with more or less ovarian (cortical) development and Mullerian duct differentiation. The medullary interstitial cells which are homologous with Leydig and hilus cells react normally to XY and XYY constitutions with testicular (medullary) and hence Wolffian duct differentiation and Mullerian duct suppression. The primordial germ cell is bipotential as demonstrated by the formation of oogonia in the cortex and spermatogonia in the medulla of XX/XY ovotestes. Mutations and sex-chromosomal imbalances, i.e. aneuploidy, deletions and certain types of mosaicism may lead to the genetic forms of gonadal dysgenesis and/or intersexuality which have been classified under Turner's and Klinefelter's syndromes, true hermaphroditism and pseudohermaphroditism. Environmental factors, such as exogenous hormonal alteration can result in postgenetic intersexuality.

Interference with the inductor capacity of the primitive testis leads to various degrees of imperfect differentiation of the Wolffian ducts and incomplete suppression of the Mullerian ducts. Various examples of these conditions will be presented and discussed in the light of this theory.

OVULATION INDUCING ACTION OF 17-SPIROLACTONES AND A-NOR-PROGESTERONE IN RANA PIPIENS. William B. Langan, Department of Physiology, State University of New York, Downstate Medical Center, Brooklyn, New York.

The ability of 45 steroids to induce ovulation in Rana pipiens has been studied by the following method: Hibernating frogs were procured from Vermont during March and April and housed in pairs in plastic cages at temperatures ranging from 18° to 22° C. The steroids were carried in sesame oil and administered in a single intraperitoneal injection. Ovulation was determined by manual stripping of the eggs twenty-four hours after the injection, followed in forty-eight hours by direct inspection of the eggs in the oviducts and uteri. The effective compounds were those which possessed androgenic, progestational or mineralocorticoid activity, whereas estrogens and glucocorticoids were ineffective. Doses ranged from 0.01 to 5.0 mg.

Recently the two spirolactones 3-(3-OXO-17β-hydroxy-4-androsten-17αyl) propionic acid - y-lactone and its 19 nor-analogue and A-norprogesterone were also found to be capable of inducing ovulation in the frog in doses of 0.1-3 mg. The 17-spirolactones possess progestational activity. Since the latter compound is devoid of androgenic or progestational activity, it appears that a steroidal substance capable of inducing ovulation in Rana pipiens need not possess these biological properties.

THE INFLUENCE OF ESTRIOL AND ESTRADIOL-17B ON UTERINE WEIGHT AND ALKALINE PHOSPHATASE LEVEL. Leonard Grosso, Dept. of Biology, Long Island University, Brooklyn, New York.

It has been known for many years that uterine development, growth, composition and metabolism are greatly influenced by the estrogenic hormones. More recently the interaction of estrogens, their metabolites and steroids of the adrenal has received attention. Some investigators believe that effects previously thought to be due to one hormone may represent the net activity of a number of hormones and possibly their metabolites.

The present report concerns itself with the weight and alkaline phosphatase titer of the uterus under the influence of two estrogens, estriol and estradiol-17B, administered separately and together.

Rats of the CD strain weighing approximately 135 grams were utilized. Three major groupsof animals were established as follows: 1) ovariectomized; 2) ovariectomized - adrenalectomized; 3) hypohysectomized - ovariectomized - adrenalectomized. Each group was subdivided so as to include members receiving either: 1) 0.1 ug estradiol-17B; 2) 1.0 ug estriol; 3) 0.1 ug estradiol-17B plus 1.0 ug estriol; 4) sesame oil without hormone; subcutaneously administered once a day for three days starting seven days after surgical procedures. All animals were sacrificed 72 hours after the start of hormone or oil injection. Immediately after killing, uterine wet weights (after blotting) were recorded and organ alkaline phosphatase determined colorimetrically.

Both estriol and estradiol-17B caused a significant increase in uterine weight in all groups. Estradiol was more effective than estriol. When the two hormones were administered simultaneously an additive effect was not seen. The alkaline phosphatase titer was significantly raised in all groups when estriol and estradiol-17B were administered singularly. The latter was more effective and caused a greater enzyme increase over the estriol caused increase. When the hormones were administered simultaneously the enzyme level was not significantly higher than the estradiol-17B induced increase.

EFFECTS OF EXOGENOUS HORMONES ON THE REPRODUCTIVE STRUCTURES OF THE FEMALE BAT RHINOPOMA. L. S. Ramaswami, Dept. of Zoology, University of Rajasthan, Jodhpur, India.

Rhinopoma kinneari Wroughton (Rhinopomatidae, Microchiroptera) is a non-hibernating bat whose breeding season is from March to July. Pregnancy is usually in the right horn and both ovaries are functional. The corpus luteum is of the extraovarian stalked type. The ovulation is induced like in the non-hibernating Pteropus (Megachiroptera).

In October, the reproductive organs of Rhinopoma return to normalcy after the previous pregnancy. This is also the month when the hibernating bats enter into their winter sleep thus enabling us to establish a comparision in the reproductive behaviour between the hibernating microchiroptera and the non-hibernating Rhinopoma and Pteropus. During October, Rhinopoma females were injected with small doses of various mammalian hormones and were examined after 2-8 days. Estrogen (Ovocyclin P: Ciba), 19-nortestosterone (Parke Davis), methyl testosterone (Biorganic Lab. USA), human chorionic gonadotrophin (Primogonyl: Schering) and luteinizing hormone (NIH) were capable of inducing ovulation in either or both ovaries; stalked corpora lutea were noticed in all these cases. The small dose of 19-nortestosterone in addition to inducing ovulation also caused the development of a small testis-like structure in the medullary region on one side of some ovaries. Progesterone (Lutocyclin: Ciba), adrenocorticotrophic hormone (Crookes), lactogenic hormone (Panlitar: Armour), testosterone propionate (Perandren; Ciba; Biorganic Lab: USA), hydrocortisone (Ciba), follicle-stimulating hormone (NIH), pregnant mare serum gonadotrophin (Priantin: Schering), Norlutin (Parke Davis), Nilevar (Searle) and Enovid (Searle) did not bring about ovulation.

In the experiments conducted during February, Rhinopoma receiving 5 mg of 19-nortestosterone (administered in 1 mg dose every alternate day) and those receiving HCG (50 I.U.) were examined after 15 days. The androgen treated ovaries showed corpora lutea. In the HCG treated animals, the ovaries hypertrophied and showed many corpora lutea atretica but no corpora lutea.

The uterus of the treated animals also showed changes in the myometrium and endometrium. The myometrium appeared comparatively thinned out in the Nilevar, Enovid and Norlutin injected specimens; the endometrium showed hardly any change in estrus animals and FSH, ACTH, PMS and hydrocortisone injected animals while the others exhibited varying amounts of change.

ON THE PER IODIC ACID-SCHIFF REACTIVE NEUROSECRETORY MATERIAL AND ITS VARIATION DURING THE REPRODUCTIVE CYCLE OF THE INDIAN BAT, RHINOLOPHUS ROUXI (TEMN.). Navanit Laxman Shah, Indian Cancer Research Centre, Parel, Bombay, India.

The neurosecretory material (NSM) found in the neuro= secretory cells, the eminentia mediana, the stalk, and in the infundibular process of the bat shows a positive per iodic acid Schiff (PAS) reaction. The PAS reaction is positive even after the saliva digestion of paraffin sections. It is absent after acetylation of sections. The NSM shows toluidine blue meta= chromasia. Therefore, the NSM of the bat has a mucoprotein component. In the light of recent controversy over the presence of a mucoprotein component of NSM this aspect has been studied in detail. Strong as well as weak PAS reactive NSM stains strongly with alcian blue, aldehyde fuchsin and chrome hemato= xylin. The chrome hematoxylin staining reveals more NSM than that after the PAS staining of the adjoining section. The near absence of the NSM has been observed only in the PAS stained neurohypophyses of some female bats. The rostral part of the median eminence and the processus infundibuli of the bat show the same morphology, quantity and the intensity of the PAS reaction of the NSM.

For a quantitative study of the PAS reactive NSM of the bat during its annual reproductive cycle, the processus infundibuli of 95 ♀ and 22 ♂ hypophyses have been examined. The females in pro-oestrus and pregnancy (February-June) have a small quantity of the PAS +ve NSM. The NSM is negligible in amount at the time of early pregnancy (April), but thereafter it increases until parturition. The lactating females (July-August) have a fluctuating quantity of the NSM. At the end of the lactation period the quantity of the NSM is fair. It then gradually increases from the time of the beginning of anoestrus (September) and reaches a maximum at the end of anoestrus (January). Preliminary observations on the males indicate that the quantity of the NSM increases from the month of June to January. However, a sudden reduction in the quantity of the NSM observed in December is followed with a remarkable increase in January. The quantity decreases once again in February and is the least in males in the month of May when they are resting.

Prior to the commencement of the active sexual phase, i.e. in January, both the sexes show a very high content of the PAS +ve NSM in the processus infundibuli. At this time there are numerous peripheral mucoid (FSH) cells. A gradual decrease in the quantity of the NSM in both the sexes takes place at the time of the increased sexual activity (February-March), when numerous actively secreting FSH cells and vesiculate mucoid cells are seen in the anterior lobe. The presence of a negli= gible to fair amount of NSM during the early pregnancy of the females (April) and the active spermatogenesis in Males (December) coincides with the presence of numerous brick-red LH secreting mucoid cells in the pars distalis.

PSEUDOPREGNANCY IN OVARIECTOMIZED FERRETS. G. Dale Buchanan, Department of Anatomy, University of Tennessee Medical Units, Memphis, Tennessee.

Pseudopregnancy in ferrets lasts as long as pregnancy and is characterized by marked folding of the uterine epithelium and enlargement of the glands. As in other mustelids, the decidual response is epithelial, and consists of the development of large, frequently multinucleated cells and areas of plasmodial tissue. These changes occur first in the luminal cells and subsequently spread to the deepest parts of the glands. This histological picture is identical to that seen in the inter-embryonic areas of the gravid uterus.

To study the effect of ovariectomy on pseudopregnant ferrets, estrous females were mated to infertile males during the normal breeding season. Bilateral ovariectomy was performed 6 or 8 days (in one case 10 days) post coitum. This is 1 to 2 weeks before the appearance of the epithelial changes which characterize pseudopregnancy. Left and right uterine horns were removed separately at selected times after ovariectomy. Histological examination of the uteri showed normal pseudopregnant changes in the endometria, which were comparable to synchronous stages in intact animals.

The failure of ovariectomy to prevent pseudopregnancy in the ferret is difficult to explain since, presumably, functional luteal tissue is required for its manifestation. Certainly in the rat, mouse, rabbit and guinea pig, ovariectomy shortly after ovulation prevents the development of pseudopregnancy. It is possible that by the time of ovariectomy, adequate amounts of progestins have already been secreted to bring about uterine proliferation. This view is difficult to accept since the ovaries were excised some days prior to the appearance of the epithelial changes. Furthermore, uteri removed 28-31 days after ovariectomy still showed extreme plasmodial involvement. It is also possible that non-ovarian tissues can secrete sufficient progestins to sustain pseudopregnancy. There is no direct evidence for this hypothesis, but some data exist which do suggest that pseudopregnancy may proceed in animals with hypofunctional corpora. Hill & Parkes (Proc. Roy. Soc. 107B:39, 1930)secured estrus, mating and ovulation in anestrous ferrets with HCG. Some animals showed pseudopregnant uteri 21 days after the last hormone injection. In this laboratory, estrus was induced with PMS and after mating, ovulation secured with HCG. Uteri which looked grossly to be pseudopregnant were obtained 30-35 days after the hormone injections. The presence of the ovaries in these cases complicates the picture, but does suggest that continued pseudopregnancy may be possible in spite of reduced progestin titers.

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THE EFFECT OF GONADOTROPHIC HORMONES ON THE IMMATURE RABBIT OVARY. Richard R. Fox, M. X. Zarrow, and J. L. Cavanaugh, The Jackson Laboratory, Bar Harbor, Maine, and Purdue University, Lafayette, Indiana.

Wilson and Zarrow (J. Reprod. Fertil. 3:148-158, 1961) and Zarrow and Wilson (Endocrinology 69:851-855, 1961) report precise quantitative studies of superovulation for the mouse and the rat. They list optimal hormone doses, time intervals between administration of PMS and HCG, and the effect of age on ovarian response. Similar studies on the rabbit are in progress using sib comparisons for treatment groups. Data from strain III (New Zealand White origin) females ranging in age from 50 to 150 days indicate that with 200 iu PMS the ovary commences to increase in weight at about 65 days of age with a peak occurring at 80 to 95 days. Following the peak the response decreases until 110 days and then plateaus. The response to 50 iu of PMS is similar but of less magnitude and is delayed approximately 10 days. Two effects of the exogenous hormones were noted. Macroscopically a change in follicle size with some follicular hemorrhage occurred. Microscopically thecal disorganization and an increase in granulosa cell size were evident. The magnitude of these changes, the follicle size, and the granulosa cell size parallel the gross effect on ovarian weights. Preliminary data indicate the optimal time interval between PMS and HCG to be about 72 hours. A maximum of 74 fertilized eggs were recovered from one 87 day old female (84 days at time of PMS).

GONADAL HORMONES: EFFECTS OF HYPOTHALAMIC IMPLANTS ON REPRODUCTION IN THE RAT. Robert D. Lisk, Dept. of Biology, Princeton University, Princeton, New Jersey, U.S.A.

Gonadal hormones can effect reproduction in the rat through interaction at two discrete loci in the hypothalamus. Estradiol implants in the preoptic-anterior hypothalamus of castrate females results in return to full estrous behavior. Similar implants in the arcuate-mammillary region of intact rats inhibit gonadotrophin release resulting in atrophy of the gonads. The present report deals with behavioral responses, pregnancy, delivery, and rearing of the young after estradiol, progesterone or testosterone have been implanted in the gonadotrophin inhibiting center of intact female rats.

The hormone was implanted either as a ball fused to the end of 30G tubing or contained within the lumen of 27G or 30G tubes. In all cases, the vaginal cycle was followed one week prior to hormone implant and throughout the experiment. One week following hormone implant the animals were placed with males and left together for 4 weeks.

After implant of a ball of estradiol the vaginal cycle was displaced towards estrous. When the males were introduced, all 10 animals immediately mated, repeatedly, every day for the next 4 weeks. When smaller implants of estradiol were used, the vaginal cycle tended to diestrous for 3 weeks. After this, full cycles of irregular length commenced and the rats began mating, accepting the males during all phases of the cycle. No pregnancies occurred.

Following implant of a ball of progesterone, no change in the estrous cycle was noted; the animals mated, gave birth 23 days later and reared their young.

Following the implant of a ball of testosterone, the vaginal smear tended to diestrous for a week, then irregular cycles commenced. These animals mated a number of times at estrous; about one half never showed signs of pregnancy, the other became pregnant but died during labor.

When an implant of estradiol was embedded in the anterior pituitary the rat became pregnant. Pregnancies, followed by the birth of normal young which were successfully raised, occurred when the testosterone implant missed the arcuate region.

Thus it appears that in the interactions of the gonadal hormones at the arcuate nucleus in the female rat, estrogen levels are most critical, both in the regulation of behavioral and physiological mechanisms. Testosterone appears to interfere with the proper functioning of the pituitary, while progesterone appears to be of little importance in the feedback system through the arcuate region of the rat.

CORRELATIONS BETWEEN DECIDUOMATA, INTRAOCULAR CORPORA LUTEA, PITUITARY PROLACTIN LEVELS AND DURATION OF PSEUDOPREGNANCY IN *MESOCRICETUS AURATUS*. George C. Kent, Jr., Dept. of Zoology, Louisiana State University, Baton Rouge, Louisiana, U.S.A.

The several studies were independent of one another. Pseudopregnancy (PS) was induced by sterile mating (SM), deciduomata (DCR) by 2 thread loops 48 hrs. later, prolactin (LTH) levels were assayed by crop sac method, corpora lutea (CL) were intraocular autotransplants, one CL per animal. PS was deemed to have terminated when psychic estrus was manifest. In the following chronology numerals indicate total hours elapsed since SM. Approximately 6 hrs. may be subtracted to determine hours elapsed since ovulation. 12: luteinization has commenced. 36: CL exhibits peak vascularity. 48: CL fully luteinized, LTH at peak level. 84: frank DCR. 96: glycogen apparent in DCR. 108: initial decrease in vascularity of CL, accelerated decrease in diameter of CL. 144: DCR reaches peak differentiation, CL exhibits vascular stasis, lutein color less intense, LTH still relatively high. 156: CL white, swells. 186: DCR shows initial necrosis. 204: PS terminates.

Previous work in our laboratory has shown that exogenous LTH induces larger DCR, and that normal DCR cannot be prolonged by exogenous progesterone administered 1 mg daily from day of trauma. Although the CL commences to retrogress at 108 hrs. pituitary LTH levels remain near peak and the DCR continues to develop, remaining healthy 3¼ days after start of CL retrogression. We have not yet correlated the CL of the intact ovary with the other observed phenomena. LTH assays beyond 144 hrs. of PS are now in progress. The possibility of an effect of LTH not mediated through the ovary in the maintenance of DCR will be investigated. That prolactin is luteotropic in the golden hamster has not yet been firmly established to our knowledge. All observations were made in our own laboratories. Published data cited herein: Kent and Atkins, Proc. Soc. Exper. Biol. & Med., 1959, v. 101, 106-107; Turnbull and Kent, Anat. Rec., 1963, v. 145, 97-100. (Prolactin supplied by the Endocrinology Study Section, N.I.H.).

ACTION D'UNE EXPOSITION AIGUE A UN FROID RIGOUREUX SUR LA FONCTION ENDOCRINIENNE DU TESTICULE. Marcel J. Perrault et Louis-Paul Dugal, Département de Biologie, Université d'Ottawa, Ottawa, Canada.

Chez le rat mâle exposé chroniquement (40 semaines) à un froid modéré (2°C), il s'opère une véritable auto-castration: destruction du tissu interstitiel du testicule et atrophie des accessoires sexuels. Après 52 semaines, malgré la dégénérescence avancée du testicule, le poids des accessoires retourne à la normale et, dans la surrénale, apparait une zone nouvelle rappelant la zone-X.

Les même phénomènes sont télescopés en 12-15 jours, si on expose des animaux à une température de -5°C. L'effet du froid sur le testicule augmente avec la durée de l'exposition et après 16-18 jours, le poids des accessoires rejoint les valeurs caractéristiques de l'animal castré. L'action du froid peut être dissociée en deux composantes: l'une, non-endocrinienne, de type métabolique, associée à l'inanition contrôlée ou de type neural, tel le cryptorchidisme réflexe avec ses effets sur la lignée germinale; l'autre composante, nettement endocrinienne: dépression de la fonction androgénique du testicule démontrée par la dégénérescence des accessoires: prostate et vésicules séminales.

Le testostérone administré à des castrés, exerce son action réparatrice sur les accessoires, indépendamment du froid, mais d'autre part, montre une toxicité inattendue, en ce qu'il retarde la croissance et diminue la survie. Enfin, l'action androgénique, minimale mais caractéristique, du cortex surrénalien, devient observable chez le castré exposé au froid.

Ainsi, l'animal exposé à un froid rigoureux pour une courte durée, subit une castration physiologique au cours de l'adaptation protectrice de l'organisme mâle contre une agression aigue et intense.

TESTOSTERONE-INDUCED INCUBATION PATCHES AND NUPTIAL PLUMAGE IN MALE AND FEMALE PHALAROPES. Jerry E. Johns and E. W. Pfeiffer, Dept. of Zoology, Montana State University, Missoula, Montana.

In phalaropes the role of the sexes is reversed during most of the breeding season, and the female possesses brilliant nuptial plumage but lacks a brood patch, while the male's nuptial plumage is dull and he possesses a brood patch. During formation of the pair bond the female takes the active role in courting the male, and the male subsequently broods the eggs and rears the young.

In order to determine the endocrine factors involved in these unusual anatomical and behavioral characteristics, 67 adult phalaropes of both sexes (*Steganopus tricolor* and *Lobipes lobatus*) have been captured during the breeding season and during the fall southward migration. These birds were maintained in captivity, some for many months, and testosterone-propionate, estradiol-benzoate, and prolactin were injected individually and in different combinations. Only testosterone-propionate and prolactin in combination produced incubation patches in birds of both sexes. Testosterone-propionate injected into phalaropes in winter plumage, whose humeral pterylae had been plucked unilaterally, produced regrowth of feathers similar to those of birds in nuptial plumage. Estradiol-benzoate, alone or in combination, had no apparent effect upon incubation patch formation or development of nuptial plumage. The implications of these findings are discussed with respect to "sex reversed" characters of wild phalaropes.

EFFECT OF NINE-HOUR SHORT-DAYS ON THE SEXUAL CYCLE OF LAL MUNIA, ESTRILDA AMANDAVA.

THAPLIYAL, J.P. & TEWARY, P.D., Department of Zoology, Banaras Hindu University, India.

Lal munia is a seasonally, sexually, dimorphic finch that breeds during late summer and early autumn months when days are decreasing in length. Birds were subjected to continued 9-hour short-days(8 a.m. to 5 p.m. of day-light) treatment during the regressive and progressive phases and regular observations were made on the plumage changes and the gonad size. One group of males was subjected to continuous 9-hour short days treatment, beginning December 21st,1961, when the birds are in eclipse plumage and the gonad is decreasing in size. Under the treatment, the gonad continued to regress reached the minimum in January, started developing in June and attained maximum volume in September when bunched spermatozoa were observed within the tubules. Regression started in October and the minimum size was registered once again for January. The plumage cycle, which reflects the hypophysial cycle,(Thapliyal,J.P. & Tewary, P.D.,1961, Science, 134, 738-739) precedes the gonadal cycle. Nuptial plumage is put-on in March/April and is maintained till October/November when it is changed to contra-nuptial type.

The second-group was set on June 21st when the birds are in breeding plumage and the gonads have started developing. Under the continued 9-hour short-days schedule this group followed plumage and gonad cycles similar to the December-group. Testis continued increasing in size and activity through July/August, attained the maximum volume in September and then involuted, reaching the miminum condition in January,

The fact that both the experimental groups follow plumage and gonad cycles which, in all details, resemble the cycles of control-group of birds held under normal day-lengths, suggests that these cycles in this species are independent of day-length and are regulated by a self-regulating internal rhythm.

SYNTHETIC HORMONAL EFFECTS ON CHICK EMBRYOS

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Depo-testosterone was injected into chicken eggs which had been incubated for 5 and for 7 days. Eggs were candled and embryos which died during the first two weeks of incubation were excluded. Measurements of length of oviducts, and surface area of all gonads were recorded on the 21st day.

Two responses are currently being exhibited as the result of the action of testosterone on the growth of the female reproductive organs. (a) When embryos are treated at 5 days and 7 days incubation time with doses of testosterone ranging from 0.05 to 0.10, there is a differential response of the gonads and oviducts to the treatment. Data indicate that the embryos at 5 days show a greater overall rate of growth than those injected at 7 days. (b) In the hormone treated embryos both the right and the left ovary and oviducts continue to develop. At hatching time considerable parallelism exists between the left and right sides. Several aspects of the chemical action of testosterone in mitotic inhibition will be considered.

INCREASE OF β-GLUCURONIDASE IN TADPOLE TAILS DURING METAMORPHOSIS. Helga Kuebler and Earl Frieden, Dept. of Chemistry and Institute of Molecular Biophysics, Florida State University, Tallahassee, Florida.

One of the most striking morphological changes which accompanies anuran metamorphosis is the resorption of the tadpole tail. Increases in hydrolytic enzymes of the tail such as cathepsin, peptidases, phosphatases, and deoxyribonuclease during metamorphosis have been previously reported. To study the soluble β-glucuronidase, tails were homogenized and the 105, 000 g supernatant was analyzed for the specific activity of the enzyme using phenolphthalein β-glucuronide as substrate. It was found that the soluble β-glucuronidase of the tadpole tail undergoes a 20 fold increase in specific activity during spontaneous metamorphosis and a 2-3 fold increase in specific activity during thyroxin induced metamorphosis. After centrifugation at 6, 500 g and 105,000 g only the β-glucuronidase of the 6, 500 g residue was solubilized by osmotic shock and freezing and thawing.

No appreciable change in the specific activity of the soluble β-glucuronidase of tadpole liver or muscle was noted.

The possible relation between these results and the lysosomal release of hydrolytic enzymes as a mechanism for tail resorption will be discussed.

(Aided by USPH grant C-3006).

DEVELOPPEMENT DES BATRACIENS ET TEMPERATURE - COMPARAISON ENTRE POICILOTHERME CHAUFFE ET SOURIS EN HYPOTHERMIE. Janine Flatin, laboratoire de Biologie générale, Université Catholique de Lyon, France.

Le développement des Poïcilothermes est très sensible à l'action de la température; leurs réactions métaboliques sont beaucoup plus lentes que celles des Homéothermes.

Nous avons cherché à explorer cette sensibilité au cours de leur développement et à savoir si la lenteur de leurs réactions endocriniennes n'était pas due en partie aux basses températures auxquelles vivent ces animaux qualifiés de Vertébrés à sang froid.

a) Le froid n'arrête pas complètement le développement embryonnaire mais prolonge considérablement la vie larvaire. Nous avons conservé à 4° au frigidaire pendant près de 2 ans de jeunes têtards d'Alytes obstetricans sans observer d'évolution métamorphique appréciable. Remis à la température du laboratoire, ces animaux se sont métamorphosés en suivant la même courbe que des animaux témoins. La diapause due au froid semble n'avoir pas modifié leurs potentialités métamorphiques.

Par contre, on déclenche rapidement la métamorphose de ces mêmes têtards d'Alytes, à toute époque de l'année, par simple chauffage.

b) Nous avons examiné, à différentes températures, chez le têtard d'Alytes : la réaction du testicule à l'hormone gonadotrope, de la thyroïde à l'hormone thyréotrope, la croissance du canal de Muller à la folliculine; ces réactions sont d'autant plus fortes et rapides que la température d'expérimentation est plus élevée.

c) L'hormone thyréotrope entraîne l'augmentation maxima de hauteur de l'épithélium thyroïdien de Batracien ou de Poisson 3 ou 4 jours après l'injection à température normale; ce maximum a pu être atteint en 40 heures en opérant à 30°.

Chez la souris, c'est 30 heures après l'injection que la réaction atteint son maximum dans des conditions normales alors qu'elle est seulement amorcée si les animaux sont maintenus en hypothermie à 20°.

La réaction - qualitative et quantitative - des endocrines paraît donc fortement conditionnée par la température interne des animaux.

La réponse endocrinienne du Poïcilotherme chauffé se rapproche de celle de l'Homéotherme et inversement.

XVI INTERNATIONAL CONGRESS OF ZOOLOGY

CONTRIBUTED PAPERS SESSION

11C. ENDOCRINOLOGY

BASES ULTRASTRUCTURALES DES RELATIONS HYPOTHALAMO-HYPOPHYSAIRES CHEZ QUELQUES ESPECES DE POISSONS TELEOSTEENS.

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L'étude au microscope électronique des deux principaux noyaux neurosécréteurs diencéphaliques (NPO+NLT) qui établissent la liaison avec l'hypophyse a été entreprise chez plusieurs espèces de Téléostéens (Perca fluviatilis et Salmo irideus). La cytologie fine des neurones des deux noyaux présente de nombreux points communs : disposition et importance des formations ergastoplasmiques périphériques ; aspect de la zone centrale du cytoplasme qui contient de nombreuses mitochondries et plusieurs amas de vésicules golgiennes.

Les neurosécrétions du NPO et du NLT se forment au voisinage immédiat des formations golgiennes. Le diamètre des grains élémentaires de neurosécrétion diffère dans les neurones des deux noyaux. La comparaison de leur taille nous a permis d'établir que les grains élaborés par les neurones du NLT présentent un diamètre moyen 30 % plus faible que celui des grains du NPO. Leur osmiophilie est également moins accusée.

Par ailleurs, la comparaison de la taille des grains élémentaires trouvés dans les péricaryons du NPO avec ceux situés dans la partie distale neurohypophysaire du même système permet de s'assurer qu'au cours du transport intraaxonal il ne se produit pas d'accroissement de leur diamètre. Dans ces conditions, il est peu vraisemblable qu'il puisse y avoir synthèse de neurosécrétion dans les axones. Les formations de Golgi sont en effet localisées dans le péricaryon et les grains élémentaires conservent le même diamètre au cours de leur trajet intraaxonal.

En partant de ces données il est possible de distinguer, dans certaines conditions, les axones des deux faisceaux grâce au diamètre des neurosécrétions qu'ils transportent et d'en suivre ainsi le trajet dans leur partie terminale.

L'étude de la partie terminale des deux faisceaux dans la neurohypophyse nous a permis de mettre en évidence des contacts neurovasculaires et neuroglandulaires dans tous les lobes hypophysaires. La présence de contacts neurovasculaires sur les capillaires cheminant dans la région proximale de la neurohypophyse confirme l'hypothèse de Green sur l'intervention du réseau vasculaire dans le mécanisme de régulation des fonctions hypophysaires chez les poissons.

La disposition du réseau vasculaire dans l'hypophyse nerveuse et glandulaire, étudiée chez les Cyprinodontes, chez Perca et chez Salmo irideus permet, en effet, de concevoir le transport et la distribution de substances d'origine nerveuse libérées dans sa partie proximale vers les cellules glandulaires de la pro et de la méso-adénohypophyse. La région proximale de la neurohypophyse des Poissons correspondrait du point de vue fonctionnel au système porte de l'éminence médiane des Vertébrés supérieurs.

De nombreuses fibres neurosécrétoires se terminent soit sur la membrane basale limitant le lobe nerveux et ses digitations (Perca-Salmo), soit sur les cellules glandulaires (Lebistes Phoxinus). Ces observations confirment l'existence simultanée d'un contrôle nerveux indirect par voie neurohumorale et d'un contrôle direct par des terminaisons neuroglandulaires, chez les Poissons.

ELECTROPHYSIOLOGY OF NEUROSECRETORY NEURONS OF THE LEECH THEROMYZON RUDE. Kinji Yagi, Howard A. Bern, and Irvine R. Hagadorn, Department of Zoology and its Cancer Research Genetics Laboratory, University of California, Berkeley, California, and Department of Zoology, University of North Carolina, Chapel Hill, North Carolina, U.S.A.

Spontaneous spike discharges were recorded extracellularly from the neurohemal area of the brain in the duck leech, Theromyzon rude. Spike discharges induced by antidromic stimulation of the neurohemal area with a capillary electrode were also recorded extracellularly from the soma of neurosecretory cells in the supraesophageal ganglion. Since the neurohemal area in Theromyzon consists entirely of the terminals of neurosecretory neurons, it is apparent that in this invertebrate species, at least, the neurosecretory neurons have the ability to conduct impulses. The observations support the concept of the neurosecretory cell as a neuron that also possesses glandular activity.

Comparison of the durations of spikes recorded from the neurohemal area with those recorded from the neuropil indicates that the action potential generated by neurosecretory neurons may be of longer duration than that of ordinary neurons. This difference also appeared in records obtained from the somata of neurosecretory and non-neurosecretory neurons both in the supraesophageal ganglion and in the segmental ganglia. This finding is consistent with results obtained by other workers from vertebrate neurosecretory cells. (Aided by NSF Grant G-8805).

SUR LE SYSTEME NEUROSECRETEUR DES MYRIAPODES DIPLOPODES. François Sahli, Laboratoire de Biologie animale et générale, Faculté des Sciences, Dijon, France.

Outre les éléments neurosécréteurs du système nerveux central, trois formations sont connues que nous appelerons "organe de Gabe", "corps paraoesophagien" et "corps connectif" et qui n'existent pas simultanément chez tous les Diplopodes.

Chez Tachypodoiulus (Iulide) nous distinguons six groupes pairs de cellules neurosécrétrices (dont 3 protocérébraux),caractérisées par leur affinité à l'égard de la fuchsine paraldéhyde et de l'hématoxyline chromique.Ces colorations généralement excellentes chez les Iulides ne donnent pas les mêmes résultats chez les Polydesmoïdes. Chez Jonespeltis (Pratinide),PRABHU distingue trois groupes et plusieurs sous-groupes; certaines homologies existent entre ses données et les nôtres (vraisemblablement A 1 = groupe des globuli I).

L'organe de Gabe,du nom de l'auteur qui l'a découvert (= "glande cérébrale" des auteurs),est une formation connue chez huit familles et qui existe probablement chez tous les Diplopodes, bien que son emplacement soit variable selon les groupes. Il ne s'agit nullement,comme le pensent certains auteurs,de l'organe mentionné par HOLMGREN chez les Iulides (l'organe de Gabe est ici en position ventrale).Il contient du matériel cyanophile et phloxinophile;des intermédiaires existent et,chez les Iulides,les mêmes produits s'observent dans le nerf afférent.

Le corps paraoesophagien (= "formation hypocérébrale", "ganglion viscéral") a été mis en évidence chez les Iulides,les Pratinides et les Strongylosomides.Ses cellules ont une activité sécrétrice propre et on y observe des boules et des flaques extracellulaires. PRABHU en fait des cellules neurosécrétrices (A 2) déversant leur produit par voie axonale dans l'organe de Gabe;appliquée aux Iulides,cette interprétation n'explique pas tous les faits observés.

Le corps connectif,terme emprunté à PRABHU, a été signalé chez les deux Strongylosomoïdes Jonespeltis et Orthomorpha. Il s'agit indubitablement d'un organe d'accumulation de matériel neurosécrété d'origine cérébrale, protocérébrale selon nous, mais qui, selon PRABHU, serait élaboré par ses cellules C 1 .

RELATİONSHİP BETWEEN THE HYPOTHALAMİC NEUROSECRETORY SYSTEM AND CERTAİN ADENOHYPOPHYSEAL BASOPHİLES OF PLATYFİSH. Nezihe Öztan, Dept. of General Zoology, Istanbul University, Turkey.

Xiphophorus maculatus bearing tumorous thyroid, fertile F_1-female hybrids obtained by crossing X.helleri females and X.maculatus males, and sterile F_2R-female hybrids obtained by backcrossing of F_1-males with X.helleri females were used. All of them were 1½ year old. Their ovaries, thyroids, pituitaries and hypothalamic neurosecretory systems were studied.

About 20 macroscopic eggs were usually found in the ovaries of fertile fishes whereas in sterile ones all the ovocytes were microscopic and the mean diameter of the ovocytes was (65± 5.0) micra. Atretic follicles and many young ovocytes with scanty ooplasm were seen in fish with tumorous thyroid.

Thyroid gland of fertile and sterile fishes were composed of similar ovoid or rounded follicles with low epithelium and with a colloid in their lumen. In fish bearing tumorous thyroid hypertrophic empty follicles consisted of columnar cells were seen.

According to cytomorphological signs three different types of periodic acid-Schiff positive cells (type 1, type 2 and type 3) were observed in the adenohypophysis of fertile fish. The last two were stained dark purple with aldehyde fuchsin. Moderate granulation and degranulation was seen in type 2 (thyrotropic) and type 3 (gonadotropic) cells of fertile fish whereas the cells of their preoptic nuclei and of nuclei lateralis tuberis were full of neurosecretory granules. In thyroid tumor bearing fish hypertrophy, hyperplasia, degranulation followed by a new granulation was prominent in type 2 basophiles although type 3 basophiles were rather reduced in size and some of them were vacuolated. The cells of their preoptic nuclei pars magnocellularis and those of nuclei lateralis tuberis were similar to that of fertile fishes. On the other hand, in sterile fish type 2 basophiles did not show an obvious alteration, but, type 3 cells were very few in number and reduced in size with shrunken cytoplasm and irregular picnotic nuclei. So it may be suggested that they are inactive and such sterile fishes were made fertile by treatment with gonadotropic hormone. Further, in sterile fishes the cells of nuclei lateralis tuberis appeared to be inactive whereas a high secretory activity was observed in the cells of preoptic pars magnocellularis. If this high activity is related directly to the hypophyseal gonadotropic function it might be expected an active gonadotropic effect of pituitary and as a result of it the maturation of the gonad, which is not the case. Therefore, keeping in mind the obvious inactivity of the cells of nuclei lateralis tuberis and the atrophic appearance of type 3 cells which results the gonadotropic inactivity it might be suggested that there is a causal relationship between the activities of the cells of nuclei lateralis tuberis and the hypophyseal type 3 basophiles.

FINE STRUCTURE OF THE FROG MEDIAN EMINENCE. Hideshi Kobayashi and Yoshihiko Oota. Zool. Institute, Faculty of Science, University of Tokyo, Tokyo, Japan.

The median eminence of the frog, Rana catesbiana, is divided into two portions. The upper portion consists of ependymal cells, glial cells and preoptico-hypophysial tract proceeding to pars nervosa. The lower portion is mostly occupied by glial cells and many neurosecretory axons which branch off from the tract. The capillaries of the primary plexus of the hypophysial portal vein cover the outer surface of the median eminence, and frequently they penetrate into the lower portion of the median eminence. Near the capillaries there are both neurosecretory and non-neurosecretory axon endings. The former contain neurosecretory granules and synaptic vesicles among axoplasmic inclusions and the latter only synaptic vesicles and no neurosecretory granules. The synaptic vesicles in the endings containing few neurosecretory granules are larger than those in the endings filled with the granules. The median eminence of the frog has essentially the same structure as that of the pars nervosa, except for the difference in size distribution of the neurosecretory granules between them. In the hilar region of the median eminence are many synapses characterized by clusters of synaptic vesicles. Synapses between neurosecretory axons and dendrites of non-neurosecretory neurons are occasionally present. The neurosecretory axons are either pre- or post-synaptic. (Supported in part by Grant A-3678, from the USPHS)

HYPOTHALAMO-HYPOPHYSEAL AND GONADAL RESPONSE IN RELATION TO THE DAILY DARK PERIOD DURING THE PREPARATORY PHASE OF THE AVIAN REPRODUCTIVE CYCLE. Albert Wolfson and Shayna J. Johnson, Dept. of Biological Sciences, Northwestern University, Evanston, Illinois.

Previous studies had established that completion of the preparatory phase in the annual gonadal cycle of *Junco hyemalis* requires exposure to an effective light-dark cycle for approximately six weeks. To examine more precisely the relation between the length of the daily dark period and the preparatory phase, two extensive experiments were performed in two successive years using the same species. In the first year the experiment began on July 15, which is the usual time for regression of gonadal activity in the laboratory under natural day lengths. The birds were divided into two groups at the beginning of the experiment and were exposed to 6L-18D cycles and 12L-12D cycles. Subsequently, after periods of 3, 6, and 9 weeks of treatment smaller groups were transferred to stimulatory light-dark cycles (16.75L-7.25D) to test the extent of preparation. The following year, similar groups were formed and treated identically, but the experiment began on September 4. Observations on the gonads and Gomori-positive material in the median eminence were made initially and at regular intervals during exposure to long days.

Data from the testicular response confirmed that the preparatory phase is regulated by short days. Probably involved are a) reactions related to completion of the preparatory phase, which appears to be an all-or-none phenomenon, and b) reactions related to degree of preparation, which is reflected in differences in rate and amplitude of growth. Comparisons of the long dark (18D) and short dark (12D) groups for each duration of treatment in each year showed that the longer dark period was significantly more effective in inducing completion of the preparatory phase. Six weeks was sufficient for 100% response in the 6L-18D groups. In the 12L-12D groups the response varied between the two years with a maximum of approximately 62%. The highest rates of growth occurred in the 12L-12D group prepared for 6 weeks in 1959 and in the 6L-18D group prepared for 6 weeks in 1960.

During the periods of preparation there was no clear-cut increase in Gomori-positive material in the median eminence. During the subsequent progressive phase there was considerable variation in the amount of Gomori-positive material in relation to gonadal response. Only about a third of the twelve groups showed gradual depletion of the Gomori-positive material during testicular growth. Of greatest interest were cases of gonadal response without prior or concomitant increase in Gomori-positive material and cases of refractoriness without accumulation of large stores of material in the median eminence.

THE INFLUENCE OF THE FOETAL THYROID OF THE RABBIT ON CHOLESTEROL METABOLISM STUDIED BY DIRECT SURGICAL INTERFERENCE ON THE FOETUS

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The experimental work of Jost and Wells has demonstrated the functional interrelationship of the pituitary and thyroid in the rabbit and rat foetus, and that thyroid hormones are present in foetal blood. However, little is known as yet of the functional and developmental importance of thyroid hormones in the foetus. Since in postnatal life it is well recognised that the thyroid hormones influence cholesterol metabolism, a series of experiments have been carried out to determine whether this influence is already present in the foetus.

The experimental studies to be described are based exclusively on the rabbit foetus. The methods used for the removal of the foetal pituitary and thyroid are similar to those described by Jost, although modifications of these techniques have been developed. Hypophyseoprivia at the 21st. to 22nd. day of development was performed in two ways. First, by high decapitation of the foetus, which leaves behind the lower jaw and neck of the foetus thus sparing the thyroid. Second, by low decapitation through the neck, which removes the thyroid with the pituitary. The decapitated foetus continues to grow within the uterine cavity following these operations and at the 29th. day, just before term, the foetuses are extracted by Caesarian section and blood samples taken from both operated and litter-mate control foetuses for cholesterol determination.

A highly significant increase in blood cholesterol was found in the operated foetuses compared with the litter-mate controls. This increase was found in those foetuses in which the pituitary alone had been removed as well as in those in which both the pituitary and thyroid had been removed.

A second series of experiments was carried out to remove the thyroid alone without removing the pituitary. This was accomplished initially by removing the larynx, upper trachea and the thyroid in one block. This operation interfered with foetal swallowing in utero since the stomach of these foetuses was found to be empty at term. Normally the stomach of a full term foetus is filled with what can be presumed to be amniotic fluid. Later it was found possible to dissect the thyroid off the trachea and larynx, leaving these intact, and the foetal stomach contents were then found to be normal. With both these methods of foetal thyroidectomy, a highly significant rise in blood cholesterol was found in the operated foetuses.

A third series of experiments was carried out in an attempt to prevent this rise in blood cholesterol by injecting into the operated foetuses minute amounts of thyroxine at the time of the decapitation of the foetuses. It was found that 150µg. of L thyroxine suspended in olive oil and injected subcutaneously into the foetus at the time of the operation effectively prevented this rise in blood cholesterol.

These experiments suggest that (1) foetal thyroid in the rabbit is essential for the normal regulation of cholesterol metabolism in the foetus, (2) that the maternal thyroid secretions which are known to cross the placenta in the rabbit, will not compensate for this loss of foetal thyroid activity, (3) the foetal thyroid activity is dependent on the normal functioning of the foetal pituitary.

IODINE METABOLISM IN ADULT KING SALMON (*Oncorhynchus tschawytscha*, Walbaum). Gilles La Roche, C. L. Johnson, A. N. Woodall, and L. L. Rosenberg, Western Fish Nutrition Laboratory, U. S. Fish and Wildlife Service, Willard, Washington, U. S. A.

In early May 1962, 66 migrating male and female adults were intercepted at Bonneville Dam and held in covered cement raceways at the Willard Fish Hatchery. The animals were kept in running freshwater containing 0.25 μg I^{127}/liter at a temperature of 6 ± 1° C. On three separate occasions (late May, early August and middle of September) groups of fish received carrier-free I^{131} or I^{125} intraperitoneally. Following injection, fish were sacrificed at intervals by a blow on the head and, in rapid succession were bled, their thyroid regions and pieces of caudal muscle were removed. Whole blood, plasma and serum samples were prepared for scintillation counting and I^{127} determinations. Meanwhile in the isolated thyroid regions the ventral aorta and proximal part of the branchial arteries were rapidly exposed, dissected out with surrounding tissues, homogenized and digested for 24 hrs at 37° C in pancreatin. Aliquots of this hydrolysate were used for counting, micro-iodine determinations and chromatographic separation of thyroid metabolites. Similarly, pieces of caudal muscle were hydrolyzed prior to counting and I^{127} determinations. Between late May and middle September, as fish attain sexual maturity, the iodine contents of blood and thyroids fall to as much as a third of their original values in both males and females. During this period the total serum iodine (I^{127}) decreased from 34.0 to 11.0 μgm I per 100 ml. The organically-bound fraction of the serum decreased from 23.7 to 6.7 μgm I/100 ml. A peak of total serum radioiodine appeared at 24 hrs after injection with an average of 0.1% of the injected dose per ml in all three groups of adults. The thyroid gland I^{127} content decreased from 49.4 to 17.1 μgm I per thyroid gland. On the other hand, muscle I^{127} remained constant throughout at 0.5 μgm per gm of tissue. The I^{131} or I^{125} thyroidal uptakes indicate maxima of 0.9 to 3.6% (average at 2.0%) of the injected dose at 336 hrs (2 weeks) following injection. In May and early August the rates of uptake appeared identical and it is only in mid-September that a slowing of this rate can be noted. Paper chromatography shows a slow incorporation of labelling in intra-thyroidal iodo-amino acids as compared to mammals and chicken. In early August 10% of the glandular I^{125} is in the thyroxine fraction at 72 hrs following injection. By mid-September identical distribution of labelling can be noted eventhough the rate of thyroidal uptake appears to have dropped. There are no significant sex differences in thyroidal parameters examined.

THYROIDAL I^{131} UPTAKE DURING POSTNATAL ONTOGENESIS IN RATS.

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Several studies on the ontogenesis of thyroid in rat have reported that morphological and functional differentiation is finished already during the fetal period and that fetal thyroid is under the control of the fetal pituitary. Present work follows the growth of the thyroid and the quantitative changes in the I-131 uptake in the course of the maturation of the organism during postnatal ontogenesis from birth up to 50 days of age. The weight gain of the thyroid during the first 2 weeks remains low. A rapid increase in the values of the absolute and relative weight begins in the 3rd week; peak values observed at 20 days are 16.7 mg/100 gm of body weight. The rate of growth in 4th week declines and the progressive rise in body weight after 30 days is accompanied by a lowering in the values of the relative weight to adult level /8.13 mg per 100 gm at 40 days/. A similar trend was observed also in tests with I-131. Thyroidal radioiodine uptake is low during the first 2 weeks /mean values 1.45 % per 1 hr. per total thyroid gland/ and increases fourfold during the 3rd week; these values expressed per weight unit of gland show a twofold increase /from 11.6 % per 10 mg per 1 hour at 13 days to 21.2 at 20 postnatal days/. The values remained steady during the 4th week and after 30 days declined in the same way as in the case of the relative weight. The 24-hrs uptake takes on a similar trend only when the data are expressed per total thyroid. Mean values were: 11.5, 50.5, and 32,0 for day-1, day -20, and day-40 respectively. When expressed per 10 mg of thyroid gland weight these values showed an opposite trend, namely very high values during the first postnatal period /343.5 % per 10 mg in new-borns/ and abruptly declined in the 2nd week /112.1 % per 10 mg/. These discrepancies suggest that some extrathyroidal factors may intervene in I-131 metabolism during the 24 hours time interval. A separate experiment revealed that I-131 may be transferred from the young to the mother by the latter's sucking their urine, pointing to the possibility of I-131 recirculation via the milk. In vitro uptake of I-131 by thyroidal tissue showed a similar trend as in preceding tests, the peak values in the day 20 and again a significant decrease occured by the end of the first month /when the data were expressed as % per 1 hour per 10 mg/. All these results point to the marked increase in thyroidal activity during the 3rd postnatal week, which suggests that the maturation of regulatory mechanisms takes place at this period.

THE UPTAKE OF THYROXINE BY THE ISOLATED, PERFUSED RAT LIVER.

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The uptake of I^{131} labeled L-Thyroxine (T_4) by the isolated, perfused rat liver was followed over a 3-hour period. The perfusate consisted of whole blood diluted to 60% with Gey's Balanced Salt Solution. I^{131} labeled L-Thyroxine was added to the perfusate in concentrations ranging from 0.94 to 2.45 x 10^{-8} M. Samples of perfusate were withdrawn from the perfusing system at 1/2 hour intervals.

The uptake of T_4 by the perfused rat liver is very rapid, reaching a maximum by 90 minutes. The uptake per gram of liver (expressed as per cent of total initial dose) increased from 1.79 for the lowest concentration to 4.14 for the highest. The uptake for the whole liver ranged from 32 to 65% of the total initial dose.

Free and protein-bound T_4 and T_4 metabolites were determined in plasma samples after 0.90, and 180 minutes of perfusion by Sephadex filtration. Free and bound T_4 in plasma decreased rapidly for the first 90 minutes of perfusion and decreased only slightly for the second 90 minutes.

An almost linear increase in plasma iodide was noted. The plasma iodide did not rise above 0.14% of the total initial dose. however. Since there was an almost negligible amount of iodide in the initial perfusate, which had been incubated with the hormone 12 hours prior to perfusion, it appears that the plasma iodide is due to hepatic degradation of T_4. No other degradation products of T_4 were detected in the perfusate.

Red cell uptake of T_4 increased markedly during the course of the perfusion. A seven-fold increase was noted between 0 and 180 minutes. The total uptake by red cells was low, however, ranging from 0.22 to 0.44 per cent of the initial total dose per ml.

It is concluded that bound and free T_4 are in equilibrium in the perfusate and that the level of free hormone determines the rate of penetration. (Supported in part by grant NSF-G21534 from The National Science Foundation.)

THE EFFECT OF PROPYLTHIOURACIL AND THYROID STIMULATING HORMONE ON GLUCOSE OXIDATION IN THYROID TISSUE. Philip F. Mulvey, Jr. and D. Ward Slingerland, Radioisotope Service, Veterans Administration Hospital, Boston, Massachusetts.

Previous observations that small amounts of propylthiouracil increased theuptake of radioiodine and the consumption of oxygen by rat thyroid slices led to a study of the effect of propylthiouracil on the metabolism of C^{14} labeled glucose. In the absence of medium glucose, a high concentration of propylthiouracil (10^{-3}M) in the medium diminished the production of $C^{14}O_2$ by rat thyroid slices, at 1 hour, from both glucose -1-C^{14} and glucose -6-C^{14}. However, with small doses of propylthiouracil (10^{-8}M), the production of $C^{14}O_2$ from glucose -6-C^{14} was markedly stimulated ($p < 0.001$) whereas the production of $C^{14}O_2$ from glucose -1-C^{14} was still inhibited ($p < 0.001$). There was no significant difference in the incorporation of glucose -1-C^{14} or glucose -6-C^{14} into either thyroidal glycogen or lipid in the presence of small amounts of propylthiouracil.

In vivo results also indicate a significant increase in $C^{14}O_2$ production from the oxidation of glucose -6-C^{14}, measured in vitro, in rats pretreated with small amounts of propylthiouracil for 4 to 6 days. The production of $C^{14}O_2$ from glucose -1-C^{14} was not significantly different from the control.

Thyrotropin stimulated the oxidation of glucose -1-C^{14} to $C^{14}O_2$ but had no significant effect on the production of $C^{14}O_2$ derived from glucose -6-C^{14}. The results suggest that small amounts of propylthiouracil stimulate the production of $C^{14}O_2$ from glucose -6-C^{14} by modifying the various pathways of carbohydrate metabolism in thyroid tissue (possibly by a stimulation of the Embden-Meyerhof glycolytic pathway) and that this stimulation is qualitatively different from that of thyrotropin.

DIFFERENCES IN ACCUMULATION OF ^{131}I IN THE THYROIDS OF SHARKS AND LIZARDS

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We have demonstrated together with Drzewicki /1935,1936/ the existence of certain differences between the thyroids of dog-fish and lizard as to their efficiency to evoke the molt of lizards, the selachian thyroid being a little less effective. In preliminary experiments I have examined the ability to accumulate the radioactive iodine in order to analyze the above statement. In the majority of cases the lizard thyroid /Lacerta agilis, L. viridis/ gave remarkably more counts per minute of γ-radiation than did the gland of the dog-fish, Scylliorhinus canicula, /comp. the table/.

Animal, sex	Whole thyroid γ-radiaton, counts per minute /particular series/							
Scyll. ♂♂	14	0	16	25	101	38	92	156
Scyll. ♀♀	305	1213	52	146	373	145	159	85
Lacerta ♂♂	794	319	1423	615	-	176	1142	23
Lacerta ♀♀	720	545	1020	673	814	64	384	107

Already Gunthorpe said /comp. Allen, 1929/ that "the Thyroid gland of all forms of vertebrates studied - fish, whale, sheep... can be used to induce metamorphosis" /of amphibian larvae/, "and each has a specific potency which is not always proportional to its iodine content...". In this connection special attention must be paid, for instance, to the relative weakness of the autoradiographs of endostyle of Ciona /Barrington, 1957/, the low effectiveness of that organ of Amphioxus in evoking the metamorphosis in axolotl /Sembrat, 1952, 1953/, as well as the lack of ability to synthesize the thyroid hormones and their precursors in Glossobalanus /Roche, Rametta, Salvatore, 1961/ - at one side -, and the propriety of organic binding of iodine occurring even in lower chordates - on the other.

Endocrinologists have devoted some attention to the problem of zoological specifity of some hormones. Beach /1958/ e.g. says that "it is only reasonable to assume that some differences exist in the pituitary and gonadal hormones of the various vertebrate classes", though Medawar /1953/ is of the opinion that "endocrine evolution is not an evolution of hormones but an evolution of the uses to which they are put". At any rate, several observations seem to prove that some differences in the thyroid hormonal agents do exist, both quantitative and qualitative ones, and their bearing on development and evolution should be studied more thoroughly /Sembrat, 1962/.

PITUITARY - THYROID RELATIONS IN DEVELOPING TURTLES.

Sister Marie Therese Dimond, Department of Biology, Trinity College, Washington 17, D. C.

Developmental function of the thyroid gland has been studied in four species of turtles, *Caretta caretta caretta*, *Chelydra serpentina serpentina*, *Chrysemys picta marginata*, and *Terrapene carolina carolina*.

During the second quarter of development the thyroid cells are organized into follicles, and iodine, as tested by the use of I^{131}, is bound. Pituitary stimulation begins shortly after the mid-stage is reached, for the administration of either potassium perchlorate or thiourea results in marked thyroid hypertrophy and hyperplasia. At this time also pituitary beta cells, the presumed source of thyrotrophic hormone, can be distinguished with Gomori's aldehyde fuchsin (AF) or periodic acid Schiff (PAS) staining. Although fluorescent dyes such as acridine orange, acridine yellow, and alcian blue differentiate basophils from acidophils, they do not distinguish functional types.

Prolonged treatment with a goitrogen is accompanied by the enlargement and partial vacuolation of the beta cells and the development of intrapituitary cysts with extremely large cells filled with AF-positive and PAS-positive droplets. After nine weeks of such treatment (about five weeks beyond the normal hatching time) the thyroid shows no sign of regression nor the pituitary of exhaustion, indicating a great reserve of thyroid and thyrotrophic function during development.

RELATIONS BETWEEN THE THYMUS AND THE STRIATED MUSCULATURE.

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To the frog thymical extract perfused in 1% conc. intensificates the gastrocnemian mechanical work of 40%, yet the optimal work increases of 100 g. The secouse height is greater and the relaxation period is reduced. The extract has also the propriety of accelerating the recovery of the tired - or treated with the lactical acid - muscles but it is ineffective upon the intoxicated with monoiodacetical acid muscles. Those results are more evident for a frequency of 30 direct excitations a minute. The positiv effect of the concentration increase. In the case of indirect excitations the muscle enters in contracture for a 3% conc.,yet with direct excitations for a 5% conc. This phenomenon of proportionality is observed too, upon the reflex time, the powerful doses being curing. The dialysed extract without Kalium, has a stimulating effect upon the ergogram.

30 days after the ethymisation, the white Rats gastrocnemian muscle presents histologically a parenchymatous atrophical myopathy and the P^{32} incorporation reduces itself of 55%.

In the insulated diaphragm the glucose incorporation diminishes of 21%, yet the glycogen synthesis of 18%. The oxygen consommation (Warburg) of the gastrocnemian is reduced of 26%, but its colinesterasical activity increase of 21% opposite to the witnesses. The muscular chronaxy is reduced for the ethymised animals, but the chronical cure with extract increases it for the normal ones.

From the mecanograms analysis results: a fall of 38% of the ergogram work; a fall of the secouse height and a lengthening of the relaxation period; the mecanical work with rolling band is of 42% mean more reduced for the thymectomised animals.

COMPARATIVE CYTOLOGY AND CYCLICAL CHANGES OF THE PANCREATIC ISLETS. August Epple, Zoolog. Institut der Universität Frankfurt/Main, Germany.

The investigations deal with following questions: 1. What is the significance of the various cell types of the pancreatic islets which have been described in many species of different systematic rank: are there only 2 sources of hormones (A-cell producing glucagon, B-cell origin of insulin) which offer a series of morphologically distinct pictures depending on their functional stage or exist more than 2 hormone producing cell forms? 2. Is there an annual cycle of the pancreatic islets like in other endocrines and if so 3. how do the cellular components of the islets behave during this cycle, and 4. how is this cycle correlated with the activity of other endocrines.

Since the morphology of the bird pancreas is especially suitable for histophysiological studies the author investigated the annual cycle of the pancreatic islets in the European Blackbird (Turdus merula). It was found that the various cell types of A- and B-tissue are functional stages of the A-, B- or D-cell and that the facts speak in favour of an autonomous function of the D-cell. During the annual cycle the islets behave contrary to all previous speculations: the 3 functional independant cell forms show parallel activity with peak in postbreeding season and trough during wintry fat deposition, but other birds with 2 annual peaks of fat accumulation (preceeding migration) show other correlations between islet activity and obesity.

Further studies on the comparative islet cytology in various classes of vertebrates as well as an investigation of the annual periodicity in mammals (1 hibernating and 1 nonhibernating species) and in a teleost species are under way and will be reported at the congress.

XVI INTERNATIONAL CONGRESS OF ZOOLOGY

CONTRIBUTED PAPERS SESSION

12A. EVOLUTION

TAXONOMY AND SPECIATION IN PARTHENOGENETIC POPULATIONS OF THE FRESHWATER PLANKTON. Umberto D'Ancona, Inst. of Zoology, University of Padua, Italy.

Planktonic freshwater organisms can be easily diffused from one biotope to another by means of atmospheric agents, birds, man, and by other ways. This transport accounts for the wide distribution of lake planktonic species and the absence among them of endemic forms. Therefore, the appearance of new forms in a lacustrine biotope does not depend upon the diffusion opportunities, which in general are easy, but on their possibilities to settle in the new basin and to insert themselves in the preexisting planktonic communities. When the immigrated form meets with suitable physical environmental conditions, its development and further multiplication will depend on the competition with the preexisting forms occupying similar ecological niches.

The problem of settlement acquires peculiar aspects for the planktonic groups having a prevailing or exclusive parthenogenetic reproduction, such as Rotifers and Cladocerans. When reproduction is exclusively parthenogenetic, also in the case of diploid parthenogenesis, genic recombination is limited and genetic variability negligible for taxonomic purposes. As a consequence, rather than good species having the typical genetical structure peculiar to amphigonic organisms, the parthenogenetic ones represent only more or less independent clones. Among them breeding occurs only in connection with the amphigonic cycles, which are frequently intercalated by a long series of parthenogenetic generations.

Immigrated new clones when settled down in a preexisting community, can therefore : a) be eliminated in the vital competition, b) maintain themselves as independent forms, c) be assimilated in the preexisting forms in connection with amphigonic cycles.

In the latter case the genetic structure of a given species can be modified. Therefore, planktonic parthenogenetic species can assume in different lake-basins peculiar phenotypic aspects, and can undergo changes in subsequent times.

Such kind of modifications may occur more easily when changed environmental conditions favour the settling down of immigrated clones and rise the frequency of amphigonic reproduction. A similar condition occurred during the lowering of the water level in lake Nemi (Rome) with following changes of its thermic system and of the shore vegetation. Phenotypical changes have been observed during the same lapse of time in Cladocerans (Volterra-D'Ancona) as well as in Rotifers (Parise).

These are in our opinion the main factors responsible for the chaotic distinction of species among the above groups, as well as for their great variability and instability. The effective value of different species as taxonomic units can only be tested by experiments of amphigonic breeding.

SUBSPECIATION IN THE CAMEL CRICKET *CEUTHOPHILUS GUTTULOSUS*.

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Intensive study of 24 characteristics of *Ceuthophilus guttulosus* Walker led to the selection of ten characteristics for which geographic variation significantly exceeds local variation. The four subspecies established on the basis of these characters exist in nature; they are not artificial entities constructed by the investigator. Implications regarding subspecies concepts are discussed.

For each characteristic the range of variation for *C. guttulosus nigricans* Scudder and for *C. g. thomasi* Hubbell falls within the combined ranges of variation for *C. g. guttulosus* Walker and *C. g. angulosus* Eades. This, along with other evidence, shows that the former two subspecies arose by hybridization between the latter two subspecies. The hybrid types now represented by *nigricans* and *thomasi* became stabilized over large areas because historical events permitted them to expand into large areas of newly suitable habitat. With additional evidence, including peculiar disjuncts where one subspecies occurs on both sides of another subspecies, it is possible to reconstruct the history of subspeciation. This history is closely related to the Pleistocene distribution of the mesophytic deciduous forests of eastern North America, the habitat of *C. guttulosus*.

POLYMORPHISM IN THE PAPILIONIDAE (INSECTA: LEPIDOPTERA).

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The genus Papilio (sensu lato) has been so widely collected that adequate series are available for the study of polymorphism. Many kinds of polymorphism can be demonstrated in this genus. The most outstanding are sexual dimorphism, seasonal dimorphism, genetic polymorphism (mimicry), and geographic polymorphism (allopatric subspeciation). The extent of polymorphism is not the same in all sections (groups) of the genus, nor in various zoogeographic areas of the world. It is possible to demonstrate indices of polymorphism for species, species complexes, subgenera and zoogeographic regions. The mode of calculation is to take the number of polymorphic variants and divide by the number of species. This gives an index of polymorphism. The indices of polymorphism vary from a high of 131 in the African dardanus group to a low of 1 (i.e. no polymorphic forms). The world index for the entire genus is 4.24, for the Oriental region 5.18, Ethiopian region 6.18, Palearctic region 2.67, New World 2.51.

There is a remarkable similarity between the indices obtained for the three subgenera. The world index for the Aristolochia forms is 3.52, for Fluted forms 3.85, and the Kite forms 3.68. For the Oriental region, the indices are 4.70, 5.68, 4.94 respectively. For the African region the indices are 3.00, 7.59, and 4.23. For the Palearctic region the indices are 2.20, 2.90, and 2.40. For the New World the indices are 2.39, 2.40, and 2.81. In any region, the Aristolochia forms are always the least polymorphic. With the exception of the New World, the Fluteds are always the most polymorphic. It is noteworthy that the Fluted subgenera contain the greatest number of species.

In all three subgenera the indices are highest for the Oriental region and lowest for the Palearctic region, provided we exclude Papilio dardanus and its forms in our calculations for the Ethiopian region. If we contrast the indices obtained for species in the Oriental region with those in the entire New World (a good contrast, for the number of species in each area is nearly the same), we see how well this phenomena is developed in the Oriental region in contrast to the New World. For the three subgenera the figures are (Oriental region/New World region): Aristolochia forms 4.70/2.39, Fluted forms 5.68/2.40, Kite forms 4.94/2.81.

THE POPULATION BIOLOGY OF THE CHECKERSPOT BUTTERFLY, EUPHYDRYAS EDITHA. Paul R. Ehrlich, Department of Biological Sciences, Stanford University, Stanford, California.

Gene flow among parts of the colony under study is greatly limited by intrinsic barriers to dispersal. Population size and total area occupied seem to vary independently from year to year in different parts of the colony. The relationship between population size and overall variability is discussed.

FURTHER STUDY ON THE EVOLUTION OF THE GOLDFISH, CARASSIUS AURATUS.

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The wild form of the goldfish or "Chi-Yu " is a common silver gray colored fresh water food fish in China , and many Asiatic countries . One fossil specimen was found in the upper Pliocene at Taiku of Shansi province in the Central China. It was interested to note there were no marked differences in the skeleton between the fossil of many hundreds thousands years ago and that of the recent wild Carassius auratus .

The first reliable red colored goldfish was recorded between 968-976 of the 10th century at Kashing city in Chekiang province of East China . The earliest colored goldfish or "Chin-Chi-Yu" was but a wild form food fish in red color .

The multicolored goldfish was recorded in 1189 . The paired caudal fins (splitting of the lower caudal fin rays) forms did not exist before 1506. The protruding eyes (dragon eye) probably recorded in 1590, the slightly short bodied forms, and various paired caudal fins, near the end of the 16th century (1596). More colors and color combinations were recorded in the 16th and 17th centuries. The first transparent scale or " crystal fish " was recorded in the early of the 17th century (1621- 1627).

In the 18th century there were many records of goldfish pictures. The earliest 3 goldfish pictures in the world were printed in the Imperial Encyclopedia, Peking,in 1726. One of them was dorsalfinless. The colored goldfish pictures were first printed in Haarlem in 1765. Among them several vestigial dorsalfin forms were shown . The most valuable color goldfish pictures were the 92 drawings painted by unknown Chinese in 1772 , in Peking , and they were reprinted in color in 1780,Paris. From these pictures, the forms of fins , the normal and protruding eyes, the long and the short bodied forms , and many color patterns,could be easily identified.

In the 19th century, new mutations, lion-head (1846) , goose-head (1899) , and extra protruding eyes (skyward eye in 1895) were recorded. In the early scores of the 20th century "Narial-bouquest "(or nasal ball), "pearl scale", "out-folded-operculum" and " water-bulble-eyes " were recorded in 1925. The extra long fins and short bodied modern types also existed.

The history of the mutational changes of the goldfish , from its fossil specimen in the upper Pliocene to the modern forms of present days, is rather complete. It is the best chronologically recorded fish in the animal kingdom.

POPULATION STRUCTURE OF THE ALL-FEMALE GYNOGENETIC TELEOST, MOLLIENESIA FORMOSA (GIRARD). Klaus D. Kallman, Genetics Laboratory, New York Aquarium, New York Zoological Society, New York.

The females of *Mollienesia formosa* reproduce by gynogenesis after being fertilized by males of closely related species. The descendants of any *M. formosa* female are genetically alike and constitute a clone. In the absence of genetic recombination, new clones may arise only by the gradual accumulation of mutations. A population, therefore, should exist as "families" of clones, each of which is composed of closely related clones. The number of clones that make up such families and the degree of their genetic differentiation appears to depend primarily upon the mutation rate, the time when the population became established and the effective population size of the family involved.

Although all female *M. formosa* collected in nature look alike, the existence of clones can be demonstrated by immunological methods. Tissue grafts survive only when exchanged among members of the same clone. Within limits, the mean survival time of grafts exchanged among members belonging to different clones can serve as a measure of the genetic difference between them.

The clonal structure of a population of *M. formosa* in a small stream near Brownsville, Texas, has been investigated during the last three years (Kallman, *Evolution*, 16: 497-504, 1962). Several hundred females were collected at four points within 5 miles of each other. Cross transplantations were performed among them and their offspring. Several clones were identified, three of which were recovered at all four stations and were also represented in each collection. More than 50% of all fish collected belonged to clones V and VII and clone VI accounted for somewhat less than 8% of the total sample. Other clones were not found at all stations or were not represented in all collections.

Evidence for the existence of clonal families was obtained. For example, clones V, VI and VII reject each other's grafts within one or two weeks and probably differ by many genes. Fin grafts from another clone (identified, so far, from a single fish) into clone V hosts survive on the average for several months, however, which indicates close genetic relationship, perhaps a single gene difference.

The descendants of less than two dozen fish collected in this stream in 1954 were tested in 1960 and 1961 and identified as belonging to clones V and VII. The clonal structure of this population appears to have remained more or less constant for at least 8 years. How far these clones range geographically must be determined by further collections. (Supported by grants C-4945 and CIA 06665 from the U.S.P.H.S. and grant G 21947 from the N.S.F.)

MICROEVOLUTIONARY MECHANISMS IN SOME AMPHIBIANS AND REPTILES FROM ROUMANIA. Bogdan Stugren, Dept. of Biology, Babeş - Bolyai University, Cluj, Roumania.

Recent investigations has shown that in Amphibians and Reptiles, the variability is multifarious. Geographical races appear when spatial discontinuity is great and some phaenotypic features, of foundamental taxonomic significance, are interdependent variables. Clines are frequent in Amphibians and Reptiles (Terentjew, 1957; Kauri, 1959). Geographical races and clines are not the unique forms of variability (Stugren, 1962). Two special forms of variability were described: interclinal (Stugren & Popovici, 1961)and anticlinal (Stugren, 1961, 1962). The microevolutionary mechanisms which act on the level of those peculiar forms of variability are discussed in this paper.

When the species, like Anguis fragilis L., is divided in geographical races, the microevolutionary process follows the classic way through spatial segregation.

In cline - species, like Lacerta vivipara Jacq., (Stugren & Vancea, 1961), most phaenotypic features are independent variables. Living in the same biome - type, on a continuous area, the populations are exposed to different biotic pressures, corresponding to differences of communities. Natural selection acts here in a stabilizing form.

Interclinal variability in Bombina - species, leads in the zone of overlapping of the ranges of european species, to the rise of populations with asymmetrical and irregular (random) distribution of the traits of both species. Natural selection acts here in a dynamic form.

In Lacerta praticola Eversm., the anticlinal variability shows a convergence of evolutionary trends at the geographic extremes of species - range.

I conclude, that in Amphibians and Reptiles, microevolution is not always realized through geographical races.

EFFECTS OF INTRODUCED TROUT ON THE EVOLUTION OF NATIVE FROGS IN THE HIGH SIERRA NEVADA MOUNTAINS. Brother Lawrence Cory, F.S.C., Biology Department, St. Mary's College, California.

Previous reports (Cory,1962. Am. Zool., 2(3):401 and 2(4):515) have described evolutionary patterns in morphology, life-history and some elements of behavior in ranid frogs of the high Sierra Nevada. Interactions between these animals and trout seem evident also, and many of these interactions appear important in modifying the actively occurring changes in the frog populations. Primitively trout were native in very few places in the high Sierra, and none of these were in the broad band of lakes alongside the main Sierra crest at altitudes of roughly 9,000 to 11,000 ft. Three species of trout (the rainbow, Salmo gairdneri; the golden, Salmo aguabonita; the eastern brook, Salvelinus fontinalis) have been widely introduced in these lakes and most of them now support good fish populations.

In general wherever there are well-developed trout populations (i.e., in virtually all of the lakes big enough to maintain fishable numbers) there are few or no frogs. In the smaller bodies of water which generally maintain frogs in normal population structure fish are typically absent. This situation is attributed to predation by the fish on the frogs at some stage or stages in their life-cycle. Some exceptions have been found, however. Most frequently this involves patches of ecologically complex shore-line; this type of situation will not be considered here.

The most interesting type of coexistence is one in which the frogs show a unique behavior pattern. As with this species of frog in all of its populations, the animals spend most of the day out of water, though always close to its edge -- usually within one jump of the water, into which they plunge when disturbed. The action here described involves an instantaneous and sharp "U-turn" upon their striking the water, which brings the frogs at once in among the narrow crevices in the type of "rock-wall" structure that has always been found where this behavior occurs. That this may be a genetically determined reaction rather than a learned one or a response to the physical conformation of the immediate environment is suggested by the fact that tadpoles in these populations also show a behavior pattern that is unique and which involves instant movement into rocky crevices rather than swimming out towards deeper water, as is more generally characteristic of tadpoles of this species.

If predation on the frogs by the fish actually occurs, then the present well-nigh universal occurrence of fish in the Sierra streams reinforces isolation of frog populations by reducing potential introgression among them. That it actually occurs seems almost certain in view of the recent discovery of enormous frog populations in a series of large lakes from which fish are absent. Experimental procedures are being designed for these lakes.

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GEOGRAPHIC VARIATION AND EVOLUTION IN NORTH AMERICAN HOUSE SPARROWS (PASSER DOMESTICUS). Robert K. Selander, Dept. of Zoology, University of Texas, Austin Texas, and Richard F. Johnston, Museum of Natural History, University of Kansas, Lawrence, Kansas.

Research is currently in progress to determine the degree to which the House Sparrow, which was introduced into the United States approximately 110 years ago, has diverged morphologically from the European stock under the pressure of selective forces in a variety of climatically and otherwise environmentally different regions in North America. The study involves measurement of the degree and rate of adaptive response of populations of this species as reflected in a large number of characters of color and size in massive series of specimens collected in 20 major areas in North America and Europe and segregated with respect to season of collection, age and sex.

A preliminary analysis of variation in 2000 specimens collected in October, 1962, demonstrates that significant geographic differentiation in color and in size has occurred since the species was introduced into North America. The smallest birds in North America are from Death Valley, California, and the largest are from Lawrence, Kansas. Birds from Texas are on the average larger than those from California but smaller than those from Kansas. In color, Death Valley specimens are palest dorsally and show an unusual degree of whitening of the cheeks in the male. This population has diverged from the English and German stocks to a greater degree than has any other North American population sampled to date. Using any one of several characters of color, the specimens from Death Valley are 100 per cent separable from all specimens from England and Germany, as well as from those from Lawrence, Kansas. Birds from the San Francisco Bay area are very dark dorsally and have the flanks grayer than those of birds from Sacramento and Los Angeles. A surprising example of differentiation on a local scale is seen in Louisiana, where a sample of specimens from Zachary, 23 miles north of Baton Rouge, shows strong development of a ventral "wash" of yellow that is absent, or at best weakly indicated, in birds from Baton Rouge.

In general, geographic variation in the House Sparrow follows trends manifested by native polytypic species, and, on this basis, most of the variation is presumed to be adaptive. Particularly conspicuous is a trend for pale coloration in arid environments. It is evident from this preliminary analysis that geographic differentiation in Passer in North America has in a period of 110 years reached a level which is equivalent to that generally considered subspecific by taxonomists.

ENVIRONMENTAL DETERMINANTS OF ENDEMISM AND SYMPATRY FOR THE DARWIN FINCHES IN THE GALÁPAGOS ARCHIPELAGO. Terrell H. Hamilton and Ira Rubinoff, Department of Zoology, The University of Texas, and Harvard Biological Laboratories.

We have recently used multiple regression analysis to consider the general evolutionary problem of the relative importance of the various factors governing the proliferation of related subspecies or species on member islands of an archipelago. For the Darwin finches (Geospizinae) in the Galápagos Archipelago, we ask the question: Why, in this bird group of restricted distribution and of monophyletic origin, is there a tendency for many species and few endemic subspecies to occur on some islands, and for few species but more endemics to occur on other islands? The question relates to the negative relation (correlation coefficient or $r = -0.60$) between insular number of species and insular number of endemic subspecies.

By the method of least squares, the following factors are tested for their values in predicting either insular numbers of geospizid species or endemic subspecies: (A) insular area, (P) plant diversity as measured by insular number of land plant species, (IN) isolation as measured by distance from nearest island, and (IC) isolation as measured by distance from Indefatigable Island, near the center of the archipelago. For predicting numbers of geospizids on individual islands, these multiple regression equations have been found:

insular no. of species = 9.8 + 0xA + 0xP - 0.1xIN - 0.02xIC
insular no. of endemics = 0.2 + 0xA + 0xP + 0.09xIN + 0xIC

These conclusions are drawn from the multiple regression analysis:

1. Insular area (A) and number of land plant species (P) are of no value in accounting for insular variations in numbers of either species or endemic subspecies.
2. Only isolation (IN), as measured by shore-to-shore distance from nearest island, is of significant value in predicting insular numbers of endemics. Such is confirmed by analysis of variance. This is not surprising since r for insular numbers of endemics and IN is +0.91. For such numbers and IC, r is +0.44.
3. For predicting insular number of species, both IN and IC are useful, with the former being the more important according to analysis of variance. Together the two factors leave unexplained a significant part of the variance of insular number of species. This is attributed either to error in our measurements and techniques or to factors not here considered.
4. That isolation (IN) positively predicts best variation in insular numbers of endemic subspecies confirms Lack's conclusion (1947), and negates Bowman's differing conclusion (1961), that the more isolated islands tend to produce more endemic subspecies than the less isolated ones.
5. Our findings demonstrate the fundamental importance of geographic isolation as a factor which controls endemism and, to a lesser degree, insular variations in the number of sympatric forms for a monophyletic group radiating within an isolated archipelago.

EVOLUTION IN A MOUSE-OF-PASSAGE: DEMOGRAPHY AND THE DYNAMICS OF t-ALLELES IN SOME COMMENSAL HOUSE MOUSE POPULATIONS. Paul K. Anderson, Dept. of Zoology, University of Alberta, Calgary, Alberta, Canada.

House mice establish populations of small size in farm granaries on the Canadian prairies. These populations are isolated from each other, reproductively, through behavioral barriers. More than one-half of all populations tested, and slightly less than one-half of all individuals, carry lethal or male-sterile t-alleles with high transmission ratios.

It is postulated that these populations are short-lived and that the demographic pattern is one of continuous establishment and extermination of small isolates. Under this hypothesis, the frequency of high-transmission-ratio t-alleles would be determined by the relative frequency with which new populations were founded by carriers as opposed to mice homozygous for the wild-type allele. This amounts to intergroup selection, since infected and uninfected populations are competing in establishment of new colonies. The hypothesis is being tested by comparison of t-allele frequencies in resident and wandering mice.

X-RAY DIFFRACTION PATTERNS OF EGG SHELLS AND THEIR POSSIBLE USE IN SPECIATION. J. R. Howes, Dept. of Poultry Science & A.N.J. Heyn, Dept. of Physics, Auburn University, Auburn, Alabama.

Although the microscopic structure of avian egg shells has been extensively investigated by means of conventional light microscopy, there is a lack of information relating to the ultrastructure of egg shells as revealed by X-ray crystallography. The only reports of which the authors are aware relate to very brief studies by Mayneord (1927) and Foulkes, et al. (1958).

In 1961 a study was initiated at Auburn University to investigate the crystalline structure of egg shells by X-ray diffraction, and to determine the effects of various environmental and dietary treatments upon egg shell structure. The calcium carbonate of several species of avian egg shells was found to occur in the form of calcite. Diffraction patterns of egg shells were found to exhibit three predominant features- asterism, discontinuous Debye-Scherrer rings, and a clear orientation effect (Cain & Heyn, 1962). Asterism was often found to be associated with breaks in the Debye-Scherrer rings and its incidence was correlated with crystal size. These conclusions relating to the X-ray structure of avian egg shells have been corroborated by electromicroscopy (Heyn 1962).

Comparative X-ray diffraction patterns of the egg shells of several species are presented. The X-ray patterns were prepared using standard conditions, but the lengths of irradiation were proportional to the thickness of the shells so as to counteract absorption. Egg shells of several avian species including the ostrich, domestic goose, turkey, duck, guinea fowl, domestic fowl, coturnix quail, and humming bird were investigated together with eggs from some species of turtle and tortoise. Investigations were also made of various parts of the same egg shell and shells of different species that has been ground to equal thickness.

A comparison of the occurrence of asterism and of Debye-Scherrer rings indicated large differences in crystal size between comparative parts of the egg shells of the various species, and also between different areas within the same shell. It is expected that this technique may be of value in evaluating egg shell strength and in studies directed towards the improvement of egg shell strength for economic purposes. Furthermore, X-ray diffraction patterns of egg shell should be useful in determining genetic relationships and speciation.

EVOLUTION AND SPECIES-BOUND DIFFERENCES OF ANIMAL NEOPLASMS

Hans Elmar Kaiser, Saskatoon, Canada

The development of tissues as shown and proved by evolution and in fossils is able to give us an answer about the phylogenetic appearance of different types of tumors. According to phylogenesis we have to assume that the epithelial tissue is older than the other tissues. Indeed, the lowest metazoans are nothing but ectoderm and entoderm. In the passage of millions of years the varieties of tissues developed. A disease can never be older than the tissue in which it occurs. As special examples I would like to single out chondromas, osteogenic tumors, and adamantinomas. E.g., chondromas cannot have formed in the cambrian, osteomas could never have arisen before bone tissue made phylogenetically its appearance in the silurian age and adamantinomas were found after the arising of this kind of tissue.

All neoplasms are a phenomenon of biology and cancers may belong to the oldest diseases, may in fact have existed since metazoan life began. With this specialization in progressive development, specialization in the nature of benigne or malignant tumors appears quite logical. In addition to the phylogeny of tumor appearance species differences are very important for a general understanding of neoplasms. It seems interesting to quote here the frequent cystadenomas of ovaries in Koala bears, frequent lipomas in parakeets, the rare malignant tumors in whales or to compare the frequency of breast cancer in women (38% of malignancies) with that in cows (0.8%). The separate development of several animal groups during the millions of years gives us a part explanation to species differences in oncology and will open new avenues in research of experimental comparative oncology.

Reference: Kaiser, H.E.; Nordmann, M.: "Vergleichende Epidemiologie der pflanzlichen und tierischen Neoplasmen." (A book in preparation.)

ERYTHROCYTE ANTIGENS AND AVIAN EVOLUTION. Danilo Mainardi, Istituto di Zoologia, Università di Parma, Italy.

On the basis of their genetic determination, the red-cell antigens common to two species can be considered as substances derived directly from a common progenitor and specific antigens as a track of past evolutionary history. Consequently, the larger the number of common antigens, the closer, in a phylogenetic sense, will the two species be.

Utilizing the two ratios between the titres with homologous and heterologous cells with the sera against the species under examination, a formula (of the immunological distances) was obtained which gives valuesthat increasewith diminution of immunological affinity (that is of common antigens) and which consequently may be utilized as an index of the degree of parentage between the species examined. This value gives an indication of the sum total of a series of characters (antigens) each of identical genetic weight, and taken as a wholeit can therefore be regarded as an ideal polygenic character, since the genes which determine it are all appreciable and distributed over nearly all the chromosomes and since it has absolutely no kind of phenotipic variability. Besides, in contrast with many other characters, it does not run the risk of false interpretation due to evolutionary convergence, given the specificity of the immunological reactions.

In the main, three problems of avian taxonomy and phylogeny have been studied usingthe method of the immunological distances: the relationships of Fringillids and their kin, the phylogeny of Gallinaceous birds, the relationships of Flamingos with Ciconiiformes and Anseriformes. Immunological diagrams were constructed which served to make clear the relationship between the species under examination, and in some cases have made it possible to give, at various taxonomic levels, a more natural systematic arrangement of certain species.

In order to define subspecific entities the frequencies of blood groups can be used. Immunological studies can not give us any real indication for delimitating the species, because the present concept of species, based on criteria of reproductive isolation, can not logically be defined by other criteria, even though they are perfectly valid for defining degrees of relationship and consequently the other systematic categories. On the contrary immunological analyses are perfectly apt for recognizing a species, and so are very exact means of finding out the wild progenitor(s) of the tame races, of testing the hybridity hypotesis and of identifying the parental species of "presumed hybrids".

MORPHOLOGICAL EVIDENCE OF POPULATION INTERACTIONS BETWEEN CLOSELY RELATED PLANKTONIC COPEPODS. Abraham Fleminger, Marine Life Research Group, Scripps Institution of Oceanography, University of California at San Diego, La Jolla, California, U. S. A.

Increased interest in interactions between closely related populations has greatly accelerated progress in understanding ecological and evolutionary processes that shape the adaptive properties of species. Taxonomic studies of populations would expedite initial investigations of multiple-species systems in zooplankton. The large, temperate to tropical, calanoid copepod genus, Labidocera, provides especially favorable material; species are numerous, mostly neritic, include omnivores and carnivores and have elaborate species-specific copulatory devices.

Eleven species occuring in the Western Hemisphere are restricted to the relatively isolated warm coastal waters of the Americas. Six species occur along the Pacific coastline and five along the Atlantic-Caribbean coastline. Three Pacific and three Atlantic-Caribbean species comprise an advanced monophyletic line. The other three Pacific species constitute a more primitive monophyletic grouping. The two other Atlantic-Caribbean species are related, also primitive and apparently distant cognates of the primitive Pacific group.

These patterns probably stem from recent events. Miocene-Pliocene trans-American seaways and Pleistocene glacial and interglacial fluctuations could have afforded Labidocera circumstances for population range expansion and contractions. And the large peninsulas (Florida and Lower California) extending across contemporary biogeographical boundaries could have combined with major ocean currents to provide the isolating mechanisms permitting genetic divergence.

The advanced group is dominant on both coastlines, measuring evolutionary success by population density and geographical range. The primitive groups are comparatively rare, have smaller ranges and are restricted to more inshore waters. They suggest increased specialization and retreat into a refugium.

Character divergence within groups includes superficial differences in copulatory structures and spermatophores. Character displacement appears in several body length relationships. Feeding appendages remain essentially similar. Species within groups are essentially allopatric.

Character divergence between groups include fundamental differences in copulatory mechanisms and feeding appendages. Inter-group species can be sympatric.

Contemporary relationships among American Labidocera suggest a history of sequential divergence originating in allopatry. Character displacement completes the process of population differentiation following contact between expanding related populations.

XVI INTERNATIONAL CONGRESS OF ZOOLOGY

CONTRIBUTED PAPERS SESSION

12B. EVOLUTION

ПРОБЛЕМА РУДИМЕНТАРНЫХ ОРГАНОВ В ЗООЛОГИИ.А.В.Яблоков.Институт морфологии животных им.А.Н.Северцова.Академия Наук СССР.Москва.

Присутствие на месте развитых задних конечностей наземных млекопитающих двух небольших косточек у Cetacea служит примером рудиментации органов.На примере строения тазовых костей Delphinapterus leucas Pall. докладчиком показывается,что и у самцов и у самок эти образования принимают активное участие в функционировании совокупительного аппарата.Несмотря на значительное упрощение этих костей по сравнению с таковыми предковых форм,они не утрачивают значения для организма,а лишь изменяют свое значение.Несмотря на упрощение этих костей,их нельзя называть рудиментами.

К аналогичным выводам приводит и рассмотрение особенностей строения "остатков волосяного покрова" Mystacoceti.Исследование строения вибрисс Balaenoptera musculus L.,B.physalus L.,B.borealis,Eschrichtius gibbosus показало,что они имеют сложное строение;их распределение на голове различных Mystacoceti неодинаково у разных видов и разных полов.Аппарат вибрисс должен рассматриваться как высокоспециализированный орган чувств,развившийся как приспособление к своеобразному типу питания.

Основной предлагавшийся ранее критерий при определении органа как рудиментарного заключается в относительно меньшем развитии органа по сравнению с таковым у предковых форм.Последовательное применение этого критерия не дает возможности провести границу между органом специализированным и рудиментарным.

Так как строение органа определяется его функциями,правильнее рассматривать филогенетические изменения функции,а не строения органа,и говорить о рудиментации соответствующей функции. Связывать же изменение всего органа исключительно с изменением только одной из присущих ему функций /что предполагается при определении какого либо органа как рудиментарного/ -совершенно неверно.

LE PROBLÈME DES SOIT-DISANTES HYPERTÉLIES. Prof.dr. N.Botnariuc, Facultatea de biologie, Universitatea Bucureşti, Romînia.

L'état actuel des opinions concernant le probléme des hypertélies, malgré les discussions qui divisent les biologistes, n'a pas encore abouti à une explication satisfaisante. En effet, il est nécessaire de partir du fait que l'objet de la sélection est constitué par la population ou l'espèce, considérées en totalité et non par les individus isolés. Tandisque la variabilité est un processus au niveau de l'individu, l'évolution et donc l'adaptation sont des processus historiques, qui ont lieu au niveau de la population ou de l'espèce (bien entendu par l'intermède des individus). En adoptant ce point de vue, il devient clair que les différents traits de l'organisation individuelle, effets de l'action historique de la séleotion doivent être utiles en premier lieu pour l'espèce. Ils peuvent même, parfois être nuisibles pour les individus isolés.

La plupart des "hypertélies" sont le résultat des différentes formes de la sélection intraspécifique.La sélection sexuelle est l'une de ces formes. Certaines des formations "nuisibles" sont le résultat de l'interaction entre la sélection intraspécifique et la sélection interspécifique.

On pourrait faire une classification plus acceptable de ces catégories de phénomènes (morphologiques , physiologiques, éthologiques etc)selon les différentes formes de la sélection intraspécifique, selon le degré de nocivité individuelle de certains caractères et leur degré d'intégration et d'utilité pour l'espèce.

En conclusion, nous pensons qu'il n'y a pas d'hypertélies dans le sens strict de ce terme, c.à.d. des formations "excessives", nuisibles aux espèces dans l'état de nature. Si quelques biologistes qualifient certains caractéres d'"hypertéliques",c'est qu'ils interprètent subjectivement, voire même abusivement, des structures insuffisamment connues sous l'aspect historique, écologique, éthologique, ou bien ils confondent deux réalités très distinctes - l'individu et l'espèce les lois caractéristiques pour chacune de ces réalités, ainsi que leur interdépendance.

ANAGENESIS AND THE PROCESSES OF EVOLUTIONARY PROGRESS. Osvaldo A. Reig, Dept. of Biology, Buenos Aires University, Florida 656, Buenos Aires, Argentina.

It is possible to distinguish three different evolutionary processes in the somewhat vague idea of evolutionary progress: 1) The perfection of a given structural type within the qualitative invariance of the general morpho-physiological plan, excluding the emergence of evolutionary novelties originating radical transformations in the 'exploitive systeme' of Waddington; examples of this are the general change occurred within the Actinopterygian fishes from Palaeoniscoids to Acanthopterygii, in Primates from Tupaioids to Apes. 2) The emergence of morpho-physiological or behavioral novelties implying qualitative changes in the structural plan and opening further possibilities of evolution at higher level of biological efficiency, i.e. creating new major taxa and furthering the exploitation of radically new adaptative zones. Examples are the emergence of Labyrinthodonts from Crossopterygians, the rising of Mammals from Synapsids, of Man from advanced Hominids, etc. 3) The fitting of a particular lineage to an extreme adaptative niche, creating a specialized structural type to well defined ways of life. The ammount of evolutionary change leading from Hyracotherium to Equus is a classical example.

All these processes are included by Huxley under the concept of anagenesis. When proposing this term, Rensch excluded explicitly from it our third meaning, but involved in it both improvement of organizational types and the 'inventions' (Erfindungen) of evolutionary novelties, though he realized that perfection (Vervollkommung) and general progress (Höherentwicklung) are different phenomena. Waddington noted the need to distinguish the different aspects of anagenesis, proposing to refer to 'open anagenesis" and 'closing anagenesis'. The need of a general name involving all types of progressive evolution, makes necessary to maintain Huxley's wide extension of the concept of anagenesis. But if the three processes mentioned above are actual, objective paths of evolution, as it appears from the known record of the history of life, their disctintion in the conceptual level must be explicitly accompanied by a distinction in the semantical level.

I propose, therefore, to coin teleogenesis (i.e. the genesis of perfection) for our case 1), arogenesis (i.e. the genesis of new levels of evolution) for case 2), and idiogenesis (i.e. the genesis of specialized particularities) for case 3). These three concepts are relative and depend on the context: a teleogenetic change in the ordinal level may be arogenetic in the family level. All three may be explained by evolution through gradual change under the pressure of natural selection, without advocating any other special force. I prefer the term arogenesis to the term aromorphosis of Sewertzow (in some sense equivalent), because there are not only morphological changes involved in this process. The term arogenesis is also preferable in view of maintaining uniformity of terminology.-

ФОРМИРУЮЩЕЕ ВЛИЯНИЕ ТИПА ОПОРЫ НА СТРОЕНИЕ И ФУНКЦИЮ КОНЕЧНОСТЕЙ МЛЕКОПИТАЮЩИХ. В.Г.Касьяненко, Отдел эволюционной морфологии, Институт зоологии, Киев.

Из трех основных типов опоры современных млекопитающих /стопо-,пальце-,фалангохождение/ наиболее близким к исходному типу, свойственному тероморфным рептилиям и первичным плацентарным млекопитающим, является стопохождение. С нарастанием скорости наземного передвижения к двучленной системе рычагов стопоходящей конечности прибавляется у пальцеходящих третий и у фалангоходящих - четвертый члены, с одновременным уменьшением относительной площади опоры.

Каждому из трех основных типов опоры соответствует свой тип строения суставов, мышц и сосудов конечностей. У современных стопоходящих большое значение имеют претерпевшие смену функции рудименты исходной семилучевой кисти и стопы, различно используемые при поступательных и иных движениях. Наши теоретические построения о путях развития специфических приспособлений в скелете и мышцах при переходе к иному типу опоры в филогенезе подтверждаются экспериментами, вызывающими смену прикрепления и функции мышц и перестройку суставного рельефа в дистальных звеньях конечностей 4-5 -палых пальцеходящих. Каждому из трех типов опоры отвечает свой тип кровоснабжения конечностей. Установлено преобладание поверхностных артериальных магистралей /и в области метаподия и пальцев -дорсальных сосудов/ у стопоходящих. Преобладание глубоких сосудистых магистралей /и в области метаподия и пальцев - волярных/ плантарных /сосудов/ характеризует переход к пальце-,фалангохождению.

Изученные в нашей лаборатории зависимости между типом опоры, строением и функцией органов движения и их кровоснабжением дают возможность составить более глубокое представление об организме, как целом, а также получить новые данные о сходстве и различиях в ряде гомологичных систем в свете их адаптивной перестройки в онто- и филогенезе. /Диссертации и последние публикации Отдела- С.Ф.Манзий,П.М.Мажуга, А.Н.Щегольков,В.Г.Касьяненко, 1961, 1962/

PROBLEMS OF THE EARLY HISTORY OF THE MOLLUSCA. Ellis L. Yochelson, U. S. Geological Survey, Washington, D. C.

New classes may be necessary to accomodate early Paleozoic fossils which differ from Recent Mollusca. Viewed in retrospect from Recent time, the combined geologic record of the six extant molluscan classes shows irregular but definite decrease, both in numbers of individuals and generic diversity. Throughout their range, representatives of Pelecypoda, Gastropoda, and Cephalopoda are abundant and diverse, whereas those of Amphineura, Monoplacophora, and Scaphopoda are uncommon and restricted.

Amphineura and Cephalopoda first occur in uppermost Cambrian rocks; Gastropoda first occur in middle-late Cambrian rocks, and may be the oldest extant class. Absence of preserved soft parts raises question of phylum affinities for certain enigmatic Cambrian fossils, but their hard parts show features considered representative of Mollusca. Most Cambrian mollusks are not clearly assignable to extant classes. Approximately five percent of named Cambrian species and a smaller percentage of genera are probable mollusks.

In 1962, Cricoconarida and Calyptoptomatida, were proposed for exclusively Paleozoic mollusks. Cricoconarida, most common in the middle Paleozoic, lack many common molluscan features and may be incorrectly assigned to phylum. Calyptoptomatida do show molluscan characteristics and deserve general recognition as an extinct seventh class. Representatives of Calyptoptomatida are most common and diverse in the Cambrian, comprising more than half of the Cambrian Mollusca.

Other Cambrian mollusks are rare and show little specific and generic diversity. Unpublished information indicates that Stenothecoides (Cambrian), formerly assigned to Monoplacophora, is a bivalve, and that Matthevia (Upper Cambrian), formerly assigned to Calyptoptomatida, is a multi-valved animal. These and Pelagiella (Cambrian-Lower Ordovician), a form having a rapidly expanding coiled shell, are morphologically distinctive. Each may be the sole representative of additional extinct classes. Other atypical molluscan-like genera are too poorly known to classify.

An adaptive radiation of Early Cambrian mollusks to fill limited numbers of ecologic niches is suggested. Because of their primitive and inefficient anatomical organization, these early forms were unable to survive a Late Cambrian and Early Ordovician radiation by more advanced mollusks. If this hypothesis is correct, Monoplacophora constitute a primitive group of more advanced mollusks, and not strictly part of the basic molluscan stock.

THE EVOLUTION OF ARTHROPOD CUTICLE. M.S.Ghilarov and L.M.Semenova, Institute of Animal Morphology, Moscow, USSR.

The cuticle in Arthropods is a multifunctional structure (exoskeleton, barrier surface, surface of gas and ion exchange, surface protecting against dessiccation etc.). This is connected with its complex chemical composition and with complex differentiation of its texture. When the cuticle performs the respiratory function its texture is rather simple. If it is differentiated principally as a protective layer, especially against dessiccation, it has a complex microscopic structure. In this latter case it is differentiated consisting of the supporting endocuticle, mechanically firm exocuticle and relatively impermeable epicuticle which are discriminated by studying differentially stained sections. Water and wet soil dwelling Arthropods having soft covers and able to skin respiration have the cuticle differentiated into the endo- and exocuticle only, the same texture being characteristic of arthropod gills too. The firmness of the cuticle in Crustaceans, Diplopods and some Stratiomyid-larvae is due to $CaCO_3$ incrustation, determining convergent similarity of their tegument structure. Land Arthropods adjusted to life in deserts have the similar differentiation of the exocuticle consisting of several differently dense strata reducing the heating of body. Inferior land Arthropods connected with soil and other moist substrates have the primitive type of cuticle consisting of two layers (endo- and exocuticle). The transition to the open terrestrial life inthe atmosphere with the deficit of moisture depends on the development of the epicuticle protecting from water loss. In Arthropods able to leave shelters but temporary the epicuticle is better developped on the sclerites, especially on dorsal surface. The development of the epicuticle in higher groups of terrestrial Arthropods only and differences in its chemical composition and physical properties in different groups prove that the resemblance of this structure in different branchs of land Arthropods is of a convergent analogous character but not of homologous one; parallel series of the development of the epicuticle are to be observed in Arachnids, in Chilopods, in Collembola, in Pterygota. The use of the term "epicuticle" when applied to the outer layer of the cuticle of Malacostraca is the source of unjustified comparison with the epicuticle in insects and some other land Arthropods, because these structures are neither homologous nor analogous. The study of the cuticle in Arthropods shows that the study of convergent structures is of value for the comprehension of the regularities of evolution.

EVOLUTION OF THE FOOT COMPLEX OF CAVE COLLEMBOLA. Kenneth A. Christiansen, Dept. of Biology, Grinnell College, Grinnell, Iowa.

The collembola of caves show a wide variety in the structure of the foot complex. The existence of forms in caves showing all degrees of adaptation for cave life, combined with the striking convergence in form brought about by the unusual requirements of cave survival permits a detailed analysis of the changes which have occurred. These changes display a striking relationship among form, function and behaviour which leads to the conclusion that evolution here involves a progression of changes in behaviour and morphology. Each change in morphology is preadaptive for a change in behaviour and conversely each change in behaviour is preadaptive for a change in morphology. The possible general significance of this progressive preadaptation is discussed.

PRONOTAL WINGS IN BLATELLA GERMANICA (L.) AND THEIR POSSIBLE EVOLUTIONARY SIGNIFICANCE. Mary H. Ross, Dept. of Entomology, Virginia Polytechnic Institute, Blacksburg, Virginia, U. S. A.

The present paper describes the morphology and development of wing-like extensions (pro-wings) on the pronotum of the German cockroach, Blattella germanica. The trait is of particular interest since wing flaps on the prothorax are a characteristic of the oldest known fossil pterygotes, and their occurrence is generally accepted as evidence that wings began as extensions of the pterothoracic paranota. The possibility that the development of pro-wings recapitulates a step in the evolution of wings is strengthened by their occurrence in a Blattid, in view of the generalized morphology and long, relatively constant geologic record of this group.

A description of the basic and branch paranotal tracheation of the thoracic terga is included. The development of branch trachea in both normal pronota and wing buds is compared with that in the pro-wings. Homology is proposed between the tracheal wing loops and the pronotal trachea giving rise to the trachea and veins of pro-wings. Possible homologies of the trachea and veins of pro-wings with those of the wings are indicated. A theory for the evolution of wings is postulated on the basis of the study of pro-wings. This is compared with the more generally accepted theories of wing evolution.

THE ORIGIN AND FUNCTIONS OF THE COELOM. R. B. Clark, Dept. of Zoology, University of Bristol, Bristol, England.

There is an overwhelming correlation between the existence of a patent coelom in animals and its use as a hydrostatic skeleton. In its simplest and, it is argued, most primitive form, the coelom serves as a hydrostatic skeleton about which the body-wall muscles act antagonistically. This is exemplified by the differences in structure and movements between planarians and coelomate worms. The former can change shape in exactly the same manner as coelomates, the parenchyma being pressed into service as a fluid skeleton, but it offers resistance to deformation and changes of shape are relatively slow, and the animals are incapable of performing substantial amounts of external work. Locomotion is by cilia or waves of contraction in the ventral longitudinal muscles, neither making demands upon the fluid skeleton. In particular, planarians are unable to burrow. Coelomates, having a true fluid skeleton, can perform relatively fast changes of shape involving considerable deformation of the fluid skeleton, such as peristaltic locomotory movements, they can perform a considerable amount of external work and, above all, they can burrow in the substratum. Most coelomate worms do so. Subsequent changes of locomotory technique may result in secondary loss or occlusion of the coelom (e.g. leeches, ? molluscs) or in its adaptation to new functions (e.g. a circulatory system in leeches and brachiopods).

The evolution of a secondary body cavity, primarily with locomotory and mechanical functions had manifold consequences. The existence of this space permitted, among other things, the accumulation of gametes over a long period of time in a manner not available to non-coelomates. Coelomates were thus able to evolve brief, annual breeding seasons which could be geared to environmental events with improved breeding success without loss of breeding potential. This demanded synchronization of breeding activity within populations of a species and a delay in sexual maturation vis à vis somatic growth. It is precisely these phenomena that are under the control of hormones in the lower coelomates, and these animals appear to have the simplest endocrine systems of any we know. Indirect consequences of the evolution of the coelom may thus include the evolution of precise breeding seasons and the increased emphasis upon sexual instead of vegetative reproduction.

HOPLONEMERTINE ANCESTRY AND VERTEBRATE HEAD STRUCTURE.

Donald D. Jensen, Dept. of Psychology, Bloomington, Indiana, USA.

Currently popular theories of the origin of the vertebrates derive craniate vertebrates from acraniate chordates. These views offer no reasonable explanation for the curious and constant organization of the vertebrate head, i.e., for the presence of and relations between the preoral gut, Rathke's pouch, anterior somites, notochord, pituitary, neural plate, neural crest, paired eyes, olfactory organ, and labyrinths. An alternative view is advanced, it being suggested that vertebrate head structure is derived very directly from the structure of the head of hoplonemertines, complex free-living carnivorous worms of the phylum Rhynchocoela. The above-mentioned vertebrate head structures are held to be homologous with hoplonemertine structures, i.e., secondary stomodaeum, muscular proboscis, proboscis sheath and associated structures, glandular proboscis, frontal organ primordium, lateral placodes, inverse retina eyes, frontal organ, and cerebral organs, respectively. The relations among vertebrate head structures is phylogenetically explained by the similar relations of homologous structures in the hoplonemertines. The embryological, anatomical, and histological evidence relevant to these homologies is reviewed and the major embryological changes which would be required to produce primitive vertebrate head organization from that of the hoplonemertine are outlined. These are increased growth and backward extension of the frontal organ or neural plate, fusion of this structure with the lateral placodes or neural crest, replication of the foregut expansions which form the secondary stomodaeum in the hoplonemertines and the mouth and gills of the vertebrates, and retardation of mesodermal migration into the proboscis apparatus or notochord until after foregut expansions are developed. The most anterior foregut expansion is presumed to induce lateral growth of the neural plate material to form the optic vesicles and the lateral migration of mesodermal material to form the anterior somites. The large number of similarities between hoplonemertine and vertebrate organs and the small number of embryological alterations necessary to derive primitive vertebrate head organization from that of the hoplonemertines strongly suggests that the hoplonemertines are the invertebrates most closely related to the vertebrates. Resemblances are maximal between eumonostyliferous hoplonemertines and myxinoid cyclostomes and it is suggested that the hoplonemertines gave rise to early myxinoids, which are accordingly the most primitive of the vertebrates. This view, in sharp contrast with the theory of acraniate ancestry, connects the vertebrates with forms which show no evidence of degeneration and which are clearly related to still more primitive forms. The ancestry of vertebrates thus includes hoplonemertines, paleonemertines, and primitive flatworms.

USE OF GROWTH RATE OF PALEOZOIC AND MESOZOIC REEF CORALS AS A FUNCTION OF THE PALEOLATITUDE FOR FOLLOWING OUT CONTINENTAL DRIEF AND THE COURSE OF POLAR WANDERING. Ting-Ying H. Ma, Dept. of Geology, National Taiwan University, Taipei, Taiwan, China

Paleozoic and Mesozoic reef corals show features of seasonal growth and their annual growth values show a function to latitude similar to living reef corals. The latitudinal distribution of annual growth values of fossil reef corals of any period from the Ordovician to the Upper Cretaceous in any fossil coralline sea fragmented on the present continents are no different from growth values of similar living forms at corresponding latitudes. Only the sudden total displacements of the solid earth shell have shifted the original coralline seas of a geologic period and pushed some parts even into present polar regions that they lie no more in the tropics and subtropics, and the accompanying drifts of continents have fragmented them that they no more lie along a great circle.

With Eurasia fixed in its present mesh of longitudes and latitudes, adjusting the central line of the coralline seas of each geologic period into a great circle brings out the change of relative positions of the continents and the pole positions as follows: (1) The eastern edge of North America was originally in contact with Europe to form Laurasia and did not separate from Europe until the Upper Jurassic; the eastern edge of Africa was in contact with the western edge of India to form Indafrica separated from Laurasia by the Tethys Sea. The northeastern margin of South America was in contact with Indafrica and its east coast was still east of 5°E. in the Ordovician with its southern end contacted to the Antarctican end of Australantarctica whose Australian end was linked with the forelands of Southeast Asia, including South Africa, to form a great continental arc bounding the yet small Indatlantic Ocean Basin which expanded as this arc moved out step by step due to the shake up time and again by the sudden total displacements of the solid earth shell to diminish the Paleo-Pacific that surrounded all the continents with its more than hemisphere extent. (2) One of the two pole positions, in present longitude and latitude, for each period with respect to the equator represented by the central line of fossil coralline seas is: Ordovician, 10°S., 35°E.; Silurian, 15°S., 25°E.; Devonian, 23.5°S., 25°E.; Lower Carboniferous, 20°S., 40°E.; Upper Carboniferous, 20°S., 20°E.; Permian, 30°S., 35°E.; Upper Triassic, 20°S., 15°E.; Upper Jurassic, 45°S., 50°E.; and Upper Cretaceous, 35°S., 25°E.

The similar distribution of annual growth values of fossil and living reef corals proves the permanency of climate on the Earth. Thus zoology is the record of the time axis of existence and not only the display of life phenomena.

ON THE PHYLOGENY OF METAZOA AND THE ORIGIN OF DEUTEROSTOMIA.

Radu Codreanu, Invertebrate Zoology, Faculty of Natural Science, University of Bucharest, Rumania.

The animal kingdom developed according to a double rythm of evolution: 1) the macroevolution having generated by ontogenetic innovations the prototypes of the phyla and the ascending line of the phylogenesis; 2) the specializing evolution with disjunctive effects which begining from the generalized, synthetic potentialities of the prototypes, realizes the inner diversification of the large groups, chiefly by orthogenetic radiation producing the numberless lineages actually known. The essential of the macroevolution being accomplished before the fossiliferous periods and the most archetypes undergoing extinction, the phylogenetical reconstitutions are especially based on comparative anatomy and embryology.

The first Metazoa, issued from coenobia of heterotrophic Flagellata, were planuloid organisms, diploblastic, ciliated and phagotrophic, with monaxial symmetry, able to become bilateral, their locomotion passing from swimming to reptation. That was, though not with the same evolutionary success, the founder of Coelenterata, perhaps that of the enigmatic Porifera and Mesozoa, as well as of the bilateral, triploblastic Protostomia, at first acoelomate. The primitive genesis of the mesoblast was done by means of mesenchymal teloblasts, leading to schizocoely, and not at all by enterocoelous process, for even the Ctenophora, which are considered to be at the origin of the last, are however forming their cross shaped mesoderm from entodermic primordial cells. This is in accordance with the fact that in metazoan ontogeny, the entoderm itself arises more primitively by cell proliferation than by invagination. The sedentary state, solitary or colonial, is certainly secondary in all the Metazoa, from the Zoophyta to the Vandel's "Polypiform Coelomata" (Lophophorata, Pogonophora, Pterobranchia), a plainly polyphyletic assemblage, reductible to a pure convergence, and too highly organised for having preceded the major bifurcation into Protostomia and Deuterostomia. The origin of Deuterostomia cannot be found out at their level, no more at that of the Coelenterata (Cuénot, Beklemishev, Remane), which were directly followed only by the lower acoelomate and pseudocoelomate Protostomia, coming from the parenchimatous macrophagous Turbellaria. Just like Coelenterata, the blastopore becomes here the mouth, which is the single digestive opening.

The true Archicoelomata as the Sipunculoidea, Echiuroidea and oligomerous Archiannelida, representing the remains of an important evolutive knot, are those which engendered the polymerous Protostomia (Annelida, Arthropoda), the other Trochozoa as the Mollusca and the sessile Lophophorata, as well as the Deuterostomia, these last phyla keeping the ancestral oligomery. Here are some prooves: the blastoporal origin of the buccal and also of the anal orifices in the Polychaeta; enterocoelous formation of the coelom in the Brachiopoda, although closely related to the spiralian Phoronida; trimerous coelom in all the Deuterostomia; presence of nephridial solenocytes in Amphioxus; mesodermal metamerism in Annelida and Cordata. As far as the threshold of Cordata, the Pogonophora, Stomocordata, Echinodermata and Tunicata followed aberrant evolutionary directions. There are only the Chaetognatha and Enteropneusta having kept some features of the primary mobile Deuterostomia, which arose from trimerous eucoelomate protostomous ancestors.

A NEW MATHEMATICAL METHOD FOR THE QUANTITATIVE RESEARCH OF THE CARYOTYPE. Mihai Şerban and Cantemir Rişcuţia, Institute of Speology, Academy R.P.R. and Institute of Legal-Medicine, Bucureşti, Romania.

In a paper read at the Session of the Center for Anthropological Research, Academy RPR., Bucharest, June 21-23 1962, we analyzed the connexion between the mechanism of sexed generation and the distribution of the chromosomes (Chr.) resulted from the 4 grandparents (generation P2) in the genome of a certain grandchild F, taking into consideration that the new combinations come according to statistical laws. Hence follows the existence of qualitative diversities between the haploid sets formed by an even number (N=2n) and those formed by an odd number (N=2n-1) of Chr. Using the following formulas we have calculated some coefficients which characterise from a quantitative point of view the qualitative differences between numbers: even (1) and odd numbers (2) of Chr.

$$2/2^N \sum_{m=0}^{N/2-1} m/n.C_N^m + 1/2^N.C_N^{N/2} \; ; \; (1) \text{ in which } m+n=N$$

$$2/2^N \sum_{m=0}^{[N/2]} m/n.C_N^m \; ; \; (2) \text{ in which } [N/2] \text{ means whole part of } N/2$$

These "coefficients of stability" separate the whole numbers into two classes: even and odd numbers. According to DOBZHANSKY 1937, 2374 flowering plants present 68,5% species of haploid sets formed by an even number of Chr., while 66,5% of more then 4000 HeLa cells (E.SAKSELA 1962) present a heteroploid number which is even too. If we consider generation P4, we get 4 classes of numbers. Flowering plants as well as HeLa cells present numbers divisible by 4 in a significatively greater frequency. This analogy between the numerical structure of the caryotype in two very different biological forms is made evident by the new method, and can lead to a better understanding of the biological significance of the chromosome numbers in plants and in animals. To the qualitative differences of the numbers, taken as mathematical conceptions, there are qualitative differences between the number of Chr. as biological units. There is a mentioning of the relations between the evolution of the caryotype and ecology with an application to man, whose greater quickness of evolution may be explained partially by reduction of one unity of the caryotype from the 3 species of related apes to N=23 chromosomes.

THE NICHE AND NATURAL SELECTION. M. R. Warburg, Dept. of Zoology, University of Adelaide, Adelaide, South Australia.

As a result of studying the ecology and physiology of some terrestrial isopods an hypothesis was developed regarding two different parts of their niches. These parts were called the 'habitual' and the 'extreme' parts meaning the common and the rare situations respectively. This was based on a study of the microclimates in which the isopods live and on their reactions towards environmental stimuli as well as their body temperature and rate of water loss. The difference in these aspects are in fact differences in the way they utilise their niches. It was suggested that while one species has evolved physiological adaptations to the extreme part as well as to the habitual, the other species was able to survive in the extreme part only by means of behavioral adaptations.

It was further suggested that natural selection takes place in the extreme part of the niche where some populations survive only precariously until they have evolved suitable physiological adaptations. It can be therefore expected that divergence from the common stock will occur in that part with the ultimate result of splitting.

As a result of studies on the faunas of deserts in different regions, it was suggested that the fact of similar selective forces in the similar habitats may be the cause for similar forms by the action of natural selection taking place within the extreme part of the niche where similar extremes occur.

RECENT ADVANCES IN OUR KNOWLEDGE OF MELANISM IN THE LEPIDOPTERA.

Dr. H. B. D. Kettlewell, Genetics Laboratory, Oxford.

During the last two years I have reached tentative conclusions on two sets of experiments which have been in progress during the last seven years. The first is in connection with the "break-down" and "build-up" of dominance in Industrial Melanism. I have been able to show that there is a "built-in" mechanism in the gene-complex of British Biston (Amphydasis) betularia L. which ensures almost full dominance of the character "blackness", even in populations where the black form carbonaria was in the recent past maintained at mutation level only. Secondly, we have demonstrated that there is a clear-cut habit difference in the wild in Shetland in the moth Amathes glareosa Esp. (and its black form edda) between the northern and southern populations which are in continuity over the distance of only sixty miles.

The Build-up of Dominance

The North American Peppered Moth Amphydasis cognataria Guenee and British Biston (Amphydasis) betularia L. interbreed freely in the laboratory. If not conspecific, they are very closely related. Each has melanic forms: f. swettaria Barnes & McDunnough in America and f. carbonaria Jordan in Britain where it has largely replaced the light form in industrial populations of this species and far beyond. f. carbonaria was first reported in 1848. Some of these earliest specimens were not as extreme in their blackness as the average carbonaria today. Nevertheless they were black (not grey) and always completely clear-cut from the light f. typica.

If f. carbonaria is crossed into North American typical stock, and if the resulting melanics are outcrossed into pure American wild type for four generations, a complete gradation of insects appears in such broods. None of the melanics are as black as wild f. carbonaria, and the majority are pale grey and speckled. Some examples are hardly recognisable from cognataria f. typica. This, then, demonstrated the break-down of dominance. Attempts were made to re-build dominance by crossing intermediate melanics with British betularia f. typica. It was hoped to reconstitute dominance by introducing the original modifiers after a few generations. In all broods of the first generation near-complete dominance had been achieved - clear-cut segregation had returned. This can only mean that modifiers were present in British betularia f. typica, and furthermore that they must be dominant. As the origin of the British Peppered Moths was industrial (Birmingham), it could be argued that these modifiers could have been built up by recent Natural Selection in such an environment. Alternatively it could be that they were universal throughout the British population. To test this I repeated the experiments by crossing fifth generation "break-down melanics" with stock from Devon where f. carbonaria does not occur. About 75 offspring have already hatched this year. Once again clear-cut segregation has occurred in the first generation. Though the majority are fully black, some are slightly less so than the offspring of industrial origin: they have a few white scales on an otherwise black forewing.

It appears therefore that the modifiers are dominant and that they are probably universal throughout the British population. As in my opinion Melanism in cryptic Lepidoptera is a constant recurring need for a multitude of environmental situations, such dominant modifiers present throughout the population would ensure almost complete dominance at each inception by mutation. It provides a short cut to all-blackness without the lengthy process of build-up by Natural Selection on each occasion. It is interesting to speculate how these modifiers survive in the population during the long periods when melanic forms offer no survival advantage!

Habit Differences between the North and South Shetland Populations of the moth Amathes glareosa Esp. (and its black form edda)

Melanism in Shetland is widespread. As these Islands have changed but little in the last 1000 years, it is highly unlikely that these melanic forms have spread recently. During the last 5 years nine of us have undertaken extensive field work here on the common moth Amathes glareosa and its melanic f. edda. The two forms are distinct. A large-scale survey (of over 30,000 specimens) showed that there was a cline from North to South Shetland. edda in the North (Unst) forms 90% of the population. Conversely the light grey typica with its two disruptive black marks occurs at a frequency of 98% in the South. Theories on the underlying causes of this situation have already been published. In the course of our work when we undertook extensive mark-release-recapture experiments it became evident that northern edda were much less active than southern typica. I at first thought that diminished flight activity was associated with edda only. Subsequent work (unpublished) does not support this. In north Shetland where edda is practically invisible as it rests on peat by day but typica less so, the species is subject to quite unusual selection pressures due to the passage of large numbers of migratory birds at this season which arrive from Scandinavia and Iceland. Similar bird concentrations, awaiting favourable winds, occur in South Shetland where areas of sand dunes favor the light form.

Over-all appraisal of the situation appears to be that in North Shetland, which is subject to constant gales, flight is hazardous through the danger of being blown out to sea: edda rests secure on a universal background of peat. In South Shetland where there is less peat, more sand and fewer headlands, the insect conforms to the more usual pattern of behaviour and, as in England (which we confirmed) flies every night.

By sampling marked insects of both forms at MV traps we showed that the majority of recapture of both forms from the South took place on the first night after release. In the North, however, more individuals of both forms were recaptured on the second and third nights after release than on the first.

This polymorphism is therefore maintained by a balance of visual and flight advantages and disadvantages together, no doubt with the physiological differences which both forms confer apart from their differing coloration.

XVI INTERNATIONAL CONGRESS OF ZOOLOGY

CONTRIBUTED PAPERS SESSION

13. GENETICS

AMINO ACID INCORPORATION INTO PROTEIN BY CELL-FREE PREPARATIONS OF DROSOPHILA MELANOGASTER. Allen S. Fox and Suk-Hee Kang, Dept. of Biochemistry, Michigan State University, East Lansing, Michigan, U.S.A.

Studies of protein biosynthesis in genetically controllable, multicellular organisms would be advantageous. C^{14}- or H^{3}-leucine is incorporated into protein in cell-free preparations from D. melanogaster. Mitochondria are not required and are removed from homogenates of adults or larvae by centrifugation at 15,000 Xg. Microsomes are sedimented at 125,000 Xg. SRNA and pH5 fraction are prepared from the supernatant by the phenol method and precipitation respectively. Incorporation requires microsomes, pH5 fraction or SRNA, 20 amino acids, ATP and generating system, GTP, and Mg^{++}. Total TCA precipitable counts, hot TCA insoluble counts (protein-bound), and hot TCA soluble counts (nucleotide-bound) all increase linearly for 25 minutes and reach a plateau after 40 minutes of incubation at 37°, indicating a possible requirement for replacement of mRNA. Rate of incorporation with excess pH5 fraction is linear with microsome concentration; with excess microsomes it is linear with pH5 fraction. (Supported by grants from NIH, GM 10079-09, and Rackham Research Foundation.)

CARBOHYDRATE METABOLISM STUDIES IN ADIPOSE MUTANTS OF DROSOPHILA MELANOGASTER. Winifred W. Doane, Dept. of Biology, Yale University, New Haven, Connecticut.

Carbohydrate determinations were made of adults homozygous for the recessive mutant alleles, adp^{fs} and adp^{60} (located at 2.83 on Chromosome II), of Drosophila melanogaster. Females of the former type are completely sterile; those of the latter are mostly fertile with 45-90% egg hatchability. Larvae and adults of both types are associated with hypertrophied fat bodies that characteristically contain about twice the amount of stored lipids present in wild type flies of many strains. Total carbohydrates were measured in individual flies of both sexes ranging in age from freshly emerged to 21 days. The levels were typically lower in adp^{fs} and adp^{60} homozygotes than in +/+, except for a period centered about the second day when a reverse in this relationship held true for females, but not males. Tests made to distinguish between ethanol-soluble and water-soluble fractions indicated that the mutants possess less glycogen, as well as less sugars and other alcohol-soluble carbohydrates than +/+.

Because of the effect of the mutant genotype on carbohydrate metabolism and because of the proximity of the adp locus to the Amylase locus (approx. location at 2.80±, Kikkawa, '60), tests were made of amylase activity in both mutant types in several different residual genomes and compared with a variety of wild type strains containing the normal allele. In addition, comparisons were made between various strong- and weak-amylase strains sent to me by Prof. H. Kikkawa. Amylase activity in the adp^{60}/adp^{60} lines is of the "strong" type, while that in the adp^{fs}/adp^{fs} genotype is of the "weak" type. The low level of carbohydrates and excessive lipid accumulations typical of the adp mutants were not found in the various Amy lines received. Analysis is under way to determine whether or not the strong amylase activity phenotype of adp^{60} homozygotes can be separated from the effects on lipid and carbohydrate metabolism and, if so, what effect this may have on fertility.

The adp^{60} mutant with strong amylase activity was previously introduced into a population cage with an Oregon-R wild type strain (low amylase activity) at a gene frequency of 0.5. It has now been maintained in this cage for 35 generations in a balanced polymorphic situation. Analyses are in progress to determine if the strong amylase effect is still associated with the adp effects after this long a period and what the possible significance of this association may be. (Supported by N. I.H. Training Grant 2G-397 and grant G 14747 from the N. S. F.)

ACTION OF AMIDES ON THE DEVELOPMENT OF THE BAR EYE DROSOPHILA.

F. DeMarinis and F. Sheibley, Fenn College, Cleveland, Ohio.

Kaji (1955) showed that ureids, nucleotides, and acid amides all tend to increase the facet number in Bar eye Drosophila. Later (1956) he demonstrated with acid amides that the group $-CONH_2$ was active in increasing facet number. DeMarinis and Sheibley (1960) showed that acetamide was more general in its reactions since it affected not only eye size but forked bristles and wing shape as well. They found that the amide link (-CONH-), when incorporated in cyclic structures, was less effective than in open chain compounds.

The present work reports some effects of other amides. Two percent concentrations of each of the following compounds in the food delay the developmental period of the fly: Urea-10 days, adipamide-10½ days, malonamide-11 days, n-valeramide-16 days, n-hexanamide-22 days, uracil-24 days, control-9 days. Two percent concentrations of hydantoin, 1-methylhydantoin, acetamide, propionamide, n-butyramide, methylurea, ethylurea, acetylurea, allylurea, N,N'-acetylmethylurea, biuret, N,N-dimethylacetamide, and N-methylformamide, are found to be toxic and sometimes result in incomplete development. Corresponding concentrations of uric acid, allantoin, biurea (N,N'-dicarbamylhydrazine), and oxamide show no particular effect on either the length of the developmental period or on the size of the Bar eye. The double amides, $NH_2CO-(CH_2)_n-CONH_2$, however, are found to be more effective and less toxic. Tests with two percent malonamide, two percent succinamide, and two percent adipamide increase the size of Bar eye (from 75 ± 5.1 facets to 450 ± 27.4 facets), and in a few cases the eyes become completely phenotypically wild in size (with approximately 650 Facets). In addition it is found that these double amides can be fed throughout the complete larval period without appearing to interfere with other developmental processes.

FURTHER INVESTIGATION CONCERNING THE COMPLEX PHENOTYPIC LINEARITY OF YELLOW MUTANTS X-RAY INDUCED IN SCUTE-8 CHROMOSOMES OF DROSOPHILA.

Sara H. Frye, Dept. of Zoology, University of California, Los Angeles, California.

Frye (1962) emphasized that the dose-frequency criterion is more likely to be meaningful where the germ cell heterogeneity is reduced.

Young scute-8 Bar males were X-rayed at a relatively high dose, 8000 r. Four post-treatment matings, or broods, were made every 24 hours and consisted of treated males and virgin females containing the sex-linked marker, yellow body color, and autosomal markers, echnoid eye, dumpy wing, and clot eye. Inseminated females from each of the four broods were transferred several times into fresh bottles. The treated males were removed from the fourth brood after 24 hours and were discarded. Consequently, F_1 females were derived from eggs that had been fertilized by 1-day old, 2-day old, 3-day old, and 4-day old sperm from time of treatment.

Exceptional yellow daughters were recovered among the expected F_1 Bar females. Simultaneous controls were run, and the total induced frequency was corrected. The total frequency of exceptional yellows from each (not all) of the four broods always fluctuated in the same direction, i.e., below the expected frequency for linearity (Brood I), above the expected frequency for linearity (Brood II), and below for Brood III. However, the totals of yellow mutants (from all four broods) induced in y^+ scute-8 chromosomes of mature and nearly mature sperm by 8000 r were found to exhibit approximately a linear dose-frequency relation as reported for lower X-ray doses (Frye, ibid). Possible mechanisms as to why the curve continues to rise will be discussed.

Progeny testing and a genetic analysis of the exceptional y^- sc^8 B daughters are in progress. Those yellows that prove to be male-viable with a normal Y-chromosome will be tested for an involvement with a gross structural change.

(Acknowledgement is made to the National Science Foundation for support of this work).

THE FIRST STAGE IN RETROGRESSIVE EVOLUTION OF THE Y CHROMOSOME, AS ILLUSTRATED IN THE FISH, ORYZIAS LATIPES. Toki-o Yamamoto, Biological Institute, Faculty of Science, Nagoya University, Nagoya, Japan.

In the medaka (Oryzias latipes), where normal sex-determining mechanism is XX=♀ and XY=♂, either the gene R(orange-red) or its allele r(white) is borne by either the X or the Y. Successful artificial control of sex differentiation ad libitum by heterotypic sex hormones, renders it possible to attack on problems otherwise difficult of approach. The body of evidence indicates the rarity of viable $Y^{R}Y^{R}$ versus commonness of surviving $Y^{R}Y^{r}$, which are obtainable by mating induced $X^{r}Y^{R}$♀ with normal $X^{r}Y^{R}$ or $X^{r}Y^{r}$♂. Our experiments and considerations provide us with a new insight into the constitution of the sex chromosomes. The hypothesis formulated is the presence of an inert section (-) in the regular Y^{R} and a "viability" segment (+) in the ordinary X^{r} and X^{R}. The linear order in the regular X^{r} is (x)-r-(+) and in the ordinary Y^{R} it is (y)-R-(-), where the (x) and (y) stand for the non-homologous sex-differential segments in the X and Y, respectively. Most $Y^{R}Y^{R}$ zygotes are nonviable because of their constitution $Y^{R,-}Y^{R,-}$ in which the (-) is in the duplex condition. A few viable $Y^{R}Y^{R}$ males are considered as crossovers with the constitution $Y^{R,-}Y^{R,+}_{c}$, of which $Y^{R,+}_{c}$ stands for a recombinant, viz., the (+) being derived from the $X^{r,+}$. Most $Y^{R}Y^{r}$ zygotes are viable because of their constitution $Y^{R,-}Y^{r,+}$. The Y^{r} is known to have arisen by crossing over between the X^{r} and the Y^{R}. So that a concomitant transfer of both r and (+) is possible. Further verifications of the hypothesis come from (1) the F_1 sons ($X^{r}Y^{R}$) from $X^{r}X^{r}$♀ x $Y^{R}Y^{R}$♂ comprise two genotypes $X^{r,+}Y^{R,-}$ and $X^{r,+}Y^{R,+}$, (2) nonviability of $X^{R}_{c}Y^{R}$($X^{R,-}_{c}Y^{R,-}$) versus viability of both $X^{R}Y^{R}$($X^{R,+}Y^{R,-}$) and $X^{R}_{c}Y^{r}$($X^{R,-}_{c}Y^{r,+}$) where X^{R}_{c} stands for a recombinant, and (3) viability of $Y^{r}Y^{r}$($Y^{r,+}Y^{r,+}$). Hence, the hypothesis seems to be real. From the evolutional viewpoint, it is noteworthy to find such a fish as the medaka representing the first or a transitional stage in the gradual deterioration of the Y chromosome among animal kingdom.

POSITION EFFECT AS A FACTOR IN THE PRODUCTION OF MELANOSIS AND MELANOMA IN THE FISH XIPHOPHORUS. James W. Atz, Klaus D. Kallman and Ross F. Nigrelli. Genetics Laboratory, New York Aquarium, New York Zoological Society, New York.

Hybrids of 11 species or subspecies combinations of fishes of the Genus *Xiphophorus* (Family Poeciliidae, Order Cyprinodontiformes) exhibit 8 spotted patterns with increased expressivity, including melanosis and melanoma. In each case, this atypical pigment cell growth results from the lack of mutual adaptation between a major, dominant gene from one species and a constellation of polygenic modifiers from the other (Myron Gordon, *Growth Symposium*, 10: 153-219, 1951; Atz, *Zoologica*, 47: 153-181, 1962). Similar, but not as intense effects occur in hybrids between *X. maculatus* from different, isolated river systems (Gordon, *l.c.*).

Non-hybrid *X. variatus xiphidium* and *X. montezumae cortezi* have exhibited melanosis and melanoma on rare occasions (Mac Intyre & Baker-Cohen, *Zoologica*, 46: 125-131, 1961), most likely as a result of genetic imbalance arising from artificial selection and inbreeding in the laboratory.

Position effect can also play a role in melanosis and melanoma production. A rare cross-over within the locus of the 5 major genes in *X. maculatus*, which behave as multiple alleles, placed 2 of them, Sd for *spotted dorsal fin* and Sr for *striped sides*, on a single chromosome of a fish from the New River, British Honduras (Mac Intyre, *Amer. Nat.*, 95: 323-324, 1961). The pigmentation of the original SdSr fish looked normal, but when it was crossed with a *spotted* (SpSp) fish from the Rio Jamapa, Mexico, the SdSrSp offspring exhibited an enhancement of the Sd pattern. Greater enhancement resulted when SdSrSp offspring were backcrossed to SdSd fish from the Rio Jamapa. Still greater enhancement of the Sd that was linked to Sr resulted when SdSrSd fish were again backcrossed to the Jamapa strain. More than half of the fish's sides, as well as the entire lower portion of the dorsal fin, were melanotic, and in one fish a melanoma developed. When a New River fish carrying Sd was crossed with the same Rio Jamapa strain, however, no enhancement of Sd occurred.

The polygenic modifiers of the Jamapa strain do not appear to be able to control completely the growth of pigment cells associated with the Sd gene from the New River in its altered chromosomal position.

ON THE DETECTION OF MELANOTIC TUMORS IN DROSOPHILA. Martin L. Kaplan, Dept. of Biology, Queens College, Flushing, New York, U.S.A.

The method generally used for determining tumor penetrance in Drosophila is based on the presence of 'melanized bodies' in larvae or adults as revealed by visual inspection or dissection. This technique has been employed in genetic analyses; in the determination of the effects of environmental stress; and in evaluating the tumor inducing capacities of many substances. In this report, evidence will be presented pertaining to the importance of histological methods in establishing tumor incidence values.

Analyses of serial sections in conjunction with 'black spot' counts in the tumor strain, tu-e and in a tumor-free stock, 51-52, have revealed some of the problems inherent in tumor incidence data based on macroscopic methods alone. In the tu-e strain, 'black spot' analysis indicated that 120 hour larvae, raised on Bridge's medium at 25°C., had a tumor incidence of 62% compared with 80% in adults. Serial sections of "non-tumorous" larvae revealed the presence of typical spindle-cell hemocyte tumors in the pre-melanotic phase in all animals examined. The 'real' tumor incidence, therefore, was 100% in larvae. Pre-melanotic tumors were not found in adults.

Tu-e larvae raised on Friedman's 'low tumor medium' had a 'black spot' tumor incidence of 15%. Histological analysis indicated, however, that all of the larvae examined contained tumors in the pre-melanotic stage of development. Thus the 'real' tumor incidence did not differ from the 100% obtained in animals raised on Bridge's medium.

The 51-52 strain is tumor-free when viewed macroscopically or in section. Larvae raised on Friedman's medium to which 100 mgm% indoleacetic acid was added developed clearly visible, 'melanized bodies', in the abdominal area which were similar superficially to "melanotic tumors". Histological evaluation, however, did not provide evidence for the presence of spindle-cell tumor cells. The 'melanized bodies' consisted of hindgut epithelium which contained intracellular depositions of melanin pigment. On the other hand, 51-52 larvae which were injected with crude extracts of tu-e larvae also developed 'melanized bodies'. In this instance, however, serial sections contained both melanotic and pre-melanotic spindle-cell hemocyte tumors. The hindgut epithelium appeared normal in the larvae examined.

The reliability of 'black spot' counts in larvae or adults as a measure of melanotic tumor incidence in Drosophila as well as the potential significance of histological analysis as an additional criterion in evaluating tumor penetrance will be discussed.

GENE-ENVIRONMENT INTERACTION IN DEVELOPMENT: EFFECT OF CORTISONE ON THE PENETRANCE OF AN EYE MUTANT IN THE MOUSE.

Muriel J. Watney and James R. Miller, Depts. of Paediatrics and Zoology, University of British Columbia, Vancouver, B.C., Canada.

The mouse is normally born with its eyes closed. However, there are a number of mutations which produce the condition "eye-lids open at birth". One of these, *elo*, which has occurred recently in the $C_3H/M1$ strain, is a simple autosomal recessive with about 65% penetrance in homozygotes. In an attempt to increase this penetrance, cortisone (as a teratogenic agent) was administered to pregnant females on several days prior to the known time of fusion of the eyelids *in utero*. Rather than increase the frequency of the defect, the cortisone resulted in a dramatic decrease in its frequency (to approximately 4%). On the basis of the known effect of cortisone on palate closure in the mouse (Walker and Fraser, J. Embryol. Exp. Morph. 5: 201, 1957), it is tentatively suggested that the cortisone modifies the action of the gene *elo* through the metabolism of mucopolysaccharides.

PSEUDOGAMY IN PLANARIANS TESTED BY SPERM IRRADIATION. Mario Benazzi, Dept. of Zoology, University of Pisa, Italy.

Several studies with my colleagues Benazzi-Lentati, Lepori, etc. have shown the occurrence, in some fresh-water planarian species, of polyploid races with pseudogamous reproduction (gynogenesis). In these races the egg develops only if activated by the sperm, but the sperm head does not form a male pronucleus and karyogamy is lacking. The present work deals with pseudogamous development of eggs activated by X-irradiated sperms.

Dugesia lugubris, a commonly European species, possesses a diploid amphimictic biotype and some polyploid ones, with either synaptic or asynaptic oöcytes; all the polyploid biotypes are pseudogamous. Adult specimens have been whole-body X-irradiated with doses rates ranging from 1,000 to 70,000 r and then they have been mated to normal unfertilized specimens, used as females, and belonging both to the diploid amphimictic and to the polyploid pseudogamous biotypes.

The development of amphimictic eggs is quite normal only when the specimens used as males received X-ray doses lower than about 2,000-3,000 r; with higher doses a normal cleavage also occurs and in some cases the embryonic spheric stage is reached, but shortly after the development stops. Obviously the irradiated sperms give rise to a lethal genetic constitution.

On the contrary, the development of pseudogamous eggs is normal even following the highest sperm irradiation and the offspring is completely viable.

The same results have been obtained with Polycelis nigra, an other European species; eggs of a polyploid pseudogamous biotype develop normally after receiving sperms irradiated with up to 50,000 r.

These findings prove that X-rays do not weaken at all the activating power of the sperm. Since O. Hertwig experiments (1913), the dissociation between karyogamy and activating function in amphimictic species (chiefly Amphibia) can be obtained through high sperm irradiation. Planarians in which the dissociation between the two sperm functions occurs as a normal phenomenon, afford good opportunity to investigate the effects of sperm irradiation.

IMMUNOGENETIC STUDIES OF ELASMOBRANCH FISHES. Carl J. Sindermann, U. S. Bureau of Commercial Fisheries Biological Laboratory, Boothbay Harbor, Maine.

Systematic studies of marine vertebrates, particularly investigations of subpopulations, have been materially aided in recent years by application of immunogenetic techniques. The elasmobranch fishes were found in the present work to constitute excellent material for basic studies with blood grouping methods.

Erythrocyte antigens have been detected in spiny dogfish (Squalus acanthias) and big skates (Raja ocellata) with cross-matching tests and absorbed rabbit antisera. In dogfish, strong isoagglutinins distinguished an antigen designated S_1, while absorbed antisera distinguished a second antigen, S_2. Individual fish possessed both, either, or neither antigen. Comparative studies of gravid females and their unborn young indicated that the two antigens were part of the same blood group system. Two erythrocyte antigens have been demonstrated in big skates with isoagglutinins as well as with absorbed antiserum. Individual skate sera were found, with cross-matching and absorption analysis, to contain both, either, or neither isoagglutinin. Isoimmune antibodies with less specificity have been demonstrated one year after isoimmunization. Strong natural heteroagglutinins for teleost and human erythrocytes were found in big skate sera, and a year-round study indicated that titers varied seasonally, reaching a maximum in summer and a minimum in winter. Isoagglutinins have also been demonstrated in little skates (Raja erinacea), but have not been detected in thorny skates (Raja radiata) or in barn door skates (Raja laevis). However, both thorny and barn door skates possessed strong and specific heteroagglutinins for erythrocyte antigens of certain big skates and Atlantic herring (Clupea harengus).

The smaller elasmobranchs thus possess several desirable characteristics for immunogenetic studies. They are easily maintained in marine aquaria, possess clearly defined isoantigens and isoagglutinins, and many are ovoviviparous.

EFFECTS OF FOREIGN BLOOD INJECTION IN GENETICALLY CONTROLLED CHICKENS. Pierre Leroy and Jacques Benoit, Laboratoire de Photobiologie, C.N.R.S., Gif-sur-Yvette, France.

The experiments of russian biologists about the changing of hereditary characters of domestic fowls by means of injection of foreign blood have been successfully repeated.

Rhode Island Red chickens (strain M-44) genetically controlled for more than fifteen years were regularly injected with unfractionated guinea-fowl blood. After treatment no phenotypic difference was noticeable on any of the treated birds but their progeny showed a characteristic melanisation of the plumage more intense in the third than in the second and in the first generations. This increasing effect was observed when descendants had not been injected. In that case not only the melanin increased in amount in the tail and wings, where its presence, although reduced, is normal, but practically covered the whole body in both sexes, thus obliterating regionalization. Crossing between males and females produced melanized chicks provided at least one of the mates was treated with guinea-fowl blood. Frequently dark blue green feathers are observed especially on the wings and chest. No plumage pattern characteristic of guinea-fowls was induced after the treatment ; however both egg and body weights were lighter in the modified adults than in the controls.

When descendants were treated with foreign blood as were their parents, the melanisation reached its maximum in the second generation, decreased in the third and vanished in the fourth. As far as coloration is concerned the phenotype of birds of the fourth generation is practically normal.

Isologous blood injection (RIR→RIR) had no visible effect on the different generations of the treated chickens.

These results obtained after four consecutive years of investigation verify and complete the previous experiments of russian biologists and have been recently confirmed by others. The problem as to the mechanism(s) of modifying hereditary characters in birds by means of foreign blood so far remains unsolved.

TRANSFER TO DESCENDENCY OF ALTERATIONS INDUCED IN THE WHITE LEGHORN BY REPEATED INJECTIONS OF HETEROLOGOUS BLOOD

J. Stroun, L. Stroun-Guttières, J. Rossi and M. Stroun,
University clinic of internal medicine, Geneva, Switzerland.

The authors have repeatedly injected blood from the grey guinea fowl to successive generations of cocks and hens descending from a white Leghorn strain with stable characteristics. Concurrently with this test Leghorn group, they raised a check Leghorn group treated under identical conditions with blood from white Leghorn, and also a control Leghorn group left untreated. The blood taken from male and female birds is injected by the intra-peritoneal route every 3 to 5 days as soon as the subjects are 10 to 30 days old and for a period of 6 to 7 months. In each new generation obtained through artificial insemination, fowls remaining up to the standards of the white Leghorn are selected from both treated groups and submitted to the blood injections. Moreover, from among the F_4 birds in the control group, the authors set up three new Leghorn groups treated under identical conditions with blood from Austhralorp, Rhode Island Red and white Leghorn respectively.

Results from F_0 to F_6 (February 1963) : No alterations are noticed in either check or control Leghorn groups. In the test group, however, which was submitted to injections of blood from grey guinea fowl, there are in each generation from F_2 onward a few animals with alterations in the colour and quality of their feathers and in the pigmentation of their feet. Such alterations do not appear in the blood treated Leghorn fowls, but in birds of the following generation, independently of any injection whatsoever. They are maintained in the descendency which is no longer treated and are transmitted both by the mother and the father birds. In the groups treated with blood from Austhralorp and Rhode Island Red, alterations begin to appear as from F_2. The authors discuss the origin and meaning of such phenomena.

(C.R. Acad. Sciences 255 : 781-783, 1030-1032, 1962.
Biol. Méd. 1963, to be published.
Arch. Sc., Genève 1963, to be published).

THE RELATIONSHIP BETWEEN DROSOPHILA MELANOGASTER AND ITS PARASITE PSEUDEUCOILA BOCHEI WELD . Ilse Walker, Institute of Zoology, University of Zürich, Switzerland.

Drosophila melanogaster, like other Drosophilidae, is a host of the parasitic wasp Pseudeucoila bochei Weld. Normally one parasite develops within a Drosophila larva, which is being consumed after puparium formation (1). Occasionally the host larvae form melanotic capsules around the parasite embryo, which generally dies within the capsule (2). These capsules consist of lymphocytes of a special type ("lamellocytes"), which normally are formed after puparium formation (3). In host larvae capable of encapsulating their parasite, these lamellocytes develop already at the end of the second and at the beginning of the third larval instar (4). The percentage of Drosophila larvae disposing of this defence reaction is specific for different geographic stocks The defence mechanism seems to be dependent on a polygenic system with a strong dominance effect (2,4,5). The penetrance is influenced by the age of the host at the time of infection, by its viability and by the surrounding temperature (4,6). Relations with the hereditary melanotic tumours (Pseudotumours) are obvious (3,4,7)

One known stock of Pseudeucoila is able to inhibit the defence reaction of the host. Cross- breeding and selection experiments support the hypothesis, that this faculty depends on a single dominant gene (I = Inhibitor), which acts during oogenesis and enables the egg of the parasite to suppress capsule formation in the host larva by inhibiting the formation of lamellocytes (maternal effect; 4,8).

However, the defence reaction of the host as well as the inhibition by the parasite are only funtioning with respect to a certain partner. Thus, Pseudeucoila embryos which are highly resistent against encapsulation by Drosophila melanogaster are normally encapsulated by the larvae of Drosophila simulans(9); and the larvae of a stock of Drosophila melanogaster, which lacks defence reaction against Pseudeucoila, do encapsulate another parasitic wasp, Phaenocarpa tapida Nees (1,10).

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CHROMOSOMAL STRUCTURE OF NATURAL POPULATIONS OF DROSOPHILA FLAVOPILOSA FREY, IN RELATION TO THE ENVIRONMENT. Danko Brncic, Institute of Biology, University of Chile, Santiago, Chile.

Populations of many species of the genus Drsophila exhibit polymorphism due to the presence of chromosomal inversions.This type of variability constitutes an ideal object of study in order to understand many basic problems of evolutionary genetics. The usual method for obtaining data on the chromosomal varia - tions in populations, is the examination of the offspring of flies collected in nature, and bred in the laboratory. Unfortunately, most members of the genus Drosophila are so restricted ecologically that it is often difficult to maintain them under artificial conditions. Furthermore, if it is considered that not all the individuals captured in nature give progeny in the laboratory, one may suspect that the above mentioned method represents a certain source of error, because only a selected part of the natural population will be examined. These considerations led the author to study the chromosomal structure of D. flavopilosa Frey, a typical example of an ecologically restricted species which cannot be bred in the laboratory. Nevertheless due to the circumstance that the females lay their eggs in the flowers of the Solanaceous shrub Cestrum parqui L'Her., which is common in Chile, it was possible to study the chromosomal structure of some natural populations of this species, analyzing larvae taken to the laboratory directly from their natural breeding sites.

In Central Chile, natural populations of D. flavopilosa are polymorphic for the gene orders in their chromosomes due to the presence of inversions. Quantitative and qualitative data on the distribution of the inversions have shown that there are some seasonal fluctuations and altitudinal gradients in the frequencies of some gene arrangements. These variations are correlated with the flowering periods of Cestrum parqui, the population density of the fly, and other ecological factors.

STRATIFICATION OF STRAINS OF DROSOPHILA MELANOGASTER INBRED AT DIFFERENT TEMPERATURES. Donald J. Nash, Dept. of Zoology, Rutgers, The State University, New Brunswick, New Jersey, U.S.A.

A series of wild-type inbred strains of Drosophila melanogaster was derived by inbreeding at a constant temperature of 18°C or 26°C. Seven inbred lines were used from each temperature. In addition, two random mating populations were set up from mass matings of the seven inbred strains within each temperature. The 16 genotypes were tested at each of three temperatures, 18°C, 26°C, and 30°C. Data were obtained on pair sterility, number of adults eclosing from a 24 hour egg laying period over a span of five days, sex ratio, and thorax length. Viability, in general, was greatest at 26°C and declined at the extreme temperatures of 18°C and 30°C. The mean number of adults eclosing per day ranged from a low of 4.6 per day for the 18°C derived lines when raised at 30°C to a high of 27.2 adults per day for the 26°C derived lines when reared at 26°C. Significant differences were observed within both sets of inbred strains at all three temperatures. Of particular significance is the fact that there was no consistent adaptive response to the temperature selection of more than 100 generations of sib-mating at 26°C and more than 50 generations at 18°C. In general, the 26°C derived lines were superior to the 18°C lines in overall reproductive performance at all temperatures including 18°C. The relative difference in mean viability of the 26°C derived strains and the 18°C derived strains was greatest at 30°C and least at 18°C. There was some overlap of the strains at all temperatures however. Thorax length varied inversely as temperature with a 12 per cent increase in length having been observed between 30° and 18°. Significant genotype-temperature interactions were evident for all characters.

GENE CONTROLLED PROCESSES IN THE GENESIS AND EVOLUTION OF ANIMAL PATTERNS. K. C. Sondhi, Gerontology Branch, National Heart Institute, National Institutes of Health, PHS, U. S. Department of Health, Education & Welfare, Bethesda, and the Baltimore City Hospitals, Baltimore, Maryland.

The present paper describes the nature of developmental processes in the genesis and evolution of orderly patterns. By using a major mutant gene, ocelli-less, in Drosophila subobscura as a source of observable variation, an attempt is made to deduce the nature of developmental processes and their genetic control by examining the range of adult variation.

Most of the observable variation met in the ocelli-less populations selected for various patterns can be explained if it is assumed that there exists primarily an unvarying "prepattern" which determines the positions at which structures are formed, and an ocelli-bristle forming substance, or "precursor", which is variable and which must be present in the required amount if structures are to be induced by an underlying prepattern. On the basis of this morphogenetic model, most of the experimental results involving the origin of a novel pattern, and involving changes in the numbers and positions of structures are explained.

The prepattern is considered as the distribution of an inducing substance with regions of high and low concentration, the regions of high concentration occurring at sites where structures later form. The process whereby such a distribution could arise will be described. Evidence will be presented to show that if the amount and distribution of the precursor is altered to a certain threshold, the constancy of prepatterns can be changed.

XVI INTERNATIONAL CONGRESS OF ZOOLOGY

CONTRIBUTED PAPERS SESSION

14A. EMBRYOLOGY

SUR LA CONCEPTION DE GRADIENT DE SEXUALITÉ CHEZ QUELQUES MÉTAZOAIRES. Enrico Vannini, Istituto di Zoologia, Università di Bologna, Italia.

On peut étudier les phénomènes de la sexualité par des méthodes soit génétiques soit morphogénétiques. Par les prémiers on cherche d'établir la distribution dans la garniture chromosomique des gènes M et F des quels dépend la détermination mâle ou femelle, par les seconds on essaie d'éclaircir les processus qui pendant le développement aboutissent à la différenciation sexuelle.

Il est à rémarquer que chez plusieurs Métazoaires hermaphrodites (par exemple quelque espèce d'Hydre d'eau douce, les Plânaires, quelque Polychète, les Chaetognathes etc.) il peut arriver que la différenciation des cellules germinales primitives en gonocytes mâles ou femelles, se fait à des niveaux différents sur l'axe principal du corps. Nos observations et expériences nous font soupçonner ,en ces cas, la présence d'un gradient morphogénétique de sexualité, le long de l'axe céphalocaudal ou apicobasal des animaux. En correspondence des différents niveaux de ce gradient, la capacité d'expression des gènes de sexualité mâles ou femelles est différente, même si ces facteurs sont en équilibre dans le génotype.

Tout à fait semblable serait la condition de quelques Métazoaires ,chez lesquels on observe un hermaphroditisme potentiel: par exemple les Bufonidae.

MORPHOGENESE DE L'ORGANE DE BIDDER CHEZ BUFO BUFO.

Elvezio Ghirardelli, Istituto di Zoologia, Università, Trieste, Italia.

Chez les Métazoaires hermaphrodites dont les gonades mâles et femelles sont à des niveaux différents sur l'axe céphalocaudale du corps, la différenciation des cellules germinales primitives est due vraisemblablement à l'action d'un gradient morphogénétique. La même chose devrait se passer aussi chez Bufo bufo où on a un hermaphroditisme rudimentaire. Au fin de confirmer cette hypothèse on a substitué, chez des embryons de Bufo bufo au stade de bourgeon caudal (stade III_3 selon Cambar et Gipouloux), le territoire présomptif de l'organe de Bidder avec le territoire postérieur de la crête génitale d'un autre exemplaire au même stade. A ce moment là, la migration des cellules germinales de l'entoderme au mesoderme génital présomptif n'est pas encore commencée.

Dans une autre série d'expériences le territoire bidderien de la crête génitale à été implanté (toujours homoplastiquement) au lieu du territoire présomptif des gonades.

Par ces expériences de greffe on a pu mettre en évidence deux facteurs, au moins, qui agissent pendant la différenciation de la crête génitale chez Bufo bufo, c'est à dire: une migration différentielle des gonocytes qui ont la tendance à se concentrer en nombre plus élevé à l'extrémité céphalique de la crête génitale, en correspondence de la quelle se développe l'organe de Bidder et une action inductrice du territoire bidderien présomtif qui agit selon un gradient céphalocaudale. En dehors de la crête génitale cette action inductrice ne s'exerce pas du tout ou bien induit une gonade sterile.

TRANSFORMATION METAMORPHIQUE DES ORGANES LARVAIRES DE BATRACIEN, ISOLES EN SURVIE. Michel Delsol, laboratoire de Biologie générale, Université Catholique de Lyon. France.

Le déterminisme thyroïdien de la métamorphose des Batraciens est connu depuis longtemps. On sait aussi que, par greffe, plusieurs organes du têtard peuvent se métamorphoser indépendamment. Il était donc logique de chercher à observer des métamorphoses sur des organes isolés en survie. Nous avons expérimenté sur divers organes de têtards d'Alytes obstetricans ou de Rana temporaria.

La peau donne des multiplications cellulaires peu caractéristiques. Les nageoires présentent des modifications qui correspondent simplement à des pertes d'eau. L'intestin - tube très long formé d'un épithélium monocellulaire - isolé et maintenu entier dans du Tyrode oxygéné présente très vite des transformations qui paraissent correspondre exactement à celles de la métamorphose naturelle : sa longueur se réduit de moitié, le nombre de spires diminue, leur calibre augmente; la proportion de régression des différentes parties correspond à celle de la métamorphose naturelle.

Histologiquement, on note la multiplication des cellules basales et l'apparition des plissements de la muqueuse. Ces images apparaissent en premier - toujours comme dans la métamorphose naturelle - dans trois régions préférentielles : estomac-duodénum, point de rebroussement, début du gros intestin.

Ce phénomène est visible quelques minutes après la mise en survie; la thyroxine, ajoutée au milieu de culture, l'active légèrement; le froid l'arrête totalement.

L'intestin des Anoures adultes - comme celui des têtards évolués d'Urodèles qui correspond histologiquement à celui d'un Anoure métamorphosé - ne présente en survie aucune modification.

Ces expériences suggèrent que l'isolement de l'intestin libère une capacité de multiplication des cellules basales; il existerait donc dans l'organisme, jusqu'à la métamorphose, un inhibiteur de cette multiplication cellulaire. Des expériences d'isolement partiel ne nous ont pas encore permis de vérifier cette hypothèse.

THE GROWTH OF AVIAN WING BUDS TRANSPLANTED FROM ONE SPECIES TO A DIFFERENT SPECIES. John M. Cairns, Biological Station, Roswell Park Memorial Institute, Springville, New York.

Transplantation of whole limb buds between avian embryos of two species which differ in the timing of morphogenetic processes or in the size attained after the same incubation period may provide clues to the regulation of the rates of growth and differentiation. Japanese quail, chick, Mallard and Muscovy duck embryos hatch after 16, 21, 28, and 35 days of incubation, respectively. Morphogenesis, as indicated by the appearance of feather germs and by ossification of the long bones, is scaled to the total incubation time. Thus limb bud stages (18 to 19) are reached after 66, 72, 96, and 120 hours incubation respectively. The quail embryo is smallest at all stages. The chick is larger than duck embryos during the interval from 10 to 16 days, the period when comparison were usually made. Reciprocal orthotopic transplantationsof whole wing buds were made between these various species at stages 18 to 20.

The maximum size difference is between quail and chick. Provided the graft heals on and is revascularized within a few hours, as frequently happens, a quail wing which has grown for 10 days on the chick may in some cases have long bones about 20% longer than those of the control left wing of the donor embryo. These are still not as long as those of the host left wing. The chick wing on the quail host is correspondingly reduced in size. The differences in appearance of the feather germs and of ossification between these 2 species are slight.

The most striking differences in stage of development between host and graft occur in chick-duck and in quail-duck combinations. The wing of a 13-day chick embryo is covered with long feather germs, while those on the 15-day Muscovy are just becoming apparent. The Muscovy wing which grows for 10 days on the chick host maintains its own rate of differentiation. In contrast, the chick wing on the Muscovy host is distinctly slowed in comparison with the donor left wing. A quail wing that has grown for 10 days on a Mallard host is particularly striking, for the long feather germs are heavily pigmented by host melanocytes, while the host left wing still has no sign of color

The increase in size observed in quail wings growing on the chick does not occur in Muscovy wings growing on the chick. In this combination the graft wing is approximately the size of the donor left wing, while the host left wing is distinctly larger.

In some of the above combinations the union of graft with host is indicated within the shoulder tract by the presence of host size feather germs medially and of graft size feather germs distally. The length of the humerus in the grafted wings is relatively short in comparison with that of the ulna and third metacarpal. This suggests some failure in adjustment between graft and host. (Supported by Grant E-276B from the American Cancer Society.)

GRAFT-HOST CELL INTERACTION IN THE SPLENOMEGALY REACTION IN THE CHICK EMBRYO. A. M. Mun, Department of Zoology, University of Maine, Orono, Maine.

In a recent study we observed, following serial transplantation of embryonic chick spleen, an increase in the spleen weight of the subsequent hosts in several transfer "lines" of the series. This suggested the transfer of "immunologically incompetent" embryo cells from the donor graft to the host spleen as well as their maintenance in the embryonic environment permitting the maturation of mechanisms of immune response (Mun, Tardent, Errico, Ebert, DeLanney and Argyris, 1962.)

To determine first whether the transferred embryo cells are capable of interaction in the embryonic environment, a piece of 17-day old embryo spleen from an inbred line (Line 15, Regional Poultry Research Laboratory, East Lansing, Michigan, B. Winton, Director) together with a piece of adult spleen from another inbred line (Line 7) were implanted on the chorioallantoic membrane (CAM) of a 10-day host embryo from the same line (Line 7). In control groups, the adult and embryo donor spleen and the host embryo were derived from the same inbred line (Line 7). After 7 more days of incubation, a significant increase in weight of the host spleen over that of controls was observed. This suggests the transfer of embryonic donor spleen cells from the graft to the host spleen and the interaction with adult spleen in the embryonic environment.

To determine next the role of host spleen cells in the splenomegaly reaction and whether host spleen cells can be similarly transferred, adult spleen from a Line 7 chicken (A7) were implanted on the CAM of 10-day old Line 7 ($E7_1$) and hybrid (EH_1) embryos. After 7 more days of incubation the host spleens were removed, weighed, cut in half and transferred to new 10-day old Line 7 ($E7_2$) and hybrid (EH_2) embryos. Thus 4 groups are established: (I) $A7 \rightarrow EH_1 \rightarrow EH_2$, (II) $A7 \rightarrow EH_1 \rightarrow E7_2$, (III) $A7 \rightarrow E7_1 \rightarrow EH_2$, and (IV) $A7 \rightarrow E7_1 \rightarrow E7_2$. The spleen weight of embryos in the group IV series served as controls.

The mean spleen weight of the first hybrid host (EH_1) was significantly greater than that of the first inbred host ($E7_1$). The mean spleen weight of the second host in groups I and III (EH_2) were significantly greater than that in the control group ($E7_2$). This demonstrates the transfer of adult spleen cells. However, the mean weight of the second host spleen in group II ($E7_2$) was also significantly greater than that of the control group although it was derived from the same line as the adult donor. This suggests the simultaneous transfer of both adult and embryo host spleen cells.

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THE IMMUNOLOGICAL SPECIFICITY OF EMBRYONIC CHICK HYPERSENSITIVITY.

Pierson J. Van Alten, Dept. of Anatomy, University of Illinois, Chicago 12, Illinois, U.S.A.

The present study was undertaken to determine the degree of immunological specificity in the hypersensitive state generated by the chick embryo. Chick embryos of 12 days of incubation were injected intravenously with rabbit serum, bovine serum or with blood serum derivitives such as bovine gamma-globulin (BGG) or human gamma-globulin (HGG). A second (shocking) injection of the same antigen or different antigen was given. Chicks which had been sensitized with rabbit serum when challenged with fresh rat, guinea pig or adult chicken serum failed to exhibit any anaphylactic response. When bovine serum had been used as the sensitizing antigen anaphylactic responses were observed following the shocking injection of bovine serum, BGG and HGG. When HGG was given following sensitizing with BGG a little less than 50% of the newly hatched chicks gave a mild anaphylactic response. Somewhat similar results were observed when BGG was given as the shocking injection and HGG as the sensitizing injection. In this case it was just a little over 50% of the chicks which manifested a mild anaphylactic reaction. In all cases where fresh whole serum was not used as the shocking injection a small amount of complement was added to the antigen. On the basis of these observations it is proposed that the cells responsible for embryonic-hypersensitivity are not fully differentiated and thus do not manifest as great a specificity as do adult cells which are responsible for antibody production. (Supported by National Science Foundation Grant #18693)

PROTEIN SYNTHESIS IN RELATION TO THE DEVELOPMENT OF SKIN AND FEATHERS. Sarah Ben-Or, Pearl Goodwin and Eugene Bell, Dept. of Biology, Massachusetts Institute of Technology, Cambridge, Mass.

This report deals with 1) the problem of when the back skin of the chick begins to synthesize keratin and 2) keratin synthesis in fast growing cultures of skin cells.

Problem one has been approached serologically; Two types of antisera have been used, one directed against a relatively pure protein fraction derived from 13 day embryonic feathers, and another directed against 7 day back skin ectoderm. Data to be presented indicate that 7 day ectoderm differs from ectoderm of earlier stages, a change which may be correlated with the beginning of keratin synthesis. Biochemical data concerned with the same questions and based on incorporation of labelled amino acids into ectodermal protein will also be presented.

When 8 day primary dissociated embryonic skin cells were grown *in vitro* as a fast growing monolayer culture (they were replated when cells approached confluence) keratin synthesis stopped abruptly but was resumed within 5 days and reached a peak at about 22 days. Cultures have been carried several months. From amino acid incorporation data there appears to be little difference in the rate of protein synthesis between cells grown in bottles as monolayer and in aggregates of cultured cells grown in diffusion chambers on the chorioallantoric membrane. Addition of vitamin A to cultures for 3 hours promptly reduces incorporation of cystine into protein. There is evidence that keratin polymerizes in cultured cells returned as an aggregate to an embryonic inviornment but that it does not polymerize in cells growing rapidly *in vitro*. In the former it is demonstrable cytochemically while in the latter it is not.

The relation of keratin synthesis to the induction of feathers and to morphological differentiation will be discussed.

GENETIC VARIATION IN THE MATRIX OF MOUSE MELANOSOMES ANALYZED BY HIGH RESOLUTION ELECTRON MICROSCOPY. Frank H. Moyer, Dept. of Zoology, University of Illinois, Urbana, Illinois, USA.

The fine structure of mouse melanosomes is controlled in part by alleles at the B locus, the C locus and the P locus. High resolution electronmicrographs show that these three loci alter the structure of the matrix on which melanin is deposited.

The matrix is formed of concentric sheet-like lamellae consisting of parallel fibers cross-linked to one another. The fibers show a first order axial periodicity of approximately 85 Å in animals homozygous for B (black) and approximately 65 Å in animals homozygous for b (brown).

The orderly arrangement of sites of melanin deposition on the matrix superimposes a second order axial periodicity along the fibers as melanin is produced. The second order periodicity is approximately 200 Å in B homozygotes and approximately 100 Å in b homozygotes. In animals homozygous for c (albino), the second order periodicity is completely absent since no melanin is formed.

In animals homozygous for p (pink-eyed dilution) the cross-linking of fibers to form the matrix sheet is extremely irregular leading to a distorted matrix. Nevertheless the axial periodicities of fibers in these distorted matrices, as determined by the genotype at the B locus, are not altered.

These observations indicate that the architecture of the melanosome matrix is dependent upon the interaction of proteins controlled by three different genetic loci. Several mechanisms of interaction are possible and will be discussed with reference to data on genetic variation in the activity of tyrosinase and DOPA oxidase.

PHYSIO-GENETICAL *IN VIVO* STUDIES ON THE BLASTOCYST OF THE RABBIT, *Oryctolagus cuniculus*.

E. S. E. Hafez, Dept. of Animal Science, Washington State University, Pullman, Washington.

Two studies were undertaken. In the first study, nulliparous New Zealand Large rabbits were bred to fertile bucks, ovariectomized at 2 days *post coitum* and injected with 4 or 40 mg. progesterone daily and autopsied at 6 days *post coitum* to examine the viability and measure the diameter of blastocysts. Untreated intact does were bred and used as control. The size of the blastocyst in the progesterone-treated ovariectomized does was similar to that in the control. The treatment had no effect on survival of blastocyst as shown by subsequent transfer to 6-day pseudopregnant recipients and the production of viable offspring. Blastocysts degenerated when the weight of the uterus was less than 9 gm. and the weight of the female was less than 7 lbs. The 6-day-old blastocysts had an outer zonal diameter ranging from 0.56 to 4.04 mm. with an average of 2.70 mm. and an inner zonal diameter from 0.56 to 4.00 mm. with an average of 2.58 mm. The diameter of inner cell mass ranged from 0.41 to 1.34 mm. with an average of 0.94 mm. and was positively correlated with the outer zonal diameter of blastocyst. The dose of progesterone had no effect on the size of blastocysts in ovariectomized does. When estradiol was injected together with the progesterone, with a ratio of 1 estrogen: 400 progesterone, the number of recovered embryos as well as the survival of embryos were decreased presumably due to the faulty tubal transport of embryos. In the second study, thirty to forty 4- to 8-blastomere embryos were recovered from superovulated donors and transferred to both uterine horns of pregnant recipients at 2 days *post coitum*. The maximum implantation capacity at 9 days *post coitum* was 45 for both horns, the average being 30. High embryonic mortality occurred following implantation. There was no indication that maximum implantation capacity of the blastocyst decreased in the first four pregnancies. There was no evidence that implantation capacity was a characteristic of the individual female. Periods of temporary infertility occurred when the whole inoculum of blastocysts did not implant. (Supported by a PHS research grant GM 09994-01 from the National Institutes of Health.)

GRADIENT TRANSFORMATION OF FREEMARTIN GONADS IN BOS TAURUS. E. Rajakoski and E. S. E. Hafez, Dept. of Animal Science, Washington State University, Pullman, Washington.

The anatomy of reproductive organs and histology and cytology of the gonads of 1-year-old bovine quintuplets were investigated. These comprised four freemartins and one fertile bull with normal sexual libido. In all cases of freemartins the cervix was absent; the vestibule and the uterine horns were present in all cases while the other reproductive organs showed different degrees of development. The highest degree of histological transformation of gonad, to the male, was characterized by absence of germinal epithelium, secondary tunica albuginea, ovarian cortex, and follicles; and by presence of mesothelium, primary tunica, tunica vasculosa, interstitial cells, primary sex cords, centrally located rete, epididymis and vas deferens. The lowest degree of transformation was characterized by partial presence of ovarian cortex with germinal epithelium, secondary tunica and primordial, growing and Graafian follicles with oocytes and nuclei in prophase of heterotypic division and more peripherally located rete. Histological transformation of gonads was parallel to anatomical transformation of reproductive organs. One male embryo was able to masculinize four quintuplet females at a less degree than that generally reported in the literature for twins and triplets. The gradient transformation of the quintuplet gonads, to the male, can be attributed to differences in concentration of hormones from male embryo, relative position of male embryo to female embryo *in utero*, surface area of anastomosis between placentae, and to stage of embryonic development at the time of vascular anastomosis of allantoida. The asymmetry in the histological characteristics of the right and left gonad of one freemartin may be due to differences in the degree of embryonic development at the time of vascular interchange or to differences in the sensitivity of gonadal tissue to circulating male hormone. Seven types of lutein cells were identified according to relative size of cytoplasm; nucleus, granulation and staining affinity. These types, most of which were comparable to those found in normal ovaries represent immature, secretory or degenerating stages. The presence of oocytes in the luteal structures may indicate that the lutein cells of such structures originate from luteinized unruptured follicles rather than from the interstitial cells. (Supported by a PHS research grant GM 10282-01 from the National Institutes of Health.)

DEVELOPMENT OF THE EXTERNAL EAR IN THE DACHS RABBIT. Dorcas D. Crary, Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine.

In the development of the external ear in man the tragus is generally considered to arise from the mandibular arch and the antitragus from the hyoid. However, because of the very rapid growth of this region at that time, the first manifestations of the developing ear are described as (1) a gill slit open ventrally, and (2) a completely closed slit, with no intermediate steps to show the nature of this closure. The hillocks of His, described from this latter stage, can be found in birds, reptiles, and mammals generally as well as in man, indicating that development of the external ear exclusive of the auricle is probably very similar in all forms.

In the adult rabbit a study of cartilage preparations shows that tragus and antitragus are parts of a single projection attached to the posterior helix and are more properly called anterior and posterior cornua of the tragus. Origin of these parts is more clearly revealed in dachs (chondrodystrophic) rabbits, where intermediate steps in early development result from generalized growth retardation. At 11 days the gill cleft is broad and still open ventrally, with a large appendage projecting from the hyoid arch. This is the anterior cornu of the tragus. In succeeding stages, it migrates around the open end of the gill slit. Because of the relatively broad cleft of the dachs, it forms a distinctive papilla in the center of the groove. In normal animals with narrow grooves it comes to lie in proximity to the anterior helix and is obscured by developing skin folds. The heterozygote is intermediate. Variations in expression of this region in heterozygous and normal animals may be attributed to slight differences in early growth rate and development.

DEVELOPMENTAL ASPECTS OF THE CHOROID PLEXUS. Arthur LaVelle, University of Illinois College of Medicine, Chicago, Illinois, U.S.A.

The brains of a series of hamsters ranging from fetal ages to the adult were perfused with cold isotonic saline, quick-frozen in liquid nitrogen, and sectioned in a cryostat. After drying *in vacuo*, fragments of choroid plexus were dissected out and weighed on a quartz fiber balance, before and after extraction of lipids with a mixture of chloroform and methanol, followed by hexane. Analysis of the extracts was made by ascending thin-layer chromatography. Choroidal mitochondria were also observed after fixation with Regaud's fluid and staining with basic fuchsin and aniline blue.

During the eleventh day of fetal development the choroid plexuses are in the process of invaginating as folds into the lateral and fourth brain ventricles. Throughout the series, from the 11½ day fetus to the adult, total extractable lipid from choroid plexuses fell into the relatively narrow range of 24-29% of the dry weight. Qualitative examination of chromatograms of plexuses of newborn animals show a phospholipid "pattern" already similar to that of the adult. In the newborn animal mitochondria in the choroidal epithelium appear numerous, are filamentous, and are apically oriented in a fashion similar to that of the adult; further staining of *in utero* specimens is in progress. These preliminary observations, in a study aimed at a structural-chemical correlate for the choroid plexus, suggest thus far that certain features of the choroid plexus have mature characteristics surprisingly early in development.

MORPHOGENESIS OF THE VERTEBRATE EYE. Alfred J. Coulombre, and Jane L. Coulombre, Laboratory of Neuroanatomical Sciences, National Institute of Neurological Diseases and Blindness, National Institutes of Health, Bethesda, Maryland, U.S.A.

This study identifies some of the factors which coordinate changes in the size, shape, and position of the tissues of the developing eye of the chick embryo.

Previous work has demonstrated that: 1. the neural retina and lens grow relatively independently of the vitreous body; 2. the change in volume and shape of the pigmented epithelium, choroid coat, and sclera, as well as the gross increase in eye size, depend in large measure upon the accumulation of vitreous substance within the vitreous cavity.

The experiments we are currently reporting demonstrate the following. When the embryonic retina is removed no vitreous substance is produced, and the lens ceases its growth. When the embryonic lens is removed the neural retina continues to grow, vitreous substance ceases to accumulate, and the eye stops growing. If the lens is removed and immediately replaced the growth of the eye is essentially normal. If the lens is removed and replaced by a boiled lens the growth of the eye ceases.

We interpret these results as follows. The neural retina grows independently of the rest of the eye. It regulates the growth of the lens. It also produces vitreous substance but does so under the influence of the lens. The accumulation of vitreous substance, in its turn, generates forces in the superficial coats of the eye which adjusts their growth to the geometry of the lens and retina.

A STUDY ON MITOCHONDRIA GENESIS IN RETINAL ROD INNER SEGMENT.

E. R. Berger, Dept. of Zoology, University of California, Los Angeles.

An electron-microscopic study of the development of a particular cell type which in the adult tissue contains a large number of mitochondria in a rather well defined space lacking other cytoplasmic components might possibly yield information concerning the morphogenesis of mitochondria. The photoreceptor in vertebrate retina is such a cell type in that the ellipsoid body, which consists of tightly packed mitochondria, nearly fills the entire inner segment of the adult retinal rod.

Retinae of neonatal and adult *Lebistes reticulatis* (common guppy) were fixed in OsO_4, acetone dehydrated, Vestopal embedded, sectioned on an LKB Ultrotome, stained with uranyl acetate and observed with an RCA EMU 3 electron microscope.

The adult rod inner segment was found to be nearly filled by a rather homogeneous population of tightly packed mitochondria of the ellipsoid body with little or no non-mitochondrial cytoplasmic space between it and the nucleus.

In the neonatal rod inner segment the developing ellipsoid body is separated from the nucleus by a ribosome-rich cytoplasmic space containing membranes and a Golgi zone. The mitochondria of the ellipsoid body are arrayed in a linear gradient with respect to size with the larger, adult-like mitochondria at the outer segment end and the smaller, less mature-looking mitochondria toward the nuclear end.

Partial reconstruction of the rod inner segment from serial sections seems to support the hypothesis of a *de-novo* synthesis of mitochondria.

ETUDE COMPAREE DES RAPPORTS VASCULAIRES MATERNO-FOETAUX AU COURS DES PERFUSIONS PLACENTAIRES CHEZ CERTAINS MAMMIFERES. Maurice Panigel, Unité de Physiologie placentaire (I.N.H.) et Laboratoire d'Evolution (Biologie Générale), Faculté des Sciences, Paris, France.

La classification de GROSSER qui caractérise les modes de placentation chez les Mammifères par le nombre d'assises cellulaires séparant le sang maternel du sang foetal, a donné lieu ces dernières années à de nombreuses critiques résultant non seulement de l'étude minutieuse des placentas d'un nombre toujours croissant de Mammifères mais surtout des nouvelles interprétations du rôle assumé par chacun des éléments constituant la "barrière placentaire" pendant la vie foetale.

Le placenta chorioallantoïdien des Mammifères est un excellent matériel pour étudier au cours d'expériences de perfusion vasculaire, l'hémodynamique foeto-placentaire dans des conditions physiologiques de maintien en survie. Ces expériences permettent d'interpréter avec précision l'influence sur la perméabilité placentaire des rapports de contiguité qui s'établissent au cours du développement embryonnaire entre les circulations maternelle et foetale dans les espèces rangées par GROSSER dans différentes catégories placentaires.

La microscopie électronique permet,par l'étude de l'évolution des microvillosités ou du chondriome par exemple, de suivre mieux que par toute autre méthode, l'état physiologique des structures utérines et foetales en survie au cours des expériences prolongées de perfusion placentaire. Elle constitue également, après perfusion de colorants vitaux, un moyen d'étude de la physiologie et de la perméabilité des structures paraplacentaires chez les Rongeurs.

Nous présentons ici de nouvelles observations sur l'anatomophysiologie comparée de la vascularisation utérine et chorioallantoïdienne chez les Bovidés, les Rongeurs et l'Homme. L'importance du flux sanguin et la direction préférentielle des courants circulatoires dans le disque ou les cotylédons placentaires, la vasomotricité au niveau du cordon ombilical, de la vésicule ombilicale et des formations allantoplacentaires, la spécialisation fonctionnelle dans les échanges materno-foetaux de zones bien localisées des vascularisations foetale et maternelle, la sensibilité particulière de chacune de ces régions aux stimuli humoraux, sont des critères physiologiques qui permettent de mieux interpréter les observations sur la structure histologique et l'ultrastructure de la membrane placentaire.

XVI INTERNATIONAL CONGRESS OF ZOOLOGY

CONTRIBUTED PAPERS SESSION

14B. EMBRYOLOGY

DETERMINATION OF CELL SYSTEM IN HYDRA. Sivatosh Mookerjee, Dept. of Zoology, Presidency College, Calcutta, India.

Determination in Hydra takes place in terms of two opposite cell systems of the hypostome and basal disc. Along its apico-distal polarity, the entire length of the organism is not uniformly determined. Middle regions remains in an indetermined state because it carries the potentialites of oral and aboral trends of determination simultaneously. Transplantation of a piece of middle region to the hypostome region forms a basal disc. Similar experiment at the posterior region will show the formation of a hypostome near the aboral and of the host. The middle region thus has a dual capacity of determination which is expressive differently at the different levels of the host.

Time factor for determination in oral and abroal cell systems from a piece of the middle region has been analysed. Length of time in the hypostome determination is invariably more than that of the basal dics. Determination of a basal disc takes about 32 hours at 24°. At the same timeperature, however, the hypostome determination is not complete by 46 hours. Before the final hour of determination, a successive transplantation of already grafted material from one level to its opposite level, will induce a change-over of determination to opposite cell system.

A contrasting property in the form of a limiting factor is under way of determination in cell system of Hydra. While the cells of the middle piece undergo basal disc type of determination, the hypostome forming ability in them is eliminated much earlier. Where as this is not quite similar when the middle piece cells start for hypostome determination. Because during this type of determination, capacity for both types is retained for much longer time, until finally cells differentiate into hypostome.

Above data have been discussed in terms of inhibitor-complex.

TECHNIQUES FOR THE ANALYSIS OF CELL FUNCTION AND DIFFERENTIATION, USING EGGS OF THE HONEYBEE. E.J. DuPraw, Dep't. of Zoology, University of California, Davis, California, U.S.A.

Embryonic stages of the honeybee have proved well-suited for morphological and physiological analysis of developmental processes at the cellular level. Success has been obtained in transferring nuclei-containing plasm micrurgically from pre-blastoderm to post-zygotic embryos, with subsequent normal development and hatching of recipient eggs. In this technique, exact equalizing of pipette and egg temperatures at the optimum of 35.5° C. is a prerequisite for survival of operated individuals.

It has been found that honeybee embryos complete the entire course of development, including hatching, during immersion in paraffin oil. Under these conditions, the eggs are transparent, making morphological events easily observable in the living egg. Time-lapse microcinematography permits exact comparison of events in unoperated embryos and in embryos undergoing regulative changes after ligature by the technique of Schnetter.

It has also been found that hatching of the honeybee egg occurs by means of a hatching enzyme, which is preserved in immiscible droplets when the embryos hatch under oil. New-laid eggs are lysed by the enzyme when brought into contact with such "hatching puddles". This phenomenon has been used as an assay technique for the enzyme, and its inactivation by 30 minute exposure to temperatures in the vicinity of 61-65° C. has been demonstrated.

Successful thin-section preparations of early honeybee embryos have been made, using osmium tetroxide fixation and methacrylate embedding. Improvement in the preparation technique, by varying fixatives and embedding media, is now in progress.

ON THE CONTROL OF THE CYTODIFFERENTIATION. Silvio Ranzi, Dept. of Zoology, The University, Milano, Italy.

Ribonucleoproteins (RNP) were extracted from liver, heart, muscles, kidney and glandular stomach of adult chicks. Two kinds of experiments were performed. A piece of coagulated albumin was soaked with these samples or these samples were introduced into an allantois explanted from a 4 day old chick embryo. The piece of albumin or the allantois were then grafted on the chorio-allantoic membrane of a 8 day chick embryo.

Liver RNP induce the formation of typical glandular cells, in which glycogen is stored, from the entoderm of grafted allantois.

Skeletal muscle RNP induce the transformation of mesenchyme cells into muscular elements, in which striated structures may also appear.

Heart RNP induce the transformation of mesenchyme cells into multinucleate elements with fibrillar cytoplasm.

Glandular stomach RNP induce the transformation of ectodermic or entodermic cells in a glandular epithelium.

Kidney RNP induce the appearence in the mesenchyme of structures related to the blood vessels. These structures are tentatively interpreted as glomeruli.

THE COMPETENCE AND DIFFERENTIATION OF CELLS PRODUCING KYNURENINE DURING DEVELOPMENT. T. M. Rizki and Rose M. Rizki, Department of Zoology, University of Michigan, Ann Arbor, Michigan, U. S. A.

The cells of the anterior region of the larval fatbody of the Ore-R normal strain of *Drosophila melanogaster* accumulate kynurenine globules at the end of larval development. Intracellular localization of this fluorescent metabolite is achieved with fluorescence microscopy, and chromatographic identification of kynurenine from the anterior fat cells has verified this distribution pattern. The *vermilion* mutant lacks kynurenine, and the fat cells from the anterior region do not display the characteristic autofluorescence of kynurenine. Implantation of normal anterior fat cells prior to their period of kynurenine accumulation into adult *vermilion* hosts has demonstrated the autonomy of these cells with respect to kynurenine production. When the mutant gene, *suppressor of vermilion*, is combined with the mutant gene *vermilion*, kynurenine production in the fat cells is restored. However, the distribution pattern of kynurenine producing cells in this mutant combination is different from that of the normal genotype (Ore-R). In addition to the normal pattern of anterior fat cells with kynurenine, some of the cells posterior to this region also become incorporated in the mutant pattern. These observations indicate that the absence of kynurenine in these posterior cells in the normal strain does not necessarily represent a loss of genetic potential to produce kynurenine, but rather indicates that the expression of this potential is under the control of the normal allele at the *suppressor* locus.

A similar, but more extensive, increase in the number of cells containing kynurenine is achieved in the normal strain by supplementing the diet with the precursor of kynurenine, tryptophan. Cell-free extracts of these tryptophan-fed larvae show a considerable increase in tryptophan pyrrolase activity. It is thus demonstrated that in this instance, an adaptive increase in enzyme activity is associated with an increase in the number of kynurenine producing cells.

Kynurenine production by the posterior cells can also be demonstrated by "in vitro" incubation of groups of isolated cells in L-tryptophan. D-tryptophan is not utilized to form kynurenine in the "in vitro" system, although larvae fed D-tryptophan will show an increase in the number of fat cells containing kynurenine. These experiments will be discussed together with a sequence of experiments determining the time and duration of tryptophan feeding necessary to induce kynurenine production in the larval fat cells posterior to the gonads.

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DIFFERENTIATING POTENCY OF ERYTHROCYTES IN VERTEBRATES.

Kikuo Chishima, Laboratory of Zoology, Department of Education, Gifu Univ. Gifu, Japan.

Erythrocyte has been regarded as so highly differentiated a cell that it can not be differentiated into any other kind of cell, and as the element which undergoes fragmentation and then eliminates from circulation after fullfilling its function as a gas transportor.

The following results, however, were obtained by my studies on the behaviors and functions of erythrocytes in amphibia, birds and mammals by means of histological sections and phase microscopic observations of living erythrocytes.

Mitotic indices in muscle, adipose tissue, liver, brain, bone marrow, ovary and Wolffian body in the normal subjects showed extremely low value.

Furthermore, sometimes the mitotic figure does not necessarily follow the cell division. On the contrary, extravascular erythrocytes scattering in the interstices of various tissues can easily be seen. And these erythrocytes show transitions, depending upon their cellular environment, respectively, into each cellular element of various tissues including the tissues of regeneration and wound healing, through the transitional phases (disintegrated erythrocytes-mass or isolated spherical erythrocytes → Synthesis of DNA → lymphocytoid or mesenchymal cells). These facts suggest that the mitosis may not be considered as an only mechanism of cell-proliferation in the various tissues. Consequently, so far as the present studies are concerned, it may be said that the erythrocytes endowed with pluripotency, play an important role as a supplying source of cellular elements of various tissues. Moreover, the following methods of cell-proliferation were found; (a) Cytoplasm-extrusion from erythrocytes. (b) Budding-like process of erythrocytes in the depleted or starved bone marrow. (c) Reverse differentiation from adipose tissue into blood cell under the sturved condition. (d) New-formation of blastomeres and embryonal cells by means of aggregation, fusion and the differentiation of yolkspheres in amphibian embryo. Though the observations and interpretation mentioned above may be seen peculiar, I intend to demonstrate and explain it by the aid of microphotographic slides.

A CONTRIBUTION TO THE HISTOCHEMICAL DEVELOPMENT OF THE TWENTY MILLIMETER PIG EMBRYO (SUS SCROFA). John C. Bartone, Department Of Anatomy, The George Washington University, School of Medicine, Washington, D.C.

Tables of the level of the histochemical development of the pig embryo have been formed containing comparative values of glycogen content and distribution in the derivatives of ectoderm, mesoderm and endoderm.

Glycogen was stained by the periodic acid Schiff (PAS) technique and selectively removed in controls by diastase digestion. Reactions for glycogen were tabulated as intense, moderate, light, scant or none. Non-glycogen staining was regarded as a carbohydrate-protein complex.

Generally, glycogen was more prevalent in the derivatives of ectoderm and endoderm than those of the mesoderm. This continues to reflect the pattern established in a series of younger embryos although with more reactivity and diversity as compared with a ten millimeter embryo.

Ectodermal derivatives revealed an intense localization of glycogen in the epidermis, nose, mouth and adrenal medulla. A light reaction was observed in the ear and hypophysis. Variations were seen in the brain and spinal cord. Glycogen was not observable in nerves or ganglia of this embryo although those of younger ones were positive.

Intense glycogen deposits in mesodermal structures were visible in cartilage, adrenal cortex, myocardium and cells of the notochord. Moderate quantities were contained in muscle, gonads and metanephros. Other structures were scarcely reactive. No glycogen was exhibited by the notochordal sheath, endothelium and mesonephros. Intense reactions in mesodermal derivatives were not observed, thus the biochemical level of carbohydrate activity is maintained similarly as in younger embryos. The relative equilibrium of activity as compared with ectoderm and endoderm derivatives is also maintained.

Endodermal derivatives were observed to contain intense reactions in the pharynx, tongue root, thymus, trachea, bronchii, esophagus, stomach and urogenital sinus. Earlier studies showed an intense glycogen stain only in the liver of the ten millimeter pig embryo. Many of the structures of the ten millimeter embryo that were moderate in reaction became intense in this older embryo. Presently exhibiting moderate glycogen quantities are the thyroid, parathyroid, auditory tube and intestine. Only the liver at this stage of development revealed a slight amount of glycogen present coupled with a similar estimated quantity of carbohydrate-protein(non-glycogen) complex. Other structures contained traces of glycogen.

Discrete changes are evident throughout this embryo in the chemical differentiation that precedes the biochemical evolution of histogenesis and organogenesis.

HISTONES IN THE DEVELOPING CHICKEN: REGULATORS OF GENE FUNCTION? David T. Lindsay, Department of Zoology, University of Georgia, Athens, Georgia

Histone proteins have frequently been suggested as possible regulators of gene function because of their presence in a wide variety of adult tissues as different groups of molecules associated with DNA. If the various histone molecules in a cell selectively influence gene function, their molecular heterogeneity would be expected to reflect the state of the cell's differentiation. Thus, changes occurring in a variety of developing tissues would be reflected by a changing array of histone proteins during development.

To test this idea, histone proteins are being prepared from several adult and embryonic chicken tissues and their molecular heterogeneity demonstrated by starch gel electrophoresis. Thus far, preliminary results demonstrate ten to fourteen histone components in adult and embryonic tissues. Several components are present in about equal amounts in adult and embryonic tissues. Other components may be missing from either adult or embryonic tissues. Such changes in histone proteins permit an estimate of the variety and proportions of histone molecules available for possible gene regulation during differentiation.

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DNA SYNTHESIS, MITOSIS, AND DIFFERENTIATION IN PANCREATIC ACINAR CELLS STUDIED WITH AUTORADIOGRAPHY AND METABOLIC INHIBITORS.

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Eleven day mouse pancreatic epithelium forms extensive acinar, zymogen-containing tissue when grown in vitro across a thin (TH) Millipore filter from mouse salivary mesenchyme. Labeling such developing tissues with tritiated thymidine (10uc/ml; 1 hour) on each of 5 days of culture reveals that the population of DNA-synthesizing cells is largely limited to the outermost cell layer and outer acini of the compact epithelial mass. Most mitotic figures, however, are found in a region beneath these outer acini. Experiments in which labeled tissues were reincubated suggests that cells move inward to the division zone after synthesizing DNA. Overt zymogen granule accumulation begins at about 4 days in vitro in the central parts of the epithelium. Through the 4th and 5th days, zymogen appears in successively more peripheral cells. These results suggest that the DNA-synthesizing,mitotic, and differentiating cells are localized in distinct regions or compartments of the epithelial population. Furthermore, a temporal interior-exterior gradient exists, in which cells which cease synthesizing DNA and dividing earliest are the first to differentiate.

If the thymidine analogue 5-bromodeoxyuridine (BUDR) is supplied to the developing epithelia during any 24hr segment of the first 72hrs in vitro, no subsequent zymogen formation occurs despite continued mitosis and healthy appearance of the cells. Conversely, if cultures are treated with the analogue for the 72-120hr period, the central regions of the explants proceed to form zymogen in a normal manner, whereas the outer acinar cells fail to differentiate. The BUDR-insensitive cells correspond roughly in position to the initial non-thymidine incorporating, central population first seen with regularity at 72hrs. These results would be expected if the central population is post-mitotic; hence it does not synthesize abnormal DNA, containing BUDR. Thus, these cells can differentiate normally in contrast to the more peripheral, dividing cells which are presumed to synthesize abnormal DNA that is unable to participate in normal differentiation. This, in turn, would suggest that DNA is influential in effecting synthetic product until at least the stages immediately before overt differentiation.

ETUDE HISTOCHIMIQUE DE L'EVOLUTION DES ACIDES NUCLÉIQUES SUR UN ORGANE EMBRYONNAIRE DE POULET IRRADIE ET PROTEGE CONTRE L'ACTION DES RAYONS X. Jean-Michel Kirrmann, Laboratoire d'embryologie expérimentale du Collège de France, Collège de France, Paris, France.

Nous avons montré qu'il est possible d'empêcher l'apparition de certaines malformations produites par les rayons X chez l'embryon de Poulet en lui injectant, avant l'irradiation, certaines substances radioprotectrices comme la cystéamine. On obtient le même résultat si on incube l'embryon dans une atmosphère d'azote quelques heures avant l'irradiation. L'étude histochimique de l'évolution des acides nucléiques après l'irradiation permet de savoir si le traitement radioprotecteur empêche l'apparition des radiolésions au niveau des ADN et des ARN.

La réaction de Feulgen, qui met en évidence les ADN et le vert de méthyle-pyronine, qui colore les ADN et les ARN montrent, sur une coupe de la région céphalique d'un embryon de Poulet de 48 heures d'incubation, un epithélium neural très développé où les mitoses sont nombreuses (31,82 mitoses par coupe en moyenne), un mésenchyme céphalique lâche et un épiblaste.

Si cette région céphalique est soumise à une irradiation de 5000 r., les premières pycnoses apparaissent 45 minutes après l'irradiation, tandis que les mitoses ont pratiquement disparu (0,64 mitose par coupe en moyenne). Deux heures après l'irradiation, la moitié des cellules sont en pycnose et après 24 heures les tissus sont détruits.

Si cette même région a été protégée, soit par une injection localisée de cystéamine (MEA), soit par une incubation de l'embryon dans une atmosphère d'azote ou d'hydrogène, avant une irradiation de 5000 r., ces radiolésions ne s'observent pas. Les pycnoses n'apparaissent à aucun moment, le nombre des mitoses reste sensiblement normal (25,34 mitoses par coupe en moyenne). La protection obtenue est aussi bonne pour les ARN que pour les ADN.

Ces données montrent que l'effet radioprotecteur s'exerce d'emblée sur les acides nucléiques, qui ne sont l'objet d'aucun processus de destruction suivi de restauration ; tout se passe comme si la cellule avait reçu une dose de rayons X beaucoup plus faible que la dose réellement administrée.

AUTODIFFERENCIATION ET INDUCTION DE CARTILAGE A PARTIR DE MESENCHYME SOMITIQUE DE POULET CULTIVE IN VITRO. Georges Strudel, Laboratoire d'Embryologie expérimentale du Collège de France, Paris, France.

In situ en l'absence des organes inducteurs - tube nerveux et chorde - le mésenchyme somitique du Poulet ne se différencie en cartilage qu'à partir du stade de 28 à 30 somites. Par contre, in vitro, l'autodifférenciation cartilagineuse sur un milieu à base de jus d'embryon de Poulet dilué ou pur se fait à partir de mésenchyme du stade de 27 paires de somites. Si le mésenchyme est entouré d'un fragment de membrane vitelline, le cartilage apparaît dans du mésenchyme du stade de 17 paires de somites. Si de l'extrait des organes inducteurs est incorporé au milieu et selon la composition du milieu, la chondrogenèse peut se faire à partir de mésenchyme prélevé sur des embryons de 20, 13 et 8 paires de somites.

Ces résultats montrent : d'une part, que la chronologie de la chondrogenèse à partir de mésenchyme somitique cultivé in vitro varie avec la composition du milieu, la méthode de culture et l'incorporation d'un extrait inducteur actif et, d'autre part, que l'aptitude du mésenchyme somitique à se différencier en cartilage doit être très précoce.

ANALYSIS OF AN EPIDERMAL MELANOPHORE PATTERN IN PELOBATES (ANURA)

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In an earlier paper (1929) it was shown for Bombina, that the adepidermal (corial) melanophore strands ran parallel to the orthogonally orientated fibers of the basement lamella. This structure can best be explained in assuming, that the melanophores creep actively along the orientated lower surface of the basement lamella. In Pelobates a similar orientation is found in the epidermal melanophores. These large (up to 260 micra long) filiform pigment cells are found between the basal and tecterial layers of the epidermis, but never come in contact with the basement lamella itself. The question arises what causes this orthogonal orientation. No correlation exists between this orientation and the boundaries of the epidermal cells or the orientation of the "filaments of Eberth", but electronmicropscopic observations show an oriented cytoplasmatic ultrastructure in the basal epidermis cells. Firstly the dense concentrations of cytoplasma at the lower border of the cells ("bobbins" of Weiss and Ferris 1954) are arranged in rows are elongated or are forming crests parallel to the fiber directions of the basement lamella. Furthermore the epidermal cytoplasmatic fibers bordering the melanophore run parallel to the longtudinal axis of the latter. The orientation of the melanophore therefore depends on a similar orientation in the basal epidermal layer, which itself depends on the orientation of the basement lamella.

The connective tissue strands running on the lower surface of the basement lamella run parallel to the fibers of the latter and are formed by the uppermost layer of mesenchymal cells of the corium, which show the same orientation. Not only the axis of the nuclei are elongated in the direction of the fibrillae of the basement lamella, but also their endoplasmatic reticulum is oriented in the same direction. This shows, that the orthogonally oriented structures in the skin depend on the mechanical influences exerted on it by the different parts of the growing body.

INDUCING ABILITY OF THE COMBINED MESODERMAL AND NEURAL FACTORS AND THE MECHANISM OF ACTION OF INDUCING FACTORS. Heinz Tiedemann, Hildegard Tiedemann, Ursula Becker, Heiligenberg-Institut Heiligenberg/Baden, Western Germany.

The "mesodermal" inducing protein and a preferentially "archencephalic-neural" inducing ribonucleoprotein were combined in the range 1 : 1 up to 1 : 50 by homogenization and then tested on Triturus alpestris (Einsteckmethode). At the ratio 1 : 1 tails with neural tube and to a particularly high percentage fin structures are formed. At the ratio 1 : 10 spinocaudal - deuterencephalic, and at 1 : 50 deuterencephalic structures with a small amount of head muscle arise. These experiments show that complex spinocaudal and deuterencephalic inductions are formed by a cooperation of inducing factors. But no conclusion can be drawn about their absolute ratio.

The direction of differentiation at a distinct ratio depends also on the environment of the cells and the interaction of cells in different positions. For the formation of complex inductions morphogenetic movements are also necessary. If these movements are inhibited in tissue culture, no harmonious organs are formed.

Further experiments indicate that the removal of a repression may be the first step towards differentiation at the cellular level. In Triturus and chick embryos the inductive factors are partially found in an inhibited state. The mesodermal factor can be activated in the embryos and in extracts from embryos by treatment with phenol. As phenol denatures high molecular proteins but not ribonucleic acid, it may be that a repressor is present which is protein in nature. Furthermore it can be supposed that the activation of the mesodermal factor is caused by the inactivation of the repressor.

PIGMENT CELL MORPHOGENESIS AS INFLUENCED BY THE TISSUE ORGANIZATION. Willie M. Reams, Jr., Dept. of Zoology, Louisiana State University, Baton Rouge, Louisiana, U.S.A.

Pigment cells can be observed to undergo differentiation at different times in different tissue locations. The extent of branching by pigment cells of a given genotype also shows a diversity in different tissue locations. In an attempt to determine factors which influence pigment cell morphogenesis in the mouse and chick, a comparative analysis is being made using techniques of auto-, homo-, and xeno-plastic tissue transplantation. The data indicate that the morphogenesis of a pigment cell in one tissue as compared to that of another tissue is not related to the arrival time of the migratory melanoblasts into the respective regions. Rather, the time of onset of differentiation is specific at a defined stage in differentiation of the particular tissue. Pigment cells will continue to proliferate and even produce melanin until triggered to branch at a critical stage in the development of the tissue community in which they reside. Once triggered, although removed from the initiating site into a relatively neutral environment, the morphogenesis of a pigment cell proceeds to completion. The definitive morphology of the pigment cell is restricted by the extent of differentiation of the tissue fabric at the time of morphogenesis of the pigment cell.

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DISTRIBUTION OF EMBRYONIC INDUCING AGENTS AMONG CELL COMPONENTS.

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Knowing the distribution of the agents responsible for embryonic induction among the cell components of heterogeneous animal tissues, and isolating them in a pure state may contribute to an understanding of the nature of such agents and the mechanism of embryonic induction. The results which we have so far obtained in pursuing this objective may be summerized as follows: cytoplasmic and nucleal ribosomes are solely neural inductive, and the non-PNP isolate from the microsomal fraction yields a mixed type of mesodermal and neural structures.

These findings were mainly obtained by using growing tissues such as 9-day chick embryo and ascites hepatoma, but in regenerating rat liver, even the non-PNP fraction had a strong neural inducing effect. An electrophoretically homogeneous component, which was solely mesoderm-inducing, was isolated from the DOC-soluble non-PNP microsomal fraction of chick embryos by the use of a DEAE-cellulose column. The same components, however, showed a neural and/or spinocaudal effect in some experiments, suggesting the unstable nature of the mesoderm-inducing agents.

At present, we assume that the mesoderm-inducing agents are some newly synthesized structural proteins, and that their change in inductive capacity, from mesodermal to neural, occurs not only as a result of the extraction procedure,but physiologically. Comparing the inductive capacities of ascites hepatoma and regenerating liver should provide some hints as to the cytophysiological nature of the mesoderm-inducing non-PNP, which we assume to be structural ones.

MALIGNANT CONVERSION VERSUS EMBRYONIC INDUCTION.

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A quest for possibilities of future histological evolution in the descendants of recent man may lead a zoologist to the study of cancer. With this problem in mind, a large number of human primary liver cell tumors were examined by means of three-dimensional reconstruction from serial sections. Primary carcinoma of the liver produces structures similar to embryonic and adult livers from every vertebrate class, an observation which leads us to assume that a great variety of morphogenetic possibilities is still available in our genome.

During this study devoted to problems of evolution, an additional and presently more important phenomenon was found incidentally (and this finding pertains to human carcinoma only and not to sarcoma nor to animal tumors). The observations were extended to carcinomata of various origin and to the influence which they exert on surrounding healthy cells in the organ of the primary site as well as at metastatic locations.

A carcinoma lodged in certain epithelial organs (liver, kidney, lung, adrenal cortex) does not destroy or replace the normal cells of the host organ, as had been assumed previously, but it exerts a converting influence on them. The normal cells become cancer cells acquiring the staining qualities, structure and behavior of the carcinoma cells. For example: Liver cells adjacent to metastatic adenocarcinoma of the colon characterized by ciliated follicles are incorporated into the tumor-follicles, acquire cilia and lose their hepatic character. Cytochemical changes demonstrable by special stains and fluorescence precede the morphological transformation.

Morphological characteristics of the parent organ (if noticeable in the carcinoma) as well as the malignant mode of growth are transmitted to normal parenchymal cells.

This phenomenon of conversion was demonstrated in 100% of 26 cases of metastatic carcinoma from many sites to liver, kidney, lung and adrenal. (Elias, Sherrick and Bouldin, Acta Hepatosplenologica, November 1962.)

Nucleic acid transfer has been suggested as the cause of this cellular assimilation. (Elias, Nature, 195:819-820, 1962; Experientia, 18:407-410; Die Naturwiss., 49:401-402.)

One is reminded of embryonic induction. However, in embryonic induction, an organizer influences adjacent cells to undergo a specific change; but this change never involves a complete assimilation and incorporation of cells into the organizer.

Nevertheless, there is a similarity of the two phenomena.

XVI INTERNATIONAL CONGRESS OF ZOOLOGY

CONTRIBUTED PAPERS SESSION

14C. EMBRYOLOGY

ALPHA AMYLASE IN DEVELOPING OYSTER EMBRYOS. Robert E. Black and E. T. Pengelley*, Dept. of Biology, College of William and Mary, Williamsburg, Virginia, and Virginia Institute of Marine Science, Gloucester Point, Virginia. Supported by grant G-9847 from the National Science Foundation. *Present address: University of California, Riverside, California.

The activity of alpha amylase was determined colorimetrically, using dinitrosalicylate, in homogenates of eggs and embryos of *Crassostrea virginica*, made in 2% NaCl buffered to pH 6.2 with 0.02M phosphate. The enzyme was first detectable in the early veliger stage (14 hours after fertilization at 28° C.), and it showed a near-linear increase in activity up to 37 hours of development. At this time the larval intestine was well-developed, showing distinct regions, internal ciliation, and muscular movements. After the peak of activity had been reached, the enzyme dropped sharply in activity, presumably because the larvae had not been fed.

The substrate specificity, pH optimum, and Q_{10} of the amylase in veliger homogenates were compared with those of the amylase in extracts of the crystalline style of the adult oyster. Both enzymes have a pH optimum of 6.2, and attack substrates in the order: potato starch soluble starch oyster glycogen. The Q_{10} for both enzymes is 2.3 between 10° and 20° C., and 1.8-2.0 between 20° and 30° C.

It appears likely that the development of amylase is an indication of the biochemical differentiation of the larval intestine; however, no data are yet available as to the localization of the enzyme in the larva. It is of interest that the appearance of amylase in the larva is not accompanied by the development of a crystalline style. It is not known whether amylase is extracellular in the larval intestine; however no enzyme is released into the sea water in which highly concentrated suspensions of larvae are incubated.

Amylase may have a digestive function for polysaccharides eaten by the veliger, or it may be involved in the metabolism of stored glycogen.

IMMUNOBIOLOGICAL STUDIES OF FERTILIZATION IN THE FROG. J. R. Shaver and S. H. Barch, Department of Zoology, Michigan State University, East Lansing, Michigan, U.S.A.

Experiments in which the sera of rabbits immunized against the egg-jelly capsules and oviducal tissues, as well as against other tissues of frogs, were reacted with various combinations of antigens, have established the tissue-specificity, and, in the case of four species of Rana, the species-specificity of egg-jelly components. The treatment of gametes of the frog with antibodies against components of the jelly-coats of the eggs, prior to insemination, has demonstrated that the fertilizing capacity of spermatozoa and the fertilizability of eggs, is significantly reduced, as compared with treatment with control serum or with antisera against non-jelly secreting tissues.

An analysis of oviducal tissue by means of the double-diffusion technique, utilizing antisera and antigens from the whole oviduct, as well as from segments representing upper, middle and lower thirds of this organ, has revealed the presence of components, the localization of which appears to overlap in the middle portion; i.e.: one component is found in the upper and middle portions, which is not found in the lower portion; another is found in middle and lower portions which is not found in the upper portion. It is possible that these components may represent, in part, the different antigenic configurations discovered by Shivers (1960), who described some components as being shared between two of three species of Rana, and others which were species-specific. Shivers (1962) has localized the species-specific component of egg-jelly of R. pipiens in the outermost layer of the jelly capsule. Since, by means of selective absorption techniques employed on anti-egg jelly sera, we have ascertained that the configurations complementary to species-specific components are most inhibitory to the fertilization reaction, when applied to gametes prior to insemination, it is possible that a fertilizin-anti-fertilizin-like system providing a barrier to cross fertilization between species resides in the jelly-coat of frog eggs and on the surface of spermatozoa.

Antibodies are present in sera prepared against jelly-capsules and oviducal tissue which exhibit disaggregative effects on egg-masses, possibly by means of precipitation membranes produced on outer surfaces of the jelly-capsules. That the effect on fertilizability of eggs produced by treatment with anti-jelly serum may not be due to the production of a lattice-barrier in the jelly, however, is suggested by experiments by Shivers and Metz (1962) which demonstrated that univalent form of the jelly antibodies are equally efficient in inhibiting fertilization. These results rather suggest sperm-egg receptor sites, similar to those which have been postulated for the gametes of species of Echinodermata.

THE EXTENSION OF FERTILIZABILITY IN SAND DOLLAR EGGS. Olin Rulon, Dept. of Biological Sciences, Northwestern University, Evanston, Illinois, U. S. A.

At 18°C. the eggs of the sand dollar (*Dendraster excentricus*) become unfertilizable within 24-48 hours after being removed to sea water from the ovaries of ripe females. Cobaltous chloride (M/400 - M/800 in sea water) will extend the fertilizability of 50% of the eggs to as much as 8 days. When sulfhydryl compounds (glutathione, cysteine, thioglycolic acid), sodium thiosulfate, and versene are added to the cobaltous chloride solutions the fertility of the eggs is extended even more (50% of the eggs are fertile after 25 days in a sea water solution of 50 cc. M/200 cobaltous chloride plus 50 cc. 0.01% thioglycolic acid).

The work has suggested that cobaltous chloride (alone and in combination with the above compounds) inhibits the deterioration of fertilizability by preventing destructive oxidations (*i.e.* sulfhydryl to disulfide) at the egg surface. This hypothesis is strengthened by the recent use of mercaptoethylgluconamide (a -SH agent reputed not to penetrate the cells) which in combination with cobaltous chloride (50 cc. M/100 mercaptoethylgluconamide plus 50 cc. M/200 cobaltous chloride) extended the fertile period 6 days beyond that of cobaltous chloride alone.

РАЗНОКАЧЕСТВЕННОСТЬ ЛИЧИНОК РЫБ, ВЫКЛЕВЫВАЮЩИХСЯ ИЗ ИКРЫ ОДНОЙ САМКИ.

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Период инкубации икры от оплодотворения до выклева первых личинок длится у осетровых и весненнерестующих костистых рыб лишь несколько дней. Однако весь выклев из икры от одной самки продолжается ряд часов и даже несколько суток. Вследствие этого для личинок последнего срока выклева продолжительность инкубационного периода будет значительно большей, чем для первых.

Личинки разных сроков выклева не одинаковы: по мере выклева они становятся все более крупными и развитыми. На протяжении периода выклева эмбрионы, остающиеся в оболочках, продолжают расти и развиваться, и эмбрионы, вынутые из оболочек в конце периода выклева, отличаются от вынутых в его начале, примерно в той же степени, что и личинки соответствующих сроков выклева. Это и является причиной того, что в каждом последующем сроке выклева выходят все более развитые личинки.

Известно, что степень дифференцировки личинок рыб в большой мере скоррелирована с их общей длиной. Однако, одноразмерные личинки разных сроков выклева не идентичны - выклевывающиеся позже могут быть несколько более развитыми.

Изменение условий инкубации изменяет ход эмбрионального развития. Однако реакция на эти измененные условия не идентична со стороны всей массы икринок от одной самки: одни из них реагируют в большей, другие в меньшей степени. Это является одним из показателей исходной неоднородности икры. Измененные условия инкубации могут вести к увеличению диапазона изменчивости эмбрионов и личинок.

Поскольку степень развития выклевывающихся личинок одного вида рыб колеблется в значительных пределах нельзя, как это делается многими авторами, определять стадию "выклевывающейся личинки" очень узко. В понятие "выклевывающаяся личинка" должен быть включен весь диапазон изменчивости.

Разница между личинками разных сроков выклева более резко выражена у костистых рыб (окунь, уклея) сравнительно с осетровыми (осетр, севрюга). Это в равной мере относится и к эмбрионам.

ALTERATIONS IN THE SERUM PROTEINS OF RATS FOLLOWING TREATMENT WITH TRYPAN BLUE. Allan R. Beaudoin and Dorothy Kahkonen, Department of Anatomy, The University of Michigan Medical School, Ann Arbor, Michigan.

Blood serum was obtained from untreated and treated pregnant Wistar rats on days 0, 5, 10, 15, and 20 of gestation. Blood was also obtained from the fetuses of untreated and treated mothers at the time of autopsy on the 20th day. Serum was obtained from pooled blood samples of 2 or 3 fetuses. Paper electrophoresis of the serum was carried out in a Spinco model R system using barbital buffer (pH 8.6) and a potential gradient of 3.2 V/cm. The paper strips were subsequently stained with bromphenol blue, and their density measured in a Spinco Analytrol to determine the relative concentration of each component.

A 2% solution of trypan blue was injected intraperitoneally on the 8th day of pregnancy (10 mg/100g). Maternal serum from rats so treated analyzed 48 hours after treatment (10th day) showed an elevation in the beta globulin. This elevation did not persist, for at 15 days the concentration of beta globulin had returned to a near normal level. Fetal serum analyzed from normal fetuses of untreated mothers and from grossly abnormal fetuses of mothers treated with trypan blue showed no marked differences in relative concentration of the serum proteins.

The rise observed in beta globulin occurs during the period the rat embryo is most susceptible to the teratogenic action of trypan blue and may be causally related. (Supported by N.I.H. grant AM 05603-03)

STUDIES ON THRESHOLD TEMPERATURES IN INSECT DEVELOPMENT. A.Glenn Richards, Department of Entomology, University of Minnesota, St. Paul 1, Minnesota, U. S. A.

Determinations were made on O_2 consumption and rate of development of eggs at various constant temperatures for the grasshopper Melanoplus, the bug Oncopeltus, the moth Pyrausta, the beetle Tribolium, the mosquito Culex and the fly Musca. When the data are plotted on an Arrhenius-type graph, O_2 consumption gives a straight line throughout the viable range but the rate of development (reciprocal of time required for development) always gives a curved line. It follows that one should expect more weight loss and more oxygen consumption for total development at temperatures approaching the temperature threshold. Quantitative determinations support this and show that more energy is required for full development at minimal temperatures than at favorable temperatures. The data suggest that this is merely the result of a delay in developmental rate at minimal temperatures.

The reasons for the above are unproven but the data are consistent with the suggestion that constant minimal temperatures upset metabolic balances and lead to some metabolic deficiency. In some but not all of the species the disturbance is so deleterious that larvae that do hatch at minimal temperatures invariably die even if placed under optimal conditions immediately after hatching. However, if the eggs being incubated at minimal temperatures are placed at a favorable temperature for only a few hours each day the deleterious effect is overcome but the developmental delay is not. It is postulated that some essential metabolic reaction cannot proceed adequately at temperatures that will permit hatching.

"PRÉDISPOSITIONS CYTOPLASMIQUES" ET FORMATION DE LA BLASTULE CHEZ LEPTINOTARSA . André HAGET, Faculté des Sciences de Bordeaux, 40 rue Lamartine, Talence (Gironde) France.

Par brûlure du plasme de maturation et de son contenu peu après la ponte, on peut obtenir des oeufs activés, mais dépourvus de pronucleus ♀. Les noyaux d'origine spermatique déjà présents dans ces oeufs dégénèrent et le cortex ovulaire ne peut être colonisé par des énergides centrifuges.

En l'absence de tout noyau, le cortex des opérés se révèle capable de recruter du cytoplasme intervitellin puis d'effectuer, avec un certain retard, une sorte de perlage très régulier conférant au germe l'aspect d'un stade épithélium primaire. Ce phénomène se manifeste pratiquement au même instant dans toutes les régions de l'oeuf et la taille des perles corticales, très constante, est en quelque sorte spécifique.

La comparaison des opérés et des témoins montre qu' en l'absence des noyaux les "perles" corticales ne sont pas isolées du vitellus par des cloisons basales. La formation de ces dernières dans le développement normal devrait par conséquent dépendre d'une action nucléaire.

La pellicule corticale superficielle, qui joue un rôle capital dans le perlage, semble posséder en outre quelques prédispositions aux mouvements de type gastrulaire.

En résumé, le système cytoplasmique de l'oeuf de Leptinotarsa témoigne d'une réelle aptitude à exécuter de façon "autonome" une partie des processus caractéristiques du développement précoce. Une telle aptitude a été déjà signalée chez quelques insectes ; elle est connue depuis longtemps chez les Echinodermes et les Amphibiens. Les résultats expérimentaux obtenus chez Leptinotarsa sont discutés en fonction des données de la bibliographie.

ORIGINE DES CROCHETS BUCCAUX CHEZ LA LARVE DE CALLIPHORA:ETUDE EMBRYOLOGIQUE.Jeanne Schoeller,Labo. de Zoologie,Faculté des Sciences de Bordeaux,France.

L'interprétation de l'extrémité antérieure des larves de Cyclorrhaphes reste très discutée et la signification des crochets buccaux n'est pas définitivement élucidée.Cependant,on considère souvent les crochets buccaux comme les homologues des mandibules ou tout au moins comme des sclérites mandibulaires.

J'ai essayé de démontrer l'origine des crochets buccaux par une étude embryologique.

L'ablation élective des différents segments gnathaux au stade bandelette embryonnaire segmentée montre que les crochets buccaux de la larve de Calliphora ne peuvent être considérés comme les homologues des mandibules,mais qu'ils sont d'origine maxillaire.

Par des ablations partielles du segment maxillaire, j'ai pu préciser que le territoire présomptif du crochet buccal est situé dans l'ébauche de la mâchoire elle-même.

Un examen approfondi du développement normal m'a permis de vérifier ces résultats expérimentaux.Le territoire présomptif du crochet buccal est formé de cellules épidermiques plus grandes que les autres,à noyau doté d'un nucléole volumineux.Ce marquage naturel m'a permis de suivre les mouvements du segment maxillaire au cours de la céphalogenèse,jusqu'à l'éclosion de la larve.

DEVELOPMENT OF COMPOUND EYES IN PIERIS RAPAE L. (LEPIDOPTERA).

Chang-Whan Kim, Dept. of Biology, Korea University, Seoul, Korea.

Epidermal cells round the larval lateral ocelli in Pieris become elongated and differentiated into elements of ommatidia during prepupation, nuclei of which are arranged at different levels. Large amount of pigment granules has already occurred around the secretory cells in the periopticon near proximal ends of the optic nerves at this time. After the ecdysis neuroglial cells grow along the existing optic nerves to be thicker, the distal ends of which extend under the basement membrane of the sensory epidermis with tracheoles. The precursor of the sensory elements is innervated by nerve fibre ramifications of the optic nerves. First of all the elongated elements of ommatidia become thinner progressively and organise into units, ommatidia, in about 15 hour-old pupae. In 30 hour-old pupae retinular cell bundles spindle-shaped, corneagenous cells and cone cells which take place at the distal ends of the spindles, and the iris cells are differentiated. Two crystalline cones develop in ommatidium at about 45 hour stage and then the secondary pigment cells, nuclei of which have migrated from the bottom to the distal portion a little over the middle, become vacuolated at about 60 hours. At this stage each ommatidium is most depressed, about 30 μ in length, just half of the initial length of the sensory epidermal cells immediately after ecdysis but after then begins to elongate again. At about 90 hour-old pupae the crystalline cones of an ommatidium unit to be a heart form and later a cone form. As the optic lobes grow out and the elements of ommatidia become elongated, optic nerves thicken and shorten and clots of pigment granules retained in the periopticon are drawn near the compound eyes. The granules, however, spread through neuroglial cells to the secondary pigment cells from about 140 hours.

NATURALLY OCCURRING DEVELOPMENTAL ARREST IN TELEOST FISHES WITH ASSOCIATED MODIFICATIONS OF EPIBOLY AND EMBRYOGENESIS. John P. Wourms, Jr., Department of Biological Sciences, Stanford University, Stanford, California, U.S.A.

Eggs, capable of extended developmental arrest, afford the sole means of continued survival for populations of oviparous cyprinodont fishes (*Cynolebias*, *Nothobranchius*, and some *Aphyosemion* species) from temporary bodies of fresh water in South America and Africa. Eggs of this type possess a developmental pattern quite distinct from that of other teleost fishes. Cleavage of the blastodisc is typical. At the end of the cleavage stages, the blastomeres undergo a striking change in shape, from essentially spherical cells somewhat deformed by contact with adjacent cells, to discoidal cells. These discoidal cells separate from one another, and assume a fibroblast-like shape with abundant filopodia. Then, as individuals rather than as a mass the cells proceed to move over the yolk surface. The yolk surface is covered by individual cells, uniformly dispersed so that each cell is separated from adjacent cells by a distance of several cell diameters and as judged by light microscopy is not in contact with them. The process of embryo formation occurs considerably after this dispersed stage has been attained, and appears to differ significantly from that reported in any other teleost. Developmental arrest occurs at the dispersed cell stage (primary arrest). Such eggs may survive and remain suspended in this pre-embryonated state for over one year under conditions of partial anhydrobiosis. This contrasts to the normal duration of the dispersed state of 36-48 hours at 25° C. in eggs which have not been subjected to environmental stress. Photographs of the normal stages of development of *Nothobranchius guentheri* (Pfeffer) serve to illustrate this type of development.

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NEED FOR CONTROLLING THE BIOLOGICAL "QUALITY" OF AVIAN EGGS INTENDED FOR EMBRYOLOGICAL STUDIES. Igor L. Kosin, Laboratory of Developmental Genetics, Department of Poultry Science, Washington State University, Pullman, U.S.A.

Avian eggs have been widely used in experimental embryology. A comparison of the data resulting from such experiments, however, usually requires criteria to measure embryonic development. A staging series, fitted to a particular species, e.g. the Hamburger-Hamilton series for the chicken (J. Morphol. 88: 49. 1951), satisfies this requirement. At this laboratory, a staging procedure was recently developed for the turkey (Biol. Bul 119: 90. 1960).

To apply the criteria of such a procedure, however, it is necessary first to provide adequate safeguards to control the biological "quality" of hatching eggs. Otherwise, both the rate of embryonic development and the frequency of deviants can become so atypical as to make difficult a meaningful interpretation of results. Data bearing on the necessity of such safeguards have been collected at this laboratory as a byproduct of a study of genetic and environmental factors affecting hatchability in the turkey. The factors are listed below in the decreasing order of importance. The observed effects were particularly marked in 3 to 4-day-old embryos.

(1) The time interval between oviposition and incubation. Embryos from eggs held more than 4 days, even under optimal pre-incubation storage conditions (12.5°C, 85% relative humidity), invariably showed depressed growth and higher incidence of abnormalities.

(2) Heredity. Selection for high hatchability evolved a population phenotype in which embryonic development was characterized by rapid rate and by low incidence of abnormalities. The eggs showed greater resistance to the effects of prolonged storage. See (1) above. Selection for large mature body size resulted in embryos characterized by lower rate of development and higher incidence of abnormalities.

(3) The time of year egg laid. Embryos from eggs laid in the middle of the spring breeding season developed faster. As the season progressed growth was depressed.

(4) The size of egg clutch (i.e. the number of eggs laid on consecutive days). Single-clutch eggs produced embryos which developed more rapidly. However, embryos from multiple-clutch eggs had lower frequency of abnormalities.

(5) The time of day egg laid. Eggs laid near the end of a 14-hour day (between 7 and 9 p.m.) produced embryos with the highest rate of development.

(6) Miscellaneous factors. Embryos from eggs with defective shells (thin, rough, atypically colored) or from eggs of abnormal shape and size developed more slowly and showed a higher frequency of abnormalities.

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"A STUDY OF REPRODUCTION AND OVARIAN HISTOLOGY IN MICE TREATED WITH CHEMICAL CARCINOGENS".

B. Kumari Batra, Department of Experimental Embryology, Indian Cancer Research Centre, Parel, Bombay 12, India.

Abstract

Ovaries of Swiss/Icrc strain mice were painted with a single dose of 0.25% 20:methylcholanthrene in benzene, to study the effect of this substance on the different cellular elements of the ovary and on fecundity in these animals. Solvent controls and untreated controls were also maintained.

The immediate effect on the ovaries in animals autopsied within 15 days of treatment show no apparent change from the normal having growing follicles and corpora lutea. However, many of the ova in the primary follicles have less definition. The cytoplasm appears hazy and the nuclear membrane ill-defined. Haemorrhagic follicles have also been observed.

In animals autopsied 30 days following treatment there is extensive destruction of ova, and the cytoplasm is as in the 15 day animals. In many of the ova shrunken nuclei and early pycnotic changes were observed. Signs of abnormal division were not uncommon.

In these two groups the other cellular elements appeared normal having no discernible differences from the benzene and untreated controls.

In the animals bred and maintained over the age of 12 months uniform proliferation of the granulosa cell elements leading to lesions were noted. The effect on the luteal cell element is not as extensive. Follicular activity compared with the controls is much decreased.

With regards fecundity many animals either did not breed or had only one litter each, though they were kept continuously with their mates.

In the young born to these mice several abnormalities were observed, which corroboratesthe histological observation on the damage of the ova. However, it also confirms that whether to a lesser or greater extent, all the ova are affected by the single treatment.

The results are discussed in the light of the evidence that attributes sustained effects of the single dose of this chemical substance on the female reproductive cell.

EFFETS PHYSIOLOGIQUES PRECOCES DE L'IRRADIATION DU COEUR EMBRYONNAIRE. Georges Le Douarin, Laboratoire d'Embryologie expérimentale du Collège de France, Paris, France.

L'irradiation aux rayons X, localisée à l'ébauche cardiaque, de l'embryon de Poulet au troisième jour de l'incubation, provoque une série de modifications de la physiologie circulatoire. En premier lieu on observe une accélération du rythme cardiaque, pour des doses de rayonnement comprises entre 3000 et 8000 r. Cette tachycardie survient dans les deux premières heures qui suivent l'intervention, reste modérée pour les doses considérées et est réversible. Par ailleurs la puissance des pulsations cardiaques est diminuée et il en résulte un affaiblissement de la circulation de retour. Des stases veineuses se produisent dans l'aire vasculaire extra-embryonnaire. Elles débutent dans la partie antérieure de l'arcade veineuse marginale puis gagnent la veine vitelline antérieure et les ramifications des branches antérieures des veines omphalo-mésentériques. Ces troubles circulatoires sont le plus souvent réversibles et la circulation redevient normale en quelques heures. Lorsque la veine vitelline antérieure est restée longtemps non fonctionnelle, elle peut se résorber et être remplacée par des suppléances formées par les veines omphalo-mésentériques. L'irradiation de fragments de coeur embryonnaires, cultivés in vitro, permet d'obtenir également une accélération du rythme des battements. Pour des doses de rayonnement élevées, de l'ordre de 300 000 r, la fréquence des pulsations atteint en quelques minutes un maximum (150 à 200 % du rythme initial) puis décroit rapidement.

L'étude de l'activité électrique de coeurs embryonnaires de Poulet, explantés en culture organotypique et recevant une irradiation de 10 000 r. a permis d'observer des perturbations de l'électrogramme. Ces perturbations surviennent dans les premières heures qui suivent l'irradiation. L'amplitude des ondes rapides diminue, l'onde lente de repolarisation se complique et l'espace iso-électrique séparant les pulsations se réduit et peut même disparaître. La morphologie du complexe QRS est moins fréquemment perturbée que celle de l'onde T, ce qui montre que ce sont les processus de repolarisation qui sont le plus affectés par l'irradiation. L'irradiation de fragments de coeur en culture histiotypique permet d'obtenir des modifications analogues de l'électrogramme. En utilisant de fortes doses de rayonnement on peut également modifier l'onde rapide dont le sens peut s'inverser.

Les résultats obtenus montrent que l'irradiation aux rayons X du coeur embryonnaire perturbe le fonctionnement physiologique de cet organe bien avant que les radiolésions histologiques ne se soient établies. Lorsque les doses de rayonnement administrées sont modérées, ces phénomènes précoces sont réversibles.

INFLUENCE DE L'ENCÉPHALE ET DE LA CHORDE SUR LA MORPHOGENÈSE DU CRANE EMBRYONNAIRE DE POULET. Jean SCHOWING, Laboratoire d'Embryologie expérimentale du Collège de France, Paris, France.

Suivant l'exemple de nombreux auteurs, précurseurs de l'analyse expérimentale des facteurs qui président à l'organogenèse embryonnaire chez le Poulet, nous avons étudié le rôle de la chorde et de l'encéphale dans la morphogenèse du crâne.

L'excision du rhombencéphale entraîne une atrophie des os pariétaux. Ceux-ci disparaissent si l'on supprime le rhombencéphale en même temps que le mésencéphale. L'excision du prosencéphale entraîne des modifications des os frontaux, tandis que l'excision du prosencéphale et du mésencéphale entraîne l'atrophie des os frontaux et du plancher cranien.

Si nous excisons la chorde seule, la plaque basilaire et le basioccipital sont réduits. L'excision de la chorde et de l'encéphale affecte la voûte cranienne, de même que les palatins et le rostre du parasphénoïde.

Si de plus nous excisons la moelle antérieure jusqu'à la 9e paire de somites, le complexe occipital disparaît.

Si l'on excise seulement le rhombencéphale, la chorde et la moelle antérieure, seul le complexe occipital disparaît en totalité, les pariétaux sont réduits, les autres os ne sont pas altérés.

Si l'on épargne la chorde, le basioccipital et la plaque basilaire se différencient mais restent petits.

Il semble donc que l'encéphale agisse directement sur la différenciation de la voûte cranienne, et concurremment avec la chorde sur celle du plancher cranien.

MODIFICATIONS DE LA COMPETENCE DE L'ECTODERME DE JEUNE GASTRULA DE BATRACIEN, APRES IRRADIATION AUX RAYONS X.

Mme Marguse REYSS-BRION. Laboratoire d'Embryologie Expérimentale du Collège de France et du CNRS, PARIS.

Après des irradiations aux rayons X variant entre 400 et 50 r, il se produit une perte progressive de compétence de l'ectoderme.

Un ectoderme irradié peut donner des formations spinicaudales alors qu'il est incapable d'édifier des structures céphaliques et il peut différencier des structures archencéphaliques alors que les structures deutérencéphaliques sont encore absentes ou rudimentaires.

XVI INTERNATIONAL CONGRESS OF ZOOLOGY

CONTRIBUTED PAPERS SESSION

15A. CELL BIOLOGY

POLYMORPHIC SPERMATOZOA IN GENERAL AND THESE OF CREPIDULA FORNICATA IN PARTICULAR. Jan Z. Wilczynski, Biolog.Department,Lebanese State University,BEIRUT,Lebanon.

Although the different types of both-human and animal- spermatozoa were long time ago described, their presumed dymorphism as connected with the chromosomal male heterogamety remains cytologically mostly unsubstantiated.

More comlicated , however, turned to be the real polymorphism of spermatozoa found in many Gasteropods / i.e. Paludina,Janthina, Bythinia,Goniobasis etc./ where their different forma had to be divided at least into two types, i.e. the presumably normal-eupyren and the much larger and so far mysterious- oligo and apyren-spermatozoa. The problem in question seems all the more interesting that it seems to be in contradiction with the theory of the chromosome individuality or their genotypical differentiation usually takenas the very base of the ensuing phenotypical differences.

A still higher grade of spermatic polymorphism was found by the author in his studies on the snail Crepidula fornicata from both American and English shores. Here spermatozoa of at least five different types have been recognized: / I / a spermatozoon with one or sometimes two tails, probably but not necessarily, representing two spermatozoa constantly with their heads stuck together, / 2 / a long spermatozoon with a small head extending into a long perforatorium, / 3 / a spermatozoon with a large thick head / 30-50 times normal / and a comparatively short thick tail, its perforatoriumbeing shorter than in other forms, / 4 / a rare type representing an only developmental type and, finally / 5 / a spermatozoon which is possibly a modification of an apyren spermatozoon.

In spite of this remarkable differentiation in so many types, no differences in their respective spermatogonies or spermatocytes were found, just as no differences in the external appearances of their veliger larvae were noticed.

The author tentatively suggests that the apyren spermatozoa with many tails could perhaps be considered as a peculiar polyspermatic mother cells, being in the same time a transporting carriers of the normal spermatozoa , which, still unripe, are included in their cytoplasm where they undergo their final development.

LENGTH MEASUREMENTS OF THE DIPLOID KARYOTYPE OF THE MEXICAN AXOLOTL (Siredon mexicanum) WITH REFERENCE TO A POSSIBLE SEX DIFFERENCE.* Victor V. Brunst and Theodore S. Hauschka, Dept. of Experimental Biology, Roswell Park Memorial Institute, New York State Dept. of Health, Buffalo 3, New York.

The diploid somatic chromosome set of the Mexican axolotl was examined in tail regenerates of young adult animals. Metaphase karyotypes fixed by the acetic-orcein squash technique were analyzed in 25 male cells and 15 female cells. In order to avoid contraction artifacts, no colchicine was used. As correctly observed by Fankhauser and Humphrey (1942, 1950) in the crowded chromosome plate of whole tissue mounts, the diploid chromosome constitution is 28. This number was confirmed in 83 well-spread metaphases. One axolotl apparently had a somatic constitution of 26 and another of 30 chromosomes. The frequency of tetraploid metaphases in our regenerates was approximately 9%; polyploid cells were equally frequent in both sexes.

No obvious morphological sex chromosome difference was found. Variations in chromosome size and morphology were therefore examined quantitatively with the aim of establishing a possibly more subtle karyotypic sex difference: The length of every chromosome in each karyotype was measured. These measurements focused attention on an ephemeral difference between the relative lengths of the 4 smallest chromosomes in the two sexes. The ratio of the combined lengths of the 4 smallest chromosomes to the total karyotype length was identical in both sexes. Thus, the degree of relative chromosome contraction in male and female metaphases is comparable and is probably not responsible for the length difference observed. Among the 4 smallest chromosomes within this quartet, the ratio of the total length of the 3 larger units to the length of the smallest unit averaged 3.62 ± 0.07 in the female and 4.39 ± 0.08 in the male. The difference of 0.77 ± 0.11 is significant ($P = <0.01$). These measurements indicate (but do not prove) a "kryptostructural" sex chromosome difference in the axolotl, discernible only by statistical evaluation of chromosome lengths. Meanwhile, Weiler and Ohno (1963, in press) have demonstrated a pronounced sex dimorphism in the African frog *Xenopus laevis*, contrary to earlier findings disclaiming sex chromosome differences in Amphibia.

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CHROMOSOME ABERRATIONS IN A CHINESE HAMSTER STRAIN OF CELLS BY THE USE OF EXTRACTS OF IDENTICAL CELLS.

D.STONE AND Y.Y.KANG*

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Cultures of Chinese hamster cells(strain FAF 28)were grown in the presence of cell-free extracts obtained from similar cells. In the control population the normal diploid stem-line(22 chromosomes) was found in156 of 200 cells observed, with an average chromosome number of 24,the lowest and highest levels being 22 and 61,respectively.On the other hand,in the treated group 23per cent of the cells contained 22 chromosomes and 21 per cent contained 44, with an average chromosome number of 38.In the latter case the lowest and highest number of chromosomes per cell being 21 and 143,respectively.

In the untreated cultures,cell squash preparations reveal the following four groups of chromosomes; 4pairs of large and metacentric,6 pairs of medium and metacentric,6 pairs of small and metacentric, and 6 pairs of small and subtelocentric chromosomes.In the regular tetraploid patterns these proportions are; 8:12:12:12.In comparison,the ratios found in the tetraploids of the treated cells were; 7.83:11.61:10.87:12.57.The latter cultures also exhibited morphological aberrations,wherein chromosomes were found of large size and subtelocentric,large size with two constrictions, and large size with three constrictions.Such morphological species were absent in the control cultures.

It is not clear whether the above changes in chromosome numberare due to cell selection,or whether the morphological aberrations were due to translocation.These data will be discussed.

GIANT POLYTENE CHROMOSOME DEVELOPMENT IN CELLS OF THE HYPODERMIS AND HEART OF FLY PUPAE. Joan M. Whitten, Dept. of Biol. Sciences, Northwestern University, Evanston, Illinois.

Trichogen and tormogen cells of the fly hypodermis contain polytene chromosomes, but do not reach great proportions. Considerably larger hypodermal cells possessing giant polytene chromosomes have been found in pupae--in the developing pulvilli of the pre-tarsus. Each cell secretes an extensive area of adult cuticle. The cells develop from small unspecialised hypodermal cells which were themselves concerned in the earlier secretion of pupal cuticle over the pre-tarsal lobes. Growth of the giant cells is rapid; during this phase a large nucleolus is evident. Polytene chromosomes appear as cell growth ceases, reaching maximum size shortly before adult cuticle secretion; puffing in the chromosomes coincides with cuticle formation. Of particular interest are the large size of the cells and their chromosomes, their rapid growth, superficial position, and specific activity in apparent response to humoral factors concerned in moulting.

Cells having giant polytene chromosomes are also found associated with metamorphosis of the heart. In the mid-pupa, the wall of the heart increases in thickness; this is brought about by the activity of individual heart cells. These cells, whose nuclei are characterised by a large nucleolus, suddenly develop giant polytene chromosomes; the latter reach their maximum size shortly before secretion of the additional material to the heart wall. It would appear that subsequent to initial heart formation in the embryo, the heart cells remain dormant until stimulated, presumably by hormones, to enter this second phase of secretory activity in the pupal stage.

The appearance of giant polytene chromosomes in these hypodermal and heart cells, is seen to be associated with specific secretory activity. In both instances, the product is measurable and forms a permanent structure, in contrast to the secretions of salivary gland, malpighian tubule, and ovarian nurse, cells.

CYTOLOGICAL AND CYTOCHEMICAL RESEARCHES IN *Peripatus acacioi* MARCUS & MARCUS.

Giorgio Schreiber, Luiz Carlos Gomes Simoes and Iolanda Marques da Silva. Institute of Biology, University of Minas Gerais, Belo Horizonte, Brazil. (*)

The following researches have been made in a new species of *Peripatus* discovered in Brazil in 1954.

1. Caryotype in spermatogenesis shows 28 chromosomes like *Peripatus capensis*. The chromosomes of spermatogonial mitoses are more contracted than those of somatic mitoses.

2. Some groups of cells in the celomic sacs of embryos show the chromosomes shorter and more contracted than those of other tissues of embryos. Probably they are primary gonocytes.

3. Cytophotometric studies on DNA content in nuclei Feulgen stained revealed a clear differentiation between some of the organs by endopolyploidy: hypoderma and neuroblasts are always 2n, the cells of the glue gland present an extraordinary variability starting from 4n to 8n. 4n ploidy was established with first spermatocites.

The cytophotometric data of DNA plotted against the corresponding values of nuclear size, show a very good linear regression with groups of concentration of data respectively at 2n, 4n, 8n.

Some nuclei of the glue gland recognizable by lower stainability, with Feulgen, present a nuclear size higher than those correspondent to the DNA value of ploidy and gave a great dispersion toward the right side of the regression line.

These nuclei however are situated mostly at the 4n and 8n and some at 6n (sesquiphase - Schreiber, 1949), level of DNA and not at any other intermediate value.

This fact seems to be interesting if interpreted according to the theory of the auto and heterosynthetic interphasic growth of Bloch. Heterosynthetic growth (greater nuclear size) takes place not at any value of DNA but only at the modal one, that is, during the resting phase between the successive endopolyploidic cycles. This suggests that heterosynthetic activity of the genome is an alternative status with the autosynthesis.

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STRUCTURE AND FUNCTION OF THE GIANT CHROMOSOMES. Atıf Şengün, Dept. of General Zoology, İstanbul University, Turkey

The giant chromosomes exhibit a lateral and a longitudinal pattern of organization. The longitudinal pattern of organization is represented by bands, interbands and by heterochromatic regions where bands are not developed clearly. This structure of homologous chromosomes shows variation: a- from cell to cell in the same tissue, b- from tissue to tissue in the same larva, c- from larva to larva of the same as well as of different ages, d- under different external conditions.

Regarding the lateral pattern of organization the giant chromosomes are composed of a lot of strands or of 4 chromatids as is seen in suitable cases. But the existence and the number of the strands is still questionable for they are sometimes not visible and their number seems to be not constant. Therefore the giant chromosomes should consist -at least- of the two homologous -of the maternal and paternal chromosomes which are divided into the two daughter chromatids at the interphase of the last mitotic division. But in the cytogenetical experiments the daughter chromatids of one homologues conduct so as if the two sister chromatids both are united intimately in one chromosome and therefore only one homologous chromosome is visible.

The application of isotopes in cytology seems to be promising to demonstrate the phsiological condition of chromosomes or of their parts for the incorporation of the labelled substances is correlated with the activity of the hereditary units. It could be shown by this method that the physiological condition of a chromosome along its length is not the same and that the uptake of substances such as the H^3-thymidine, H^3-adenine, P^{32}, S^{35} etc. varies a: from region to region in the same chromosome, b: from chromosome to chromosome in the same nucleus and -if the homologous chromosomes are compared - c: from nucleus to nucleus in the same tissue, d: from tissue to tissue in the same larva, e: from larva to larva of the same as well as of different ages. Concerning the lateral organization of the giant chromosomes it seems so as if each homologue is acting as a physiological unit whereas the activity of each daughter chromatids is generally masked for they have the same gene loci.

The varying degree of incorporation of the labelled substance is correlated in some cases with the morphologically distinct parts of the chromosomes such as the heterochromatic and the euchromatic regions. But there are chromosome regions with similar disc pattern, -e.g. the homologous regions of the two homologues combined in one giant chromosome- which show a different activity.

The cytological studies with different methods have shown that very small regions of chromosomes such bands and interbands secrete substances. The secretes of DNA containing bands give Feulgen positive reaction whereas those of the interbands do not.

CRISTALLOIDES ERYTHROPHILES INTRANUCLEAIRES DANS L'ORGANE DE VAN DER HOEVEN DE NAUTILUS UMBILICATUS LAM. (CEPHALOPODA).

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Faite au cours d'un travail histologique d'ensemble sur le Nautilus umbilicatus Lam., l'étude cytologique et histochimique de l'organe de Van der Hoeven a révélé une abondance extraordinaire de cristalloïdes intranucléaires.

L'histologie de l'organe de Van der Hoeven est bien connue grâce à Griffin (1900) ; on sait que c'est un organe à symétrie bilatérale, formé de lamelles et de tubes glandulaires, l'ensemble étant recouvert d'un épithélium.

Les tubes glandulaires sont constitués par deux types de cellules ; les unes très grandes, cylindriques, les autres petites et coniques dont les noyaux contiennent des cristalloïdes. Les lamelles ont des cellules à noyaux pourvus de cristalloïdes alternant avec des cellules glandulaires à noyaux basaux sans cristalloïdes. Tous les noyaux des cellules épithéliales de recouvrement ont des cristalloïdes.

Ces derniers sont homogènes, octoédriques ou rhomboédriques, réfringents. Ils occupent la plus grande partie du noyau et sont distincts des nucléoles. Ils retiennent l'éosine, la safranine, l'azocarmin, la fuchsine basique, l'hématoxyline au fer. Leurs affinités tinctoriales sont exactement les mêmes que celles des cristalloïdes érythrophiles du mésentéron de Gryllus (Insecta Orthoptera) ou de la glande digestive et du pénis de Ptérotrachea (Mollusca Heteropoda).

Les cristalloïdes sont APS-négatifs et n'ont ni fer, ni calcium, ni lipides figurés, ni acides nucléiques, ni mucopolysaccharide, ni protide sulfhydrilé, histochimiquement décelables. Leur point isoélectrique est celui des protides ; ils sont riches en α-amino acides et en acides aminés aromatiques.

Les cristalloïdes ne semblent pas présenter de rapport direct avec le fonctionnement des cellules glandulaires de l'organe de Van der Hoeven.

CELLULAR AND SUBCELLULAR ORGANIZATION OF THE OSPHRADIUM IN BUSYCON[1]

Everett Anderson, Dept. of Zoology, University of Massachusetts, Amherst, Massachusetts

The osphradium is an organ of unknown function; however, it has been viewed as a respiratory organ, dye secretory organ and a chemoreceptor. Macroscopically, it is composed of a variable number of leaflets each of which extends laterally from a rather broad central ganglion. In the center of each leaflet are muscle fibers, connective tissue elements and nerve fibers which originate from the central ganglion. The epithelium of the leaflet rests on a well developed basement lamina and consists of pigmented, ciliated and mucuous cells. The plasma membrane at the base of the epithelial cells displays processes of varying length. Beneath the electron opaque basement lamina is a rather lucid substance through which weave numerous collagen fibers.

The pigment cells contain large amounts of rough endoplasmic reticulum, multivesicular bodies, mitochondria and Golgi complexes. The dense pigment bodies are limited by a thin membrane; some of these appear to be in various stages of "maturation". Other membrane limited spherical structures are also present in pigment cells. Internally, these bodies consist of three parts: (1) vesicles 600-800 A in diameter, (2) closely stacked smooth membranes which show a curvilinear or circular pattern and (3) dense membrane bounded bodies of varying diameters. Microvilli as well as pseudopods extend from the apical portions of the pigment cells.

The ciliated cells, which occupy definite positions on the leaflet, display no unusual structure. Their cilia show the characteristic structural pattern when viewed in cross section. Arising from the base of the kinetosomes are long striated filaments which sometimes extend to the level of the nucleus. The cytoplasm of these cells show the regularly occuring organelle systems. In addition there are a host of 600-800 A vesicles and a few multivesicular bodies.

The nerve fibers of the central ganglion and those within each leaflet are of the unmyelinated type and contain large neurotubules. Some of these are found running between the cells of the epithelium.

[1] Supported by a grant (GM 08776-02) from the National Institutes of Health, U.S.P.H.S.

CYTOLOGICAL AND CYTOCHEMICAL OBSERVATIONS ON THE ATRIAL GLAND OF MILAX GAGATES (DRAP).

by Aristeo RENZONI, Dept. of Zoology, Siena (Italy)

Milax gagates (Pulmonata Sthylommatophora) is notable for its complicated reproductive system,in particular for the stimulator and the atrial gland.

The stimulator,which is lodged in the atrium,is mostly muscular tissue.It is conical,slightly curved at the apex and with three longitudinal rows of papillae and a papilla at the tip.

The atrial gland is posterior to the stimulator.It rests on the terminal portion of the hermaphrodite duct and is made up of about 30-40 tubules.

The atrial gland can be subdivided into three sections: proximal,medial and distal.

In the proximal portion the walls of the tubules consist of two layers:the internal one a simple cuboidal epithelium, the external one made up of pearform gland cells.These have their tips wedged between the cells of the cuboidal lining epithelium and secrete directly into the lumen of the tubules.

In the medial portion the walls of the tubules are noticeably thinner due to the total disappearance of the gland cells, which have been substituted by circular muscular cells.The cells of the cuboidal epithelium are smaller than in the preceding portion.

In the distal portion the tubules enter the stimulator individually and open into the papillae.The muscular cells of their walls merge with those of the stimulator.

The gland cells are the only part of the tubules that undergo clear morphological variations in relation to age and functional activity.

In sexually mature adult individuals the gland cells become considerably enlarged.They are full of granules and drops of secretion that give a strongly positive PAS reaction,are not metachromatic and do not stain with dyes for mucin.

Only the cuboidal epithelium of the tubules,at least at the apical portion of its cells,gives a somewhat positive reaction to dyes for mucin.

These facts lead to the conclusion that even though a small quantity of mucin is found in the secretion of the atrial gland, it cannot be considered a mucous gland.

HISTOCHEMICAL STUDIES OF YOLK SYNTHESIS IN DYSDERCUS CINGULATUS.

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Each of the ovaries of Dysdercus cingulatus consists of seven telotrophic ovarioles. Each ovariole can further be divided into an anterior part, the germarium with oogonia, trophocytes and the interstitial nuclei, and a posterior part, the vitellarium with oocytes in various stages of growth. The trophic tissue of the germarium can be divided into three zones; (i) anterior most zone of the trophic tissue with distinct cell boundries; (ii) zone II where the cell boundries first disappear and the nuclei tend to fuse; (iii) zone III with larger and fewer nuclei. There are three kinds of nuclei in the trophic region of the ovariole; small migratory nuclei; large migratory nuclei and the giant nuclei.

In the telotrophic ovarioles of Dysdercus cingulatus there are present three types of lipid bodies, namely L_1, L_2 and L_3. The L_1 bodies are in the form of granules consisting of phospholipids. The L_2 bodies are in the form of vesicles and crescents having a phospholipid sheath and a triglyceride core usually is masked. The L_3 bodies are composed of triglycerides only. The lipids in the trophic tissue are of L_1, L_2 and L_3 types: in the nutritive cords these are of L_1 type only while in the follicular epithelium lipids are composed mainly of L_1 and L_2 types. A fully mature oocyte has all three types of lipids mentioned above. In such oocytes the L_1 and L_2 bodies are usually cortical in disposition while the L_3 bodies are centrally distributed. In the early growth stages of the oocyte lipids are contributed by the trophic tissue mainly as L_1 bodies while in the late stages of vitellogenesis lipids are also contributed by the follicular epithelium.

The yolk spheres are acid mucopolysaccharide in nature and arise from the precursors in the cortical ooplasm. These precursors are actually the nucleolar extrusion bodies. No precursors are present either in the trophic tissue or the follicular epithelium.

RNA is contributed to the oocyte by the trophic tissue through the nutritive cords. With the growth of the oocyte there is also observed a gradual decrease in the cytoplasmic RNA. DNA of the giant nuclei in the trophic region is also believed to pass down the nutritive cords as some Feulgen negative material.

STRUCTURE ET ULTRASTRUCTURE DES ORGANES LUMINEUX CHEZ QUELQUES TELEOSTEENS. Jean-Marie Bassot, Institut Océanographique, 195 rue Saint-Jacques, Paris, France.

L'étude de plusieurs espèces de téléostéens Stomiatidae (Argyropelecus, Chauliodus, Cyclothone, Ichthyococcus, Maurolicus, Stomias, Vinciguerria) permet de reconnaître un plan commun dans l'organisation de leurs photophores. Ceux-ci comprennent:

1° Des tissus hautement spécialisés dans leur rôle dioptrique : reflecteur à paillettes de guanine orientée, couche pigmentaire, lentille sertie d'un anneau lamellaire permettant peut-être de varier sa courbure.

2° Deux catégories bien individualisées et constamment présentes de cellules glandulaires .

Les cellules glandulaires "A" sont caractérisées par un abondant ergastoplasme; elles élaborent des grains glycoprotidiques dont les caractères histochimiques et tinctoriaux sont strictement semblables chez les espèces lumineuses étudiées. Leurs caractères cytologiques (localisation plus ou moins basale de l'ergastoplasme par exemple) varient selon les espèces, mais d'une manière que l'on peut tenter de classer . Qui plus est, chez Argyropelecus et Maurolicus, les cellules "A", très nombreuses, montrent une sériation dans l'espace des étapes de formation du produit de sécrétion. Cette disposition privilégiée a permis de préciser les aspects successifs et les relations des organites cytoplasmiques au cours du cycle sécrétoire .

Les cellules glandulaires " B " présentent des aspects cytologiques variables selon les espèces . Chez Argyropelecus, les cellules "B" les plus distales possèdent un chondrioconte unique et géant enchassé dans une dépression du noyau .

Des micrographies optiques et électroniques montreront différents types de photophores, l'aspect de leurs structures annexes et de leurs cellules glandulaires dont le cycle sécrétoire sera discuté .

THE SIGNIFICANCE OF VARIATION IN INTERVALS BETWEEN SUCCESSIVE OVARIAN OVA IN CHICKENS AND TURKEYS. E. G. Buss, The Pennsylvania State University, University Park, Pennsylvania, (USA)

Ovarian ova from 107 chickens and 213 turkeys have been weighed to provide further information on the nature of the variable interval between successive eggs within a clutch and between clutches. In both species, evidence was found for weight differences between successive ova. In some cases, the difference between two successive ova was nearly double that which was characteristic for the respective birds. The data are interpreted to indicate that, in these cases, there was an exceptionally long delay in the initiation of the accumulation of yolk by the smaller of the two sequential ova.

A few cases were observed in various stages of growth in which two ova had the same weights. These data are interpreted to indicate that double-yolked eggs occur because the two ova started accumulating yolk simultaneously, hence reach maturity and are released at the same time. Thus, if the infundibulum successfully picks up both ova at nearly the same time, a double-yolked egg is formed.

In turkeys, three cases where two eggs were passing through different parts of the oviduct at the same time were observed.

It is concluded that the interval or lack of interval between maturing ova in chickens and turkeys is a consequence of the variation in the initiation of the rapid growth phase by immature ova. This condition suggests a rhythmic variation in the effective level of the follicle-stimulating hormone.

STUDIES ON SPERMATOGENESIS AND FINE STRUCTURE OF SPERM IN DIDELPHYS MARSUPIALIS. Edward D. DeLamater, Section on Cytology & Genetics, Dept. of Physiology, School of Medicine, and John D. Biggers, King Ranch Laboratory of Reproductive Physiology, University of Pennsylvania, Philadelphia.

Stages in the development of sperm and aspects of the fine structure of mature sperm of the opossum (Didelphys marsupialis) have been recorded photographically. Histochemical approaches and electron microscopical techniques have been combined. Photographic evidence will be presented.

The cap and acrosomal granule appear to form via the Golgi opposite a nucleolus adherent to the nuclear membrane, associated with the formation of a complex ring not previously recorded. As the nucleus condenses perpendicularly to its original axis, the acrosome is composed of two parts; the manchette appears to originate from this ring, or below it from the nuclear membrane, and defines the area within which the tail and mid-piece develop. The condensing nucleus flattens, with acrosome above and manchette below, into a palmate-ovate structure. The subsequent U-shape is formed by an outgrowth of an arm from the more rounded border of the flattened nucleus. The ring described above appears to circumscribe an area on or in the nuclear head, including a complex vesicle with included granules. The flagellum develops from a centriole lying in an indentation in a nuclear portion of the head lying in this vesicle. Electron micrographs indicate the complexity of this part. The origin, nature and subsequent fate of the large chromatoid body are obscure. The mid-piece is formed from mitochondria into a complex spiral. This membrane complex of the mid-piece is multilayered, each layer being of unit membrane organization with opposing granules intrinsic in the two dense leaves of the membrane. The axial filament complex is of the 9 + 9 + 2 arrangement with complicated interconnections between the fibers. The distal ring at the end of the mid-piece appears to be composed of a complex of fine fibrils which envelop the principal piece of the tail, and are external to the sheath. There is evidence of distinctive ultrastructure in the external "homogeneous" fiber complex, as well as in the so-called "spiral sheath".

Comparison of the sperm of related forms has been initiated and will be commented upon.

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DELAYED CLEAVAGE IN ACTINOMYCIN D-TREATED ARBACIA EGGS.

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Unfertilized eggs of Arbacia punctulata were placed in 25 μg/ml actinomycin D in sea water for 3 to 4 hours, thoroughly washed and transferred to sea water, where they were fertilized. The fertilization reaction (membrane elevation) occurred promptly and normally but the formation of the first cleavage furrow was significantly delayed. Under the conditions of the experiments the completion of the first cleavage furrow occurred in the control eggs 40 (±3) minutes after fertilization. In eggs pre-treated with actinomycin D it was delayed by 10 to 18 minutes compared with non-treated control eggs of the same parentage. (The comparisions were made between the first appearances and the control eggs were kept in sea water before fertilization for the same length of time as the experimental ones were kept in actinomycin D, to eliminate any influence of eventual ageing on the results). No cleavage delay was observed when sperm suspensions were pre-treated with actinomycin D in a similar manner and used afterwards to fertilize non-treated eggs. It can be therefore concluded that the causes of the cleavage delay are associated with cytoplasmic constituents (presumably engaged in RNA synthesis), rather than with the nucleus (Wolsky and Wolsky, Biol.Bull., 121: 414, 1961).

Earlier work by Henshaw (Am.J.Roentgenol., 27: 890, 1932 and 43: 899, 1940) has demonstrated a similar cleavage delay in Arbacia eggs if either these or the fertilizing sperm were exposed, immediately before fertilization, to ionizing radiations. Henshaw has concluded that the delay must be due to effects on nuclear DNA. Studies are now in progress in order to compare and correalate the radiation induced and the actinomycin induced cleavage delays.

Slides were prepared from eggs showing the actinomycin induced delay. They were fixed in Bouin's fixative at the time when the control eggs completed the first cleavage furrow, sectioned (7 μ) and stained with Heidenhain's iron hematoxylin. The sections have shown that the pronuclei were in most cases not yet fused. Even in the most advanced cases the amphimixis of the pronuclei was still incomplete and there was only a single, rather small aster in close vicinity of the male pronucleus. In view of the known role of RNA in the formation of the kinetic apparatus (and the probable role of this in the movements of the pronuclei) it is likely that this situation is the outcome of inhibited RNA synthesis in the cytoplasm of the actinomycin D-treated eggs.

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THE MECHANISM OF FROST-RESISTANCE IN EGG-CELLS OF THE SEA URCHIN.

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It was found in several species of the sea urchin that soon after fertilization the frost-resistance in the egg cell to rapid rate of cooling and to limit of low temperature at which the egg cell could endure freezing for more than several hours increased remarkably. Also in the egg cells in which cortical change had been induced by urea-treatment a remarkable increase in frost-resistance was found.

Intracellular ice formation was found to be fatal in these egg cells at least at a climatic low temperature. The initiation of intracellular freezing takes place as a result of inoculation from the outside of cells with ice crystals. For protection against such ice seeding into the cell, the protoplasmic surface layer in the egg cell is the best barrier. Consequently, in an extracellularly freezing cell having still a lot of easily freezable intracellular water, every factor which can reduce the rate of cooling at the cell surface is supposed to be effective in prevention of an intracellular freezing. In fertilized or urea-treated egg cells, both the permeability of the cell to water and the ductility of the cell surface is remarkably higher than in unfertilized ones. These two characters, by increasing the velocity of extracellular crystallization of water from the freezing cell, can reduce the cooling rate at the cell surface where ice forming and therefore the liberation of latent heat successively occur.

After an extracellularly frozen cell has reached a steady state at a given low temperature, the cell protoplasm is still resisting a severe dehydration and concentration for a certain period of time. For the preventive of this kind of frost-injury, polyol production in overwintering insects has been well known. In the egg cell of the sea urchin, however, a rapid increase of small molecular substance was not found. A cytochemical observation suggests that the increase in cellular frost-resistance is expected in the egg cell under a certain cytoplasmic condition being rich in protein-bound SH groups. Since quantitative studies on SH groups in the sea urchin eggs revealed that SH in total protein remained constant up to the 2-cell stage (Sakai, H. 1960), the protoplasmic factor responsible for the frost-resistance capacity might not be the amount of protein SH in the whole cell, but the concentration of SH in some cell component in the cytoplasm.

CHANGES IN FLUID COMPARTMENTS OF RAT LIVER MITOCHONDRIA FOLLOWING COLD EXPOSURE.[1]. Joseph B. Boatman, Adeta Riner and Richard D. Prather, Laboratory of Environmental Physiology, Midwest Medical Research Foundation, Wichita, Kansas, U.S.A.

Recent studies have indicated that cold exposure resulted in maximal tissue hydration at 12-24 hrs. (Am. J. Physiol. 199: 256, 1960) and that absorbance changes of rat liver mitochondria are more rapid in hypotonic media after 24 hrs. cold exposure (Am. J. Physiol. 202: 1037, 1962). Malamed and Recknagel (J. Biol. Chem. 234: 3027, 1959) reported that with hypotonic sucrose, all of the mitochondrial space which sucrose did not enter behaved as an ideal osmometer.

Four liver mitochondrial pellets were prepared from each animal of groups of 4 room-temperature (23-25°C) and 4 cold-exposed (3-5°C) adult female rats. The individual pellets were separated in 0.44 M sucrose by differential centrifugation, and then exposed to 0.44 M, 0.25 M, 0.15 M and 0.05 M sucrose media, employing essentially the methods of Malamed and Recknagel. C^{14}-carboxyl-labelled sucrose was used to measure sucrose dilution volumes, H^3 labelled water as well as wet weight-dry weight differences were used to measure water content, and I^{131}-labelled l-thyroxine (10^{-9} M) was used to measure binding affinities of mitochondrial membranes. All isolation and incubation procedures were conducted at 3-5°C.

Liver mitochondria from cold-exposed rats showed significantly increased sucrose-accessible space, decreased sucrose-inaccessible space, and increased water content both on a wet weight-dry weight basis and with tritiated water dilution volumes expressed on a volume per dry weight basis, compared to room-temperature rats. These differences were progressive with decreasing osmolarity from 0.44 M to 0.05 M sucrose media. Mitochondrial pellets from cold-exposed rats bound 5x the amount of I^{131}-thyroxine as the amount bound by the pellets of room-temperature animals, suggesting a greater surface area available, and showed no relationship of amount bound to the osmolarity of the media.

Evidence from this study indicated that liver mitochondria from cold-exposed rats had already undergone changes in permeability before isolation, resulting in altered compartmentalization with *in vitro* osmotic gradients. It was concluded that hydration effects of short term cold-exposure shown previously in liver tissue slices, extends to intracellular effects upon the liver mitochondrion.

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XVI INTERNATIONAL CONGRESS OF ZOOLOGY

CONTRIBUTED PAPERS SESSION

15B. CELL BIOLOGY

GOLGI FINE STRUCTURE IN A RADIOSENSITIVE GIANT AMOEBA. Edward W. Daniels and Nancy Reed, Division of Biological and Medical Research, Argonne National Laboratory, Argonne, Illinois, and Harvard University, Cambridge, Massachusetts.

Amoebae of the radiosensitive species, *Pelomyxa illinoisensis*, were fixed in OsO_4 during interphase and in various stages of mitosis. Thin sections from Epon blocks containing single organisms were studied with an RCA EMU 3F electron microscope. Randomly distributed Golgi bodies were found, many of which resemble small pyramids or cones, somewhat smaller than the adjacent mitochondria. At the base and in the mid-portion of each Golgi body there are flattened vesicles while at the apex there are rounded vesicles. A number of the latter have an internal fringe similar to that on the plasmalemma and on the interior surface of fresh pinocytosis vesicles that originally come from the plasmalemma. Partly flattened, club-shaped vesicles have been found in some Golgi bodies and also singly in the cytoplasm. These may represent intermediate stages of flattening between the apical and basal components. The flattened vesicles of a Golgi body often display small lateral blebs which occasionally contain minute electron-dense particles. It is our present interpretation that pinocytosis vesicles, and possibly other small vesicles, collect at the apex of the Golgi body and gradually become integrated and flattened. The formation and fate of Golgi bodies in these amoebae is being studied by electron microscope methods during the feeding and starvation cycle, and at different mitotic stages. Pairs of Golgi bodies were observed in relatively high frequency in some amoebae during mitosis. The frequency and significance of this is being investigated further. (Work supported by U. S. Atomic Energy Commission.)

FUNCTION OF MASTIGONEMES OF A MARINE CHRYSOMONAD. Theodore L. Jahn, Michael Landman and James Fonseca, Department of Zoology, University of California, Los Angeles.

Mastigonemes occur on flagella of many species of Protozoa, but the only function so far proposed for the mastigonemes is to increase the effective surface of the flagellum. Locomotion of a marine chrysomonad resembling Chromulina, approximately 6.4 micra by 4.7 micra with one long and one short anterior flagellum, was examined by means of high speed microcinematography up to 600 pictures per second. In addition, analyses were performed on the movement of the organism in a suspension of polystyrene spheres (1.17 micra in diameter).

The films clearly show that the organism moves by means of a planar sine wave in the long flagellum which is directed anteriorly. The wave starts at the basal end of the flagellum and progresses distally. It is apparent that the organism is pulled forward by the action of the long flagellum, and that the flagellum does not push backward as would ordinarily be expected with a forwardly directed sine wave.

It is well known that flagellar sine waves usually start at the base, move distally, and produce a push toward the point of attachment. It also is known (Bovee, Jahn, Fonseca and Landman, 1963, Abstracts, Biophysical Society) that in Mastigamoeba a sine wave may start at the tip, progress proximally toward the base, and pull the organism. However, in the present organism the wave begins at the base, moves distally (i.e., forward), and pulls the organism.

The theoretical calculations of Taylor (1952 Proc. Roy. Soc. A 214:158-183) show that if a thin cylinder, undergoing sinuous movements produced by a traveling sine wave of active bending, has a roughness of surface which is sufficiently great and has a certain directional character, propulsion may be achieved in the direction opposite to that expected of a smooth cylinder bending in exactly the same manner.

It is known that the long flagellum of the chrysomonads is pantonematic, i.e., has numerous lateral mastigonemes on at least two and probably all sides. It is proposed that one function of these mastigonemes is to provide this roughness of surface and thereby to bring about this type of reversal. This proposition is being investigated in other chrysomonads.

FINE STRUCTURE OF THE BUCCAL APPARATUS IN PARAMECIUM. Dorothy R. Pitelka, Cancer Research Genetics Laboratory, University of California, Berkeley.

The buccal cavity of Paramecium multimicronucleatum, like the body surface, is lined by an uninterrupted unit membrane beneath which is a mosaic of flat, membrane-limited alveoli. As previously described (1, 4), buccal ciliary organelles include the ventral peniculus, dorsal peniculus, quadrulus and endoral membrane. The endoral membrane is limited to the right side of the buccal overture; each of the others consists of four rows of cilia extending the length of the buccal cavity. Each row of the quadrulus is accompanied on its right by a row of parasomal sacs. Three rows of parasomal sacs parallel the two peniculi, one row between them and two rows flanking them to right and left. Kinetosomes of these buccal cilia bear no kinetodesmal fibrils, but from at least the right-hand row in each organelle arise ribbons of fine (about 20 mμ), tubular fibrils that may extend for some distance within shallow ridges of the adjacent non-ciliated wall. Ventrally, the wall bears high, well-marked ribs, beneath which run orderly bundles of similar fibrils; these descend posteriad past the cytostome as microscopically visible postesophageal fibers. In addition to the tubular-fibril systems, tracts of very fine filaments interconnect buccal kinetosomes and weave an extensive subpellicular network, enclosing dense nodes, around much of the buccal cavity.

Both the tubular-fibril systems and the filamentous network resemble structures associated with the buccal cavities of unrelated ciliates (2, 3). Both also resemble elements of the ciliary apparatus on the body surface of Paramecium. The ribbons of tubular fibrils are similar to short ribbons arising at the right posterior edge of each body kinetosome (or of one of each pair), while the filaments of the buccal network resemble those composing the infraciliary lattice. Moreover, each kinetosome (or pair) on the body surface has at its right one parasomal sac. Thus, similar units are combined and oriented with equal precision but in strikingly different patterns in the two body zones.

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THE STRUCTURAL CHANGES OF THE MACRONUCLEAR CONSTITUENTS DURING THE DIVISION CYCLE IN CILIATED PROTOZOA. Hidemi Sato, Department of Cytology, Dartmouth Medical School, Hanover, New Hampshire.

Spirostomum ambiguum, Tetrahymena pyriformis and Paramecium caudatum were used as material.

The structural changes of the chromatin during the division cycle of S. ambiguum were studied with emphasis on the duplication cycle. During the macronuclear morphogenesis (amitosis), the chromatin aggregates of the interphase macronucleus become fibrous and in many instances appear paired. This fibrous chromatin is irregularly distributed within the newly formed nodes. In each growing node of the daughter cell, the chromatin aggregates increase in number and have a stranded, twisted or paired appearance. Most of the chromatin lie across the long axis of the node, and they are distributed in the daughter nodes without the mutual separation of the paired strands.

The duplication cycle of the macronuclear chromatin in T. pyrifornis (Sato and Saito, '59) and P. caudatum (Saito and Sato, '61) is similar to that of S. ambiguum.

Based on the duplication cycle and the structure of the chromatin, it is probable that the macronuclear chromatin corresponds to "macronuclear chromosomes".

The relation of the nucleolar cycle to the division cycle is best observed in T. pyriformis. The nucleoli increase in number through the early prefission stage and then migrate to the peripheral region of the macronucleus during the late prefission stage. Some of them are extruded through the membrane. No special configurations of the nucleoli occur during the remainder of the division cycle.

THE CHROMOSOMES IN THE CILIATE MACRONUCLEUS.

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Two views have been held in regard to the ciliate macronucleus: (a) it is compound, with many sub-nuclei; (b) it is polyploid. Confirmation of either view would obtain support from a demonstration of chromosomes in it. In a few ciliates, chromosome-like bodies have been observed, but in the large majority of them, a convincing demonstration of chromosomes in the macronucleus has not been possible. DNA is consistently present, and in every known case, the macronucleus is brilliantly Feulgen positive. Division, however, is byamitosis.

Experiments with the macronucleus of two spirotrichous ciliates, Blepharisma and Spirostomum, have shown that (a) it is highly extensile and can be pulled out to a length about 15 times the normal, and (b) centrifugation and treatment with despiralizing agents like KCN and NaCN produce from it a mass of long and fine Feulgen positive filaments. In species of Spirostomum where the macronucleus is beaded, the beads expend themselves in the process of filament formation. Recent examination with the electron microscope has shown that in both ciliates, the macronucleus contains a large number of filaments of different sizes disposed in it in a random manner. These correspond to the filament bundles obtained by centrifugation and cyanide action. The filaments are the chromosomes of the macronucleus. Electron micrographs of sections of filaments resemble in every respect those of chromosomes of higher animals. Each seems made of many microfibrils about 150 A in diameter. The fibrils are disposed along the filament in a random manner but with a slight orientation in its long axis. It would therefore appear that the macronucleus owes its size to the large number of long Feulgen positive filaments, which represent the chromosomes. On this view, the chromosome-like bodies reported by earlier workers could not be whole chromosomes. Chromosomes in the macronucleus of the ciliates under study are greatly elongated bodies, quite unlike those described by other workers.

VARIATIONS IN ENDOPLASMIC RETICULUM AND MITOCHONDRIA OF PROTOZOA IN RELATION TO THE CHARACTER OF THEIR METABOLISM.

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The endoplasmic reticulum of the ciliates having a buccal apparatus differes from that of the ciliates lacking a buccal apparatus and feeding osmotically by the level of its organization. The endoplasmic reticulum of the former is rather developed and represents a very broad system of chanals, vacuoles and cysterns connected with digestive vacuoles and with the body surface. The most highly developed chanals of endoplasmic reticulum of ciliates has aquired the possibility to secret protein shells on the body surface(Colpidium, Ichthyophthirius). In Astomata group the endoplasmic reticulum is rather poor and this might be connected with osmotic type of their nutrition. Intracellular parasitic protozoa(Coccidia) as well as extracellular saprozoic intestinal protozoan parasites(Lamblia, Trichomonas, Opalina, Gregarina etc.)possess rather poor developed endoplasmic reticulum chanals which also can be connected with the type of their nutrition. Endoplasmic reticulum takes part in an active transport of some substances as well as in the secretory process and is most highly developed in protozoa with an active protein metabolism.

The chanals of endoplasmic reticulum are able to take part in an active water metabolism being in close connection with the "nephridial plasm" surrounding contractile vacuoles. In absence of contractile vacuoles(Loxodes, Bursaria and others), the endoplasmic reticulum forms large cysterns. In the cytoplasm the richest system of smooth membranes appears to be developed, the latter form the chanals possessing the function of the "nephridial plasm". Thus a continuous system arises for taking off the water from the body through the chanals of the endoplasmic reticulum opening on the body surface. The chanals of the "nephridial plasm" have smooth walls and are connected with contractile vacuoles. This chanals differ from flattened sacs forming dictiosomes of Golgi complex in the same degree as their function differ from each other. At the same time the smooth lipoprotein osmiophylic membranes of both structures just mentioned have much in common as concerns their origin from endoplasmic reticulum.

Intestinal Protozoa living in deficient oxygen media have either no mitochondria at all(Entamoeba and other)or their mitochondria number very little membranous structures(Lamblia). In the ciliates adapted to aerobic existance the mitochondria are found to have a large number of closely packed membranous tube shaped structures, the walls of which are formed by two more densitive outer layers (per 20 Å) and the third intermediate layer of less density(unit membrane). This structure corresponds to the mollecular organization of the cell membrane postulated by Robertson. While some obligate aerobes(Paramecium, Loxodes for example)are acted upon by different inhibitors of respiration, some disordes in mitochondria structures occur but the number of tubules inside the mitochondria does not reduce.

Analysis of the Antigens of Balantidium caviae (?) with the aid of Electrophoretic methods.

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The first data concerning the species specificity in Balantidium caviae (?) as well in B. wenrichi were reported by Krascheninnikow and Jeska, 1961. The present paper further analyzes the antigenic material using the electrophoretic method.

Ciliates were obtained from guinea pigs and purified according to the procedure described by Krascheninnikow (1958). Aqueous buffered extracts were used for paper and agar-gel electrophoresis at pH 8.6 and 7.5.

The paper electrophoresis showed only one component corresponding to protein. The immunoelectrophoresis five antigen-antibody precipitin bands appeared but only one of these was electrophoretically mobile. The cross reaction with B. coli antiserum was observed but no electrophoretic mobility was revealed in these components.

The testing of extracts with the Millon and Molisch reactions, precipitation of these with trichloroacetic acid, dialysis, and the subsequent resting, indicates that probably only one of the antigenic components is protein-others are polysaccharide moieties. The lack of electrophoretic mobility in the components cross-reacting with the B. coli serum may indicate the presence of polysaccharide antigens in other species of Balantidium. This paper confirms the previous data of Krascheninnikow and Jeska (1961) in regard to number of precipitating antigens in B. caviae.

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VELOCITY PROFILES OF AMOEBOID MOVEMENT. Robert A. Rinaldi, Department of Zoology and Entomology, The University of Tennessee, Knoxville, Tennessee.

A technique has been developed by this author to plot movements of individual granules in *Amoeba proteus*. With this method, termed the pictograph technique, one does not follow individual granules through consecutive frames of a motion picture film, but one takes a single time exposure photograph of an amoeba illuminated by darkfield microscopy. Those granules that are moving rapidly record as long streaks, those moving less rapidly appear as shorter streaks, and granules that are not moving register as dots. These amoeba pictographs have revealed:

1. Regions of the gel move at different rates.
2. There exists a greater granular movement at the anterior and posterior regions of the amoeba.
3. There exists a forward flow of protoplasmic granules through the interstices of the gel of an advancing amoeba.
4. There is a uniform protoplasmic flow in the central region from the anterior tip to the tail of the advancing amoeba.

The velocity profile of the gel region is two-phased in that some granules are motionless, whereas other granules flow forward through this gel region. The velocity profile of the centrally moving protoplasm is truncated as would be expected from a complex chemical and physical mixture like protoplasm, but nonmoving "gel" islands do occur which impede the uniform flow of the centrally moving protoplasm.

The data are consistent with the sol-gel interconversion (contraction-hydraulic) theory of amoeboid movement advanced by S. O. Mast.

SYNTHESIS OF HIGH ENERGY PHOSPHATE BOND IN THE FROG LIVER.

Wiktor W. Nowinski and William C. Mahaffey, Dept. of Surgery, University of Texas Medical School, Galveston, Texas.

Studies were carried out on mitochondrial fractions of livers from winter frogs (Rana pipiens) in order to establish whether the synthesis of high energy phosphate bond within the citric acid cycle (as Adenosine Triphosphate, ATP) in poikilothermic animals is different from that of homeothermic (rat liver). The estimated figures for P/O ratios were as follows: Pyruvate 2.4 ± 0.64, Isocitrate 2.5 ± 0.59, α-Ketoglutarate 2.3 ± 0.48, Succinate 1.7 ± 0.06, Malate 2.3 ± 0.64, which in the cell corresponds to P/O 3.0, 3.0, 3.0, 2.0 and 3.0 respectively. These results will be discussed in detail and the conclusion reached that there is no difference in ATP synthesis in the livers of warm-blooded and cold-blooded animals, and that as far as formation of high energy bonds is concerned, no special adaptations exist either in homeothermic, or in poikilothermic animals. (Supported by USPHS Grant CA 02296-13 CB)

CYTOCHROME C OXIDASE AND RESPIRATION IN EMBRYONATED AND UNEMBRYONATED *ASCARIS* EGGS. L. C. Costello, H. Oya and W. N. Smith, Department of Physiology, University of Maryland, School of Pharmacy, Baltimore, Maryland.

The presence of cytochrome oxidase was determined in homogenate preparations of unembryonated (one-cell stage) and embyonated (infective stage) eggs. Cytochrome c oxidase was readily demonstrated using spectophotometric assay in embryonated eggs. The amount of oxidase activity obtained was more than the necessary amount to account for oxygen consumption of the eggs. It was further observed that in the presence of substrate (malate or succinate) the homogenates of embryonated eggs appeared to oxidize the substrate by way of the cytochrome system. In the case of unembryonated eggs the presence of cytochrome oxidase could not be demonstrated although in the presence of substrate cytochrome c was readily reduced. Considering the amount of respiration of unembryonated eggs cytochrome oxidase, if present, should have been detected by the techniques employed. It appears that cytochrome oxidase does not account for the respiration of unembryonated eggs which is contrary to the conditions observed in the embryonated stage.

The authors propose the following possible pathways of terminal oxidation in the embryonated and unembryonated eggs:

Embryonated

$$\text{Substrate} \rightleftharpoons (\text{DPN}) \rightleftharpoons \text{FAD} \rightleftharpoons \text{Cyt.c}_{\text{red.}} \rightleftharpoons \text{Cyt.c}_{\text{ox.}} \xrightarrow[\text{oxidase}]{O_2} H_2O$$

Unembryonated

$$\begin{array}{l} \text{Substrate} \rightleftharpoons (\text{DPN}) \rightleftharpoons \text{FAD} \rightleftharpoons \text{Cyt.c}_{\text{red.}} \\ \qquad\qquad\qquad\qquad\quad \downarrow \text{oxidase?} \\ \qquad\qquad\qquad\qquad\quad H_2O_2 \end{array}$$

O_2

The authors have no evidence for the presence of an oxidase resulting in the oxidation of reduced flavin with peroxide being the product. This proposal is suggested only as a possibility since respiration and cytochrome oxidase cannot be reconciled at present in the unembryonated eggs although the relationship has been established in the embryonated stage. The authors have observed a marked increase in cytochrome oxidase activity in morula eggs during which respiration begins to sharply increase in developing eggs. It appears that the unembryonated eggs are initially dependent upon more anaerobic activities than the embyonated eggs. It should be pointed out however that as of this time the identification of cytochromes in *Ascaris* eggs has been completely elusive.

THE APPEARANCE OF A UNIQUE ALKALINE PHOSPHATASE IN THE INTESTINE OF THE INFANT MOUSE. Florence Moog, Harvey R. Vire, and Robert D. Grey, Dept. of Zoology, Washington University, St. Louis 30, Missouri, U.S.A.

Just before the infant mouse is ready to be weaned, the alkaline phosphatase activity of the duodenum undergoes a 15 to 20-fold increase. Studies of crude homogenates of developing duodenum have previously shown that the increase of phosphatase activity is accompanied by a rising preference for phenylphosphate (PhP) relative to beta-glycerophosphate (bGP) as substrate. This change in PhP/bGP ratio suggested the possibility that the increase in activity represents the appearance of a new phosphatase (NP) not present in earlier stages. By chromatography on DEAE columns and by electrophoresis on starch gel, it has now been possible to isolate the NP in partially purified preparations (specific activity 40 mg P/mg N/min) from butanol extracts of intestinal homogenates. At pH 8.0 the enzyme is eluted at low salt concentrations, and its mobility in a potential field is lower than that of other isozymes of mouse intestinal phosphatase. The NP is quite inactive on bGP, having a PhP/bGP ratio as high as 9.9 at 20-22 days, when duodenal phosphatase activity is maximal. The high ratio clearly distinguishes NP from other alkaline phosphatases in intestine and other organs, since non-specific phosphomonoesterases generally hydrolyze PhP and bGP at roughly equal rates. With PhP as substrate at pH 9.6, NP is also characterized by a high K_m (more than 10 mM, as compared with less than 1 mM for ordinary jejunal phosphatase). No trace of NP is found in butanol extracts of duodenum or jejunum of mice of 5-6 days or 11-12 days of age. At 15-16 days, however, when duodenal phosphatase activity is increasing, about 35% of the activity recovered after DEAE chromatography is of the NP type in the duodenum, but none is found in the jejunum. At 20-22 days about 70% of the activity recovered from the first 3 cm of the duodenum is NP; in cm 4-7 about 20% of the activity is NP, and in the jejunum less than 10%. In the adult (2 month old males) NP comprises approximately 40% of the recovered activity in the first 3 cm of the duodenum, but in the 4-7 cm segment it still makes up more than 20% of the total activity. Only a trace is found in the adult jejunum.

Supported by research grant 3937 from the National Institutes of Health (U.S.A.).

PROTEIN-BINDING REAGENTS IN ULTRASTRUCTURE RESEARCH: A STUDY OF EHRLICH ASCITES CELLS. B. Mundkur, Dept. of Zoology, University of Connecticut, Storrs, Connecticut, U.S.A.

Mouse Ehrlich ascites cells were frozen-dried with adequate safeguards to ensure a minimum of post mortem morphological and cytochemical changes in the protoplasm, as well as of ice crystal artifacts. Following heat-denaturation *in vacuo*, the specimens were exposed to numerous reagents which specifically bind certain functional groups of protein side chains. These reagents were applied either in solution or as anhydrous vapors immediately following removal of the free water from the specimens. In certain cases, basophile staining was superimposed on specimens previously subjected to specific protein-binding compounds. These procedures have involved numerous control experiments designed to establish the specificity of the methods, especially following enzymatic digestions. The principal findings from these experiments include the disclosure of the submicroscopic organization of the nucleus, particularly the nucleolus and the chromatin. These observations will be illustrated and discussed with the aid of electronmicrographs.

A CUMULATIVE ERROR IN LABELLED AMINO ACID STUDIES OF KERATINIZING TISSUES. Roy T. Sims, Anatomy Dept., Cambridge University, England.

An ideal pulse dose of a tracer is available to the tissues of an animal which receives it for one short period: none of the tracer is available before or after the pulse. A single injection of a labelled amino acid does not behave as an ideal pulse dose because small amounts of it continue to be available to the tissues after the pulse has gone. The incorporation of these small amounts of label reach significant proportions in hair because they are collected over a long period.

Hooded rats were given 10/u c/g. body weight of tritium labelled tyrosine. The tyrosine was generally labelled and had a specific activity of 434mc/mM. The animals were killed in pairs 10 min., 30 min., 12hr., 36 hr., and 7 days after injection. Skin which had actively growing hair at the time of injection was fixed in Bouin and radiographs of it prepared. A numerical expression of the degree of radioactivity was obtained by counting the number of silver grains per unit area in the emulsion over the hair cortex.

At 10 and 30 min. after injection radioactivity was present along the hair from the matrix to the beginning of the fully keratinized part of the shaft. No activity was found in the fully keratinized region until 12 hr. after injection. By 36 hr. the activity was highest in the fully keratinized region at the level of the skin surface, but significant activity was present in the growing part of the follicle. The amount of activity expected in the matrix and supra-bulbar regions at 12 and 36 hr. was calculated, assuming an ideal pulse dose situation, from that found at 30 min. The actual activity exceeded the expected activity of (1) the matrix by 10% at 12 hr. and 0% at 36 hr., (2) the supra-bulbar region by 40% at 12 hr. and 100% at 36 hr.

The results show that labelled amino acid is incorporated into protein of the hair after the pulse dose injected has been cleared from the body fluids. The process is important in keratinizing tissues because it is cumulative. Its consequences are that protein turnover is concealed, and a continuous delayed incorporation of labelled tyrosine appears to occur from the pulse injected. It may be possible to detect the accidental exposure of laboratory staff to radioactive amino acids by monitoring their hair.

ELECTRON MICROSCOPE OBSERVATIONS ON PERICARDIAL CELLS FROM "STARVED" GRASSHOPPERS INJECTED WITH PEPTONE AND BEEF BLOOD SERUM. Richard G. Kessel, Department of Zoology, State University of Iowa, Iowa City, Iowa.

Previous studies with the phase contrast microscope (Kessel, Jour. Morph., 110; 1962) indicated that some correlation existed between the nutritional state of grasshoppers and the activity of the pericardial cells. Further, the injection of proteins, albumins, and amino acids into the hemocoel of starved animals also affected the secretory activity of the cells. These observations have been extended to include those at the ultrastructural level of organization. Organisms deprived of food for varying lengths of time were subsequently injected with a solution of peptone or beef blood serum and, after three or twenty-four hours, most of the pericardial cell tissue was fixed in buffered osmium tetroxide and embedded in epoxy resins for electron microscope study.

The plasma membrane of the pericardial cell shows a complicated system of invaginations which are extremely numerous and, at various points, desmosome-like structures are associated with them. The structure of pericardial cells taken from "starved" animals which had been injected with beef blood serum or peptone differs from the cells of control animals in the following ways: (1) In the former case, material of low density is observed within the invaginations of the plasma membrane and (2) the number of caveolae formed from the surface of the invaginated plasma membrane is much greater. Also, (3) the number of isolated spherical or elongate pinocytotic vesicles and (4) the number of inclusions of various shapes and sizes are much greater in the experimental than in the control cells. Fusion of pinocytotic vesicles appears to occur progressively resulting in vacuoles of different sizes with different density characteristics. The morphological variations observed at various times after injection of the materials suggest that the pericardial cells are very active in a process of pinocytosis and that it is presumably the injected materials or a slightly modified form that are being taken up and concentrated by the cells. The specializations of the plasma membrane in this cell thus appear to be involved with increasing the formative surface area for the production of pinocytotic vesicles and to thus affect a more rapid uptake of material.

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RADIATION-INDUCED SYNDROME AT THE CELLULAR LEVEL.[1] Roland M. Nardone, Biology Dept., Catholic University of America, Washington, D.C.

The effect of ionizing radiation on the cytology, growth, division rate, protein synthesis, nucleic acid synthesis, and respiration of Strain L monolayers is being investigated in an attempt to establish the nature of a radiation-induced syndrome at the cellular level.

A source of cobalt-60 housed in an incubator-irradiator was used to irradiate the cells with doses varying from 100 to 3000 r. These doses have resulted in effects ranging from temporary mitotic arrest to complete mitotic arrest (accompanied by giantism) to metabolic degeneration and death. Doses which are responsible for the production of large numbers of nuclear blisters and cytoplasmic vacuolization permit nucleic acid and protein synthesis to continue at a reduced rate. The relative sensitivity of protein and nucleic acid synthesis in the nucleus and cytoplasm is being established by tracer and spectrophotometric methods. The incorporation of C^{14}-labeled uracil, glycine, histidine, methionine, and glucose will be discussed.

1. Supported, in part, by contracts with the U.S. Atomic Energy Commission and the Department of the Army.

MELANOCYTE PERFORMANCE DURING RADIATION-INDUCED TANNING OF MURINE SKIN. W. C. Quevedo, Jr., R. M. Brenner and P. Kechijian, Dept. of Biology, Brown University, Providence, R. I., U.S.A.

The plantar skin of genetically-competent pigmented mice becomes intensely darkened during 16 daily exposures to ultraviolet light (UV) (Quevedo and Smith, 1961). The radiation-induced hyperpigmentation results from an increased number of epidermal melanocytes engaged in melanin synthesis and, as a consequence, from the increased amounts of melanin pigment incorporated into epidermal cells. In some strains of mice, irradiation of the plantar skin elicits as much as a thirty-fold rise in the numbers of epidermal melanocytes identifiable with DOPA-reagent. Detailed analysis of the tanning response in C57BL mice indicates that there is a gradual increase in melanogenically active melanocytes beginning by the sixth exposure to UV, and that the peak population is established between the tenth to sixteenth treatment. Examination of skin specimens from irradiated C57BL mice treated with colcemid prior to sacrifice on the sixth to twelfth day suggests that irradiated epidermal melanocytes may increase in numbers. Similarly, preliminary analysis of radioautographs of skin from irradiated mice treated with thymidine-H^3 and sacrificed during the same period indicates that UV may stimulate division of epidermal melanocytes. Attempts to identify amelanotic melanocytes by use of vital dyes, osmic iodide and gold impregnation techniques, although not uniformly successful, lend support to the presence of these cells in non-irradiated plantar skin. Accordingly, both melanocyte "activation" and proliferation may account for the characteristic increase in melanogenically-active melanocytes within irradiated murine plantar skin; the relative contribution of each process to radiation-induced tanning remains to be established. (Supported by U.S.P.H.S. grants, CA 06097-02, from the National Cancer Institute, and A-3786(C2), from the National Institute of Arthritis and Metabolic Diseases.)

XVI INTERNATIONAL CONGRESS OF ZOOLOGY

CONTRIBUTED PAPERS SESSION

16. GENERAL PAPERS

SOME SPECIFIC APPROACHES TO INTERNATIONAL COOPERATION IN AQUATIC SCIENCES COMMUNICATIONS. Sidney J. Holt, Fisheries Branch, Biology Division, FAO of the United Nations, Rome, Italy; and Saul B. Saila, Graduate School of Oceanography, University of Rhode Island, Kingston, Rhode Island, U.S.A.

Preliminary steps are being taken in the aquatic sciences to establish a communication network designed to aid in rationally directing information flow between the originator and the user. These steps include: (1) collaboration in the continuing development of a truly comprehensive abstract journal to provide a current awareness function (2) incorporation of an information retrieval system as a network component to aid in the rapid search of past literature and (3) conventional and unconventional means for improving communication channels other than technical literature. Details concerning these approaches and the agencies involved are described, and recommendations for further developments are outlined.

THE ZOOLOGICAL RECORD. Marcia A. Edwards, The Zoological Society of London, Regent's Park, London, England.

The Zoological Record, a publication of the Zoological Society of London, was started in 1865 with the idea of making available to zoologists a list of papers and books of zoological interest published in any one year, with a brief note of their contents.

The method of compilation is essentially simple and depends on a system of colour coded cards. Literature received in the major scientific libraries in London is searched and cards made with the reference and title of suitable papers. These cards are given to the appropriate recorder who extracts the information he requires from any article and divides it into a subject and systematic section. The subject index is arranged in broad groups of "Structure, Physiology, Ecology, Geography and Geology", which in turn are sub-divided into many smaller sections. The systematic index contains information relevant to the particular species or groups of the Phylum concerned, together with details of new Taxa. By employing this arrangement, it is possible for a worker wishing to obtain information on a particular aspect of an animal or group of animals, to look in the appropriate sections of the subject and systematic indices and to find references to the papers he requires.

The work of searching and recording is largely carried out by the compilers outside their normal working hours, a fact which is possibly not always appreciated. As the volume of literature increases year by year, so their task becomes correspondingly greater and zoologists are much indebted to their diligence and perseverence.

The Zoological Record may be regarded as one of the most important sources of information available to the research worker and is of particular value to the systematist. Although every effort is made to ensure wide coverage, this is extremely difficult. Authors are asked to help by sending reprints or details of their papers to the Editor, and it would be appreciated if workers finding papers which have not been recorded, would give the appropriate information to the Editor.

During recent years, two zoologists have been employed full time on the preparation of some sections of the Record and it is hoped that if sufficient funds are available in the future, more permanent staff will be engaged.

PERSPECTIVES DE LA PEDOZOOLOGIE EXPERIMENTALE.

Madame C. JEANSON - Centre National de Recherches Agronomiques
Versailles - FRANCE -

La faune du sol est un facteur biologique de pédogénèse relativement peu étudié, son action est difficile à préciser dans le milieu naturel étant donné l'interaction de nombreux facteurs. Une étude expérimentale de cette action pourrait ouvrir à la zoologie un nouveau domaine d'investigation.

L'activité des Lombricides dans le sol est prise comme exemple pour essayer de comprendre les contradictions de la littérature : la stabilité structurale des terres, propriété physique importante pour la pratique agricole, n'est pas modifiée selon TEOTIA 1950 et augmente selon GUILD 1955 sous l'action des lombrics. Les difficultés d'étude du milieu naturel sont dues à l'interaction des facteurs en présence et à l'interférence des phénomènes bioclimatologiques.

La méthode expérimentale mise au point en 1957 par la reconstitution au laboratoire d'un milieu artificiel controlé, limite les facteurs, permet l'étude d'un phénomène particulier et facilite la mise en évidence de l'importance relative des divers facteurs.

Les premiers résultats lèvent des contradictions sur l'action de Lumbricus terrestris Linné et montrent que la stabilité structurale des turricules augmente de 40%, par rapport à la terre initiale, en présence de matière organique alors qu'elle diminue de 16 % en l'absence de matière organique.

Cette variation de la stabilité structurale est en relation directe avec la dégradation et l'évolution de la matière organique. La méthode expérimentale permet de différencier la part de l'humification due aux Lombricides de celle due aux microorganismes, ce qui n'est pas possible dans le milieu naturel. L. terrestris augmente la fixation des matières humiques sur le complexe organo minéral du sol de 25 % par rapport à la fixation due aux fermentations.

On peut donc espérer que la généralisation de cette méthode expérimentale, à l'étude de l'action de tous les groupes de la faune du sol, permettra de reposer certains problèmes pédologiques et agronomiques sur des données plus scientifiques : la formation, l'évolution et la fertilisation des sols sous l'action des populations terricoles, par exemple.

Cette nouvelle méthode présente, en outre, un intérêt pour l'écologie et l'étude du comportement animal dans un milieu déterminé.

RATE OF GROWTH IN DEVELOPMENTAL STAGES OF PROTEUS-GROUP OF AMOEBA. Monica Taylor, Convent of Notre Dame, Glasgow, Scotland.

This paper proposes for problem the rate of growth in the developmental stages of the Proteus-type of amoeba. It emphasizes the necessity of procuring experimental cultures which copy exactly the natural habitat of the amoeba. This involves procuring a food chain from bacteria through small flagellates, ciliates, rotifers to amoeba, supported by a pabulum sufficient to maintain this chain. The various appearances of the amoeba thus cultivated are described: the fragile young adult, the large easily spreading amoeba in its prime, the smaller non-spreading, old adult over packed with inclusions. Their respective ages are responsible for their varying appearances.

It is then shown that a culture full of old adults gives place to one devoid of them which eventually becomes peopled by young adults.

A search made during the 'Depression' period reveals developmental stages but without the aid of two techniques a sufficiency of material could not be forthcoming. Changing the pH kills off adults but not spores which hatch out as amoebulae. The same result can be obtained by drying and wetting a culture. But the continuity of growth is arrested by a series of encystments, which also account for the varying sizes of the developmental stages revealed by this change of pH. This habit of encystment must be broken before a straight forward attack on developmental rate can be made.

THE SUBMICROSCOPIC CHANNELS IN BONE TISSUE. C.A. Baud and P.W. Morgenthaler, Institut de Morphologie, Université de Genève, Ecole de Médecine, Genève, Switzerland.

The submicroscopic interfibrillar spaces of bone substance representing the nutritional pathways of 5th order in bone have been studied quantitatively using polarization microscopy, electron microscopy and X-ray diffraction. This investigation (Morphol.Jb., 1963, in press) has been done on undecalcified sections of compact bone tissue from adult long bones of humans and mice.

To establish the volume of spaces, we have examined the curves of form birefringence of total bone. The retardations have been measured in dependence on refractive index of a hydrophilic imbibition liquid series ranging from 1.333 to 1.595 (water + ethylene glycol + cadmium iodide). It was possible to calculate from the value of form birefringence the volume of spaces according to Wiener's formula. This volume represents on an average 7.36 per cent for the human tibia.

Informations on space shape and orientation could be obtained from observations done by electron and by polarization microscopy. The spaces are spared between collagen fibrils in close packing and constitute narrow channels parallel to the fibril axis, seen as curvilinear triangles in transverse sections.

The space dimensions have been measured indirectly by a particular radiocrystallographic process. By artificial means we have introduced a foreign substance into the interstitial spaces where, after crystallization, it could be submitted to X-ray analysis. For this purpose, we have reduced silver which had diffused in ionic form in the bone. The size of the cubic silver crystals incorporated into the submicroscopic spaces was calculated according to the relation existing between crystal size and breadth of the X-ray diffraction lines. The average particle size, which indicates the minimum diameter of interfibrillar spaces, is 124 Å in human bone.

On the whole, these observations enabled us to calculate the exchange area represented by the 5th order pathways per unit of volume. It means 35 000 mm^2 per mm^3 of bone substance, i.e. a considerable area for exchanges between bone substance and interstitial liquids.

(Supported by a grant from the Fonds National Suisse de la Recherche Scientifique, Commission pour la Science Atomique.)

GENETIC VARIANCE IN SELECTED LINES OF DROSOPHILA MELANOGASTER AND D. SIMULANS AT DIFFERENT TEMPERATURES. Abd-El-Azim Osman Tantawy. Faculty Of Agriculture, University Of Alexandria, Alexandria, Egypt, U.A.R.

An experiment was designed to study the effects of selection for wing length in Drosophila melanogaster and D. simulans at two temperatures; 18°C. and 28°C. Two selected lines, in each species at each temperature, were carried out, one for long wing and the other for short wing length for twenty five generations. Mating system and the technique used for the maintainance of these lines were essentially the same as that reported by TANTAWY (1956). The genetic variance was estimated first in the initial foundation populations as well as at intervals of five generations before and after relaxation of selection. At the twentieth generation of selection, the selected lines were transferred to the reverse temperature for another five generations before and after relaxation of selection. At the twentieth generation of selection, the selected lines were transferred to the reverse temperature for another five generations. Heritability of wing length was estimated in each of the selected lines, at the reverse temperature, as well as after selection for further five generations at this temperature. Progeny tests carried out to estimate the heritability values, were the same as given by TANTAWY (1957).

Heritability estimates indicate that the populations of Drosophila melanogaster and D. simulans carry considerable genetic variability. Such estimates were 39.27, 31.74 percent at 25°C. in both species, respectively. At higher or lower temperature, heritability estimates were found to be 31.91, 26.29 percent at 18°C., 25.48, 23.44 percent at 28°C., respectively.

Selection was effective in both species of Drosophila; low lines show usually more response than high lines. The response to selection is more pronounced in D. melanogaster than in D. simulans, and in all lines selection at 18°C. is more effective than selection at 28°C.

Heritability estimates in the selected lines at different temperatures indicate that selection at either temperature leads to an apparent increase in the genetic variance-even though the selection response has ceased in long wing lines-and in all lines short wing lines display greater estimates than that found in long selected lines.

Another point considered was the effect of reversing the temperature on the heritability estimates, compared with that found under the original temperature of the selected lines. The results show clearly that the genetic variance, under the new environmental conditions, decreases at the new temperature of 18°C. and increases at the new one of 28°C. Such results are in agreement with those reported by TANTAWY (1961) on another populations of Drosophila pseudoobscura and attributed such behaviour to genotype-environment interaction.

TANTAWY, A.O., 1956 Response to selection for wing length in Drosophila melanogaster with different systems of mating. Genetica, 28 : 231 - 262.

TANTAWY, A.O., 1957 Genetic variance of random inbred lines in Drosophila melanogaster in relation to coefficients of inbreeding. Genetics, 42 : 121 - 136.

TANTAWY, A.O., 1961 Effects of temperature on productivity and genetic variance of body size in populations of Drosophila pseudoobscura. Genetics, 46 : 227 - 238.

RECHERCHES ENTOMOLOGIQUES DANS LES ILES DU GOLFE DE GUINEE (Principe, Sao Tomé et Annobon). P. Viette, Entomologie, Muséum National, 45 bis rue de Buffon, Paris 5, France.

Les îles importantes du Golfe de Guinée sont au nombre de quatre : Fernando-Po, Principe, Sao Tomé et Annobon. La mission du Muséum national d'Histoire naturelle de Paris, organisée avec le concours du Centre national de la Recherche scientifique et du navire océanographique "La Calypso" (bien connu grâce au film "Le Monde du Silence") ne devait visiter que les trois dernières îles, à l'exclusion de Fernando-Po. Partie de Douala, au Sud-Cameroun, le 2 juin 1956, la mission devait revenir dans ce port le 9 juillet, après être restée trois semaines environ dans l'île de Sao Tomé.

Après avoir envisagé la position des trois îles par rapport à Fernando-Po et au Mont Cameroun, on parlera de la végétation de Principe, d'Annobon et surtout de Sao Tomé qui, atteignant 2.024 m d'altitude au Pico de Sao Tomé, présente, notamment au-dessus de 1.400 m environ, une succession de climax fort intéressants pour le Naturaliste.

En ce qui concerne la faune entomologique, celle-ci est une faune : (a) montrant de grandes affinités avec la faune de la pluvisilva africaine, ce qui n'est pas pour nous surprendre, (b) appauvrie, (c) avec un pourcentage élevé d'endémiques au niveau spécifique. Ces espèces endémiques appartiennent à des genres continentaux et sont proches d'espèces continentales. Les genres endémiques sont peu nombreux.

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