THE AVIAN EMBRYO RESPONDING TO MICROGRAVITY OF SPACE FLIGHT

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INTRODUCTION

Central questions and paradigms of developmental biology have in recent decades evolved to stress gene regulation. (See Moore, 1987 for an excellent review). However, during fertilization and the pre-embryonic cleavage stages, cells of identical genome are responding to multiple microenvironmental factors, e.g., pH, spatial position, temperature, osmotic and barometric pressure, light, electrical field charge, and the extracellular concentration of substrates, cellular byproducts, gases, and electrolytes. These cells commonly retain their full potential until chance of spatial positioning alters their access to the internal or external environment. (The commercial exploitation of these techniques at the 8-cell stage is now well-developed in beef and dairy breeding.) Importantly, these factors are exerting their influence prior to the genome repression/activation, all of which leads to germ layer definition and basic tissue differentiation. Of all the many potential and real microenvironmental influences, only gravity would appear to have remained relatively constant and ubiquitous for developing organisms. Histo- and organogenesis as well as differential growth of the embryo and fetus may have evolved with a constant environmental factor of gravity ($G=6.7 \times 10^{-8}$ c.g.s. units).

Embryos of 2-d and 9-d stage of incubation were flown in an incubator on the Space Shuttle during a 5-d mission. Significant differences in embryo response to this microgravity environment were observed. This paper offers an analysis and suggests mechanisms which may contribute to these results.

MATERIALS, METHODS, RESULTS

In an experiment designed and carried out by a team of ten scientists, domesticated chicken (*Gallus domesticus*) embryos were exposed to the microgravity environment (Hullinger et. al., 1990). This experiment measured the effects of near zero gravity upon a developing vertebrate system. Our decision to utilize embryos not younger than 2-d, was determined by our ability to candle them for fertility. Otherwise we selected ages to include otolith formation and osteogenesis for the domesticated chick.

Results of this Shuttle STS-29 mission (flown March 1989) revealed that none of the sixteen 2-d chick embryos survived a 5 d incubation in earth orbit. All sixteen 9-d embryos and ground controls of both ages survived and hatched following the mission. Significantly, however, the younger space flight embryos died at different ages (3 - 6.5 d). This susceptibility to microgravity and the significant difference in embryonic response is the subject I intend to address.

In our study, half of the eggs, flight and control were opened at mission's end; half were incubated to the presumed hatch date. All eight of the younger experimental embryos, opened after a 7-d incubation, had stopped development. These young embryos died during the 5-d mission, but they did so at different ages/stages: one at 6.5-d; two at 4-d; two at 3.5-d; one at 3-d; and two eggs revealed no embryos. The eight younger experimental embryos, incubated until hatch date, did not pip and, when opened, had stopped development at these stages: two at 5-d; two at 4-d; and four eggs revealed no embryos (Table 1).

Table 1: Shuttle STS 29
2-d Chick Embryo Mortality

Age in days at death	# embryos
7	-
6.5	1
6	-
5.5	-
5	2
4.5	-
4	4
3.5	2
3	1
2.5	-
2	-
*No embryos	6

* Eggs revealing no embryos showed evidence of embryo autolysis and resorption.

When the 2-d flight embryos were examined grossly, they appeared normal for the age/stage at which they had died. It was possible to age them without ambiguity by classical standard criteria of crown-rump length, facial development, distal limb differentiation, eye

pigmentation, feather papillae, etc. (Fig. 1). When examined in section by light microscopy, no differences were detected in organ topography, organ organization, tissue and cellular structure, or routine and selected histochemistry.
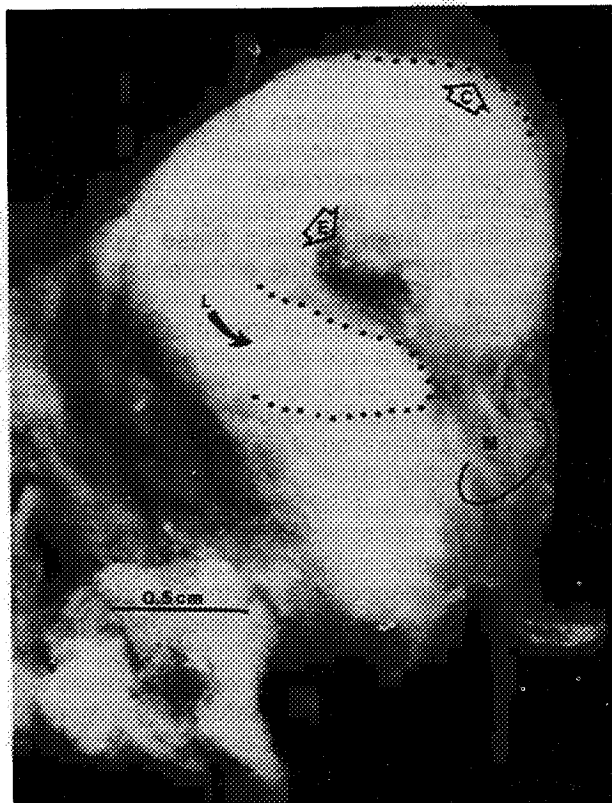

Figure 1. Surface features flight embryo, 2-d group, development arrested 5-d age/stage, examined at 21st d incubation. Crown (C), eye (E), forelimb (L), extraembryonic membranes (M). Formaldehyde fixation.

DISCUSSION

I assume for this discussion that the hypergravity, vibration, and noise of launch are not detrimental factors, our preflight testing having controlled for these. Similarly, I assume that cosmic radiation nor the physical shock and hypergravity of re-entry are not detrimental, the re-entry factors having occurred after the death of the embryos.

The important individual variation in age/stage of death of the younger embryos could be related to slight, but normal, differences in 1) age/stage at egg laying, 2) interval spent at room temperature before routine refrigeration, or 3) time of pre-launch holding in refrigeration. None of these variables have yet been excluded. I assume

this variation was due to individual responses to the microgravity environment.

Flights of other avian (Japanese quail) have experienced similarly high mortality in the young embryos (Sabo, 1980; Meteshko et al, 1991; Boda et al., 1992). The flights of 1979 and 1989 together reported 85% mortality with the highest mortality within the first four days of the normal 17-d incubation. Significantly, in their 1989 flight, 8 quail reached full term development and 6 hatched while in orbit. These birds were judged normal according to many structural/functional criteria.

There is a large and compelling literature concerning the influence of ionic currents upon development. Jaffe and Stern (1979) with chick embryos demonstrated current driven in at the primitive knot and leaking along the primitive streak, thus serving as a current source. They described these events related to the development of occluding junctions between surface ectoderm cells. It is now clear that epithelial differentiation creates the possibility of selective permeability, ionic pumps, and facilitated diffusion. These features coupled with the appearance of the zonulae occludens and adherens of the junctional complex creates the morphology required to establish an ionic gradient and membrane potential (Borgens, 1992). Nuccitelli and Erickson (1983) and Erickson and Nuccitelli (1984) correlate these physiologic currents with fibroblast movement in the extracellular matrix of quail. Robinson (1985) reviews the response of the basic tissues and neural crest to electrical fields and relates an electrotaxis to cytoskeleton, membrane charges, and ionic channels. Stern (1981) argues that electrical field effects upon mesenchymal cell movement in chick mesoderm are a consequence of effects upon the extracellular matrix, perhaps via the polarization of fibronectin. Borgens (1986) has demonstrated, in extensive studies of neurite outgrowth in response to applied electric fields, changes in these endogenous currents in response to injury.

The histogenesis of epithelium is preceded in ontogeny by the development of the embryoblast and trophoblast, limiting the blastocoel. Here too is an epithelial envelope, a structural basis for driving current. Wiley and Nuccitelli(1986) describe ionic currents in the 8-cell cleavage stage of the mouse. Differentiating cells have been demonstrated to be especially sensitive to altered gravity (Cogoli et al., 1984).

However, all of our embryos were 48 hours (assuming plus or minus no more than 4 hours) development. By this stage significant organogenesis has occurred in the nervous system and cardiovascular system; all histogenesis is well-advanced.

The death of the younger embryos and at different ages/stages is an intriguing problem. It suggests that embryonic physiology during 2-7 d is susceptible to hypogravity, unlike in those embryos of 9-14 d. I would like to propose that the intrinsic environment of the embryo, given the advanced organogenesis and physiology of 9 days, is sufficient to remain independent of hypogravity influences.

Consider: If ionic, substrate, or byproduct gradients are critical throughout development to hatch, by 9 d there may be sufficient internal/intrinsic current flow to assume the regulative role for growth and differentiation. Before 9 d, or perhaps only during d 2-7, such a gradient may be superimposed upon the embryo, directly by gravity or as a consequence of gravity regulating the conditions of electrical field. In the absence of gravity, the embryo in the interval 2-7 d may not itself be able to control these organizing processes. As Cameron et al. (1985) suggest and Spooner (1991) relates to the macromolecular level, the effect may exists only during a limited time, a "window of sensitivity."

With differentiation of a limb, the synthesis of protocollagen, its release and organization to form tropocollagen, and, in turn, its polymerization and alignment in the intercellular space will typically result in the formation of dense regular collagenous connecting tissues of tendon. The regulation of the initial polymerization, alignment of a single reticular fiber along the axis of the parent fibroblast, and the alignment of the fibroblast relative to the axis of the limb, may be determined by an electrical field or current being driven proximal-distal within the limb. However, after the initial alignment, association with adjacent skeletal myoblasts and myotubules, and association with periosteum, the subsequent polymerization, differentiation, and growth of tendon may be less dependent or perhaps independent of the existent electrical field or current being driven.

The location of the first polar body, first mitotic spindle, cytoskeletal organization, and yolk distribution in the zygote may be dependent upon the site of contact and adhesion of the spermatozoon. Subsequent cleavage divisions may be affected in rate and degree of daughter cell differentiation as a consequence of that, but may no longer be dependent upon this site. Allarets (1991) discusses this "positional information" and compartmentalization as they may be affected by gravity and influence cytoskeleton and cell shape.

It is possible that, when in low-earth orbit, the embryos are free of not only normal gravity, but also free of other environmental influences, e.g., electrical fields. If that is determined to be the case and the influence is detrimental, the establishment of that other "epifactor" could be easier/more economical than establishment of a gravitational field in orbit or during an interplanetary mission (Lewis, 1990).

Microgravity does have whole-organism effects in embryos of other species. Vernos et. al. (1989) suggested these negative effects were due to altered distribution of maternal components, but 75% of Drosophila embryos still reached the final stages of development. Beetschen and Gautier (1987) have shown the importance of "tilt" in axolotl development. In Xenopus, yolk distribution was undisturbed in clinostat studies conducted by Smith and Neff (1986); but Cooke (1985) determined gravotrophism to be important in determining "spatial information." These effects have been demonstrated in meso- and microlecithal ova and cleavage stages. In macrolecithal avian species these "positional" effects upon the cells of the embryoblast may be less important.

Little direct effect is known of zero gravity. Much evidence of a role of gravity is derived from work with adaptation to hypergravity. Boďa and colleagues have published extensively of these studies in Japanese quail (Boďa, 1984). Clearly the focus of microgravity investigations should extend, as Allaerts (1991) and Spooner (1991) suggest, to the macromolecular level to study the extracellular matrix, cytoskeleton, cell membrane, and such phenomenon as exocytosis, endocytosis, cell movement, microciliary and microvilliary movement, as well as mitosis.

Surely these studies in weightlessness will contribute much to the understanding of normal development, wound healing, and the neogenesis of tumor formation. It could also lead to a unifying theory of development, what Moore (1987) calls a "basic theory of development."

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