"Gravity and Embryonic Development"

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The relationship between the developing embryo (both plant and animal) and a gravitational field has long been contemplated. The difficulty in designing critical experiments on the surface of the Earth because of its background of 1 G, has been an obstacle to a resolution of the problem. Biological responses to gravity (particularly in plants) are obvious in many cases; however, the influence of gravity as an environmental input to the developing embryo is not as obvious and has proven to be extremely difficult to define. In spite of this, over the years numerous attempts have been made using a variety of embryonic materials to come to grips with the role of gravity in development. Three research tools are available: the centrifuge, the clinostat, and the orbiting spacecraft. Experimental results are now available from all three sources. Some tenuous conclusions are drawn, and an attempt at a unifying theory on gravitational influence on embryonic development is made.

1. Introduction

Does gravity exert a definitive influence on embryogenesis? The literature on the effects of gravity on animal development

has been reviewed recently by Pitts (1). His review includes clinostat and centrifuge studies on amphibian and a variety of invertebrate eggs, as well as whole animal (turtles, mice, rats and chickens) studies on the centrifuge. It also briefly describes the orbital-weightless studies done to date. Rather than re-review these largely observational papers, it is the purpose of this paper to describe one of the fundamental problems in the field of embrogenesis (induction) and to propose that the use of weightlessness as an experiment research tool, can contribute to the formulation of testable theories about the nature of certain cellular processes, such as induction and differentiation. Unfortunately, most early biological experiments flown in orbital spacecraft have been exploratory and observational in nature and have perhaps lacked the sophistication of design and rationale which the rigorous application of the "scientific method" demands. In fact, some have been poorly conceived and performed, to the extent that the "scientific community" has had a negative reaction to the scientific utility of space flight experimentation. The high cost of space flight research demands that a maximum effort be put into experimental design and background study and that rigorously testable hypotheses underlie flight experiments. Purely observational experiments are probably an ill-advised luxury in todays' environment.

2. The Problem

I view the egg as a single cell containing sufficient information to develop into a complex multifunctional mass (the organism) upon proper stimulus (fertilization), which does not necessarily include the introduction of new genetic material. The processes by which the egg undergoes this transformation are enormously complex and only partially understood. As cells develop into a multicelled organism, they become morphologically and functionally different from one another even though they are provided with identical genomes. As Jacobson (2) points out, the mechanism of cell differentiation offers to the investigator some of the most provocative problems of modern biology. The mechanism by which many, if not all, animal embryos differentiate is called induction. Induction refers to the interaction between tissues in which one set of cells responds to the presence or juxtaposition of another to proceed on a course of development different from that which it would have followed if the interaction had not occurred. Spemann (3) postulated an organizer tissue in very early embryos which served as a primary inductor. This primary inductor has been traced in transplantation experiments by Curtis (4) to reside in the cortical cytoplasm of the gray crescent region of the undivided egg. Upon fertilization, visible changes occur in the cortical cytoplasm and the inductive properties appear to be quickly spread through the cortex, thus

being imparted to the daughter cells in subsequent divisions. The physical-chemical nature of the inductor is not understood. It appears that at least some of the response and inducing capacities are formed during oogenesis, but the unfertilized egg is morphologically dormant until triggered by the act of fertililzation. Most embryonic induction studies have been done with amphibian material and it is such work that will be concentrated on in this paper.

The question is, does gravity play a determinative role in the process by which the fertilized egg juxtaposes certain cells or intracellular material in such a way as to determine irrevocably, or at least temporarily, the future role of those cells? The morphogenetic movements which bring inductor and reacting tissues together with such precision must be accounted for in any satisfactory explanation of embryogenesis.

3. The Evidence

3.1 Centrifuge

Gravity has long been suspected of playing a role in development. Most experimental work has been done on the amphibian egg, probably because of its availability and ease of handling in the laboratory and because of its obvious response to gravity. The amphian egg is telolecithal- that is, the heavier yolk is located in the vegetal hemisphere, which in turn forces the nucleus and much of the cytoplasm into the other (animal) hemisphere. The animal hemisphere then is characterized by its

darkly pigmented cytoplasm. The line running between the two poles is the polar axis which is uppermost at the animal pole and lowest at the vegetal pole. During oogenesis in the frog then, the eggs are already oriented with respect to gravity and polarity and metabolic gradients established thereby.

Mature eggs, removed by artificial means from the frog are randomly oriented with respect to gravity and become "frozen" in position rapidly by the imbibition of water by the surrounding jelly mass secreted with the eggs. When fertilized, the first sperm to penetrate the jelly and the vitelline membrane of the egg triggers a number of rapid responses. First the vitelline membrane is elevated and becomes the fertilization membrane, which prevents the entrance of any more sperm. This membrane lifting creates a perivitelline space within which the egg may rotate. At the same time there are metabolic changes, and increase in cytoplasmic viscosity and membrane permeability. Migration of cortical material is also seen at this time. The most striking events, however, are the onset of rotation of the egg so that it becomes reoriented with respect to gravity in a matter of minutes, and the movement of cortical cytoplasm and pigment granules toward the point of sperm penetration with the concurrent appearance of a crescent-shaped area where the pigment is less dense (gray crescent) opposite the point of sperm entry. At this time bilateral symmetry is established.

This rotation of the frog egg was probably the original stimulus for studies on the role of gravity in development. Schultze (5) held fertilized frog eggs between two glass slides in the inverted position and found that a large number of abnormalities developed including a fair proportion of twins. He noted that these results were seen primarily when the experiment was performed at around the two cell stage. Penners and Schleip (6) extended Schultze's experiments in some detail and with similar results. Tschou Su (7) reported twinning and other abnormalities in frog eggs contrifuged before cleavage, as did Kas' Yanov (8). At the same time, Young et al (9) using Xenopus eggs showed that the sensitivity of these eggs to gravitational influence was very time dependent (Figure 1). In these studies several experiment protocols were used. Fertilized eggs were placed on the centrifuge within 5 minutes of fertilization and runs made at 500g for 1½ min., and 20 and 40g for 1 hour 15 min. respectively. Runs made at 20g for 1 hour or more were lethal regardless of the developmental stage up to the 8-16 cell stage when significant survival was observed. Abnormalities in general decreased in these experiments as the age of the embryos used increased, dropping to very low levels by the early blastula stage. Runs made at 40g for 14 min. showed that these eggs were most sensitive to gravity between fertilization and 35 min. after fertilization. This time period was also the only one in which twins were produced. By 40 min. after fertilization the eggs

XENOPUS EMBRYOS CENTRIFUGED AT 40 G FOR 15 min

TIME (IN min) AFTER FERTILIZATION	NORMAL	ABNORMAL	DEATH	TWINS (percent LIVE)
15-35	25	60	15	41
30-50	40	25	35	0
45-65	25	15	60	0
60-80	20	15	65	0

FIGURE 1

were relatively insensitive and a high percentage of normal embryos resulted. At the time of first cleavage a second period of sensitivity was seen with many abnormalities produced, not including twinning. 500g for 1½ min. produced essentially the same results. After first cleavage the amphibian egg becomes progressively less sensitive to gravitational influence. These experiments were run at 22°C at which temperature first cell division occurred at 70-75 min.

3.2 Clinostat

In addition to centrifuge studies a considerable effort has been put into clinostat research; particularly with plant material and also with amphibian eggs. The clinostat has the capability of systematically and continuously disorienting the organism with respect to gravitational stimuli by slow rotation. Early studies (10,11,12) showed no increase in abnormality as a result of slow rotation of frog eggs. On the other hand other workers (13,14) found increases in abnormalities. Tremor and Souza (15) in detailed clinostat studies found abnormalities in Rana pipiens and Xenopus laevis eggs and determined that the speed of rotation was a critical factor. The eggs must be rotated at a speed which was properly suited to compensate for the normal rotational speed of the egg. These workers found that rotation at ½ rpm was optimal for the production of abnormalities in R. pipiens, and ¼, 1, and 2 rpm were most effective for X. laevis which has a

faster rate of rotation. Rates greater than 2 rpm were not effective in producing abnormalities. They also found that in R. pipiens, the period of greatest sensitivity was before the two cell stage. It was concluded that at higher rotational speeds, the eggs remained in a fixed position with respect to gravity since the directional force of gravity at any point in time was, in effect, counteracted at another. At lower than optimum speeds, the eggs were able to rotate and maintain a normal "yolk-down" position with respect to gravity, at least through first cleavage. The centrifugal force in such experiments is very low (10^{-2}-10^{-3}g). Thus, although the mechanism is different, the displacement of cytoplasmic material in eggs maintained in a position abnormal to the earths' field by slow rotation can be compared, in effect, to that obtained by high speed centrifugation. The key to such experiments is in rotational timing in the case of the clinostat, to speed and duration of centrifugation and in both cases to the stage of egg development at which treatment begins. This latter observation is consistent with the centrifuge studies of Banta and Gortner (16).

3.3 Weightlessness

Yet another type of experiment has been performed in orbital space flight by Young and Tremor (17,18). In these experiments, near weightlessness was experienced for 4 days in the Gemini 8

and 12 flights and for over 2 days in Biosatellite 2. In all cases, eggs of R. pipiens were used and the effects of orbital flight on cell division and subsequent embryogenesis were studied. From such experiments, it was concluded that a gravitational field is not necessary for the egg to divide normally, nor is it necessary for differentiation and morphogenesis. In the Gemini 12 flight, normal swimming tadpoles were recovered. Unfortunately, in all these cases, the eggs were fertilized on the ground and did not experience the weightless condition until after the 2-cell stage, which virtually all ground based studies show to be critical. Such results are consistent with the studies of Vinnikov et al (19) on the vestibular development of R. Temporaria in weightlessness. Here too, normal development was observed, but only later stages of development were exposed to orbital flight. To date, technical difficulties in experimental implementation have made it impossible to do the crucial experiments, namely, getting embryonic material into the weightless condition before fertilization so that all phases of development are devoid of gravitational input.

4. Discussion

The experimental observations briefly described above seem to suggest rather strongly that embryonic material is especially sensitive to the influence of gravity during the earliest stages of development, including oogenesis; see for example, the work

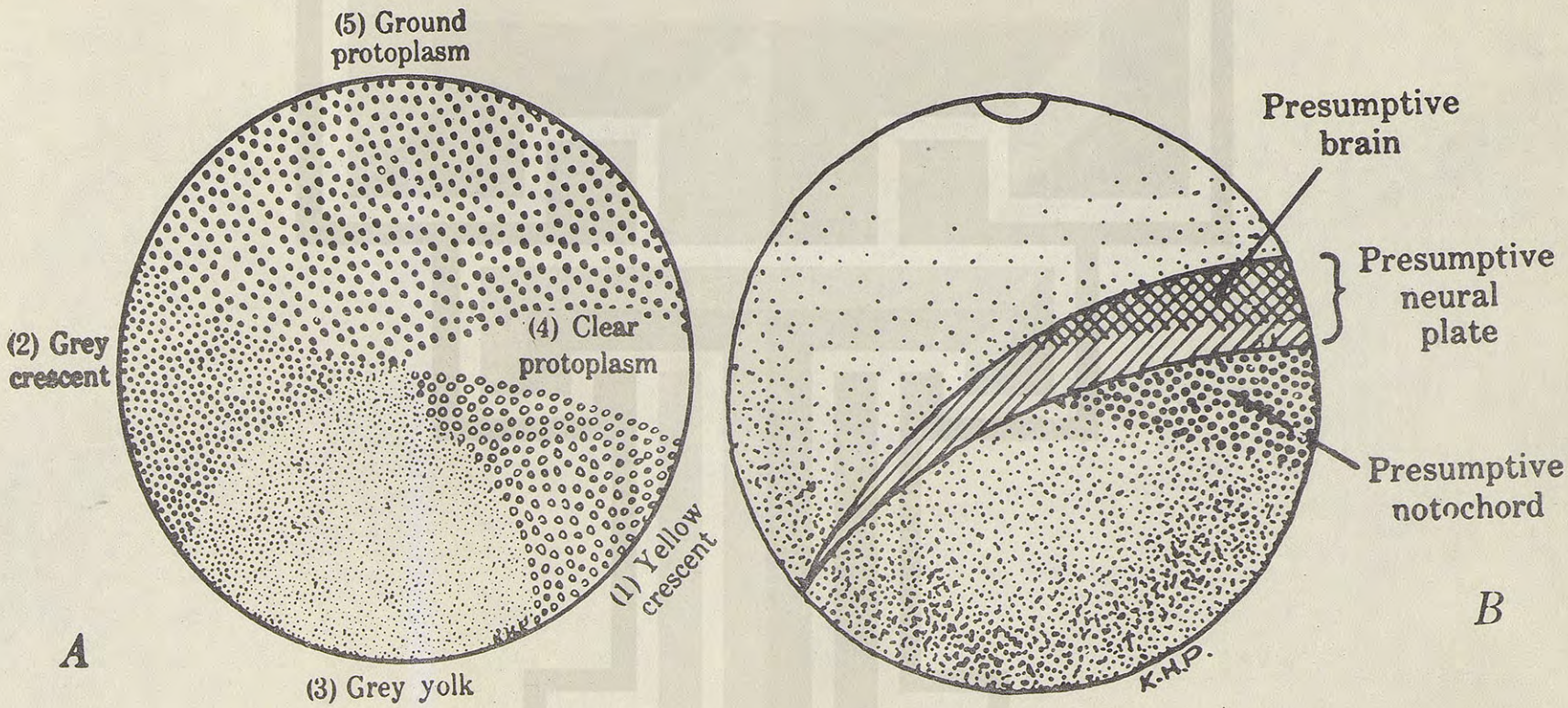
of Kochav and Eyal-Gilali (20). By the time of first cleavage, the embryo has become much less responsive to gravitational input although, of course, physical damage can be done to the developing embryo with very high g loading. The centrifuge, clinostat, and weightless results seem quite consistent with the concept of induction. Developmental programming begins during oogenesis when the cytoplasm of the egg apparently becomes regionally differentiated to some degree, depending on the egg. At fertilization, dynamic processes are triggered which further program the fate of the various regions of the cytoplasm, so that by the time of first cleavage the two daughter cells are already differentiated to a degree and a great deal of the future history of the embryo is imprinted. Organs arise as a result of such early determination and the process of induction. Steinberg (21) feels that the mechanism underlying morphogenetic movements which bring inductor and reactive tissues together may be a simple hierarchy of adhesives in a number of randomly distributed adhesive sites on cell surfaces. Certainly, the first visible suggestion of induction is seen in a behavioral change in the reacting cells, that is, shaping or clumping or a variety of movements. In most systems in which the chemical basis of induction was studied, it has turned out that most determination has already occurred before the study began - usually before cleavage. As development proceeds with time, the responding cells become more and more firmly committed to a particular

course of differentiation, perhaps ultimately with the production of cell-specific proteins.

There would seem to be sufficient experimental evidence to suggest very strongly that an embryo, after a few cell divisions, has already been sufficiently differentiated so that external influences will have little, if any, effect on subsequent morphogenesis. Gravity seems to play no determinative role after a few cleavages as illustrated in centrifuge, clinostat and space flight experiments. In fact, if determination occurs as early as most data indicate, one should not expect a gravitational influence on later developmental processes and continued attempts to fly such experiments in spacecraft may indeed be wasteful.

On the other hand, there is clearly a gravitational influence on morphogenesis before first cleavage, with perhaps the most sensitive period being shortly after fertilization, at least in the amphibian egg. It is also clear that there are considerable differences between eggs of different organisms. These differences may well be due to differences in the structure of the egg as it is formed in the ovary or later in the oviduct. The amphibian egg has a great deal of yolk in the cytoplasm. This yolk is of greater density and responds accordingly to gravity. Thus this type of egg is gravity oriented from the beginning. This is also true of the avian egg where bilateral symmetry is established by the gravitational forcing of the egg (and blastodisc) into an oblique position in the uterus (20).

It seems safe to assume that differences in density of cytoplasmic constituents is responsible, during oogenesis, for the earliest events related to subsequent differentiation. A localization of certain materials in the cortical cytoplasm of the egg as a response to gravity should be expected. That such localizations exist can be seen from early studies (22,23,24). In some cases (e.g., extremely teleolecithal eggs) such localizations are already sufficient to serve as determinants, in a limited sense, for future development (e.g., bilateral symmetry in the chick egg), however such early determination can be altered in many cases by external means. At the time of fertilization, a mechanical stimulus (e.g., sperm entry) is the event which actuates the system now poised for a rapid sequence of physical-chemical changes (Figure 2). By inducing changes in solubility, permeability and viscosity of egg components, fertilization causes the egg to respond very quickly to external input (e.g., gravity) again. This response is very complex, but among other things, cytoplasmic material undergoes chemical reaction and change and is relocated in the egg, very likely in response to gravitational input. At this time the egg is most susceptible to external input (centrifuge, clinostat, weightlessness, etc.) and is very labile. Once the reaction to the stimulus of fertilization is complete the situation tends toward stability once more, and considerable stability is evident by



Organ-forming areas in tunicate eggs. *A*, Fertilized egg of *Styela*, from left side (*after Conklin*). *B*, Unfertilized egg of *Ascidiella*, from right side (*after Vandebroek, in Dalcq*). Approx. ×250.

FIGURE 2

the time of first cell division. By this time presumptive material has been relocated and has begun its job of induction on receptor material and the process of determination becomes less and less reversible or labile.

It should be pointed out that the time schedule for transition from lability to stability in the process of induction will vary from organism to organism and will very likely be found to be related to density differences within the egg. In this context, it would be interesting to restudy the literature on echinodern development, since these eggs have relatively little yolk. Experiments on Ascaris, such as those of Schatz and Teuchert (25), should also be reconciled with these concepts.

I would suggest that weightless flight offers a unique opportunity to test the hypothesis that gravity indeed plays a determinative role in embroyogenesis, but that experiments to date have not had the proper design to answer the question, in that flight experiments to date have not placed material into the weightless condition well before first cell division. I would further suggest that the whole animal (frog, etc.) should be used so that the process of oogenesis can also be experienced in the virtual absence of gravitational stimuli. A method should be devised to go through the entire gamut of development from oogenesis, ovulation, fertilization, to subsequent embryogenesis. It is not clear that centrifuge and more particularly clinostat studies can be considered adequate substitutes for weightlessness,

but in any case, both centrifuge and clinostat studies should be extended backward to include the period of oogenesis to complete the picture, if such experiments are feasible. The feasibility of flight of the whole animal (Xenopus) has been explored by the author. The flight of the whole animal, with the induction of ovulation and fertilization appears to be possible, but very difficult. On the other hand, such an experiment has the potential of resolving one of the fundamental problems of developmental biology.

Bibliography

1. G.C. Pitts, Life Science and Space Research XI, 171 (1973).
2. A.G. Jacobson, Science 152, 25 (1966).
3. H. Spemann, Zool. Jahrb. Abt. Allgem. Zool. Physiol. Tiere 32, 1 (1912).
4. A.S.G. Curtis, Endeavour 22, 134 (1963).
5. O. Schultze, Arch. Entwicklungsmech. Organismen 1, 269 (1894).
6. A. Penners & W. Schleip, Z. Wiss-Zool. 130, 305 and 131, 1 (1928).
7. Tchou-Su, Comptes Rend. Soc. Biol. 122, 1043 (1936).
8. V.L. Kas'yanov, Dokl. Akad. Nauk. SSSR 180, 1008 (1968).
9. R.S. Young, P. Deal, K. Souza & O. Whitfield, Exper. Cell. Res. 59, 267 (1970).
10. W. Roux, Bresl. Aertzt. Zeits. 6, 57 (1884).
11. C. Kathariner, Archiv. Entwick.-Mech. 12, 597 (1901).
12. T. H. Morgan, Anat. Anz. 21, 313 (1902).
13. O. Schultze, Phys. Med. Ges. 4, 41 (1897).
14. S. Gordon, Personal Communication (1969).
15. J. W. Tremor & K.A. Souza, Space Life Sc. 3, 179 (1972).
16. A.M. Banta & R.A. Gortner, J. Exp. Zool. 17, 433 (1915).
17. R.S. Young & J. W. Tremor, Life Sci. & Space Res. 10, 87 (1968).
18. R.S. Young & J. W. Tremor, Bioscience 18, 609 (1968).
19. Y. A. Vinnikov, et al, Zhurnal, Evol. Biok. i Fiziol. 8, 343 (1972).

20. S. Kochav & H. Eyal-Giladi, Science 171, 1027 (1971).

21. M. Steinberg, in Cellular Membranes in Development, Academic Press, New York, 321 (1964).

22. P. Vintemberger & J. Clavert, C.R. Soc. Biol. Paris 154, 1072 (1960).

23. P. Ancel & P. Vintemberger, Bull Biol. Suppl. 31, 177 (1948).

24. J. Pasteels, Arch. Biol. 52, 321 (1941).

25. A. Schatz & G. Teuchert, Aerospace Med. 43, 614 (1972).