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AVIAN EMBRYONIC DEVELOPMENT IN HYPERDYNAMIC ENVIRONMENTS

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ABSTRACT:

This project was undertaken to examine the effects of hyperdynamic fields upon embryo formation and survival in chicken eggs and throughout the period of development.

Prerequisite to the research was the development of an incubator/hatcher that could be mounted upon an operating centrifuge, and suitably equipped for controlling and monitoring the centrifuge incubator/hatcher environment. No guidelines existed for such an apparatus, particularly in choosing among the variety of alternative operational principles and structural characteristics. Two models were developed, one with 0° and the other 1° of freedom -- the latter proving more satisfactory.

The avian embryo is in a buoyant environment over most of its development so it is essentially free of a net load. Gravitational influences upon embryos depend largely upon density gradients (both in the egg and in the embryo) so that the acceleration effects are zonal. Studies of the influence of hyperdynamic fields upon embryogenesis must ultimately be related to the sites of gravitational load within the embryo. Preliminary observations indicated that substantial density gradients exist in embryos, and that they change over the developmental period.

The first 24 hours of development of the chick embryo occur while the egg is still forming in the oviduct -- so studies of this developmental period require centrifugation of laying hens. Embryos which developed for 24 hours in the oviduct of hens maintained at 2 G and which were subsequently incubated at earth gravity had a 14% reduction in hatchability. Increased mortality during the first 4 days, and an increase in embryonic abnormalities were of the types usually found during the first mortality peak (2-3 days).

Embryos in eggs that were produced at earth gravity and continued their development on the centrifuge at fields of 2 G or less did not appear to be greatly affected by the treatment. At 4 G, 91% of the embryos died, mostly on the first and second days of incubation. Abnormalities prominent in the centrifuged eggs include: (a) a failure of the primitive streak to develop; (b) interference with the development of the axial skeleton; (c) multiple hemorrhages, mostly petechial which is consistent with capillary fragility; and (d) retardation of embryo growth.

The growth retardation caused some concern since it intimated an inadequate thermal control (e.g., zonal temperatures in the incubator) and required a considerable amount of trouble-shooting. However, subsequent consideration indicated that this slowing of development may have been caused by an inteference with gaseous diffusion, the result of an acceleration-induced increase in gas density in the centrifuging incubator.

BACKGROUND:

Earth gravity has been found to affect embryonic development -- the initial observations being made by Pfluger in 1883 (11). He found that in developing amphibian eggs the first cell division was parallel to the field of gravity -- and that abnormal orientation interfered with embryogenesis. Later investigations (8,10) indicated that the mechanism was indirect -- that abnormal orientation led to turbulence in the cytoplasm (the result of the interaction of gravity with density gradients in the egg material), which mechanically disturbed the materials of early the embryo, interfering with development.

There have been only a few studies of the influence of gravitational fields upon embryonic development in avian eggs. Besch (3,4) centrifuged fertile Coturnix and chicken eggs, prior to incubutation, in fields up to 150 G, finding a greater embryo mortality (when incubated subsequently at earth gravity) that was proportional to field strength -- the effect was greater in the larger chicken egg. The lethal mechanism was the same as that in the abnormally oriented amphibian eggs -- turbulence in the yolk mechanically dispersing the embryonic cells, interfering with further development.

Redden (13) studied bone development in hen eggs at fields of 4 and 5 G (by putting eggs in a small centrifuge, 0.74 m diameter, and then operating it in a large incubator). Changes in bone conformation appropriate to the increased field were noted -- they became longer and more slender, a function of tensile forces, a phenomenon discussed by Galileo. Unfortunately, no observations were reported on embryonic mortality, terata, etc., and the treatment did not include the first 72 hours of development.

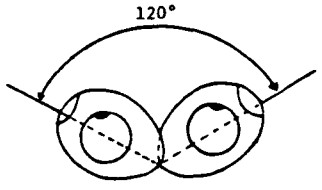
Atherton (1,2) in a study of avian embryonic erythropoieses, centrifuged incubating eggs for 24 or 72 hours (in fields of 2.1 to 7.8 G) after 8, 9, 13, 14 or 15 days of development. The study involved few eggs and a centrifuge 0.63 m diameter, which was enclosed in an incubator. Mortality data was reported, but with small sample sizes it is highly variable. However, the 8-9 day old embryos were more affected than the 13-15 day old embryos. It appears that in a 2 G field, 24 hours of centrifugation leads to a mortality of 25.7% in 13/15 day embryos, as compared to 31.6% for 8/9 day old embryos.

In 1978, we initiated this study to determine the effects of gravitational fields on avian embryonic development and also the changes produced in surviving and non-surviving embryos.

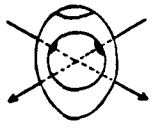
CENTRIFUGE INCUBATORS:

An existing centrifuge (7) was adapted to carry several incubators large enough to accommodate about 50 eggs each. This capacity provides statistically sufficient numbers for the various studies. Provision for turning the eggs is particularly critical. The usual method is by rocking the eggs -- however, in the centrifuge this has the disadvantage of potentially changing the radius of rotation, producing an undesirable cycling of the G-field. To prevent this,

we first experimented with incubators in which the eggs had 0° of freedom. The eggs were mounted with their major axes held parallel to the centrifuge's axis of rotation. When the eggs were rotated around their major axis, the yolks reoriented to the net field -- which achieved the same changes between yolk and shell obtained by rocking eggs at earth gravity:



A. Egg turning at Earth gravity.



B. Egg turning at 2 G.

However, experience with this 0°-freedom arrangement indicated some limitation, especially at low-G fields. In fields less than 2 G the inertial field did not provide sufficient deflection of the yolk for the turning to change the location of the embryo with respect to the shell. Also at very high fields and in the early stages of development there was a probability that the gradient of increasing positive buoyancy between albumen, yolk and embryo could provide an excessively strong contact between the embryo and shell -- significantly deforming the embryo. Consequently, after some 18 months of development, we abandoned the 0°-freedom principle.

We then developed two incubators that would operate at 1°-freedom -- retaining the instrumentation for environmental control and monitoring. A hatching tray was arranged to hold rows of eggs -- arranged along the radius of rotation. These were rocked 5 times daily in a direction tangential to the rotation. This produced a variation of only 0.05 cm in the radius of rotation -- and a difference in the acceleration field of 0.03%. Further studies indicated that this had no significant effect on the embryos. By comparison, a 4 cm displacement along the radius of rotation would have changed the field strength ±5%, which would be much more likely to affect the incubation results.

Constructional details of the two incubator models and the instrumentation for thermoregulation and humidity control have been described in detail in the annual progress reports.

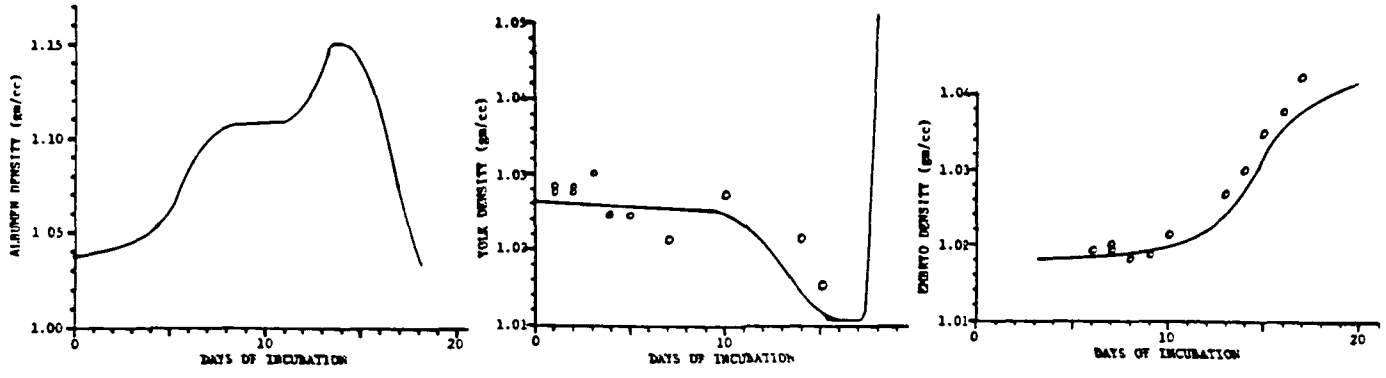
EGGS:

Up to August/September 1980, all incubation experiments were done with eggs from acceleration tolerant line. At that time a mold contamination (Aspergillis) appeared in the eggs which was not immediately corrected. Later experiments were done with eggs from a high-hatchability commercial line (Donsing Hatchery, Rio Linda). Some of the results with the Donsing eggs were dissimilar from those obtained with the acceleration-tolerant line. The discrepancy may have been due to a strain effect, modifying response to acceleration -- or it may have been related to the mold problem in the acceleration tolerant line. The basis for the difference was not resolved -- and results from the two kinds of eggs were summarized separately.

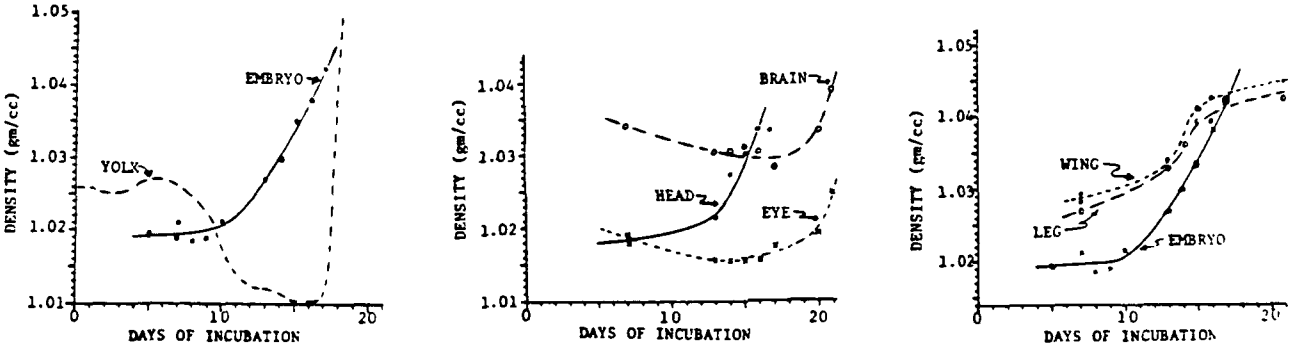
GRAVITATIONAL LOADING OF THE EMBRYO:

The load imposed upon the embryo is a function of the embryo mass and the difference in densities between it and the surrounding fields. Although direct determinations of the densities of the egg contents are not available, the general relationships were calculated from their chemical composition as reported in the literature (see figure 1).

Figure 1. Density changes (gm/cc) in egg contents and developing embryo over the period of incubation. Solid lines for egg contents and whole embryo were calculated from the chemical composition. Points (o, •, x) indicate measured densities.



Around 11 days of incubation, the density relationship between the embryo and yolk is reversed. However, throughout incubation, the albumen appears to have a much greater density than either the yolk or the embryo -- so the yolk and embryo have positive buoyancy throughout the aquatic phase of development.



There also are changes in the densities of larger body parts in the developing embryo. The density of the head increases sharply after 12 days and that of the leg and wing a day or so later. This results from calcification, and the time differences represent growth gradients in the skeleton.

We measured the densities of yolk, albumen, embryos, and embryo parts with standard $CuSO_4$ solutions -- a procedure commonly employed by hematologists (12). Our preliminary results are summarized in figure 1. They indicate a complex pattern of gravitational loading within the developing embryo. Up to 10 days, the head has a positive buoyancy as compared to the rest of the embryo -- and later, a negative buoyancy. Wing and leg have a negative buoyancy with respect to the embryo up to 16 days of development, and a positive buoyancy thereafter. These studies were discontinued when we could no longer support the necessary assistance.

GRAVITATIONAL EFFECTS DURING EGG FORMATION:

In the 24 hours between ovulation/fertilization and oviposition, the embryo develops to the early gastrula stage -- with about 50,000 cells. Studies of acceleration effects in this period requires the centrifugation of laying hens. This was done with eight sets of eggs from group-eta hens (S_{21}) and two from group-theta hens (S_{22}). The results (Table 1) do no include data from four earlier sets from the eta-group (sets 89, 96, 103 and 110) in which normal eggs were removed for examination during incubation -- nor for two later sets from the theta-group (sets 196 and 204) in which there was evidence of a fungal infection.

The results are summarized in terms of survival by periods (as a % of viable embryos entering that period) -- Table 1. It is apparent that centrifugation during egg formation affected embryo survival in the earliest periods.

TABLE 1. Embryonic survival at 4-day intervals during incubation at earth gravity (1 G). Survival is calculated as % of viable embryos ± standard deviation entering each period.

EGGS FROM CENTRIFUGING HENS (2 G; 8 sets; 376 fertile eggs)

	Eta group (S_{21}) 6 sets; 308 eggs	Theta group (S_{22}) 2 sets; 68 eggs	ALL 8 sets; 376 eggs
Fertilization to day 4	86.5 ± 6.54	93.4 ± 2.26	88.2 ± 6.44
days 5 to 8	92.6 ± 3.25	96.5 ± 5.02	93.5 ± 3.79
days 9 to 12	98.5 ± 1.21	99.2 ± 1.20	98.7 ± 1.15
days 13 to 17	98.1 ± 1.93	100.0 ± 0.00	98.6 ± 1.86
day 18 to hatch	84.5 ± 5.43	76.9 ± 0.64	82.6 ± 5.80
Fertilization to hatch	65.1 ± 3.72	68.6 ± 0.42	65.9 ± 3.55

EGGS FROM CONTROL HENS (1 G; 8 sets; 396 fertile eggs)

	Eta group (S_{21}) 6 sets; 265 eggs	Theta group (S_{22}) 2 sets; 131 eggs	ALL 8 sets; 396 eggs)
Fertilization to day 4	93.9 ± 1.78	91.3 ± 3.11	93.4 ± 2.12
days 5 to 8	96.4 ± 3.10	100.0 ± 0.00	97.3 ± 3.11
days 9 to 12	99.6 ± 1.06	100.0 ± 0.00	99.7 ± 0.92
days 13 to 17	99.5 ± 0.87	100.0 ± 0.00	99.6 ± 0.78
day 18 to hatch	90.0 ± 4.94	89.4 ± 4.17	89.8 ± 4.47
Fertilization to hatch	80.6 ± 4.41	82.0 ± 1.13	81.0 ± 3.80

The significant reduction from fertilization to hatch was principally the result of the increased early embryo mortality enhanced by the accumulation of the lesser embryo mortality in other periods.

Period	Effect of Centrifugation; % reduction in survival (p)
Fertilization to 4 days	-5.5% (<0.05)
5 to 8 days	-3.9% ns
9 to 12 days	-1.0% ns
13 to 17 days	-1.0% ns
18 days to hatch	-8.1% (<0.02)
Fertilization to hatch	-18.5% (<0.001)
n sets	8 centrifuge, 8 control
n eggs	376 centrifuge, 396 control

The greater early mortality also is apparent when the distribution of embryo mortality (as % of total mortality) is compared for eggs produced in different gravitational fields:

Incubation Period (days)	Gravitational field during egg formation	
	2 G	1 G
Fertilization to 4 days	37.6%	28.0%
5 to 8 days	17.6%	15.5%
9 to 12 days	3.6%	4.7%
13 to 17 days	3.3%	3.4%
18 days to hatch	37.9%	48.4%
Fertilization to hatch	100.0%	100.0%

The greater susceptibility of the embryo in the first four days of incubation reflects the importance of the embryogenic processes taking place. This is the time of development of the brain and the anlage for all other organs.

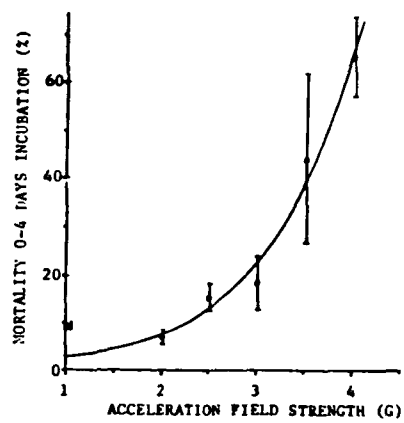
TABLE 2. Embryonic survival at 4-day intervals during incubation in several gravitational fields. All of these incubations used commercially available eggs (Donsing). Survival is calculated as percent of viable embryos (± standard deviation) entering each period.

	FIELD STRENGTH (G)							
	Static Control	10 rpm	1.5 G	2 G	2.5 G	3 G	3.5 G	4 G
Sets	4	2	1	5	2	2	2	4
Eggs	377	71	29	193	64	60	46	115
Fertilization to day 4	90.6 ±5.4	83.4 ±2.7	82.8	92.9 ±2.8	84.4 ±4.4	81.5 ±7.9	55.4 ±25.3	34.0 ±17.2
days 5-8	98.8 ±2.4	98.3 ±2.3	100.0	100.0	98.1 ±2.7	100.0	97.8 ±3.2	79.4 ±19.5
days 9-12	98.4 ±1.3	100.0	100.0	96.5 ±3.5	98.0 ±2.8	100.0	100.0	100.0
days 13-17	98.7 ±2.0	100.0	95.8	99.2 ±1.9	96.5 ±5.0	100.0	100.0	100.0
day 18 to hatch	89.5 ±7.8	45.5 ±6.4	100.0	84.3 ±9.9	6.1 ±3.2	35.7 ±13.9	10.8 ±8.4	38.4 ±30.2
Fertilization to hatch	77.5 ±3.8	37.3 ±5.6	79.3	74.1 ±8.8	4.7 ±2.3	28.6 ±8.4	4.8 ±2.1	8.4 ±5.5

Kinetics of the 0-4 days mortality were calculated from the 2-4 G incubation data:

$$\text{Mortality (\%)} = 2.5e^{1.1(G-1)}$$

[$r = 0.985$ $(p<0.001)$]



Also the heart commences to function and the extra embryonic membranes form, which compartmentalizes the egg contents, form. The second, and higher mortality peak involves failures in one or more of the processes associated with hatching, i.e., yolk retraction, uptake of fluids, regression of the allantoic circulation, onset of pulmonary respiration, embryo position changes and pipping.

Although a variety of abnormalities were encountered in eggs laid during centrifugation they resemble those found in control eggs laid at earth gravity. Although an increased gravitational field during egg formation significantly reduced early embryonic survival, it did not impart any specific debility to those developing beyond the early stages.

EFFECT OF ACCELERATION IN EGGS LAID AT 1 G:

Our studies of gravitational affects on embryonated eggs following egg formation were carried out principally with commercially available eggs (Donsing). The results of this treatment are presented in terms of embryo survival by incubation periods in Table 2. The experiments conducted with the centrifuge operating at 10 rpm had the lowest hatchability of eggs incubated at 2 G or less. This is considered to result from the 0°-freedom configuration -- which produces only an insignificant deflection of the yolk, such that turning the egg does not change the embryo-shell relationship. However, since none of the other sets incubated in fields of 2 G or less exhibited systematic differences from the controls, it appears that non-acceleration machine effects did not contribute significantly to the results.

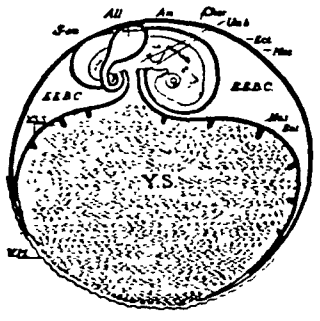
When the experiments with 2 G and greater fields are examined the only difference is the marked early embryo death at 4 G. This is particularly impressive when the distribution of embryo mortality (as % of total mortality) is compared to field strength:

	Distribution of embryo mortality at indicated field of incubation			
	1 G	2 G	3 G	4 G
Fertilization to 4 days	39.3%	27.4%	23.3%	90.5%
5-8 days	5.0%	2.7%	0	1.8%
9-12 days	6.2%	12.0%	0	0
13-17 days	3.3%	2.7%	0	0
18 days to hatch	46.2%	55.2%	76.7%	7.7%
Fertilization to hatch	100%	100%	100%	100%

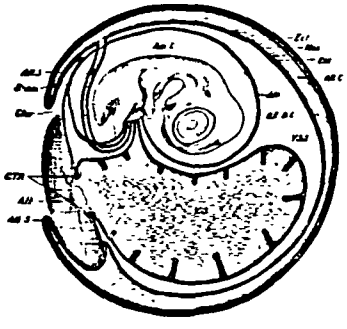
Similar mortalities were not reported by the other two investigators who have centrifuged eggs at 4 G and greater fields (1,13) -- however, neither centrifuged eggs before 48 hours of incubation.

The principal mortality at 4 G occurs prior to 48 hours -- with very little mortality in the intermediate period, 5-17 days. Over the first 48 hours, the extra-embryonic membranes form, initiating a compartmentation of the egg contents. On the third day the amnion closes and begins to fill with a low

density fluid. Consequently from the third day on, the embryo is located in the "bottom" of the amniotic sac which provides some protection from the mechanical forces developed during centrifugation by the higher density yolk and albumen. This relationship is indicated by the following schematic diagram (6):



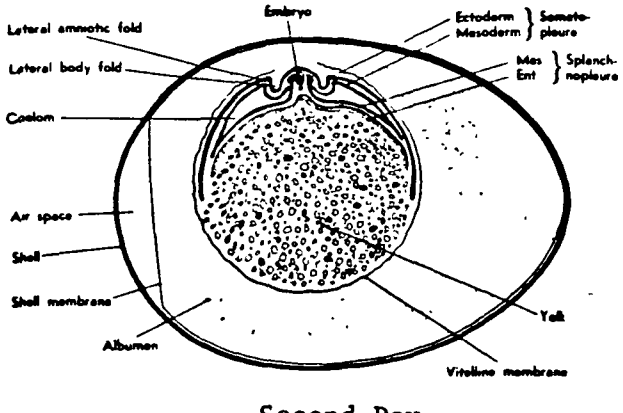
Fourth day



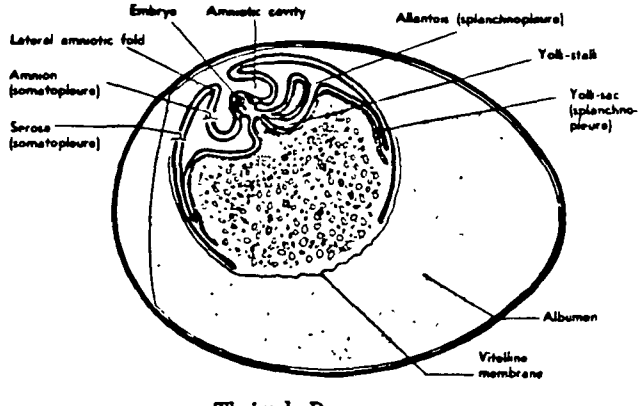
Ninth day

It is possible that the 0°-freedom arrangement may have contributed to this mortality since with it the contact would be between embryo and shell (vice embryo and air cell with one degree of freedom). This was examined in another 4 G experiment with incubation providing one degree of freedom. If shell contact were the mechanism for the early embryo mortality at 4 G, then the 20% survival represented embryos located epicentrically from the pole of minimum density. Besch (5) observed that 25% of the blastodiscs in fertile eggs are located more than 45° from the region of minimum density. Since little difference was observed between embryos incubated with 0° or 1° of freedom at 4 G, the mechanical basis for the early embryo death does not seem to have been a factor.

It appears more likely that the early mortality at 4 G resulted only from interference with tissue movements involved with primitive streak and embryonic axis formation. The spatial relationships at this period are indicated by the following diagrams (schematic) of the chick embryo at the second and third days of incubation (9):



Second Day



Third Day

Motive forces for these tissue changes may be biological or physical in nature. Forces could develop biologically from the proliferation of tissue, or the action of specific tissue. Late on the third day, the amnion develops a musculature which starts rhythmic contraction late on the fourth day (14).

TABLE 3. SUMMARY CODING SYSTEM FOR EMBRYONIC OBSERVATIONS.

This system summarizes several categories of direct observations, which appeared in earlier progress reports [code numbers of direct observation categories are indicated in brackets]. The summary categories are not exclusive, and one embryo could contribute to more than one of them.

1. Early death, no detectable abnormalities [02,03,04,05,09]
2. Hemorrhage [21,22,23,24]
3. Edema [20]
4. Anemia [06]
5. Beak abnormalities [41,42,43]
6. Eye defects [32,33,34,35,36,37,38,39,47]
7. Head defects [45,29,30,31,44]
8. Trunk defects [10,54,55,59]
9. Limb defects [62,76a]
10. Visceral abnormalities [10,19]
11. Abnormalities affecting entire embryo [08,27,40,50]
12. Dead in shell/pipped (alive and dead) [X6,X8,99]
13. Malposition [93]
14. Hatched with abnormalities [91,96,98]
15. Hatched with leg abnormalities
16. Temporary balance problem

TABLE 4. FREQUENCY OF OBSERVED ABNORMALITIES.

Abnormalities noted in eggs laid at earth-gravity (Donsing) and incubated at the indicated field strength are summarized as % of initial embryos (i.e., fertile eggs).

Field Strength	1 G	2 G	3 G	4 G
Number of Initial Embryos	202	193	60	57
Abnormalities:				
1. early death	5.0	4.2	13.6	36.8
2. hemorrhage	-	-	-	5.3
3. edema	0.5	0.6	-	-
4. anemia	-	2.4	-	1.8
5. beak	-	-	-	-
6. eye	-	-	-	5.5
7. head	-	1.2	1.7	15.8
8. trunk	-	1.2	15.0	21.1
9. limb	-	0.6	-	-
10. viscera	-	-	-	-
11. entire embryo	5.4	0.6	1.7	28.1
12. dead in shell/pipped	1.5	13.5	56.7	42.1
13. malposition	-	1.8	16.7	24.6
14. hatched/abnormal	-	-	1.7	-
15. hatched/leg defects	-	-	1.7	1.8
16. balance problems	-	-	25.0	3.5

TABLE 5. Frequency of observed abnormalities in eggs centrifuged during only part of the incubation period (hatching indicates the incubation period beyond 18 days of development).

Field, incubation	2 G	2 G	4 G	4 G	At 4 G only 24-48 hours incubation
Field, hatching	1 G	2 G	1 G	4 G	
Number of initial embryos	66	193	56	57	55
Abnormalities:					
1. early death	6.1	4.2	73.2	36.8	16.4
2. hemorrhage	-	-	16.1	5.3	-
3. edema	-	0.6	-	-	-
4. anemia	-	2.4	-	-	-
5. beak	-	-	-	-	-
6. eye	-	-	5.4	-	-
7. head	-	1.2	16.1	15.8	-
8. trunk	1.5	1.2	12.5	21.1	-
9. limb	-	0.6	-	-	-
10. viscera	-	-	-	-	-
11. entire embryo	-	0.6	7.1	28.1	-
12. dead in shell/pipped	16.7	13.5	10.7	4.2	10.9
13. malposition	-	1.8	1.8	24.6	3.6
14. hatched/abnormal	3.0	-	-	-	-
15. hatched/leg defects	-	-	-	1.8	-
16. balance problems	-	-	-	3.5	-

Gravitational loading could interfere with these biological motive forces. Physical motive forces could develop from gravitational interaction with the density gradients which exist in the contents. The lack of any proportionate effect with incubation at 2 G tends to discount a physical basis for the observed gravitational effects. However, if gravitational loading (of the albumen-yolk-embryo system) is a factor, and a normal field is required for movement of the developing tissues, this early stage of embryogenesis will also be disrupted during incubation in a weightless environment.

Observations also were made on non-surviving embryos which indicated a very wide variety of abnormalities -- some of which were unique. A summary coding system developed for the analysis of the direct observations is included as Table 3. Abnormalities observed in non-surviving centrifuged embryos are summarized in Table 4. Several embryonic abnormalities appear to be enhanced in frequency by acceleration field -- some only appearing in the 4 G field:

Field Strength	Frequency (% of initial embryos)			
	1 G	2 G	3 G	4 G
Abnormalities noted:				
1. early death	5.0	4.2	13.6	36.8
2. hemorrhage	-	-	16.1	5.3
7. head defect	-	1.2	1.7	15.8
8. trunk defect	-	1.2	15.0	21.1
11. entire embryo defect	5.4	0.6	1.7	28.1
12. dead in shell/pipped	1.5	13.5	56.7	42.1
13. mal position	-	1.8	16.7	24.6
15. hatched/leg defects	-	-	1.7	1.8
16. hatched/balance problems	-	-	25.0	3.5

Early deaths (the failure of the blastoderm to form an embryo) and late deaths (the inability of completely formed embryos to complete the hatching process, although in some the shell may be pipped) are the kinds of embryonic death encountered in normal incubation. However, with incubation in a hyperdynamic environment, early and late death incidence increased, and proportionally to field strength. Abnormalities involving the entire embryo (incomplete development, delayed development, dwarfing, diminished/lack of pigment, etc.) also are encountered in normal incubation, but at 4 G their incidence was increased 5-fold.

General retardation of embryo growth is encountered in all fields, but it is relatively frequent only in the highest field. This phenomenon was the cause of some concern, since it was an indication of inadequate temperature control. However, later consideration indicated that it also could result from changes in gaseous diffusion, caused by selective changes in air density in the centrifuge [i.e., there would be no significant change in barometric pressure]. At 4 G air density would be 4-fold that of air at one atmosphere pressure and normal gravity. This would reduce the gaseous diffusion (for O_2 and CO_2) by half [since the diffusivity is inversely proportional to the square root of gas density]. This would impose no limitation on oxygen transfer, since the concentration difference (molar basis) between atmosphere and embryo would be increased 4-fold. However, it would impose a severe limitation upon CO_2 transport, and CO_2 would accumulate in the egg.

Other defects encountered only rarely in normal incubation (hemorrhage, head and trunk defects, etc.) were prominent only in the more intense fields -- 3-4 G. The hemorrhagic abnormalities were generally multiple and discrete.

Their nature is compatible with a mechanism of capillary fragility. There is an indication that frequency of this abnormality may be enhanced in fields as low as 2 G. Generally, hemorrhagic events result from defects in the hemostatic process or in the thrombocytes (in mammals, platelets). Abnormalities of the axial skeleton are not prominent in embryos incubated in fields less than 4 G. The "head only" and "elongated trunk" are ordinarily quite rare, seen in only one out of perhaps 1,000 dead embryos.

Abnormalities in chicks hatched after normal incubation also are encountered occasionally (usually designated as weak or crippled). However, an unusual balance problem (postural instability) was observed in some chicks produced at 3 and 4 G. This phenomenon was transient, disappearing during the first day, and it resembled the unusual behavior (somersaulting) seen in normally hatched chicks after a few days of centrifugation (15). Consequently, the balance problems may represent the result of exposure of chicks (or completed embryos) to acceleration, rather than a developmental abnormality.

PERIODIC CENTRIFUGATION:

Originally it was proposed to carry-out selective centrifugation, particularly over periods that appeared to be sensitive, on the basis of time of embryo death. Only a few of these were done, and the results are summarized in table 5.

The most acceleration sensitive period of embryogenesis appears to occur around 48 hours. Exposure of embryos to 4 G from 24-48 hours of incubation substantially increases early mortality -- and it also substantially increases late mortality (dead in shell/pips). So some acceleration injury incurred early in embryogenesis is not manifest until late in the process.

The transfer of eggs to a static incubator after 18 days (so the hatching period, 18-21 days, is done at 1 G) does not reduce mortality (dead in shell/pips), but it does reduce the frequency of malpositions. This reduction in malpositions at 1 G may reflect a lack of interference with the physiological process involved in orientation, rather than any influence upon the developmental process. This is consistent with the absence of balance problems in chicks hatched at 1 G. If this reduction in malposition represents a geo-orientation (which seems likely), then this mode of late death will probably be equally important in hatching eggs in weightlessness.

CONCLUSIONS:

1. What appeared at the outset to be a simple and discrete project developed into a much larger and more complex program. Many aspects of gravitational influence upon embryogenesis were neither anticipated nor apparent without some experience. However, progress was made and several gravitational effects upon embryogenesis were identified and evaluated.

2. Several alternate methods for developing a centrifuge-incubator were investigated and tested. Eventually we settled upon a 1° freedom device which was workable -- but not optimum. The technology of a centrifuge-incubator constitutes a separate problem -- particularly regarding the physical nature of the gaseous environment. For example, a suitable instrument for measuring gas density in a high-G environment is not currently available.

3. The complexity of the interaction of the embryonated-egg and the acceleration field was not anticipated. Until the last few days of incubation, the embryo is buoyant, and as such does not develop any net load during centrifugation. Consequently any gravitational influence upon the embryo will result from the presence of density gradients — and the differential interaction of body-parts with the field. Some measurements of embryo body and organs were made which indicated that the relationships were complex -- also these changed in the course of development. Developing a suitable description of the density, and other physical relationships in the incubating egg constitutes a separate, and essential project. This should include studies with several species (quail, pheasant, chicken, turkey, etc.) to identify scale relationships.

4. On the positive side, it was established that hyperdynamic fields affect embryogenesis in incubating eggs, and the degree of effect varies substantially at different levels of development. The most susceptible developmental period is that which occurs between oviposition and 4 days of incubation. This is the period of formation for the extra-embryonic membranes and the anlage for most organs. It would be desirable that any future studies of this sort be conducted with pedigreed eggs -- so that any inter-individuality would not become compounded with variation in field intensity. A high degree of individuality would indicate a genetic basis, and a likelihood of altering the response through selection.

Interference with embryogenesis may have a physical or a biological basis. Further understanding of the nature of the mechanism might be gained by: (a) repeating the study with eggs of different size -- quail, goose, etc.), since physical relationships should be scale related; and by (b) selecting a line with embryos tolerant to hyperdynamic environments. The data in table 2 indicates that this would be feasible. An inverse correlation of degree of acceleration effect with embryo size would support a physical basis -- whereas an improvement with selection would support a biological basis.

5. One part of the original proposal was a quantitative study (anatomic and chemical composition) of viable embryos at varying ages and acceleration fields. From the chemical studies of the embryo and remaining egg materials, it would be possible to evaluate the over-all metabolic function and the energetic efficiency of embryonic development. These studies, deferred until the completion of the gravitational influence on embryo abnormalites, were not undertaken.

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