ORIENTATION IN SYSTEMS WITH ASYMMETRIC DENSITY DISTRIBUTION

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ABSTRACT The hen's egg is a convenient and suitable model for biological systems in which mass is asymmetrically distributed. Under the influence of an acceleration field (such as provided by gravity on earth), such systems will become oriented, and this may have biological significance. However, depending upon the viscous and elastic properties of the system, some minimal force, i.e. a threshold, will be necessary for movement in the system. This threshold, and the restraining properties of the yolk-albumen boundary, have been evaluated for the hen's egg and are reported herein.

INTRODUCTION

Recently, the effect of accelerative forces upon biological systems has attracted considerable attention. Studies of short term exposures have been made, largely for their interest to aviation medicine (reviewed recently by Gauer and Zuidema, 1961). Exposures of much greater duration, to the extent of being considered as "chronic acceleration," have also been studied partly as an aid in understanding the effects of gravity and also regarding prolonged exposure to other accelerative force fields, anticipated in bioastronautics (Matthews, 1953; Oyama and Platt, 1963; Smith and Kelly, 1963; Wunder, 1962). Generally, the effects observed with prolonged treatment are considered to result from changes in the weight-to-mass (W:M) ratio and also to have a continuous relationship with the ambient accelerative force. Since these studies have been made only at greater gravity than earth gravity, the continuity into subgravity conditions is assumed. However, in the one case where such observations (short term) have been made from weightlessness to 3 *G*, (Roman et al., 1962) no discontinuities were observed.

In some systems, specifically those which are non-rigid and with a non-uniform distribution of mass, the effects of changing the W:M ratio may have a discontinuous relationship with the ambient accelerative force. Such systems may require a minimum acceleration field for orientation and at lesser intensities the positioning would be random and unpredictable. Thus a "threshold" would exist for acceleration effects depending, quantitatively, upon the degree of asymmetry of mass and the relative properties of viscous and elastic components. Several biological structures share these properties including the sensory receptors that respond to accelerative forces (e.g. gravity). However, the most convenient model for such systems is the avian egg, which is geometrically simple, abundantly available, and readily subject to a variety of experimental procedures.

In the avian egg (Fig. 1) a lower density yolk floats in albumen and is loosely restrained by the chalazas. The yolk also has a density gradient, the "light pole" (T in Fig. 2) being located in the region of the blastoderm (germinal tissue). Consequently, in an acceleration field, there will be a gross movement of the yolk (a_2 in Fig. 1) as well as a rotation (a_1 in Fig. 1). Both of these motions will be restricted by the chalazas as well as the shear stresses on the yolk surface, and enhanced by differences in specific gravities of yolk and albumen, and by the distribution of yolk mass. The blastoderm (germinal cells) does not necessarily coincide with the "light pole," T in Fig. 2, however it generally lies within a cone that is 30° ± 1.95°[1] from the vertical axis at normal gravity (Besch, 1964). If the "light pole," T, is deflected from its vertical position, it will always tent to return to that position if a large enough acceleration field exists. This effect is motivated by a stabilizing movement of the asymmetric density of the yolk.

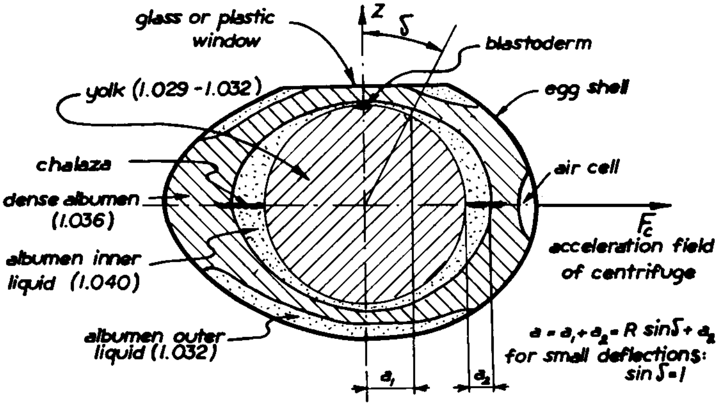

FIGURE 1 Schematic diagram of an egg prepared for acceleration studies. Specific gravities of various components are indicated in parentheses. For further information on the composition and structure of the avian egg refer to the book by Romanoff and Romanoff (1949).

Deflecting T in plane of the Y, Z axes (Fig. 2) by an angle, δ, a stabilizing moment will develop around the X axis, which can be calculated, if the density distribution $\rho\ (x, y, z)$ is known.

In equilibrium (where: $\delta = 0$) the stabilizing moment is zero. In this position a

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[1] Arithmetic mean and its standard error.

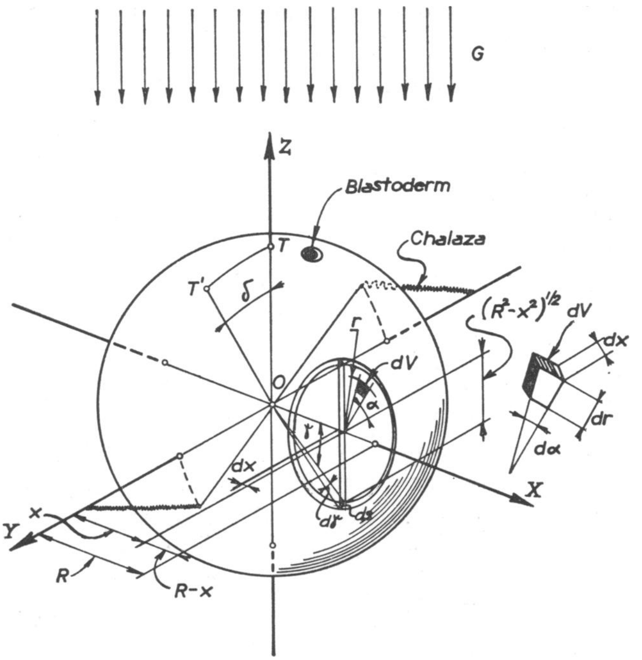

Figure 2 Schema of the forces acting upon the yolk. T is the "light pole" and T' is its location after being displaced through the angle δ by an acceleration field parallel to the Y axis. R is the yoke radius, V is a small volume which is in an angle α from the plane of X, Y axis.

thin (dx) disc of yolk parallel with the plane of the Z, Y axes, and x distance from the center, contains a small volume (Fig. 2):

$$dV = r \cdot d\alpha \cdot dr \cdot dx \quad [\mathrm{cm}^3]$$

The weight of this element of volume, dV, in a G[2] acceleration field is:

$$dW = G\rho(\alpha, r, x) r \cdot d\alpha \cdot dr \cdot dx \quad [\mathrm{gm}]$$

The moment of the element around X axis becomes:

$$dM_\rho^X = r \cos\alpha \cdot dW = G\rho(\alpha, r, x) r^2 \cos\alpha \cdot d\alpha \cdot dr \cdot dx \quad [\mathrm{dyne} \cdot \mathrm{cm}]$$

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[2] Notations of accelerative forces can be in absolute units (e.g. dyne) or relative terms (e.g. multiples of the earth's gravitation). Gravitational acceleration is conventionally designated g.

$$F_{(\mathrm{absoltue})} = m \cdot a$$

$$F_{(\mathrm{relative})} = m \cdot \frac{a}{g} = m \cdot G$$

Since G is a ratio of accelerative forces it becomes dimensionless. Detailed discussions of units and terminology regarding accelerative forces are available (*Nat. Acad. Sc.—Nat. Research Council, Publication,* 913, 1961; Dixon and Patterson, 1953).

Integrating all such elements for the entire yolk, (of radius, R) the stabilizing moment resulting from this asymmetric density distribution (which must be zero in an equilibrium situation) can be obtained:

$$M_\rho^X = \int_0^{\alpha=2\pi} \int_0^{r=(R^2-x^2)^{1/2}} \int_0^{x=R} G\rho(\alpha, r, x)r^2 \cos\alpha \, d\alpha, \, dr, \, dx = 0 \qquad (1)$$

Deflecting the yolk by an angle, δ, in the plane of the Y, Z axes the stabilizing moment would become:

$$M_\rho^X = \int_0^{\alpha=2\pi} \int_0^{r=(R^2-x^2)^{1/2}} \int_0^{x=R} G\rho(\alpha, r, x)r^2 \cos(\alpha + \delta) \, d\alpha, \, dr, \, dx \ [\text{dyne}\cdot\text{cm}] \qquad (2)$$

Another stabilizing effect, the chalazas[3] holding force, can be taken into consideration when the deflection is in the Y, Z axes plane. Besch, (1964) found that when the acceleration field is parallel with Y axis (instead of Z axis, as shown in Fig. 2) the blastoderm lies within a cone 26.8° ± 1.3°[1] from the Y axis. However, when the isolated yolk (disconnected from the chalazas) is placed in glycerine-saline, the blastoderm lies within a cone 9.0° ± 1.4° from vertical (i.e. from the Y axis if this is the direction of the acceleration field). This difference in orientation results from the restraining influence of the chalazas. In an idealized system the restraining moment of the chalazas would be:

$$M_{ch}^X = 2RF(\delta) \cos\left\{\left[\text{arc } tg \frac{l + R - R\cos\delta}{R\sin\delta}\right] - \delta\right\} \qquad (3)$$

Where: $F(\delta)$ is the chalazas restraining force; l is the chalazas length; δ is the chalazas strain.

$$\left|\delta = \frac{[(l + R - R\cos\delta)^2 + (R\sin\delta)^2]^{1/2} - l}{l}\right|$$

However, the chalazas are very loose and at small deflections of the yolk can be neglected; moreover, rotation of the yolk around the Y axis does not involve any resistance to movement by the chalazas.

A third consideration, regarding orientation of the yolk in an acceleration field, is the resistance to movement resulting from shear stress between the yolk and the surrounding medium. The moment resulting from these stresses around the X axis can be described as:

$$M_F^X = \tau 2\pi R^3 \int_0^\pi \sin^2\gamma \, d\gamma \qquad (4)$$

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[3] The chalazas are fibrous, albumen elements firmly attached over the surface of the yolk in its equatorial region. They form a fibrous capsule, inseparable from and imbedded in a layer of dense albumen surrounding the yolk membrane. This capsule, together with the albumen envelope make up the chalaziferous layer of egg albumen (about 2.7% the total volume of albumen). Their function is to stabilize the position of the yolk near the geometric center of the egg (Romanoff and Romanoff, 1949).

Where: $2\pi r \, ds = 2\pi R^2 \sin\gamma \, d\gamma$ is the surface element; $2\pi R^3 \sin^2\gamma \, d\gamma$ is the moment of the surface element; and τ is the restraining fluid shear stress between the yolk and the surrounding medium. Adding all the moments together the final stabilizing moment can be defined:

$$M_s^X = M_\rho^X + M_{\text{ch}}^X - M_F^X \quad [\text{dyne}\cdot\text{cm}] \tag{5}$$

With small deflections of the yolk, δ, $M_{\text{ch}}{}^X$ can be neglected, so in such cases, the stabilizing moment becomes:

$$M_s^X = M_\rho^X - M_F^X = \iiint G\rho(\alpha, r, x)r^2 \cos\alpha \, d\alpha, dr, dx - \tau 2\pi R^3 \int \sin^2\gamma \, d\gamma \quad [\text{dyne}\cdot\text{cm}] \tag{6}$$

The stabilizing moment, M_s^X, is dependent upon the position, δ, and the acceleration field, G; but the fluid shear stress moment, M_F^X, is independent of these two parameters. Consequently, some minimum acceleration field, G_{min}, will be required for a deflection, δ, in order for the stabilizing moment, M_s^X, to equal or exceed the fluid shear stress moment, M_F^X, and reorient the yolk. This minimum acceleration can be calculated, assuming $M_s^X = 0$:

$$G_{\text{min}} = \frac{2\pi\tau R^3 \int \sin^2\gamma \, d\gamma}{\iiint \rho(\alpha, r, x)r^2 \cos(\alpha + \delta) \, d\alpha, dr, dx} \quad [G] \tag{7}$$

If the acceleration field is angular, as generated by a centrifuge:

$$G = 1.118 \times 10^{-5} n^2 R_c$$

the minimum rotation rate (RPM) to provide G_{min} is:

$$n_{\text{min}} = \left[\frac{2\pi\tau R^3 \int \sin^2\gamma \, d\gamma}{1.118 \times 10^{-5} R_c \iiint \rho(\alpha, r, x)r^2 \cos(\alpha + \delta) \, d\alpha, dr, dx} \right]^{1/2} \quad [\text{RPM}] \tag{8}$$

Where n is the minimum requisite rotation rate (RPM); R_c is the length of the centrifuge arms (cm); and δ is the displacement of the yolk.

Both G_{min} and n_{min} can be determined experimentally.

METHOD

Eggs for these experiments were laid by single comb white leghorn (SCWL) chickens under optimum husbandry conditions. The hens were injected with Evans Blue dye (T-1824) which become deposited upon the yolk surface—except in the region of the blastoderm—increasing visual contrast. This treatment did not otherwise affect the yolk. After collection they were stored at 50 to 55°F until usage—usually within 48 hr. A hole, 7/8 in in diameter was cut in the side of each egg and covered with transparent material (glass cover slip or polyethylene film, Fig. 3) to permit visual observation. It

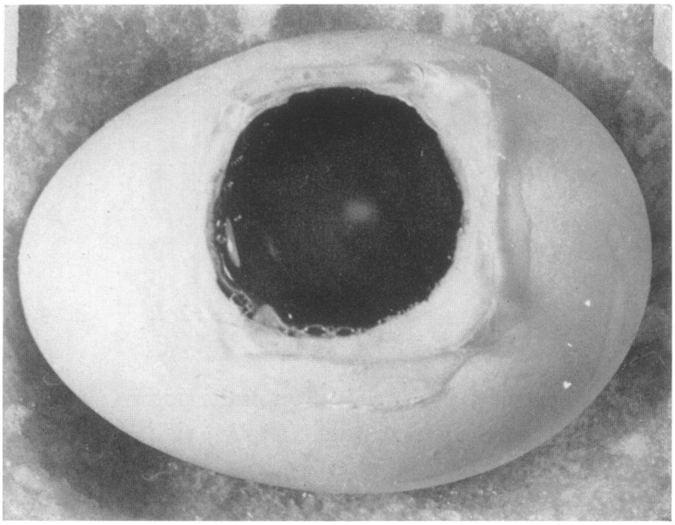


FIGURE 3 Egg with the observation window. The blastoderm can be seen as a pale region on the surface of the dyed yoke.

was necessary that all the air space be eliminated from the egg, otherwise the meniscus would become displaced with acceleration, producing a third and undesirable form of yolk movement. In practice, the air was displaced with oil, which prevented the yolk from contacting the window insuring all moving boundaries were "liquid-to-liquid."

Prepared eggs were mounted in depressions on a polystryene foam, "Styrofoam," panel (Fig. 4) which restrained them, with "no degrees of freedom" during centrifugation. The panels were then mounted on a centrifuge (Kelly et al., 1960) (Fig. 5), with provision for lighting and photographic recording of yolk displacement. With this arrangement, the centrifugal field was perpendicular to the field of gravity. However, both fields affected orientation of the egg.

During centrifugation, where the yolk becomes displaced from the position at normal gravity (Fig. 6), the moment around the center, 0, is:

$$\Sigma M_0 = (R^2 - a^2)^{1/2} N_{F_c} - aN_g$$

The resolution of the two fields, centrifugal and gravitational, can be considered as a field, F, parallel to the centrifugal field, the moment for this resultant field would be:

$$\Sigma M_0 = (R^2 - a^2)^{1/2} N_F$$

Adding the two equations together,

$$(R^2 - a^2)^{1/2} N_{F_c} - aN_g = (R^2 - a^2)^{1/2} N_F$$

and expressing the forces with mass (m) and acceleration (F_c, g, F),

$$(R^2 - a^2)^{1/2} mF_c - amg = (R^2 - a^2)^{1/2} mF$$

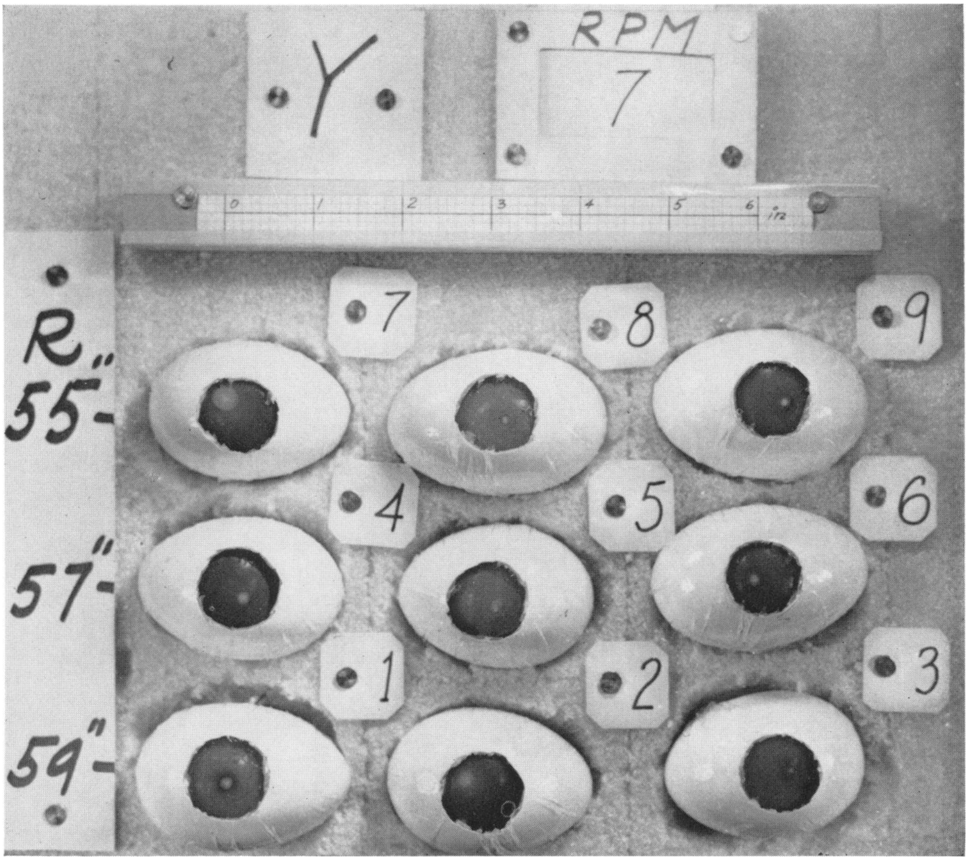


FIGURE 4 Panel for holding eggs during centrifugation.

the resultant field becomes:

$$F = F_c - \frac{ga}{(R^2 - a^2)^{1/2}} = F_c - \Delta F \quad [G] \tag{9}$$

ΔF can be called the "field correction" which reduces the angular acceleration field by the opposing gravity effect. At small yolk deflections (up to 0.5mm) this correction can be considered as linear (Fig. 6).

At normal gravity the stabilizing force of the yolk also was measured by a different method. For this, yolks were separated from the egg and placed in a glycerine-saline solution which had the approximate specific gravity of the inner thin albumen (i.e. 1.040; Romanoff and Romanoff, 1949). Small iron weights were placed at the pole of least density (i.e. the point contacting the surface). When such weights equalled the stabilizing force, the yolk behaved as if the mass distribution were symmetrical, and it would retain any position into which it was placed (i.e. indicated by the angle of the blastoderm, δ). The balanced stabilizing moments have the relationship:

$$M_s = BR \sin \delta$$

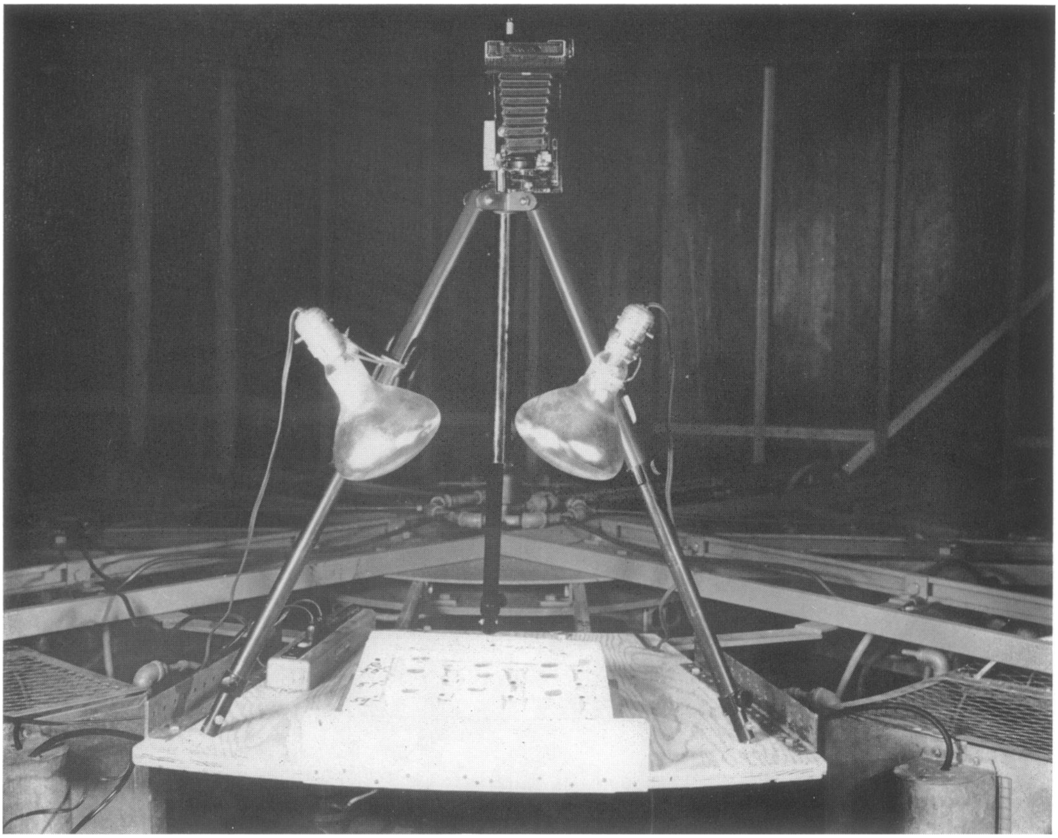


FIGURE 5 Centrifuge prepared for acceleration studies with eggs.

where B is the balancing weight and R is the radius of the yolk.

This new expression of the stabilizing moment can be substituted into equation (6):

$$M_s = BR \sin \delta = \iiint G\rho(\alpha, r, x)r^2 \cos (\alpha + \delta)\, d\alpha, dr, dx - \tau 2\pi R^3 \int \sin^2 \gamma\, d\gamma \quad [\text{dyne} \cdot \text{cm}] \qquad (10)$$

From which, the fluid shear stress, τ, can be obtained:

$$\tau = \frac{\iiint \rho(\alpha, r, x)r^2 \cos (\alpha + \delta)\, d\alpha, dr, dx - BR \sin \delta}{2\pi R^3 \int \sin^2 \gamma\, d\gamma} \quad [\text{dyne/cm}^2] \qquad (11)$$

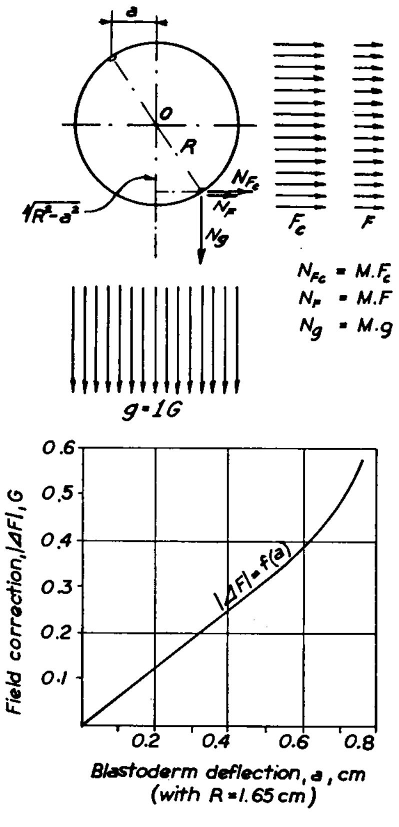
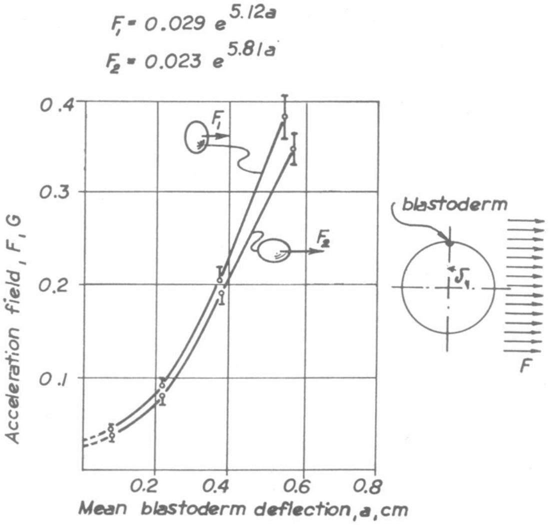


FIGURE 6 Calculation of the orienting force on egg yolks during centrifugation. In certain yolk positions gravity counteracts the effect of the centrifugal field. This effect is proportional to the degree of yolk displacement. A correction factor, Δ F, is calculated and used as a negative correction factor on the centrifugal field to calculate the orienting field.

RESULTS

A series of 99 eggs from 12 hens were examined at centrifugal forces of 0.08 to 0.76 G (corresponding to 7 to 22 RPM at radii of 132 to 150 cm). Movement of the yolk was determined photographically, using the displacement of the blastoderm as an index. In this way both the displacement and rotation of the yolk (a_2 and a_1 in Fig. 1) would be combined. With a greater exposure of the yolk, so that its perimeter would be visible, the two quantities could have been separated. However, such treatment would involve a considerable modification of the albumen structure which was considered undesirable.

Yolk movement was determined for each egg in two positions; with the major axis parallel to the centrifugal force, and also perpendicular to it. In these two positions, the restraining influence of the chalazas, if a factor, would be quite different (see Fig. 1). The yolk deflection in each position is presented as a function of the orienting force, in Fig. 7. The curves are exponential and quite similar, the principal difference being a greater mobility when the major axis of the egg is



F_1	G	0.0413	0.0897	0.2030	0.3810
a	cm	0.074	0.213	0.376	0.551
±SE	cm	0.005	0.010	0.015	0.023
±SD	cm	0.036	0.089	0.135	0.188
F_2	G	0.036	0.080	0.190	0.348
a	cm	0.079	0.216	0.363	0.561
±SE	cm	0.002	0.008	0.010	0.018
±SD	cm	0.028	0.068	0.084	0.150

FIGURE 7 Deflection of yolk as a function of the orienting field.

parallel to the centrifugal field (i.e. when the movement is in the plane of the chalazas):

$$F_1 = 0.029\ e^{5.12a}$$

$$F_2 = 0.023\ e^{5.81a}$$

Where F is the displacing acceleration field in G; F_1 with the major axis of the egg is perpendicular to the centrifugal field, and F_2 parallel to it; a is the displacement in cm.

The intercept values, 0.029G and 0.023G represent the minimum field for deflection; i.e., they are the "threshold" for orientation effects. Fields of lesser intensity will not move the yolk because of the restraining fluid shear stresses.

The proportionality coefficients, 5.12 and 5.81, relating field strength to yolk displacement, have an inverse relationship to the threshhold. Thus, with the egg perpendicular to the field (position F_1), a greater threshold is involved, and above this intensity there is lesser yolk displacement with a unit increase in field strength.

Consequently, similar factors would appear to regulate both the threshold, and also subsequent movement of the yolk.

When the yolk mobility is examined for individual hens (Table I, mean for both positions) rather marked differences are evident. Orientation thresholds vary from 0.0175 to 0.032G, and deflection constants from 3.99 through 8.02. The orientation threshold does not appear to be correlated with either yolk or egg size (correlation coefficients, r, are 0.02 and 0.06 respectively). However, there is a significant and inverse relationship between the orientation threshold and the displacement coefficient ($r = -0.64$, significant at the 5% level).

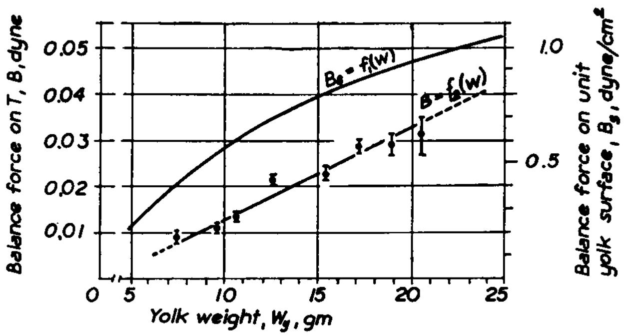
The relationships between the stabilizing force, B, (as measured by "weighting" the yolk with iron-wire fragments), and size of yolks (Wy, yolk weight) also was investigated (Fig. 8). The stabilizing balance force increases linearly with the weight of the yolk:

$$B = (2.03\,Wy - 7.8) \times 10^{-3} \quad \text{[dyne]} \tag{12}$$

The stabilizing balance force can also be related to yolk surface, B_s, and this quantity also increases with yolk size (which may relate to differences in lipid content):

$$B_s = \frac{2.03\,Wy - 7.8}{4\pi\left(\dfrac{3\,Wy}{4\pi\rho_{\text{avr}}}\right)^{2/3}} \quad [\text{dyne/cm}^2] \tag{13}$$

Where ρavr. = yolk average density.



B	dyne	0.0090	0.0108	0.0143	0.0213	0.0222	0.0288	0.0292	0.0308
W_y	gm	7.6	9.6	10.7	12.5	15.3	17.2	18.8	20.4
±SE	dyne	0.00136	0.00065	0.00068	0.00082	0.00133	0.00169	0.0023	0.00408
±SD	dyne	0.00236	0.00292	0.00447	0.00141	0.00548	0.00794	0.01012	0.01000

FIGURE 8 Yolk size and stabilizing moment. The degree of weighting (B, gm) of the light pole (T in Fig. 2) necessary to compensate the yolk stabilizing moment is compared with yolk size—both directly /$B = f(W)$ / and per unit surface /$B_s = f(W)$ /. (The values of B_s are calculated from B.)

From the results of centrifugation and weighting studies, the restraining fluid shear stress can be obtained. The centrifuge method indicated a minimum field, $G_{\min}$, of 0.023 at $\delta = 90°$ to orient the yolk (average of 99 eggs, with mean yolk weight of 18.90 gm, and mean radius, 1.65 cm). The weighting indicated a mean balance force, B, of 0.03 gm for yolks of similar size. When these data are applied to equations (6) and (10):

$$0 = 0.023 \iiint \rho(\alpha, r, x)r^2 \cos(\alpha + 90°)\, d\alpha, dr, dx - \tau 2\pi \times 1.65^3 \int_0^{\pi} \sin^2 \gamma\, d\gamma$$

$$0.03 \times 1.65 = 1 \iiint \rho(\alpha, r, x)r^2 \cos(\alpha + 90°)\, d\alpha, dr, dx - \tau 2\pi \times 1.65^3 \int_0^{\pi} \sin^2 \gamma\, d\gamma$$

And solving the above equations, the restraining fluid shear stress and the moment of the asymmetric density distribution can be expressed:

$$\tau = \frac{0.03 \times 0.023 \times 1.65}{(1 - 0.023) \times 37.6} = \underline{\underline{2.6 \times 10^{-5}}} \quad [\text{dyne/cm}^2]$$

$$\frac{M_\rho^X}{G} = \iiint \rho(\alpha, r, x)r^2 \cos(\alpha + 90°)\, d\alpha, dr, dx = \underline{\underline{0.05}} \quad [\text{dyne} \cdot \text{cm}]$$

The stabilizing moment at higher acceleration field, G, for an average egg:

$$M_\rho^X = 0.05G \quad [\text{dyne} \cdot \text{cm}]$$

DISCUSSION

The hen's egg appears to be a good model for biological systems in which density gradients may have a functional importance. Other such systems (e.g. sensory organs responding to accelerative forces) are less accessible, more complex in structure, and less amenable to manipulation. However, similar behavior (i.e. threshold effects) have been noted for the acceleration sensing organs. Walsh (1957) in reviewing the physiology of the labyrinthine organs cites several estimates of threshold stimulii, and these approximate 0.012 G for the otolith, which is of the same order as the forces required to displace the hen's egg yolk.

The results of our studies, concerning orientation of the yolk, also have important implications for experiments in which bird eggs would be incubated in weightlessness (e.g. in an orbiting satellite). Chicken embryos assume a characteristic position within the egg prior to hatching in which the beak is located underneath the right wing, and at the air cell end of the egg (Landauer, 1961). Under certain circumstances these embryos may assume a "malposition" which is different from this "normal position" and as a result there is a decrease in hatching (Sanctuary, 1924 to 1925). Several investigators (Taylor, 1932; Cavers and Hutt, 1934; Byerly and Olsen, 1936) have shown that these malpositions are related to the orientation of the early embryo and to the position of the egg during incubation. Byerly and Olsen (1936) have also found that the increase in malposition II (head in small end of egg) can be caused by incubating eggs with small end up or by covering large (air cell) end with paraffin and incubating with large end up. They concluded that the occurrence of this malposition is influenced by gravity but that hypoxia is the regulating factor.

Consequently, in a weightless situation, it would be very important to orient the blastoderm towards the air cell, to prevent the confounding of malpositioning and weightlessness phenomena. This could be done simply by using a cylindrical incubator with the "blunt" ends (containing the air cell) pointed inwards. A brief spinning of this device would suitably orient the yolks of most of the eggs. However, in those in which the blastoderm was located 180° from the air cell, a very great force would be needed (see Fig. 9). This condition could be avoided if the egg holder was arranged so that the major axis of the egg would be initially oriented 30° from the acceleration field, and during the spinning gradually became coincident with the field—then all eggs would become suitably positioned.

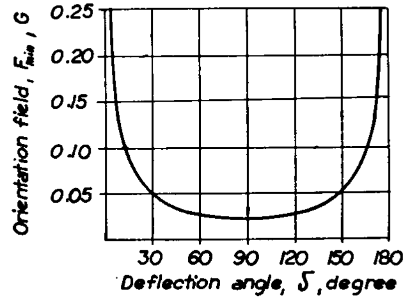


FIGURE 9 Relationship between blastoderm position and acceleration field necessary to orient blastoderm towards air cell.

TABLE I

DATA OF INDIVIDUAL BIRDS

Hen no.	Acceleration vs. deflection	Mean egg weight	Mean yolk weight	Yolk of egg
	$F = G_0\, e^{ka}[G]$	W_e[gm]	W_y[gm]	%
225	$F = 0.0175\, e^{6.81a}$	57.72	19.14	33.16
228	$F = 0.0197\, e^{8.02a}$	53.25	17.87	33.56
231	$F = 0.0205\, e^{5.43a}$	61.02	19.01	31.15
213	$F = 0.023\, e^{7.68a}$	55.27	18.18	32.89
267	$F = 0.027\, e^{3.99a}$	60.87	20.10	33.02
268	$F = 0.028\, e^{4.98a}$	58.97	18.86	31.89
226	$F = 0.028\, e^{4.62a}$	63.96	20.68	32.33
232	$F = 0.031\, e^{5.40a}$	56.80	18.61	32.76
227	$F = 0.032\, e^{3.99a}$	62.80	19.36	30.82
269	$F = 0.032\, e^{5.93a}$	49.49	17.96	36.29

Relationships between acceleration threshold and other egg parameters: $G_0 = 0.0225 + 0.000616W_e$; $G_0 = 0.0100 + 0.000838W_y$; $G_0 = 0.0373 - 0.001976k$.

Also it should be noted that the density gradient of the yolk and the viscous properties of the system vary between eggs from particular hens (Table I). In the event that an orienting system were to be applied to hen's eggs, they should be selected so as to be suitable to the acceleration field developed.

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