THE INFLUENCE OF CLINOSTAT ROTATION ON THE FERTILIZED AMPHIBIAN EGG

JOHN W. TREMOR and KENNETH A. SOUZA
Ames Research Center, NASA, Moffett Field, Calif. 94035, U.S.A.

(Received 11 August, 1971)

Abstract. Unrestrained, fertilized eggs of *Rana pipiens* and *Xenopus laevis* were rotated in a plane parallel to the normal gravity vector. In *R. pipiens* rotation at 1/4 rpm for 5 days at 18°C produced a significantly increased number of commonly occurring abnormalities. Rotation at 1/15, 1/8, 1, 2, 5 and 10 rpm did not significantly affect normal development. *X. laevis* eggs reacted similarly. *R. pipiens* eggs were most sensitive to rotation at 1/4 rpm when exposure was initiated before first cleavage. Mixing of intracellular constituents apparently occurred only at 1/4 rpm in *R. pipiens* (of the clinostat speeds studied), and may have been the cause of the increased abnormality observed at this rate.

1. Introduction

The advent of space science has reawakened an interest in the biological effects of gravity, both from the standpoint of weightlessness as a natural consequence of space flight and as an available research tool. Studies of geotropisms have contributed extensively to a knowledge of plant physiology, and basic cell biology has benefited from the use of the centrifuge. Comparatively few studies, however, have been concerned with basic animal processes as affected by the absence of the orienting effect of gravity.

The clinostat, by slowly rotating a tropistically reacting plant in a horizontal plane, systematically and continuously disorients the organism in respect to gravity. Plant workers have termed this process 'gravity compensation'. The effects of such rotation are apparently similar if not identical to effects generated by the weightless condition when imposed upon a plant (Lyon, 1968; Johnson and Tibbitts, 1968). These orbital flight experiment results support the proposal (Thimann, 1968) that, recognizing the limitations imposed by slow horizontal rotation on the simulation of weightlessness (Brown, 1968), the clinostat and gravity compensation can be used effectively in studying a possible influence of weightlessness on at least some developmental mechanisms in both animal and plant material.

The frog egg is sensitive to the force of gravity. In normal development, after fertilization and the lifting of the vitelline membrane, the heavy yolk end descends, orienting the egg in a vertical animal-vegetal polarity. Disturbances of this polarity by certain altered gravity conditions (e.g. inversion, centrifugation) have been shown to affect normal development (see Discussion). On the other hand, clinostat rotation (Roux, 1884), slow rotation by a water current (Kathariner, 1901; Morgan, 1902) and orbital weightlessness (Young and Tremor, 1968a, b) have been reported as having no influence on the development of the unrestrained frog egg. The early studies,

Space Life Sciences **3** (1972) 179–191.

however, appear not to have been critically performed with respect to appropriate periods of rotation and to a statistical analysis of results. Also, in regard to the effect of weightlessness, the stage of development at which the embryos were exposed, after first cleavage, may have been relatively insensitive to this environment. Therefore, in order to better define a possible effect of gravity compensation in a developing vertebrate system a series of clinostat experiments was conducted with the fertilized egg of *Rana pipiens* and, in one series of experiments, the toad *Xenopus laevis*.

2. Materials and Methods

The use and theory of the clinostat have been recently reviewed (Larsen, 1962; Werber, 1969). The instruments used here were of a simple design (Figure 1), the single axis of rotation driven by an interchangeable synchronous motor. Clinostat speeds of 1/15,

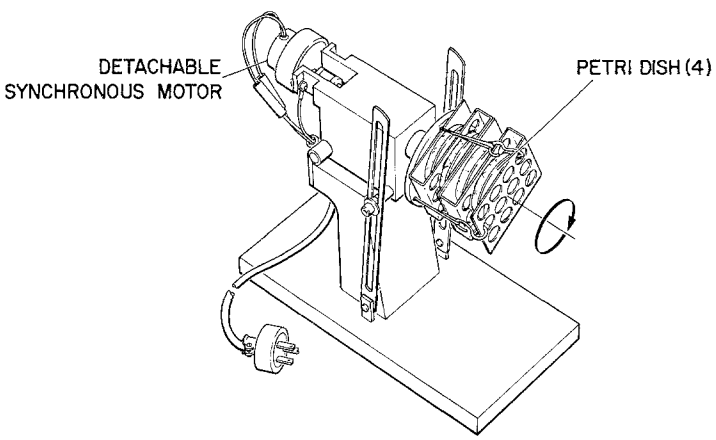

Fig. 1. Clinostat with egg-containing petri plates and synchronous motor attached.

1/8, 1/4, 1, 2, 5, and 10 revolutions per minute (rpm) were utilized in initial experiments to test the effect of different rotational speeds on normal development. They were selected to include the range of 0.5 to 2 rpm, the rate at which the inverted egg, over the pre-first cleavage period, returns to a normal position. To provide rotation in a plane parallel to the normal gravity vector clinostats were fixed in the horizontal position. The rotational axis could be adjusted vertically to provide a control condition. As many as four 30-ml capacity plastic petri dishes could be affixed by rubber bands to the rotating plate, each dish containing eggs and spring water covered by parafilm and capped by the plastic lid.

Immediately upon receipt from the supplier, frogs (as many as 200) were stored until use in 6 × 2-ft stainless steel tanks containing 5 gal of 5 °C spring water, changed daily. Ovulation was induced by an intraperitoneal injection of anterior lobes of frog pituitary glands (2–5, depending on the season) according to the method of Rugh (1962).

The sperm suspension used consisted of macerated testes at a concentration of 1 pair per 5 ml of commercial spring water. Female donors were selected on the basis of a pre-experiment fertilization test of 4 to 8 frogs. The frog yielding the highest percent of fertilization was selected for an experiment.

After stripping groups of eggs onto the center of a dry petri dish, all eggs in excess of 10 were selectively removed. Eggs were immediately exposed to the sperm solution, the sperm remaining for $1\frac{1}{2}$ to 3 min before being rinsed off with spring water. This allowed a very early exposure (within 5 min) to clinostat rotation. The eggs used in any one experiment were donated by a single frog. Upon addition of the sperm solution, the jelly capsules, swollen by water uptake, adhered tenaciously to the bottom of the dish, independent of dish position. The enclosed eggs were free to rotate within the vitelline membranes. Plates were affixed to the clinostats so that no egg was more than 7 mm from the axis of rotation.

All operations and subsequent clinostating were carried out at 18 °C in a walk-in cold room subject to variable ambient lighting conditions. Equal numbers of unclinostated control plates were prepared. Eggs were observed, after hatching in Shumway stage 20–21, at 5 days of development under the experimental conditions and, for analytical purposes, a dish of 10 eggs was considered a sample unit.

When *X. laevis* eggs were used, the above conditions were, in general, applicable. Amplexus and fertilization were induced by injection of both male and females with 350–650 IUs of chorionic gonadotropin into the dorsal lymph sac on the day preceding use. Within 15 to 20 min after fertilization by the male, the eggs extruded in amplexus by the female were placed on the clinostat. Hatching occurred after 3 days incubation at 24 °C.

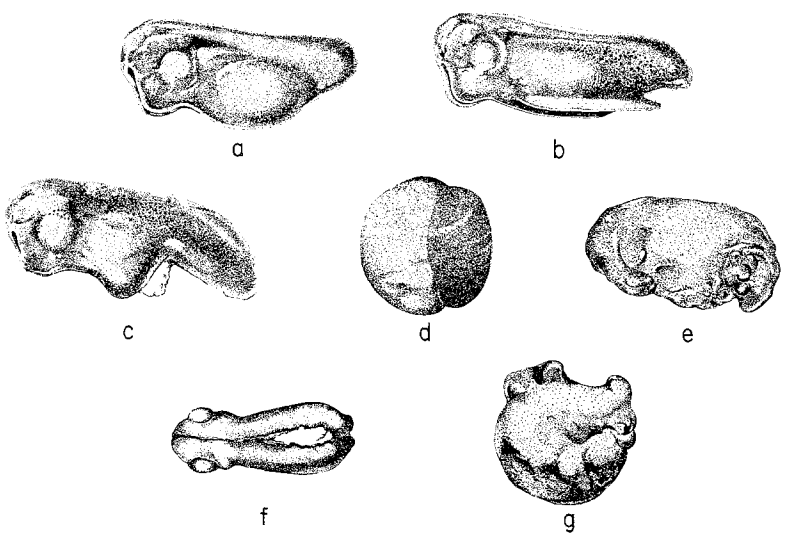

Fig. 2. Types of abnormalities scored. See text for definitions. (a) Normal; (b) Projection; (c) Incomplete invagination of yolk; (d) Exogastrula; (e) Caudal and ectodermal abnormalities; (f) Spina bifida; (g) Microcephaly and ventral bulge.

Three principal categories of development were recorded: normal, abnormal, and dead. The latter category comprised the unfertilized eggs as well at those dying before neural plate formation since it was most difficult to distinguish between these at the time observed.

The abnormal embryos were further classified into types and severity of anomalies: mild, moderate, and severe. Definitions follow and are illustrated in Figure 2: microcephaly (m) – reduced head development ranging from incomplete mouth formation to complete absence of cephalic differentiation; projections (p) – projection from various parts of the body; ventral bulge (v.b.) – edematous condition causing embryo to appear bloated, abdomen extended; ectodermal abnormality (e.a.) – wrinkling and convolutions of the ectodermal surface usually in the abdominal region; spina bifida (s.b.) – incomplete closure of the neural folds, resulting in a split spine and in many cases twin tails; incomplete invaginations of yolk (i.i.) – failure of the yolk plug to close, resulting in varying degrees of yolk exposed at the anus; exogastrula (ex) – extremely abnormal gastrulation, dorsal lip of blastopore folding outward toward animal pole leaving yolk exposed (the difference between an exogastrula and a severe i.i. is very subjective due to the difficulty in scoring a yolk-filled disintegrating mass; these latter cases are usually termed severe i.i., especially if any sign of neural or cephalic differentiation is discerned); caudal abnormality (c.a.) – abnormal tail development usually bent dorsoventrally, and often related to i.i.; stunting (st.) – reduced growth; other (o) – all other abnormalities, no one of which comprising more than 1% of the total.

3. Results

A. EFFECT OF RATE OF ROTATION

Initial experiments were conducted with *R. pipiens* to determine the rate of horizontal rotation that might be most effective in influencing development. Synchronous motors rated at 1/15, 1/8, 1/4, 1, 2, 5, and 10 rpm were attached to the clinostats and 6 experiments run. The female donors provided 360 eggs apportioned by dish to each of the 7 rates of rotation and the static control. Figure 3 represents the results in terms of percent normality. The one clinostat speed yielding a significant ($p<0.01$) decrease in normality was 1/4 rpm. Consequently, in all subsequent experimentation with *R. pipiens*, clinostats were rotated at this rate. The low normality (48%) observed in the control group resulted from poor fertilization, and not abnormal development. Subsequent experiments in which the controls averaged 65–75% normal development also exhibited significant decreases in normality at 1/4 rpm. This rate, at a maximum radius of 7 mm, effected an acceleration of approximately 10^{-6} g in the plane of rotation.

Eggs of *X. laevis* reacted similarly to *R. pipiens* eggs when exposed to varying rates of horizontal rotation. Over the course of 5 experiments, a sample of about 67 dishes of eggs was exposed concurrently to the rotational speeds of 1/4, 1, 2, 5, and 10 rpm while the static control was represented by 128 dishes. In increased order of clinostat velocity, observation of hatching revealed normality rates of 76.8%, 75.1%, 76.2%,

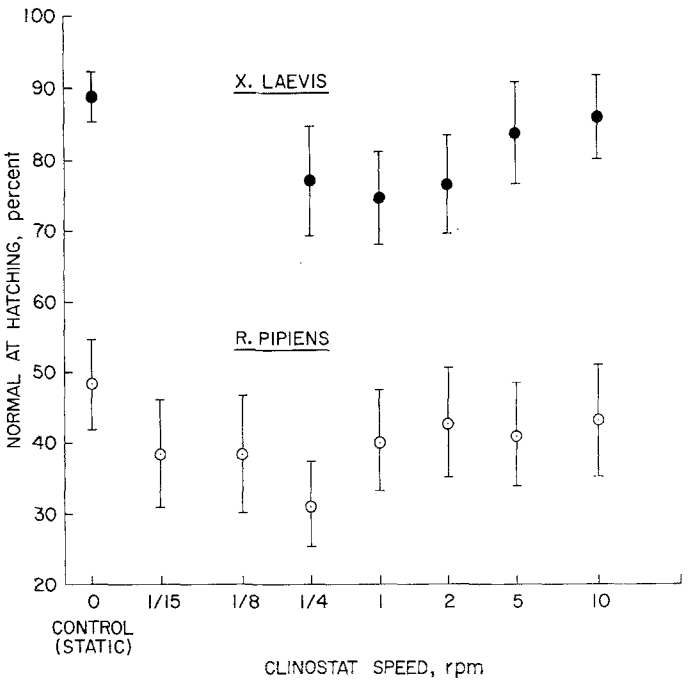

Fig. 3. Normality of embryos of *R. pipiens* and *X. laevis* when exposed to horizontal clinostat rotation at various speeds (from fertilization to hatching); two standard errors plotted on each side of mean percent ($n = 36$ dishes/treatment for *R. pipiens*, $n =$ ca. 67 dishes/treatment for *X. laevis*).

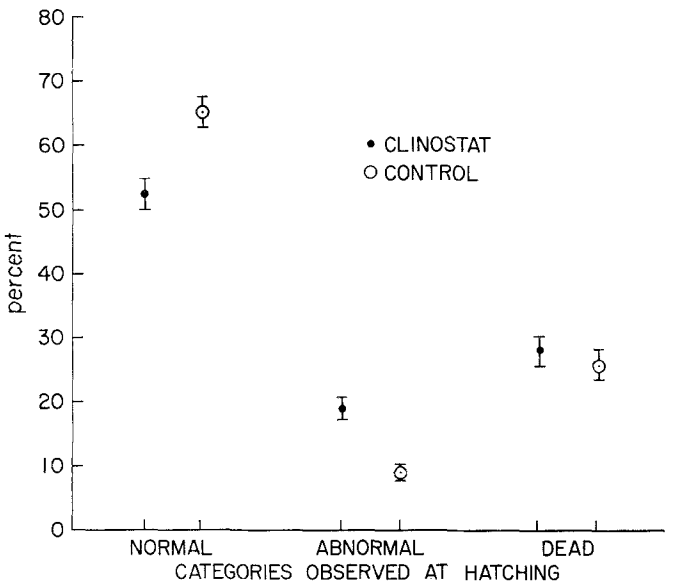

Fig. 4. Summary of experiments conducted on 1/4-rpm horizontal clinostat, *R. pipiens*; plotted as described in Figure 3 ($n = 314$ dishes/treatment).

84.0%, and 85.8%, compared to a control value of 88.8% (Figure 3). The speeds of 1/4, 1, and 2 rpm are effective in significantly ($p<0.05$) influencing normality. Due to the relative difficulty of obtaining *X. laevis* egg preparations, all further experimentation was carried out with the more easily controlled *R. pipiens* material.

The data gathered from all the experiments involving eggs of *R. pipiens* placed immediately post-fertilization on the 1/4-rpm clinostat are summarized in Figure 4. It is evident that the categories of normality and abnormality were affected significantly by rotation in a plane parallel to the normal gravity vector. The control groups exhibited a normality of 64.9% compared with 52.9% for the rotated specimens ($p<0.01$). Abnormality increased to 19.1% from 9.0% ($p<0.01$) with clinostating while death increased insignificantly to 28.1% from 25.8%, rate of unfertilization probably contributing equally to both treated and untreated eggs.

B. HORIZONTAL VERSUS VERTICAL ROTATION

Since it was necessary to dispose of rotational speed per se, vibration, and thermal characteristics of the clinostat (1/4 rpm) in contributing to the progress of development, a series of experiments with the axis of clinostat rotation in the vertical plane was conducted. Five experiments with *R. pipiens* yielded a total of 42 dishes per sample apportioned equally among static control, clinostat in vertical plane of rotation, and clinostat in horizontal plane of rotation.

Figure 5 is a graphic representation of the results. Normality of development was exactly the same (72%) for those embryos exposed to rotation, axis in the vertical

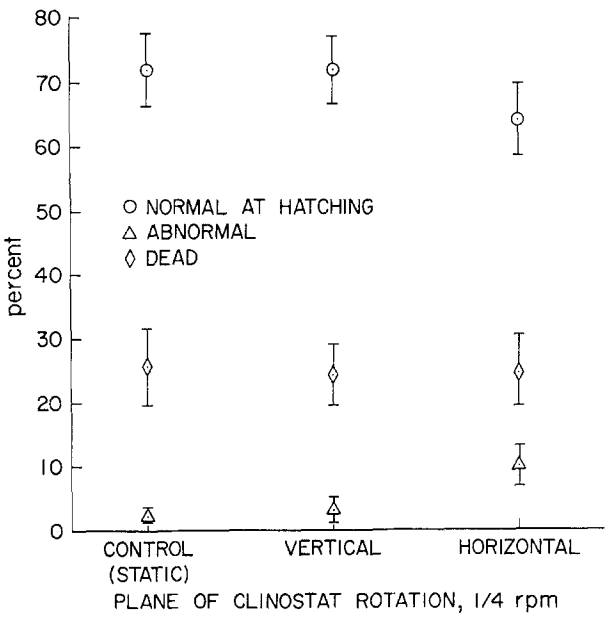

Fig. 5. Percent normality, abnormality, and death as affected by plane of clinostat rotation (*R. pipiens*); plotted as described in Figure 3 ($n=42$ dishes/treatment).

plane, as for the static controls (maintained in normal position on the bench top). Normality was lower (64.5%) in the eggs rotated in the horizontal position, approaching significance at the 5% level. Death (or failure of fertilization) remaining essentially the same (ca. 25%) over the three conditions, abnormalities of eggs horizontally rotated increased significantly ($p < 0.01$) to 10% from the values (2–3%) resulting from the other two conditions. Vertical rotation, with concomitant clinostat thermal and vibration characteristics, was indistinguishable from the static condition in affecting development.

C. STATE OF DEVELOPMENT AND SENSITIVITY

In attempting to define relative stages of sensitivity to rotation, eggs were placed on the clinostat at different times after fertilization (4 experiments, 40 dishes per sample). Placed on the clinostat immediately after fertilization and at 0.5, 1, 1.75, 2.5, 3.5, and 15 h post-fertilization, mean normality at hatching after 5 days of exposure was 63.0% 60.2%, 65.0%, 66.5%, 67.0%, 72.5%, and 77.6%, respectively. These data compared with a control (unrotated) value of 74.5%. The points are presented in Figure 6.

The variability of the data and relatively small sample size did not permit significant separation of these points from each other. There was, however, an obvious trend in decreased sensitivity as the egg was exposed at succeeding stages of development, rapidly losing sensitivity as the 2-cell stage ($2\frac{1}{2}$–3 h) was passed.

In experiments (5 frogs, 69 dishes) where eggs were placed on the clinostat immediately after fertilization and removed at the 2-cell stage, hatching revealed (Figure 7) a decrease in normality compared to the control organisms and to those placed on at

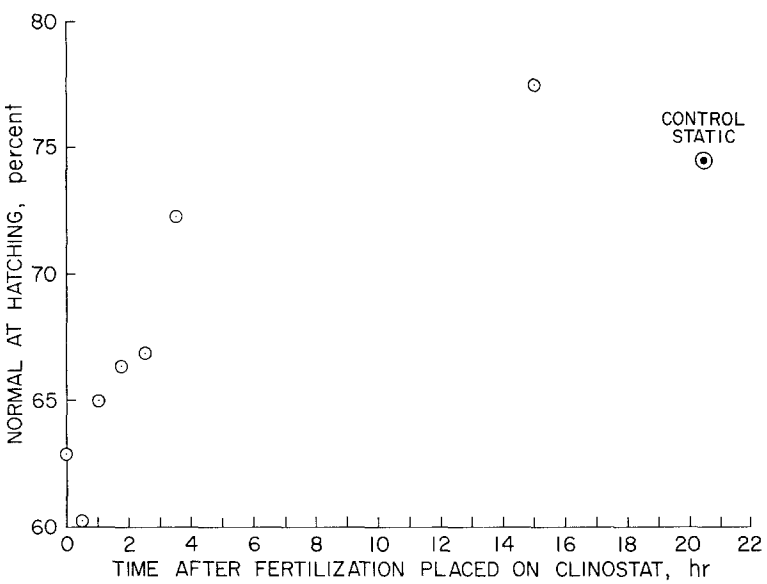

Fig. 6. Relation of normal development in *R. pipiens* to time of initial exposure to horizontal rotation (1/4 rpm) of clinostat; each point represented by 40 dishes.

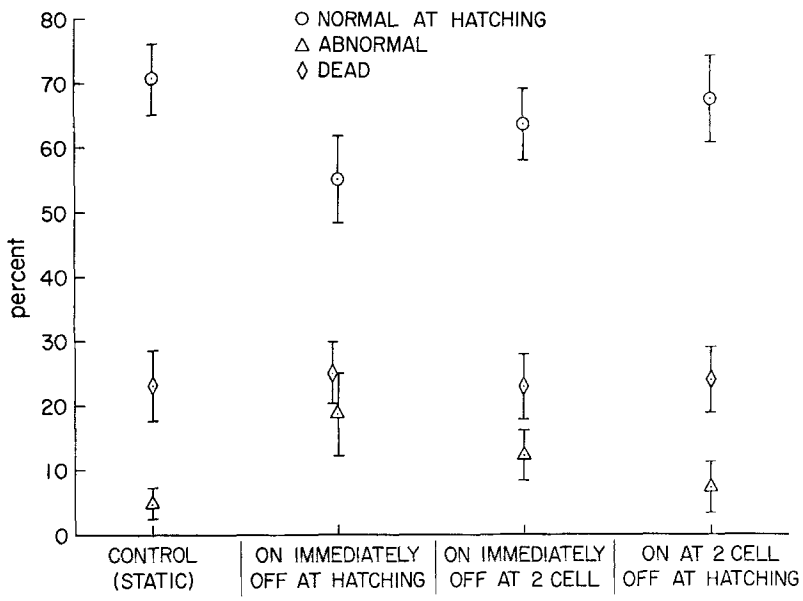

Fig. 7. Effect of horizontal clinostat rotation (1/4 rpm) on fertilized eggs of *R. pipiens* as influenced by phase of exposure after fertilization; plotted as described in Figure 3 ($n = 69$ dishes/treatment).

TABLE I

Types of abnormalities in *R. pipiens* resulting from 1/4 rpm clinostat rotation compared with those produced in unrotated (static) development; expressed as ratio (%) of number of embryos of each specific type to number of total abnormalities observed at hatching in rotated (total of 842 abnormalities occurring in 615 abnormal embryos) and in static (total of 368 abnormalities occurring in 284 abnormal embryos) classes; total treated in each class was 3300 eggs; c.a. = caudal abnormality, m = microcephaly, p = projections, i.i. = incomplete invagination of yolk, v.b. = ventral bulge, e.a. = ectodermal abnormality, s.b. = spina bifida, st = stunted growth, ex = exogastrula, o = other. See text for definitions of these terms.

Type	Rotated	Unrotated
c.a.	7.2	7.7
m	4.2	3.4
p	6.2	8.1
i.i.	18.7	22.8
v.b.	13.1	13.3
e.a.	17.5	14.1
s.b.	2.3	3.7
st	23.2	21.2
ex	2.4	2.1
o	4.8	3.4

the 2-cell stage and removed at hatching. These decreases, however, were not as much as that evidenced by the eggs exposed continuously through hatching (15% decrease from the control, $p<0.01$). Further, abnormalities were significantly ($p<0.01$) higher in those eggs removed at the 2-cell stage as compared with controls, with continuous exposure yielding yet more abnormalities. From this it may be deduced that a greater relative sensitivity existed prior to first cell division and the manifestation of decreased normality was enhanced by continued exposure to clinostat rotation beyond the 2-cell stage. The rate of abnormalities resulting from these treatments was inversely proportional to the normality values, consistent with this conclusion.

In only a few cases throughout this investigation were deaths, as defined above, influenced by clinostating. In these cases, the eggs usually gave some evidence of abnormality before fertilization, which in turn was usually comparatively low in rate.

D. ABNORMALITY TYPES

Table I gives a quantitative comparison between control eggs and those exposed to the 1/4-rpm clinostat regarding the kinds of abnormalities produced (see above). Each embryo was scored for all types of abnormalities described (842 abnormalities by rotation compared to 368 in the controls, resulting from a total of 3300 eggs treated in each group). It appeared that gravity compensation did not specifically influence a particular anomaly but had rather a general effect.

4. Discussion

The force of gravity alone or centrifugal force affects normality of development when experimentally imposed. When the frog egg was inverted and held by pressure with the yolk-end up, as in Schultze's early experiments (1894), abnormal development followed. This treatment was most effective when applied at the 2-cell stage, although Penners and Schleip (1928) reported sensitivity extending through the 8-cell stage. Here the abnormalities, chiefly twinning phenomena, were thought to proceed by a separation, due to cleavage, of the gravity-caused flow of vitelline material down through the cytoplasm. Normal development then was affected by an abnormal effect of gravity.

Again, as noted above, experiments conducted on the clinostat with eggs fixed within their shells at slow speeds of rotation (Schulz, 1897; Gordon, 1969) and with eggs unrestrained but subject to higher speeds on the centrifuge (Kas'yanov, 1968; Banta and Gortner, 1915; Todd, 1940; Pasteels, 1941; Hertwig, 1887; Young *et al.*, 1970) resulted in increased death and abnormalities. The direction and intensities of the forces applied to the captive egg effected a critical rearrangement of cellular material. When, however, eggs left free to rotate within their capsules were either placed on a slowly rotating clinostat (Roux, 1884) or were slowly and randomly turned by a current of water (Kathariner, 1901; Morgan, 1902), no increased abnormality was observed. These speeds of rotation varied from 1 to 84 rpm at radii yielding 10^{-5} to 2 g. It should be noted that forces had to exceed 5 g before effects due to centrifu-

gation imposed before first cleavage could be observed in *R. pipiens* although 3 g continuously applied over the first 6 to 8 h of development was sufficient to decrease normality significantly (Souza, unpublished results). In *R. fusca*, forces had to exceed 6 g through hatching to produce abnormal development (Moszkowski, 1902).

The effectiveness of clinostat rotation in influencing the development of the frog egg escaped notice due principally to the differing objectives of preceding investigations. These objectives, for the most part, grew out of the turn-of-the-century controversy occasioned by interpretations cast upon experiments designed to elucidate the role of gravity in early development. Chief protagonists of a theory claiming the indispensability of gravity in determining normal development were Pflueger (1884) and Schultze (1900), while Roux (1884) and others who followed set forth experiments and proposals in disagreement. See Moszkowski (1902) and Kathariner (1901) for reviews of this work.

In reference to the results reported here, it was reasonable to assume that no increased abnormality would have been detected by earlier workers if (1) the maximally effective clinostat speed was not employed, or (2) sample size was not sufficient for statistical analysis, or both. In any of these investigations, with the eggs of other species (*R. fusca*, 'toad'), rotational speed was not deemed critical except insofar as it did not allow rotational readjustment within the capsule, and indeed the much slower speeds were not utilized. Further, there was no evidence of a statistical analysis of the usually quite variable data. In any case, clinostat speed of rotation was critical and may be related to the period of rotation of the unrestrained fertilized egg (*X. laevis* eggs, which rotate more quickly than *R. pipiens* eggs, respond in increased abnormality to higher clinostat speeds). Roux (1897) reported that the egg in its rotational speed within the capsule was responsive to the amount of fluid within the vitelline space. Physiological differences, then, within a species could account for the variability observed.

The mechanism by which the unrestrained egg responds to slow rotation is not fully understood. Rotation at the higher speeds (1, 2, 5, and 10 rpm) seemed to have no effect on the initial random orientation of the eggs in *R. pipiens*, although in *X. laevis* there was an orientation of the egg axis to the resultant of the force of gravity and the very small centrifugal force (10^{-2}–10^{-3} g). In both cases gravity compensation may be said to have operated at these higher speeds since the egg remained fixed in position throughout clinostat rotation, the directional force of gravity at any one point in time being counteracted at another. At the lower speeds (1/15, 1/8) the egg maintained, by its natural rotational capability within the capsule, a yolk-down position through each clinostat rotation, at least through first cleavage. There was thus no gravity compensation here, rather an active and constant orientation to gravity. The situation, however, was quite different in *R. pipiens* at the intermediate speed of 1/4 rpm.

Eggs were fertilized and allowed to rotate before being placed on the 1/4-rpm clinostat at 30 min post-fertilization and the response of the vitelline material recorded through first cleavage. The yolk masses moved down from the original (animal-vegetal pole in horizontal position) 'yolk-in' positions over approximately 30 min to a 'yolk-

down' position, but somewhat oblique to the vertical. This oblique position, maintained nearly to first cleavage through each clinostat rotation, resulted in a mixing of cytoplasmic constituents. A gray area appeared near the top of the yolk mass, possibly resulting from a downward movement of yolk granules from the upper boundary of the vegetative pole in response to gravity. These were superficial observations. Internally, a newly formed vertical gradient may have been effective in maintaining a gravity responsiveness. At approaching first cleavage, the eggs lost their ability to 'keep up' with the clinostat rotation and began to assume random positioning similar to that of the eggs exposed to the higher rpm. No further mixing would, therefore, be expected to occur beyond first cleavage, while the eggs truly gravity–compensated during the sensitive period before first cleavage were not susceptible to intracellular mixing and developed normally. At 1/4 rpm, then, in *R. pipiens*, and at 1/4, 1, and 2 rpm in *X. laevis*, abnormality production was increased significantly over the control. It would appear that these results were in effect analogous to the restrained egg and centrifugation experiments where abnormalities were produced by an abnormal effect of gravity (i.e., displacement of cellular material occurred in any egg maintained counter to the normally acting gravity field or in eggs exposed to hypergravity forces).

In any event, it was apparent that the time at which exposure of the fertilized egg to clinostat rotation was initiated was critical. That the increased abnormality rate of those exposed early was more than the result of an increased exposure time is indicated in Figures 6 and 7. There was little diffrence in normality resulting in those eggs placed on the clinostat near the 2-cell stage as compared with unrotated control eggs. Those placed on the clinostat immediately after fertilization and remaining to hatching were maximally subject to an increased abnormality rate, while there was even a significant ($p<0.01$) effect on those eggs exposed immediately after fertilization and removed at the 2-cell stage (Figure 7). The period of greatest sensitivity then appeared before the first cell division. Centrifugation studies (Banta and Gortner, 1915), where sensitivity was inversely proportional to stage of development, were consistent with this finding.

It has been reported that weightlessness does not affect the development of *R. pipiens* eggs when imposed from the first cleavage through the neural plate stage (Young and Tremor, 1968a). The experiment reported here suggests that exposure to weightlessness through the evidently more sensitive pre-first-cleavage period would also produce no increase in abnormalities if, as in plants, gravity compensation yields results similar to the effects of weightlessness exposure. It would still be difficult to predict a disturbing effect of weightlessness on the normally cohesive constituents of the cell realizing that the weightless egg is subject to forces (or lack of forces) quite different (Pollard, 1965; Tyler, 1966; von Borstel and Kondo, 1969) from those effective in the gravity-compensated egg.

Under normal conditions, however, bilateral symmetry, progress initiated soon after fertilization, may be determined by a variety of factors in the amphibian egg. Roux's rule (sperm copulation path predominating in determining bilateral symmetry) and Hertwig's rule (position of nuclear spindle, itself dependent on gravity, assuming predominancy) have both been defended and attacked by a variety of workers. Ancel

and Vintemberger (1948) have shown the position of the plane of bilateral symmetry to be dependent on a combination of the action of the orientation rotation, sperm path, and gravity. Under conditions of gravity compensation, where normal development followed, it is not known whether or not there was developed a normal gray crescent (associated in position with the plane of bilateral symmetry). Under these conditions the sperm path may well have been the principal determinant of bilateral symmetry in a flexibly responding egg and remains a subject for morphological and histological study. In this respect, weightlessness may provide a tool for studying questions arising from the long-standing controversy regarding predeterminism of polarity and bilateral symmetry in the amphibian egg.

5. Summary

(1) *R. pipiens* eggs exhibited a significantly reduced (ca. 13%) normality from the control at a rotation of 1/4 rpm, while rotational speeds of 1/15, 1/8, 1, 2, 5, and 10 rpm were not significantly effective.

(2) *X. laevis* eggs did not respond in decreased normality at 5 and 10 rpm on the clinostat but yielded significantly less normality (ca. 13%) as compared with controls at the speeds of 1/4, 1, and 2 rpm.

(3) In *R. pipiens* there was a greater sensitivity to clinostat rotation at 1/4 rpm when the fertilized egg was exposed before first cell division as compared with initial exposure after the 2-cell stage.

(4) While there was a significant increase in abnormalities at 1/4 rpm in *R. pipiens*, there was no increase in specific category of abnormality but rather a general increase.

(5) The speeds of clinostat rotation causing increased abnormality production were believed to be effective by mixing of cellular constituents due to maintenance of the eggs (through inability of the eggs to rotate completely to a yolk-down position with each clinostat rotation) in oblique positions. Gravity compensation was effective at higher speeds, while the slower speeds permitted maintenance of normal equilibrium.

References

Ancel, P. and Vintemberger, P.: 1948, *Bull. Biol. France Belg.* **31**, 1–24.

Banta, A. M. and Gortner, R. A.: 1915, *J. Exp. Zool.* **17**, 433–451.

Brown, A. H.: 1968, 'Relationship of Clinostat and Spacecraft Research on Biological Effects of Gravity', Statement by the Space Biology Subcommittee, Space Science Steering Committee, Feb. 1968.

Gordon, S. A.: 1969, Personal communication.

Hertwig, O.: 1897, 'Über einige am befruchteten Froschei durch Centrifugalkraft hervorgerufene Mechanomorphosen', *Sitzungsber. Preuss. Akad. Wiss.* pp, 14–18.

Johnson, S. P. and Tibbitts, T. W.: 1968, *Bio Sci.* **18**(6), 655–661.

Kas'yanov, V. L.: 1968, *Dokl. Akad. Nauk S.S.S.R.* **180**(4), 1008–1011.

Kathariner, L.: 1901, *Archiv. Entwick.-Mech.* **12**, 597–609.

Larsen, P.: 1962, in W. Ruhland *et al.* (ed.), *Encyc. Plant Physiol.* **17**(2), 34–73, Springer, New York.

Lyon, C. J.: 1968, *Bio Science* **18** (6), 633–638.

Morgan, T. H.: 1902, *Anat. Anz.* **21**, 313–316.

Moszkowski, M.: 1902, *Arch. Mikrosk. Anat.* **60**, 17–65.

Pasteels, J.: 1941, *Arch. Biol.* **52**, 321–339.

Penners, A. and Schleip, W.: 1928, *Z. Wiss. Zool.* **130**, 305–454 and **131**, 1–156.

Pflueger, E.: 1884, *Arch. Ges. Phys.* **34.**
Pollard, E. C.: 1965, *J. Theor. Biol.* **8**, 113–123.
Roux, W.: 1884, *Bresl. Aertzt. Zeits.* **6**, 57–62.
Roux, W.: 1897, *Arch. Entwick.-Mech.* **5**, 387–388.
Rugh, R.: 1962, *Experimental Embryology*, Burgess, 3rd ed.
Schultze, O.: 1894, *Arch. Entwicklungsmech. Organ.* **1**, 269–305.
Schultze, O.: 1897, *Phys. Med. Ges.* **4**, 41–48.
Schultze, O.: 1900, *Arch. Mikrosk. Anat.* **56**, 309–334.
Thimann, K. V.: 1968, *Proc. Nat. Acad. Sci.* **60**, 347–361.
Todd, R. E., Jr.: 1940, *J. Exp. Zool.* **83**, 359–377.
Tyler, A.: 1966, *J. Theor. Biol.* **11**, 59–62.
Von Borstel, R. C. and Kondo, S.: 1969, 'Biological Instability in the Absence of Gravitational Forces', in manuscript.
Werber, M.: 1969, 'The Clinostat', Biological Sciences Communication Project, George Washington Univ. Med. Ctr.
Young, R. S., Deal, P. H., Souza, K. A., and Whitfield, O.: 1970, *Exptl. Cell Res.* **59**, 267–271.
Young, R. S. and Tremor, J. W.: 1968a, *Bio Science* **18**(6), 609–615.
Young, R. S. and Tremor, J. W.: 1968b, *Life Sciences and Space Research*, North-Holland, pp. 87–93.