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SEA URCHIN MITOSIS IN HIGH MAGNETIC FIELDS

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SEA URCHIN MITOSIS IN HIGH MAGNETIC FIELDS*

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SUMMARY PAGE

THE PROBLEM

Precise characterization of the effects of high magnetic fields upon living systems is of paramount importance in evaluation of the potential hazards to man should he be exposed to such an environment. The extensive descriptions of mitosis in the sea urchin egg available in the literature provide the norms necessary to a better understanding of these effects as they influence the processes of cell division so vital to life.

FINDINGS

Mitosis in sea urchin eggs was retarded following their exposure to magnetic fields higher than 70,000 gauss which had gradients greater than 4200 gauss/centimeter. The degree of retardation was correlated simultaneously to both the field strength and the field gradient. Division delay in fields ranging from 100,000 to a maximum of 120,000 gauss was independent of the gradient.

Differences in division delay were found which may be a reflection of the differential migration of oxygen and nitrogen due to their para- and diamagnetic properties.

An hypothesis is given concerning the biochemical processes affected by magnetic fields.

ACKNOWLEDGMENTS

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INTRODUCTION

The primary objective of this investigation was the precise characterization of the effects of high magnetic fields upon cell division in the hope that the scope of future studies may be legitimately limited to specific parameters of these effects.

Living organisms are generally most susceptible to injury by physical agents during their developmental periods. This well-known fact coupled with the extensive background of literature concerning mitosis in the sea urchin egg led to its choice as the model system for these studies.

Perakis (1) exposed the eggs of the sea urchin Echinus melo to both homogeneous and inhomogeneous magnetic fields which ranged from 8,000 to 43,000 gauss. The inhomogeneous fields caused a delay in egg development and an excess of abnormal cleavage. No such effect was found in eggs exposed to homogeneous fields of 33,000 gauss. Beischer and Knepton (2) followed Arbacia eggs through the first three divisions after exposure to fields between 88,500 and 140,000 gauss. Although a retardation in the cleavage rate was noted following exposure, these workers were unable to detect a difference between the effects of homogeneous and inhomogeneous fields.

The specific selection of Strongylocentrotus purpuratus rather than Arbacia as the organism to be studied was made in view of the transparency of the egg in this species. This characteristic increased, sequentially, the microscopic resolution of the mitotic apparatus, the accuracy of the observations, and the validity of the classifications which form the basis for the conclusions. It should be pointed out, however, that any system of classification of cells with respect to their instantaneous position within the mitotic cycle is somewhat arbitrary since the division process is one of continuous change rather than the step-like progression implied by the system.

PROCEDURE

MAGNETIC FIELDS

Fields of various intensities and gradients were generated by two modified Bitter magnets made available through the courtesy of the Naval Research Laboratory, Washington, D. C. Magnet A was so designed that the field had an axial uniformity to within 1 per cent over a 2-inch span at the geometrical center of the coil. This was accomplished by the insertion of a "shorted turn" at the center which created a dip in the field peak at that point. Magnet B had no such "shorted turn" so that the field peaked smoothly in a manner characteristic for this type of solenoid (Figure 1).

APPARATUS

The specimen support consisted of 14 pairs of plastic containers so arranged that opposing members were equidistant from the geometrical center of the structure. Plastic tubing from each container to one of a remote bank of hypodermic syringes

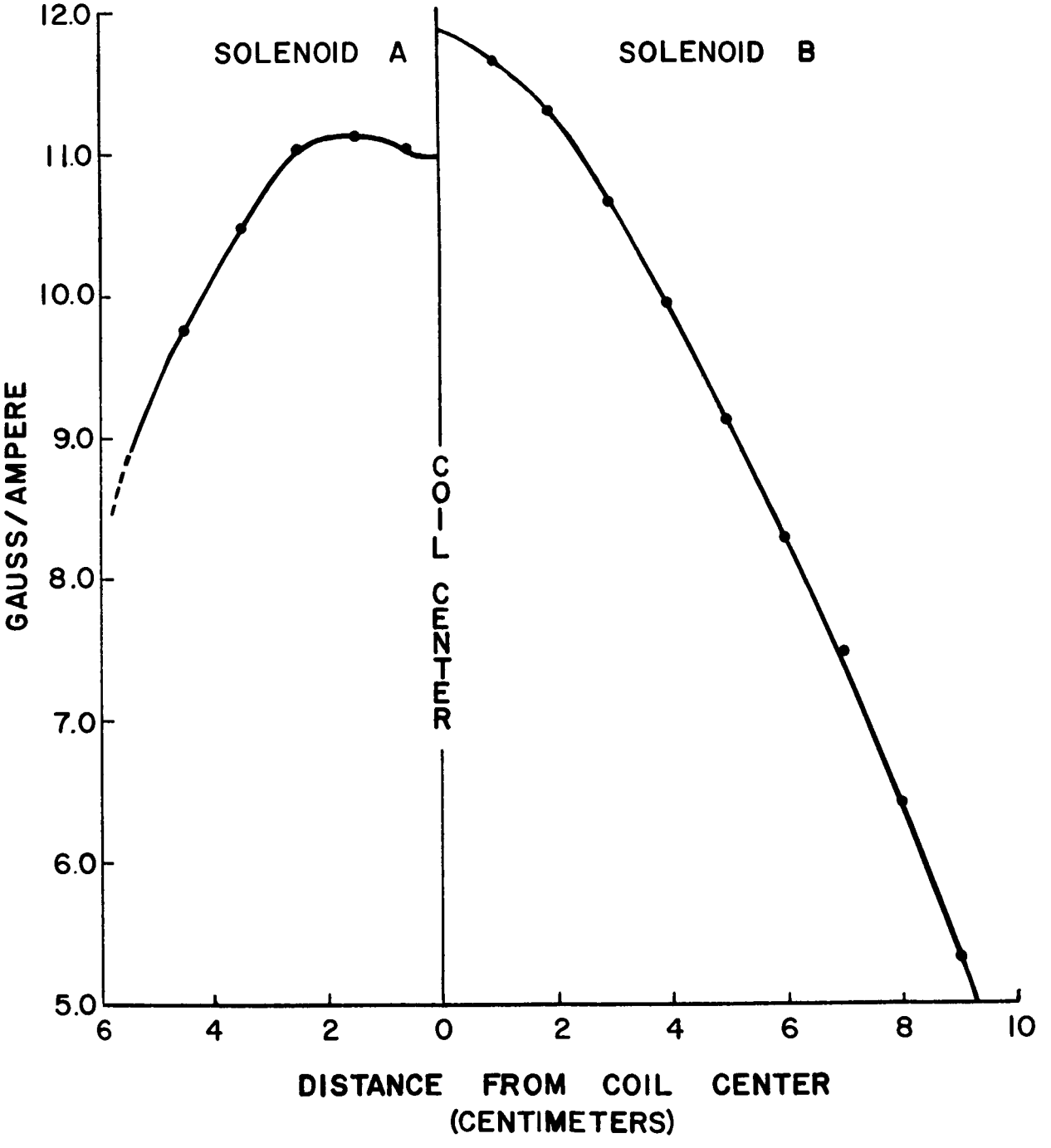

Figure 1

Magnet Characteristics

made it possible to add known amounts of fixative to any selected group of cells within the solenoid during operation (Figure 2).

An identical apparatus held the control cells in corresponding positions in a plastic tube having the same internal diameter as that of the magnet. The control tube was wrapped with opaque tape to equate the conditions of illumination to those affecting the experimental cells.

Temperature regulation within the solenoid was provided during operation by means of a brass water-jacket through which water from a manually controlled mixing valve was circulated. A steady stream of air at room temperature flushed both the experimental and control chambers to minimize local heating. Reference to the temperature readings provided by a sensor within the solenoid determined the rate of flow of the coolant.

METHOD

Division processes were arrested during exposure by the simultaneous addition to the experimental and control cells of equal quantities (1.0 cc) of acetic-alcohol fixative (1:3). Cells in the containers aligned vertically along one side of the axial center of the solenoid were fixed at the earlier of two time intervals while their opposites were allowed to progress further through the division cycle before fixation (Figure 2).

Visualization of the mitotic apparatus necessary for classification presented several problems. A number of nuclear stains were tried with little success. Direct examination of the cells by phase-contrast microscopy seemed to be the method of choice and was adopted for this investigation. Cells from each of the specimen containers were suspended in 45% acetic acid, flattened by pressure on the coverglass, and classified according to their stage of division until a total of 100 had been examined. Such a procedure resulted in the individual study of some 14,000 ova.

The control of, or compensation for, temperature in any investigation based upon the rate of division in sea urchin eggs is of vital importance to a reasonable interpretation of results. Although differences in temperature between the experimentals and the controls throughout a single experiment were so low as to be immeasurable, they reached a maximum of 3.7°C during the entire series. Since a difference of this magnitude could invalidate a direct comparison of results, it was necessary to devise a procedure by which all data could be normalized with respect to the effects of the existent, rather than the optimum, temperature. The rationale and mechanics of this method of temperature compensation are given in the Appendix.

All subsequent conclusions are based upon data normalized by this procedure. The results of one experiment were discarded in their entirety after the temperature analysis showed a large discrepancy between the expected and observed values in the controls. Reference to observations made at the time of the experiment indicated a question of possible overfertilization which could account for the condition.

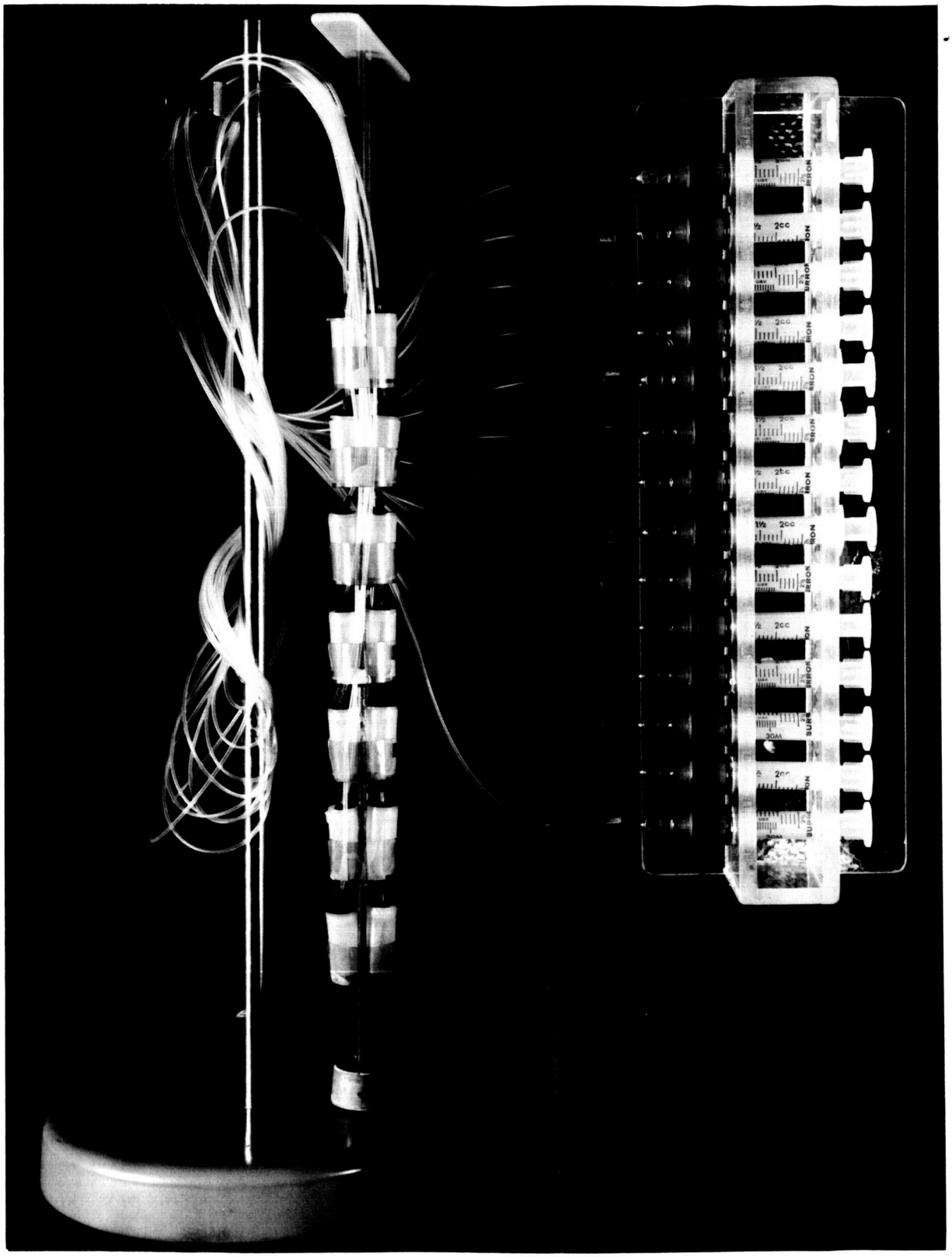

Figure 2

Fixation and Positioning Apparatus

RESULTS

Several parameters of the effects of high magnetic fields upon cell division in Strongylocentrotus were characterized by this series of experiments. The degree of division delay in the exposed cells was correlated simultaneously to both the field gradient and the field strength. The ratios of cells delayed to the total number of cells exposed to the given field conditions are indicated numerically in Figure 3. Fields higher than 100 kilogauss caused a delay in division regardless of the gradient. Between 80 and 100 kilogauss the influence of the gradient became apparent in that division delay was generally more pronounced in the fields of higher gradient than in those of corresponding strength with a lower gradient. Below 80 kilogauss the delay appeared to be related more directly to the gradient than to the field strength. Fields of less than 70 kilogauss with a gradient of about 4200 gauss/centimeter or less had no detectable effect.

Earlier stages of the division cycle were more susceptible to the magnetic field than were later stages within the same cycle. In seven of the eight pairs of specimens indicated in Table I those fixed at the earlier time interval showed the greater delay. The table includes only those values for cells exposed to fields of 100 kilogauss or lower in which an effect was detected and for which data were available permitting direct comparison of the two time intervals.

Table I

Effect of Time of Exposure on Division Delay

Time of Exposure (Fertilization to Fixation)			
38 Minutes		43 Minutes	
Specimen*	Degree of Delay#	Specimen*	Degree of Delay#
F1	>D	F8	D
C5	>D	C12	O
C4	D	C11	O
C2	D	C9	O
C6	D	C13	O
F6	D	F13	O
F7	D	F14	O
E4	O	E11	D

*Each specimen designation represents an aliquot of 100 cells counted.

#Arbitrary units O = No delay

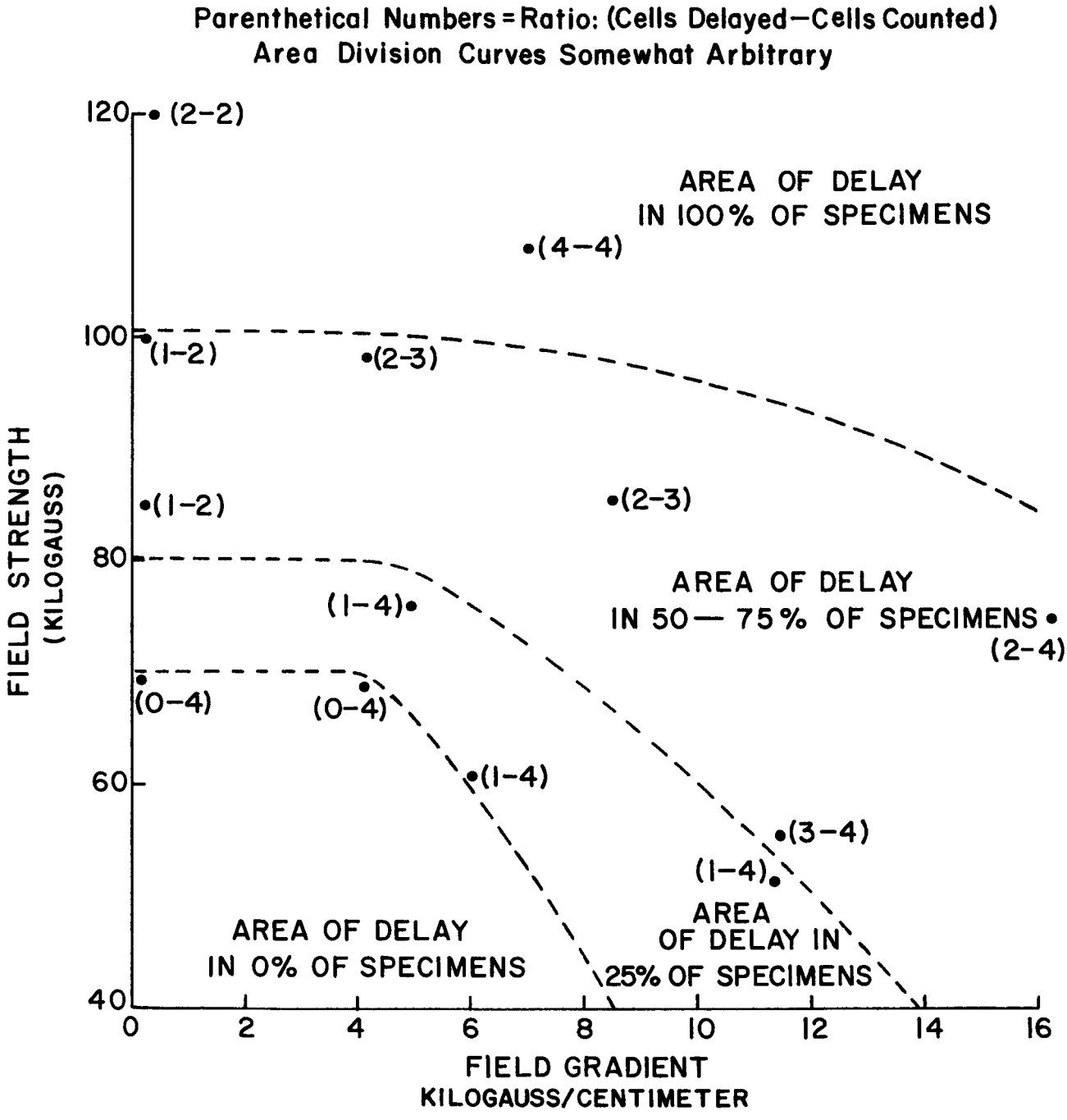

Figure 3

Relationships Between Division Delay and Field Parameters

Comparison of the degrees of division delay suffered by cells exposed to equivalent field conditions but probable differences in concentrations of oxygen and nitrogen indicates a greater delay in those exposed to a nitrogen-rich atmosphere (Table II).

Table II

Effect on Division of Migration of Gases

Division Delay			
Above Coil Center (Excess O_2)		Below Coil Center (Excess N_2)	
Specimen*	Degree#	Specimen*	Degree#
F5	D	F3	>D
F12	D	F10	>D
F7	D	F1	>D
F14	O	F8	>D
F13	O	F9	D
C5	>D	C3	D

*Each specimen designation represents an aliquot of 100 cells counted.

#Arbitrary units O=No delay

DISCUSSION

One anticipated condition incidental to the primary investigation was the possible differential migration of gases due to their particular magnetic properties. Since this condition coexists with those necessary to attain the high magnetic fields required, it cannot be eliminated as a potential factor in the results obtained. The discussion of this phenomenon is given prior to that of the principal investigation in order to preserve the continuity of the latter.

If a mixture of paramagnetic and diamagnetic components is exposed to a magnetic field, it will obey fundamental physical laws stating that any system tends to assume the lowest possible energy. The net result is a movement of the paramagnetic entities toward and the diamagnetic ones away from the area of the greatest field (3). The paramagnetic nature of molecular oxygen is well known and is so pronounced that its reaction to a magnetic field forms an integral part of the principle of operation of the Pauling oximeter (4).

Since the gaseous atmosphere within the solenoid and in each specimen container was composed primarily of paramagnetic oxygen and diamagnetic nitrogen, an increase in the partial pressure of oxygen with a concurrent decrease of nitrogen could be expected as the geometrical center was approached. Gravity held the eggs at the lowest point within each receptacle; therefore, those eggs above the coil center were exposed to an increasingly oxygen-rich environment as the center was approached, while those below experienced one of increasing nitrogen (Figure 4). Since each

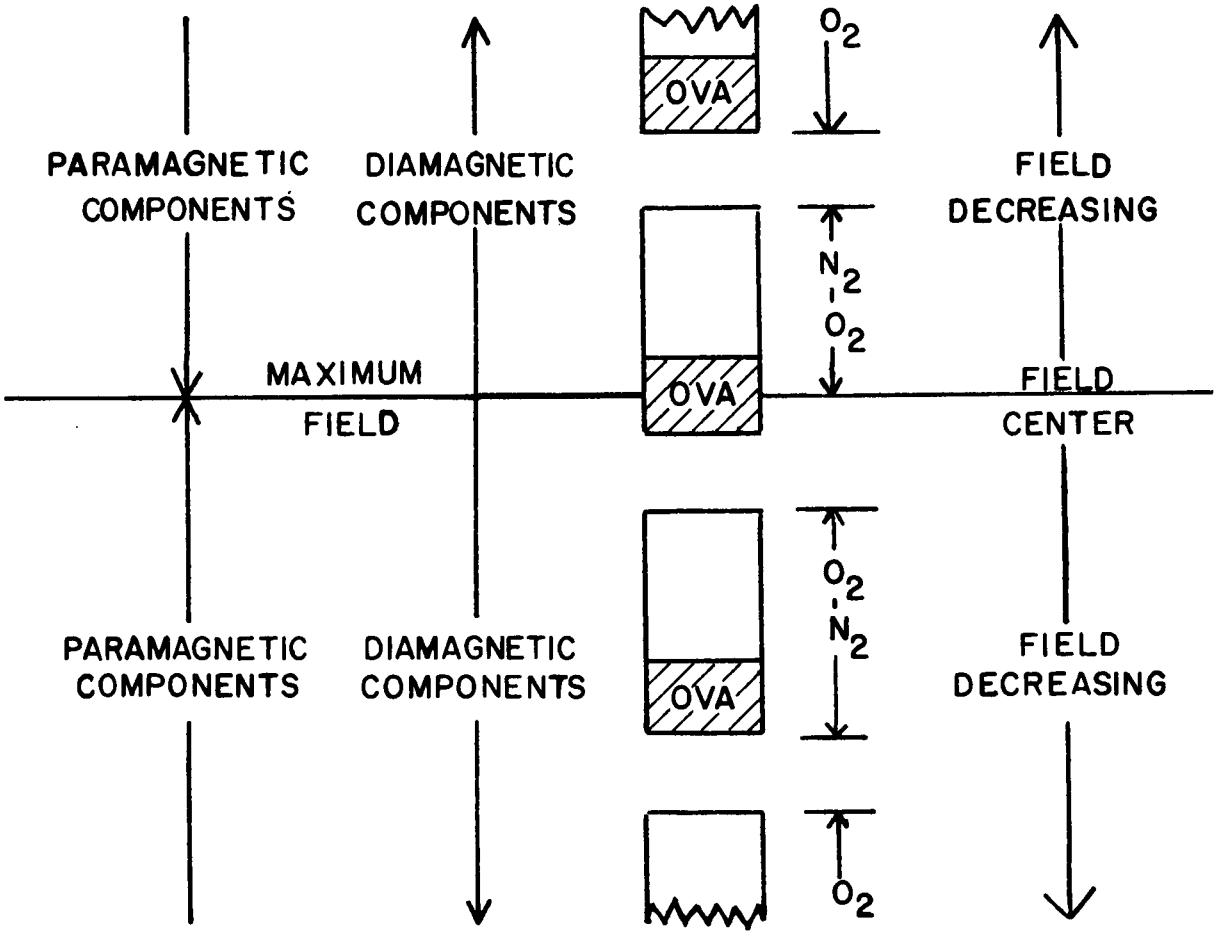

Figure 4

Differential Migration of Gases During Magnet Operation

container and its homologue at the opposite end of the solenoid functioned as an isolated system under equivalent field conditions, any difference detected between opposing pairs could be due to a change in gas ratios caused by the field. This condition was reflected as the increased delay in division shown in Table II and is in keeping with the known (5, p. 544) aerobic nature of the division process in marine eggs.

Basic to the following discussion of cell division as it is influenced by high magnetic fields is the premise that such fields are physical agents capable of generating responses within living systems analogous to those evoked by other such agents.

Two general methods by which cell division may be suppressed by physical agents were given simultaneous consideration during the design and interpretation of this series of experiments: 1) a disorientation in the formation or movements of the elements of the mitotic apparatus, and 2) interference with one or more of the biochemical steps involved in the preparations of the cell for division.

The first of these possibilities usually results in a visible alteration of the mitotic apparatus. Mazia (6), for example, in speaking of cells in general, pointed out that visible disordering usually follows functional blockage of the mitotic apparatus. More specific to the case at hand is a recent exhaustive study of sea urchin eggs by Rao which, as cited by Failla (7), implicates a delay in the visible condensation of the chromosomes as being responsible for mitotic inhibition following irradiation.

The fact that no gross disarrangement or abnormalities of the mitotic apparatus was evident throughout the described studies contraindicates a purely mechanical effect of the field upon the mitotic apparatus as the determinant in the observed division delay. Planned phase- and electron-microscope studies on the isolated mitotic apparatus should assist in a more definitive evaluation, if not complete elimination, of this possibility. The bulk of the positive evidence, on the other hand, favors interference at the chemical level to be the resultant of exposure of the cell to a high magnetic field.

The degree differences in effect noted between the lower and upper "thresholds" (of no delay and complete delay, respectively, Figure 3) implicates a chemical mechanism since an all-or-none, not a graded, response would be expected should the mitotic apparatus be damaged.

The dependence of division on gas ratios produced by the field (Table II) also argues against mechanical influences, since such influences would not be contingent on environmental gases, and for a chemical change in that altered gas ratios could profoundly affect many chemical reactions taking place during division.

The results found with sea urchin eggs are comparable to those obtained in earlier studies (8,9) in which actively-dividing, mammalian tissue had a lower rate of oxygen uptake than did mitotically-inactive adult tissue of the same type exposed to the same magnetic field.

The observations in both the current and the earlier studies support the conclusion that it is one or more of the biochemical steps intimately associated with the preparation of the cell for division, rather than the mitotic apparatus itself, which serves as the target for the magnetic field.

One of the more labile of the many essential preparations for division is the synthesis of desoxyribonucleic acid (DNA). This is particularly true in regard to its response to injury by physical agents, a circumstance leading to a voluminous literature concerning the effects, for example, of radiation on this system. Should an unwinding of the double helix take place during replication of DNA in the manner recently proposed by Cairns (10), the net effect of a magnetic field could be expressed as a resistance to the rotational movements involved. The magnitude of this resistance would be a function of the para- and diamagnetic properties of the molecules, and would be dependent upon both the gradient and the absolute strength of the field. It seems reasonable, therefore, to speculate that it is this process, the synthesis of DNA, which may be affected by the field.

This hypothesis for a magnetically-sensitive system was tested experimentally in other tissues by following DNA synthesis in ascites tumor cells exposed to magnetic fields. Autoradiographic detection of the tritiated thymidine used as a tracer indicated a definite retardation in the rate of DNA synthesis in these cells (9).

It is generally recognized that DNA synthesis takes place either during or before the very early stages of the mitotic cycle of the cell. In most cases it is in interphase immediately preceding actual mitosis (5 p. 119; 11). Heinegardner et al. (12) have recently shown that the first major synthesis of DNA in Strongylocentrotus purpuratus begins about thirty minutes after fertilization, lasting for a period of about ten minutes at 15°C. On the basis of experiments conducted at 5° and 15°C these workers estimate that the rate limiting reaction had a Q_{10} of 2. Extrapolation of this value to the temperature range in which the present experiments were conducted (25°-28°C) would predict a period of DNA synthesis of approximately five minutes.

The lack of precise, continuous regulation of temperature and of synchrony in the fertilization of the eggs precluded such precision in our experiments. The times of fixation were, therefore, so chosen that the first of the two might be expected to be within but near the end, and the second shortly after the period of DNA synthesis. Values obtained at the forty-three-minute fixation period provided the basis for comparison with those from cells fixed at thirty-eight minutes. If it is the process of DNA synthesis which is affected, the cell might be expected to show a greater sensitivity to the field at the earlier time interval. Just such an increased sensitivity was reflected in these experiments since those cells fixed at the earlier of the two intervals suffered the greater delay (Table I).

Figure 5 diagrammatically illustrates a scheme for the population kinetics involved which simultaneously satisfies the requirements of linearity with time of the stages of the division cycle (see Appendix) and the observed increase in division delay noted at the earlier of the two periods of fixation.

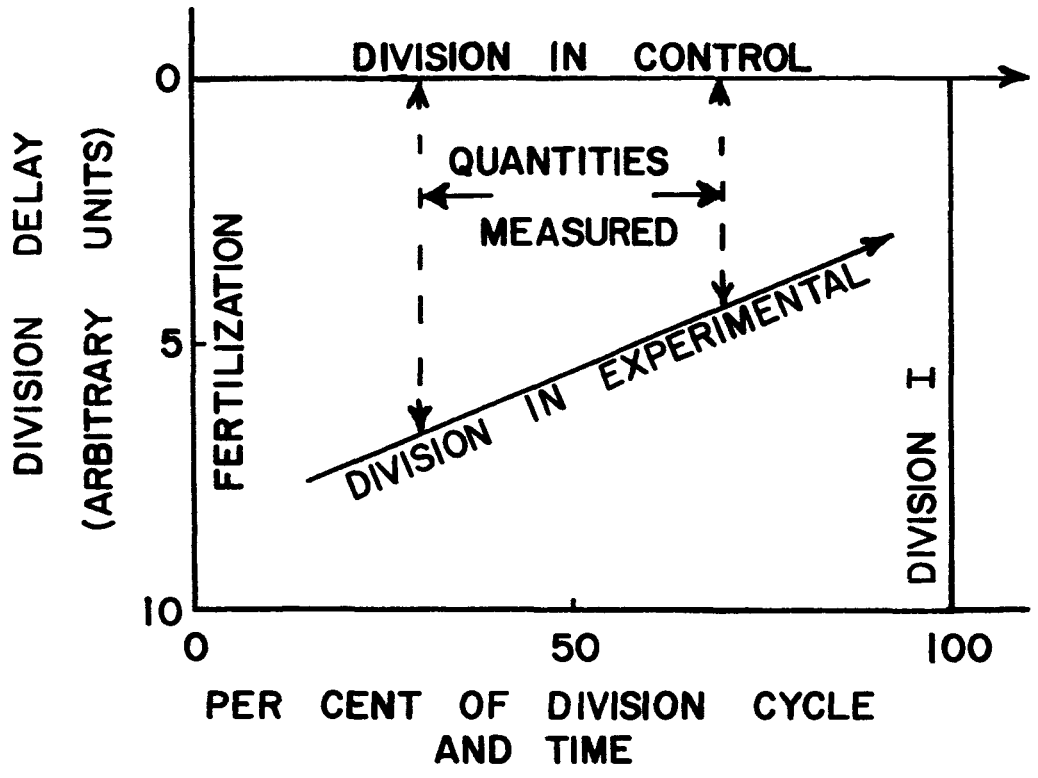

Figure 5

Relationship Between Delayed Division Measured at Two Time Intervals

The observed results also indicate a kind of recovery or compensating mechanism on the part of the cell. If this were not the case, the degree of delay at the later period of fixation could only equal or exceed that found earlier. This ability of the cell to repair itself has been widely studied with respect to injury by other physical agents. Failla (7) cited several workers who have noted recovery from radiation damage in Arbacia eggs then showed that this repair mechanism required neither oxygen, DNA synthesis, nor protein synthesis to function. In her discussion of the recovery of cells from radiation damage she pointed out that although the ultimate damage may be to nuclear components, it is the cytoplasm which is required for recovery. There is no reason at present to doubt that the same generalizations may be valid with respect to recovery from injury by high magnetic fields. Even though this type of injury proves to be reparable, however, its significance cannot be disregarded.

The serious consequences to the cell and to its progeny of tampering with a process so vital to its well-being as the synthesis of DNA make it imperative that any future exposure of man to high magnetic fields be made with an awareness of the potential hazards involved.

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Appendix A

Temperature Compensation Procedure

Curve A (Figure A1) represents the relationships between temperature and time from fertilization to first cleavage in Arbacia punctulata as published by several workers and compiled by Harvey*. Implicit in the construction of a parallel curve for Strongylocentrotus purpuratus is the assumption that division within the two species is equally affected by temperature, and the necessity for one point through which the curve may pass. Such a point was provided by the determination of the time of first cleavage in S. purpuratus at 25°C as noted independently by two observers and listed in the following table:

Minutes from Fertilization to First Cleavage at 25°C

Trial 1	Trial 2	Trial 3
53	51	51

Mean: 52 minutes

Curve B (Figure A1), constructed parallel to Curve A through this mean value of 52, represents the effect of temperature on the division time of the S. purpuratus used in these experiments. The validity of both the original assumption and the consequent curve is attested to by the fact that the curve passes through a value published by Tyler# for the time of division in S. purpuratus at 20°C.

A nomogram (Figure A2) which provided for the determination of the effect of temperature on each stage in a single division cycle as a function of time was constructed by expressing the times given for Arbacia at 23°C by Harvey (page 97) for each division stage as a percentage of the total division time. It was found that a linear relationship existed between these parameters, and upon the assumption that a similar relationship existed in S. purpuratus it was possible to construct parallels representing the corresponding parameters in this species. Reference to Figure A1 (Curve B) provided the point necessary to locate each parallel as a function of the time of division at a given temperature. By means of this nomogram it was possible to predict which stage of division should be reached at any given time under existent conditions of temperature.

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*Harvey, E. B., The American Arbacia and Other Sea Urchins. Princeton: Princeton University Press, 1956. P 98.

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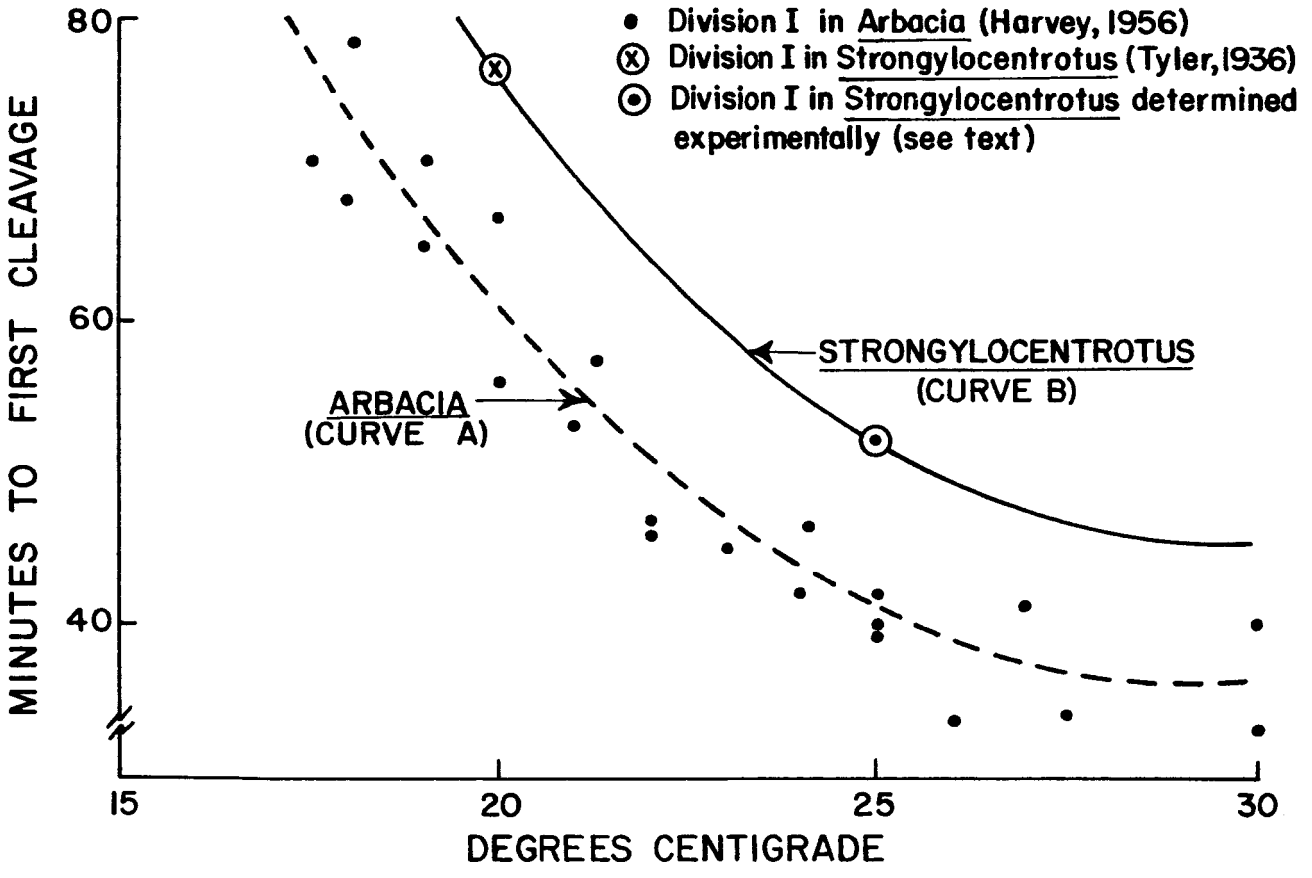

Figure A1

Effect of Temperature on Cleavage

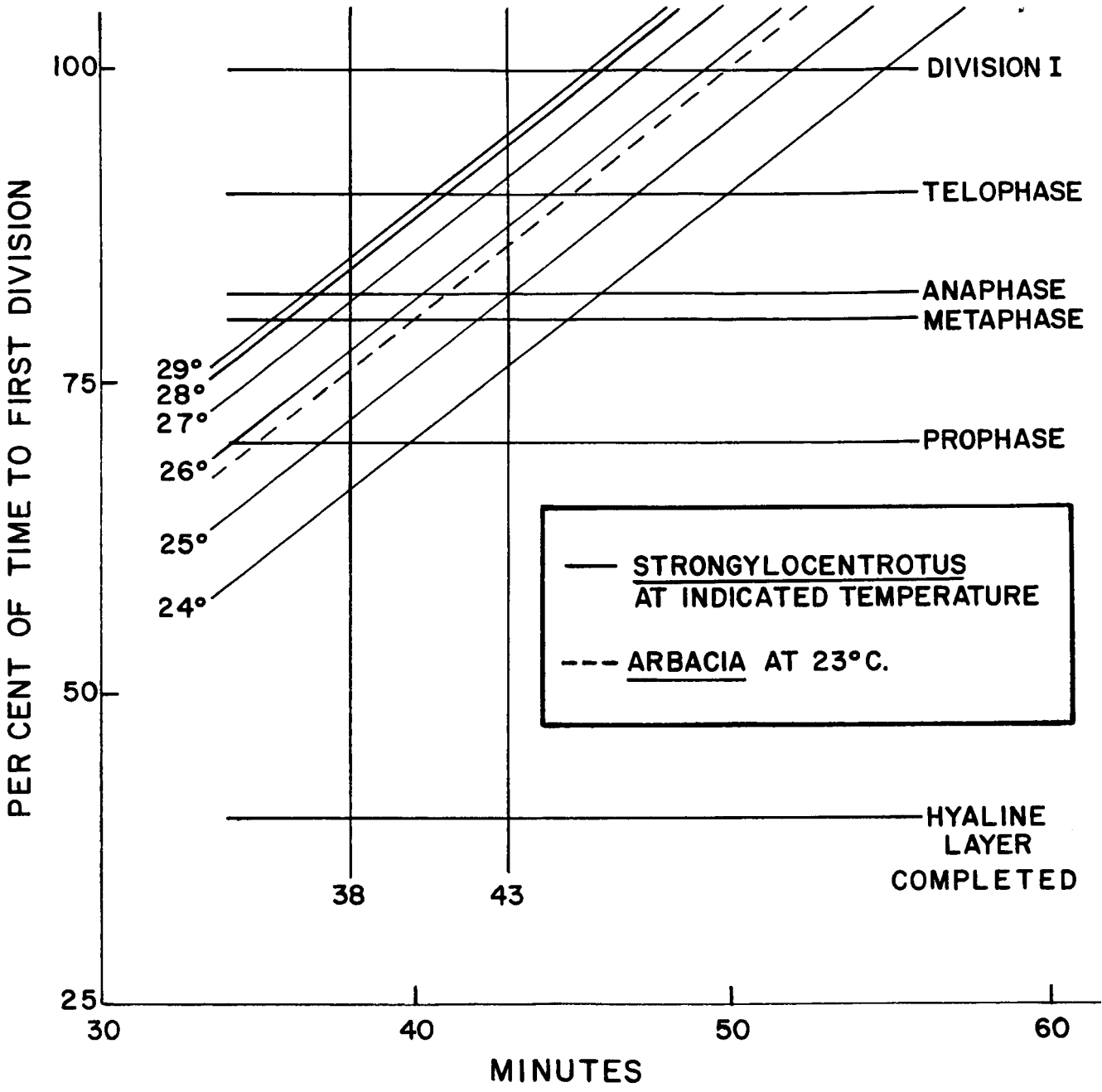

Figure A2

Effect of Temperature on Individual Division Stages

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Sea urchin						
Ova						
Strongylocentrotus purpuratus						
Paramagnetic						
Diamagnetic						
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Nitrogen						
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Mitosis in sea urchin eggs was retarded following their exposure to magnetic fields higher than 70,000 gauss which had gradients greater than 4200 gauss/centimeter. The degree of retardation was correlated simultaneously to both the field strength and the field gradient. Division delay in fields ranging from 100,000 to a maximum of 120,000 gauss was independent of the gradient.

Differences in division delay were found which may be a reflection of the differential migration of oxygen and nitrogen due to their para- and diamagnetic properties.

An hypothesis is given concerning the biochemical processes affected by magnetic fields.

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Biological

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Reno, V. R. 1966

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