TIME RELATIONSHIPS IN THE CLEAVAGE OF THE NORMAL FERTILIZED EGG OF ARBACIA PUNCTULATA

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Cleavage of the egg of the sea urchin *Arbacia punctulata* has been used as the end point in so many physiological studies that quantitative data on the normal time of cleavage and its variability may be useful to many workers. Such data were obtained from samples of eggs which served as controls for the experiments on the effects of ultraviolet radiation described in the preceding paper (Blum and Price, 1950), and are summarized here.

Methods of Counting

In the previous paper two methods for determining cleavage time were described, but the methods for counting the eggs were not included.

Method 1

In this method samples of eggs were fixed at various times in the course of cleavage and stored in small vials. The percentage of eggs in a given stage of cleavage was determined for each vial by counting with the microscope. By plotting the percentage of eggs cleaved against the time from fertilization, the time of cleavage of 50 per cent of the eggs may be determined. This method is generally satisfactory for dealing with normal cleavage, but when cleavage is interfered with; *e.g.*, by dosage with ultraviolet radiation, it may be unreliable. Moreover, this method gives no information regarding the behavior of individual eggs.

Method 2

Successive photographs of a sample of 60 to 100 eggs in various stages of cleavage were obtained with the apparatus described in the preceding paper (Blum and Price, 1950). Each cleavage was represented by about 25 exposures on a strip of 35 mm. film. The exposures, taken at ½ or 1 minute intervals, were numbered successively. To determine the cleavage time of the eggs, the image of the first exposure on a strip of negative film was projected upon a sheet of graph paper; circles were drawn to represent all the eggs in the field and each egg given a number. The successive exposures on the film were then superimposed on this "map" of the field, and for each egg the number of the exposure in which cleavage occurred was recorded in the circle representing that egg on the projection sheet. Since the time corresponding to each frame was recorded during the experiment, the time of cleavage could be determined for each egg by reference to the projection sheet.

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Some abnormal cleavages were found. For instance, an occasional egg goes directly into the four-celled stage. Occasionally, too, an egg changes its position so that it is impossible to be sure of the time of a particular cleavage. These or any other occurrences which made the determination of one cleavage uncertain caused the whole record for the particular egg to be discarded. Assuming that the events which lead to the discarding of certain eggs are random in occurrence, the remaining eggs should constitute a random sample.

TABLE I

Time Relationships for Cleavage as Determined by Method 1 (Based on Cleavage of 50 Per Cent of the Eggs)

Experiment	Time			
	Fertilization to 1st cleavage	1st to 2nd cleavage	2nd to 3rd cleavage	3rd to 4th cleavage
	min.	*min.*	*min.*	*min.*
M-47	52.9	30.3	30.8	30.0
D-47	50.4	29.4	31.6	30.6

TABLE II

Time Relationships for Cleavage, as Determined by Method 2 (Based on Times of Cleavage of Individual Eggs)

Experiment	No. of eggs	Time							
		Fertilization to 1st cleavage		1st to 2nd cleavage		2nd to 3rd cleavage		3rd to 4th cleavage	
		Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
		min.	*min.*	*min.*	*min.*	*min.*	*min.*	*min.*	*min.*
E-48	70	47.6	1.50	31.3	1.21	30.4	2.29	30.4	2.56
H-48	87	50.5	3.03	31.0	1.27	29.0	1.24	29.6	1.47
I-48	99	46.2	1.14	30.0	1.02	29.1	1.63	28.7	1.86
N-48	83	49.6	2.45	31.9	2.13	29.8	2.56	29.3	3.01

Knowing the times for the four cleavages of any given egg, the length of the intercleavage intervals could be determined, and these compared within the population of eggs used in each experiment.

RESULTS

Table I summarizes data for two experiments with Method 1; Table II for four experiments with Method 2. The data obtained with the two methods are in good agreement. The time from fertilization to first cleavage is about 50 minutes. The three later cleavages then follow at nearly equal intervals; approximately 30 minutes at the temperature range (20 to 23°C.) of our experi-

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ments.[1] This constancy of the intercleavage interval after the first cleavage agrees with findings for other echinoderm eggs; those of Gray (1926–27) for *Echinus miliaris*, and of Moore (1933) for *Strongylocentrotus franciscanus*, *Dendraster eccentricus* and cross-fertilized eggs of these two species. In all these cases the interval between fertilization and first cleavage is longer than the intervals between the next three or four cleavages, the latter being very nearly equal.

The interval between fertilization and first cleavage includes events that do not occur in the subsequent intercleavage intervals. First, time is required for the fertilization of the eggs, although this is short; most of the eggs show fertilization membranes by the end of 2 minutes under the conditions that we have used. After fertilization, some time must be required for the fusion of the pronuclei, and possibly for other events, before the cleavage cycle proper begins. Gray (1926–27) has made the assumption that after the fusion of the pronuclei the process of cell division goes on at the same rate as is observed for the later cleavages. In the case of the *Arbacia* egg, this puts the time of fusion of the pronuclei at about 20 minutes after fertilization of the eggs. This extrapolation should, of course, be accepted with reservation.

The data obtained with Method 2 permit an estimate of the variability of the cleavage intervals among individual eggs; this is measured by the standard deviations, which are presented in Table I. The standard deviations are fairly consistent for a given sample of eggs, but vary somewhat more for different sets of eggs. For reasons obvious from the above discussion the intervals between fertilization and first cleavage should be treated separately from the intervals between later cleavages. A histogram based on the latter only—that is, including for each of the four experiments represented in Table I, the three intercleavage intervals following the first cleavage—is presented as Text-fig. 1. It indicates a fairly normal distribution with some skew.

These data give an idea of the variability to be anticipated in cleavage times of individual eggs, since about one-third of the eggs can be expected to deviate from the mean cleavage time by more than the standard deviation. Thus, while it may not be necessary to observe a very large number of eggs in order to get a reasonably accurate figure for the intercleavage interval, considerable error may be introduced if observations are based only on single eggs. This is particularly important to keep in mind when the normal cleavage rate is subjected to experimental interference.

When the standard deviation for the first cleavage is compared with those for the second, third, and fourth, general agreement is found. This suggests, though it does not prove, that the principal variability in the first cleavage is

[1] The temperature fluctuation during any given experiment followed that of the sea water used to cool the moist chamber; this was usually not over 1°, and whatever fluctuations occurred should have affected equally all the cells in a given experiment.

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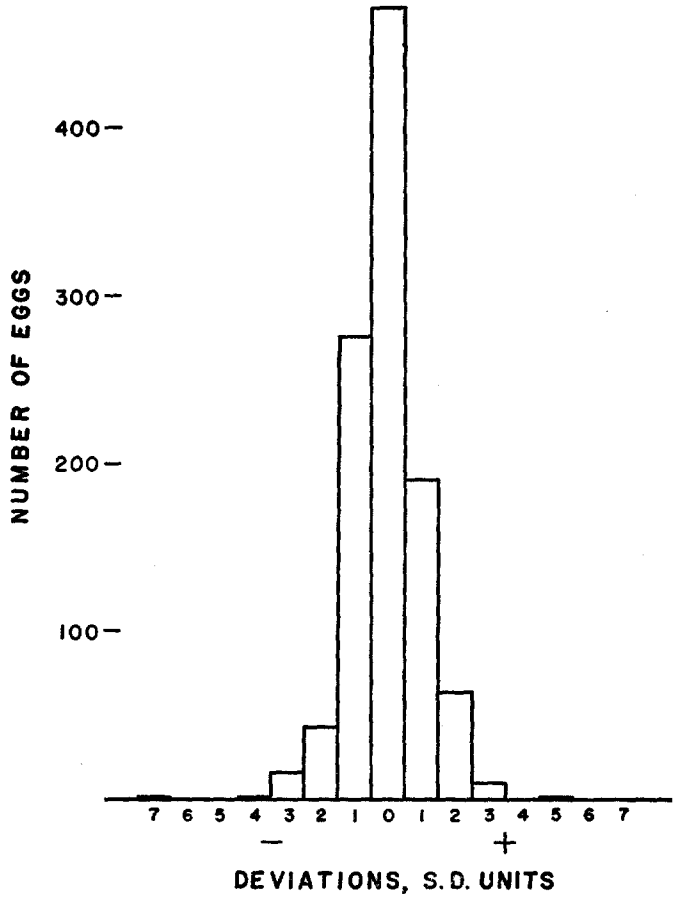
due to variability in the same processes that are concerned in the second, third, and fourth cleavages; that is, the events, such as fertilization and fusion of the pronuclei, do not contribute greatly to the variability.



TEXT-FIG. 1. Distribution of frequency of intercleavage intervals, calculated from group means.

For all cleavages there is a certain interval of time between the cleavage of the first and of the last eggs. This difference may be greatly increased when there is experimental interference (see Blum and Price, 1950, Figs. 4 and 5). It is important to know whether the earliest eggs to cleave are those with an inherently faster rate of cell division, the last to cleave those with a slower rate; or whether their position in the cleavage of the population as a whole is accidental; *e.g.*, due, say, to differences in time of fertilization. Plotting the time

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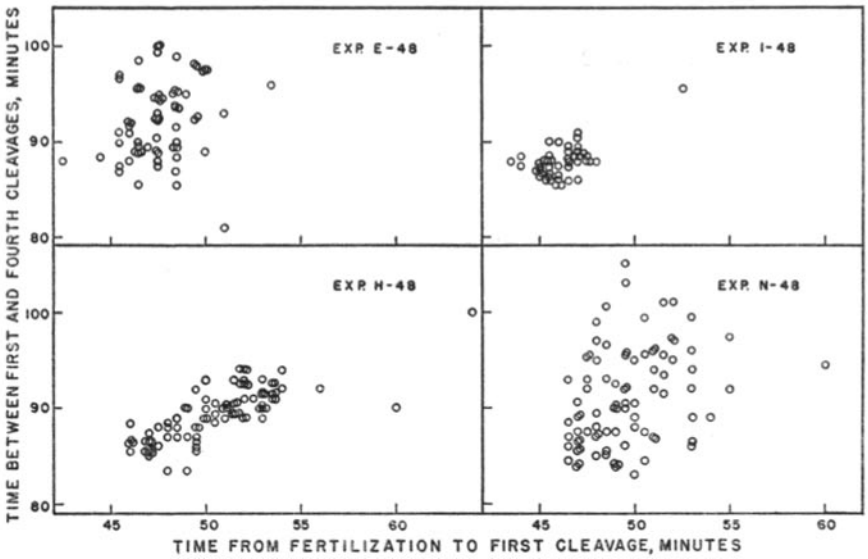
of the first cleavage against the interval between the first and fourth cleavages would seem the best index of a correlation, if one exists. Data for the four experiments listed in Table II are plotted in this way in Text-fig. 2. In one case the correlation seems clear cut, although there is considerable scatter; in the others there is little or no evidence of a correlation. It may be concluded that, while there is some correlation between order of cleavage within the population and inherent rate of cell division, this correlation is usually of a low degree. There do seem to be occasional cells which are very slow in cleaving, but these may be



TEXT-FIG. 2. Scatter plots for individual eggs, for the four experiments of Table II. (See text.)

moribund cells that will tend to be thrown out in counting since they fail to go through four cleavages within the time of the experiments. Such cells might introduce considerable error into measurements with the first method, however. The causes of variation of the individual eggs in a sample we have not attempted to assess. It obviously differs somewhat with the sample of eggs, and this may possibly be due in part to slight differences in treatment, although we believe the differences cannot have been greater than those introduced in other methods commonly used.

SUMMARY

Quantitative data are presented indicating the extent of variability in the time of cleavage of normal fertilized eggs of *Arbacia punctulata*.

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