

DELAY OF CLEAVAGE OF THE ARBACIA EGG BY  
ULTRAVIOLET RADIATION

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PLATE 2

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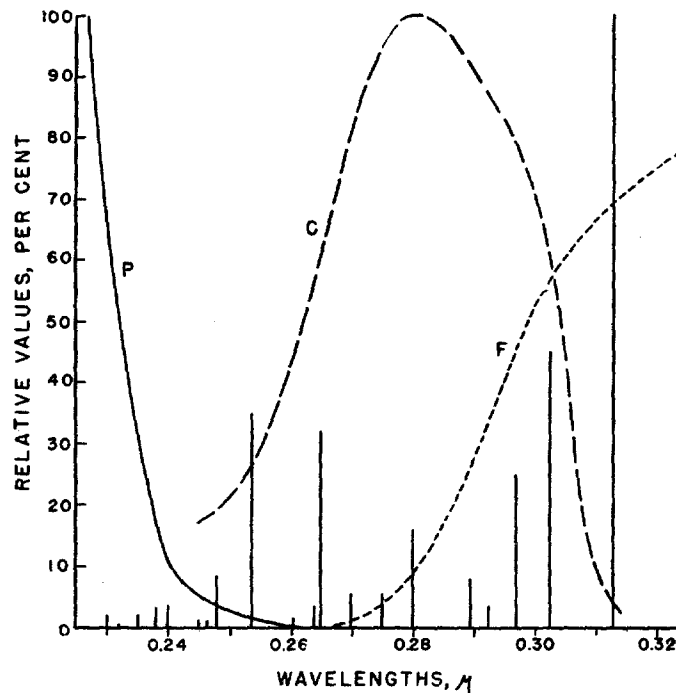
This investigation was undertaken as part of a study of the effect of ultraviolet radiation on cell division, the echinoderm egg being well adapted for certain purposes because the time of cleavage can be accurately followed through the first four divisions. The experiments were carried out on the eggs of *Arbacia punctulata* at Woods Hole during the summers of 1947 and 1948. Doses of ultraviolet radiation were applied at various times relative to fertilization, and their effect on subsequent cleavages followed.

It is possible for ultraviolet radiation to exert its effect on various components of the cell, and at various places in the cell. In the case of the sea urchin's egg it is known to produce two effects which do not appear to be closely related. One of these is the raising of the fertilization membrane when the ultraviolet radiation is applied to unfertilized eggs (artificial parthenogenesis); this is followed by a few cleavages of the egg, often rather irregular (Harvey and Hollaender, 1938). Only wave lengths shorter than about  $0.26\mu$  elicit this response, as is indicated in Text-fig. 1 on which curve P is the action spectrum obtained by Hollaender (1938) for this process in the *Arbacia* egg. A second effect of ultraviolet radiation on the sea urchin's egg is the delay of cleavage of the fertilized egg. An action spectrum for this effect obtained by Giese (1938 a, 1946) with eggs of another species, *Strongylocentrotus purpuratus*, shown as curve C in Text-fig. 1, serves to illustrate the difference in the wave lengths which elicit the two effects. In the present study attention focuses on cleavage, and the effect of ultraviolet radiation in raising the fertilization membrane has been virtually eliminated.

In the interim since these experiments were carried out, papers by Kelner (1949) and by Dulbecco (1949) have appeared, showing that light from the visible spectrum tends to enhance the recovery of fungi and bacteria from heavy doses of ultraviolet radiation. Publication of the present paper was therefore delayed in order to determine whether a similar phenomenon occurs in the case of the *Arbacia* egg. Preliminary experiments have now shown that light from the short wave length end of the visible spectrum ( $\sim 0.4$  to  $0.5\mu$ ) markedly accelerates the recovery of normal cleavage rate after delay by ultra-

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violet radiation (Blum, Loos, Price, and Robinson, 1949), and is in fact responsible for a considerable part of the recovery we have observed in our experiments. Our general conclusions remain essentially unaltered by this finding,



TEXT-FIG. 1. Spectral Relationships. The vertical lines represent the position of the mercury lines; the height of each line represents the relative intensity emitted by the type of arc used in these experiments. Curve P, action spectrum for the raising of the fertilization membrane in eggs of *Arbacia punctulata* (Hollaender, 1938). The curve represents incident intensities required to raise membranes on 50 per cent of the eggs. Curve C, action spectrum for the delay of cleavage of eggs of *Strongylocentrotus purpuratus* (Giese, 1946). Curve F, per cent transmission of cortex D filter used to limit the spectrum in some of our experiments.

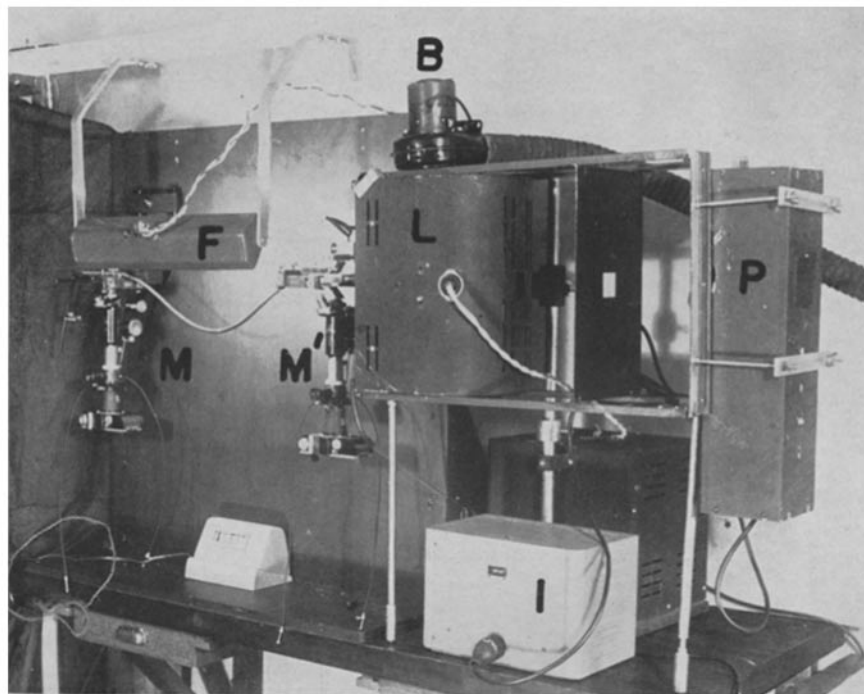
and it seems desirable to withhold publication no longer; but to describe the effects of visible light at a later time.

#### Method

*Ultraviolet Radiation.*—The source of ultraviolet radiation was an “intermediate” pressure mercury arc.<sup>1</sup> The type of arc and the measurement of dosage were the same as those used several years ago in studies of the induction of

<sup>1</sup> Hanovia Chemical and Manufacturing Company, analytic model.

cancer by ultraviolet radiation. They have been described elsewhere in detail, including the method of calibration (Blum, Kirby-Smith, and Grady, 1941), and only a brief description will be given here. The apparatus is shown in Text-fig. 2, where the various parts are indicated. The type of mercury arc employed



TEXT-FIG. 2. Apparatus used for irradiating and observing cleavage in the *Arbacia* egg. *M* and *M'*, inverted microscopes each equipped with micro-ibso and Argus cameras. *F*, fluorescent lamp used for illumination of both microscopes for photography. *L*, housing containing the mercury arc, equipped with exhaust blower, *B*. *P*, photocell arranged to receive a sample beam from the back of the arc. *I*, impulse counter for recording the impulses from the photocell.

has the advantage that the relative intensity of the various lines does not vary greatly with changes in temperature or voltage; that is, although the intensity as a whole fluctuates, the spectral quality of the light does not change. This makes it possible to measure the dosage of ultraviolet radiation by means of a photocell. The photocell was one devised by Rentschler (Rentschler, Nagy, and Mouromseff, 1941), used with a counting device developed by Kuper, Brackett, and Eicher (1941), it is activated only by wave lengths shorter than about  $0.32\mu$ . The apparatus is so arranged that, each time a given quantity of

such radiation has impinged upon the cathode of the photocell, an electrical pulse activates the counting device and is recorded by a telephone relay. By appropriate calibration, one can measure the dosage of ultraviolet radiation incident on the eggs, each impulse recorded by the relay representing a known quantity of energy delivered. From the rate at which the impulses are recorded, one may also obtain the average intensity. This arrangement permits the dosage of radiation to be reproduced very accurately from one experiment to the next, but estimates in terms of absolute energy values involve a certain degree of error entering from the various steps of the calibration.

In Text-fig. 1 the vertical lines represent the positions of the lines of the mercury spectrum, the height of each line indicating the relative intensity of the corresponding wave lengths. When passed through window glass, this radiation was ineffective in delaying cleavage; hence, all wave lengths greater than  $0.313\mu$  were regarded as ineffective. When the full spectrum was used, as in the 1947 experiments, the delay in cleavage was accompanied by raising of fertilization membranes in a small percentage of eggs, interfering with certain experiments. For this reason, in the experiments carried out in 1948 the radiation was restricted to the longer wave lengths by interposing a corex D glass filter having the transmission indicated by curve F in Text-fig. 1.

#### *Irradiation of Eggs and Determination of Cleavage Time*

The exposure of the eggs to ultraviolet radiation and the determination of the times of the various cleavages were accomplished by two different methods, which served somewhat different purposes.

#### *Method 1*

The method used for the determination of cleavage time in the summer of 1947 is one in fairly common use. Washed eggs in quantity sufficient to form a single layer, covered by a few millimeters of sea water, were placed in a large flat-bottomed dish and fertilized by adding a few drops of a dilute suspension of sperm. The temperature was maintained approximately constant by placing the dish in running sea water. The time of fertilization was recorded, and samples of eggs taken at appropriate times thereafter were fixed immediately by placing them in vials containing a few drops of 5 per cent formalin. The fixed eggs were then counted to determine the proportion which had undergone cleavage at the time of fixation. By plotting the time after fertilization against the percentage of eggs cleaved, curves such as those in Text-figs. 4 and 5 were obtained. Cleavages up to the 16 cell stage may be readily followed in this way, but after this, counting becomes difficult. Irradiation was carried out in a chemical hood in order to avoid accumulation of gases formed by the arc. The eggs were exposed in the dish in which they were fertilized, the arc being at 50 cm. distance with no filter interposed. Under these conditions, the intensity of the radiation was approximately  $4 \times 10^4$  ergs  $\text{cm.}^{-2}$   $\text{sec.}^{-1}$  for the total of wave lengths  $0.313\mu$  and shorter. The doses ranged from 5 to 60 seconds.

*Method 2*

For reasons which will become clear later, the above method leaves considerable to be desired (see also Blum and Price, 1950) when the action of an agent such as ultraviolet radiation is to be studied. A second method was therefore employed in the summer of 1948, which made it possible to follow photographically the first four cleavages in a sample consisting of 60 to 100 eggs. This method has the advantages that the timing is more accurate for photographing than for fixation; and, more important, the time of cleavage can be followed for individual eggs. Text-fig. 2 is a photograph of the apparatus, showing two inverted microscopes *M* and *M'* with cameras attached.<sup>2</sup> The sample of eggs to serve as a control was placed on the stage of one microscope (*M*), that to be irradiated on the stage of the second (*M'*).

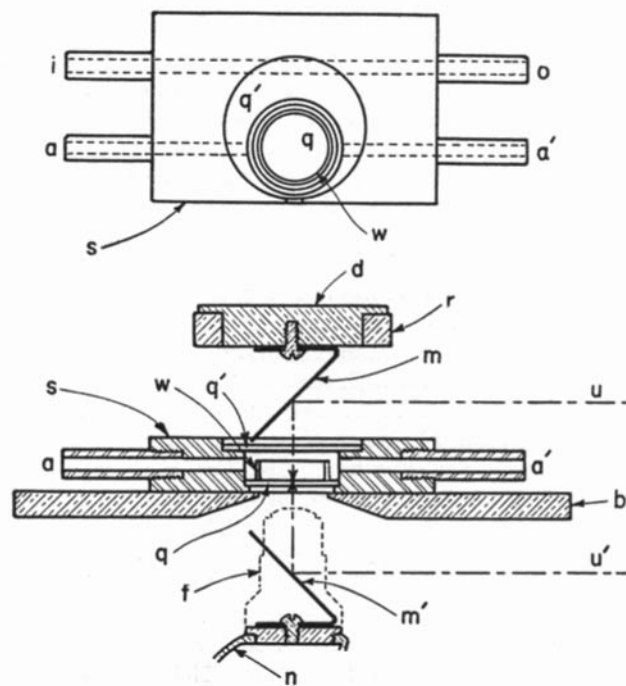
The vessels and moist chambers which contained the eggs were of identical pattern, constructed as shown in Text-fig. 3. Each vessel for holding the eggs was made from an optically flat plate of fused quartz 1 mm. thick onto which a glass ring 5 mm. high and 17 mm. in diameter was sealed by means of beeswax and vaseline. The chambers in which the vessels containing the eggs were placed were constructed of stainless steel and each was provided with a tubular opening through which sea water could be passed. The same stream of sea water passed through both chambers, which were thus maintained at the same temperature, the relatively large mass of metal serving to buffer against any sudden changes in the temperature of the room air. An optically flat plate of fused quartz formed the cover of each chamber. Inlet and outlet were provided for passing a stream of moist air through the chamber but this was not found to be necessary under the conditions of humidity at Woods Hole, and they were merely left open to the outside air. The suspension of eggs was adjusted so that no more than a single layer settled to the bottom of the containing vessel; under these conditions they cleaved normally, and if left in the chamber developed into normal plutei. Samples of identically treated eggs from the same animal behaved in identical fashion in the two chambers, indicating the uniformity of the conditions.

For observation and photography, an ocular 10 X and objective 10 X were used. A "fluorescent" lamp with two 15 watt burners, indicated by *F* in Text-fig. 2, served as illumination for observation and photography. The light was reflected into the microscope with a concave mirror, no condenser being used.<sup>3</sup> Photographs were made with Argus cameras adapted for use with the Leitz micro-ibso attachment, using 35 mm. panatomic-X film. Exposures were for 1 second..

The eggs were fertilized in a large dish as in the previous method; then after allowing about 5 minutes for complete fertilization, a sample was placed in the quartz-bottomed vessel on the stage of the microscope. A field containing 60 to 100 eggs in a single layer was then found for each of the microscopes. About 25 photographs were taken during each cleavage, usually at  $\frac{1}{2}$  minute intervals.

<sup>2</sup> The microscopes were standard Bausch and Lomb models which were inverted by means of a few simple attachments without any alteration of the microscope itself. The design and construction were carried out by Mr. Russell Mycock.

<sup>3</sup> It was this light which was found, subsequently, to have a marked effect in accelerating recovery of the eggs after exposure to ultraviolet radiation (Blum, Loos, Price, and Robinson, 1949).



TEXT-FIG. 3. Diagram of chamber holding eggs for observation, and arrangement of mirrors for exposing the eggs to ultraviolet radiation. A cross-section of the apparatus is shown below; above a top view of the chamber holding the eggs. The vessel holding the eggs consists of a quartz plate, *q*, forming the bottom, with a glass ring, *w*, sealed to it to complete the chamber; the eggs settle onto the quartz plate, *q*, at the position indicated by the converging arrows. A stainless steel chamber, *s*, encloses the vessel holding the eggs. This chamber, *s*, moves about freely on the stage of the microscope, *b*, being manipulated by a mechanical stage not shown in the diagram. A quartz plate, *q'*, serves as cover for the chamber which is open to the outside air through the tubes *a* and *a'*. A stream of sea water is passed through a tubular opening in the stainless steel chamber by means of the inlet and outlet, *i* and *o*; the same stream of water passes through both the control and experimental chambers, which are exact duplicates.

Two aluminum mirrors, *m* and *m'*, serve to direct beams indicated by *u* and *u'*, from the mercury arc onto the eggs. The dotted outline, indicated by *f*, represents the objective of the microscope when this is in position for observation and photography. The lower mirror, *m'*, is mounted on the revolving nosepiece, *n*, of the microscope in the position that would ordinarily carry a second objective; thus, this mirror, and the microscope objective can be quickly interchanged. The upper mirror, *m*, is mounted on a brass disk, *d*, which fits into the ring ordinarily holding the condenser of the microscope, adjustable vertically by means of a ratchet. By placing a grease spot on top of the quartz plate, *q'*, at the level of the eggs to serve as a photometer the upper mirror may be adjusted to a position so that the incident radiation from top and bottom is equal at the center of the field where the eggs under observation are located.

Photographs showing the eggs in different stages appear in Figs. 1 to 4. The eggs retain their positions remarkably well once they are settled on the bottom of the dish, as can be judged from comparison of the photographs, so that it is never difficult to identify a particular egg even after the fourth cleavage. The detail of the photograph is sufficient to permit identification of the stage of cleavage up to the fourth. The asters can be observed as they form and divide. The method of counting is described in the following paper (Blum and Price, 1950), which deals with the normal behavior of these eggs, their variability, and the accuracy of these measurements.

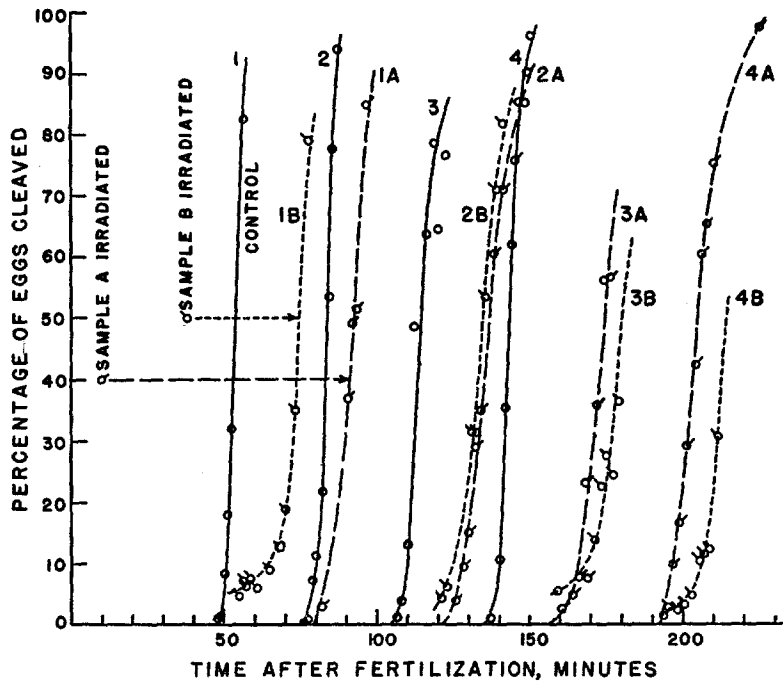
The eggs could be irradiated at any desired time without disturbing their positions on the microscope stage, by means of two plain aluminum mirrors placed so as to direct one beam from the mercury arc upon the eggs from above, another beam from below as shown in Text-fig. 3. The lower one of these mirrors was mounted in the place of an accessory objective on the revolving nosepiece of the microscope, so that it could be thrown quickly into position for the irradiation. The upper mirror was mounted on the ring which ordinarily carries the microscope condenser, the position of which is adjustable by means of a ratchet. The mirrors were adjusted so that the beams impinging upon the eggs from top and bottom were of the same approximate intensity, as determined previous to the experiment by "grease spot" photometry. The dose delivered to the eggs including that incident from both sides was  $\sim 150$  ergs per egg, of radiation of wave lengths 0.27 to 0.313 $\mu$ . This was delivered in approximately 70 seconds.

#### RESULTS

Certain general relationships are illustrated in Text-figs. 4 and 5, which represent experiments done with Method 1. In Text-fig. 4 the solid line represents the times of cleavage of normal eggs not subjected to ultraviolet radiation. These curves show that fifty per cent of the eggs had undergone first cleavage at 53 minutes after fertilization and that cleavages 2, 3, and 4 followed at intervals of 30 minutes thereafter. This is normal behavior for the fertilized *Arbacia* egg (see the following paper).

The other curves in Text-fig. 4 represent two samples of eggs from the same animal, which were each subjected to the same dose of ultraviolet radiation, but given at different times in the period between fertilization and first cleavage. Sample A was irradiated 10 minutes after fertilization with a dose of ultraviolet radiation of 11 seconds' duration. The result is a very considerable delay of the first cleavage, which occurs about 40 minutes later than in the control. The second cleavage is also delayed, the third to a less extent, while the fourth occurs at about the normal interval, 30 minutes, after the third. Recovery had obviously taken place; rate of cell division tending toward return to the normal. In Experiment B, the dose of ultraviolet radiation was introduced 28 minutes after fertilization, not long before the time at which the first cleavage would occur in untreated eggs. The first cleavage is not so much delayed as in the first sample, but the second cleavage is delayed to a greater extent so that

it occurs at about the same time as in sample A. Again as in sample A, the third and fourth cleavages tend to catch up, and to regain the normal rate of cell division. Ultimately, eggs treated with such doses of ultraviolet radiation develop into normal plutei, the only apparent difference from the control being that they are somewhat delayed.



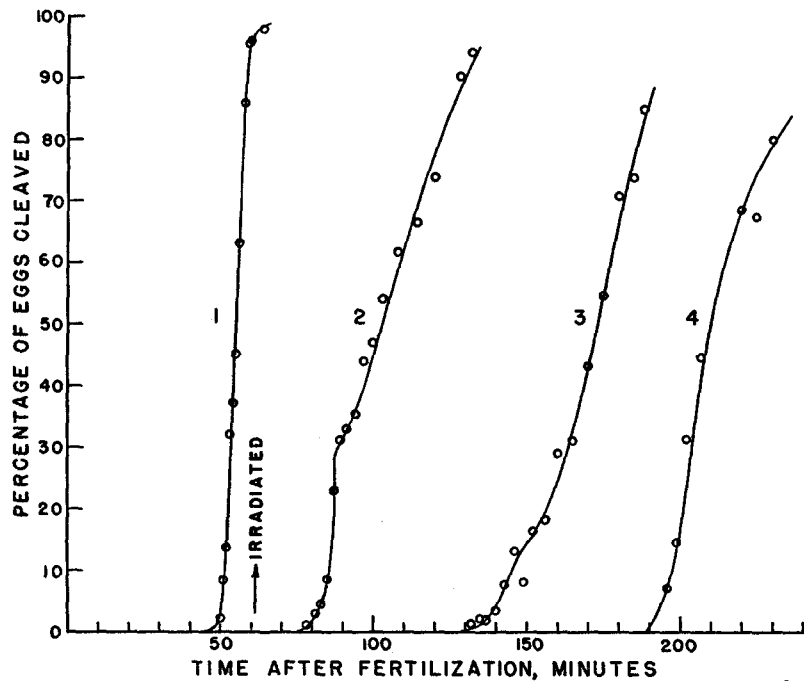
TEXT-FIG. 4. Effect on cleavage of ultraviolet radiation applied between fertilization and first cleavage. Percentage cleavage curves obtained by Method 1, showing the time sequence for the first four cleavages of fertilized *Arbacia* eggs. Control and two experiments A and B in which doses of ultraviolet radiation were applied 10 minutes and 20 minutes after cleavage, respectively. The dose in each case was approximately  $5 \times 10^6$  ergs persq. cm., inclusive of wave lengths  $0.313\mu$  and shorter, delivered in 12 seconds.

Text-fig. 5 represents an experiment in which a comparable dose of ultraviolet radiation was introduced just after the first cleavage. Thus, the curve for the first cleavage is normal and serves as a control which may be compared with those for the later cleavages. Referring to the curve for the second cleavage, it is seen that the early part of the curve is very little affected, but the later part is considerably delayed beyond what would be expected for untreated eggs. The third cleavage is on the whole delayed more than the second, but return toward the normal cleavage rate is evident in the fourth cleavage.

The results represented in Text-figs. 4 and 5 may appear confusing upon first



examination, but become readily interpretable in terms of the results obtained later with the second method. It is obvious from examination of these figures that when a dose of short duration is applied to a population of eggs it does not find them all in exactly the same stage of the cell division cycle, there being a difference of 10 minutes or more between the cleavage of the first and last egg

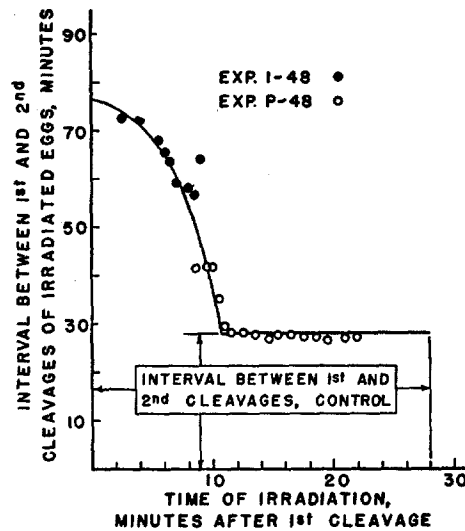


TEXT-FIG. 5. Effect on cleavage of a dose of ultraviolet radiation applied after the first cleavage. Percentage cleavage curves obtained by Method 1. A dose of  $2 \times 10^6$  ergs per sq. cm., inclusive of wave lengths  $0.313\mu$  and shorter, delivered in 8 seconds was applied 61 minutes after fertilization. Note the extreme distortion of the curves after the irradiation.

in a normal population which has not been irradiated. With our second method it is possible to follow the time of cleavage of individual eggs, and so to know just when a particular egg cleaves and when the dose of radiation is received by this egg. The relationships are clearest when the radiation is applied after the first cleavage, since one can use the time at which the egg has undergone first cleavage as a point from which to measure. Knowing from the control the average time between the first and second cleavages, one can estimate with reasonable accuracy (see Blum and Price, 1950) the time at which the second cleavage would have occurred had no radiation been given. Thus, the time of

application of the radiation can be established with reference to the normal intercleavage interval.

The effect of a dose of ultraviolet radiation falling between the first and second cleavages is illustrated in Text-fig. 6 by typical results from two comparable experiments. It is seen that if the dose of radiation is applied in the latter part of the normal intercleavage interval the second cleavage is not delayed, but if applied during the first part of the normal interval the second cleavage is delayed. The earlier the dose is applied, the greater is the delay of the second cleavage.

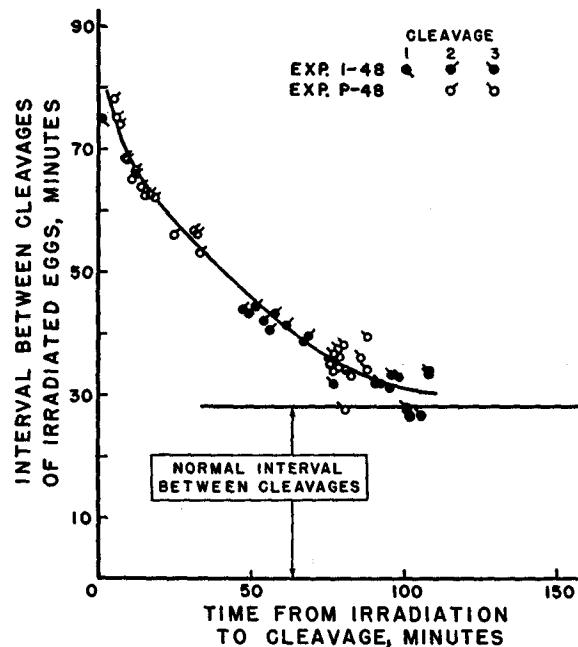


TEXT-FIG. 6. Effects on the intervals between first and second cleavages, of ultraviolet radiation introduced between first and second cleavages. Data obtained by Method 2. Two experiments are represented. Each point represents one or more eggs.

That the effect of the radiation persists after the end of the exposure is shown clearly by the experiments previously discussed. Since the processes of cell division proceed normally up to the time the radiation is applied, it is to be expected that the delay of cleavage would be greater when the dose is applied earlier. Since, however, the effect of the radiation is recovered from, and quite rapidly at first, as we shall see, the shape of the curve indicated in Text-fig. 6 cannot be assigned quantitative significance.

If the progress of recovery from the effects of irradiation is to be studied, the delay of successive cleavages must be compared. It is necessary, however, to compare the length of the successive cleavage intervals in terms of the time elapsed after the application of the radiation, and there is difficulty in choosing the point in the cell division cycle which should be compared. The effect of the

changes brought about by the radiation must be integrated over the cell division cycle, but since the effect is not uniform throughout this cycle (see Text-fig. 6) there is no very sound basis on which to make this integration. The next best thing is to choose an accurate point in the cell division cycle for purposes of comparison. The best criterion available seems to be the completion of cleavage, since this marks the beginning of the next cell division cycle. Hence, in Text-fig. 7, we have chosen to plot the length of the interval between any



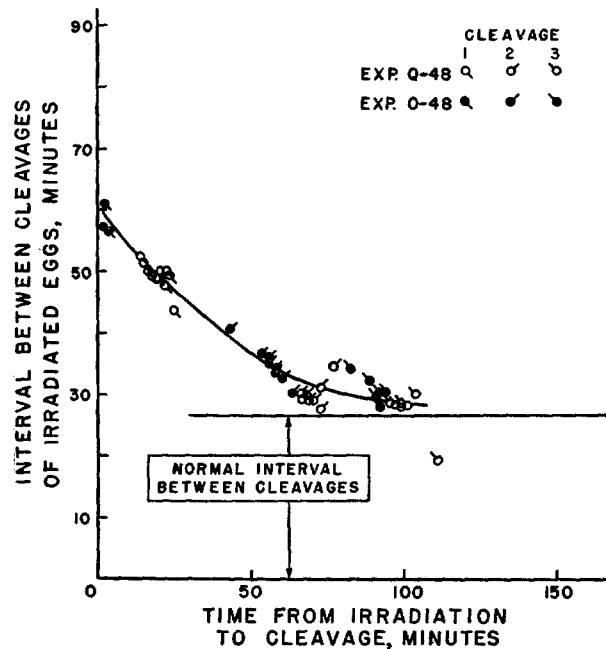
TEXT-FIG. 7. Recovery from the effects of ultraviolet radiation applied between first and second cleavages. Data obtained by Method 2. The same two experiments are represented as in Text-fig. 6, and the same groups of eggs.

two successive cleavages, *vs.* the time from irradiation to the cleavage marking the beginning of that interval. The data plotted in this figure represent later cleavages in the same experiments as those described in Text-fig. 6. A number of points which represent the same groups of cells can be identified in the two figures.

Plotted in the above way the points fall along a smooth curve, the interval between cleavages falling off progressively with the time elapsed after the application of the radiation. For reasons which should be obvious from the above discussion, no rigidly quantitative significance can be attributed to the shape of this curve, but that the effect of the radiation falls off progressively

in a regular manner is clearly shown. It would have been difficult to demonstrate this with Method 1, since the points obtained represent the average effect upon a population, which, because of uncontrollable time relationships, has not all been treated in exactly the same way.

Recovery followed much the same course when the ultraviolet radiation was applied between fertilization and first cleavage, as when it was applied between the first and second cleavages. Text-fig. 8 represents data from two typical

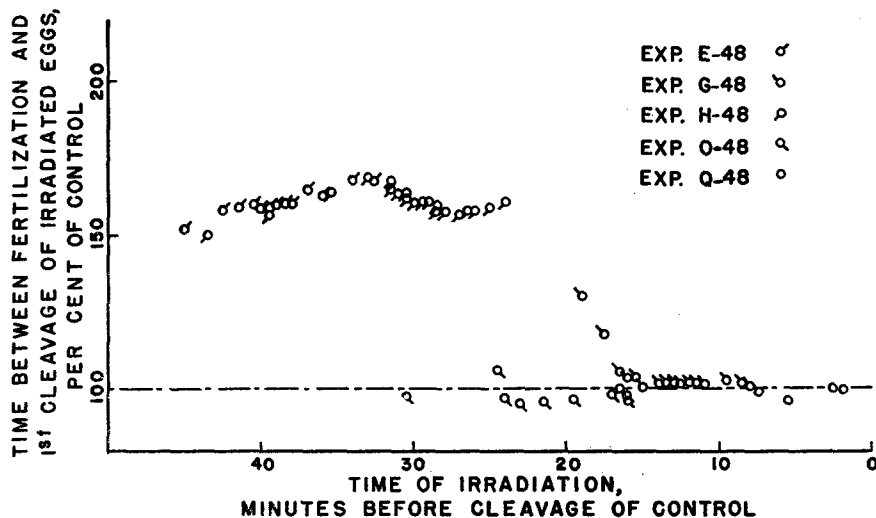


TEXT-FIG. 8. Recovery from the effects of ultraviolet radiation applied before first cleavage. Data obtained by Method 2. Two experiments are represented.

experiments of this type; the plotting is the same as that in Text-fig. 7, the only difference being that three cleavage intervals are involved instead of two. Recovery is of the same order, the normal cleavage interval being reached about 100 minutes after irradiation, no matter whether this occurs before or after the first cleavages.

The relationships described in Text-fig. 6 are also found when the ultraviolet radiation is introduced before the first cleavage, but are not so clearly demonstrable. It is to be remembered that the interval between fertilization and first cleavage includes certain events not represented in the interval between the first and second cleavages. A part of the time is required for the sperm to reach and penetrate the egg, although this is probably short for the majority

of the eggs. Following this, time elapses until the fusion of the pronuclei. Whether or not one can assume that subsequent events correspond quantitatively with those that follow the first cleavage, as assumed by Gray (1926-27), is difficult to judge. Under the circumstances there is no way of knowing the point in the cell division cycle at which a given dose of ultraviolet radiation finds the egg; and any comparison is, under these conditions, more or less arbitrary. In Text-fig. 9 the time from fertilization to first cleavage of the irradiated eggs is plotted as the percentage of the same interval for the untreated eggs *vs.* the time of irradiation as measured back from the cleavage time of



TEXT-FIG. 9. Effect on the interval between fertilization and first cleavage, of ultraviolet radiation introduced during this interval. Data obtained by Method 2. Five experiments are represented including the two included in Text-fig. 8.

the control. It is evident that there is a period, say, of 20 to 30 minutes, just prior to the first cleavage during which application of ultraviolet radiation does not affect that cleavage, although there is considerable quantitative disparity between the different experiments. Part of this disparity may result from differences in the samples of eggs and part from inherent uncertainties in measurement, which will now be discussed.

When a dose of radiation is introduced after the first cleavage, one has an exact point from which to measure, that is, the completion of first cleavage. On the other hand, when the radiation is introduced between fertilization and cleavage, in experiments such as ours, there is no way to determine the stage in the cell division cycle at which a given egg finds itself at the moment of irradiation. The data represented in Text-fig. 6 show that the magnitude of the

delay varies with the point in the cycle of cell division at which the radiation is applied. The earlier it is applied, the greater is the delay. Suppose, now, the irradiation falls some time, say 30 minutes, before the normal cleavage time. In the population of eggs irradiated, some are in one stage of cell division, some in another. Those that are in earlier stages when irradiated should be delayed more than those that are in later stages, but there is no way of knowing whether or not the latest cleaving eggs are those which were the most affected by the radiation, and a degree of uncertainty is involved which obscures the quantitative relationships. Because of this uncertainty, no attempt has been made to draw a curve through the points shown in Text-fig. 9. Qualitatively, at least, the results of irradiation within the interval between fertilization and first

TABLE I  
*Relative Effects of Ultraviolet Radiation Applied before and after Cleavage. (Based on Weighted Averages)*

Time of irradiation* relative to fertilization	Time from irradiation to first cleavage	Time between fertilization and first cleavage of irradiated eggs (per cent of control)
	<i>min.</i>	
8.5 min. after	77.5	173
3.0 min. before	67.5	145
29.0 min. before	97.0	136
70.0 min. before	131.0	109
120.0 min. before	175.0	117

\* ~ 150 ergs of radiation of wave lengths  $0.27\mu$  to  $0.313\mu$  incident per egg. Applied from top only. Duration of dose approximately 2 minutes.

cleavage are similar to those when the radiation is introduced between the first and second cleavages, and although we did not test the possibility it seems certain that the same is true for subsequent cleavages.

The uncertainty involved in studying the effect of ultraviolet radiation in delaying the first cleavage—where one deals with a composite effect, and where he lacks a proper point of comparison—emphasizes that caution is necessary in the interpretation of results obtained with this or any other agent when only the effect on the time to first cleavage is measured. More favorable for studies on cell division is the interval between the first and second cleavages, and it is obvious that much may be learned by following the effect on subsequent intercleavage intervals.

Studies of the effect of irradiation before fertilization required a slight modification of method, in that the whole sample of eggs in the chamber had to be irradiated and then fertilized at the desired time. After this a field was selected for study. Thus, the identical group of eggs could not be followed before and after irradiation, as was the case when the radiation was applied after fertili-

zation. In addition, restrictions in the shape of the chamber made it advisable in this case to irradiate the eggs from one side only. The same dose was given, but since it was applied from only one side the duration had to be twice as long. Hence, these experiments are not strictly comparable with those that have been described above, although, within the limits of error, the delay in cleavage is the same whether the eggs are irradiated from both sides, from top or from bottom, so long as the total dose applied is the same in each case.<sup>4</sup>

In Table I are presented data from several experiments in which the ultraviolet radiation was applied before or just after fertilization. For reasons similar to those discussed above, there is uncertainty as to the correct basis of comparison, and some arbitrariness is unavoidable. Experimentally, fertilization is the best point for comparison, and the time of irradiation relative to this event is given in the first column of the table. The time from irradiation to first cleavage of the irradiated eggs is shown in the second column. In the third column the delay of the first cleavage is treated in the same way as in Text-fig. 9; that is, as relative to the time required for the normal first cleavage. It is evident from the values in this last column that recovery takes place in the egg previous to fertilization, since the longer before fertilization the radiation is applied the less is the delay of first cleavage.

#### DISCUSSION

In the phenomenon under study, the first act must be the absorption of ultraviolet radiation by some component of the egg; but our experiments furnish no direct information regarding the nature of the light-absorbing substance nor of the resulting photochemical reaction. Only the effect of these events in delaying cell division is reflected in our measurements.

Whatever the nature of the photochemical changes, their effect is reversible, in a biological sense, since the egg recovers completely, returning to its normal rate of cell division and subsequent normal development. This recovery, insofar as the experiments show, is quite independent of cell division itself. The smooth curves in Text-figs. 7 and 8 indicate this independence; and, furthermore, recovery takes place before fertilization when cell division is not going on, as is shown in Table I.

The nucleus undergoes dramatic changes during the cell division cycle, and it seems difficult to believe that these would not affect recovery if the immediate effect of the radiation were on the nucleus. Although adequate absorption data are lacking, it may be estimated that only a small fraction of the incident radiation reaches the nucleus. Thus, while the exact locus of action of the

<sup>4</sup> With this method the unilateral effects on fertilization membrane, etc., described by Spikes (1944) would not have been observed. Since he used a shorter wave length (2537Å), it is doubtful that these effects would have been induced in our experiments (see Text-fig. 1).

ultraviolet radiation cannot be definitely decided without further information, the cytoplasm seems the more probable.

Delay of cell division appears to be secondary to the immediate effects of the radiation. From the curves in Text-figs. 7 and 8 it may be deduced that the amount of delay is proportionate to the extent to which the changes produced by the ultraviolet radiation persist at the time a particular cleavage cycle is going on; but reference to Text-figs. 6 and 9 shows that there is a sharp change during the cell division cycle in the sensitivity of the egg to these changes; if irradiation occurs after a certain point, the cleavage immediately following is not delayed.

While it has not been possible to follow the nuclear changes in detail with our method, the time of onset of the insensitive period seems to correspond roughly with the beginning of mitosis, as though once the cell division process has gone beyond a certain point it is difficult to hinder it. This indication of a correlation between sensitivity to the effects of radiation and nuclear changes supports the idea that the delay in the cell division process has its origin in the nucleus; that is, as regards the mechanism of cell division, the nucleus is the point of impingement of the effect of changes resulting from the photochemical reaction. On the other hand, while there is no change in the cytoplasm so overt and dramatic as the initiation of mitosis in the nucleus, changes do occur there at about the same time, as is indicated by the sudden decrease in viscosity which occurs in both cytoplasm and nucleus (Heilbrunn, 1920, 1921; Heilbrunn and Wilson, 1948; Carlson, 1946).<sup>5</sup>

Without knowing the exact locus of these effects in the cell, the mode of action of the ultraviolet radiation can only be treated in a general way. At least some of the changes which take place during cell division involve the expenditure of free energy which is derived from stored chemical potential released by metabolic activity. Anything which interferes with the effective utilization of this free energy might be expected to slow cell division without bringing about permanent changes in the cell; energetically this would amount to the "wasting" of a certain amount of the stored chemical potential. The transient slowing of cell division without detectable permanent alteration in the organism, which is observed in the *Arbacia* egg after exposure to ultraviolet radiation, is in keeping with such a point of view. The idea that the ultraviolet radiation interferes in some way with the utilization of the energy resources of

<sup>5</sup> Cleavage of the egg and nuclear division have been spoken of collectively above, because in our experiments they appeared closely coordinated, nuclear division always preceding cleavage by a relatively short interval. Examination of Figs. 1 to 4 indicates no disparity between the number of nuclei present and the stage of cleavage of the egg. Discrepancies between nuclear and cytoplasmic division were observed by Nebel, Harvey, and Hollaender (1938) but their doses of ultraviolet radiation were so high that the eggs did not develop beyond the third cleavage.



the cell is an attractive one; but such an effect could be brought about in a number of ways, and the adoption of this hypothesis provides no direct explanation of the primary effect of the ultraviolet radiation.

To what extent may our findings on the eggs of a single species be applied to cell division in general? As regards other echinoderm eggs, the experiments of Giese (1938 *b*) on *Strongylocentrotus purpuratus*, and those of Chase (1938) on *Dendraster excentricus*, when viewed in the light of the present results, seem to demonstrate comparable effects. Chase's observations on the worm *Urechis caupo* suggest the same thing, but the relationships are more obscure. In a much different kind of cell, the grasshopper neuroblast, Carlson and Hollaender (1944) found a period of insensitivity to ultraviolet radiation during the latter part of mitosis, which seems comparable to our findings for the *Arbacia* egg. They also found evidence of recovery, but this appeared to be slower than in these eggs. Thus, their results seem to be in general agreement with ours. It may be pointed out that the shape of the sensitivity curve as illustrated in Text-fig. 6 is determined in part by the shape of the recovery curve, illustrated in Text-fig. 7. If there are, as we postulate, two semi-independent mechanisms involved, one might expect to find a variety of pictures when one studies the effect of ultraviolet radiation on different kinds of cells. It seems reasonable to think that the results of Carlson and Hollaender may be explained on the basis of the same fundamental mechanisms which we postulate, although quantitatively the picture is somewhat different.<sup>6</sup> Giese (1947) reviews other studies of the kind on various types of cells, which offer no obvious contradictions to the findings or interpretations which we present.

#### SUMMARY AND CONCLUSIONS

While our data do not permit us to state the exact locus or mode of action of ultraviolet radiation in the *Arbacia* egg, certain general conclusions may be reached. The amount of delay of cleavage of these eggs is determined by two principal factors: (1) The extent of an effect, resulting from photochemical action induced by ultraviolet radiation, which is reversible in a biological sense, the reversibility not being directly dependent upon the process of cell division.<sup>7</sup> (2) The sensitivity of the cell division process to the effects of the ultraviolet-induced photochemical reaction. This factor varies with the stage of cell division, the cell being insensitive during a period corresponding to most of mitosis.

<sup>6</sup> Since illumination plays such an important role in recovery under the conditions of our experiments (Blum, Loos, Price, and Robinson, 1949), it is possible that differences in this factor might account for any apparent discrepancies between Carlson and Hollaender's (1944) results and our own.

<sup>7</sup> But being influenced by a second photochemical process involving light from the "visible" spectrum.

It seems likely that these findings may apply to cell division in general, but, since the quantitative relationships observed must, in this case, reflect the integration of two semi-independent factors, the over-all picture may appear quite different for different kinds of cells.

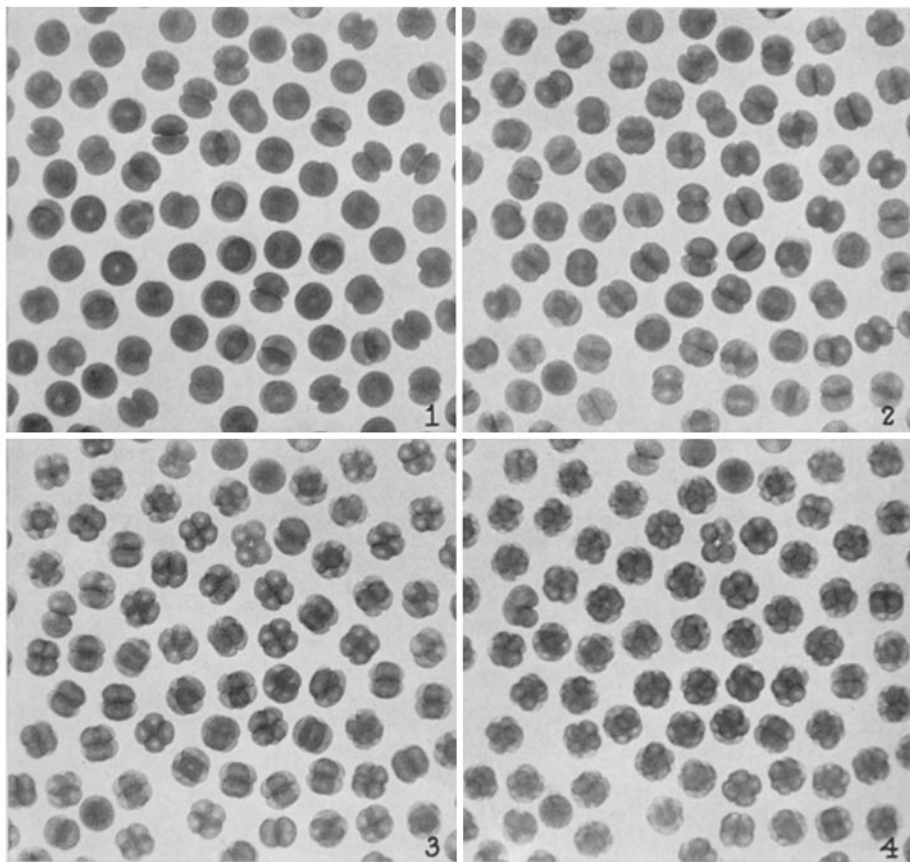
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## EXPLANATION OF PLATE 2

FIGS. 1 to 4. Cleavage stages of eggs of *Arbacia punctulata*. Photographs obtained by Method 2. The same field of eggs is shown about midway in the first, second, third, and fourth cleavages. Note that the position of the eggs relative to each other is virtually the same in all the photographs, and that there is no difficulty in identifying a particular egg in all four, or assigning its stage of cleavage. There are a few unfertilized eggs in this field. Note that the number of nuclei and the number of blastomeres correspond.



(Blum and Price: Cleavage of *Arbacia* egg)