ULTRAVIOLET LIGHT AND THE MITOTIC CYCLE IN THE SEA URCHIN'S EGG

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PLATE 5

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The amount of delay in cell division brought about by ultraviolet radiation or x-rays varies with the time in the cell division cycle at which the exposure is made. This commonly has been related to the events of the nuclear changes of mitosis, but such interpretation often fails to take into account certain complicating factors. If the dose of radiation is given too late in the cell division cycle there may be no delay of the cell division at the end of that cycle (Giese, 1938; Henshaw and Cohen, 1940; Yamashita, Mori, and Miwa, 1939; Blum and Price, 1950 *a*). The radiation is not without effect during this "refractory period," however, because later divisions are delayed. Recovery from the effects of the radiation follows the same type of curve regardless of the point in the cell division cycle at which the dose is applied, whether it falls in the refractory period or not (Blum and Price, 1950 *a*). These matters raise the question whether there is a very direct relationship between the events of the nuclear changes in the mitotic cycle and the sensitivity of the cell to radiation. This paper represents an attempt to reexamine this question, using the egg of *Arbacia punctulata* as experimental material.

EXPERIMENTAL

Method

The method of exposing eggs to ultraviolet radiation and of following cell cleavage photographically has been described in detail elsewhere (Blum and Price, 1950 *a*). An innovation in the present study was the use of infrared sensitive film (Eastman infrared, 35 mm.) to obtain clearer pictures of the nuclei. The illumination was from a 60 W tungsten filament lamp, passed through a Corning No. 2424 red filter. The filter and the spectral sensitivity of the film limit the photographic range to wave lengths 0.58μ to 0.86μ. The cytoplasm of the egg is relatively transparent in

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this spectral range, in which the echinochrome pigment does not absorb strongly, and the structures associated with nuclear division show in greater contrast than when a wider range is used (see Harvey and Lavin, 1951). The spectrum of the illuminating light is outside the range which causes photorecovery, so the eggs remain in effective darkness after the exposure to ultraviolet radiation. Under these circumstances the eggs eventually return to their normal rate of cleavage, but much more slowly than if they are exposed to wave lengths between 0.3μ and 0.5μ (Blum, Loos, and Robinson, 1950).

The control and irradiated samples to be compared were always from the same female and male, fertilization being at the same time in both samples. The photographs were made as routine at intervals of 2 minutes. Selected photographs from a series illustrating the extent to which mitotic changes can be followed are shown in Figs. 1 to 4. In some of the experiments three samples were examined simultaneously, using three microscopes; in some experiments only two samples were studied.

To measure changes during the first cleavage cycle we have determined the time of four events, beginning with fertilization of the eggs by the sperm. The second event is the appearance of the *streak*, which is illustrated in Fig. 1 in some of the cells. This stage seems to correspond approximately with the time the egg and sperm nuclei combine to form the fusion nucleus.[1] The third event is the appearance of what we call the *dumbbell*, illustrated in Figs. 2 and 3; the beginning of the dumbbell corresponds approximately with late anaphase. The fourth event is the cleavage of the egg, which is shown in Fig. 4.

For making measurements the photographic negatives were projected successively on a screen and the time of occurrence of streak, dumbbell, and cleavage determined for as many eggs as possible. The time of cleavage can be determined with considerable accuracy, but assigning the time of reaching the streak and dumbbell stages is more difficult, for several reasons. The streak, whatever its morphologic character, may be regarded as an elongated structure lying approximately along one diameter, and so has a different appearance depending upon its orientation with respect to the plane of the photograph. End on, the streak appears as a dark spot. If it lies in the plane of photograph it displays its greatest length; if it lies at an intermediate angle the streak appears intermediate between these two extremes. All these positions may be detected in Fig. 1. In counting, those eggs in which the streak could not be seen to develop clearly were neglected. Similar difficulties arise in determining the time of appearance of the dumbbell, which is also an elongated structure that may present different aspects in the photomicrographs (see Fig. 2), and a considerable number of eggs have to be eliminated from the count of this stage.

[1] Professor Allan C. Scott prepared stained whole egg samples in the course of one of our experiments. Comparison of these with our photographs helps to establish the approximate time of these events.

When comparisons of the times of the three stages, streak, dumbbell, or cleavage are to be made (as for the data in Table I) it is necessary to eliminate every egg in which any one of these stages cannot be seen clearly. This method of elimination seems a fair procedure since the principal source of error relates to the position of the egg, which is a random matter. By such elimination the total number of countable cells is greatly reduced. Subjective judgment plays a greater role in estimating time of appearance of streak and dumbbell than in the case of cleavage. Different observers tend to select appearance of the streak

TABLE I

Comparison of Events in the Fertilization to Cleavage Cycle in Normal and Irradiated Cells. Data of 1951

Experiment	Conditions*	No. of eggs	Temperature	Fertilization to streak	Streak to dumbbell	Dumbbell to cleavage
			°C.	*min.*	*min.*	*min.*
I (51)	Normal	15	20.0	19	20	9
	75 units, unfertilized eggs	13		"	42	12
K (51)	Normal	35	"	23	18	9
	100 units, fertilized eggs	7		"	51	10.1
Z (51)	Normal	32	23.0	17	22	11.5
	10 units, sperm	25		"	44	15
V (51)	Normal	19	"	14	24	11.5
	10 units, sperm	16		20	50	19.5
X (51)	Normal	15	22.5	20	20	10
	5 units, sperm	17		17	44	13
Y (51)	Normal	13	24.0	21	21	10
	5 units, sperm	"		22	43	12.5

* In the tables radiation units $\sim 6 \times 10^4$ ergs cm.$^{-2}$ of $\lambda \leqq 0.313\mu$.

and dumbbell stages consistently earlier or later; but a given individual reproduces his results wth reasonable accuracy, although this accuracy is considerably lower than for estimation of time of cleavage.

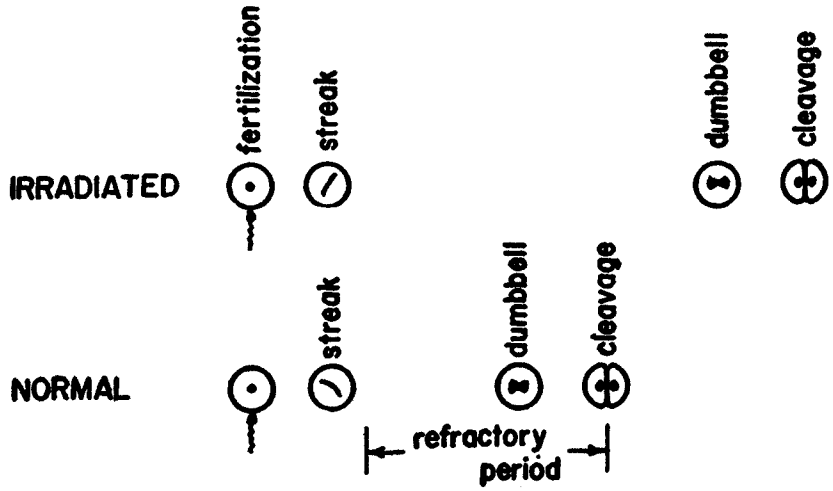
RESULTS

Table I summarizes results of a number of experiments in which ultraviolet radiation was applied in different ways; to the eggs or to the sperm before fertilization, or to the eggs just after fertilization. The diagram in Text-fig. 1 may help the reader to understand the timing of the events under discussion.

Streak to Dumbbell (Fusion Nucleus to Anaphase).—The greater part of the

increase in the time from fertilization to first cleavage is in the streak to dumbbell interval, as is indicated in Table I. Thus the principal delay in the cell division cycle occurs between the fusion of the sperm and egg nuclei and anaphase of the mitotic cycle.

Fertilization to Streak (*Fertilization to Fusion Nucleus*).—The time from fertilization to formation of the streak seems not to be altered by ultraviolet radiation. The data in Table I vary considerably in this regard, but there seems to be no trend relating changes in this interval to the part exposed to the ultraviolet radiation; *i.e.*, the sperm, unfertilized egg, or fertilized egg. It seems safe to say that doses of ultraviolet radiation sufficient to cause considerable



TEXT-FIG. 1. Diagram of the events of the cell division cycle in the *Arbacia* egg, and the approximate time of the refractory period.

delay in cleavage have little or no effect on the rate of formation of the fusion nucleus.[2]

Dumbbell to Cleavage (*Anaphase to Cleavage*).—The question whether or not ultraviolet radiation increases the interval between time of appearance of dumbbell and cleavage seems particularly important, since this interval falls entirely within the period of refractoriness to radiation. If applied during this refractory period, radiation does not delay the cleavage immediately following (Blum and Price, 1950 *a*), and presumably does not alter the timing of the mitotic events which precede that cleavage. On the other hand, if radiation is applied before the beginning of the refractory period cleavage is delayed, and there must be some disturbance of the timing of mitotic events. The action of

[2] Henshaw (1940) reported the same for x-ray, and that all the other stages of the cycle were delayed, particularly prophase, but no statistical analysis is given.

the radiation is centered in the nucleus (Blum, Robinson, and Loos, 1951), and this suggests that the refractory period is related to events of the mitiotic cycle taking place in that locus. It is conceivable that once mitosis has reached a certain critical point, radiation cannot interfere with its continuing to completion at its normal rate; this point would then mark the beginning of the refractory period, which corresponds roughly to the beginning of prophase (see Text-fig. 1). In such case, delay resulting from the introduction of radiation before the beginning of the refractory period might be expected to delay events

TABLE II

Comparison of the Dumbbell to Cleavage Interval in Normal and Irradiated Cells. Data of 1951

Experiment	Conditions*	No. of eggs	Dumbbell to cleavage	Standard deviation dumbbell to cleavage	Probability that normal and irradiated are the same
			min.		
I (51)	Normal	42	10.5	2.52	
	75 units, unfertilized eggs	35	12.8	1.69	<0.01
K (51)	Normal	"	9.3	2.42	
	100 units, fertilized eggs	30	10.0	2.86	0.3–0.2
Z (51)	Normal	83	12.7	1.75	
	10 units, sperm	55	12.5	2.57	0.6–0.5
V (51)	Normal	35	10.3	2.35	
	10 units, sperm	23	18.8	11.02	<0.01
X (51)	Normal	15	9.7	3.08	
	5 units, sperm	17	13.2	4.49	0.02–0.01
Y (51)	Normal	13	10.2	2.26	
	5 units, sperm	"	12.5	4.22	0.1–0.05

occurring before this critical point in mitosis, but not those events occurring after. In that event the streak to dumbbell interval, part of which is outside the refractory period, should be lengthened, as is found to be the case (see Table I), whereas the cleavage to dumbbell interval, which falls within the refractory period, should remain unaffected.

The measurements recorded in Table I suggest the contrary; that the dumbbell to cleavage interval is consistently lengthened. But the differences are not in all cases statistically significant, as is indicated in Table II, in which some of the counts have been extended by disregarding the streak stage and including all the cells in which the time of appearance of both dumbbell and cleavage could be determined. Because of the variation among these experiments, it was

thought advisable to make additional measurements in the summer of 1952, taking particular care with regard to certain technical details. The results, which we believe to provide the most reliable data we have, are assembled in Table III, which includes two experiments (Y 52 and V 52) with normal eggs only. Calculated statistics indicate that normal samples from the same male and female may be considered identical, and that the conditions are comparable for

TABLE III

Comparison of the Dumbell to Cleavage Interval in Normal and Irradiated Cells. Data of 1952

Experiment	Conditions*	No. of eggs	Temperature	Fertilization to cleavage	Dumbbell to cleavage	Standard deviation dumbbell to cleavage	Probability that dumbbell to cleavage is the same for normal and irradiated
			°C.	*min.*	*min.*		
Y (52)	Normal	41	20.0	57.5	10.9	1.83	
	"	40	"	57.9	11.3	1.62	
	"	37	"	57.4	11.1	1.92	
V (52)	Normal	90	20.0	54.1	10.5	1.84	
	"	85	"	53.8	11.1	1.55	
	Normal	54	21.0	47.1	11.1	1.90	
P (52)	175 units, unfertilized eggs	47	"	68.6	11.0	2.04	0.8–0.7
	175 " , fertilized "	39	"	72.8	12.5	2.74	<0.01
U (52)	Normal	30	20.0	62.0	12.3	1.93	
	10 units, sperm	19	"	82.2	13.0	2.43	0.3–0.2
	175 " , fertilized eggs	31	"	92.4	15.3	6.94	0.05–0.02
N (52)	Normal	39	20.0	46.0	10.5	1.68	
	200 units, unfertilized eggs	25	"	76.2	12.8	2.07	<0.01
L (52)	Normal	38	21.5	46.2	9.5	1.81	
	175 units, fertilized eggs	33	"	67.5	9.6	2.08	0.9–0.8
DD (52)	Normal	57	20.5	58.5	10.6	2.51	
	10 units, sperm	62	"	74.8	12.2	2.39	<0.01

the different microscopes. In the remainder of the experiments the significance of the difference between normal and irradiated samples varies widely. In three cases there is no change in the dumbbell to cleavage interval; in three cases this interval is significantly lengthened; in the other two cases there is an increase but not a statistically significant one. In some, but not all, cases the standard deviation is high in the irradiated sample as compared to the normal controls. The standard deviation also varies widely among an additional number of experiments listed in Table IV, in which there were no controls, but only irradiated samples.

When all the data represented in Tables II, III, and IV were examined in detail it was found that for the normal cells the distribution of the length of the dumbbell to cleavage interval was approximately normal and that the value of 18 minutes was never exceeded. Among the irradiated samples, on the other hand, there were 13 out of 24 cases in which this period was exceeded in some of the cells. In the experiments listed in Table IV the cells were followed through four cleavages, and in these it was possible to determine that the cells with abnormally long dumbbell to cleavage interval (over 18 minutes) went on to cleave normally and so could not be regarded as moribund cells. When a statistical analysis of all the data was made it was found that there is a slight

TABLE IV

Dumbbell to Cleavage Interval in Irradiated Cells. Data of 1951

Experiment	Conditions*	No. of eggs	Fertilization to cleavage	Dumbbell to cleavage	Standard deviation of dumbbell to cleavage
			min.	*min.*	
U (51)	50 units, fertilized eggs	83	84.5	12.8	4.31
	100 " , unfertilized "	72	78.3	12.5	4.03
T (51)	5 units, sperm	55	82.2	10.1	1.67
	10 " , "	47	89.6	10.5	2.01
S (51)	5 units, sperm	34	67.6	10.4	2.59
	10 " , "	52	73.8	12.1	2.39
R (51)	20 units, sperm	27	103.2	12.7	2.70
	40 " , "	26	121.3	11.0	1.61
P (51)	75 units, unfertilized eggs	42	70.3	10.9	2.48
	150 " , " "	27	71.0	10.8	2.26
K (51)	100 units, fertilized eggs	30	83.5	10.0	2.86

tendency for the dumbbell to cleavage interval to increase with the fertilization to cleavage interval (about 7 per cent of the increase in fertilization to cleavage appearing as an increase of dumbbell to cleavage). This tendency still appears after eliminating those samples which contain an excessive number of cells with dumbbell to cleavage intervals greater than 18 minutes (Experiments S 51, 10 units sperm, and DD 52) and certain others (V 51 and X 51) in which the dumbbell to cleavage interval showed what was thought might be an abnormally high correlation with fertilization to cleavage interval.

It may be concluded from the analysis that there is a tendency for cells which cleave late to have a long dumbbell to cleavage interval. Since the cells that have been irradiated cleave later than normal cells it seems probable that irradiation of the cells before the beginning of the refractory period tends to

lengthen the dumbbell to cleavage interval. This is not an inescapable conclusion; but if correct, the hypothesis that the period of refractoriness to radiation represents a period during which the timing of mitotic events cannot be altered is negated.

DISCUSSION

The above experiments are somewhat disappointing in not giving us a clear cut indication of the meaning of the "refractory period," during which the cell is not immediately responsive to the action of ultraviolet light. They illustrate some of the difficulties that confront attempts to explore the mechanism of cell division in the sea urchin's egg, and indicate that any interpretations based on experiments with this or other material should be made with caution. It is obvious that the complexity of the time relationships, including the refractory period, makes studies in which individual cells are not followed liable to misinterpretation. Observations made on too small numbers of cells may be misleading because of the wide variability in the timing of the events of the mitotic cycle, which parallels what has already been shown for the fertilization to cleavage interval (Blum and Price, 1950 *b*).

SUMMARY

The effect of ultraviolet light in delaying certain events in the cell division cycle has been examined. The time to fusion of the egg and sperm nucleus is not affected by doses of ultraviolet that cause considerable delay in other parts of the cycle. The principal delay occurs before anaphase. Between anaphase and cleavage there is only slight delay. The "refractory period" during which the radiation does not delay the immediate cycle of cell division, does not seem to represent complete refractoriness of the mitotic cycle to interference during this period.

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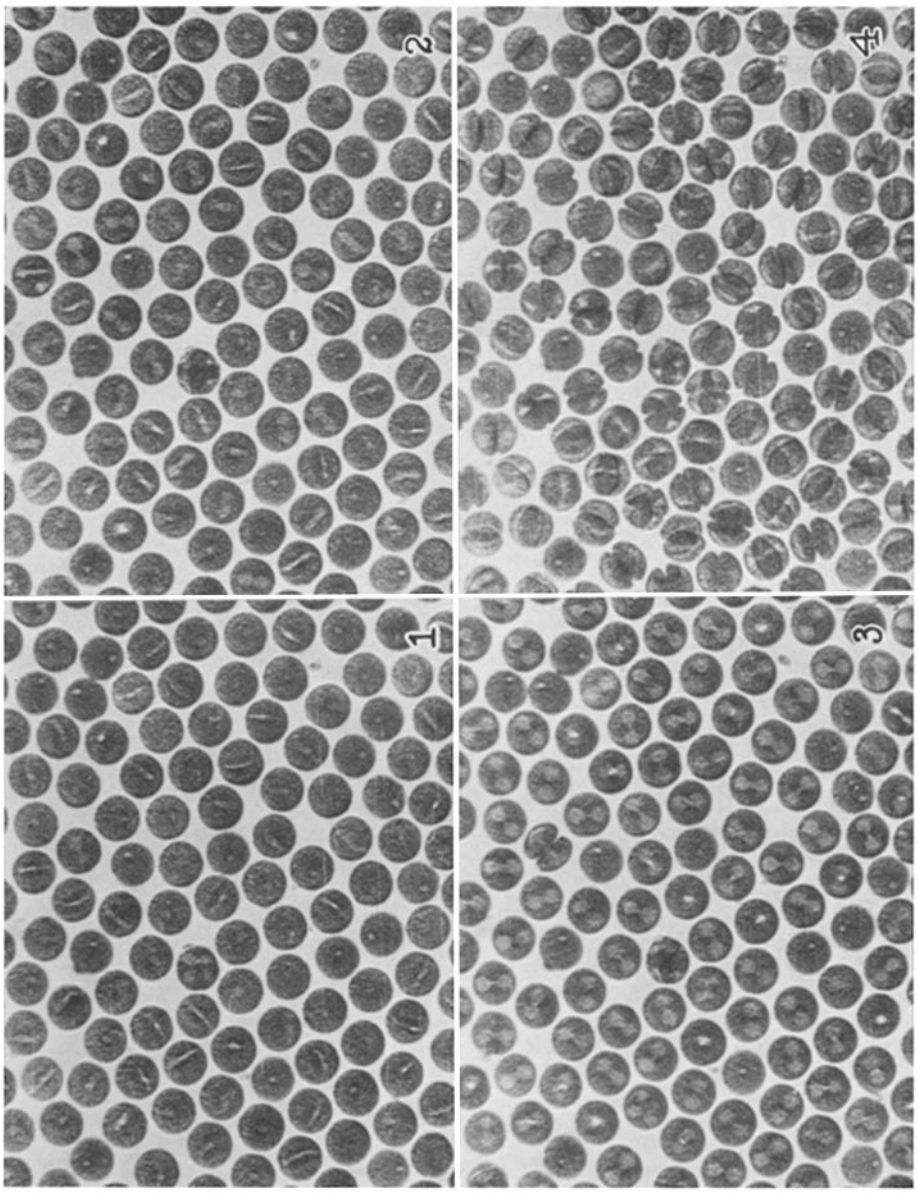
EXPLANATION OF PLATE 5

FIGS. 1 to 4. Successive stages in the cell division cycle of *Arbacia* eggs. In Fig. 1 a number of the eggs show streaks. In Fig. 2 some of the eggs show dumbbells; some are still in the streak stage. In Fig. 3 most of the cells show well formed dumbbells. In Fig. 4 the majority of the cells have cleaved. Note that one cell shows a double dumbbell in Fig. 1 and goes directly into a four celled stage in Fig. 2; this is the result of polyspermia; such occasional cells were eliminated in counting. The magnification is approximately 75 times.



(Blum *et al.*: Ultraviolet and mitosis)