THE LOCI OF ACTION OF ULTRAVIOLET AND X-RADIATION AND OF PHOTORECOVERY IN THE EGG AND SPERM OF THE SEA URCHIN ARBACIA PUNCTULATA

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PLATE 1

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This paper describes experiments on the effects of ultraviolet radiation and of x-rays on the eggs and sperm of the sea urchin *Arbacia punctulata*, carried out during the summer of 1950.[1] These, together with earlier findings already described (Blum and Price, 1950 *a*, *b*; Blum, Loos, and Robinson, 1950) permit certain conclusions to be reached regarding the locus of action of these radiations, and that of photorecovery after ultraviolet radiation. To aid in understanding the rationale of the experiments and their interpretation the results will be briefly recapitulated.

Locus of Delay of Cleavage

In moderate doses both ultraviolet radiation and x-ray delay cell division (cleavage) of the eggs of the sea urchin. To determine the locus of this action advantage was taken of the fact that the eggs of *Arbacia* can be separated by centrifugation into nucleate and enucleate halves. Thus whole eggs, nucleate, or enucleate halves may be exposed to ultraviolet or x-radiation, either before or after fertilization with normal sperm. Or the sperm may be exposed to the radiation before it is used to fertilize the eggs or halves. The various combinations are indicated in Text-fig. 1 A, which also summarizes our findings with regard to delay of cleavage by ultraviolet radiation. Cleavage is delayed in all cases except when the enucleate half is exposed to ultraviolet radiation before fertilization with normal sperm. This is also the only case in which the part that receives the radiation contains no nucleus. If the sperm nucleus is introduced into the enucleate half by fertilization before exposure, or if the sperm itself is exposed to ultraviolet radiation, there is delay of cleavage. We conclude that the locus of action of the radiation is the nucleus or something closely associated with the nucleus.

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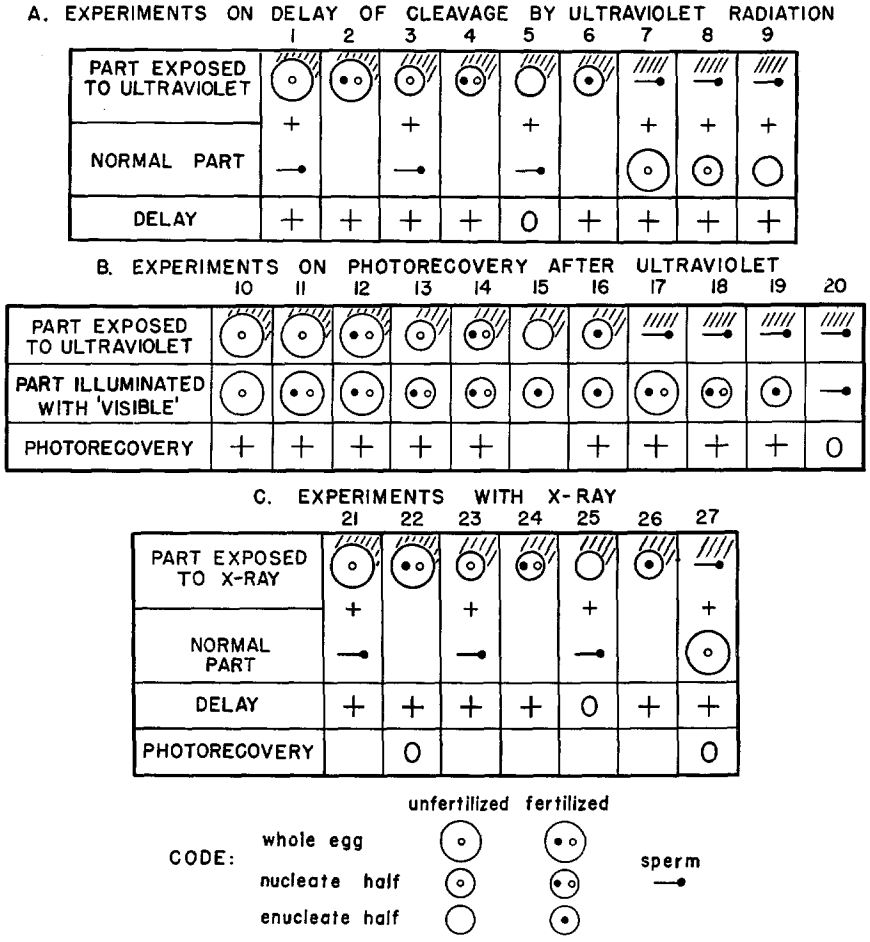
[1] Reported in preliminary form by Blum, Robinson, and Loos (1950).

Text-fig. 1 C summarizes results with x-ray, including some obtained by other investigators. The results parallel those with ultraviolet as far as delay of cleavage is concerned, indicating that the locus of action is the nucleus.



TEXT-FIG. 1. Summary of experiments.

Locus of Photorecovery

After the initial delay there is a gradual return toward the normal cleavage rate. In the case of ultraviolet radiation this recovery process is greatly accelerated by illumination with "visible" radiation.[2] To determine the locus of the

[2] The effective wave lengths range from the near ultraviolet into the visible, ~ 0.3 to $0.5\ \mu$ (Blum, Loos, and Robinson, 1950).

photorecovery process experimental combinations, similar to those already described, were employed. The results are summarized in Text-fig. 1 B. Photorecovery cannot, of course, be demonstrated in the enucleate half exposed to ultraviolet before fertilization because cleavage is not delayed (see Text-fig. 1 A). Photorecovery was observed in all other cases except that of the sperm irradiated and illuminated before introduction into the egg. Eggs fertilized with irradiated sperm recovered more rapidly if subsequently illuminated with visible radiation. Nucleate or enucleate halves may take the place of the whole eggs in such experiments. Egg cytoplasm is essential for the photorecovery process. The sperm lacks the ability to recover, whether in light or darkness.

Recovery after x-ray is associated with egg cytoplasm, as in the case with ultraviolet radiation. But acceleration of recovery by visible light does not occur after x-ray (see Text-fig. 1 C).

STUDIES WITH ULTRAVIOLET RADIATION

Methods

The method permits individuals of a population of fertilized eggs or egg "halves" to be observed through the early cleavage stages, and a photographic record obtained. Dosage with ultraviolet radiation and other aspects of the experiments have been described in earlier publications to which the reader is referred for all details (Blum and Price, 1950 *a*, *b*; Blum, Loos, and Robinson, 1950). In 1950 an additional refinement was added in that the temperature of the eggs was maintained at 22° C. by a flow of water from a constant temperature bath instead of by running sea water. The mercury arc radiation was filtered through a corex D filter as in the experiments of Blum and Price (1950 *a*). This eliminates wave lengths shorter than 0.27 μ, which cause artificial parthenogenesis.

RESULTS

To facilitate discussion the different types of experiments outlined in Text-fig. 1 A, B, C, have been numbered serially. These numbers will appear in parentheses at appropriate places in the text, and in the lower left hand corner of the diagrams to which they apply. Each experiment has been carried out at least twice. There have been no conflicting results.

Irradiation of Whole Eggs (Experiments 1, 2, 10 to 12)

Our experiments with whole eggs have already been described (Blum and Price, 1950 *a*, *b*; Blum, Loos, and Robinson, 1950) but some will be mentioned briefly because of certain apparently contradictory results that have been reported.

That there is photorecovery from the effects of ultraviolet radiation applied to the fertilized egg of *A. punctulata* (Experiment 11) is reported by Marshak (1949 *a*, *b*) as well as by ourselves. Wells and Giese (1950) have found the same

for the sea urchin *Strongylocentrotus purpuratus*. By following through four cleavages we find some recovery of cleavage rate in the dark. This is comparable to the case of x-ray delay, in which recovery is seen, but in which there is no acceleration of recovery by light (see below).

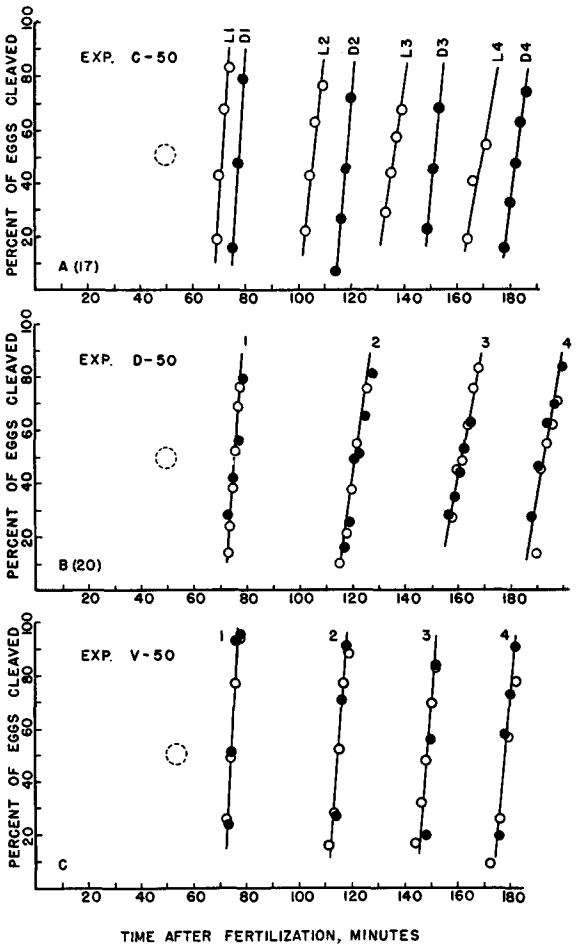
We also found that photorecovery may occur in the egg of *Arbacia* prior to fertilization (Experiment 10), as is clearly illustrated in the paper in which we described these experiments in detail (Blum, Loos, and Robinson, 1950, *e.g.* Fig. 4). Results published in an earlier article (Blum and Price, 1950 *a*, Table I) also show that such recovery occurs. Marshak reported that he found no photorecovery under these conditions (compare Blum *et al.*, 1949; Marshak, 1949 *a*). We are unable to account for Marshak's findings, which he supports by only a single described experiment. His method depended upon direct counting and estimation of the time required for 50 per cent of the eggs to undergo first cleavage, whereas ours permits the course of cleavage to be followed photographically through four cleavages. There are other points in Marshak's paper with which our results are in disagreement and we can only attribute these discrepancies to the limitations of the method he used. Marshak used radiation principally of wave length 0.2537 μ whereas we have used longer wave lengths, but this seems of minor consequence for reasons discussed elsewhere (Blum and Price, 1950 *a*; Blum, Loos, and Robinson, 1950; see also Wells and Giese, 1950). Wells and Giese (1950) have since reported findings on *Strongylocentrotus purpuratus* in accord with ours. They find no recovery in the dark in the unfertilized egg of *Strongylocentrotus*, but we have not done the comparable experiments with *Arbacia*. Henshaw (1932) has, however, shown that unfertilized eggs of *Arbacia* recover from x-ray (see below).

Irradiation of Sperm (Experiments 7, 17, 20)

If sea urchin sperm is exposed to ultraviolet radiation and then used to fertilize normal eggs (Experiment 7), these eggs cleave later than do eggs fertilized with normal sperm (*e.g.*, Giese, 1939, 1946; Marshak, 1949 *b*). The dose required to produce comparable cleavage delay is considerably lower for sperm than for eggs. The delay of cleavage is indicated in Text-fig. 2, A to C, in which the approximate time of normal first cleavage is represented by the dotted circles.

Figure 2 A illustrates an experiment showing photorecovery in eggs fertilized with ultraviolet-irradiated sperm (Experiment 17). A sample of dilute sperm suspension was exposed to ultraviolet radiation and then used immediately to fertilize normal eggs. One sample of the fertilized eggs was placed in effective darkness,[3] the other was illuminated with visible light. The figure shows that

[3] Effective darkness is obtained by removing all wave lengths shorter than 0.5 μ by means of a red filter (*e.g.* Corning 2424). This still permits observation and photography (see Blum, Loos, and Robinson, 1950).



TEXT-FIG. 2. Experiments with ultraviolet irradiated sperm.

Sperm in dilute suspension exposed to 1×10^4 ergs cm.$^{-2}$ sec.$^{-1}$ of radiation of wave lengths 0.27 to 0.313 μ (mercury arc radiation filtered through corex D, see Blum and Price, 1950 *a*). Doses are indicated for each of the experiments. Intensity of illuminating radiation about 10^3 ergs cm.$^{-2}$ sec.$^{-1}$ of wave lengths 0.4 to 0.5μ (see Blum, Loos, and Robinson, 1950).

A. Normal eggs were fertilized with sperm which had received 10^5 ergs cm.$^{-2}$ of radiation. One sample in the dark (solid disks), another illuminated (open circles). D1, D2, D3, D4, first four cleavages of eggs in the dark. L1, L2, L3, L4, first four cleavages of illuminated eggs.

B. Sperm received 10^5 ergs cm.$^{-2}$ of radiation. One sample of the sperm was kept in the dark (solid disks), another illuminated (open circles). Normal eggs were fertilized with these samples of sperm 1 hour after the sperm had been exposed to ultraviolet radiation. Both samples of eggs were kept in the dark after fertilization. 1, 2, 3, 4, first four cleavages of the eggs.

C. One sample of sperm received 2×10^5 ergs cm.$^{-2}$ 1 hour before it was used to fertilize normal eggs (solid disks). Another sample of sperm received the same dose immediately before it was used to fertilize normal eggs (open circles). Both samples of eggs were in effective darkness after fertilization. 1, 2, 3, 4, first four cleavages of the eggs.

photorecovery occurs under these conditions. The difference between the light and dark samples at first cleavage is not great, but increases in later cleavages as is to be expected if light accelerates the recovery process (see Blum, Loos, and Robinson, 1950). In normal eggs the intervals between cleavages 1 and 2, 2 and 3, and 3 and 4 are equal, each being around 30 minutes at the temperatures we have used (Blum and Price, 1950 *b*). The progressive reduction of the interval between cleavages in the dark sample shows that there is some recovery under these conditions. This behavior is comparable to that seen when the egg is irradiated, the sperm normal (Blum, Loos, and Robinson, 1950).

The sperm of *Arbacia* is itself not capable of photorecovery (Experiment 20). Text-fig. 2 B illustrates an experiment in which a suspension of sperm was first dosed with ultraviolet radiation, then divided into two parts, one being placed in visible light, the other in darkness. At the end of 1 hour two samples of normal eggs were fertilized with the irradiated sperm, one with the sample of sperm which had been illuminated with visible light, the other with the sample of sperm which had been kept in the dark. The fertilized eggs were kept in the dark during the remainder of the experiment. The figure shows clearly that there has been no photorecovery of the sperm. The first cleavage is delayed—controls cleaved in about 50 minutes. Subsequently the cleavage intervals diminish in the same way as in the dark sample in Text-fig. 2 A. Exposure of the sperm to light has not increased the rate of recovery either before or after its introduction into the egg. Marshak (1949 *a*, *b*) reported similar results.

That the recovery in the above cases is exclusively a function of the egg cytoplasm is indicated by the following type of experiment which shows that the sperm has no ability to recover. A sample of sperm was exposed to ultraviolet radiation and then kept in darkness for 1 hour. At the end of that time another sample of the same sperm was exposed to an equal dose of ultraviolet radiation and two samples of normal eggs were immediately fertilized with the two samples of sperm. Both samples of fertilized eggs were kept in the dark. The cleavage times of these eggs are shown in Text-fig. 2 C. Cleavage has been delayed by the irradiation of the sperm but the four cleavages occur at the same time in both samples. Experiments by other investigators on x-rayed sperm, which will be cited later, also indicate that the sperm has no ability to recover.

This seems a point of considerable importance, as will appear in the discussion, and it is necessary, therefore, to examine carefully the report of photorecovery in the sperm of *S. purpuratus* by Wells and Giese (1950). Their experiments seem to have been carried out in essentially the same manner as ours, but the figure they present shows only the first cleavage of the eggs. This figure indicates that illumination with visible light hastens the first cleavage to a small extent. Wells and Giese report that exposure of the sperm of *Strongylo-*

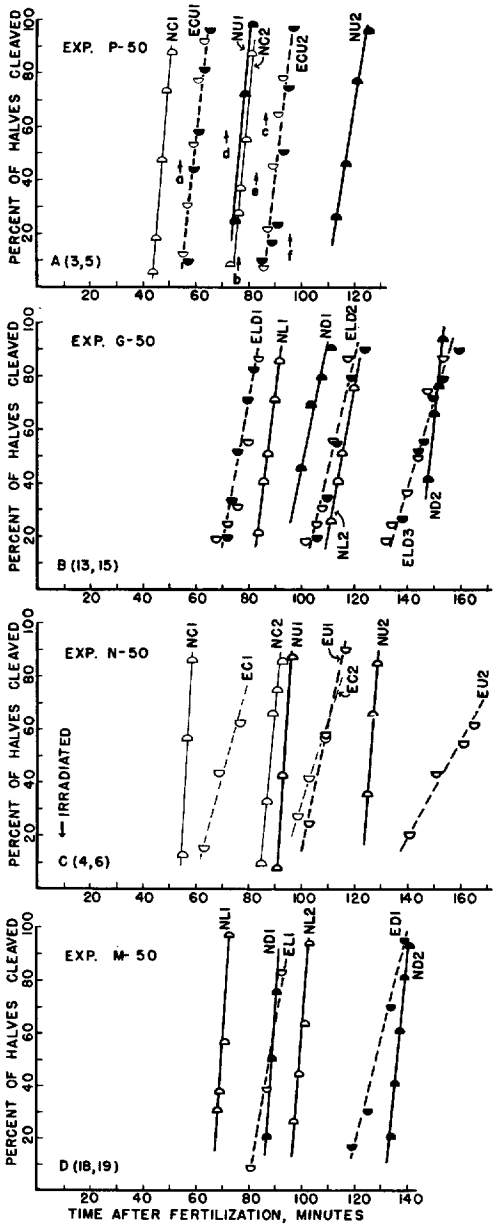
centrotus to visible light reduced the ability to fertilize the normal egg. This we also observed in at least one case in the *Arbacia* sperm, but we did not make a systematic study. Whatever the effect of visible light in reducing the fertilizing power of the sperm, it seemed to have no effect on the subsequent rate of cleavage once fertilization has been accomplished. Thus it should not have influenced our results which are based upon the total number of cells fertilized and undergoing cleavage. Experiments of the type illustrated in Text-fig. 2 B show that this is true.

Experiments with "Halves" (Experiments 3 to 6, 13 to 16)

If the *Arbacia* egg is centrifuged at high gravity for a short time it splits into two "halves." One, the nucleate half, contains the nucleus and certain cytoplasmic material (this is the "white" half). The other, the enucleate half, contains most of the granular material including all of the red echinochrome pigment (this is the "red" half). Usually a fairly high percentage of the nucleate halves will, if fertilized with normal sperm, undergo cleavage. They behave much as the whole egg even in late stages of development (Harvey, 1949). The enucleate halves are more variable in their behavior, although these too, after fertilization with normal sperm, have been occasionally carried as far as the pluteus stage (Harvey, 1940). The rather unpredictable behavior of the enucleate halves makes them difficult to work with, and a certain number of the experiments were for this reason unsuccessful. Also their cleavage is sometimes irregular, but we have found no difficulty in making comparative measurements; while some samples cleave better than others, exposure to ultraviolet radiation does not contribute to the irregularity. As a rule it was not feasible to follow them further than through the second cleavage of the halves because it became difficult to observe the cleavage planes.

In our studies the eggs were centrifuged through a density gradient formed between one molar sucrose solution and sea water. The centrifuge was an air turbine type, operated at forces between 40,000 and 65,000 $\times$ g, for periods from 30 to 90 seconds exclusive of the times required for accelerating to the maximum speed and for deceleration. Under these conditions the nucleate and enucleate halves had about the same volume (see Harvey, 1941). The percentage of enucleate halves which could be fertilized and would proceed through two cleavages ranged from zero to 88 per cent. We found no index for predicting whether the enucleate halves would be useable or not. No attempt was made to separate the enucleate from the nucleate halves, our aim being to have a mixture of the two types in the same microscopic field; an example is shown in Figs. 1 to 6.

Exposure before Fertilization (Experiments 3, 5, 13, 15).—In Text-fig. 3 A are shown results of an experiment in which a mixture of nucleate and enucleate halves was exposed to ultraviolet and then fertilized with normal sperm (Ex-


EXP. P-50
NCI
EGUI
NUI
NC2
EGU2
NU2
A (3,5)
EXP. G-50
ELDI
NLI
NDI
ELD2
NL2
ELD3
ND2
B (13, 15)
EXP. N-50
NCI
EGI
NC2
NUI
EUI
EG2
NU2
EU2
← IRRADIATED
C (4,6)
EXP. M-50
NLI
NDI
ELI
NL2
EDI
ND2
D (18,19)
PERCENT OF HALVES CLEAVED
TIME AFTER FERTILIZATION, MINUTES

TEXT-FIG. 3

periments 3, 5). An unirradiated sample fertilized at the same time served as control. Both samples were placed in effective darkness after fertilization. In the controls the "enucleate"[4] halves cleaved somewhat later than the nucleate. This is consistently the case with samples of halves not exposed to radiation. In the sample exposed to ultraviolet radiation the cleavage of the nucleate halves was delayed (Experiment 3), the first cleavage in the irradiated halves occurring at about the same time as second cleavage of the nucleate controls. This behavior is comparable to that of whole eggs when exposed to ultraviolet radiation.

In contrast to the nucleate halves, the cleavage of the enucleate halves is not delayed by exposure to ultraviolet radiation (Experiment 5). The enucleate

[4] For convenience we will refer to the red half as the "enucleate" half even though it has received the sperm nucleus by fertilization. In none of our experiments did the enucleate halves cleave without fertilization.

TEXT-FIG. 3. Experiments with egg "halves" exposed to ultraviolet radiation. Intensity of the illuminating radiation as indicated in Text-fig. 2.

A. Unfertilized halves irradiated with ultraviolet. One sample of eggs received 2.5×10^6 ergs cm.$^{-2}$ of radiation just before fertilization with normal sperm (solid symbols). Control sample not exposed to ultraviolet, fertilized at the same time (open symbols). Both samples kept in the dark after fertilization. NC1, NC2, first two cleavages of the nucleate halves in control sample. NU1, NU2, first two cleavages of the nucleate halves in irradiated sample. ECU1, ECU2, first two cleavages of the enucleate halves in control and in irradiated sample.

B. Unfertilized halves irradiated with ultraviolet. All halves received 2.5×10^6 ergs cm.$^{-2}$ of radiation just before being fertilized with normal sperm. One sample of the halves was kept in the dark after fertilization (solid symbols), another sample was illuminated (open symbols). ND1, ND2, first two cleavages of the nucleate halves in dark sample. NL1, NL2, first two cleavages of the nucleate halves in illuminated sample. ELD1, ELD2, ELD3, first three cleavages of the enucleate halves in light and dark.

C. Fertilized halves irradiated with ultraviolet. One sample of halves received 2.5×10^6 ergs cm.$^{-2}$ of radiation 10 minutes after fertilization. An unexposed sample of the fertilized halves served as control. Both samples were illuminated after fertilization. NC1, NC2, first two cleavages of the nucleate halves in the control sample. NU1, NU2, first two cleavages of the nucleate halves in the irradiated sample. EC1, EC2, first two cleavages of the enucleate halves in the control sample. EU1, EU2, first two cleavages of the enucleate halves in the irradiated sample.

D. Unexposed halves fertilized with irradiated sperm. The sperm received 2×10^5 ergs cm.$^{-2}$ of radiation. One sample of the halves fertilized with this sperm was kept in the dark after fertilization (solid symbols), another sample was illuminated (open symbols). ND1, ND2, first two cleavages of the nucleate halves in the dark sample. NL1, NL2, first two cleavages of the nucleate halves in the illuminated sample. ED1, first cleavage of the enucleate halves in the dark sample. EL1, first cleavage of the enucleate halves in the illuminated sample.

halves in the irradiated and control samples cleaved at approximately the same time. This is true for both the first and second cleavages. Failure of ultraviolet radiation to delay the cleavage of enucleate halves when they are irradiated before fertilization was not only observed in two other experiments of the type just described, but is obvious in numerous others discussed below.

Six selections from the photographic record of the above experiment are presented in Figs 1 to 6. These show stages in the cleavage of the control (Figs. 1 to 3), and of the irradiated sample (Figs. 4 to 6). The times at which these photographs were obtained are indicated in Text-fig. 3 A. In Fig. 1 is the control sample 54 minutes after fertilization. The nucleate halves (light in the photograph) have completed the first cleavage. The enucleate halves (dark in the photograph) have not yet cleaved. At the end of 76 minutes (Fig. 2) the red halves have also undergone first cleavage. At the end of 87 minutes (Fig. 3) the nucleate halves have completed the second cleavage, the enucleate halves are for the most part still in the two celled stage. In the irradiated sample the picture is quite different. At 71 minutes (Fig. 4) after fertilization, the enucleate halves have already cleaved, since the irradiation has not affected them. The nucleate halves, on the other hand, have not yet begun to cleave. In Fig. 5, taken at 83 minutes, the nucleate halves are cleaving, but in Fig. 6, taken at 95 minutes, they are still in the two celled stage whereas the enucleate halves have undergone second cleavage.

Text-fig. 3 B represents an experiment in which a sample of nucleate and enucleate halves was exposed to ultraviolet radiation just before fertilization. One half of the sample was then kept in the light and the other half in dark (Experiments 13, 15). There is no difference in the time of cleavage of the enucleate halves whether in the dark or in light. Again, the ultraviolet radiation applied to enucleate halves before fertilization has not delayed cleavage. Obviously no photorecovery can be demonstrated in the enucleate halves under these conditions because there has been no delay.[5] On the other hand cleavage of the nucleate halves is delayed so that they divide after the enucleate halves. Visible light accelerates the recovery of cleavage rate in the nucleate halves, those in the light cleaving well before those in the dark.

Exposure after Fertilization (Experiments 4, 6, 14, 16).—Text-fig. 3 C represents an experiment in which nucleate and enucleate halves were first fertilized with normal sperm. One part of the sample was then exposed to ultraviolet radiation, the other part serving as a control (Experiments 4, 6). Cleavage is delayed in both the nucleate and enucleate halves, as compared with the control. We see here strong evidence that the delay of cleavage is due to action of the ultraviolet radiation on the nucleus. By fertilization the sperm nucleus has

[5] Visible light is without effect on cleavage rate unless cleavage has been delayed by ultraviolet radiation (Blum, Loos, and Robinson, 1950).

been introduced into the enucleate half, the cleavage of which now is delayed by exposure to the radiation, whereas no delay occurred when the enucleate half was exposed before a nucleus was added (compare with Text-fig. 3 A and B).

Experiments comparable to that described in Text-fig. 3 B showed that photorecovery takes place in fertilized halves (Experiments 14, 16). The halves were fertilized before exposure to ultraviolet radiation. After exposure one portion of the sample was kept in the dark, the other in the light. Cleavage of both the fertilized nucleate and enucleate halves occurred later in the portion kept in the dark. Diagrams of such experiments are omitted for brevity.

Irradiation of Sperm before Fertilizing Halves (Experiments 8, 9, 18, 19).— When sperm was exposed to ultraviolet radiation and then used to fertilize samples of halves (Experiments 8, 9) there was delay in both the nucleate and enucleate halves, as compared to unexposed controls. A diagram of such an experiment, which would be similar to Text-fig. 3 C, is omitted for brevity.

When one sample of halves fertilized with irradiated sperm was kept in the light and another in the dark (Experiments 18, 19), cleavages of both nucleate and enucleate halves occurred earlier in the illuminated sample, as is shown in Text-fig. 3 D. The behavior of both nucleate and enucleate halves is comparable to that of whole eggs fertilized with irradiated sperm. This result is the same as that obtained when whole eggs were fertilized with irradiated sperm, which is illustrated in Fig. 2 A. The demonstration of photorecovery in the enucleate half fertilized with irradiated sperm, whereas there is no photorecovery of the sperm itself, seems conclusive evidence that egg cytoplasm is concerned in this process.

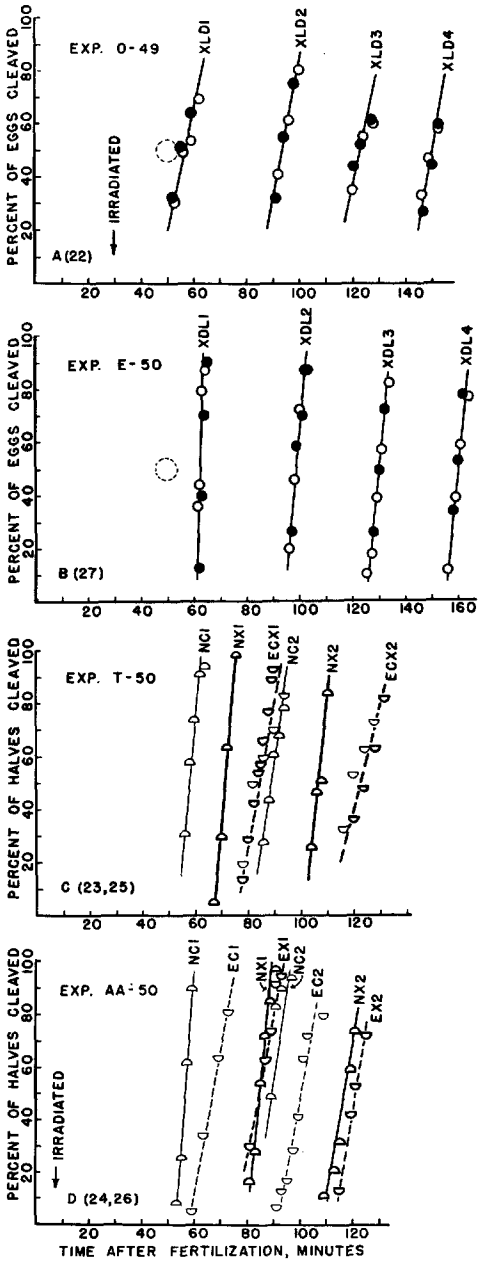
STUDIES WITH X-RAYS (EXPERIMENTS 21 to 27)

The scheme in Text-fig. 1 C includes our experiments and some performed by others which we have not repeated.

Experiments on Whole Eggs

Text-fig. 4 A illustrates an experiment in which eggs were exposed to x-ray[6] after fertilization, one sample being placed in the light, the other in the dark (Experiment 22). Cleavage is delayed and there is recovery, illustrated by return toward the normal cleavage rate. The first cleavage was not greatly delayed because the exposure to x-ray came near to the normal cleavage time, showing as do other of our experiments that there is a "refractory" period comparable to that found for eggs irradiated with ultraviolet (Blum and Price

[6] The source of x-rays was the high intensity apparatus available at the Marine Biological Laboratory, Woods Hole. Doses of 1000 to 3000 r were used for the eggs and halves, doses of 3000 to 7000 r for the sperm.


EXP. 0-49
XLD1
XLD2
XLD3
XLD4
IRRADIATED
A(22)
PERCENT OF EGGS CLEAVED
EXP. E-50
XDL1
XDL2
XDL3
XDL4
B (27)
PERCENT OF EGGS CLEAVED
EXP. T-50
NC1
NX1
EGX1
NC2
NX2
EGX2
C (23,25)
PERCENT OF HALVES CLEAVED
EXP. AA-50
NC1
EC1
NX1
EX1
NC2
EC2
NX2
EX2
IRRADIATED
D (24,26)
PERCENT OF HALVES CLEAVED
TIME AFTER FERTILIZATION, MINUTES

TEXT-FIG. 4

1950 *a*). Yamashita *et al.* (1939) also recognized the existence of such a refractory period in the eggs of the sea urchin *Pseudocentrotus depressus* exposed to ionizing radiation.

The absence of photorecovery is clear since the eggs in the dark cleave at the same time as those in the light. Dulbecco (1950) has reported a slight amount of photorecovery after x-ray in the *E. coli*–bacteriophage system, but if such occurs in the *Arbacia* eggs it is very slight and is not measurable by our method (Blum and Price, 1950 *b*).

We did not carry out any experiments in which the eggs were x-rayed previous to fertilization (Experiment 21), but extensive studies on *Arbacia* by Henshaw (1932) have shown that cleavage is delayed when the eggs are treated in this way and that there is recovery before fertilization. Similar findings are reported by Miwa *et al.* (1939 *a*), and Mori *et al.* (1939) for the eggs of *Pseudocentrotus depressus* exposed to ionizing radiations. This is comparable to our findings for delay by ultraviolet radiation (Experiment 10).

Experiments with Sperm

Henshaw (1936, 1938) found no recovery of sperm of *Arbacia* after treatment with x-ray. This was also found to be the case for sperm of *Pseudocentrotus de-*

TEXT-FIG. 4. Experiments with x-rays.

A. Eggs received 1000 r 25 minutes after fertilization with normal sperm. One sample was kept in the dark after irradiation (solid disks), another was illuminated (open circles). XLD1, XLD2, XLD3, XLD4, first four cleavages of eggs in darkness and in the illuminated samples. The large broken circle indicates the approximate time of first cleavage of normal eggs.

B. Normal eggs were fertilized with sperm which had received 3000 r. One sample was kept in the dark after fertilization (solid disks), another was illuminated (open circles). XDL1, XDL2, XDL3, XDL4, first four cleavages of eggs in darkness and in the illuminated samples. The large broken circle indicates the approximate time of first cleavage of eggs fertilized with normal sperm.

C. Unfertilized halves exposed to x-ray. One sample of halves received 1500 r; they were then fertilized with normal sperm. A control sample of unexposed halves was fertilized with normal sperm at the same time as the irradiated sample. Both samples were illuminated after fertilization. NC1, NC2, first two cleavages of the nucleate halves in the control sample. NX1, NX2, first two cleavages of the nucleate halves in the irradiated sample. ECX1, ECX2, first two cleavages of the enucleate halves in the control and irradiated samples.

D. Fertilized halves exposed to x-ray. One sample of halves received 1500 r 8 minutes after fertilization with normal sperm. An unexposed sample of the fertilized halves served as control. Both samples were illuminated after the irradiation. NC1, NC2, first two cleavages of the nucleate halves in the control sample. NX1, NX2, first two cleavages of the nucleate halves in the irradiated sample. EC1, EC2, first two cleavages of the enucleate halves in the control sample. EX1, EX2, first two cleavages of the enucleate halves in the irradiated sample.

pressus treated with β-radiation (Miwa *et al.* (1939 *a*) and Mori *et al.* (1939)). This is comparable to what we have found with ultraviolet radiation.

We had thought it possible that sperm irradiated with x-ray might show photorecovery when introduced into the egg, in a manner comparable to that which was found for ultraviolet radiation. This is not the case, however, as is shown by the experiment illustrated in Text-fig. 4 B, in which normal eggs were fertilized with sperm that had been exposed to x-ray (Experiment 27). One half the sample of fertilized eggs was kept in the light, the other in effective darkness. There is clear evidence of recovery, as shown by the diminution of the intercleavage interval with time. We take this as evidence that while the sperm alone is incapable of recovery, it does recover in the presence of egg cytoplasm. To this extent the findings are comparable to those for ultraviolet radiation. But there is no evidence of photorecovery since, as Text-fig. 4 B shows, the light and dark samples cleaved at the same time.

Experiments with Halves (Experiments 23 to 26)

Text-fig. 4 C illustrates an experiment in which halves were subjected to x-radiation before fertilization with normal sperm (Experiments 23, 25). An unexposed sample of halves served as control. The result is comparable to that with ultraviolet radiation illustrated in Text-fig. 3 A. The cleavage of the nucleate halves is delayed by x-ray. The cleavage of the enucleate halves is not delayed. Here, as in the case of ultraviolet radiation, the effect appears to be upon the nucleus. Henshaw (1938 *a*, *b*) also found that there is no delay of cleavage of enucleate halves when they are exposed to x-ray before fertilization.

We exposed fertilized halves to x-ray (Experiments 24, 26) and found that, as in the case of ultraviolet radiation, the cleavages of both the nucleate and enucleate halves were delayed. This is illustrated in Text-fig. 4 D which may be compared with Text-fig. 3 C.

No experiments on photorecovery were made with halves, since this effect was not found in x-rayed whole eggs.

DISCUSSION

We find it difficult to interpret our results in any other way than that outlined in the introduction. It seems clear that the action of either ultraviolet radiation or x-rays in delaying cleavage of the *Arbacia* egg has its locus in the nucleus. Only when a nucleus is present do these agents affect the rate of cleavage. It makes no qualitative difference whether the nucleus be that of either gamete or of the zygote. When the part irradiated has no nucleus, as in the case of the enucleate half before fertilization, cleavage is not affected. Our findings make clear the results obtained by Harding and Thomas (1950) who exposed to ultraviolet radiation *Arbacia* eggs stratified by centrifugation. The nucleus in such eggs lies in the upper clear zone close to the surface, where it

is more readily reached by ultraviolet radiation incident from above than that from below which has to pass through considerable material. They found that radiation from above was more effective in delaying cleavage than radiation from below. Studies by Miwa *et al.* (1939 *b*) of the effects of α-rays on eggs of the sea urchin, *Strongylocentrotus purcherrimus*, led them to conclude that the effect is at least in great part on the nucleus.

On the other hand photorecovery after exposure to ultraviolet radiation appears to be associated exclusively with the egg cytoplasm. The sperm itself displays no ability to recover from the effects of ultraviolet radiation whether it is in the light or in the dark. Yet in the presence of egg cytoplasm there is photorecovery from the effects of ultraviolet radiation whether this has been applied to the sperm alone or to the egg, sperm, or fused nucleus when in the egg.

Although in delaying cleavage the ultraviolet radiation and x-ray act upon nuclear material, one cannot say that such radiation has no effect whatever on the cytoplasm. The radiation has to pass through a considerable amount of cytoplasm in order to reach the nucleus, and there are substances in the cytoplasm, *e. g.* ribosenucleic acid and proteins, which absorb ultraviolet radiation, and which may be expected to undergo photochemical change. X-rays are also absorbed within the cytoplasm and might well have some effect thereon. We note, however, that the experiments of Reed (1948) with ultraviolet radiation, and those of Lucké, Ricca, and Parpart (1951) with x-ray indicate that permeability of the cell membrane is not altered by these agents. The thing that seems clear from our experiments is that whatever alterations may occur in the cytoplasm they do not demonstrably affect the tempo of cleavage of the nucleus or of the cytoplasm.

There is a striking parallelism between these results and those obtained by Dulbecco (1950) on bacteriophage and *Escherichia coli*. When inactivated by ultraviolet radiation bacteriophage shows no photorecovery. Photoreactivation of the bacteriophage occurs, however, if the host upon which the bacteriophage is adsorbed is exposed to visible light. That essentially the same photorecovery process is involved in both cases seems clear since they have several critical characteristics in common[7] (compare Dulbecco, 1950; and Blum, Loos, and Robinson, 1950). But the analogy we wish to draw goes further.

If one thinks of the *Arbacia* sperm as comparable to the bacteriophage and egg cytoplasm as comparable to the *E. coli*, analogy is readily seen. Neither bacteriophage nor *Arbacia* sperm is able by itself to recover from the effects of ultraviolet radiation. Visible light does not promote recovery in either case. The introduction of the irradiated sperm into the egg induces effects, the recovery from which is accelerated by visible radiation absorbed by the egg cyto-

[7] The demonstration of this phenomenon in a vertebrate animal (see Blum and Matthews, 1950) indicates its widespread distribution in the living world.

plasm, just as the bacteriophage is reactivated when in intimate association with the illuminated host cell, *E. coli*. Recovery from the effects of ultraviolet radiation on either *E. coli* itself (Kelner, 1949; Johnson, Flagler, and Blum, 1950) or on the *Arbacia* egg is enhanced by illumination with visible light.

Without attempting to explain the mode of action of the ultraviolet radiation we may point out that in both the bacteriophage–*E. coli* system and in the *Arbacia* sperm–egg system the effect appears to be upon nucleoprotein. *E. coli* bacteriophage is almost pure nucleoprotein, and the *Arbacia* sperm head contains little else. In the case of bacteriophage and of sperm we may assume that some alteration takes place in the nucleoprotein which the bacteriophage or the sperm alone cannot "repair." On the other hand this damage can be repaired when *E. coli* or egg cytoplasm is present, since photorecovery does occur when the bacteriophage or sperm is introduced into the cell.

On the basis of this analogy we may make tentative suggestions regarding the nature of the effects of ultraviolet radiation and of the process of photorecovery. If we think of the action of ultraviolet radiation as altering some component of an organized nucleoprotein system, and photorecovery as the "repair" of this system we are struck by the fact that repair only takes place in systems in which active reproduction of parts occurs. Neither bacteriophage nor *Arbacia* sperm can reproduce itself and it seems reasonable to think that in neither case is there a mechanism present for the synthesis of new nucleoprotein. Such synthesis goes on in the host cell of the bacteriophage, *E. coli*, or in the egg of *Arbacia* in the case of *Arbacia* sperm and these are the same systems in which photorecovery from the effects of ultraviolet radiation takes place. There seems then an association of the ability to repair the damage done by ultraviolet radiation with the synthetic processes concerned in reduplication of cellular patterns. We have suggested, therefore (Blum, Robinson, and Loos, 1950) that the repair of damage by ultraviolet radiation in these instances involves synthetic processes in the cell.

Swenson and Giese (1950) have clearly shown that ultraviolet radiation inhibits the ability of the yeast cell to adapt itself to the oxidation of galactose, and the recovery of that process in the presence of visible light. It appears that the ultraviolet radiation in some way inhibits the adaptive formation of the enzyme galactozymase, since the authors present evidence that the action of the radiation is not directly on the enzyme. They suggest that the locus of action is the nucleoprotein component of the cell concerned in synthesis of the enzyme. Following our line of reasoning, photoreactivation would in this case seem to be concerned with the "repair" of this nucleoprotein component. Dulbecco finds evidence on the basis of results not yet published that the photoreactivation of bacteriophage is intimately connected with the metabolism of the *E. coli* cell (personal communication). While none of these pieces of evidence excludes alternative hypotheses, they all lend support to the view that

photorecovery from the effects of ultraviolet radiation involves synthetic processes in the living cell.

The present authors also suggested—with great caution—that visible light may supply energy to the synthetic processes concerned in the damage brought about by ultraviolet radiation. These synthetic processes in general are—at least in an over-all sense—endergonic. In this case the photorecovery process might be considered as a kind of "photosynthesis" widely distributed among living organisms, and such a mechanism could have interesting evolutionary aspects.[8] For the time being, however, such ideas belong to the realm of speculation although they are in line with the idea that the recovery process is associated with synthetic properties of the living organism.

SUMMARY

Various experiments are described which show the locus of action of ultraviolet radiation and of x-ray in delaying cell division of the fertilized egg of *Arbacia punctulata* to be in the nucleus.

Photorecovery following ultraviolet radiation has its locus in the egg cytoplasm.

Analogy is drawn with the action of ultraviolet radiation and photorecovery on the *E. coli*–bacteriophage system, and certain suggestions are made regarding a common mechanism.

Photorecovery after x-ray exposure could not be demonstrated.

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[8] *E.g.*, see Blum, H. F., Time's Arrow and Evolution, Princeton University Press, 1951.

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EXPLANATION OF PLATE 1

Figs. 1 to 6. Selections from the photographic record of Experiment P-50, illustrated in Text-fig. 3 A.

The enucleate (red) halves appear dark, the nucleate (white) halves appear light. There are a few halves which do not cleave and these serve as markers.

Control sample:

FIG. 1. 54 minutes after fertilization (*a*, in Text-fig. 3 A).

FIG. 2. 76 minutes after fertilization (*b*, in Text-fig. 3 A).

FIG. 3. 87 minutes after fertilization (*c*, in Text-fig. 3 A).

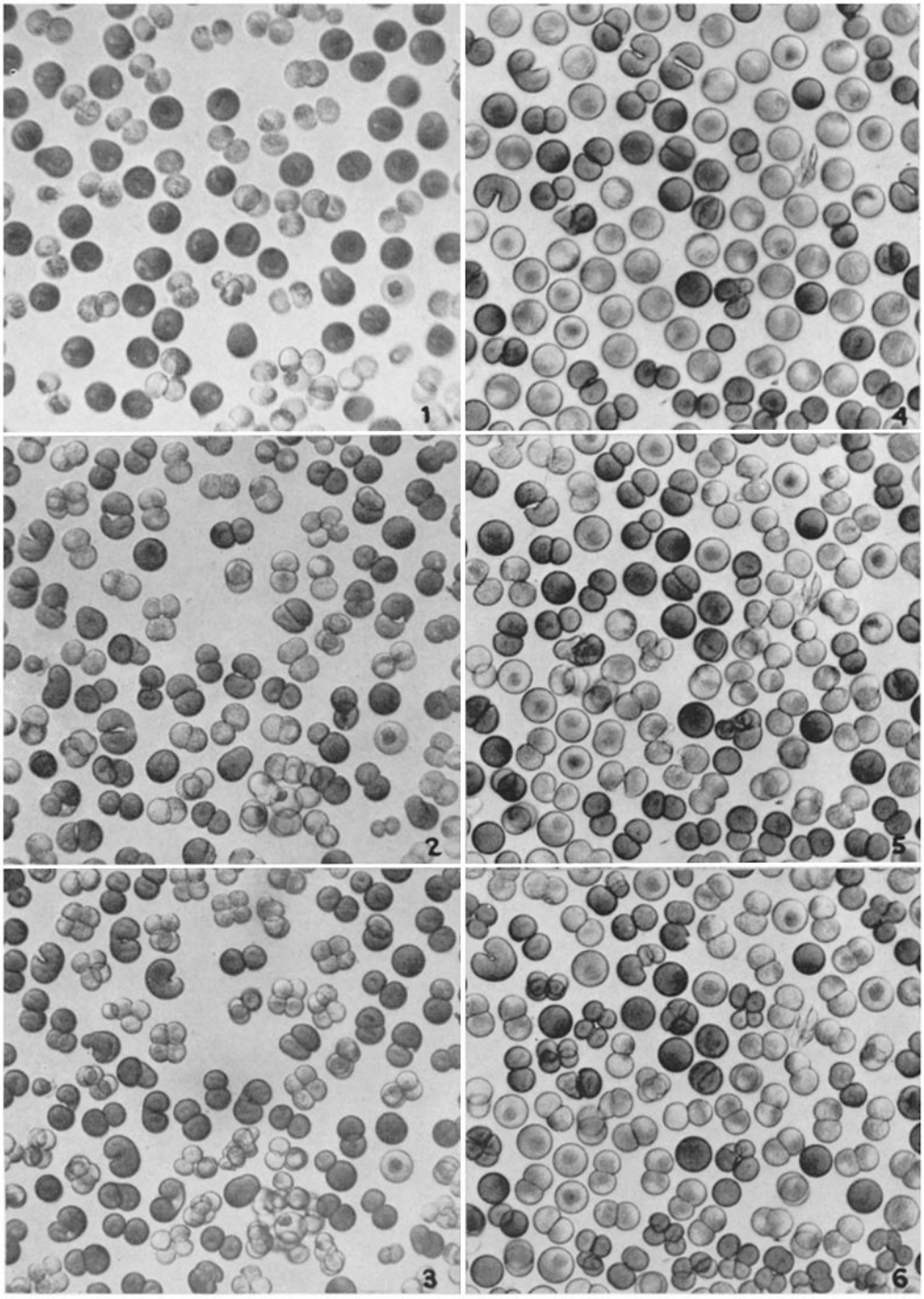
Irradiated sample:

FIG. 4. 71 minutes after fertilization (*d*, in Text-fig. 3 A).

FIG. 5. 83 minutes after fertilization (*e*, in Text-fig. 3 A).

FIG. 6. 95 minutes after fertilization (*f*, in Text-fig. 3 A).

Note that in the control sample the nucleate halves cleave before the enucleate, whereas in the irradiated sample the enucleate halves cleave before the nucleate.



(Blum *et al.*: Radiation and photorecovery)