

A COMPARISON OF FIVE EFFECTS OF ULTRAVIOLET LIGHT ON THE ARBACIA EGG

BY HAROLD F. BLUM,* JOHN S. COOK, AND GORDON M. LOOS

(From the National Cancer Institute, National Institutes of Health, Public Health Service, Department of Health, Education, and Welfare, Bethesda; the Department of Biology, Princeton University, Princeton; and the Marine Biological Laboratory, Woods Hole)

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Ultraviolet radiation is absorbed by various kinds of molecules in the cell and hence it is possible that a number of photochemical reactions may be forwarded. If we study only one aspect of the cell's behavior, however, we may see only the result of one of these reactions, and are likely, then, to think that ultraviolet radiation has only one specific effect on the cell. This may lead to confusion, particularly when different investigators study different aspects. There is the possibility, moreover, that one effect of the radiation may be modified by another, so we should be aware of the possibility of confusion by "mixed" effects. This is more likely to occur with polychromatic radiation than with monochromatic; but where action spectra overlap the use of monochromatic radiation does not in itself eliminate the possibility of mixed effects.

In the present study five separate effects of ultraviolet radiation on the *Arbacia* egg are examined and compared.

EXPERIMENTAL

Methods

The methods differ little from those described in earlier papers from this laboratory. The source of ultraviolet radiation was an intermediate pressure mercury arc which emits its radiation in a series of "monochromatic lines." In some of our experiments wave lengths shorter than 0.26μ were filtered out by means of a Corning glass filter 9700 (corex D); this will be referred to as *filtered* radiation. In other experiments the arc was used with no filter so that wave lengths as short as 0.23μ were incident on the eggs; this will be referred to as *unfiltered* radiation. The intensity of the various lines, and the transmission of the filter have been described in a previous paper (Blum and Price, 1950), to which the reader is also referred for details regarding the methods of dosage, etc. The "dosage units" referred to here are based on the amount of energy required to activate the photocell with which the dosage is

* Present address: Department of Biology, Princeton University, Princeton, New Jersey.

monitored. For unfiltered radiation each unit represents $\sim 5.6 \times 10^4$ ergs cm.^{-2} of wave lengths 0.23μ to 0.313μ at intensity $\sim 1.2 \times 10^4$ ergs cm.^{-2} sec.^{-1} incident on the eggs; for filtered radiation the same unit represents $\sim 2.0 \times 10^4$ ergs cm.^{-2} of wave lengths 0.26μ to 0.313μ at intensity $\sim 0.5 \times 10^4$ ergs cm.^{-2} sec.^{-1} incident on the eggs. Wave lengths longer than 0.313μ are without appreciable effect, as shown by interposing a window glass filter which cuts off this and shorter wave lengths.

The eggs were exposed to the radiation in flat dishes, after they had settled to form a single layer on the bottom. Since the eggs were not stirred the radiation impinged directly on one hemisphere only. Comparisons were always made on samples of eggs from a single female, each sample subjected to a certain dose of ultraviolet radiation. In the majority of experiments the eggs were not fertilized; in some they were fertilized before, and in some after, the exposure to ultraviolet radiation; in a few experiments the sperm was irradiated before being used to fertilize the eggs.

Quantitative measurements were based on successive photographs of the same population of eggs with low power microscopy, using the technique described by Blum and Price (1950). The range of observations was extended by adding a third microscope to the battery, and further amplified in some experiments by using multiple chambers on each microscope. These were made by attaching short lengths of glass tubing or of "soda straws" a few millimeters in diameter to glass disks, the multiple chamber being substituted for the single larger chamber ordinarily used. In many experiments, however, samples of eggs were examined at intervals without attempting to follow the behavior of the individual eggs. In such cases the television microscope was sometimes used for detailed examination at higher power.

RESULTS

The kinds of changes brought about by ultraviolet radiation we designate as follows: (1) delay of cell division (cleavage); (2) cytolysis; (3) fixation; (4) activation; and (5) removal of the jelly membrane.

Delay of Cleavage.—Ultraviolet radiation delays the cleavage of fertilized *Arbacia* eggs. After the initial delay the eggs return gradually to their normal rate of cleavage and go on to form normal larvae. The recovery of cleavage rate is accelerated by exposure to "visible" light; that is, there is photorecovery. This phenomenon has been the subject of previous studies in this laboratory (Blum *et al.*, 1950, 1951).

Cytolysis.—We apply the term *cytolysis* to a process which ends in the egg breaking up into small globules. The first observable changes occur in the cytoplasm within a few minutes to a few hours after exposure to ultraviolet radiation, depending upon the dose. There may be rapid churning or cyclic movement in the cytoplasm, observable with the television microscope. Characteristically, the echinochrome pigment accumulates in a limited region at the periphery of the cell, forming a spherical red mass with a diameter approximately $\frac{1}{3}$ to $\frac{1}{4}$ that of the cell. When the cell breaks up it forms globules; the volume containing the pigment usually constituting one of these, so that

in viewing a field of cytolyzed cells one sees numerous ruby-like, gleaming spheres accompanied by a much larger number of smaller "colorless" ones. The cell nucleus is usually lost track of in this mass of cellular debris. Much the same changes take place in fertilized as in unfertilized cells.

Cytolysis may be brought about by either filtered or unfiltered radiation. The same type of cytolysis occurs occasionally in cells that have not been exposed to ultraviolet radiation. We have seen the concentration of pigment in untreated eggs and it was not always followed by cytolysis. We have seen fertilized eggs show pigment concentration after exposure to ultraviolet radiation, then go through several cleavages, the pigment remaining in one part of the egg.

Fixation.—When eggs are subjected to high doses of unfiltered radiation they may not break up into globules, but remain intact, retaining the approximate volume of normal eggs or showing moderate increase in some cases. However, there are as a rule extensive changes in the interior of the cell. Usually the pigment concentrates to form a ruby-like spot just as in eggs about to cytolyze, the remainder of the egg also showing changes similar to those that go on prior to cytolysis. Sometimes there is superficial resemblance to a fertilized egg that has gone through many cleavages. This phenomenon, which we have termed *fixation*, may occur in either fertilized or unfertilized eggs.

Activation of the Egg.—Unfiltered radiation may bring about another series of changes in unfertilized eggs; furrows develop on the surface, often followed by more or less normal cleavage. Occasionally one finds such a cleavage in which the nuclei can be detected in the two divided cells, but this is not always observable. For the most part the cleavages are irregular. Usually the eggs break up after going through one or a few cleavages. Rarely such changes occur among normal populations of unfertilized eggs.

Such behavior is commonly recognized as *activation* of the egg, the first step in artificial parthenogenesis. It has been described following exposure to ultraviolet radiation by a number of investigators, including Loeb (1914), Lillie and Baskerville (1922), Heilbrunn and Young (1930), Hollaender (1938), and Harvey and Hollaender (1938). In most cases the eggs did not go beyond one or a few cleavages, but Lillie and Baskerville report that some blastulae were formed when the eggs were treated with hypertonic sea water after exposure to ultraviolet radiation, and Young found development to the gastrula stage in some cases (Heilbrunn and Young, 1930). In our experience the proportion of activated eggs varied widely among samples from different females. Always present with the activated eggs were cells which were distorted in shape but did not display a tendency to cleave, and also a certain number of cytolyzed or fixed cells.

In the course of activation the eggs sometimes develop a membrane which is usually assumed to be identical with the fertilization membrane that appears

after the entrance of sperm. Since in our experiments only one hemisphere was exposed directly to the radiation, the membranes, when they appeared, tended to be one-sided, as Spikes (1944) has previously reported in *Lytechinus*. In his experiments Hollaender (1938) caused the cells to be rotated while they were being exposed to the radiation and this produced more uniform membranes. We have found the raising of membranes quite a variable thing; sometimes we could not detect it. There appears to be a membrane formed around the cytolyzing egg before it breaks up, and membranes are also apparent around fixed eggs. We have been unable to assure ourselves, even after observation with the television microscope, which reveals a good deal of detail, that the membranes we observed in activated eggs were different from those around cytolyzing or fixed eggs.

Removal of the Jelly Membrane.—The *Arbacia* egg is surrounded by a tenuous membrane of jelly, which varies in thickness according to the batch of eggs. It is rather easily removed by shaking or other means, among them ultraviolet radiation. We have studied the effect of the latter agent, using Janus green B after exposure to the radiation to detect the presence or absence of the membrane. Unfiltered radiation readily removes the membrane (all or in part); filtered does not. The removal of the membrane does not seem to affect any of the other phenomena here described.

Dose Relationships.—Fig. 1 serves to illustrate difficulties encountered in trying to separate cytolysis, fixation, and activation in terms of the effective dosage. In the particular experiment described, which was carried out late in the season, all three effects were produced by relatively low doses of unfiltered radiation. It is seen that a few of the eggs cytolized in the control, and that the per cent cytolized increased sharply with dosage of ultraviolet radiation up to a maximum at 20 dosage units. Above this value the incidence of cytolysis fell off parallel to the increase of fixation, which first appeared a little above 10 dosage units. The percentage of eggs activated reached a maximum at about 30 dosage units, falling off again with increased dosage.

Many experiments of this general type were carried out during the course of the summer; but while the same three phenomena were always observed, their incidence with respect to dosage varied widely. The experiment illustrated in Fig. 2, which was performed early in the season, contrasts sharply with that described in Fig. 1. In the former no fixation was produced by a dose of 56 units, 99 per cent of the cells being cytolized or activated at this dosage, whereas in the latter over half the eggs were fixed by this dose. In other experiments, fixation of virtually all the cells occurred at doses above 100 units although a high percentage of the eggs cytolized at lower doses. Activation varies even more widely than cytolysis and fixation. The eggs seem to become more sensitive to radiation as the season progresses, but the differences between batches of eggs are so great that it might be difficult to define a trend.

As described above, the fixed egg usually shows internal changes similar to those seen in eggs about to cytolize; as though a sort of case hardening had occurred which arrested the course of cytolysis by preventing the cell from breaking up into globules. The probability that fixation prevents cytolysis is indicated in Fig. 1, where cytolysis falls off as fixation increases; and there is other evidence of a reciprocal relationship. Fixation is not brought about by

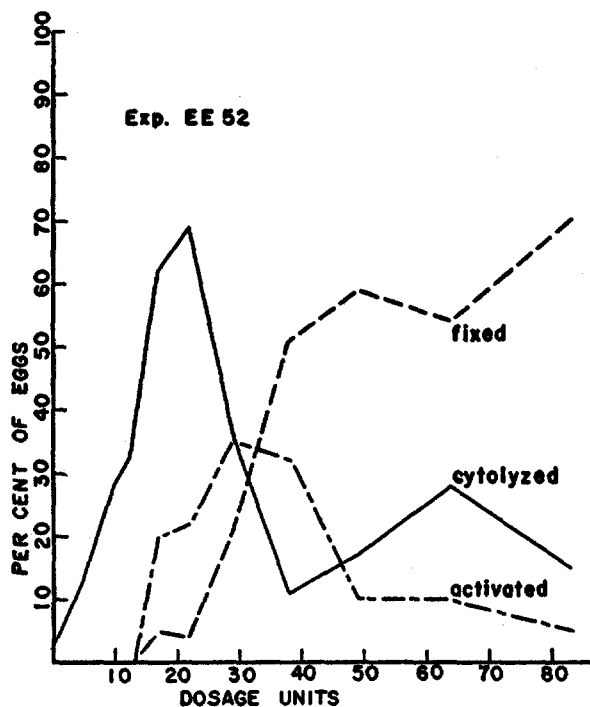


FIG. 1. Incidence of cytolysis, fixation, and activation 5 hours after exposure to ultraviolet radiation. The experiment was carried out on August 18, 1952, with unfertilized eggs from a single female. Dosage units represent $\sim 5.6 \times 10^4$ ergs cm^{-2} , λ 0.23μ to 0.313μ .

filtered radiation, that is, it depends upon wave lengths shorter than 0.26μ , whereas cytolysis is also produced by filtered radiation, although much higher doses are required. It seems that we deal with two essentially different processes, but that there is interplay between them which may depend upon the rates at which certain reactions occur and hence should be influenced by the intensity of the radiation. Activation is also brought about only by wave lengths shorter than 0.26μ , but whether there is any direct relationship between activation and fixation is uncertain.

Delay of cleavage is brought about by doses of filtered radiation much smaller than those that are ordinarily required to produce cytolysis, for example, 100 dosage units of filtered radiation produces marked delay, but ordinarily at least ten times this amount is required to bring about cytolysis.

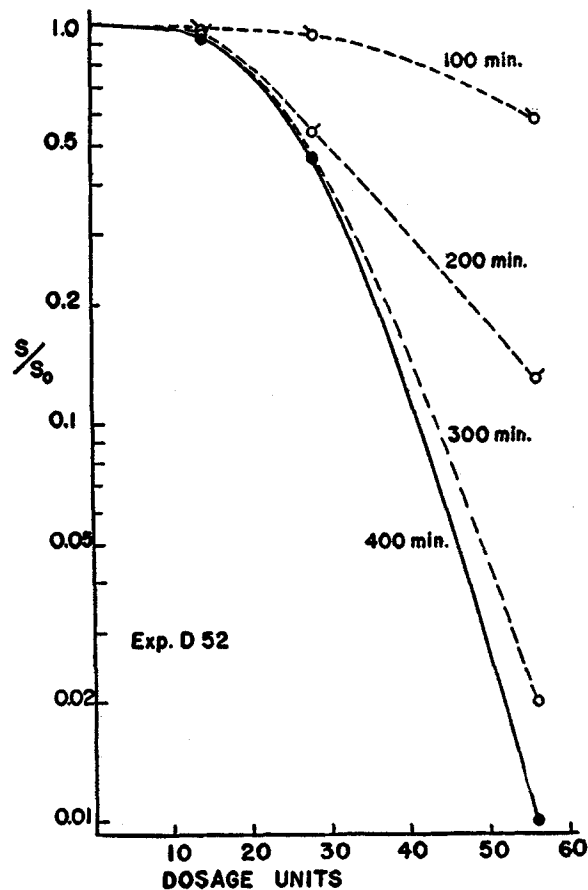


FIG. 2. Survival curve for unfertilized eggs exposed to ultraviolet radiation. The experiment was carried out on July 4, 1952, with unfertilized eggs from a single female. Dosage units represent $\sim 5.6 \times 10^4$ ergs cm^{-2} , λ 0.23 μ to 0.313 μ .

Again, the delay of cleavage is quite uniform—both qualitatively and quantitatively—for eggs from different females and throughout the season, in contrast to the variability of the other effects.

It is difficult to study the removal of the jelly membrane quantitatively; the dose required seems to vary widely from one batch of eggs to another.

Loci of Action.—That there is more than one light absorber concerned in

the photochemical reactions underlying these changes is clear from the respective ratios of effectiveness of filtered and unfiltered radiation which are shown in Table I. The first two ratios are only rough estimates, but they are suggestive as regards the loci of action, and adequate for general comparative purposes.

In the case of cleavage delay it seems clear from other evidence that the nucleus is the locus of action, and that the light absorber is probably nucleic acid (Blum, Robinson, and Loos, 1951). If we calculate the relative number of quanta absorbed by a thin layer of nucleic acid when exposed to filtered and unfiltered radiation we find the ratio 1/29. The ratio for cleavage delay given in Table I is reasonably close to this, and so is consistent with the other evidence converging to indicate nucleic acid as the light absorber for this process.

The lower ratio in the case of cytolysis is difficult to interpret. Calculation of the relative numbers of quanta absorbed by a typical protein gives a ratio of about 1/10 for filtered as against unfiltered radiation. This ratio is higher than

TABLE I

Effect	Effectiveness of filtered radiation (0.26 μ to 0.313 μ) Effectiveness of unfiltered radiation (0.23 μ to 0.313 μ)
Cleavage delay	1/25
Cytolysis	1/100
Activation	0
Fixation	0?
Removal of jelly membrane	0

that for nucleic acid, and altogether out of line with the ratio of effectiveness in producing cytolysis. It should be remembered, however, that the intensity of the filtered radiation is much lower than that of the unfiltered, though we have no way of estimating accurately the relative effective intensities without knowing the spectrum of the light absorber. It seems likely that the rate of photochemical change may be important in determining whether cytolysis occurs, and hence intensity may have influenced the ratio of effectiveness shown in the table. We are left with no real evidence as to the nature of the light absorber for cytolysis. That this effect is based on cytoplasmic rather than on nuclear changes is suggested by experiments in which normal eggs were fertilized with sperm that had been exposed to ultraviolet radiation. The eggs did not cytolysis (at least not for many hours). If cytolysis were due to production of a cytolytic agent in nuclear material one would expect it to occur in this case.

Table I shows a ratio of zero for activation; that is, filtered radiation, which contains only wave lengths longer than 0.26 μ , is without effect. This agrees with the action spectrum measured by Hollaender (1938) which shows no

activation by wave lengths 0.265μ and longer; the action spectrum rises at shorter wave lengths, gradually at first and then more steeply with no maximum out to 0.226μ beyond which the measurements were not carried. The action spectrum for hemolysis, obtained by Sonne (1928), is of the same character and has approximately the same spectral position. Sonne suggests that ultraviolet radiation may act upon the lipid fraction of the red cell surface to produce hemolysis, and that the action spectrum reflects the absorption spectrum of that fraction. One is tempted to carry this explanation over to the activation of the *Arbacia* egg, particularly since it is thought that activation involves changes in the lipids of the cell membrane.

Harvey and Hollaender (1938) demonstrated activation of enucleate halves of *Arbacia* eggs with ultraviolet radiation, showing clearly that the locus of action is outside the nucleus. More definite evidence regarding the nature of the light absorber is lacking, but it is certainly different from those concerned in delay of cleavage and in cytolysis.

In all but one experiment fixation was produced only by wave lengths shorter than 0.26μ . We are left uncertain as to the relationship of this to the other phenomena.

Only wave lengths shorter than 0.26μ are effective in removing the jelly membrane. The locus of this effect may be in the jelly membrane itself, or possibly at the surface of the cell. In the latter case the light absorber might be the same as that for activation.

Photorecovery.—That there is photorecovery after cleavage delay by ultraviolet radiation has been clearly shown (see Blum *et al.*, 1950, 1951). The possibility of photorecovery in the case of the four other phenomena was examined by running parallel series in the dark and under "fluorescent" lamps (daylight type), but we were unable to demonstrate any significant difference in the degree of cytolysis, fixation, activation, or jelly removal. Since photorecovery following cleavage delay was so easily demonstrable under the same conditions, we have had to conclude that it does not occur in the other cases. This is again evidence of the fundamental difference in the mode of action of ultraviolet radiation in delaying cleavage, and in producing the other four effects here discussed. This seems to bear importantly upon the theoretical interpretation of experiments, since the carrying over of evidence from one of these phenomena to another would not seem justified.

It is of particular interest that eggs activated by ultraviolet radiation do not cleave more rapidly or more regularly when illuminated with visible radiation than when in the dark, since this may indicate that the mechanism controlling cleavage in the artificially activated egg is different from that controlling cleavage in the normally fertilized egg. The cleavage of normally fertilized eggs is delayed by the action of ultraviolet radiation on the nucleus, photorecovery manifesting itself by more rapid return to normal cleavage rate

when the eggs are illuminated with visible light (see Blum, Robinson, and Loos, 1951). In the course of activation with unfiltered ultraviolet radiation the dose received by the egg nucleus should delay the cleavages if they were controlled by the same mechanism as in the normally fertilized egg, and so subsequent illumination with visible light should accelerate cleavage of the activated eggs. This is not, however, the case.

The objection might be made that photorecovery is inhibited by the heavy doses of unfiltered ultraviolet radiation used to activate the eggs. This is apparently ruled out, however, by experiments in which eggs subjected to doses of unfiltered radiation sufficient to activate were fertilized with normal sperm immediately after exposure to the radiation. Most of these eggs cleaved normally, but much later than normal eggs fertilized at the same time, indicating that the ultraviolet radiation had affected the egg nucleus. Subsequent illumination with visible light greatly accelerated the return to normal cleavage rate, indicating that the mechanism for photorecovery was intact and functioning, and that the egg nucleus was capable of recovering. The sperm nucleus was, of course, not exposed to ultraviolet, and so the part it contributed to the fusion nucleus was presumably not subject to photorecovery (see Blum, Loos, and Robinson, 1950; Blum, Robinson, and Loos, 1951).

It would seem that the failure of illumination to speed up cleavage of the activated egg indicates that the nucleus has little or no influence on the timing of cleavages resulting from activation. Since enucleate halves of *Arbacia* eggs may be activated by ultraviolet radiation and undergo subsequent cleavage (Harvey and Hollaender, 1938), it seems that the locus for the timing of cleavage of activated eggs is in the cytoplasm. Other experiments with halves show clearly that the timing of cleavage of the normally fertilized egg is associated with the nucleus (Blum, Robinson, and Loos, 1951). It is tempting to suggest that the timing mechanism in the cytoplasm is a primitive one, whose function is normally taken over by the more recently evolved nucleus.

"*Survival Curves.*"—In the study of the effects of ultraviolet radiation on microorganisms one of the things most commonly measured is the survival curve; that is, the relationship of the number of organisms surviving (usually measured as the number able to form colonies) to dose. The simplest type of curve is first order or one quantum, commonly called a "single hit." It is described by the equation

$$\frac{S}{S_0} = e^{-kD} \quad (1)$$

in which $\frac{S}{S_0}$ is the fraction of the microorganisms surviving a given dose D , and

k is a constant characteristic of the particular organism. This relationship yields a straight line when the logarithm of the fraction of survivors is plotted against

dose, the origin on the logarithmic axis being 0. The best interpretation of this type of curve is that the death of the organism is brought about by a single quantum of ultraviolet radiation, which means that the primary photochemical change leading to death is in only one molecule in the cell. The most reasonable interpretation is that the quantum of ultraviolet radiation obliterates some pattern, comprised in a single molecule, which is necessary for the reproduction of substances vital to the cell. Lea (1947) has pointed out the analogy to lethal genes.

If more than one of these patterns were present in the cell, all would have to be obliterated before the cell dies, and this would give a curve of another shape, convex upward at the beginning and then falling off along a straight line on the semilogarithmic plot. The straight line part of such a curve is described by the equation

$$\frac{S}{S_0} = \frac{1}{n} e^{-kD} \quad (2)$$

The number of quanta (n) required to kill should be obtainable by extrapolating to zero dose (see Atwood and Norman, 1949; Norman, 1951, for a discussion of the theoretical implications).

In Fig. 2 the logarithm of the fraction of eggs surviving is plotted against dosage, as is common practice for survival curves for microorganisms. The curve labelled 400 minutes represents eggs surviving 400 minutes after exposure to ultraviolet radiation; the curve did not change appreciably after that time. The count of survivors was based on normal appearance of the cells in photomicrographs, the non-survivors being those eggs that cytolysed or underwent changes interpreted as activation. When sperm was added to corresponding samples of these eggs, the number which underwent normal cleavage paralleled roughly the microscopic count, so it may be assumed that the numbers plotted in the curve represent those eggs that could have "survived;" *i.e.*, developed normally. It is obvious that this curve would be classed as multiquantum or multihit type, although there are not enough points to tell whether the latter part of the curve is a straight line, or to define its slope. The exact interpretation of the curve is uncertain, but it is obvious that many quanta have to be absorbed by the average egg before it is rendered non-viable. On the other hand, if the first part of the curve for cytolysis in Fig. 1 is plotted in the same way as is Fig. 2, it suggests that only a few quanta are required to destroy the cell; this is not so surprising when we note that in Fig. 1 some of the cells have cytolysed without being exposed to radiation, for we may assume that the eggs used in this experiment were more susceptible to injury than those represented in Fig. 2. A multiquantum type of curve is to be expected if the destruction of the cell depends upon extensive changes, such, for example, as alterations in the cell surface.

Are we justified in comparing these curves with survival curves for microorganisms? Certainly not with single hit curves, where, as previously explained, the mechanism of killing must be very different, since it involves a change in only one molecule in the cell. But single hit curves are relatively rare, and may not some of the multihit curves that are reported for microorganisms be influenced by mechanisms similar to those that produce the type of curve shown in Fig. 2? The killing of a fraction of the organisms by multiple hit injury of a non-specific nature comparable, say, to cytolysis of the *Arbacia* egg, could distort a single hit curve to give it an apparent multihit character. If this were the case, the single hit, or small number of hits, type of curve might often be obscured. This seems important to remember in the interpretation of survival curves. It might explain apparent changes in the survival curves observed with change in environmental conditions.

The type of curve shown in Fig. 2 illustrates what is often called a "threshold" for such effects. Obviously the threshold is not very definite and the curves for times earlier than 400 minutes indicate the need of waiting long enough to reach a final condition.

SUMMARY AND CONCLUSIONS

The demonstration of five different effects of ultraviolet radiation on the sea urchin's egg indicates that more than one basic photochemical process goes on there. Photorecovery is observed in only one of these. The need for caution in interpreting such effects is obvious.

Evidence for a different mechanism for the timing of cleavage in eggs activated by ultraviolet radiation as compared to normally fertilized eggs is presented.

The bearing of these studies on survival curves for microorganisms is discussed.

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