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8. Approximately the same side of the moon faces the earth at all times. To a stationary observer on the moon the earth would therefore neither rise nor set, but, except for librational effects, would maintain a fixed position in the sky. If an "earth illusion" were visible to him, it would normally be due only to his movement between points where the earth is seen on the horizon and in elevation. But the moon's curvature being greater, its horizon is consequently less distant than the earth's.

15 October 1962

Recovery from Radiation-Induced Delay of Cleavage in Gametes of Arbacia punctulata

Abstract. A decrease in delay of cleavage, when irradiated sperm fom *Arbacia punctulata* were allowed to "recover" inside eggs, occurred when cell division was interrupted by the removal of oxygen soon after fertilization. The recovery mechanisms in irradiated eggs of this species before and after fertilization have been compared and appear to differ.

Exposure to ionizing radiation of either the egg or sperm of *Arbacia punctulata* quantitatively retards the time of first cleavage of the fertilized egg (*1*). Early experiments by Henshaw demonstrated that the magnitude of the cleavage delay induced by radiation decreases with the length of time that the irradiated eggs remain in sea water after irradiation but before insemination (*1*). Henshaw found that with time this decrease was exponential and termed the phenomenon "recovery." No such recovery could be demonstrated in irradiated sperm, which consist mostly of nuclear material. It has been assumed, therefore, that certain cytoplasmic components are necessary for the recovery mechanism to operate.

In a recent reinvestigation of aspects of this recovery process, the recovery rate of irradiated eggs in sea water was independent of oxygen (*2*). When irradiated eggs were kept, prior to fertilization, in deoxygenated sea water the same decrease in delay of cleavage was observed as when they were kept in fully aerated sea water for the same period. It was decided, therefore, to look for recovery in irradiated sperm by allowing the sperm to enter the egg and then blocking cell division for various lengths of time by removing oxygen. This technique demonstrated that recovery does indeed occur in irradiated sperm if the sperm is inside the egg during the recovery period.

The criterion of effect was the delay in the time of first cleavage of fertilized eggs induced by exposure of either the eggs or sperm to a dose of 10,000 r. The time of cleavage was measured, in minutes, from the time of fertilization to the time when cleavage took place in 50 percent of the eggs. The time of cleavage is considered to be good to about $\pm$ 1 minute; hence the values for cleavage delay are accurate to about $\pm$ 2 minutes. Irradiation was delivered by a 5000-curie, cesium-137 gamma-ray unit, at a rate of 5000 r/min.

For the investigation of sperm recovery, irradiated and nonirradiated sperm from the same animal were used to fertilize normal eggs in test tubes. Within a few minutes after fertilization, nitrogen was bubbled continuously through the fertilized egg suspensions to displace the oxygen. Samples were removed thereafter at intervals to finger bowls which contained fresh sea water, and the time of cleavage was measured. The time of cleavage of the unirradiated samples treated with nitrogen was compared with that of the normal controls to give values for the prolongation of the cell division cycle caused by the removal of oxygen. These prolongations are plotted as "recovery periods" in Fig. 1.

The results of two experiments shown in Fig. 1 illustrate the two types of recovery curve observed. In two out of four cases, the radiation-induced delay of cleavage decreased exponentially with recovery time (curve *A*). In the other two cases there was an initial sudden drop followed by a slower exponential decline (curve *B*). The similarity in the terminal slopes of curves *A* and *B* is coincidental; some variation in recovery rate occurs when the sperm are obtained from different animals.

It was then decided to apply the same technique to irradiated eggs and to compare the curves for eggs allowed to "recover" before and after fertilization. The results of two such experiments are shown in Fig. 2. In the case

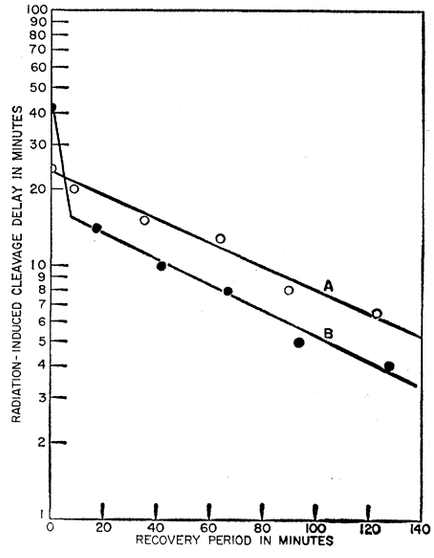

Fig. 1. Recovery of *Arbacia* sperm inside the egg after exposure to 10,000 r. Curves *A* and *B* show results of two experiments and represent the two types of response found. Abscissa, prolongations of cell division are plotted as "recovery period" intervals. Ordinate, time delays of first cleavage, logarithmic scale.

of the unfertilized eggs the recovery period represents the time interval between irradiation and fertilization; for the fertilized eggs, inseminated immediately after irradiation, the recovery period represents the time interval that cell division was retarded when oxygen was replaced by nitrogen. The two forms of recovery curves were the same as the forms of the curves with irradi-

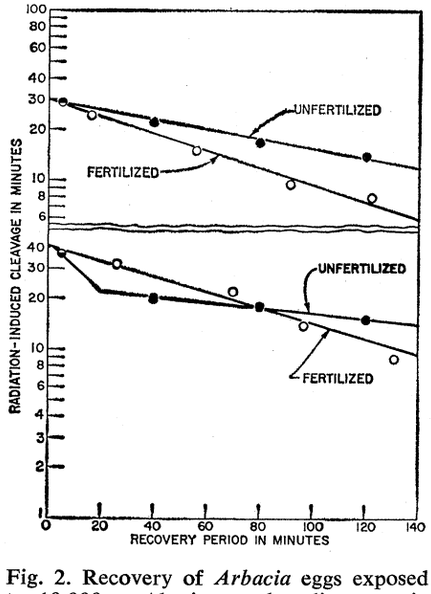

Fig. 2. Recovery of *Arbacia* eggs exposed to 10,000 r. Abscissa and ordinate as in Fig. 1. Results of two experiments indicate the types of response found.

ated sperm, and in four experiments all four possible combinations of the two curve shapes were observed. In each of these cases, however, the terminal portion of the recovery curve for the fertilized eggs was steeper. Therefore, it appears, that the kinetics of the recovery processes before and after fertilization of irradiated eggs from the same animal differ and that the post-fertilization recovery process is somewhat more efficient.

The cleavage delay induced by exposing the gametes of *Arbacia* to radiation has been interpreted as the time required for the zygote to repair or replace some constituent (of the nucleus) destroyed by radiation, which was necessary for cell division (*3*). Since recovery from this delay was demonstrated in unfertilized eggs but not in sperm, it was postulated that some cytoplasmic component was essential for repair. The current experiments confirm this view since they demonstrate that recovery can take place in irradiated sperm if the sperm is inside the egg where it can presumably mobilize the resources required for repair. It has also been shown that the recovery mechanism which operates in the irradiated unfertilized egg is not necessarily the same in the zygote (*4*).

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Heterokaryon-Incompatibility Factor Interaction in Tests between Neurospora Mutants

Abstract. Studies on presumably isogenic *ad-3A* and *ad-3B* mutants of the ascomycete *Neurospora crassa* derived from the same wild-type strain have revealed that the negative heterokaryon tests shown by certain pairwise combinations can be attributed to interaction of incompatibility factor mutations.

Heterokaryon tests on mutants with identical biochemical requirements induced in the same or isogenic wild-type strains of *Neurospora* have been used widely to group such mutants into series of presumptive alleles, and to test within each group for allelic complementation. Since such mutants are essentially isogenic, it is generally assumed that they are fully heterokaryon-compatible (*1*) and that a negative test is indicative of functional allelism (*2*). Mutation of genes affecting both heterokaryon formation and subsequent growth can be expected to occur simultaneously with mutation of genes governing nutritional requirements in some percentage of the mutants recovered from forward-mutation experiments. Heterozygosity for incompatibility-factor mutations can be generally recognized by the failure of a given mutant isolate to show positive heterokaryon tests with both allelic and nonallelic testers induced in the same genetic background (*3*).

In the present experiments evidence has been found for incompatibility-factor mutations with more subtle effects, which do not prevent positive heterokaryon tests when they are heterozygous individually but which do prevent positive heterokaryon tests when they are present in some heterozygous combinations.

Previous data from crosses and heterokaryon tests (*4*), tetrad analysis (*5*), an insertional translocation (*6*), and x-ray-induced recessive lethal mutation in the *ad-3* region (*7*) have shown that the *ad-3A* and *ad-3B* mutants are functionally distinct and in separate cistrons. In extensive tests on samples of *ad-3* mutants induced in mating type *A* wild-type strains by x-ray or ultraviolet treatment, no mutants were found that gave negative heterokaryon tests with all *ad-3A* or *ad-3B* testers. However, some mutants were found that gave negative heterokaryon tests only with certain *ad-3A* and *ad-3B* testers so that they were represented initially on the complementation map of the *ad-3* region as partial overlaps into the a*d-3A* or *ad-3B* cistrons. The existence of such a class of mutants in the absence of a class noncomplementing with all *ad-3A* and *ad-3B* testers was not expected. Because of this, experiments were planned to determine whether the negative tests, given by those mutants represented as partial overlaps, could be explained on any other basis. If, for example, such negative heterokaryon tests were due to the presence of incompatibility-factor mutations at other loci, segregation would be expected in the F_1 progeny from a cross of such a mutant to wild type.

The interaction patterns of three *ad-3A* mutants, three *ad-3B* mutants, and of mating type *A* F_1 progeny from a cross of each mutant to wild-type strain 74-OR8-1a (mating type *a* strain essentially isogenic with 74A) are given in Table 1. Three types of tests were made as described previously (*4*): (i) original isolate + original isolate, (ii) F_1 progeny + original isolate, or (iii) F_1 progeny + F_1 progeny. Under these conditions three of the nine combinations involving the original isolates gave a weak but positive response. Heterokaryon formation in such cases was delayed for many days and subsequent growth was at less than wild-type rate. Marked variation in the ability to form heterokaryons was also observed among the F_1 progeny from each cross. In some instances, the F_1 progeny gave either a negative test or a vigorous positive test, but in most crosses those combinations giving positive tests showed variation both in the time of heterokaryon formation and the type of response. By selection among the F_1 progeny, however, derivatives of each of the six mutants were obtained that gave vigorous heterokaryon tests (no delay in heterokaryon formation and growth at wild-type rate) for all of the *ad-3A* + *ad-3B* mutant combinations recorded as negative in the same type of test on the original isolates.

This analysis shows that the failure to obtain positive heterokaryon tests in certain pairwise combinations of presumably isogenic *ad-3A* and *ad-3B* mutants can be attributed to the interaction of extrinsic factors affecting heterokaryon formation and subsequent growth. Studies on known incompatibility factor mutations in *Neurospora* (*1, 8, 9*) have shown (i) that there are many different stages involved in the formation and subsequent growth of heterokaryons that can be affected by mutation, and (ii) that incompatibility is found only when the tested strains are heterozygous for such incompatibility mutations. If there are a large number of incompatibility loci in the *Neurospora* genome, then the presence of incompatibility mutations in large samples (> 100) of allelic mutants as a consequence of simultaneous mutational events is not unexpected. The mutations encountered in this study are somewhat atypical of those previously described, in that none prevent hetero-