Communications

Tests for Photoreactivation in Gametes of *Urechis caupo*

Ultraviolet-induced injury to the sperm of the sea urchin, *Arbacia punctulata,* is not reversed by subsequent illumination with visible light, although eggs are readily photoreactivated (*1*). Since the point is one of considerable theoretical interest, it seemed desirable to compare photoreactivation in the gametes of another animal. Studies were therefore made on the sperm and eggs of the echiuroid worm *Urechis caupo* for a comparison of photoreactivation in these gametes.

The worms were collected in Elkhorn Slough on Monterey Bay, California, and kept in running sea water. After withdrawal from the gonosac of the worm, the sperm were diluted 1:400 in 0.05*M* glycine (*2*) in sea water, but the eggs were kept in sea water. By running sea water around the syracuse dishes containing the eggs or sperm, all samples were kept at 16° ± 2°C. Only combinations of gametes giving 90 to 95 percent fertilization and good development were used for experiments. The appropriate gametes were exposed to the radiations of a Sterilamp (mainly wavelength 2537A), the dosage being determined by a Hanovia UV meter. Photoreactivation was accomplished with a G.E. CH-4 Mercury Spotlamp 2 ft from the samples and filtered through 2 to 5 in. of water and a Corning No. 3060 filter to remove heat and ultraviolet radiations, respectively. A 1-hr exposure to the white light alone was not injurious to either sperm or eggs, although it is injurious to the sperm of some animals (*3, 4*). The samples were stirred by playing a jet of air on the surface of the water. The time required for 50 percent of the zygotes to reach the two-celled stage was used to measure the effect of the different treatments, and each of the experiments was repeated at least three times.

Eggs irradiated with a dosage of 3000 erg/mm^2 of UV and fertilized with normal sperm were delayed in cleavage, a span of time 32 percent longer than the controls being required for half of the eggs to reach the first division. When ultraviolet-injured eggs were treated with white light for 15 min, the delay was reduced by 64 percent, indicating an average of 64 percent photoreactivation. The results were comparable, whether the eggs were illuminated before or after fertilization with untreated sperm.

Dosages of UV from 40 to 480 erg/mm^2 had no effect on sperm, since eggs inseminated with them were fertilized and cleaved at times comparable to those of controls. Only 50 percent of the eggs were fertilized with sperm given a 3200 erg/mm^2 dosage of UV, and cleavage was delayed. Sperm subjected to UV dosages of 6400 to 12,800 erg/mm^2 were generally incapable of fertilizing eggs, only a small percentage of the eggs being activated. These ultraviolet-induced injuries were in no case reversed by illumination with white light; in fact, the injuries were exacerbated. The sperm of *Urechis,* therefore, resemble those of *Arbacia* (*1*) in being incapable of photoreactivation by white light after ultraviolet-induced injury.

Since the possibility exists that a maximal amount of photoreactivation is achieved by the visible light present as an impurity in the spectrum of the Sterilamp, the UV used for irradiating sperm was passed through a visible-light-absorbing filter (*5*) ($CuSO_4$ and $NiSO_4$), which transmits about 70 percent of the UV at 2537A. The results were essentially the same as in the afore-described experiments. Ultraviolet-induced injury to the *Urechis* sperm nucleus therefore appears to be irreversible and is not susceptible to photoreactivation under the conditions tested.

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References

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Remarks on Fischer's Article, "Stress and the Toxicity of Schizophrenic Serum"

We wish to put on record some facts concerning the investigation of Roland Fischer (*1, 2*) and to state our disagreements with his conclusions. Fischer acknowledges that his work with *Xenopus laevis* tadpoles was *started* under the direction of one of us (F. G.); but since his published figures are identical with those that he summarized in our laboratory in 1949–50, we are driven to believe that they form the sole basis for his conclusions. We regret that Fischer failed to communicate with us before publishing his paper and that we are thus obliged to object in print to his interpretations:

1) After reexamining statistically all the experimental material, we came to the conclusion that, despite considerable differences in some individual experiments, the results as a whole are *not significant.* This opinion together with other biological experiments has recently been published by one of us (H. P. R., *3*).

2) In this acticle (*3*) no mention is made of Fischer's hypothesis that sudden changes in cold and warm weather would influence the results. In order to survey the extremely complex material, we divided the experiments into three groups: positive, indifferent, and negative, according to the hypothesis under discussion, that schizophrenic body fluids are more toxic than normal ones. Admittedly, a comparison of the