30 min; however, after 30 min no further accumulation of dye was observed, while control tubules continued to accumulate CPR. In addition, tubules from two fish were incubated with 10^{-} M CPR plus either 1.4 x 10^{-4} M (50 ppm) DDT in 1% N, N-dimethylformamide (NNDF) in Forster's medium or 1% NNDF alone. Neither NNDF alone nor NNDF with DDT inhibited dye uptake at any time.

To further quantitate the degree of inhibition, isotope experiments were conducted using 10^{-7} to 10^{-5} M ^{3}H-CPR (Bull. MDIBL 9:30-31, 1969) with 10^{-8} to 10^{-4} M unlabeled DDA. At t end of 2 hours the tissue fragments were removed, blotted, weighed, dissolved in 1 ml of Solu ene (Packard Instrument Co.), and counted in 10 ml of toluene scintillation fluid (5 g/liter PP 250 mg/liter POPOP) on a Nuclear Chicago scintillation counter. Aliquots of medium were treated similarly. Tissue to medium concentration ratios and percent inhibition were calculat Holding ^{3}H-CPR constant at 10^{-7} M, inhibition ranged from 18 to 53% and tended to increase higher DDA concentrations (30% inhibition at 10^{-8} M DDA, 18% at 10^{-7} M, 32% at 10^{-6} M, 39% at 10^{-5} M, and 53% at 10^{-4} M). Inhibition also increased when ^{3}H-CPR concentrations were d creased while holding DDA constant at 10^{-4} M (34% inhibition at 10^{-5} M CPR, 48% at 10^{-6} M, and 53 and 70% at 10^{-7} M). Finally, net efflux of ^{3}H-CPR was examined using tissue pre-incu bated for 2 hours in 10^{-7} M ^{3}H-CPR, rinsed three times, and incubated for one hour in Forst alone, 10^{-4} M DDA, or 10^{-4} M CPR. In all cases somewhat less than half the counts were released from tissue to medium.

These results show that DDA inhibits renal tubular transport of the organic acid, CPR. F thermore, the degree of inhibition increases as DDA concentration rises and falls as CPR con centration rises. In addition, the parent pesticide, DDT, which lacks the carboxyl group, does not inhibit organic acid transport. Finally, DDA does not damage the cells so that CPR leaks out rapidly. The explanation most consistent with these results is that DDA is a competitive i hibitor of the organic acid transport system.

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1970 #35

PROTEIN SYNTHESIS, DNA SYNTHESIS AND CLEAVAGE DELAY IN Echinarachnius parm ZYGOTES: THE EFFECT OF ULTRAVIOLET RADIATION

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These studies were undertaken to further clarify the effect of an imposed ultraviolet (UV lesion into gametes and zygotes of E. parma on the "division related" protein synthesis durin the early synchronous cleavages. We have previously shown (Bull. MDIBL 9:54, 1969) that thi protein synthesis is delayed in irradiated zygotes and that this phenomenon is photoreversibl This reversibility indicates that the observed delay is DNA related.

Sperm were irradiated with 2.47 x 10^{3} ergs/cm^{2} at 254 nm which is about 80% lethal whe photoreactivation is not used. These irradiated sperm were used to fertilize non-exposed egg and the subsequent extension of cycloheximide sensitivity was used as a measure of synthesis delay. Cleavage delay and DNA synthesis were determined. In order to determine the effect o photoreversibility on DNA synthesis zygotes were radiated at 60 min post fertilization with s

sequent aliquots taken for the same above determinations.

When sperm were irradiated the "division-related" protein synthesis was delayed as is indicated by the extension of cycloheximide sensitivity as well as a cleavage delay (Figure 1). Also, the observed delay was reduced when these zygotes were maintained under conditions for photoreactivation.

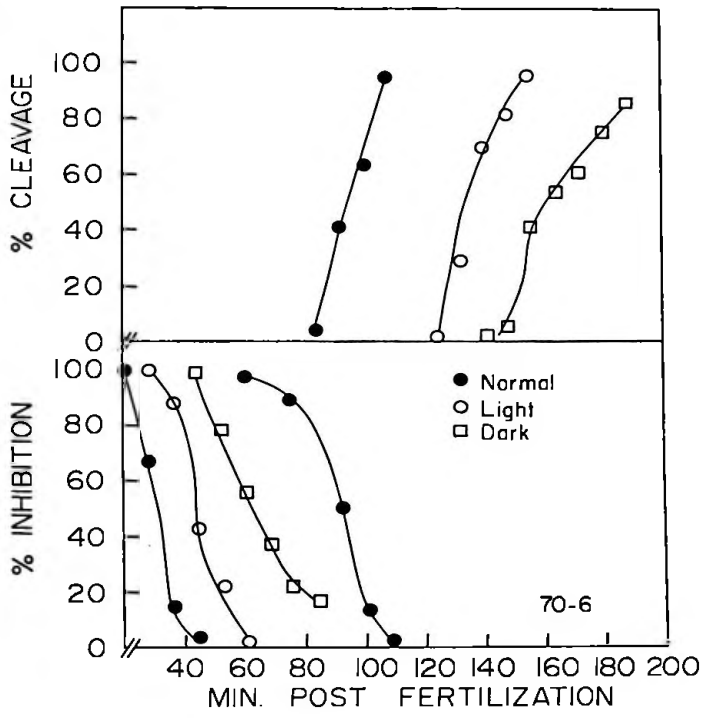

Figure 1.

Figure 2 shows that S_1 was not delayed, with both the non-irradiated and exposed ^{3}H-thymidine uptakes being coincident, but the S_2 was delayed. This is an expected result, since the protein synthesis which is a determinant of the S_2 occurs at 30-40 min post fertilization and had been delayed (Figure 1).

Zygotes irradiated 57 min post fertilization and subjected to photoreactivation illumination will not be delayed for any subsequent cleavages. Those zygotes kept in the dark, however, will not show a cleavage delay for the period immediately following cleavage but will be delayed during the next division (Figure 3). Photoreactivation also deletes any extension of cycloheximide sensitivity. This indicates that the UV lesion was made at a point in time after the first "division related" protein synthesis and that photoreactivation removed the defect before the next synthetic period. In such a circumstance it would be expected that DNA synthesis in irradiated and photoreactivated zygotes would occur at the same time as normal. As shown in Figure 4, such does occur. The timing of the S periods is coincident but there is a discrepancy in total uptake which cannot be explained at present.

The fact that the delays in protein synthesis, DNA synthesis and cell division can be photo-

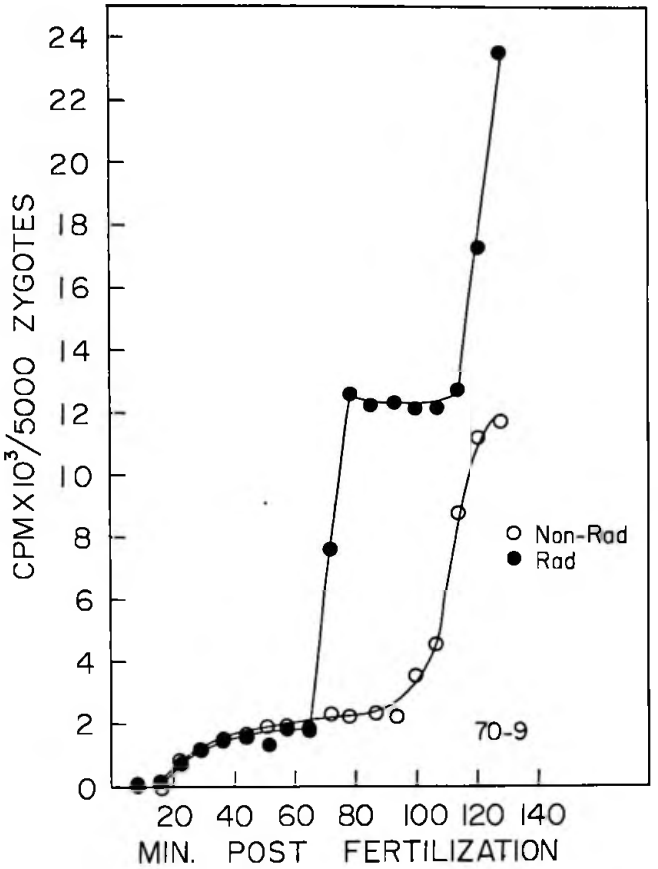

Figure 2.

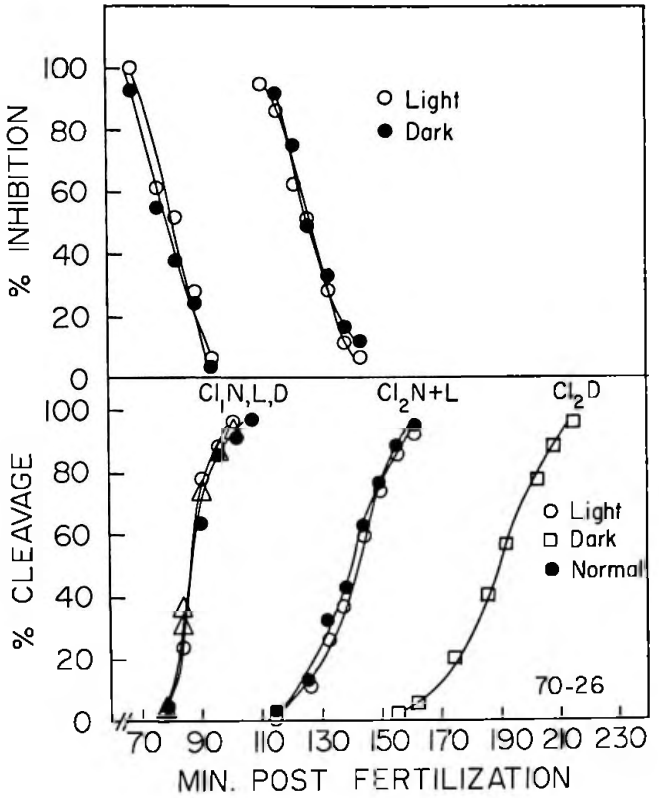

Figure 3.

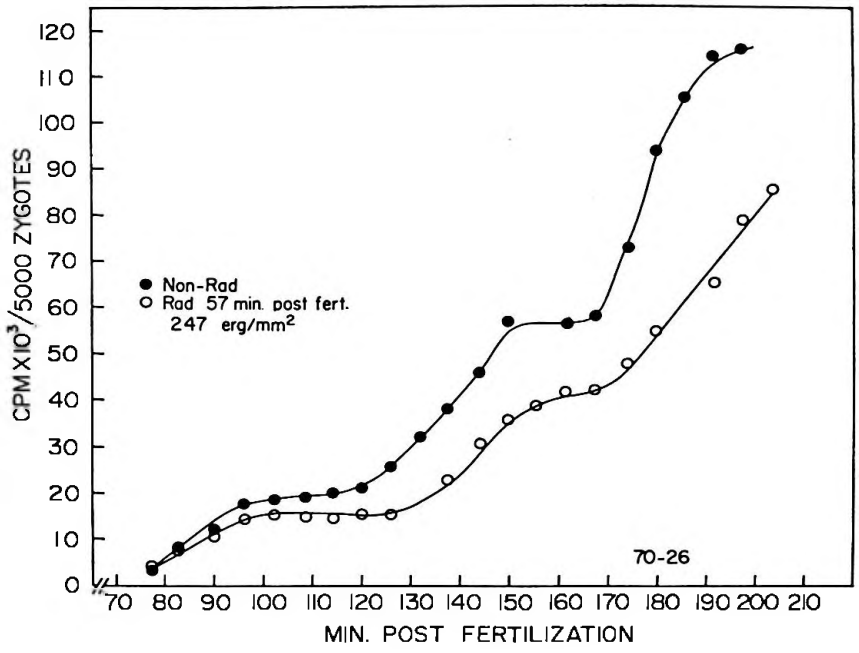

Figure 4.

reactivated indicates that the lesion is most probably a cyclobutane type dimer in the DNA molecule which has been monomerized.

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1970 #36

OSMOTIC AND DIFFUSIONAL WATER PERMEABILITY IN METAMORPHOSING Rana clamitans TADPOLES

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Tadpoles of Rana clamitans metamorphose during the month of July on Mount Desert Island. As the hind legs grow, the tail atrophies. At the stage when the hind legs are about the same length as the tail, the tadpole suddenly changes from gill to lung respiration. Previous experiments on metamorphosing tadpoles showed that the diffusional water permeability is considerably lower in newly metamorphosed frogs than in tadpoles prior to metamorphosis, but insufficient data were obtained to define the change that takes place during metamorphosis (Mackay and Schmidt-Nielsen, Bull. MDIBL 1969). Furthermore, in order to compare the diffusional and the osmotic permeabilities, it was necessary to obtain better data on urine flow.

Rana clamitans tadpoles in various stages of metamorphosis were caught in local ponds during the months of July and August. R. catesbeiana tadpoles were also caught locally, but the data obtained on these, although similar to the data from R. clamitans, are not included here.