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1968 #31

QUANTITATIVE STUDIES ON THE ULTRAVIOLET SENSITIVITY OF SAND DOLLAR SPERMATOZOA

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Frequent references are made in the literature regarding the extremely high sensitivity of sperm to ultraviolet (U.V.) irradiation. However, the damage is most usually measured by cleavage delay or abnormal development produced when irradiated sperm are used to fertilize normal non-irradiated eggs. These methods give no indication of the lethality of any given exposure.

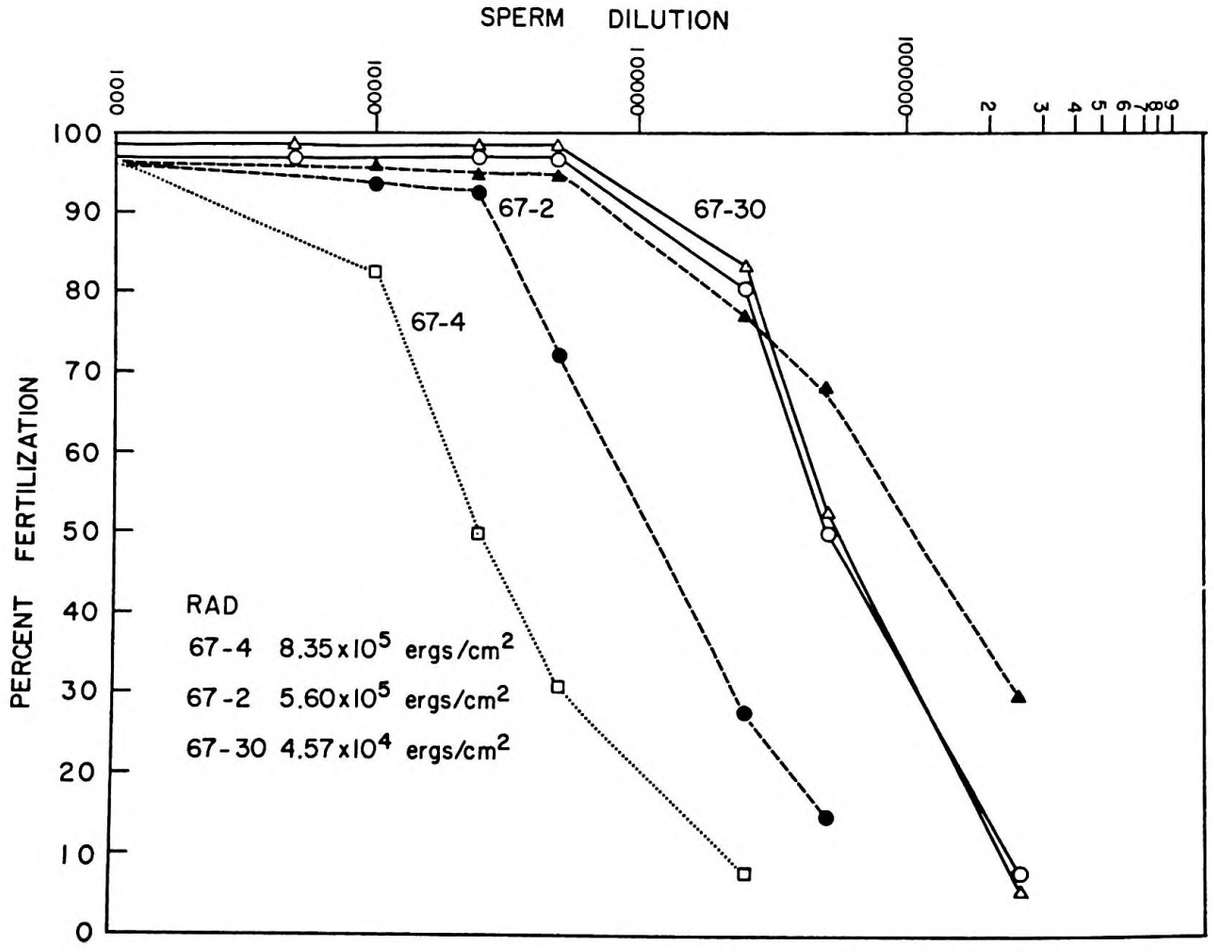

Figure 1

A variety of dilutions of Echinarachnius parma sperm were exposed to U.V. of wave length 2537 Å at various dose levels ranging from 0.145 to 1.12 x 10^6 ergs/mm^2. The effect of the irradiation on fertilization capacity was measured by mixing serial dilutions of control or exposed

sperm with a standard number of unfertilized eggs. The percent fertilized were counted in each sample and plotted as in Figure 1. Sperm counts were made and the dilution was transformed to a sperm concentration. From such a plot one could determine the percent of surviving sperm at a given dose level by comparing non-irradiated with exposed sperm of the same population.

The degree of dilution of sperm during irradiation had no effect if the concentration did not exceed 4.96×10^6/ml. Difference in dose-rate had no effect on inactivation. Figure 1 indicates that at 4.57×10^2 ergs/mm^2 no inactivation occurs, but that as the dose increases the percent inactivation increases. The 50% inactivation dose is approximately 1.12×10^4 ergs/mm^2. A dose-survival curve was plotted from numerous experiments as shown in Figure 2. The first

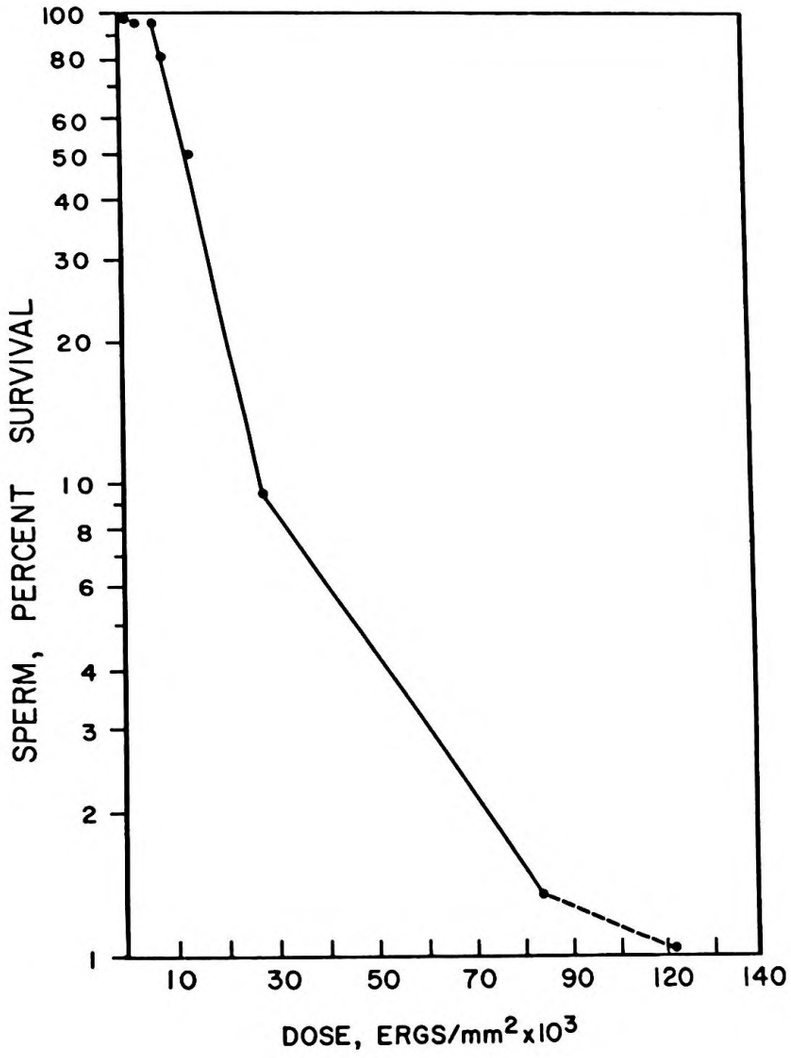

Figure 2

portion was exponential with a slight shoulder, but at higher doses this relationship did not hold. At low dose levels, where killing of sperm was not observed a biological lesion could be demonstrated when these exposed eggs were used to fertilize normal eggs. Radiation below 6.40×10^2 ergs/mm^2 caused only cleavage delay, whereas energy levels above this produced asynchronous unequal cleavage, the percent increasing with increasing dose. In those instances where cleavage was unequal the zygote would not develop beyond the early blastula stage.

These experiments show that a biological lesion can be produced by U.V. without killing any

sperm in the population. Also, a quantitative method of measuring the lethal effect of U.V. on spermatozoa has been devised. The type of damage for sperm killing is most probably of a different nature than the lesion for a developmental effect.

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1968 #32

WHY VERTEBRATE EXTRACELLULAR AND INTRACELLULAR pH VALUES ARE APPROXIMATELY 7—PHYSICAL-CHEMICAL BASIS OF THE EVOLUTION OF CO_2 TRANSPORT

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Previous work from this laboratory suggests that the general shape of vertebrate CO_2 titration curves is similar among all vertebrates. Edsall and Wyman have analyzed the physicochemical basis of CO_2 titration curves and have derived an expression for the relationship between PCO_2 and H^+ as a function of the pK of the buffer systems available for the buffering of H_2CO_3:

$$PCO_2 = \frac{1}{{}_{\alpha}CO_2} \times \frac{K_B}{K_{H_2CO_3}} \times \frac{(HCO_3^-)\ (HCO_3^- - D)}{C\text{-}HCO_3^- - D}$$

Where PCO_2 = CO_2 tension in mm Hg.
${}_{\alpha}CO_2$ = Solubility Coefficient for CO_2 in M/L/mmHg.
K_B = Dissociation constant of a given buffer.
$K_{H_2CO_3}$ = Dissociation constant of H_2CO_3.
$[HCO_3^-]$ = Concentration of $[HCO_3^-]$ M/L.
D = Difference between fixed cations and anions M/L.
C = Concentration of the buffer M/L.

Using this expression it is possible to calculate CO_2 titration curves that would result from buffers with different dissociation constants. The figure shows 3 such curves with CO_2 titration curves calculated for buffers of pK values of 6.0, 7.0, and 9.0 respectively assuming that all HCO_3^- present in the system arises from buffering of H_2CO_3. The lowermost curve (pK = 6) would present 3 difficulties in terms of vertebrate survival. The animal would be acidotic at any PCO_2. Under conditions associated with hypercapnia there would be limited buffering of H_2CO_3. Because of the small change in total CO_2 with large changes in PCO_2, CO_2 transport from the tissues to the external gas exchanger and from the external gas exchanger to the ambient environment would require huge and unrealistic cardiac outputs. The uppermost curve (pK = 9) would permit adequate tissue and external CO_2 exchange only at low PCO_2s (aquatic vertebrates). However, CO_2 exchange at PCO_2 greater than 5 torr would require huge and presumably unattainable levels of cardiac output. Thus survival during hypercapnia in aquatic forms would be difficult. The transition from aquatic to terresterial vertebrates with attendant high PCO_2 values presumably could not have occurred. It therefore appears that the development of oxidative phosphorylation with substantial CO_2 generation requires the presence of significant concentrations of buffers in body fluids with pK values approximately 7.