

# STUDIES ON THE MECHANISM OF ACTION OF IONIZING RADIATIONS. V. THE EFFECT OF HYDROGEN PEROXIDE AND OF X-RAY IRRADIATED SEA WATER ON THE RESPIRATION OF SEA URCHIN SPERM AND EGGS

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It has been known for some time (Risse, 1929; Fricke, 1934) that when water is irradiated with x-rays in the presence of oxygen there is formation of  $H_2O_2$ . Since  $H_2O_2$  is a powerful oxidizing agent and it easily oxidizes sulphydryl groups, it was reasonable to assume that this substance, if formed on irradiation, would contribute to the biological effects of ionizing radiations. In fact, Barron and Dickman (1949) on studying enzyme inhibitions by ionizing radiations, and Barron and Flood<sup>1</sup> on studying the oxidation of 2,3-dithiopropene and of glutathione by x-rays, were able to distinguish the  $H_2O_2$  contribution to this oxidation by the use of catalase. The French investigators Loiseleur, Latarjet, and Caillot (1941), and Loiseleur and Latarjet (1942) have postulated that the primary effect of irradiation in aqueous solutions is  $H_2O_2$  formation, which would thus become of importance in the interpretation of the mechanism of ionizing radiations. The same view is held by Evans (1947) who found that the fertilizing power of sea urchin sperm is decreased when suspended in sea water irradiated with large doses of x-rays. Evans attributed this inhibition to the action of  $H_2O_2$  seemingly formed on irradiation of sea water. In living cells the role of  $H_2O_2$  becomes more complicated because the sulphydryl groups which might be oxidized by this agent not only are present in the protein moiety of enzymes, but also exist as non-protein sulphydryl groups.

We present in this paper experiments on the effect of  $H_2O_2$  and of x-ray irradiated sea water on the respiration of sea urchin sperm. They do not support the belief that  $H_2O_2$  is an important factor in x-ray toxicity on sea urchin sperm.

## EXPERIMENTAL

Sea urchin sperm was obtained as described previously (Barron et al., 1949), and in all experiments a dilution of 1:200 was used. Freshly filtered sea water was irradiated at room temperature in large cellophane dishes and immediately after irradiation the sperm suspension was added, enough to make the desired dilution of 1:200.

The catalase added to irradiated sea water was prepared from beef liver according to Sumner and Dounce (1939).  $H_2O_2$  was determined by the colorimetric method of Bonet-Maury (1944). An aliquot of the solution was taken (up to

<sup>1</sup> Unpublished experiments.



3 cc.) to which was added 0.5 cc. of 20 per cent  $\text{H}_2\text{SO}_4$ , 5 drops of the titanium sulfate reagent (10 g.  $\text{TiSO}_4$  ground in mortar with 50 cc.  $\text{H}_2\text{O}$  and 20 g.  $\text{H}_2\text{SO}_4$ ,  $D = 1.84$ , let stand 24 h., centrifuge, take the supernatant and add 20 g.  $\text{H}_2\text{SO}_4$ ), and distilled water to 5 cc. The yellow color produced which is stable was read in a Beckman spectrophotometer at 4000 Å. With this method amounts of  $\text{H}_2\text{O}_2$  from 0.5 micrograms to 25 micrograms could be determined. The respiration measurements were made at 25°.

*Effect of  $\text{H}_2\text{O}_2$  on the respiration of sea urchin sperm*

Barron et al. (1948) have shown that sulfhydryl reagents when used in small concentrations increase the respiration of sea urchin sperm, whereas they inhibit it when the concentration is increased. To explain these opposite effects it was postulated that the cell contains two kinds of sulfhydryl groups: the non-protein sulf-

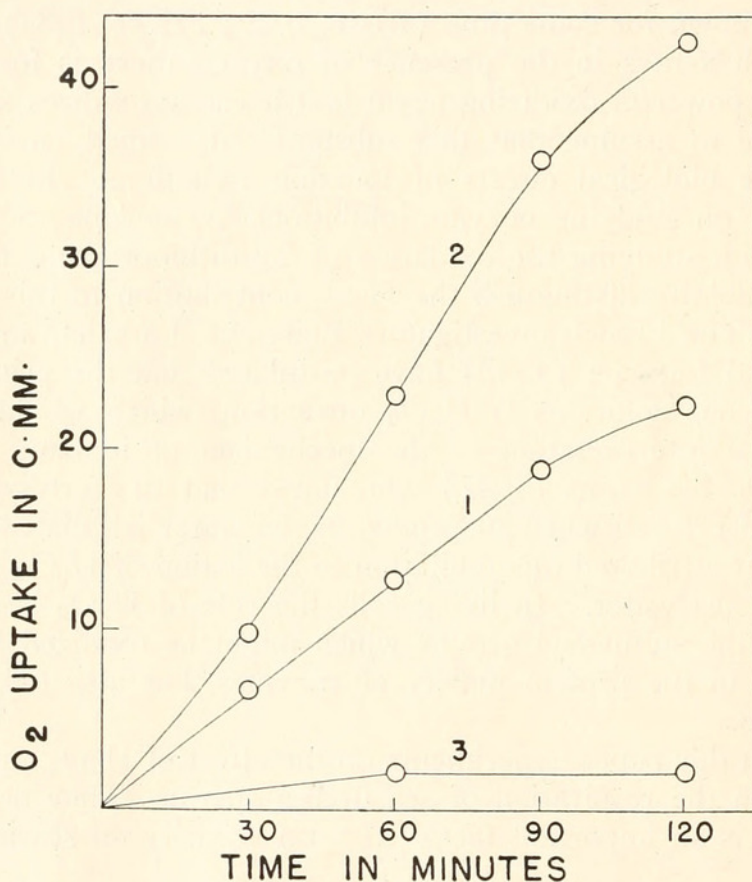


FIGURE 1. The effect of  $\text{H}_2\text{O}_2$  on the respiration of sea urchin sperm. 1. Control; 2.  $\text{H}_2\text{O}_2$ ,  $1 \times 10^{-5}$  M; 3.  $\text{H}_2\text{O}_2$ ,  $1 \times 10^{-3}$  M.

hydryl groups (namely glutathione), which regulate the rate of respiration, and the sulfhydryl groups in respiratory enzymes. Destruction of the first would increase respiration, while destruction of the second would inhibit it.  $\text{H}_2\text{O}_2$ , an oxidizing agent of sulfhydryl groups, behaves in the same manner. At a concentration of  $1 \times 10^{-5}$  M it increased respiration, while  $1 \times 10^{-3}$  M inhibited it almost completely (Fig. 1). By altering the concentration of  $\text{H}_2\text{O}_2$  between these two limits the effect on respiration is changed accordingly (Table I). In fertilized sea urchin eggs  $1 \times 10^{-4}$  M  $\text{H}_2\text{O}_2$  increased the  $\text{O}_2$  uptake 25 per cent (Fig. 2). If the effects of x-ray irradiation were mainly due to  $\text{H}_2\text{O}_2$  formation, as postulated by



TABLE I

*Effect of  $H_2O_2$  on the respiration of sea urchin sperm. Sperm dilution, 1:200.  
 $Q_{O_2}$ , c.mm.  $O_2$  uptake per mg. dry weight per hour*

$H_2O_2$ Concentration (M)	$Q_{O_2}$ values		Inhibition (–) or increase (+) (per cent)
	Control (c.mm.)	$H_2O_2$ (c.mm.)	
0.01	22.3	0	– complete
0.001	22.3	2.1	– 90
0.0005	16.0	15.3	No effect
0.0001	20.2	33.7	+61.5
0.0001	22.0	42.0	+91

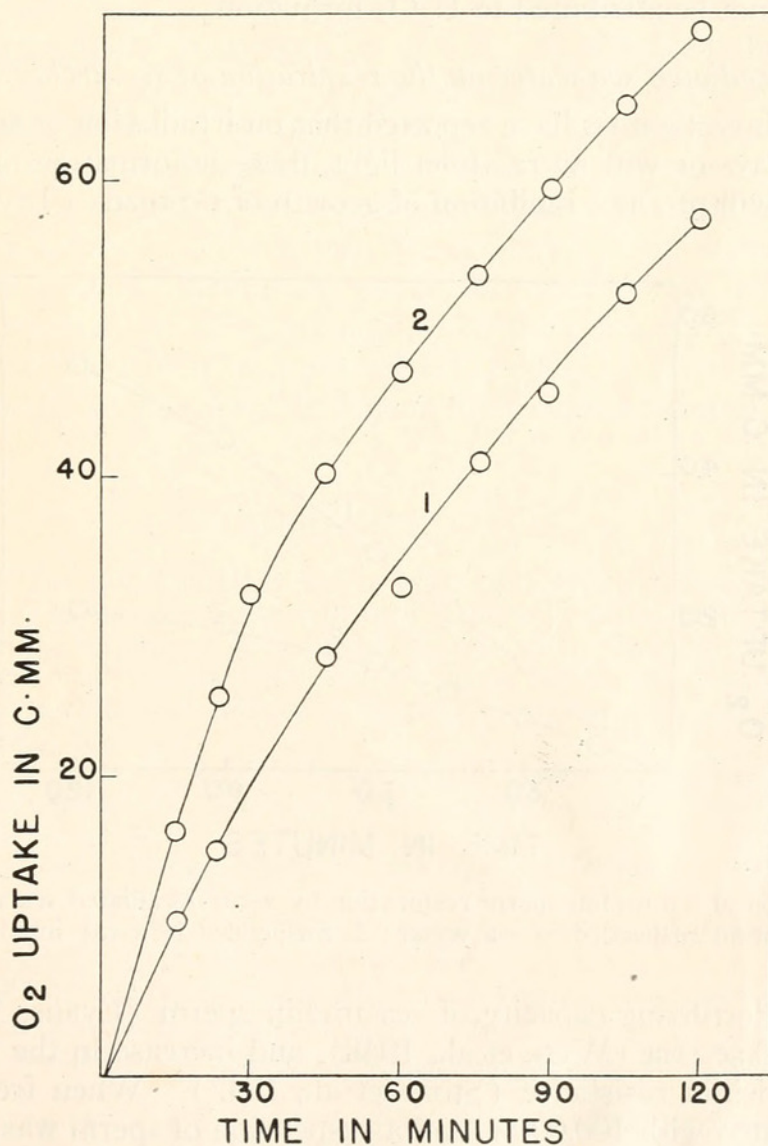


FIGURE 2. Effect of  $H_2O_2$  on the respiration of fertilized sea urchin eggs.  $H_2O_2$  concentration,  $1 \times 10^{-4}$  M; 1. Control; 2.  $H_2O_2$ .



TABLE II

*Inhibition of sea urchin sperm respiration by x-ray irradiated sea water. X-ray dose, 100,000 r. Liver catalase (0.2 cc.) added immediately after irradiation; sperm suspension 5 minutes later*

Experimental conditions	O <sub>2</sub> uptake		Inhibition (per cent)
	First hour (c.mm.)	Second hour (c.mm.)	
Control	29.7	53.5	
X-ray irradiated sea water	14.9	33.1	38
X-ray irradiation + catalase	16.2	36.6	34

Loiseleur et al. (1941, 1942) and by Evans (1947), x-ray irradiation at a dose of 1000 r would produce an increase in cell respiration.<sup>2</sup> Barron et al. (1949) have shown that on the contrary, respiration is inhibited. It must be concluded from these experiments that the effects of x-ray irradiation on the metabolism of sea urchin sperm cannot be attributed to H<sub>2</sub>O<sub>2</sub> formation.

*Effect of x-ray irradiated sea water on the respiration of sea urchin sperm*

A number of investigators have reported that on irradiation of aqueous solutions, whether with x-rays or with ultra-violet light, there is formation of some unknown substance which will produce inhibition of growth of protozoa (Taylor et al., 1933),

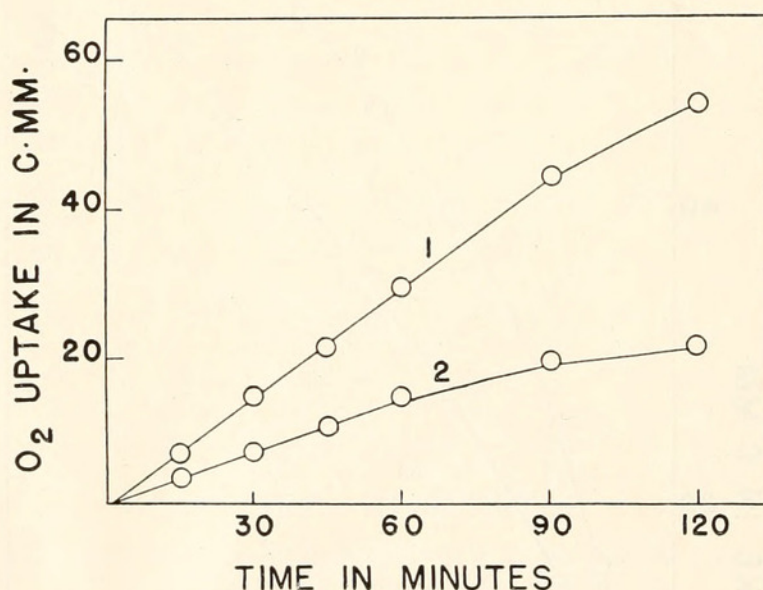


FIGURE 3. Inhibition of sea urchin sperm respiration by x-ray irradiated sea water. Irradiation, 200,000 r. 1. Sperm suspended in sea water; 2. Suspended in x-ray irradiated sea water.

inhibition of the fertilizing capacity of sea urchin sperm (Evans, 1947), inhibition of the growth of bacteria (Wyss et al., 1948), and increase in the mutation rate of *S. aureus* to penicillin resistance (Stone et al., 1947). When freshly filtered sea water was irradiated with 100,000 r, and a suspension of sperm was added to it soon

<sup>2</sup> From Bonet-Maury and Frilley's data (1944) it can be calculated that 1000 r would produce on irradiation of water about  $1 \times 10^{-5}$  M of H<sub>2</sub>O<sub>2</sub>.



after irradiation, there was an inhibition of respiration of 38 per cent. The inhibition was not affected by previous addition of catalase (Table II), an indication that the inhibition was not produced by  $\text{H}_2\text{O}_2$ . The inhibition increased when the dose of x-rays rose to 200,000 r (Fig. 3). Further evidence that this phenomenon was not produced by  $\text{H}_2\text{O}_2$  was obtained by its detection with the titanous sulfate colorimetric method. While distilled water irradiated with 100,000 r gave 30 micrograms  $\text{H}_2\text{O}_2$  per cc., filtered sea water irradiated with the same dose of x-rays gave no color at all. The lack of color formation was not due to the salt concentration of sea water, for on addition of  $\text{H}_2\text{O}_2$  to sea water the color reaction appeared. We believe that the inhibition of respiration is due to the formation of stable organic peroxides formed on oxidation of the organic matter contained in sea water. It is quite possible that similar stable organic peroxides are formed on irradiation of biological fluids and that they contribute to the toxic effects of ionizing radiations. This problem is now under investigation.

#### SUMMARY

Hydrogen peroxide at a concentration of 0.001 M produced almost complete inhibition of the respiration of sea urchin sperm suspended in sea water. At a concentration of 0.0005 M it had no effect. When the concentration was diminished to 0.0001 M it increased the respiration from 60 to 100 per cent.

When sperm was added to sea water irradiated with 200,000 r, there was a marked inhibition of respiration (about 60 per cent). Sea water irradiated with 50,000 r produced small inhibition (10 per cent). Addition of catalase previous to the addition of sperm had no effect at all on this inhibition. Furthermore, sea water irradiated with 200,000 r gave no positive test for  $\text{H}_2\text{O}_2$ . It is postulated that inhibition is due to the action of stable organic peroxides produced on irradiation of sea water.

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Barrón, Eleazar S. Guzmán., Flood, Veronica, and Gasvoda, Betty. 1949.  
"STUDIES ON THE MECHANISM OF ACTION OF IONIZING RADIATIONS. V. THE  
EFFECT OF HYDROGEN PEROXIDE AND OF X-RAY IRRADIATED SEA WATER ON  
THE RESPIRATION OF SEA URCHIN SPERM AND EGGS." *The Biological bulletin*  
97, 51-56. <https://doi.org/10.2307/1538093>.

**View This Item Online:** <https://www.biodiversitylibrary.org/item/17396>

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