

7414 P

Nature of the Hemolytic Agent in Previously Irradiated Solutions of Eosine.

H. F. BLUM AND C. R. SPEALMAN.

From the Division of Physiology, University of California Medical School, Berkeley.

One of us¹ demonstrated that red blood cells are hemolyzed by solutions of fluorescein dyes which have been previously irradiated with the visible portion of sunlight. Finding that such solutions contained a small concentration of peroxide,¹ and for reasons which seemed consistent with the then known facts of photodynamic action, we assumed that the hemolysis was probably due to the activity of this peroxide, which we suspected of being an organic peroxide. Recently,² however, we have offered evidence to show that this peroxide is H_2O_2 .

We have determined the quantity of peroxide and dye in solutions which are just capable of producing hemolysis, and have found that mixtures of eosine and H_2O_2 do not produce hemolysis at nearly such low concentrations of these reactants as do irradiated eosine solutions. Table I indicates this discrepancy.

Furthermore, in most cases, we have found that when $\text{Na}_2\text{S}_2\text{O}_3$ is added to the irradiated solutions in concentrations just equivalent to the concentration of H_2O_2 present, hemolysis is not abolished nor in most cases perceptibly diminished.

TABLE I.
Concentrations of eosine and H_2O_2 in irradiated and non-irradiated solutions just producing hemolysis.

Eosine millimols per l.	H_2O_2 milli-equi- valents per l.	Increased hemolytic activity	Increased hemolytic activity per milli- equivalent H_2O_2	Quantity of eosine bleached millimols per l.
Non-Irradiated Dye.				
5	6	2.5	0.4	
2.5	8	5	0.6	
0.6	11	21	2	
0.15	45	83	2	
0.07	90	166	2	
Irradiated Dye.				
0.43	0.17	29	170	0.07
0.9	0.1	14	140	0.1
0.475	0.3	13	42	0.025
0.34	0.1	17	170	0.17

¹ Blum, H. F., *Biol. Bull.*, 1930, **58**, 224.

² Blum, H. F., and Spealman, C. R., *J. Phys. Chem.*, 1933, **37**, 1123.

An amount of bleaching of the dye solutions occurs which is detectable spectrophotometrically; the quantities are indicated in Table I. We have been forced to conclude that the products of this bleaching, and not peroxide are responsible for at least the greater part of the increased hemolytic properties of our solutions. Further experiments are in progress.

7415 P

Observations on the Treatment of Infertile Rabbits with Antuitrin "S".

PAUL D. ROSAHN, HARRY S. N. GREENE AND C. K. HU.

From the Laboratories of The Rockefeller Institute for Medical Research.

Prolonged experience with a large breeding colony of rabbits has revealed that a small proportion of sexually mature females repeatedly fail to become pregnant after being served by known fertile bucks. In general, such does are in excellent physical condition and show no external evidence of disease, although a few have a tendency to obesity characterized by a shoulder girdle adiposity and an abnormal accumulation of fat in the abdomen. Recently, certain of these persistently infertile does carried genetic factors which were under investigation, and progeny from them were desired in order to carry on the genetic studies. Accordingly, they were submitted to different therapeutic procedures, with a view to developing a method which would render them fertile. Since all the animals were of value because of their genetic constitution, operative interference and experiments hazardous to life were of necessity avoided. The present report is concerned with one of the methods employed in the treatment of these does.

In the rabbit, ovulation occurs about 10 hours after copulation. It was thought that the threshold necessary to induce ovulation in refractory females was higher than the stimulus afforded by the act of coitus. A simple method of augmenting the copulation stimulus lay in the observation of Friedman¹ that the parenteral administration of human pregnancy urine induces ovulation in the rabbit. The method employed in the present investigation was to inject a single dose of 40 Rat Units per kilo body weight* of Antuitrin "S"

¹ Friedman, M. H., *Am. J. Physiol.*, 1929, **90**, 617.

* This dose was suggested by the work of Snyder, F. F., *Bull. Johns Hopkins Hosp.*, 1934, **54**, 1.