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Influence of X-rays on Respiration of Nuclei of Fowl Erythrocytes.*

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In recent years a series of publications have appeared dealing with the influence of X-rays on respiration. Williams and Scheard[1] demonstrated increased oxidation in irradiated frog's skin. Lacassagne[2] working with yeasts also observed increased O_2 consumption after irradiation.

In a previous paper[3] we have demonstrated that fowl-erythrocytes irradiated with X-rays consume more oxygen than do unirradiated red cells. The purpose of the present study was to investigate the respiration of the irradiated nuclei of fowl erythrocytes and to compare the results with those obtained with whole erythrocytes.

Methods. (a) Preparation of nuclei. Nuclei were prepared according to the technique of Dounce and Lan. White Leghorn hens were bled by cardiac puncture and 20 cc of blood were sterilely withdrawn into 2 ml of 3% solution of Na citrate. The erythrocytes were centrifuged twice washed in 0.9% saline and the sedimented cells were resuspended in saline to the original volume of the blood. In order to induce hemolysis a solution of 0.15 g of saponin in 5 ml of an 0.11 M solution of phosphate (pH 7) was added to each 100 ml of erythrocyte suspension. After hemolysis was completed (within 2-3 minutes), the hemolysate was centrifuged and subsequently washed 5 or 6 times by centrifuging in 0.9% saline and was finally resuspended in this solution up to a concentration of approximately 10-18 mill/mm^3. The nuclei were then counted in a counting chamber.

(b) Irradiation. The X-ray tube used in these experiments was operated at a tension of 35 KV at a current of 15 mA with a copper anticathode. The window was aluminum foil 30 μ in thickness. Absorption analysis showed that the rays which penetrated through the window were mainly copper X-rays. The distance between the vessel in which the nuclei were irradiated and the focus was 50 mm and the X-ray intensity was about 60,000 r/min. Irradiation was performed on 0.5 ml portions of the nuclei suspensions in flat-bottomed, shallow glass dishes of 15 mm diameter. The suspension layer did not exceed 3 mm in thickness. Irradiation doses of 0.5 Mil., 1 Mil., and of 1.5 Mil. were applied.

(c) Measurement of respiration. Respiration was measured in a Warburg apparatus at 39-40°. Two ml of suspension were used per vessel for each estimation. Five to six 0.5 ml portions had, therefore, to be irradiated consecutively and combined. Control estimations of non-irradiated nuclei from the same sample of suspended nuclei were carried out in every case. The samples to be irradiated, as well as those already irradiated and the controls were kept under identical conditions of temperature throughout. The first reading was made 20 minutes after inserting the manometers in the water-bath (about half an hour after the termination of irradiation). Readings were taken initially at intervals of 30 minutes and later at intervals of 60 minutes.

Results and Discussion. As Dounce has already shown chicken erythrocyte nuclei continue for some days to exhibit a weak but demonstrable respiration. In our study this respiration of isolated nuclei was 1/8 to 1/10 of that of whole erythrocytes. Absolute values varied with individual chickens. X-rays had

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1 Williams, M. M. D., and Scheard, C., *Protoplasma*, 1932, **15**, 396.

2 Lacassagne, Schoen et Beraud, *Ann. des Fermentations*, 1939, **5**, 124.

3 Frankenthal, L., and Back, A., *Biochem. J.*, 1944, **38**, 351.

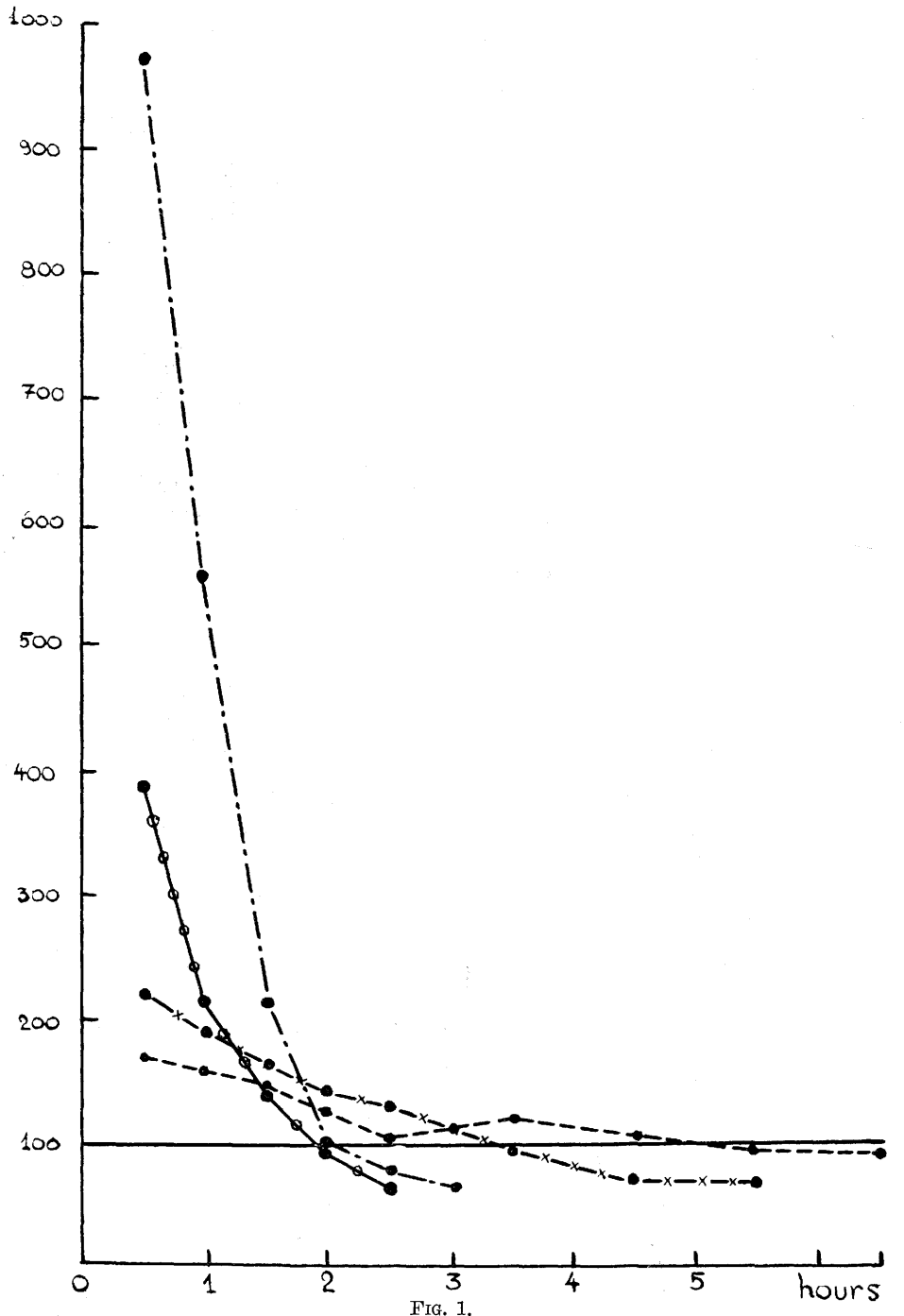

FIG. 1.

Effect of X-radiation on the respiration of nuclei of fowl erythrocytes and of whole erythrocytes. The data given are the O_2 consumption in the experimental vessels, expressed as a percentage of the O_2 consumption observed in the control vessels at a given time.

———— Non-irradiated nuclei or erythrocytes.
O—O—O Nuclei irradiated with 500,000 r.
——·—— Nuclei irradiated with 1,000,000 r.
- - - - - - - Erythrocytes irradiated with 500,000 r.
——×—— Erythrocytes irradiated with 1,000,000 r.

TABLE I.
Influence of Irradiation on O_2 Consumption of Nuclei of Fowl Erythrocytes.
μl O_2 Consumed by 2 ml of Nuclei Suspension 10 Mill Nuclei/1 mm^3 After 30 to 180 min.

Irradiation dose (in millions r)	30 min.	60 min.	90 min.	120 min.	150 min.	180 min.
0.5	22.9	41.5	52.1	59.1	64.4	
0	5.9	14.4	22.0	29.6	38.1	
1	68.8	101.2	120.5	131.2	140.7	148.9
0	7.1	14.7	23.6	34.8	47.1	59.6
1.5	16.7	28.6	35.9	41.2	46.6	50.5
0	2.0	4.0	8.0	12.6	17.8	23.9

an accelerating effect on the respiration of nuclei and this effect was far more pronounced than that on whole erythrocytes as may be seen in Fig. 1. Thus, the effect of ½ million r on isolated nuclei is about twice as great and the effect of one million r is more than 4 times as great as the corresponding effects on whole erythrocytes.

In Fig. 1 we give the rate of O_2 consumption of normal and of irradiated nuclei and of whole erythrocytes irradiated and non-irradiated. The rate of respiration of non-irradiated erythrocytes and that of non-irradiated nuclei was arbitrarily designated as 100 (the horizontal base line). Values for irradiated nuclei observed at the same times were computed relatively to this normal value. The curves thus represent percentage increase or decrease in the respiration of irradiated nuclei compared with non-irradiated controls, plotted against time. The same figure also presents the curves describing the intensified respiration of whole erythrocytes irradiated also with identical doses of ½ and one million r. The fact that the curves for nuclei for both doses fall sharply and that in both cases the normal is reached simultaneously is striking.

Table I presents a series of typical experiments with different doses of X-rays. These figures show that after exposure to any of the given doses the intensity of respiration of the irradiated nuclei is initially high, becomes gradually weaker and falls to below normal after 3 hours. A similar, though somewhat delayed course is taken by the respiration of whole erythrocytes after irradiation, in which case the process of inhibition sets in much later.

It is still impossible to explain the process of X-ray activation described by various authors. The observed difference between the respirations of irradiated erythrocytes and irradiated nuclei alone, however, may be due to the protective effect of cytoplasmic proteins against the influence of X-rays. In order to verify this assumption we added 0.5% albumin or hemolysate to the nuclei before irradiation, thereby considerably weakening the stimulation.

Dale,[4] Friedewald,[5] Evans[6] and others have described such a protein effect on viruses, enzymes and sea-urchin sperm. It may be assumed that the proteins present in erythrocytes (globulin, hemoglobin, etc.) also exert a similar protective effect on the influence of X-rays on the respiration of whole erythrocytes.

Summary. 1. The effect of X-ray irradiation on the respiration of the nuclei of fowl erythrocytes has been investigated. 2. The respiration of these nuclei was temporarily stimulated by treatment with doses of 500,000 to 1,500,000 r. This stimulation of nuclear respiration was more pronounced than that obtained with whole erythrocytes. 3. The addition of hemolyzed blood or albumin to the suspension of nuclei before irradiation has a protective effect against the activating influence of X-rays.

[4] Dale, W. M., Meredith, W. J., and Tweedie, M. C. K., *Nature*, 1943, **151**, 280.

[5] Friedewald, W. F., and Anderson, R. S., *J. Exp. Med.*, 1941, **74**, 463.

[6] Evans, T. C., Slaughter, J. C., Little, E. P., and Failla, G., *Radiology*, 1942, **39**, 663.