2. Chemical and enzymic methods have been developed which, together with the protein precipitation test, make it possible to distinguish between pyrophosphoric acid and metaphosphoric acid.

3. The mycelium of *A. niger* also contains a

difficultly acid-hydrolyzable phosphorus compound which has been obtained in the form of a water-soluble Ba salt.

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The Effect of X-rays on the Respiration of Fowl Erythrocytes

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This paper deals with experiments concerning the effect of high doses of X-rays on the respiration of fowl erythrocytes.

METHODS

Preparation of material. Fowl erythrocytes were chosen for this study in view of their high O_2 consumption compared with non-nucleated erythrocytes. Six chicks aged 6–8 weeks were used alternately for all the present experiments. The blood was taken from the heart, defibrinated, filtered through sterilized glass wool and the filtrate centrifuged for 5 min. at 2500 r.p.m. The sediment of erythrocytes was then suspended in physiological saline (neutralized with 0.1N-NaOH to phenolphthalein; Siebeck, 1923) and again centrifuged. This procedure was repeated three times in order to eliminate serum and leucocytes as completely as possible. After the third washing the sediment of erythrocytes was suspended in neutralized saline at a concentration of about 2×10^6 erythrocytes cu. mm. as estimated in a blood-counting cell.

Irradiation. The X-ray tube used in these experiments was operated at a tension of 35 kV. and a current of 15 mA. with a copper anticathode. The window was aluminium foil 30μ 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 blood was 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 erythrocyte suspension in flat-bottomed shallow glass dishes of 15 mm. diameter. The blood layer did not exceed 3 mm. in thickness.

Respiration. The respiration was measured in the Warburg apparatus at 39–40°. 1 ml. of suspension was

used for each vessel for each estimation; three 0.5 ml. portions had therefore to be irradiated consecutively and afterwards combined. Parallel estimations of the respiration of non-irradiated (normal) erythrocytes of the same blood sample were carried out in every case. The portions to be irradiated, as well as those already irradiated, and controls were kept during the whole time of irradiation under equal conditions of temperature, etc. The first reading was made 20 min. after inserting the manometers in the water-bath (or about half an hour after the termination of irradiation). Readings were then taken, initially at intervals of 30 min. and later at intervals of 60 min.

Haemolysis. This was determined by the acid haematin method (Hawk & Bergeim, 1938). The erythrocyte suspension to be tested was centrifuged (5 min., 2500 r.p.m.), the supernatant solution was diluted (according to its haemoglobin content) with 0.1N-HCl and the extinction of the resulting solution was determined photometrically. The percentage haemolysis was calculated from the haemolysis-grade/extinction curve which was obtained for every blood sample by the following method:

0.1 ml. of the whole erythrocyte suspension was haemolyzed with 9.9 ml. 0.1N-HCl, and several known dilutions of the haemolysate in 0.1N-HCl were measured photometrically. The curve obtained by plotting the extinction against haemolysate concentration formed a straight line at this level of concentration.

RESULTS

Three doses of X-rays were examined: 500,000, 1,000,000 and 2,000,000 r. The results of typical experiments are presented in Table 1.

Table 1. *Influence of irradiation on O₂ consumption of erythrocytes*

Irradiation dose (r.)	μ l. O ₂ consumed by 1 ml. erythrocyte suspension after (min.)										
	30	60	90	120	150	180	210	270	300	330	360
0	6.3	15.4	24.5	—	32.9	—	44.1	56.0	—	70.7	77.0
500,000	14.0	26.6	36.5	—	44.9	—	56.8	70.1	—	86.2	92.5
0	11.3	21.1	33.7	43.5	53.3	60.8	67.1	81.5	86.5	—	—
1,000,000	25.4	43.4	60.1	72.1	83.4	91.4	96.0	105.5	108.5	—	—
0	18.9	33.4	44.7	60.5	73.7	85.0	—	104.5	—	126.5	134.1
2,000,000	35.5	52.2	56.9	69.6	81.0	84.3	—	95.0	—	102.4	106.4

The rate of respiration of normal erythrocytes in saline decreases with time. In Fig. 1 the value for the O₂ consumption rate of normal blood at any specified moment was taken as 100 (horizontal line). Values for irradiated erythrocytes at the same time points are rates relative to this unit. The curves thus show plots against time of the percentage increase or decrease of the respiration of irradiated erythrocytes in comparison with non-irradiated controls.

Fig. 1. Effect of X-radiation on the respiration of fowl erythrocytes. The data given are the O₂ consumption in the experimental vessels, expressed as a percentage of the O₂ consumption observed in the control vessels at a given time. ——— non-irradiated erythrocytes. —○— erythrocytes irradiated with 500,000 r. —×— erythrocytes irradiated with 1,000,000 r. —●— erythrocytes irradiated with 2,000,000 r.

Haemolysis. It is known that respiration is manifested by intact erythrocytes only, and not by their haemolysates. Effects due to haemolysis must therefore be taken into account.

The spontaneous haemolysis of a suspension of fowl erythrocytes in saline was found to be negligible even after 6 hr. of constant agitating at 39–40° (Table 2). Experiments showing positive results of haemolysis obtained with X-rays are very rare (Levin & Piffault, 1934, man, guinea-pig, with

1,200,000 r.); Halberstaedter & Goldhaber, 1943, 1944 (rabbit and fowl, with 2,000,000 r.).

Upon irradiation with doses of 500,000 and 1,000,000 r. no immediate haemolysis was observed. The supernatant in such cases is in fact paler than in non-irradiated controls; even with 2,000,000 r. immediate haemolysis did not exceed 4–5%. However, blood incubated at 39–40° after irradiation begins to haemolyze. The speed of this haemolysis increases with the dose of X-rays for any given blood sample and varies from sample to sample; and with any particular sample it increases also with the storage time.

Table 2. *Haemolysis on incubation of irradiated and non-irradiated erythrocytes*

Irradiation dose (r.)	Haemolysis after incubation for (min.)	
	30 (%)	330 (%)
0	0.9	2.5
500,000	1.2	23.2
0	0.9	1.7
1,000,000	3.5	73.1
0	0.8	1.5
2,000,000	15.6	71.5

It should be noted that the post-irradiative course of haemolysis was not followed continuously for technical reasons. Haemolysis was determined on three 0.5 ml. portions which were irradiated consecutively, pooled (as in respiration experiments), and shaken at 39–40°. This quantity permitted only two haemolysis estimations. One was carried out after 30 min. incubation (corresponding to the first reading in the respiration experiments) and the other after 6 hr. incubation. The intermediate values of haemolysis were calculated by interpolation from these readings; for the purposes of this approximation the haemolysis-time curve was considered to be linear. The values representing averages of several experiments with blood of various chicks were used for the correction of the results of the respiration experiments presented in Fig. 1 (which are likewise averages). Correction of the data of each respiration curve by parallel values for haemolysis obtained with the same blood sample would obviously have

been preferable. However, since storage time seriously affects the haemolysis rate, correction on this basis was not in practice feasible. A corrected percentage respiration-increase/time curve is plotted in Fig. 2.

Fig. 2. Effect of X-radiation on the respiration of fowl erythrocytes, after correction for haemolysis. 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 erythrocytes; —○— erythrocytes irradiated with 500,000 r.; ---○--- erythrocytes irradiated with 500,000 r., corrected for haemolysis; —×— erythrocytes irradiated with 1,000,000 r. ---×--- erythrocytes irradiated with 1,000,000 r., corrected for haemolysis; —●— erythrocytes irradiated with 2,000,000 r.; ---●--- erythrocytes irradiated with 2,000,000 r., corrected for haemolysis.

DISCUSSION

From the results in Table 1 and Fig. 1 it may be concluded that X-ray treatment of fowl erythrocytes in physiological saline stimulates their respiration to a high degree. The stimulation increases with the dose in the interval of 500,000–1,000,000 r.

The fact that the average stimulation effected by 2,000,000 r. is almost equal to that of 1,000,000 r. (Fig. 1) might be due to some destruction in the respiration system caused by the larger dose. In experiments on the effect of radiation on various cells, Wels (1924 *a, b*) found that the respiration of fowl erythrocytes was not stimulated by X-rays; however, the doses administered were very small in comparison to ours. Hazama (1924), on the other hand, who worked with γ -rays, found that irradiated fowl erythrocytes manifested a stimulated respiration and in this case the stimulation increased with the dose up to a certain limit above which it disappeared altogether.

As may be seen from Fig. 1 the stimulation is in all cases greatest at the beginning of the measurements, i.e. at about 30 min. from the termination of the radiation. In some instances stimulation records of 200–300% are obtained. The initial

stimulation immediately after the termination of the irradiation may confidently be expected to be still higher. Its value can be approximately estimated by extrapolation of the curve to zero on the time co-ordinate (dotted lines of Fig. 1). As the respiration proceeds the stimulation curve falls off, the slope increasing with the irradiation dose administered. It intersects the horizontal curve in all cases and at later stages the respiration rate of irradiated cells seems to be lower than is that of the normal cells. Intersection occurs sooner with larger doses, with which both initial increase and subsequent decrease are more pronounced. For the case of 2,000,000 r. intersection also occurs in the total respiration-time curves (data of Table 1). This shows that the decrease, at least in this case, is not due to lack of substrate or accumulation of reaction product and, at least in part, must be due to haemolysis, the latter being almost negligible in the controls, but increasing in relation to the dose in the irradiated suspensions.

To arrive at the real stimulation of O_2 uptake by irradiation, the calculation must be based not on the initial number of cells irradiated, but, at every given moment, on that fraction which has escaped haemolysis. Correction of the values on this basis causes an increase in the general level of the stimulation values obtained. The increase, moreover, is especially marked for the later stages of the experiments when haemolysis is well advanced. For doses of 500,000 and 1,000,000 r., and most clearly for the latter, the correction raises the relative curves wholly above the level of the normal (horizontal curve of Fig. 2).

It is therefore suggested that X-ray treatment of fowl erythrocytes causes a stimulation of respiration rate which decreases slowly and can be noted as long as the cells escape haemolysis within the duration of our experiment. Integration of the curves within the interval of its terminal points is, of course, necessarily arbitrary. Discussion on this point is impossible, however, since only approximate haemolysis corrections have been available.

A dose of 1,000,000 r. is optimal for the stimulation of the respiration of fowl erythrocytes. At 2,000,000 r. the rays exert probably also a damaging effect upon the respiration system, since in this case inhibition in the later stages of respiration measurements remains manifest even after the introduction of the necessary corrections for haemolysis (Fig. 2).

Fowl erythrocytes thus constitute one of the few known examples in which irradiation causes a marked and persistent stimulation of a biological process. Stimulation of respiration by ray treatment has previously been observed by Stoklasa (1930) by β - and γ -rays in plants, and by Williams & Sheard (1932) for frog skin treated with fairly small X-ray doses.

SUMMARY

1. The effect of X-radiation on the respiration and haemolysis of fowl erythrocytes has been investigated.

2. It was found that the respiration of fowl erythrocytes is markedly stimulated by treatment with doses of 500,000–2,000,000 r.

3. The stimulation increases with the dose up to 1,000,000 r. The initial effect of 2,000,000 r. does not exceed that of 1,000,000 r.

4. If radiation is administered in optimal doses the stimulation induced persists as long as the erythrocytes escape haemolysis within the duration of these experiments (6 hr.).

5. Fowl erythrocytes, incubated at 39–40° (with shaking) following irradiation, undergo progressive haemolysis, the velocity of which is proportional to the X-ray dose previously applied. The haemolysis of non-irradiated erythrocytes under equal conditions is negligible even after 6 hr. incubation.

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