THE EFFECT OF X-RADIATION ON THE OXYGEN UPTAKE OF EMBRYONATE EGGS*

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X-radiation causes profound metabolic changes within cells as a result of ionization. An important effect appears to be the "activation" of water, with the formation of free H and OH radicals, which disappear rapidly by recombination and the formation of hydrogen peroxide (1, 2). Known specific effects are the depolymerization and deamination of nucleic acid (3, 4), the inactivation of $-SH$ enzymes, (5, 6), the interference with nucleic acid synthesis, (7) and the conversion of RNA to DNA (8–10). Changes probably resulting from direct ionization of biochemical entities have also been observed (7, 11).

Previous studies on the effect of x-radiation on the oxygen uptake of living organisms have given varied results. In rats, Kirschner *et al.* (12) found an increased uptake, occurring within 24 hours after radiation when lethal doses were given, but appearing more slowly following sublethal doses. Tahmisian and Adamson, (13), using the grasshopper embryo, observed a period of decreased oxygen consumption for several days after radiation, followed by a period of increase (13).

Embryonate eggs offer certain advantages for the study of this problem. They can be maintained at constant temperatures, and their convenience makes it possible to obtain data at frequent intervals on groups large enough to average out individual differences. The observations to be reported here were made not only for their intrinsic interest, but also to establish data which might be correlated with the effects of radiation on the growth of rickettsiae and viruses in embryonate eggs.

Material and Methods

In the first experiment 700 fertile eggs, after incubation for 10 days at 37.5°C., were divided into 5 groups of 140 each. One group served as controls, while the other 4 groups were placed in open egg cartons with the air space uppermost and given, respectively, 250, 500, 750, and 1000 roentgens of x-radiation. The distance from the tops of the eggs to the tube

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target (tungsten) was 50 cm. No filter was used. A 200 kv. deep therapy machine was used which delivered radiation at the rate of 118 r per minute. The filter factor of the shell was not determined. The incubation temperature throughout the experiment was 37.5°C.

The oxygen uptake of all groups of eggs was determined 10 hours before irradiation and 4, 14, 24, 34, 42, 66, and 90 hours after irradiation. The methods used for these determinations and for their statistical evaluation were those previously described in detail (14). Briefly, the eggs in groups of 20 were placed in sealed aluminum desiccators equipped with fans to keep

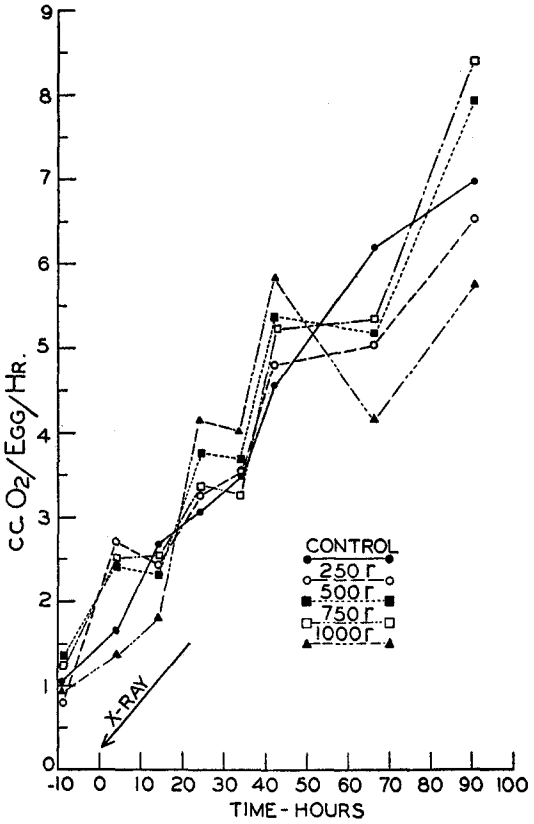

FIG. 1 The effect of x-radiation on the oxygen uptake of embryonate egg.

the air stirring, and allowed to come to equilibrium in a constant temperature water bath. Samples of gas were then removed at suitable intervals and analyzed for oxygen by means of a Haldane-Henderson gas analyzer.

The second and third experiments were repetitions of the first except for the fact that the number of eggs in each group was 125 and 118 respectively.

RESULTS

The oxygen consumption curves obtained in all 3 experiments were remarkably similar. The results of the first experiment are shown graphically in Fig. 1. The variations in the rates of oxygen consumption of all 5 groups *10*

hours before irradiation were statistically insignificant. *4 hours after irradiation* the 250 r, 500 r, and 750 r groups were consuming O_2 at rates significantly higher than the control group. (In the second experiment, all 4 radiated groups showed at this point rates of O_2 uptake significantly higher than the controls.) *At 14 hours*, the rates for all irradiated groups had either levelled off or fallen slightly and were about equal to the rate in the control group, which had continued to rise. This was the first step in a staircase effect which was noted in all irradiated groups. *Between the 14th and 24th hours*, all irradiated groups showed sharp rises in the curves of O_2 consumption, and the rates for the 500 r and 1000 r groups were significantly higher than the rate for the control group.

Between the 24th and 34th hours, the second levelling off period occurred, but *at 42 hours* the rates for the 500 r, 750 r, and 1000 r groups were again significantly higher than the rate for the controls. A third levelling off period occurred *between the 42nd and 66th hours. At 66 hours* the respiratory rate of the 1000 r groups had fallen to a level well below that of all other groups. *At 90 hours*, the descending order of the rates of O_2 uptake was 750 r, 500 r, control, 250 r, and 1000 r. The most striking effect, seen in the chart, is the irregular "staircase-like" curves of all radiated groups, as contrasted with the relatively "straight line" curve obtained in the control group. This difference was equally marked in the other 2 experiments, and in all 3 experiments the levelling off periods were noted during the same time intervals.

DISCUSSION

An explanation of these findings cannot at present be given. It is probable, however, that single dose x-radiation causes several different types of enzymatic alterations, which are for the most part stimulative and which become effective at different time intervals. In an organism with a constant respiratory rate, this would result in an undulatory curve, parallel to the base line, but in fertile eggs, in which the rate rises steadily with embryonic development, a staircase-like curve would result. In studies of respiratory rates of eggs infected with influenza virus (15), we have attempted to explain a similar staircase effect on the basis of the periodic release of toxin following phases of intracellular virus multiplication. A similar explanation might be considered for the results reported here, if it could be shown that different types of cells set free toxic degeneration products at different time intervals after the initial injury.

SUMMARY AND CONCLUSION

Groups of 10 day old embryonate eggs were given 250, 500, 750, and 1000 r of x-radiation, and oxygen consumption determinations were made subsequently at various intervals during a period of 90 hours. In general, the effect of radiation was moderately stimulatory, but after 90 hours the respiratory rates of the 250 and 1000 r groups were well below those of the control groups. The most

striking effect, noted in 3 separate experiments, was the occurrence of a "3 step staircase" type of oxygen consumption curve in all irradiated groups, the levelling off periods apparently occurring between the 4th and 14th, the 24th and 36th, and the 42nd and 66th hours after exposure. These curves were in striking contrast to those shown by groups of non-irradiated eggs, which were practically of the straight line type.

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