THE EFFECTS OF RADIATIONS ON BIOLOGICAL SYSTEMS

II. Immediate and Subsequent Effects of X-Ray Irradiation Upon Respiration of Drosophila Larvae

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In gathering data concerning the effect of radiations on biological systems we have proceeded in our study from a simple system, enzymes in solution,[1] to a more complex system, *Drosophila* larvae.[2–4] As a result of irradiation with x-ray radiation the prepupal period of the life cycle of the *Drosophila* is prolonged. This effect serves as a convenient measure of relative effectiveness of radiations.[4] In the hope that *Drosophila* larvae might prove to be a convenient system to study more satisfactorily the immediate as well as more remote effects of irradiation previously attempted in the rabbit[5] and dog, we determined to observe the influence of x-rays on metabolic activity as measured by respiratory rates. Since no satisfactory method was available for such observations, it was necessary to develop one, which end was attained by the construction of a respirometer capable of use under all conditions of constant humidity. The apparatus and the principles

[1] Hussey, R., and Thompson, W. R., *J. Gen. Physiol.*, 1922–23, **5,** 647; 1923–24, **6,** 1, 7; 1925–26, **9,** 211, 217, 309, 315. Thompson, W. R., and Hussey, R., *J. Gen. Physiol.*, 1931–32, **15,** 9. Thompson, W. R., and Tennant, R., *Proc. Soc. Exp. Biol. and Med.*, 1932, **29,** 510.

[2] Hussey, R., Thompson, W. R., and Calhoun, E. T., *Science*, 1927, **66,** 65.

[3] Tennant, R., *Science*, 1931, **73,** 567.

[4] Hussey, R., Thompson, W. R., Tennant, R., and Campbell, N. D., *J. Gen. Physiol.*, 1932–33, **16,** 207.

[5] Hussey, R., *J. Gen. Physiol.*, 1921–22, **4,** 511. Sprunt, D. H., *J. Biol. Chem.*, 1931, **92,** 605.

of refractovolumetric respirometry, applied especially to the measurement of CO_2 and O_2 changes, are described elsewhere.[6–8]

Technique

Larvae for experiments were obtained in the same manner as in the work recently reported[4] except that seedings were made upon a preparation called Y. B. A., No. 3, instead of yeast-agar as before, and larvae were transferred in the usual manner[4] at a median age, *a*, of 2.5 days in random samples of approximately equal numbers to ordinary tapering drinking glasses, each of which contained 25 ml. of a special food, Y. B. A., No. 2; Y. B. A., No. N (Yeast-banana-agar No. N) being a mixture in the proportion: 1 gm. of over-ripe banana pulp; +3 gm. Fleischmann's yeast; +5 ml. of *No. N* agar mixture in the proportion: 9 N gm. agar-agar; +1 gm. sodium acetate; + enough water and acetic acid to make the volume equal 1 liter and the pH = 6.0.

In the present work aseptic technique was not maintained beyond this point.

These vessels were convertible into respiratory chambers suitable for sealing into the respiratory machine by fitting into the tops a large rubber stopper with inlet and outlet delivery tubes, the stopper being fitted into the tumbler about 1 cm. below the rim, the space above the stopper partly filled with mercury during observation in order to prevent leakage at the joints or through the rubber. The ends of the delivery tubes projecting into the tumbler were provided with a flange which was covered with a double-layered voile cap in order to prevent the escape of larvae. The method of estimation of CO_2 respiration was essentially that used for *Drosophila* imagos in a water-saturated environment.[8] However, no attempt was made to estimate O_2 changes in these experiments. This was made necessary by our interest in observing respiratory rates in simultaneously maintained groups of larvae from the same lot but variously treated as to irradiation. Respiration in 20 minute intervals was measured, preceded by an introduction of fresh air (approximately water-saturated) and a 10 minute mixing and adjusting period in the manner previously described.[6,8] A set of blanks was used to introduce a correction for failure to attain complete saturation with water in the 10 minutes of preliminary circulation. This correction and its standard deviation played a minor rôle in the estimation of CO_2 respiration. Indeed, the crude refractivity change observations, if plotted in place of the CO_2 change estimates, give practically the same general impression.

EXPERIMENTAL

Prior to irradiation respiratory observations were made over an interval of approximately 1 day, the age at the beginning of irradiation approximating 3.5

[6] Thompson, W. R., *J. Gen. Physiol.*, 1932–33, **16**, 5.

[7] Thompson, W. R., and Tennant, R., *J. Gen. Physiol.*, 1932–33, **16**, 23.

[8] Thompson, W. R., and Tennant, R., *J. Gen. Physiol.*, 1932–33, **16**, 27.

days. In irradiations the tumbler, food, and larvae were merely covered with perforated wrapping paper. The source of radiations was a Coolidge water-cooled x-ray tube,[4] with target directly above the centroid of three tumblers of the type just described, placed upright with rims contiguous. It was impressed with a potential difference of peak approximately equal to 188 kv. with a tube current of 30 ma. The distance from target center to objective was approximately 59 cm. with no filter except a piece of white writing paper placed across the aperture. The aperture and all other conditions were the same as in experiments recently reported.[4] Irradiations were made to start simultaneously in any given

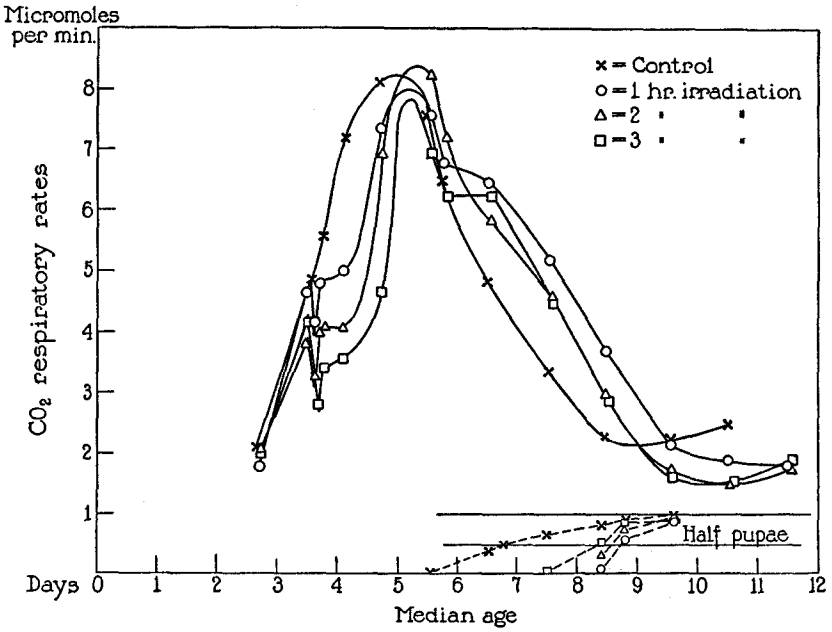

FIG. 1

experiment, the various lots being removed at the end of prescribed periods and replaced by dummy tumblers with covers of the same type. The results of two experiments where larvae were irradiated for 1, 2, and 3 hours in this manner (between 22 to 25°C) and CO_2 respiration rates contrasted with those of a control are given in Figs. 1 and 2. The larvae chambers were flushed, at all times except during transfer or irradiation, with a stream of water-saturated air at 24.2°C. which was fresh except when sealed in the respirometer.

An experiment similar to these in all respects (including a control) except that the x-ray tube was not operated yielded no significant differences between any of the lots of larvae. Thus it is indicated

that the definite differences exhibited in the former experiments were the result of irradiation.

Pupation was followed in each instance and the median time of pupation, P, estimated by means of independent inspection estimates of three observers. The coincidental estimates of the fraction, f, of individuals having pupated is given below the respiration curves, using the same abscissae. Thus P is the time where $f = 0.5$ for a given lot.

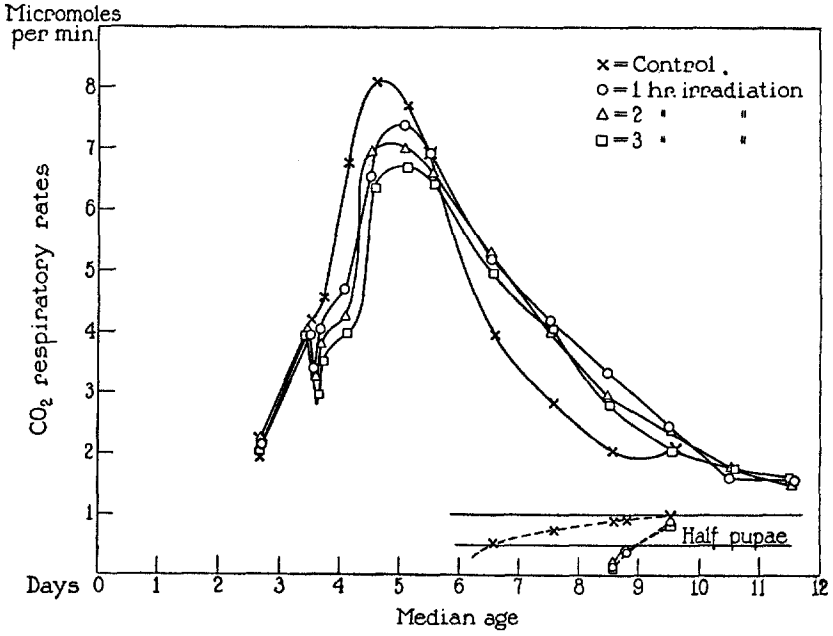

FIG. 2

The characteristic most obvious in these experiments is the decreased respiratory rates observed immediately after irradiation. It appears as if the CO_2 respiratory rate of such larvae is a decreasing function of the period of irradiation within the limits of the experiments; but upon discontinuation of irradiation this rate increases rapidly for a short time (about 0.1 day), then markedly less rapidly (with a point of inflexion), then more rapidly again up to a maximum. Apparently the control attains a maximum sooner and declines to a

minimum about a day or two before the irradiated lots—this latent decline being correlated, with the observed delay in pupation. The subsequent rise apparent in the control curves is associated with the emergence of imagos—observation being discontinued on account of their extensive drowning in the remnants of the food. The characteristic decreased rate of respiration after irradiation was observed when larvae were irradiated under essentially the same conditions of maintenance as in former work;[4] that is, in sealed wells in stearin-paraffin with perforated tops. Under such conditions, however, it was extremely difficult to measure respiration soon after or before irradiation and considerable manipulation of the larvae at the time seems objectionable and might even confuse the issues involved; hence the resort to the procedure described above. Results similar in some respects but markedly different in others have been obtained in similar manner using ultraviolet light instead of x-rays, which will be reported in another communication.

COMMENT

It should be emphasized that in these observations our interest in following changes in functional state at short intervals and in simultaneously maintained lots was of prime importance. From this point of view observations of respiration were made without a prolonged interval in which to allow adjustment of the larvae to their altered environment. In this manner slightly higher respiratory rates pertained than otherwise, but these were consistent provided at least a half-hour interval were allowed to elapse between any two observations on the same lot. Therefore, we should not strictly interpret the curves obtained as indicative of actual respiration during the intervals between observations, but we assume that they indicate this relatively.

It is of interest, however, to consider relationships between the apparent total CO_2 respiration of the different lots over the interval from the age of 2.5 days to the end of the larval stage. If the result of the action of the radiations upon the larvae were merely to uniformly alter the rates of all processes, then it would follow that the total CO_2 respired would be the same in any interval between corresponding structural states of the larvae, which obviously is not the case.

SUMMARY

By means of measurement of CO_2 respiratory rates it has been possible to observe *immediate* and *latent* effects of x-ray irradiations upon *Drosophila* larvae. The observations were extended over a period of several days, the duration of the prepupal period being observed also. In every instance a significant decrease in the rate of CO_2 respiration was observed immediately after irradiation. This decrease was univariant with the period of irradiation within the experimental limits.