Radiosensitivity of Larval and Adult Amphibia in Relation to Temperature During and Subsequent to Irradiation.* (22369)

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That the temperature prevailing in a protoplasmic system *subsequent* to exposure to ionizing radiations is an important factor in modifying the extent of damage reflected over a given period is generally acknowledged. However, regarding the effect of temperature *during* exposure, Patt has written recently and fittingly: "Evidence that relates to the effect of temperature during exposure to radiation is equivocal and virtually every conceivable effect has been described"(1). The issue is highly important toward an understanding of the nature of biological responses to ionizing radiation. Patt and Swift(2) have presented good evidence that (a) survival of frogs (*R. pipiens*) is not influenced by altering their body temperature during and/or for the first 24 hours following exposure to X-rays, and (b) survival is greatly enhanced so long as the animals are kept at 5°-6°C, continuously, following exposure. When, after 60 to 130 days in the cold these latter are returned to 23°C, there is no change in absolute survival nor any significant difference in the time-course of deaths when compared with similarly irradiated frogs which had been maintained at 23°C immediately following irradiation. It would be of interest to learn whether this pattern of post-irradiation response would be duplicated by larval frogs. In preliminary communications (3-5) we have reported that reduced temperature during exposure to X-rays was accompanied by increased radioresistance in urodele larvae (Amblystoma); this was reflected in such criteria as feeding behavior, response to tactile stimuli, swimming motions, atrophy of gills and tail, and damage to epithelia of digestive tract and tissues of the eye and brain. While no mortality studies were made, the observations are rather at variance with what might reasonably be expected on the basis of Patt and Swift's data on adult frogs. Also, the histopathologic data on these urodele larvae do not agree with the findings of Allen and his associates(6) on the radiosensitivity of hematopoietic tissue of tadpoles of *R. catesbiana* irradiated at different temperatures.

Accordingly, the present report will be concerned with: (a) influence of temperature prevailing during and after whole-body irradiation on X-ray lethality in anuran larvae; (b) influence of temperature during and after whole-body X-irradiation as reflected in certain tissues and features of behavior of larval urodeles and (c) survival characteristics of adult urodeles irradiated at different temperatures.

Materials and methods. Larvae of *R. catesbiana* (predominantly first year), larval *A. punctatum* and *A. maculatum* (15-22 mm) and *Triturus viridescens* comprised the experimental material. Temperature of the surrounding water was taken as the measure of body-temperature during and subsequent to irradiation. Animals to be irradiated at 2°-3°C or 7°-9°C were conditioned to these temperatures for at least an hour before exposure. Irradiation conditions and disposition of control groups will be more conveniently described below in connection with each experiment.

Results. Exp. 1A. *Larval R. catesbiana exposed to 1000r at 3°C, and 23°C, respectively, and subsequently maintained at ca 25°C.* Irradiation conditions: 120 kv; 9 ma; target-specimen distance, 25 cm (measured to half the 9 cm depth of water in a plastic container, 15 cm in diameter); no added filter; 200r/min.; dose, 1000r. The results, and control data are summarized in Fig. 1, It will be seen that the temperature at the

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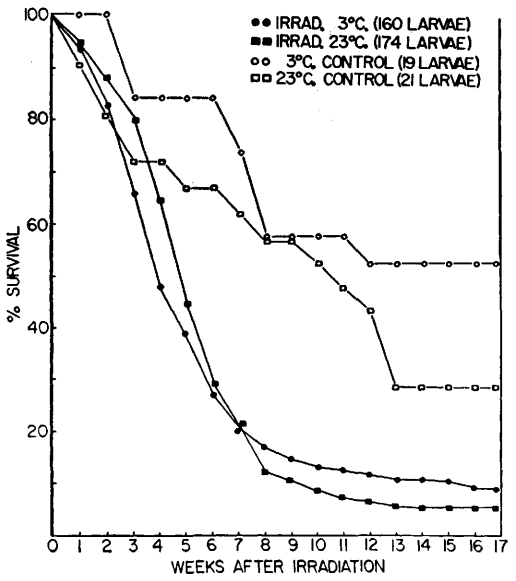

FIG. 1. Survival characteristics of larval *R. catesbiana* irradiated at 3°C and 23°C, respectively, and subsequently maintained, at 25°C; non-irradiated control groups also shown.

time of exposure had no significant effect upon the survival characteristics of larvae maintained at ca 25°C subsequent to exposure.

Exp. 1B. *Larval R. catesbiana exposed to 1000r at 3°C and 23°C, respectively, and subsequently maintained at 3°C for 8 weeks and then returned to ca 25°C.* Irradiation conditions were the same as for Exp. 1A. The results, and control data, are summarized in Fig. 2. Clearly, temperature at the time of exposure had no significant effect on survival when the animals were kept, subsequent to exposure, at 3°C for 8 weeks and then returned to room temperature; prolonged refrigeration after irradiation unmistakably fostered survival, *but only for such time as the reduced temperature prevailed.* Each control group here, and in Exp. 1A exhibited only ca 50% survival at 11-12 weeks; this will be discussed below.

Exp. 2A. *Larval A. punctatum exposed to 1176r or 3136r at 7°-9°C and 26°-28°C, respectively; after irradiation half of each group was maintained at 12°-15°C and half at 22°-24°C; appropriate control groups were observed.* Irradiation conditions: 140 kv; 5 ma; 1000r/min. Larvae were anesthetized in 1:2000 MS 222 during exposure. No survival studies were made. Observations based on 360 specimens, of which some 50 were periodically fixed in Bouin's and studied histologically. Observations on living larvae: in virtually all of the specimens studied, the following patterns of externally observable responses were clearly evident. As compared with larvae irradiated at the lower temperature those irradiated at the higher temperature exhibited: (a) markedly reduced readiness (ability?) to feed on small enchytraeid worms which are ordinarily a favorite food of such larvae; (b) decidedly more abnormal swimming movements, whether spontaneously inaugurated or in response to tactile stimuli; (c) a more pronounced curling and darkening of the extremity of the tail and also a more marked atrophy of the gills. These manifestations were not found in control groups. Histological observations: Particular attention was directed to the epithelial elements of the digestive tract, for these are generally regarded as particularly radiosensitive and their relative simplicity enhances their value in the assaying of cellular damage. Here too the damage is noticeably greater in

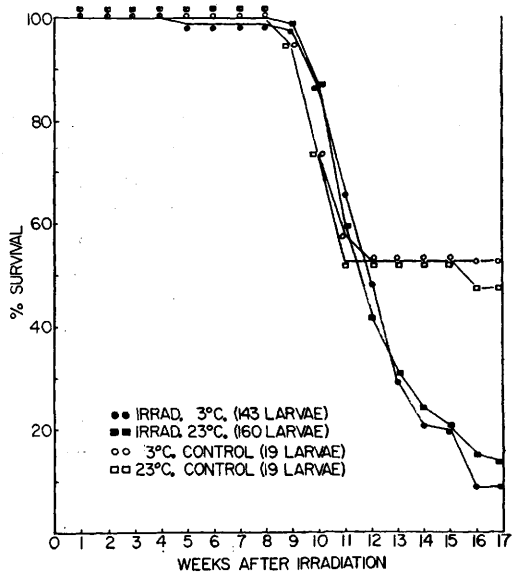

FIG. 2. Survival characteristics of larval *R. catesbiana* irradiated at 3°C and 23°C, respectively, and subsequently maintained at 3°C for 8 weeks and then returned to 25°C; non-irradiated control groups also shown.

those specimens irradiated at the higher temperature; damage is reflected principally in a variety of chromatin disarrangements, vacuolization of the cytoplasm and overall tissue degeneration.

Exp. 2B. *Larval A. maculatum exposed to 15,750r at 2°C, and 33°C, respectively, and subsequently maintained at ca 25°C; appropriate non-irradiated controls were observed.* Irradiation conditions: 140 kv; 5 ma; target-specimen distance 11.5 cm; 1050r/min. (This experiment is essentially the same as Exp. 2A except for a much heavier dose of radiation and a wider range between the two temperatures employed.) The larvae exposed at 33°C exhibited such abnormal features as curling and darkening of the extremity of the tail and abnormal swimming movements as early as 1 day following irradiation, whereas these changes appeared in the group exposed at 2°C only 3 days following exposure. A histological study was directed to the tissues of the eye and the wall of the forebrain; the damage to the larval eye and brain wall was significantly less, over a given time, in those larvae exposed at the lower temperature. The damage was most clearly revealed in the developing retinal layers of the eye.

Exp. 3. *Adult T. viridescens exposed at 3°C and 26°C, respectively, and subsequently maintained at ca 25°C.* Irradiation conditions: 220 kv; 15 ma; target-specimen distance, 25 cm (measured to half the 9 cm depth of water in a circular plastic container, 15 cm in diameter); 396r/min.; dose, 7920r. The experimental results, with control data, are summarized in Fig. 3. It will be seen that the temperature at the time of irradiation had a definite effect on the time-course of deaths; the lower exposure-temperature was accompanied by a significantly lower mortality rate during the first 5 post-irradiation weeks.

Discussion. The results of Exp. 1A demonstrate that, for the temperature points and radiation dosage employed, the temperature at which larval *R. catesbiana* were irradiated was without significant influence on the final mortality outcome. In Exp. 1B, prolonged refrigeration following irradiation was accompanied by the same enhancement of survival (during the period of refrigeration) as was described for adult frogs by Patt and Swift(2). The present results with anuran larvae are in full agreement with the observations of these authors and, we believe, are of additional value by reason of the larger number of organisms employed. The data would suggest that the primary changes, effected during the irradiation, are independent of body temperature and that the enhanced survival during prolonged refrigeration following exposure reflects a postponement or retardation of temperature-sensitive secondary reactions, rather than any real measure of "recovery."

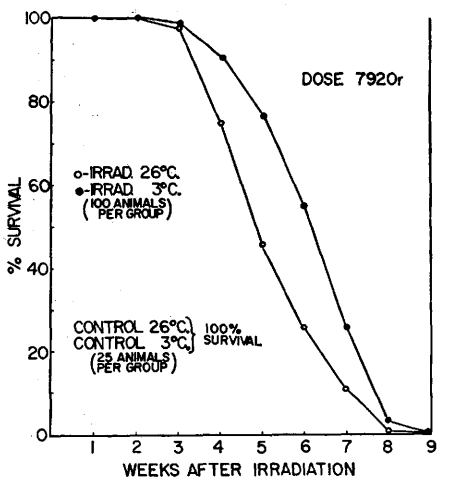

FIG. 3. Survival characteristics of adult *T. viridescens* irradiated at 3°C and 26°C, respectively, and subsequently maintained at 25°C; there was 100% survival in the non-irradiated control groups.

It will be noted that in Exp. 1A the non-irradiated control groups exhibited only about 55% survival at 11-12 weeks. While this reflects a rather high mortality, it is definitely considerably less than the approximate 90% mortality exhibited over this same period by the experimental groups; these latter showed a 50% mortality as early as 4-5 weeks following irradiation. It may also be pointed out that at about the 13th week the control and experimental groups level off and continue with relatively little change for another month at which time the experiment was terminated. While this mortality rate in the controls was higher than might be an-

ticipated, it would seem that the overall time course of deaths in the controls together with the absolute survival figure to 17 weeks are sufficiently distinctive so as to suggest that the factors involved did not essentially modify the survival characteristics of the irradiated larvae. It is important to note that in Exp. 1B the control and irradiated larvae both exhibited 50% mortality at 11-12 weeks. However, at this point the controls leveled off and maintained this 50% survival picture to 17 weeks, whereas the irradiated larvae rapidly declined to an approximate 10% survival by week 17. It would appear that under the existing laboratory conditions, 50% survival at 11-12 weeks and a leveling off from that point onward is a characteristic response of non-irradiated bullfrog larvae and need not be regarded as affecting the survival data of the irradiated animals.

The results of Exp. 2A and 2B (it will be remembered that no survival studies were made on these groups) are not easily reconciled with the present data on frog larvae, and the studies of Allen, *et al.*(6) on radiosensitivity of hematopoietic tissues in the tadpole under different conditions of temperature. They do agree, however, with data gathered by others in similar studies involving embryonic chick tissue, amphibian germs and eggs of drosophila.

The results of Exp. 3 with adult *T. viridescens* present a different picture. Here it will be seen that when the organisms are irradiated at 3°C the mortality-rate is significantly lower during the first 5 post-exposure weeks than in those animals irradiated at 26°C, both groups having been returned to room temperature following exposure.‡ More specifically, at the end of the 5th post-irradiation week, the mortality is about 50% for those animals exposed at 26°C, whereas it is only about 20% for those exposed at 3°C. These results are in agreement with the earlier noted observations on larval urodeles in Exp. 2A and 2B. They do not agree with the data of Patt and Swift on adult frogs(2). While the temperature at the time of exposure did not, in terms of absolute survival, affect the final outcome, nevertheless, the significant difference in the time-course of deaths over a representative post-irradiation period (5 weeks) is evidence that the temperature prevailing *during* irradiation is a radiosensitivity-modifying factor.

We are not prepared at this time to suggest an explanation for the difference in response, following irradiation at different temperatures, of adult and larval anurans on the one hand and larval and adult urodeles on the other.

Summary. 1. Survival of larval *R. catesbiana* is not affected by regulation of body temperature (to 3°C or 23°C) during whole-body irradiation; prolonged refrigeration subsequent to irradiation fosters survival, but only for as long as the animals are kept at the reduced temperature (3°C); upon return to 23°C the mortality characteristics develop in essentially the same pattern as larvae maintained at 23°C immediately subsequent to irradiation. 2. As compared with those irradiated at lower temperatures, 2-9°C, larval *A. punctatum* and/or *A. maculatum* irradiated at higher temperatures, 28-33°C, and subsequently maintained at room temperature, exhibit definitely more abnormal (externally observable) responses, as well as greater damage to digestive epithelium and elements of the developing eye and brain wall. 3. Adult *T. viridescens* irradiated at 3°C exhibit, during the first 5 post-irradiation weeks, a significantly lower mortality rate than those irradiated at 26°C, both having been returned to 23°C immediately after exposure.

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