Radiation-induced Biochemical Changes in the Larvae of Housefly, Musca domestica

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Radiation-induced changes in the third instar larvae of Musca domestica were investigated. Pupariation delay was evident in the irradiated larvae. Both 5 and 10 kR exerted similar pupariation delays.

At biochemical level, glycogen seems to show an enhancement in the irradiated larvae as compared to control. Free sugars register initial rise followed by decline in the irradiated larvae. Total ninhydrin positive compounds showed a similar trend as free sugars. The irradiated larvae exhibited a delayed increase in protein content as compared to control. There was no significant change in ribosidic compounds due to irradiation. These results are discussed in the light of the likely targets of radiation at the cellular level.

INTRODUCTION

The use of ionizing radiation as a source to enhance the mutation frequency was recognized as early as 1930. This has been exploited in controlling insect pests widely and the evident example is the eradication of screw-worm by releasing radiation-sterilised males. Similar approach is also being tried to control Aedes, Musca, Mediterranean fruit fly, etc.

Irradiation of insect larvae may lead to a number of responses in addition to simple lethality. These include delay in pupation, melanization of larval body, developmental abnormalities in the adult, death during pupal stage or failure of emergence and imaginal death soon after eclosion. Developing stages of insects show drastic variations in their sensitivity to radiation. However, little information is available on the biochemical alterations that lead to manifestation of physiological effects caused by irradiation in the developing insects.

In the present paper an attempt has been made to investigate the changes in glycogen, free sugars, free amino acids, soluble ribosidic compounds and soluble proteins in housefly larvae after irradiation.

MATERIALS AND METHODS

The strain of houseflies used in this study (Musca domestica nebulo Fabr.) has been maintained in our laboratory at 31±1°C. Techniques used for rearing the houseflies, collection and incubation of eggs have been the same as described earlier. Fifty
hour old larvae were taken for experiments. Larvae were irradiated with gamma-cell 4000 (5500 Ci 60Co) obtained from BARC, Bombay at a dose rate of 70 R/sec. to 5 and 10 kR. After irradiation 50 larvae were seeded in a 250 ml beaker containing milk-soaked cotton and three replicates were prepared for each treatment. The experiments were carried out at 35±1°C as this temperature supports maximum rate of development.

The larvae were then checked at 4 hour interval and number of them moulting to pupae was noted by counting the white barrel-shaped structures in which tanning has already set in. Pupariation delay was determined graphically from hours after irradiation versus the percentage of puparia formed by comparing the time taken for 50% pupariation in control as well as irradiated larvae.

For biochemical analysis, larvae irradiated with 5 and 10 kR were used. Average of three determinations was taken. There was no significant difference between control and irradiated larvae. Free sugars, free amino acids and soluble ribosidic compounds were extracted by homogenizing the larvae (about 100 mg wet weight) in acid alcohol (80% alcohol in 0.1 N PCA). The homogenate was centrifuged at 16,000 x g for 15 minutes and the supernatant was used for estimation by the methods of Plummer15), Clark16) and Painter and Kilgore17) for free sugars, free amino acids and ribosidic compounds respectively. D-Glucose, glycine and yeast RNA were used as standards.

Glycogen was extracted using the method of Ashman and Seed18). Hundred mg of larvae were ground with 5 ml of 1.5% KOH followed by centrifugation at 16,000 x g for 20 minutes. The supernatant was discarded and the pellet was incubated with 5 ml of 1.5% KOH in boiling water for 30 minutes. To this was added an equal volume of absolute methanol and was centrifuged at 16,000 x g for 20 minutes. Once again the supernatant was discarded and the pellet was dissolved in 5 ml of 1 N HCl and heated in boiling water for 30 minutes. This was filtered on a Whatman No. 1 filter paper and filtrate was used for the estimation of glycogen, according to Plummer15).

For extraction of soluble proteins, larvae were homogenised with 4 ml of Tris-HCl buffer (pH 7.3) and was centrifuged at 20,000 x g for 20 minutes. The supernatant was utilized for soluble protein estimation using the method of Lowry et al.19).

RESULTS

Fifty hour old larvae used in the present study exhibit pupariation delay markedly (Fig. 1). It is obvious from the data that the delay induced by radiation is dose-dependent upto 5 kR. There is no appreciable difference in pupariation delay induced by 5 and 10 kR. Larvae irradiated with higher doses show sluggish movements and sometimes remain motionless.

Figure 2 shows the glycogen content of housefly larvae with regard to 5 and 10 kR irradiation. Non-irradiated control larvae show an increase in glycogen content. The control larvae could not be analysed beyond 24 hours as most of them form
puparia by then. Interestingly, irradiated larvae register an enhancement of glycogen and this enhancement further seems to be dependent on post-irradiation period. The level of free sugars is high in control and irradiated larvae only during the initial period (Fig. 3). Larvae irradiated with 10 kR show the maximum content of free sugars.
sugars and the level starts declining from 24 hours onwards (Fig. 3).

The ninhydrin-reactive materials of larvae were measured and used as an indication of the free amino acids present. Figure 4 shows the total free amino acid level of non-irradiated and irradiated larvae. The changes observed are closely similar.
to free sugars level (Fig. 3). The maximum content is seen at 12 hour post-irradiation. There is no significant change in ribosidic compounds due to irradiation (Fig. 5).

Non-irradiated larvae show an increase in soluble protein content towards their
moult to puparia. In irradiated larvae, a delayed increase in protein content is evident (Fig. 6).

DISCUSSION

Biological effects of exposure to ionizing radiations are complex and vary with the type of cells in the tissue involved. In actively proliferating tissues irradiation results in destruction and loss of a large proportion of cells. The actively growing early instar larvae of Musca are markedly sensitive to radiation. On the other hand, the late instar larvae are quite resistant to 10 kR as there was no mortality observed. Of the various parameters used to measure the physiological disturbance induced by irradiation in different insects, pupariation delay is a quite sensitive index with regard to mature larvae. The extent of pupariation delay induced by radiation in Musca domestica is quite similar to the results obtained with larvae of Sarcophaga bullata and Tenebrio molitor.

An understanding of the effect of ionizing radiation on living cells requires a knowledge of the changes brought about in chemical composition and intermediary metabolism of tissues. Zdarek and Fraenkel reviewed pupariation in flies and suggest that it does not involve cell division. The matured larval tissues of insect thus represent noncytokinetic state. Hence, the observations made on late larval instar may throw some light towards understanding the manifestation of radiation injury in many post-mitotic tissues as the basic mechanisms of cellular damage were probably common between mammals and insects.

Whole body irradiation of animal alters the metabolism of various organs. In Musca larvae, both 5 and 10 kR enhanced glycogen and free sugars content. These results are similar to the observations made on Drosophila melanogaster. In both adult male and female flies, there is a sharp increase in glycogen and free sugars level after irradiation.

Generally, in insects the glycogen level increases as the larval growth proceeds with temporary drop during each moulting period. Since the glycogen stored in last instar larvae would be utilised during metamorphosis, it is not surprising to find an initial increasing trend in our studies (Fig. 2). In Musca domestica larvae, males and females could not be analysed separately as there is no satisfactory method of separating them until after their emergence as adult. However, Mayer and Bridges reported a depression in glycogen and small increase in free sugars in male horn flies (Haematobia irritans) when irradiated as pharate adults. Our observations on irradiated Musca larvae are closely similar to the reports made on mammalian liver. Regarding the enhanced glycogen content, it has been stated that it is the result of an inhibition of glycogenolytic enzymes. This view has not been supported by some other workers. Some of these processes may operate in Musca larvae resulting in an enhanced glycogen content.

Irradiation has increased the concentration of free amino acids in Musca domestica larvae (Fig. 4). Richardson and Mayer showed that large doses of irradiation (20 kR)
increased the total concentration of amino acids in the haemolymph pools of prepupae of honeybee (Apis mellifera) and last instar larvae of greater waxmoth (Galleria mellonella). Contrastingly Mayer et al. found no influence of irradiation on the total concentration of amino acids in either physiological or total amino acid analysis in adult horn flies. Also, Raghavan et al. found that whole body irradiation of rice moth larvae reduced tyrosine pool considerably. The increase in free amino acids in Musca may be derived from the radiation-induced degradation of proteins. The changes observed in total free amino acid level are closely similar to free sugars but dose-response relationship at maximum point is reversed. This, however, is difficult to explain at present and will have to be looked into further.

The increase in soluble protein content in non-irradiated larvae is consistent with the general view that the larvae before pupariation show protein biosynthesis. This increase may also be due to the appearance of some specific proteins at the time prior to puparium formation. Duke and Pantelouris found that soluble proteins increased from 1 band in the first instar larvae to a total of 11 in the late third instar larvae of Drosophila. Irradiated larvae showed higher soluble protein content at a much later stage. It is worth mentioning that Mukerjee and Goldfeder also observed an increase in protein biosynthesis in the liver of X-irradiated mice. Hidvegi et al. found an increase in mRNA as an early response to irradiation in guineapig tissues. Therefore, the initial increase in soluble protein content at 12 hour in case of 10 kR may be correlated with the initial increase in mRNA as an early response to irradiation. Further, studies involving a number of insects from different orders would enable to pinpoint the susceptibility of tissue components to radiation.

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