CHAPTER I

EFFECTS OF LOW DOSE X-IRRADIATION ON THE SUCCINATE DEHYDROGENASE ACTIVITY OF GUINEA PIG, RAT AND MOUSE TISSUES

INTRODUCTION

Radiations are known to cause disturbances in activities of enzymes or metabolic rates of tissues. Vogel (1960) and Hager et al. (1962) have shown that radiations cause the swelling and vacuolation in cell organelles, when animals are exposed even to small doses of radiations. Radiations also cause mitochondrial swelling and disorganization of cristae as observed by electron microscopy and with the production of peroxides of structural lipids of mitochondrial membranes (Jordana and Lamsfus, 1971; Jordana et al., 1971; Zys and Michalska-Kaszczynska, 1972). The formation of lipid peroxides and the swelling which accompany it may be responsible for the abnormalities seen in the hepatic and splenic mitochondria following irradiation (Benjamin and Yost, 1960; Jordana et al., 1971; Cohan et al., 1973). These changes in mitochondria may be related to altered enzymic activity and energy metabolism of these tissues. Succinate dehydrogenase (SDH) being a mitochondrial enzyme, can serve as a sensitive parameter for such studies. In this chapter we report alterations in SDH activity in some tissues of guinea pig, rat and mouse subjected to low dose X-irradiation. These changes have been studied cytochemically and biochemically. Significance of the alterations in the enzymic levels at different intervals after low dose (72 r, 120 r and 240 r) X-irradiations have been discussed.
MATERIALS AND METHODS

Animals: Normal guinea pig (Cavia porcellus L.), weighing 350-400 gm; albino rat (Rattus norvegicus L.), weighing 170-200 gm and albino mouse (Mus musculus L.), weighing 25-30 gm, were used in the present experiments. They were bred and maintained in the temperature conditioned laboratory on standard feeds (Hindustan Lever Ltd.) and water ad libitum.

X-irradiation procedure: The animals under light ether anaesthesia were X-irradiated between 11.00 a.m to 1.00 p.m. in the thoracic region (ventral side facing the source) by Copper K alpha lines (Philips model). The skin target distance was 20 cm. The diameter of the circular portion of thorax exposed to X-irradiation was 1.78 cm and the total area exposed was 5.6 square cm, at a voltage of 40 kV and 20 mA. The dosage employed was 24 r/second. The dose rate was measured with a Victorean dosimeter.

Experimental procedure: Guinea pig, rat and mouse were exposed to different doses of 72 r, 120 r and 240 r local X-irradiation and compared with those of non irradiated control animals. The cytochemical localization and biochemical estimation of succinate dehydrogenase (SDH, E.C. 3.1.99.1) were carried out on control and X-irradiated animals. The irradiation effects were studied at 1 h, 24 h, 48 h and 72 hours after irradiation exposure. As the cytochemical localization of succinate dehydrogenase (SDH) activity is not clearly manifested in all the tissues, the SDH changes were investigated only in pectoralis major muscle of these animals. However, the biochemical estimations were carried out in liver, kidney, pectoralis major muscle, adrenal, spleen, brain (cerebellum),
Modified method of George and Talesara (1961) was used for cytochemical localization of SDH. The muscle was frozen immediately after sacrificing the animals and approximately 5 µ thick hand sections were cut into the phosphate buffer (pH 7.4 to 7.6). Sections were incubated in the medium at 37°C for a period of 25, 30 and 35 minutes for rat, mouse and guinea pig respectively.

The quantitative level of SDH was determined colorimetrically by the method of Kun and Abou (1949). The control and experimental animals were decapitated at the fixed time intervals and the tissues were taken out immediately, blotted free of blood, weighed and homogenized in chilled distilled water. The liver, kidney, muscle and adrenal were incubated for one hour; spleen, cerebellum, caput and cauda epididymides and testis for two hours and ovary and uterus for 24 hours at 37°C to obtain optimum reaction. Optical density was read at 420 µ in Spectronic 20 (Bausch & Lomb).

Optical density values obtained from triphenyl tetrazolium chloride (TTC, BDH) were utilized to get absolute values of formazan formed in the samples. The enzyme activity was expressed as µg formazan formed/100 mg wet weight/1 h or 2 h or 24 h respectively.

OBSERVATIONS

A. Cytochemical:

Controls:

Guinea pig: In control animals the formazan deposition in pectoral muscle in general was localized specifically as granules
demarcating the localization of SDH activity and in turn the size, number and situation of mitochondria. The SDH activity varied according to the fibre types. In normal animals, type I fibres of the pectoralis major were deeply stained showing high concentration of SDH, whereas type II fibres showed negligible deposition of formazan showing minimum amount of enzyme (Figs. 1 & 2). The intermediate fibres have medium level of SDH concentration and the enzyme was localized towards the periphery of the fibre, leaving the core of the fibre comparatively blank. The peripheral intensity of SDH localization was much more profound in the type I fibres, as compared with the intermediate fibres.

**Rat and Mouse:** Control muscle of rat and mouse show similar fibre types and are so described together. The type I fibres of the pectoral muscle of rat and mouse possess maximum concentration of SDH, whereas the intermediate fibres showed relatively less enzymic activity and type II fibres showed minimum activity (Figs. 15, 16, 23 & 24).

**Experimental:**

**Effect of 72 r, 120 r and 240 r X-irradiation on guinea pig muscle:**

X-irradiation with 72 r did not show significant alteration in the enzyme localization upto three days.

With 120 r X-irradiation, at 1 h, there was no noticeable effect. Twenty four hours after irradiation the SDH showed increased activity in all the fibres. Type I and intermediate fibres showed formazan deposition in the central region with little distortion in the peripheral integration of enzyme activity (Figs. 3 & 4), as compared with the control ones (Figs. 1 & 2). At 48 h (Figs. 5 & 6)
SDH activity was decreased in all the fibre types. The SDH activity was much altered and the enzyme localized even outside the fibres. At 72 h (Figs. 7 & 8), the normal distribution of the enzyme was not regained as the amount of the formazan deposited was less, as compared with normal muscle fibres. However, the intermediate fibres seemed to be more affected by 120 r X-irradiation than type I and type II fibres.

With 240 r X-irradiation, the formazan deposition at 1 h did not show noticeable alteration but at 24 h the formazan deposition increased in all the types of fibres (Figs. 9 & 10). At 48 h, the SDH activity increased in the intermediate and type I fibres (Figs. 11 & 12). The size of the formazan granules as well as their number were increased and demarkation was sparse in the intermediate fibres. The enzyme showed increased activity in the central region of the intermediate fibres, altering the normal pattern of distribution, with little distortion in the peripheral integration of enzyme activity. Type I and type II fibres showed more activity, as compared with the control fibres. At 72 h, type I fibres showed seemingly normal pattern of the enzyme distribution. However, in intermediate and type II fibres, normal enzyme distribution and integration was not regained (Figs. 13 & 14).

**Effect of 72 r, 120 r and 240 r X-irradiation on rat muscle:**

At 24 h and 72 h after 72 r X-irradiation, there was markedly weak staining of SDH in type I and intermediate fibres, as compared with control fibres. But type II fibres at 72 h showed increased activity, as compared with 24 h. The formazan deposition in type I at 24 h and 72 h were not clearly discernible due to high
amount of formazan deposition but there is a little indication of diffusion in the enzyme activity (Figs. 17 - 20).

With 120 r X-irradiation, at 72 h the normal distribution of the enzyme was not observed. Type I and intermediate fibres showed weak SDH staining, in comparison with control ones (Figs. 15 & 16). The mitochondrial situation of all the three fibres was not clearly discernible and the formazan deposition appeared more diffused signifying disruption of SDH localization (Figs. 21 & 22).

X-irradiation with 240 r did not show noticeable variation in SDH localization up to three days.

Effect of 72 r, 120 r and 240 r X-irradiation on mouse muscle:

X-irradiation with 72 r, 120 r and 240 r did not show significant variation in SDH, as compared with control ones, as they showed normal distribution of the formazan granules at all time intervals studied (Figs. 25 & 26).

B. Biochemical:

The results are presented in figures 27 to 30. A minimum of five sets were performed for each tissue and treatment. The data was statistically analyzed and the standard error of each mean value has been indicated by vertical bars.

Control group:

Amongst the three animals, guinea pig ovary; rat liver, kidney, muscle, uterus and testis and mouse adrenal, spleen, brain and caput and cauda epididymides possess higher concentration of SDH.
X-irradiated group:

Liver: Guinea pig liver did not show significant variation as compared with control values up to three days after 72 r X-irradiation. After 120 r X-irradiation, 66% increase in SDH was recorded at 1 h, 50% activation at 24 h and returns to normal levels at 48 h following irradiation, whereas the SDH concentration was significantly reduced at 72 h after 120 r irradiation. Administration of 240 r irradiation resulted in a little decrease at 1 h but the SDH values were activated up to 72 h, being maximum at 48 h (50%) after treatment. In rat, after 72 r irradiation an increase in SDH was observed from 1 h to 72 h. There was significant reduction in SDH values up to three days after 120 r X-irradiation. On the contrary, 240 r irradiation caused activation in SDH levels up to 24 h, while normal levels were obtained at 48 h and 72 h after irradiation. In mouse, 72 r and 240 r X-irradiation showed significant activation in SDH levels up to 72 h after irradiation, whereas following 120 r irradiation its concentration did not show noticeable variation up to 48 h but the enzymic values were found activated at 72 h after radiation exposure (Fig. 27).

Kidney: In guinea pig kidney, with 72 r irradiation the SDH concentration was activated up to 48 h and showed normal levels at 72 h after exposure. However, with 120 r irradiation its concentration increased at 24 h and returns to normal levels at 48 h after radiation. On the other hand, at 72 h the SDH values were significantly reduced after irradiation. An increase in the SDH was recorded at all post-irradiation intervals.
Fig. 27. The concentration of succinate dehydrogenase (μg formazan formed/100 mg wet weight/hour) in guinea pig, rat and mouse tissues of normal and at 1 h, 24 h, 48 h and 72 h following 72 r, 120 r and 240 r X-irradiation. The values are mean ± S.E.M.
FIG. 27
following 240 r irradiation. The concentration being maximum at 48 h (53%). The SDH levels in rat and mouse after 72 r irradiation increased up to 72 h after exposure. In rat, with 120 r irradiation the enzymic levels declined up to three days and did not show any recovery at this time interval, while its concentrations were increased up to 72 h after 240 r irradiation, being maximum at 24 h after irradiation. In mouse, 120 r X-irradiation caused decrease in SDH up to 48 h and returns to normal levels at 72 h after radiation. On the other hand, with 240 r X-irradiation, enzymic levels increased at 1 h and control levels were recorded at 72 h after radiation exposure (Fig. 27).

Muscle: SDH concentrations in guinea pig muscle were found activated up to 48 h and normal levels were recorded at 72 h after 72 r and 240 r irradiation. Similar activation in SDH was obtained after 120 r radiation but at 72 h the enzyme was seen significantly reduced. In rat, 72 r irradiation showed activation in the enzymic level up to 48 h, while normalcy was recorded at 72 h after exposure. However, with 120 r X-irradiation significant reduction in the SDH level was recorded up to three days, while only 45% enzyme was obtained at 72 h after 120 r irradiation. With 240 r irradiation the enzyme levels gradually decreased up to three days and showed only 56% activity at 72 h after irradiation. In mouse, with 72 r and 240 r irradiation increased SDH activity was recorded. The activation was 100% at 72 h after 72 r irradiation. Following 120 r irradiation the SDH levels were gradually reduced up to 24 h, while normalcy was recorded at 48 h and 72 h after irradiation (Fig. 27).
CONCENTRATION OF SOU (μg per mg WET TISSUE)

MOUSE SPLEEN
C 1 24 48 72
NAT ADRENAL
1 24 48 72
MOUSE ADRENAL

GUINEA PIG SPLEEN
1 24 48 72
GUINEA PIG ADRENAL
1 24 48 72

HOURS POST X-IRRADIATION

FIG. 28
Adrenal: The SDH activity of guinea pig adrenal was seen significantly activated by all the three doses of X-irradiation. The manifold increase was recorded at 72 h after 72 r irradiation. The SDH activity of rat adrenal was also activated by all the three doses of X-irradiation. Fivefold increase was obtained at 72 h after 120 r and 240 r irradiation in rat adrenal. In mouse, SDH levels increased after all the three doses of radiation. The fourfold activation was obtained at third day after 72 r irradiation (Fig. 28).

Spleen: In guinea pig and rat, the SDH activity was seen significantly activated by all the three doses of irradiation. On the contrary in mouse spleen 63%, 33% and 55% decrease was recorded at 72 h following 72 r, 120 r and 240 r irradiation respectively (Fig. 28).

Cerebellum: In guinea pig, cerebellar SDH concentrations were activated by 72 r, 120 r and 240 r irradiation. In rat brain, SDH levels did not show significant variation at all post-irradiation intervals after 72 r irradiation. Administration of 120 r irradiation caused 24% activation at 24 h and normalcy was recorded at 72 h post-irradiation. However, SDH activity was successively decreased from 1 h to 72 h after 240 r X-irradiation. In mouse brain, 23% and 30% reduction was noticed at 72 h after 72 r and 240 r irradiation respectively. A dose of 120 r resulted in increased SDH values at all post-irradiation periods, being maximum at 48 h (68%) after irradiation (Fig. 29).
CONCENTRATION OF 5 OH (M q FORMAT AN FO t/M W /m OKf WETTISSUEj

M O U S e  UTERUS

C 3

XS3i

P33

r/24 4H

R A T  UTERUS

GUINEA PIG UTERUS

MOUSE BRAIN

D a S T

X - / BPAOIATION

FI G .29

CONCENTRATION OF SUN (Mq FORMAZAN FORMATION/100 MG WET TISSUE)

GUINEA PIG OVARY

RAT OVARY

MOUSE OVARY

HOURS POST XIRRADIATION

FIG .29
Ovary: In guinea pig, X-irradiation with 72 r showed subsequent decrease up to 48 h and 34% decrease was obtained at 72 h post-irradiation, whereas no noticeable variation was observed up to 48 h after 120 r X-irradiation, but at 72 h 21% decrease was noticed. Irradiation with 240 r caused significant decline in SDH values from 1 h to 72 h. In rat, SDH activity increased by 72 r, normalcy was recorded by 240 r radiation and slight decrease occurred at 72 h after 120 r radiation. SDH concentrations in mouse ovary showed 20-25% activation by 72 r and 120 r irradiation, whereas manifold activation was obtained at 72 h after 240 r X-irradiation (Fig. 29).

Uterus: In guinea pig uterus, twofold increase was recorded after 72 r and 240 r X-irradiation at 72 h post-irradiation. On the other hand, 36% decrease was noticed at 72 h after 120 r irradiation. In rat ovary, 72 r radiation showed manifold activation up to three days. Decreased SDH levels were obtained by 120 r and normal levels were recorded after 240 r X-irradiation at third day after radiation exposure. However, in mouse, uterine SDH activity was noticeably activated by all the radiation experiments studied, being maximum by 240 r irradiation (Fig. 29).

Caput epididymis: Manifold activation in guinea pig caput epididymis was recorded at all post-irradiation periods following all the three doses of radiation. In rat, 72 r and 120 r X-irradiation caused significant activation in SDH activity. On the other hand, no significant changes were seen after 120 r irradiation. In mouse, 50% activation was observed by 72 r irradiation and normal levels were recorded after 120 r and
FIG. 30

Comparison of 5-OH (N-formazan) formation in various tissues at different times post X-irradiation.
Cauda epididymis: Guinea pig and rat cauda epididymides showed significant activation by all the three doses of irradiation. In mouse, 72 r irradiation caused 50% reduction in its activity, while remaining two doses showed seemingly normal concentration at 72 h after irradiation (Fig. 30).

Testis: In guinea pig, rat and mouse, 72 r and 240 r X-irradiation showed increased SDH activity, whereas normal levels were recorded at 72 h after 240 r irradiation in guinea pig and mouse testis. In rat, after 120 r irradiation decreased SDH activity was recorded at all post-irradiation phases (Fig. 30).

DISCUSSION

It is evident from the above studies that there is significant alteration in SDH in all the tissues of guinea pig, rat and mouse. However, the pattern of recovery is widely different in these animals. Also there is a variation in response by different tissues of different animals. In guinea pig and rat, liver and kidney showed significant decrease in SDH following 120 r irradiation. Previously Dovhy et al (1971) and Shah et al (1973a) have reported a significant reduction in liver SDH levels after low dose X-irradiation. This reduction in SDH might be due to structural and/or functional changes in mitochondria, resulting into an impairment of mitochondrial oxidative phosphorylation and respiratory changes. Using ultrastructural and biochemical methods,
Jordana et al. (1971) and Zyss and Michaelska-Kaszczynska (1972) observed swelling in mitochondrial matrix, vacuole formation and mitochondrial membranes were damaged with disappearance of cristae as well as vesiculation of smooth and rough endoplasmic reticulum following high doses of irradiations. These changes in mitochondrial structure due to irradiations, might be the cause of alterations in SDH activity, which can effect the oxidative metabolism of tissues. Similarly, Alexander et al. (1975) also reported impairment of oxidative phosphorylation after irradiation.

In contrast to liver and kidney of guinea pig and rat, mouse liver and kidney showed significant increased activity of SDH after all the three doses of X-irradiation. The enhanced SDH levels may be due to the increased oxidative metabolism, which can be explained as a protective reaction of the irradiated organism. These biochemical abnormalities in the irradiated animals are believed to be secondary to endocrine disturbances. In particular, it has been suggested that the radiation effect on oxidative phosphorylation in different tissues is indirect and mediated through the hormonal response involving the pituitary-thyroid and pituitary-adrenal axis (Alexander et al., 1975).

Radiation induced changes in striated muscle have been well documented (Warren, 1943; Lewis, 1954; Zeman and Solomon, 1971; Khan, 1974). Zeman and Solomon (1971) reviewed changes in skeletal muscle following relatively small doses of X-irradiation. In the present study also, we find significant alterations in SDH localization and quantitative levels in muscle upon low doses, eventhough muscle is reported to be relatively radioresistant.
tissue. The normal localization of SDH was altered in all the three fibres and even the activity was found outside the fibre of guinea pig muscle following irradiation (Figs. 5 & 6). Thus, radiation seems to have brought out some diffusion of this enzyme. Dowben and Zuckerman (1963) also demonstrated alteration in the membrane potential and enzyme leakage. The formazan granules in the intermediate fibres of guinea pig muscle showed increased size at 48 h after 240 r irradiation (Figs. 11 & 12). The increased size of formazan granules might be a compound result involving multiple factors like, swelling of mitochondria, vacuole formation or fusion of mitochondria. The enzyme showed increased activity in the central region of the intermediate fibres of guinea pig altering the normal pattern of enzyme distribution after low dose of X-irradiation. In rat also, formazan granules were not clearly discernible at 72 h after 72 r and 120 r irradiation (Figs. 19 to 22), showing significant damage to mitochondrial organization and distribution by irradiation. This may be due to structural and functional changes in mitochondria caused by radiation. These cytochemical and biochemical changes in SDH reflect on the disturbances in energy metabolism of mitochondria following irradiation. Similar alterations have also been reported by Nair and Bhakthan (1969) and Shah et al (1976a). These structural alterations are able to cause diminution in the activity of respiratory chain or alter its ionic equilibrium or structural changes of internal membranes of mitochondria. Radiation induced skeletal muscle changes result from a combination of interrelated primary and
secondary factors, such as direct myofibre injury, microcirculatory compromise and metabolic disturbances following irradiation. It is generally recognized that the adrenal gland constitutes a buffer system against a variety of traumatic conditions. It is reported by Patt and Brues (1954) that, although the apparent resistance of adrenals to structural changes, functional responses may be elicited with relatively low doses.

In the present study SDH levels were significantly activated after 72 r, 120 r and 240 r irradiation in all the three animals investigated. The observed increase in SDH might be due to the possible ACTH action after irradiation (Yago et al, 1972; Lin et al, 1974), as ACTH stimulates some Krebs cycle enzymes. It therefore, seems possible that the response was due to the indirect effect of irradiation. The increased SDH activity after irradiation may possibly be due to the stimulatory effect of pituitary (Hameed and Haley, 1964), which probably related to the increased hormonal levels upon irradiation.

From our biochemical studies on spleen, it is clear that guinea pig and rat spleen showed increased SDH activity with all the three doses, while the enzymic levels were reduced in mouse spleen by 72 r, 120 r and 240 r irradiation, showing alteration in mitochondrial function. Reduction in SDH levels is related with increased acid phosphatase activity following irradiation (Chapter II). Release of acid phosphatase from lysosomes is associated with breakdown of cell organelles (Roth et al, 1962), as SDH activity significantly reduced in mouse spleen after irradiation. Benjamin and Yost (1960) also reported
marked changes in the oxidative phosphorylation of splenic mitochondria following irradiation. The increased SDH activity in guinea pig and rat spleen may be similar to liver and kidney after irradiation.

In guinea pig cerebellum, the SDH levels showed increased activity by all the three doses of X-irradiation. Low dose radiations, thus, resulted in an activation of brain SDH which is correlated with increased oxidative metabolism of brain cells (Manocha and Olkowski, 1971; Bhatavdekar et al, 1974). The SDH activity showed normal levels in rat cerebellum after 72 r and 120 r irradiation. The return of normal levels of SDH from irradiated brains to normal function may be the result of mitochondrial repair after low dose irradiation, as slightly damaged mitochondria yield normal measurements of oxidative function (Cohan et al, 1973). The observed decrease in rat (by 240 r) and mouse (72 r and 240 r) cerebellum SDH may be due to swelling of mitochondria (Wills and Wilkinson, 1966) or aggregation of lipid droplets into mitochondria (De Estable-Puig and Estable-Puig, 1973). We believe that the impairment of fatty acid oxidation by mitochondrial damage may be responsible for the decreased SDH activity in brain cells following irradiation. The above authors also reported intimate relation between mitochondria and lipid droplets in the irradiated brains. There are several other cases where this association of mitochondria to lipid droplets seem to be more than incidental in neural tissue (Maunsbach and Wirsen, 1966). All these facts could well serve to correlate the damage of mitochondria to low
dose of X-irradiation.

Borakar and Denham (1952) and Borakar et al. (1953) found high SDH activity in the corpora lutea. They also reported fair SDH activity in granulosa and theca interna cells of developing follicles. Baker and Franchi (1972) observed numerous mitochondria in the normal follicles. They have reported that short, radially arranged cristae of mitochondrial profiles become bulbous and occasionally appear as clear vesicles in the denser matrix of mitochondria of monkey oocytes following X-irradiation. The reduction in SDH activity in guinea pig ovary (72 r, 120 r and 240 r) and rat ovary (120 r and 240 r) by irradiation, might be due to structural and functional changes in mitochondria resulting in altered oxidative phosphorylation or destruction of ovarian follicles after irradiation. On the other hand, SDH activity increased in rat (72 r) and mouse (72 r and 240 r) ovary after irradiation. SDH activity also showed significant alteration in uterus in all the three animals following irradiation. As the functions of the uterus are dependent on the ovarian hormones, the changes in SDH levels are directly related to the altered hormonal levels after irradiation (Shah et al., 1974). Shepherd and Snart (1972) also reported increased SDH activity in rat uterus by estradiol treatment. The altered SDH activity may result in irregularities in the estrous cycle or altered oxidative phosphorylation of ovarian and uterine cells, which probably related to altered hormonal levels upon irradiation.

The highest SDH activity occurred in the spermatozoa
and by virtue of the fact that the epididymal cauda possesses vigorously motile mature spermatozoa, thus, being richer in SDH activity than the epididymal caput and testis of all the animals investigated. SDH showed decreased levels in mouse cauda epididymis after 72 r and 120 r irradiation. Similar decrease in SDH in sperm suspension from testis and epididymis of rat was also reported by Chinoy and Bach (1975) after 120 r whole-body gamma irradiation. SDH, being a mitochondrial oxidative enzyme, it is obvious that changes in SDH concentration would be the outcome of mitochondrial damage following irradiation (Hugon and Borgers, 1966; Ito, 1966). Since sperm mitochondria are responsible for their oxidative metabolism, it is obvious that any structural or functional alterations in mitochondria would consequently affect the sperm motility and metabolism.

It is known that, functional integrity of epididymis is dependent on the presence of normal spermatozoa, testicular fluids and on the normal threshold levels of circulating androgens (Gustafsson, 1966; Rajalakshmi and Prasad, 1968, 1969). Thus, if it is assumed that sperm motility is curtailed due to mitochondrial damage by radiation as mentioned above, then it follows that, due to the presence of non-motile spermatozoa, the internal milieu and functional integrity of the cauda epididymis would be affected. This, in turn, would result in an altered secretory activity of the cauda epididymis in addition to decreased sperm motility (Chinoy et al. 1975). The caput epididymal SDH was either activated or restored to normal levels
following irradiation. This is probably due to the fact that the caput epididymis would be considered as a store house for spermatozoa which are not fully motile.

The decreased SDH activity in rat testis following 120 r irradiation might have resulted in decreased secretory activity of interstitial cells (Koch and Harrison, 1971a) and this would further contribute to the damage to the spermatogonia. As SDH is known to be androgen dependent enzyme (Chinoy and Buch, 1975), thus, changes in SDH activity may be related to altered androgen secretion in irradiated testis. Guchhait et al (1963) reported the terminal methyl group of cholesterol requires the functioning of the Krebs cycle. The observed decrease in the activity of SDH can be explained as accumulation of cholesterol and which probably resulted in decreased homogenogenesis after low dose X-irradiation. The reduced activity can also be viewed to the accumulation of pyrophosphate, which is an inhibitor of SDH (Gupta and Bawa, 1974). The activity of SDH was appreciably found reduced in rat testis, their depleted levels directly reflect the curtailment of spermatogenesis and spermatogenic cell population (Blackshaw, 1970). The decreased SDH levels might be due to degenerative processes or disintegration and shrinkage of mitochondria (Gupta and Bawa, 1974) following irradiation.

The mechanism of increased SDH activity in testis and epididymides of guinea pig, rat and mouse is not understood. The increase of enzyme level in short period after irradiation can be explained as a protective reaction of the irradiated animal.
obviously upsurge of activity of SDH during the post-irradiation period may be due to altered testis-pituitary axis (Ellis, 1970; Gupta and Bawa, 1974).

The results of the present study seem to point out that the low dose X-irradiation (72 r, 120 r and 240 r) resulted in significant alteration in SDH activity. However, a conclusion is difficult because of the different LD 50 values as well as variation in the radiosensitivity of the tissues of these animals. The increase in SDH was significant after 72 r, as compared with 120 r and 240 r X-irradiation. X-irradiation showed significant damage to SDH in guinea pig and rat muscle, as normal enzyme distribution and integration was not regained even after three days. Thus, it is obvious that mitochondrial structure and/or functions are altered following irradiation. The adrenal SDH levels were found significantly activated in all the three animals upon irradiation. This is in accord with the observations on the effects of other noxious stimuli, which like X-irradiations are presumed to reflect on an increased demand for the adrenal hormones. The increase of enzyme level in short period after irradiation may be a complex process of triggering of some interacting chain reactions or increased secretions of some hormones which bring about increased rate of oxidative metabolism, which in turn cause increased SDH activity of mitochondria. The decrease of SDH activity in the post-irradiation intervals may be due to structural or functional alterations in mitochondria following low dose local X-irradiation.
SUMMARY

The effect of X-irradiation on succinate dehydrogenase (SDH) was studied cytochemically and biochemically in various tissues of guinea pig, rat and mouse.

The cytochemical changes in SDH induced by X-irradiation were studied in pectoralis major muscle of guinea pig, rat and mouse after 72 r, 120 r and 240 r X-irradiation. This study showed significant alterations in size, number and distribution of formazan granules in the muscle following X-irradiation. The normal enzyme distribution pattern was not regained even after three days of irradiation.

Biochemical studies on SDH were carried out on various tissues of these animals, which showed changes in enzymic activity after X-irradiation. Moreover, the pattern of recovery was widely different in the tissues of these animals. Adrenal of all the three animals showed significant activation in SDH after all the three doses of irradiation.

SDH, being mitochondrial enzyme, reacts to X-irradiation as a sensitive parameter. Alterations in SDH localization pattern and its quantitative levels occur following low dose local X-irradiation in various tissues. The significance of these alterations in SDH levels with regard to oxidative metabolism of tissues and the motility and metabolism of spermatozoa in these animals has been discussed.

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Figs. 1 & 2. T.S. of normal pectoralis major muscle of guinea pig showing succinate dehydrogenase localization. Note higher concentration of SDH in type I and intermediate fibres, as compared to type II ones. In intermediate fibres, the enzyme was localized towards the periphery of the fibre, leaving the core of the fibre comparatively blank.

Fig. 1. x 400.

Fig. 2. x 800.

Figs. 3 & 4. Guinea pig muscle showing increased SDH activity in all the three fibres at 24 h after 120 r X-irradiation. The intermediate fibres showed formazan deposition in the central region with little distortion in the peripheral distribution of enzyme activity.

Fig. 3. x 400.

Fig. 4. x 800.

I - Type I fibre
II - Type II fibre and
In - Intermediate fibre.
Figs. 5 & 6. Guinea pig pectoralis major muscle at 48 h after 120 r X-irradiation. The SDH activity was found decreased in all the fibres. The enzyme was localized even outside the fibres.

Fig. 5. x 400.
Fig. 6. x 800.

Figs. 7 & 8. Guinea pig muscle at 72 h after 120 r irradiation. The amount of formazan deposited was less in all the three fibres, as compared with normal muscle fibres.

Fig. 7. x 400.
Fig. 8. x 800.

I - Type I fibre,
II - Type II fibre and
In - Intermediate fibre
Figs. 9 & 10. Guinea pig muscle at 24 h after 240 r irradiation. The formazan deposition increased in all fibres.
Fig. 9. x 400.
Fig. 10. x 800.

Figs. 11 & 12. Guinea pig muscle at 48 h after 240 r irradiation stained for SDH. The SDH activity increased in type I and intermediate fibres. The size of the formazan granules were increased in the intermediate fibres. The enzyme showed increased SDH activity in the central region of the intermediate fibres altering normal localization pattern.
Fig. 11. x 400.
Fig. 12. x 800.

I - Type I fibre,
II - Type II fibre and
In - Intermediate fibre
Figs. 13 & 14. SDH activity in guinea pig muscle at 72 h after 240 r irradiation. Type I, type II and intermediate fibres did not show normal distribution of SDH at this time interval.

Fig. 13. x 400.
Fig. 14. x 800.

I  - Type I fibre.
II  - Type II fibre and
In  - Intermediate fibre.
Figs. 19 & 20. Rat muscle at 72 h after 72 r irradiation. The SDH activity increased in type I, type II and intermediate fibres. The granular deposition was not discernible.
Fig. 19. x 400.
Fig. 20. x 800.

Figs. 21 & 22. SDH activity in rat pectoralis major muscle at 72 h after 120 r irradiation. All the three fibres showed weak SDH staining, as compared with control ones.
Fig. 21. x 400.
Fig. 22. x 800.

I - Type I fibre,
II - Type II fibre and
In - Intermediate fibre.
Figs. 23 & 24. Control mouse pectoralis major muscle, showing three types of muscle fibres as that of rat muscle.
Fig. 23. x 400.
Fig. 24. x 800.

Figs. 25 & 26. SDH in mouse muscle at 48 h after 120 r irradiation. The SDH localization did not show marked alterations upon irradiation.
Fig. 25. x 400.
Fig. 26. x 800.

I - Type I fibre,
II - Type II fibre and
In - Intermediate fibre.