CHAPTER VI

STUDIES ON PROTEIN LEVELS IN NORMAL AND X-IRRADIATED GUINEA PIG, RAT AND MOUSE TISSUES

INTRODUCTION

It has been generally known that the synthesis of RNA and protein, in contrast to DNA, is relatively radioresistant (Kelly, 1961). Most of the studies, however, have been made by determining changes in the total amount of RNA or protein synthesized following X-irradiation (Payne et al., 1952; Whitemore et al., 1961). Hevesy (1949a, 1949b) was the first to observe that after X-irradiation the incorporation of labelled acetate into the proteins of liver and the small intestine was slightly increased. Though several workers confirmed Hevesy's observation (Kay and Entenman, 1956; Petrovic, 1962), others claimed that protein synthesis was either insensitive (Abrams, 1951; Holmes and Mee, 1952) or even decreased after X-irradiation (Uchiyama et al., 1966). Recently, Hidvegi et al. (1968) found enhanced protein synthesis in vitro by isolated liver ribosomes of whole-body X-irradiated rats.

All the previous studies were made on isolated ribosomal or mitochondrial protein synthesis by whole-body irradiation. Moreover, in most studies the radiation doses used were high over the LD 50 or close to it. The present study deals with the effects of low doses of local body X-irradiation on the protein levels of guinea pig, rat and mouse tissues. The data showed that biochemical changes occurred even in the tissues which are not directly exposed to X-irradiation. These changes were found significant in all the animals studied.
MATERIALS AND METHODS

Animals and irradiation conditions:

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.

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 dosemeter.

Experimental procedure: The animals were exposed to single doses of 72 r, 120 r and 240 r X-irradiation and compared with those of control animals. They were sacrificed at 1 h, 24 h, 48 h and 72 hours after X-irradiation. Biochemical estimations on protein were carried out on normal and X-irradiated animals. The estimations were carried out in liver, kidney, pectoralis major, adrenal, spleen, brain (cerebellum), ovary, uterus, caput and cauda epididymides and testis of guinea pig, rat and mouse.
Protein was biochemically estimated by the method of Cornell et al. (1949) using biuret reagent. The nucleic acids were extracted and estimated according to Swift (1955). To the remaining residue 5 ml of 1 N NaOH was added and kept at room temperature for 12 to 14 hours. Four ml of biuret reagent was added to 1 ml of 1 N NaOH digest and the mixture was incubated at room temperature for 30 minutes. The optical density was read at 540 mp in Spectronic 20 (Bausch & Lomb) against the blank. One per cent egg albumin was used as standard. The concentration of protein was expressed as mg protein / 100 mg wet weight of the tissue.

OBSERVATIONS

The results are presented in figures 1 to 4. A minimum of five sets were carried out for each tissue and treatment. The standard error of each mean value has been indicated by vertical bars.

Control group: In the control group, cauda epididymis of guinea pig; muscle, adrenal, cerebellum, uterus, caput epididymis and testis of rat and mouse liver, kidney, spleen and ovary possess high concentration of protein.

X-irradiated group:

Liver: In guinea pig, protein content did not show significant variation up to 48 h but showed 43% increase at 72 h after 72 r X-irradiation. X-irradiation with 120 r resulted in 30% decrease at 1 h, 30% increase at 24 h and normal levels were obtained at 48 h
FIG. 1
and 72 h post-irradiation, protein content did not show marked increase up to 24 h but 62% and 43% increase was recorded at 48 h and 72 h respectively.

Irradiation with 72 r in rat liver showed 19% increase at 1 h, whereafter it decreased up to 72 h and 27% reduction was recorded at 72 h post-irradiation. With 120 r X-irradiation 26% increase was observed at 1 h but its concentration was subsequently reduced up to three days. The reduction at 72 h was similar to that of 120 r irradiation. After 240 r radiation protein content showed significant reduction from 1 h to 72 h post-irradiation.

In mouse liver, 72 r and 240 r irradiation showed marked decline from 1 h to 72 h. The reduction was 57% and 77% at 72 h after 72 r and 240 r irradiation respectively. On the other hand, 120 r radiation caused decreased protein content at 1 h, which was subsequently increased and showed 17% increase at 72 h post-irradiation (Fig. 1).

Kidney: The protein content did not show noticeable variation in guinea pig kidney up to 48 h but 21% decrease was obtained at 72 h after X-irradiation. After 120 r irradiation the protein content increased at 24 h and 48 h and showed 21% reduction at 72 h after exposure to radiation. After 240 r radiation the protein concentration subsequently decreased up to 48 h and showed control levels at 72 h post-irradiation.

In rat kidney, 20%, 17% and 50% reduction was recorded at 72 h after 72 r, 120 r and 240 r X-irradiation respectively.
The protein concentration of mouse kidney showed 18% reduction up to 48 h and normal levels were observed at 72 h after 72 r irradiation. With 120 r radiation significant increase was obtained at 72 h post-irradiation. On the contrary, protein content was slightly decreased at 1 h, showed normal levels at 24 h but again 50% decrease was recorded at 48 h and normal levels were obtained at 72 h after 240 r irradiation (Fig. 1).

Muscle: In guinea pig muscle, 72 r radiation showed gradual increase up to 24 h but normal levels were recorded at 48 h and 72 h post-irradiation. However, 120 r and 240 r irradiation showed 26% and 61% increase at 72 h post-irradiation respectively.

Protein contents in rat muscle did not show marked variation by 72 r and 120 r irradiation. On the other hand, 240 r also did not show significant variation up to 48 h but 23% reduction was noticed at 72 h post-irradiation.

In mouse muscle, 72 r irradiation showed little increase up to 24 h, was reduced at 48 h and restored to control levels at 72 h after exposure to radiation. However, protein content significantly increased up to 48 h and showed 239% increase at 48 h and 33% increase at 72 h post-irradiation. With 240 r X-irradiation the protein levels gradually increased up to 48 h and showed normal levels at 72 h after radiation (Fig. 1).

Adrenal: In guinea pig, 72 r irradiation showed gradual decline up to 48 h and the concentration of protein showed control levels at
CONCENTRATION OF PROTEIN (mg/100 mg WET TISSUE)

RAT ADRENAL

GUINEA PIG BRAIN

GUINEA PIG Spleen

MOUSE BRAIN

MOUSE SPLEEN

MOUSE ADRENAL

HOURS POST X-IRRADIATION

FIG. 2
72 h after irradiation, while the protein content was unaffected at 1 h after 120 r radiation but was significantly enhanced up to 48 h and showed normal levels at 72 h after radiation. On the other hand, 240 r irradiation caused slight decrease at 1 h, was normal at 24 h and 48 h but was slightly increased at 72 h after irradiation.

In rat adrenal, 72 r, 120 r and 240 r X-irradiation resulted in significant reduction up to 72 h. The decrease was maximum after 120 r irradiation.

The protein concentration in mouse adrenal was subsequently decreased by 72 r radiation up to 48 h (50%), which was slightly increased at 72 h but did not show normal levels at this time interval. On the other hand, 120 r radiation showed slight decrease at 1 h, significantly increased up to 72 h after irradiation. X-irradiation with 240 r resulted in gradual and significant decrease up to 72 h (80%) after radiation (Fig. 2).

Spleen: In guinea pig, 72 r irradiation did not show marked variation up to three days. On the other hand, the protein concentration significantly increased up to 48 h and showed 31% increase at 72 h after 120 r irradiation. However, 240 r radiation showed 62% increase at 1 h, was normal at 24 h and 48 h but which increased at 72 h post-irradiation.

In rat and mouse spleen protein contents showed significant decrease by all the three doses of X-irradiation. The reduction was maximum by 240 r at 72 h (Fig. 2).
Fig. 4. The concentration of protein (mg/100 mg wet tissue) in guinea pig, rat and mouse tissue of normal and at 1 h, 24 h, 48 h and 72 h following 72 r, 120 r and 240 r X-irradiation. Values are mean ± S.E.M.
FIG. 4
marked reduction up to 72 h (55%) post-irradiation.

Rat caput epididymal protein showed marked decrease up to 48 h (50%) but the contents were found slightly increased at 72 h, as compared with 48 h values after 72 r radiation. X-irradiation with 120 r caused 30% decrease at 1 h and did not show variation up to 72 h. On the other hand, 240 r irradiation showed slight decrease at 1 h, which was gradually reduced up to 72 h (45%) after irradiation.

In mouse, 72 r irradiation did not show significant variation up to 48 h, while 40% reduction was recorded at 72 h post-irradiation. With 120 r X-irradiation, the protein content showed normal levels at 48 h but was reduced at 72 h after irradiation. However, 240 r irradiation showed 50% decrease in protein levels at 1 h and did not show variation up to 72 h (Fig. 4).

Cauda epididymis: In guinea pig, 72 r irradiation caused 50% decrease in protein content up to 48 h but the values were further decreased at 72 h (80%) after irradiation. X-irradiation with 120 r showed 25% reduction at 1 h and did not show much variation up to 48 h but the protein content further decreased (50%) at 72 h after irradiation. X-irradiation with 240 r the protein values were reduced at 1 h (20%) and showed no variation up to 72 h after exposure to radiation.

In rat cauda epididymis, protein content was unaffected after 72 r radiation at 1 h, but showed 35% reduction at 72 h post-irradiation. With 120 r radiation 25% decrease was recorded at 72 h.
On the other hand, with 240 r radiation the protein concentration was unaffected at 1 h but subsequent inhibition was recorded up to 72 h after irradiation.

Protein content in mouse cauda epididymis showed reduction up to 24 h but was normal at 48 h and remarkably increased at 72 h (35%) after 72 r irradiation. With 120 r radiation the protein concentration increased at 1 h and 24 h, decreased at 48 h and was again found increased at 72 h (13%) after irradiation. X-irradiation with 240 r did not show much variation up to 24 h, gradually reduced up to 72 h (52%) after irradiation (Fig. 4).

Testis: In guinea pig, 72 r irradiation showed slight decrease at 1 h, caused 60% decrease at 24 h and 50% reduction was recorded at 48 h and 72 h after irradiation. Irradiation with 120 r resulted in slight decrease up to 48 h which was further decreased at 72 h (60%) after exposure to radiation. With 240 r irradiation protein concentration reduced up to 24 h (60%), while the concentration increased at 72 h but did not show normal levels at this time interval.

In rat testis, the protein content was found significantly reduced by all the three doses of radiation. The reduction at 72 h after 72 r, and 240 r was 80%, while 120 r radiation showed 75% reduction in the protein content.

Mouse testis showed significant decrease up to 48 h after 72 r radiation, as compared with other post-irradiation intervals. Irradiation with 120 r showed normal concentration at 1 h, but
significantly reduced from 24 h to 72 h (30%) post-irradiation. A dose of 240 r caused 60% reduction at 1 h and 24 h, 80% and 60% decrease at 48 h and 72 h post-irradiation respectively (Fig. 4).

**DISCUSSION**

The results presented here show that radiation inhibits protein contents in liver and kidney of all the three animals (Fig. 1). The decrease observed could be due to any of the following factors: (a) activation of RNase (b) depletion of mRNA (c) effect on the formation and/or maturation of mRNA. Involvement of the above factors in the inhibition of protein synthesis following radiations have been reported by Warner et al. (1966), Kim et al. (1970) and Szumiel et al. (1972). We have also observed even enhanced protein content in guinea pig liver (by 72 r and 240 r) and in mouse liver (with 120 r) at 72 h post-irradiation. These can be explained that some factors might show completely reverse action in different tissues. These contentions are supported by the evidences that RNA contents in these tissues also increased following similar dose of radiations. Hidvegi et al. (1968, 1970) reported increased mRNA content of the liver as an early radiation response. Mukerjee and Goldgeder (1972) reported the increased incorporation of $^{14}$C-leucine and $^{3}$H-UTP by irradiated mitochondria. They found that the increased levels of protein and RNA syntheses were due to the increased transport of RNA and protein precursors following change in mitochondrial membrane permeability.
induced by X-irradiation.

The microsomal fraction of skeletal muscles has relatively small amount of RNA and the fraction containing myofibrils has bulk of RNA (Narayanan and Eapen, 1974). The biochemical alterations in protein content might be similar as reported for liver and kidney.

The protein content in guinea pig adrenal showed elevated levels following 120 r and 240 r and in mouse after 120 r radiation. Considerable evidence has been accumulated since Bransome and Reddy (1963) demonstrated that ACTH stimulates the incorporation of labelled amino acids into rat adrenal protein, suggesting induction of protein synthesis during steroidogenic action of ACTH. Perese et al (1969) and Castells et al (1973) have shown that ACTH, via cyclic AMP, induces protein synthesis. It seems reasonable to assume that changes in RNA synthesis would precede protein synthesis. In the present study, RNA content were elevated in guinea pig and mouse adrenals (Chapter V), presumably as stimulated by ACTH, which subsequently showed enhanced production of protein. In rat adrenal (by 72 r, 120 r and 240 r) and in mouse adrenal (after 72 r and 240 r) protein content, were reduced which might be due to the result of reduced RNA content or decreased ACTH production or alteration in the cyclic AMP after irradiation.

Brain cells are recognized to be active sites of protein synthesis. In the present study, normal rat cerebellum showed significantly high protein content, as compared with guinea pig
and mouse. Protein concentrations showed marked reduction in guinea pig cerebellum (by 240 r) and in rat cerebellum after all the three doses of irradiation, while in mouse cerebellum after 72 r and 240 r radiations at 72 h post-irradiation. Biochemical studies on brain indicate that X-irradiation inhibits protein concentrations (De Vellis et al, 1971). Bamberger et al (1967) have shown inhibition of 3H-leucine into most cellular fractions of whole cortex and brain stem on the second day after single localized exposure of X-irradiation. The decrease of protein amounts may be due to lysis of the proteins present by X-irradiation or may be at the synthesis levels, also may be by the depression of enzyme systems involved in the activation of amino acids and transferring to tRNA (Wender and Zgorzalewicz, 1969) or by the inhibition of release of synthesized polypeptides from polysomes (Kim et al, 1970). The increase of protein in guinea pig cerebellum may be due to two reasons. (1) Irradiated cells contain more mRNA and ribonucleoprotein, as a result of increased protein concentrations. Such an increase in the mRNA and ribonucleoprotein content of these cells would account for their greater protein synthesizing capacity. (2) The other possibility that has to be taken into account is that, X-irradiation results in increased stability of the polysomes already present. This may be due to certain factors that maintain the polysome integrity or to the decreased activity of enzyme in the catabolism of polysomes (Hidvegi et al, 1968).
Ovarian protein content was seen increased in guinea pig (120 r and 240 r) and in mouse after 72 r, which might be due to decreased RNA concentration or atrophy of the ovary after irradiation. Similar increase was noticed in guinea pig and mouse uterus after 120 r radiation. This increased protein content may be related to the increased estrogen production by the ovary, which stimulates nucleic acid and protein metabolism. Treatment for 4 days with either 0.05 µg or 1.0 µg of estradiol resulted in increased synthesis of DNA, RNA and protein (Miller and Baggett, 1972b; Bronson and Hamilton, 1972). The decreased protein content may be due to inhibition of RNA following radiation or irradiation caused marked atrophy of the organ and may produce temporary or permanent sterility (Warren, 1942, 1943). If sterilization is not permanent, there may be a reawakening of metabolic processes after a passage of several days. Depending on the changes produced by X-irradiation there occurs atrophy of accessory organs.

The results of the present study show that in all but three species (dog, man and guinea pig) the caput epididymis contains more protein than the cauda epididymis. Evidence is now available to suggest that the caput epididymis absorbs much of the fluid that comes from the testis and high protein content in this region, in fact, be due to contribution of the testis fluid (Riar et al., 1973). It is pertinent that, in the bull, Crabo (1965) found a high protein content in the epididymal plasma collected from the
Caput epididymal spermatozoa were more active in synthesizing proteins than cauda epididymis. Bhargava and Abraham (1963) have demonstrated the existence of protein synthesis in ejaculated spermatozoa from buffalo, goat and bull. Premkumar and Bhargava (1972) and Engel et al. (1973) have reported that in mature freshly ejaculated bovine spermatozoa, the transcription and translation is mostly mitochondrial. They also reported that the strong inhibition produced by chloramphenicol supports the conclusion that protein synthesis in rat epididymal spermatozoa is mostly mitochondrial. Epididymides of guinea pig, rat and mouse showed an overall decrease in the protein content after all the three doses of irradiation. This might be due to loss of spermatozoa from the epididymides following low dose X-irradiation or increased proteolytic activity after radiation.

The seminiferous tubules secrete a fluid which carries the spermatozoa out of the testis and into the epididymis. The protein content of the rete testis fluid of various animals is very low (Setchell, 1970). Recently, Koskimies et al. (1973) have found evidence that the protein concentration of the primary secretion of the seminiferous tubules is even lower. In the present study, guinea pig, rat and mouse testis showed noticeable reduction in protein content after 72 r, 120 r and 240 r irradiation. This reduction may be due to (1) decreased weight of the testis, (2) depletion of the spermatozoa or (3) alteration in the transcription and translation by mitochondria by low dose X-irradiation. Bloom
and Bloom (195^) reported that a single total-body exposure of rabbit to 800 r of X-rays destroy the spermatogonia. This causes an interruption in the production of new spermatocytes and consequently of spermia several weeks later.

The results obtained here clearly suggest that the biochemical alterations in protein content show a high sensitivity to X-irradiation in guinea pig, rat and mouse tissues. The declined protein contents in ovary and testis might be due to the loss of germ cells after low dose X-irradiations. This may result in atrophy of the organ and thereby resulting in temporary or permanent sterility.

**SUMMARY**

The effect of low dose X-irradiation on protein concentration was studied biochemically in guinea pig, rat and mouse tissues.

The protein level was found to be high in rat tissues followed by guinea pig and mouse. In guinea pig, the protein content decreased in reproductive tissues after 72 r, while in other tissues it remained more or less same as those of controls. X-irradiation with 120 r brought about a decrease within 72 h in kidney and reproductive tissues but liver showed normal levels. In other tissues, however, the protein levels were elevated. A dose of 240 r resulted in decreased protein content in cerebellum, uterus and male reproductive tissues but increased in other tissues.
In rat, on the other hand, all doses of radiation studied, diminished the protein contents of the various tissues and did not normalize even after three days of irradiation. Likewise, a similar decrease was obtained in mouse by 72 r and 240 r radiation, but with 120 r, an increased protein concentration was recorded. The effects of low level X-irradiation on the altered protein levels in guinea pig, rat and mouse are discussed.

Thus, we have tried to study the general metabolic pattern following various low doses of X-irradiation on different tissues. In this study we report the effect of three different doses (72 r, 120 r and 240 r) on various tissues of three different mammals i.e., guinea pig, rat and mouse and have tried to correlate the variations of the effects obtained within three days following low dose X-irradiation.

REFERENCES


