CHAPTER III A

EFFECTS OF LOW LEVEL RADIATION ON THE PECTORAL MUSCLES OF RATS AND MICE

INTRODUCTION

The effects of radiation on some oxidative and hydrolytic enzymes and metabolites of mammalian tissues have been studied both histochemically as well as biochemically (Barron, 1946; Dubois et al., 1951; Shah et al., 1973; Chinoy et al., 1973). Electron microscopic studies have revealed that mitochondrial structure is altered with formation of vacuoles in them (Nair and Bhakthan, 1969). Similarly, changes in the concentration of a mitochondrial oxidative enzyme, viz., SDH has also been reported in pectoral muscles of rodents (Shah et al., 1973). Since skeletal muscles are known to utilize both fat and glycogen as fuel for contraction (George and Berger, 1966), it was thought worthwhile to investigate the effects of low level radiation on some metabolites and an enzyme of the glycolytic pathway in pectoral muscle of rodents.

MATERIALS AND METHODS

Adult, healthy rats (Rattus norvegicus) and mice (Mus musculus) were utilized for the present study. They were
maintained in the laboratory on standard diets (Hindustan Lever Ltd., Bombay) and water ad libitum. The animals were irradiated by an X-ray source (Philips model) at a distance of 20 cm from the point source in the thoracic region at a dosage of 120 r at a voltage of 40 KV and 20 mA.

The biochemical estimations of fructose, glycogen and phosphorylase in pectoralis major muscles were carried out on rats and mice which were sacrificed immediately after radiation (1 hr) as well as at recovery phases after intervals of 24, 48 and 72 hours upon treatment. Fructose were estimated by the method of Roe (1934) and the concentration was expressed as µg/100 mg fresh weight of tissue. Glycogen (Seifter et al., 1950), Phosphorylase (Fiske and Subbarow, 1925 and Cori et al., 1943). The details are given in Chapter I A.

A minimum of 6 replicates were done for each tissue and treatment and the results were statistically analyzed using the student's 't' test.

RESULTS

In controls: The concentration of fructose was higher in the pectoralis of rat than in mice, whereas, the levels of glycogen and phosphorylase were higher in the latter.

Effects of radiation:

1 hour after radiation:

A significant increase in fructose was observed in both animals as compared with the control values and its
concentration was higher in the rat than in mice. The level of glycogen was also higher than in control. Moreover, rat muscle possessed significantly ($P < 0.001$) greater glycogen than that of the mice. Although the concentration of phosphorylase increased in both the animals yet a significant increase ($P < 0.001$) was observed in case of mice alone. (Tables I & II).

24 hours after radiation:

The levels of fructose and phosphorylase were higher in mice as compared with the white rat and higher as compared with the 1 hr values. Moreover, the concentration of glycogen in both animals was also increased in comparison with 1 hr post irradiation (Tables I & II).

48 hours after X-irradiation:

Fructose and glycogen levels were increased in both animals as compared to 24 hours post irradiation. The concentration of phosphorylase was increased much beyond the control and 24 hr after irradiation (Tables I & II).

72 hours after X-irradiation:

The concentration of fructose was slightly increased in white rat but significantly increased ($P < 0.001$) in mice. The levels of glycogen and phosphorylase were decreased in both animals, when compared with 48 hours of recovery. In
rat, while glycogen and phosphorylase had almost recovered; the recovery was incomplete in mice as evidenced by the higher values compared to controls (Tables I & II).

**DISCUSSION**

Mammalian tissues are known to be radioresistant (Fly and Ross, 1947; Rhoades, 1948 a, b; Warren and Bowers, 1950). Thus Rhoades (1948a) had reported the radioresistance of liver in rodents. Similarly, Shah et al., (1973) and Chinoy Chinoy et al., (1972 a, b) had investigated the biochemical makeup of various tissues of rodents after low level X-irradiation and confirmed their radioresistant nature.

Minor changes in hepatic injury have been observed by Brues (1948) which might be the cause of alteration in glycogen metabolism. The oxidative enzymes were also disturbed by X-rays through permanent or temporary damage to the mitochondria in mammalian tissues (Barron, 1946; Patt and Brues, 1954; Moreno and Winzenz, 1969; Nair and Bhakthan, 1969; Dohvy et al., 1970; Ellis, 1970; Shah et al., 1973). Such disturbances in oxidative enzymes in irradiated animals would result in alteration of metabolite turnover and their pool. The increased activity of a particular enzyme after irradiation would lead to decreased levels of a metabolite on which it acts unless it is mobilized from elsewhere. Thus
in the present study, along with an increase in phosphorylase activity, there is a corresponding increase in the concentration of glycogen and fructose levels, thus paving the way for enhanced glycolysis by the muscle. It is a well known fact that an increase in phosphorylase activity is associated with increased glycogenolysis (Cahill *et al.*, 1957). Furthermore, the activity of this glycolytic enzymes reflects the ability of the muscle fiber to synthesize and utilize glycogen (Dubowitz and Pearse, 1960). Phosphorylase occupies a strategic location in the glycolytic sequence, since it is the initial catalytic force in the chain of chemical events that leads to the phosphorylative degradation and utilization of glycogen (Stetten and Stetten, 1960). Thus the phosphorylase activity and the intensity of glycogen metabolism of a tissue have been positively correlated (Shapiro and Wertheimer, 1943). A similar correlation in the different regions of the rat diaphragm has been shown by Susheila and George (1963, 1966) and Palasi and Larner (1960). Likewise results of this study also reflect to a correlation between glycogen and phosphorylase activity in muscle and indicate enhanced utilization of glycogen.

In the resting muscle, the activity of phosphorylase is reported to be low (Krebs and Fisher, 1955). Leonard *et al.*, (1959) showed that glycogen and phosphorylase levels increased in the muscle of rat after arousal from hibernation.
phosphorylase is maintained at a higher active level in an active muscle. Low dose radiation too, resulted in a marked activation of phosphorylase. An inhibition of glucose absorption and diminished phosphorylation of fructose have been observed 4 hr after X-irradiation (Barron et al., 1947). The inhibition of glycogen cleavage was greater with 1200 R than 600 R, and liver glycogen was increased during the first two days after total body irradiation. This corroborates with the present results since glycogen in the pectoralis muscle was increased two days after radiation, but thereafter it decreased. Bloom and Bloom (1954) have reported an increase in fat content and changes in liver glycogen after irradiation.

Diphospho-fructose-diphosphatase and phospho-fructokinase enzymes are present in skeletal muscle to provide cycling between fructose-6-phosphate and fructose-1, 6-diphosphate during glycolysis (Newsholme and Crabtree, 1970). Thus the recycling mechanism may greatly increase following a build up of fructose as obtained in these experiments. In the present study, fructose concentration was increased throughout the period of recovery.

**SUMMARY**

A biochemical study was carried out on some metabolites and one enzyme of the glycolytic pathway in pectoral muscle of normal, radiated and recovering rodents. Low dose-
radiation did not impair muscle metabolism. The results showed the existence of a correlation between phosphorylase activity and glycogen with its enhanced utilization by the muscle. Fructose concentrations increased during the recovery phase while recovery of glycogen and phosphorylase was almost complete in both the animals. The results are discussed in the light of physiological function of X-irradiated muscle.
CHAPTER III A

REFERENCES


6. Chapter I A. Studies on adaptive modifications in vertebrate skeletal muscle. 1. Evidences for the occurrence of structural and metabolic evolutionary trends in vertebrate skeletal muscles.


### Table I

<table>
<thead>
<tr>
<th>Enzymes/metabolites</th>
<th>Normal</th>
<th>1 hour</th>
<th>24 hours</th>
<th>48 hours</th>
<th>72 hours</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fructose</td>
<td>144.83 ± 6.70</td>
<td>585.90 ± 47.45</td>
<td>632.60 ± 74.50</td>
<td>846.0 ± 40.27</td>
<td>877.20 ± 42.93</td>
</tr>
<tr>
<td>Glycogen</td>
<td>54.79 ± 1.06</td>
<td>133.1 ± 5.54</td>
<td>105.20 ± 3.06</td>
<td>144.4 ± 6.90</td>
<td>66.81 ± 7.28</td>
</tr>
<tr>
<td>Phosphorylase</td>
<td>0.018± 0.001</td>
<td>0.019± 0.001</td>
<td>0.022± 0.001</td>
<td>0.16± 0.001</td>
<td>0.011± 0.001</td>
</tr>
</tbody>
</table>

The values are mean ± S.E.
Table II

Showing the levels of fructose, glycogen and phosphorylase in the pectoralis major muscle of mice (normal and at 1, 24, 48, 72 hours post irradiation)

<table>
<thead>
<tr>
<th>Enzymes/metabolites</th>
<th>Normal</th>
<th>1 hour</th>
<th>24 hours</th>
<th>48 hours</th>
<th>72 hours</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fructose</td>
<td>127.29 ± 18.70</td>
<td>473.00 ± 47.60</td>
<td>684.20 ± 46.20</td>
<td>885.10 ± 42.2</td>
<td>1132.30 ± 32.10</td>
</tr>
<tr>
<td>Glycogen</td>
<td>58.50 ± 2.80</td>
<td>71.65 ± 3.70</td>
<td>78.44 ± 4.84</td>
<td>92.89 ± 10.50</td>
<td>75.76 ± 3.20</td>
</tr>
<tr>
<td>Phosphorylase</td>
<td>0.026 ± 0.006</td>
<td>0.071 ± 0.007</td>
<td>0.073 ± 0.004</td>
<td>0.122 ± 0.002</td>
<td>0.75 ± 0.001</td>
</tr>
</tbody>
</table>

The values are mean ± S.E.
EFFECT OF LOW LEVEL RADIATION ON THE RED AND WHITE MUSCLE REGIONS OF GUINEA PIG PECTORAL MUSCLE

INTRODUCTION

It is well known that ionizing radiations induce changes in muscle and nerve-muscle preparations of invertebrates, amphibians and mammals (Hug and Schliep, 1960; Bergeder, 1961; Conard, 1956; Bacq et al., 1949; Burn et al., 1952; Crutschkowskii, 1967; Michaelov, 1970 a, b, c) and metabolic alterations occur which would eventually lead to pathological changes. Even low dose of X-irradiation altered the carbohydrate metabolism with enhanced utilization of glycogen in the muscle of rat and mice (Chapter III A), and a number of workers have shown that some enzymes and metabolites of mammalian tissues are also affected by radiation (Abrams, 1951; Ermoleava, 1967; Makarchenko, 1968; Kleinbergs and Bernstein, 1969; Koemierska, 1970; George and Eapen, 1972; Shah et al., 1972; Bhatavdekar et al., 1973; Chinoy et al., 1973 a, b; Chinoy and Buch, 1976).

The present study deals with the effects of low level, whole-body X-irradiation on red and white muscle regions of
the guinea pig pectoral muscle with special reference to carbohydrate metabolism and alterations of the ascorbic acid turnover.

**MATERIAL AND METHODS**

Adult, healthy guinea pigs (*Cavia procellus* L.) were utilized for the present study. They were maintained in an air-conditioned animal house at a temperature of 26° ± 2°C and 14 day light hours on standard diets (Hindustan Lever Ltd., Bombay) and water given *ad libitum*.

The animals were irradiated by an X-ray source (Philips model) at a distance of 20 cm from the point source in the thoracic region at a dosage of 120 r and a voltage of 40 KV and 20 mA. A total of 80 animals were distributed in five groups. They were sacrificed at intervals of 1 hr, 24, 48 and 72 hrs post irradiation.

Biochemical estimations on the following enzymes and metabolites in the pectoralis major muscle were carried out on treated animals, as per methods described in Chapters I A and II A.

1. Fructose (Foreman *et al.*, 1973)
2. Glycogen (Seifter *et al.*, 1950)
3. Phosphorylase (Fiske and Subbarow, 1925; Cori *et al.*, 1943).
4. Citric acid' (Ettinger et al., 1952)

5. Free ascorbic acid (AA), ascorbigen (ASG), the rates of ascorbic acid utilization (AAU) and ascorbic acid macromolecule complex (AA-MM complex) (Chinoy et al., 1974).

6. Ascorbic acid free radical forming special peroxidase (AA-FR special peroxidase), (Chinoy, 1973).

A minimum of six replicates were done for each treatment and parameter and the results were statistically analysed using the student's 't' test.

RESULTS

1 hour after X-irradiation: The ascorbic acid metabolism, fructose and citric acid were on the whole decreased by irradiation. The concentration of phosphorylase and glycogen were enhanced in both red and white regions of guinea pig muscle exposed to 120 r as compared to the control values (Tables I and II).

24 hours after X-irradiation: The ascorbic acid metabolism and fructose levels were reduced as at 1 hr, while the concentration of phosphorylase, glycogen and citric acid were increased in both red and white regions (Tables I and II).

48 hours after X-irradiation: An increase in the level of free AA and the rate of AAU was observed in red muscle region
whereas in the white region the values were almost same as in normal control muscle. The other forms of ascorbic acid and AA-FR special peroxidase were less than in the control muscle. The activity of phosphorylase increased but the concentration of glycogen was reduced. The recovery in fructose level was more marked in the red muscle region than in the white. Citric acid contents in both muscle regions were nearly similar to control (Tables I and II).

72 hours after X-irradiation: The different forms of ascorbic acid recovered or increased in red muscle region than the white. The AA-FR special peroxidase was on the other hand less than in control in both regions. However increase in phosphorylase and glycogen was higher in white than in the red region but citric acid levels were greater in the latter. A decrease in fructose was observed in white region of the muscle by 72 hrs as compared to control (Tables I and II).

DISCUSSION

The guinea pig pectoral muscle is known to possess two distinct regions, viz. the red mixed and the white mixed regions respectively (Chapter I B). Low level (120 r) whole body X-irradiation produced almost the same pattern of alterations in the activity of phosphorylase and the concentrations of fructose and glycogen as those obtained in rat
and mice under the same dosages (Chapter III A), except that the fructose levels did not recover in guinea pigs. Furthermore, the effects of irradiation on the above mentioned metabolites and enzymes of the red and white regions were also nearly similar but the recovery was greater in the red region. Since the levels of fructose were reduced by radiation in both the regions suggests that instead of an active glycolysis, aerobic utilization of glycogen by HMP shunt pathway is probably more pronounced.

The ascorbic acid turnover pattern in irradiated guinea pig muscle was affected more in the white muscle region than in the red. It must be noted here that guinea pigs cannot synthesize ascorbic acid in their tissues (Sebrell and Harris, 1967). The red region is known to have a higher percentage of type I (red) fibers containing more ascorbic acid than the white region which has a predominance of type II (white) fibers (Chinoy, 1969; Chapter I B). The decreased activity of AA-FR special peroxidase in guinea pig muscle throughout the treatment, indicates that during the third day of recovery, a mobilization of bound ascorbigen to the free form takes place which is utilized via a greater rate of formation of AA-Macromolecule charge transfer complex and its subsequent breakdown yielding the free radical of ascorbic acid (see Chapter I A, Fig. 12), monodehydroascorbic acid (MDHA). An increased turnover of ascorbic acid and formation of MDHA is
indicative of a radio-protective action of ascorbic acid (i) in preventing radiation-induced oxidations of -SH to -SS (Zirkle, 1954; Ellis, 1970; Ala-Ketola et al., 1975). (ii) Another beneficial effect of ascorbic acid is the biological inactivation of radiation (stress)-induced histamine (Nandi et al., 1973, 1974; Ellis, 1970; Subramanian, et al., 1973,1974) which being a vasoconstrictor for large blood vessels and vasodilator for small arteries and venules (Rochan E. Silva, 1955; Quastel and Hackett, 1973), might otherwise alter the blood flow to the muscle.

SUMMARY

The effects of low level whole body X-irradiation on the metabolism of the red and white muscle regions of guinea pigs were studied. The glycolytic pathway in the white region of guinea pig muscle was much more affected than in the red. The radiosensitivity of guinea pig white muscle region was apparently greater than that of the red region where the recovery was more pronounced. The ascorbic acid stores were depleted by radiation in guinea pig muscle due to utilization but absence of synthesis for overcoming the radiation induced alterations in muscle metabolism.
REFERENCES


7. Chapter I A. Studies on adaptive modifications in vertebrate skeletal muscle. 1. Evidences for the occurrence of structural and metabolic evolutionary trends in vertebrate skeletal muscles.
8. Chapter I B. Studies on adaptive modifications in vertebrate skeletal muscle. 2. Regional differences in guinea pig pectoral muscle.
9. Chapter II A. Studies on the effects of drugs on muscle.
1. Tranquilisers.


### Table I

Showing the levels of fructose, glycogen, phosphorylase and citric acid in the red and white muscle regions of guinea pig pectoral muscle (normal and at 1, 24, 48 and 72 hours post irradiation)

<table>
<thead>
<tr>
<th>Enzymes/metabolites</th>
<th>Fructose</th>
<th>Glycogen</th>
<th>Phosphorylase</th>
<th>Citric acid</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Normal</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Red region</td>
<td>624.58 ± 56.58</td>
<td>62.67 ± 4.87</td>
<td>0.014 ± 0.001</td>
<td>0.70 ± 0.06</td>
</tr>
<tr>
<td>White region</td>
<td>556.98 ± 21.93</td>
<td>64.23 ± 4.42</td>
<td>0.017 ± 0.001</td>
<td>0.70 ± 0.01</td>
</tr>
<tr>
<td><strong>1 hour</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Red region</td>
<td>287.38 ± 27.88</td>
<td>233.60 ± 9.35</td>
<td>1.13 ± 0.10</td>
<td>0.42 ± 0.02</td>
</tr>
<tr>
<td>White region</td>
<td>197.40 ± 8.41</td>
<td>209.70 ± 26.76</td>
<td>4.95 ± 0.85</td>
<td>0.43 ± 0.07</td>
</tr>
<tr>
<td><strong>24 hours</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Red region</td>
<td>460.70 ± 72.96</td>
<td>118.00 ± 8.26</td>
<td>0.36 ± 0.02</td>
<td>0.91 ± 0.15</td>
</tr>
<tr>
<td>White region</td>
<td>243.60 ± 11.53</td>
<td>180.60 ± 24.11</td>
<td>0.71 ± 0.19</td>
<td>0.65 ± 0.12</td>
</tr>
<tr>
<td><strong>48 hours</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Red region</td>
<td>517.90 ± 96.78</td>
<td>41.10 ± 8.90</td>
<td>0.31 ± 0.001</td>
<td>0.60 ± 0.14</td>
</tr>
<tr>
<td>White region</td>
<td>256.10 ± 13.69</td>
<td>43.20 ± 17.99</td>
<td>0.29 ± 0.001</td>
<td>0.64 ± 0.01</td>
</tr>
<tr>
<td><strong>72 hours</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Red region</td>
<td>422.00 ± 13.69</td>
<td>43.20 ± 8.56</td>
<td>0.21 ± 0.001</td>
<td>1.47 ± 0.21</td>
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<tr>
<td>White region</td>
<td>250.30 ± 14.02</td>
<td>86.70 ± 9.26</td>
<td>0.53 ± 0.11</td>
<td>1.16 ± 0.15</td>
</tr>
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</table>

The values are mean ± S.E.
Table II

Showing the levels of ascorbic acid (AA), ascorbigen (ASG), the rate of ascorbic acid utilization (AAU) ascorbic acid macromolecule (AA-MM) complex and AA-FR special peroxidase activity in red and white regions of the guinea pig pectoralis major muscle (normal, and at 1, 24, 48 and 72 hours of the X-irradiation)

<table>
<thead>
<tr>
<th></th>
<th>AA</th>
<th>ASC</th>
<th>AAU</th>
<th>AA-MM complex</th>
<th>AA-FR special peroxidase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Red region</td>
<td>2.52 ± 0.18</td>
<td>0.60 ± 0.08</td>
<td>15.70 ± 0.90</td>
<td>0.71 ± 0.08</td>
<td>8.04 ± 0.85</td>
</tr>
<tr>
<td>White region</td>
<td>3.08 ± 0.18</td>
<td>1.40 ± 0.25</td>
<td>18.82 ± 1.93</td>
<td>1.01 ± 0.28</td>
<td>8.57 ± 0.70</td>
</tr>
<tr>
<td>1 hour</td>
<td>Red region</td>
<td>1.11 ± 0.19</td>
<td>0.11 ± 0.09</td>
<td>11.03 ± 1.10</td>
<td>0.23 ± 0.03</td>
</tr>
<tr>
<td></td>
<td>White region</td>
<td>1.53 ± 0.003</td>
<td>0.13 ± 0.02</td>
<td>12.14 ± 1.88</td>
<td>0.38 ± 0.013</td>
</tr>
<tr>
<td>24 hours</td>
<td>Red region</td>
<td>0.77 ± 0.11</td>
<td>0.67 ± 0.09</td>
<td>7.12 ± 0.85</td>
<td>0.70 ± 0.17</td>
</tr>
<tr>
<td></td>
<td>White region</td>
<td>0.90 ± 0.13</td>
<td>0.61 ± 0.18</td>
<td>8.94 ± 1.49</td>
<td>0.12 ± 0.002</td>
</tr>
<tr>
<td>48 hours</td>
<td>Red region</td>
<td>2.99 ± 0.49</td>
<td>0.31 ± 0.11</td>
<td>20.37 ± 3.04</td>
<td>0.40 ± 0.94</td>
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<tr>
<td></td>
<td>White region</td>
<td>2.94 ± 0.40</td>
<td>0.46 ± 0.008</td>
<td>18.77 ± 0.57</td>
<td>0.44 ± 0.16</td>
</tr>
<tr>
<td>72 hours</td>
<td>Red region</td>
<td>3.36 ± 1.01</td>
<td>0.67 ± 0.16</td>
<td>24.19 ± 4.20</td>
<td>1.46 ± 0.20</td>
</tr>
<tr>
<td></td>
<td>White region</td>
<td>2.32 ± 0.20</td>
<td>0.24 ± 0.009</td>
<td>16.39 ± 1.54</td>
<td>0.41 ± 0.07</td>
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</table>

The values are mean ± S.E.