CHAPTER 3

THE GLYCOGEN AND PHOSPHORYLASE LEVELS IN THE PIGEON BREAST MUSCLE UNDER DISUSE ATROPHY

When the pigeon breast muscle is subjected to disuse atrophy several structural and physiological changes take place. Recent studies conducted on the fat, lipase and water content of the muscle have shown that there is a severe disturbance in the lipid metabolism of the muscle, particularly in the initial period of atrophy (Chapter 1). A striking increase in the lipid content 24 hours after subjecting the muscle to disuse, has prompted us to suggest that fat is being synthesized in the muscle from other metabolites. It is known that there is a reduction in the muscle protein during muscular atrophy (Fischer and Ramsey, 1946). Hines and Knowlton (1935) and Lazere et al, (1943) have shown that there is a decrease in glycogen concentration following denervation of the muscle. However, the changes in the muscle phosphorylase activity are not in agreement with the changes in glycogen concentration. Aronson and Volk (1957) have reported that there is only a slight decrease in the phosphorylase activity during the first few weeks but later on it decreases rapidly. Humoller et al, (1951b) have shown a steady decrease in phosphorylase activity immediately after denervation of the muscle. Recently Hals and Leonard (1961) have shown that there is slight increase in phosphorylase activity five days after denervation and then a decrease reaching the lowest level by the 20th day. In contrast to these findings
Mitsui and Kuriaki (1959) observed an increase in phosphorylase activity up to 20 days after denervation of the muscle. Since the available data on the glycogen and phosphorylase levels in the muscle under disuse are confusing it was thought worthwhile to investigate these aspects in the pigeon breast muscle which is metabolically a highly active muscle.

Material and Methods

Fully grown, well fed, adult healthy pigeons of either sex, weighing between 290 to 320 gm. were used for experiments as well as for controls. A plaster cast was applied in the middle of the wings after keeping them in a back-to-back position as described in Chapter 1. They were sacrificed after varying periods of one day to sixty days. After decapitation a piece of the breast muscle was immediately excised, weighed and digested in 2 ml. of hot 30% KOH. Glycogen in this digested material was estimated according to the method of Seifter et al. (1950) using the anthrone reagent for the colour development. The digested material was cooled and 2.5 ml. of 95% ethanol was added and brought to boiling in order to precipitate the glycogen. It was then stored in a refrigerator for 30 minutes for the complete precipitation of glycogen and centrifuged for 15 minutes at 3000 r.p.m. After decanting off the alkali, the glycogen was twice reprecipitated with ethanol. The finally precipitated glycogen was dissolved in water and proper dilutions were made so as obtain 30 to 40 gammas per ml. From this diluted sample 1 ml. duplicate samples were transferred to test tubes.
and 4 ml. of the anthrone reagent (0.2%) prepared in 95% sulphuric acid were added to these tubes and the contents mixed well. A standard glucose solution which contained 30 gamma per ml. was always run with the samples. All the tubes were heated in a boiling water bath for 5 minutes for colour development. The tubes were then cooled and the optical density measured at 620 mp using a Bausch and Lomb "Spectronic 20" colorimeter. Glycogen in the muscle is expressed as per centage of glycogen on wet muscle.

Phosphorylase activity in the muscle was assayed by a modification of the method of Cori, Cori and Green (1943) as adapted by Cahill et al., (1957). A 2% homogenate of the muscle was always prepared in cold distilled water and was used as the enzyme material in all the cases. Samples of 0.1 ml. of this fresh homogenate were added to a cold mixture in a test tube containing 0.2 ml. of sodium citrate buffer (0.1 M) of pH 5.9 and 0.3 ml. of potassium fluoride (0.154 M) and 0.05 ml. of glucose-1-phosphate (0.2 M) (L. Light and Co., England) as the substrate. The samples were incubated for 15 minutes at 30°C and the reaction was terminated by the addition of 1 ml. of 10% trichloroacetic acid (TCA).

Controls were run with each experiment by adding 1 ml. of 10% TCA in the incubation mixture before the addition of the homogenate and incubated along with the samples. Blanks were prepared by the addition of 1 ml. of 10% TCA in the incubation medium. After the incubation period of 15 minutes the
samples and the controls were filtered into 10 ml. graduated test tubes by several washings of the filter paper to ensure complete recovery of the phosphate. The filtration procedure was carried out in a cold water bath at 10 to 15°C due to the high room temperature which was found to be interfering with the hydrolysis of glucose-1-phosphate. The inorganic phosphate liberated from the glucose-1-phosphate due to the enzymic action was estimated by the method of Fiske and Subbarow (1925) and the optical density was read on a Klett-Summerson photoelectric colorimeter using 660 mμ filter. The readings for the controls were subtracted from those of the samples to determine the phosphate moiety released by the enzymic action. The enzyme activity was calculated as μg phosphorus liberated per 10 mg. dry weight of the muscle at 30°C for 15 minutes.

Results

Table 1 shows the glycogen levels and phosphorylase activity in the pigeon breast muscle during different periods of atrophy. The results clearly indicate that there was a drastic fall in the glycogen level immediately after the first day of immobilization of the muscle. But the corresponding change in the phosphorylase activity was very negligible. However, a sharp fall was observed in the glycogen as well as phosphorylase levels after 7 days of disuse. After this period a gradual rise in the enzyme level was recorded and though it rose considerably high in 60 days it was still less than the enzyme level in controls. There was however, no striking changes
in the glycogen levels other than slight irregular fluctuations.

Figures 1 and 2 are a graphic representation of glycogen and phosphorylase levels in the pigeon breast muscle during muscular atrophy indicated for the number of days.

Figure 1. Changes in the glycogen levels of the pigeon breast muscle during induced muscular atrophy. Dots indicate the individual readings and the curve the average of the readings.
Table 1
Changes in the glycogen content and phosphorylase activity in the pigeon breast muscle during different periods of disuse muscular atrophy.

<table>
<thead>
<tr>
<th>Atrophy in days</th>
<th>Glycogen % in gm. on wet muscle</th>
<th>Phosphorylase activity µg phosphorus/ 10 mg. dry muscle</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± S. D.</td>
<td>Mean ± S. D.</td>
</tr>
<tr>
<td>1 Day</td>
<td>0.5238 ± 0.1764</td>
<td>258.60 ± 36.40 (5)</td>
</tr>
<tr>
<td>8 Days</td>
<td>0.5629 ± 0.1982</td>
<td>227.80 ± 52.85 (5)</td>
</tr>
<tr>
<td>7 Days</td>
<td>0.2466 ± 0.1670</td>
<td>87.87 ± 34.37 (8)</td>
</tr>
<tr>
<td>14 Days</td>
<td>0.5721 ± 0.1580</td>
<td>168.10 ± 28.74 (8)</td>
</tr>
<tr>
<td>21 Days</td>
<td>0.6159 ± 0.1260</td>
<td>154.30 ± 49.58 (6)</td>
</tr>
<tr>
<td>30 Days</td>
<td>0.3991 ± 0.0488</td>
<td>191.30 ± 32.49 (8)</td>
</tr>
<tr>
<td>60 Days</td>
<td>0.4262 ± 0.0730</td>
<td>249.10 ± 39.80 (5)</td>
</tr>
<tr>
<td>Normal (Controls)</td>
<td>1.4050 ± 0.2550</td>
<td>335.90 ± 54.51 (8)</td>
</tr>
</tbody>
</table>

Figures in the parenthesis indicate the number of experiments in each set.
Figure 2. Changes in the phosphorylase activity of the disused pigeon breast muscle. Dots indicate the individual readings and the curve the average.

Discussion

The importance of glycogen as a major energy source and the role of the enzyme phosphorylase in the initial reversible catalytic reaction which effects the synthesis and breakdown of the α, 1, 4 glucosidic linkages is well recognized (Stetten and Stetten, 1960). Hence, changes in phosphorylase activity could be considered as also changes associated with alterations in glycogen metabolism.
Leonard (1957), Huls and Leonard (1961) have shown that muscle phosphorylase activity is reduced in dystrophic and denervated skeletal muscle. Huls and Leonard (1961) have also reported a slight increase in the enzyme activity after 5 days of denervation and the maximum decrease in the enzyme activity has been observed after 20 days of denervation. However, in the present observation on the pigeon breast muscle the enzyme activity was found to be decreasing gradually and by the 7th day it was found to be the least. Thereafter it rose up to almost the same level as in the control birds after a period of 60 days. These differences in the enzyme activity in the different animals may be attributed to the species differences and perhaps to the methods employed in the enzyme assay.

The glycogen levels in the atrophied muscle, however, do not seem to show a proportional relationship with phosphorylase activity. The glycogen level decreases initially with the decreasing enzyme level and reaches its lowest by the 7th day when the activity of phosphorylase is also the least. The initial drastic decrease in glycogen level observed on the very first day, however, has not showed a corresponding decrease in phosphorylase activity. When the enzyme activity increased gradually after the fall in the first week reaching the peak of its activity after 60 days of atrophy, the glycogen levels in the muscle during this period still remained low and did not tend to increase. The high phosphorylase activity is indicative of high glycogenolysis and low glycogen synthesis (Cahill et al, 1957). The
decreasing level of glycogen suggests that glycogen is being utilized and the rate of glycogen synthesis is as efficient. Following denervation, muscle glycogen has been shown to undergo rapid decrease in concentration at a velocity which markedly exceeds the rate of concomitant phase changes in the tissue mass (Lazere et al., 1943). Moreover, the glycogen of atrophic muscle is not increased by glucose ingestion and is more susceptible, than that of normal muscle, to the glycogenolytic effects of exogenous adrenaline, insulin and thyroxin (Lazere et al., 1941). It could be inferred from the present studies that there is a low glycogenolytic potential in the atrophied muscle from an altered glycogen metabolism.

From the studies reported in Chapter 1 it was suggested that there is a disturbance in the lipid metabolism of the muscle during the early days of atrophy, and that the increase in muscle fat at the onset of atrophy may be possibly due to de novo synthesis of fat from other metabolites which are being broken down at a rapid rate. For the substantial increase in lipid, it is unlikely that the source is glycogen but what is most likely is that the source is protein which is being reduced. The increase in lipid may also be due to a shift in the metabolism of the muscle from fat to carbohydrate resulting in rapid utilization of glycogen and accumulation of lipid.