CHAPTER 4

ELECTROLYTE CHANGES IN BREAST MUSCLE AND SERUM OF PIGEON UNDER DISUSE ATROPHY OF THE MUSCLE

The physiological significance of electrolytes such as calcium, sodium and potassium in muscle contraction is well recognized. The contraction of a muscle fibre is believed to be initiated by the release of calcium from the sarcoplasmic reticulum which brings about a change in the action potential and an influx of sodium and an efflux of potassium ions. The excitability of a muscle cell is dependent partly on its ability to extrude sodium ions and take in potassium ions during rest. Generally the level of potassium in most excitable cells is 20-50 times more inside than in the external medium while that of sodium 3-5 times more outside than in the cell. But this also appears to be dependent upon the functional status of the muscle. In rat soleus, which is purely a red muscle, a low content of potassium was observed (Drahota, 1962). In a similar study, Pishawikar (1962) reported a high content of potassium in the tetanic fast contracting white fibres of the pigeon pectoralis. During muscular activity the loss of potassium from the cell is always balanced by the gain of sodium from the extracellular space.

It has been reported that the denervation of a muscle causes a decrease in the potassium content and a
simultaneous increase in sodium content (Drahota, 1958, 1960). In muscular dystrophy too a significant reduction in the intra-cellular potassium with a corresponding rise in sodium was reported (Howarth et al., 1955; Shy et al., 1955; Williams et al., 1957). However, it is not clear from these reports how the electrolytes are maintained at an equilibrium when there is a disturbance in the functional state of the muscle. For a better understanding of this aspect it was thought necessary to study the changes in electrolytes in the muscle and blood serum simultaneously after inducing atrophy in the muscle.

Material and Methods

The pigeon pectoralis muscle was used as the experimental material since it was found to be an ideal material for studies in disuse atrophy and also because these findings could be compared with the metabolic disturbances noted in the other studies.

Fully grown pigeons of 300-320 g. fed on a normal diet of mixed grains were selected for the study. A plaster cast was applied on the wings after keeping them in the back-to-back position as reported in chapter I. They were then left with other normal pigeons in a cage and fed on a normal diet of grains. At the end of the scheduled experimental period (1-60 days) the birds were decapitated after collecting blood from the heart with a syringe,
and a piece of the pectoralis muscle was removed. The muscle piece was then blotted free of blood and dried in an air oven at 100°C till constant weight was obtained. Blood was allowed to clot at room temperature for 10 minutes and was then centrifuged at 3000 r.p.m. for 10 minutes. The serum was collected and stored in a refrigerator.

The estimations of sodium, potassium and calcium were carried out using an EEL flame photometer. The dried muscle samples of about 100 mg. were transferred to clean crucibles and ashed in a muffle furnace at a temperature of 350-400°C till all the organic matter was oxidized and a constant weight was obtained. The ashed samples were then dissolved in 10 ml of 1N HCl and brought to a final volume of 25 ml. with glass distilled water.

For calcium, larger quantities of the samples were ashed and dissolved in 2 ml. of 1N HCl and diluted to 5 ml. or 10 ml. according to the amount of the material taken.

For sodium estimation, 0.2 ml. of serum was diluted to 50 ml. with glass distilled water and for potassium 0.2 ml. of serum was diluted to 10 ml. with water.

Calcium was estimated after precipitating the calcium as calcium oxalate by adding 3 ml. of oxalic acid-oxalate solution into 1 ml. serum. After 30 minutes the precipitate was collected by centrifugation and dissolved in 5 ml. of 0.05N perchloric acid.

Standard curves for potassium, sodium and calcium
using different concentrations of the elements were prepared. In all the experiments the reading for the maximum concentration used in the standard curve was adjusted to the 100th division of the scale on the instrument and the zero was set with glass distilled water. The reading for the experimental materials were recorded using different filters at an air pressure of 12 cubic lbs. Sodium, potassium and calcium in muscle were calculated as mg. in 100 mg. dry muscle and blood serum as mg. in 100 ml. serum.

Results

Results obtained in the present investigation are presented in table 1. It is clear from the results that there is an increase in the calcium content of the muscle. The maximum levels are recorded for the 7th and 30th days under disuse. By the 60th day the calcium level in the muscle decreased but remained slightly higher than that in the normal animals. A parallel increase of the calcium content of the serum is also noticed for the 7th day. However, this increase which was very significant on the first day itself decreased after 14 days of atrophy and by the end of 60 days it was lower than that in the normal pigeon. The sodium content of the muscle decreased and the potassium showed a steep increase by the 7th day. After the 30th day the sodium content was very low and the potassium content was considerably
TABLE 1
Electrolytes in the pectoralis muscle and serum of the pigeon during induced muscular atrophy

<table>
<thead>
<tr>
<th></th>
<th>Sodium</th>
<th>Potassium</th>
<th>Calcium</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Muscle</td>
<td>Serum</td>
<td>Muscle</td>
</tr>
<tr>
<td>Normal</td>
<td>724.44±85.65</td>
<td>384.14±16.88</td>
<td>570.00±72.03</td>
</tr>
<tr>
<td>1</td>
<td>727.5±25.86</td>
<td>421.66±30.91</td>
<td>573.33±68.00</td>
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<tr>
<td>7</td>
<td>672.00±61.45</td>
<td>380.00±159.00</td>
<td>820.00±74.50</td>
</tr>
<tr>
<td>14</td>
<td>630.00±85.29</td>
<td>380.00±9.35</td>
<td>440.00±58.95</td>
</tr>
<tr>
<td>21</td>
<td>729.00±161.70</td>
<td>359.00±13.93</td>
<td>502.00±63.52</td>
</tr>
<tr>
<td>30</td>
<td>662.50±50.18</td>
<td>386.66±36.59</td>
<td>742.50±34.91</td>
</tr>
<tr>
<td>60</td>
<td>567.50±43.23</td>
<td>349.00±8.80</td>
<td>778.00±118.00</td>
</tr>
</tbody>
</table>

The values are expressed in mg./100 g. dried muscle or mg./100 ml serum. 'f' Standard deviation.
higher than the normal. Serum sodium and potassium did not show the parallel drastic changes except for the 1st and 30th days of atrophy. Both the sodium and potassium levels on the 30th day were the same as that of the normal birds.

Discussion

The high concentrations of the electrolytes observed in the present study in the normal pigeon breast muscle is indicative of a major role of these ions in the physiology of the muscle. The higher concentration of sodium and low concentration of potassium observed may be due to the morphological differentiation of the cellular components of the muscle, in having a larger number of red fibres than the white ones. Among the two types of fibres, the white fibres possess a large concentration of potassium and very low amount of sodium (806.13 mg. K and 181.68 mg. Na/100g. wet muscle). Whereas the red fibres have lower concentration of both (103.1 mg. K and 33.53 mg. Na/100 g. wet muscle) (Pishawikar, 1961). Drahota (1960) had also reported a similar observation in the rat soleus muscle which predominantly consists of red fibres. This higher concentration of potassium in the white fibres is suggestive of being a characteristic feature of the fast contracting white fibres which are adapted for anaerobic metabolism. The relation between the potassium and the sequence of events in glycolytic
metabolism has been demonstrated by the fact that K⁺ is not only stimulatory but essential for the transfer of the phosphate from phosphopyruvate to ADP in order to form pyruvate and ATP (Kachmar and Boyer, 1953; Lardy, 1951). The increased levels of K⁺ reported in the present study on the atrophied muscle may be associated with the increased carbohydrate metabolism occurring in the muscle during atrophy. It has already been reported in the chapters 1 and 2 that during disuse atrophy of the muscle, the fat-loaded narrow red fibres tend to shift to glycolytic metabolism. This is evident from the increased concentrations of glycogen and phosphorylase in the red fibres (Chapter 1). The increase in the potassium content during atrophy is also in close agreement with that of Fenn (1938) who has reported an increase in K⁺ content of the liver parallel with the increase in glycogen when the rats were fed on a carbohydrate diet. It has also been reported that the increased K⁺ levels activate glycolysis in the resting muscle in the presence of calcium (Leon Kaye and Mommaerts, 1960). It is also known that there is a parallel relationship between the contents of calcium and the connective tissue in muscle (Hines and Knowlton, 1937). The increased calcium levels observed in the present study may thus be associated with increased glycolytic metabolism and the increase in the connective tissue as a result of atrophy. Although the activation of glycolysis in the resting muscle requires
extracellular $K^+$ and $Ca^+$, the presence of $Na^+$ not found to be that much essential (Leon and Mommaerts, 1960). This may be the reason for the low sodium content observed during atrophy.

The significant relationship between muscle and serum electrolytes as revealed from the present investigation is suggestive of the high permeability of the muscle cells established during atrophy.