CHAPTER 5

EFFECT OF DISUSE ATROPHY ON FAT METABOLISM IN THE PIGEON BREAST MUSCLE

It is well known that fat accumulates in the muscle when the muscle is put into disuse by any of the various methods of immobilization. Recently it has been demonstrated by George and Vallyathan (1962) that when the pigeon breast muscle is put into disuse by immobilization of the wings, there is marked increase in the fat content of the muscle during the early days. It is not known how this fat accumulates in the muscle. They (1962) suggested that fat is built up in the muscle from other metabolites which are broken down at a rapid rate during disuse atrophy. This suggestion was based on a quantitative assessment of fat, glycogen and protein contents of the muscle under disuse. They obtained an increase in the fat content and lipolytic activity with a simultaneous decrease in glycogen and protein contents of the muscle. Further studies (Chapter 1 and 2) on the histochemical localization of glycogen and phosphorylase and succinic dehydrogenase (SDH) in the red (fat metabolizing) and the white (glycogen metabolizing) fibres during disuse have provided evidence to indicate that during atrophy the metabolism of the muscle shifts from oxidative to glycolytic. In the normal pigeon breast muscle the red fat metabolizing fibres which predominate in number over the white glycogen utilizing ones, are well equipped for oxidative metabolism utilizing fat

(George and Talesara, 1961,1962) but during atrophy they have been shown to become adapted for glycolytic metabolism by increasing their concentrations of glycogen and phosphorylase(Chapter 1). This shift in the metabolism of the muscle might well be the primary reason for increase of lipid in the muscle. So it was thought necessary to investigate whether the increase in fat is due to lower fat utilization or to de novo fat synthesis in the muscle or both. The present study of the capacity for fatty acid (sodium butyrate) oxidation and the respiratory quotient of the muscle under varying periods of disuse atrophy was therefore undertaken.

Material and Methods

Adult, healthy pigeons, weighing 300-320 g. maintained on a standard diet of mixed grain, were used. A plaster cast was applied at the shoulder joint after the wings were placed in a back to back position as described earlier (Chapter 1). At the end of the required experimental period the pigeons were sacrificed by decapitation and pieces of the breast muscle were excised, blotted to remove the blood and used for experiments.

FATTY ACID OXIDATION: A piece of the breast muscle was homogenized in a chilled mortar for 2 minutes and further homogenated in a MSE homogenizer for 1 minute at 14,000 r.p.m. A 5% homogenate was then prepared in cold distilled water. After being freed of the cell debris and connective tissue,

this homogenate was used as the material to study the oxygen uptake with sodium butyrate as the substrate. The in vitro oxidation of sodium butyrate in the presence of a "sparker" was determined by the oxygen uptake in a standard manometric system as reported earlier (George and Vallyathan, 1964). Sodium malate was used as the"sparker", since it is known that the presence of a citric acid cycle intermediate is essential for the fatty acid oxidation system (Green,1951, 1955; Knox, et al., 1948; Weinhouse et al., 1949). The experiments were conducted at 37°C and the gas phase in the flask was atmospheric air. The total volume of the reaction mixture in the main chamber of the flask was 3ml. and 0.2 ml 20% KOH was placed in the centre well for the absorption of CO_2. This 3 ml reaction mixture contained 20 mM phosphate buffer, 7.5mM $MgCl_2$, 1.5mM ATP, 0.1125 mM cytochrome c, 1mM DPN, 0.5mM coenzyme A, 10mM sodium malate and 5 mM sodium butyrate in the samples. The manometers were shaken at 100 oscillations per minute with an amplitude of 4.5 cm and the readings were taken after every 10 minutes for 1 hour. The results are expressed as µl O_2 uptake per milligram protein for the first 30 minutes. Protein was determined in duplicate samples according to the biuret method of Gornall et al. (1949).

RESPIRATORY QUOTIENT: The oxygen consumption and carbondioxide output were measured by the Warburg direct method as described by Dixon (1943) and Umbreit et al. (1957).

A piece of the breast muscle of nearly 2 cm^2 was cut out immediately and thin slices of 0.2 mm thickness were cut on a freezing microtome and dropped in the ice cold Krebs Ringer phosphate buffer of pH 7.4 in graduated test tube. Concentrations of components in the buffer were as follows: 100 ml 0.154 M NaCl, 4ml 0.154 M KCl, 3ml 0.11 M $CaCl_2$,1 ml of 0.154 M KH_2PO_4 and 1 ml 0.54 M $MgSO_4$. After collection of the required material, the volume of the fluid was adjusted to 3 ml with more cold Ringer phosphate. Then the contents of the tube were directively emptied into the main chamber of a Warburg flask and 0.2 ml of 20% KOH was added to the central well of one of the flasks for the absorption of the carbon dioxide evolved so as to measure directly the oxygen taken up by the tissue. The second flask with the equal amount of tissue used without KOH, gave the net change in gas phase . From these readings, the respiratory quotient was calculated based on the protein content. The gas phase in the system was atmospheric air. The flasks and the manometers were shaken at 100 oscillations per minute at 37°C. and the readings were taken for 1 hour at every 10 minutes interval. The results presented in the tables are the values for the first half hour.

Protein content was determined according to the ~~methed~~ micro-Kjeldahl steam distillation method (Hawk et al., 1954). After the experiments, the contents of the Warburg flasks were directly transferred into micro-Kjeldahl protein tubes. Protein content of the digested material was determined in

duplicate and the calculation of the oxygen uptake and the carbon dioxide output were based on the average of these protein values.

Results

The results of the oxidation of sodium butyrate and sodium malate in terms of oxygen uptake per mg protein, by the homogenate of the atrophied muscle at varying periods of atrophy are given in Table 1. Table 2 represents the respiratory quotients, oxygen uptake and carbondioxide output of the muscle during the same periods of atrophy. The results presented in the tables are the values for the first half hour after a lapse of 10 minutes taken for the preparation of the material for the respective experiments. Eventhough experiments were carried out for one hour in both cases, there was a decline in the total uptake in the second half hour and hence the results are presented only for the first half hour. A difference in the oxygen uptake by the muscle homogenate used for the fatty acid oxidation system and the tissue sections used for the R.Q. studies were also observed. It was found that the muscle homogenate used for the fatty acid oxidation had a higher oxygen uptake rate per mg protein than that of the tissue sections used for the R.Q.experiments. It was also observed that when muscle homogenate was used for the R.Q. studies, the oxygen uptake was much less compared to that of the tissue sections. The differences in the two systems are probably due to the presence of added cofactors in the fatty acid oxidizing system.

TABLE I

Oxygen uptake due to the oxidation of sodium butyrate and sodium malate by the breast muscle homogenate of pigeons under varying periods of induced muscular atrophy

Days of atrophy	No. of animals used	µl oxygen uptake per mg protein/30 minutes					
		µl oxygen uptake due to malate oxidation (incomplete system)		µl oxygen uptake due to malate and butyrate oxidation (complete system)		Difference due to malate oxidation (negative difference due to butyrate	Difference due to butyrate oxidation
		Average	S.D.	Average	S.D.	Average S.D.	Average S.D.
Normals (controls)	6	4.47 ± 0.997		5.07 ± 0.979		-	0.61 ± 0.233
1 Day	4	4.66 ± 0.145		3.96 ± 0.195		0.694 ± 0.174	-
3 Days	5	3.85 ± 0.557		2.90 ± 0.779		0.949 ± 0.457	-
7 Days	6	5.06 ± 0.848		3.96 ± 0.302		1.110 ± 0.436	-
14 Days	6	3.56 ± 0.332		4.02 ± 0.461		-	0.456 ± 0.151
21 Days	4	6.17 ± 0.474		5.47 ± 0.415		0.701 ± 0.068	-
30 Days	8	6.81 ± 1.780		6.34 ± 1.880		0.471 ± 0.248	-
60 Days	5	5.51 ± 1.180		4.72 ± 1.210		0.790 ± 0.274	-

TABLE II

Oxygen consumption (QO_2) carbon dioxide output and respiratory quotient (R.Q) of the pigeon breast muscle under different durations of induced muscular atrophy

Days of atrophy	µl O_2 uptake per mg protien/30 minutes		µl CO_2 output per mg protein /30 minutes		R.Q		No. of animals used
	Average	S.D.	Average	S.D.	Average	S.D.	
Normal (controls)	0.226	± 0.064	0.248	± 0.081	0.996	± 0.094	6
1 Day	0.189	± 0.061	0.261	± 0.079	1.404+	± 0.179	4
3 Days	0.296	± 0.083	0.363	± 0.119	1.346	± 0.093	6
7 Days	0.189	± 0.044	0.241	± 0.061	1.347	± 0.276	6
14 Days	0.198	± 0.053	0.193	± 0.037	0.976	± 0.077	6
21 Days	0.304	± 0.057	0.435	± 0.104	1.359	± 0.014	5
30 Days	0.247	± 0.065	0.310	± 0.053	1.323	± 0.209	7
60 Days	0.286	± 0.130	0.328	± 0.138	1.151	± 0.133	6

The results of the experiments on the oxidation of sodium butyrate by the atrophied muscle homogenate show that the presence of sodium butyrate in the system causes an inhibition of the oxidative processes in the atrophied muscle. However, after 14 days of atrophy the muscle homogenate was found to be capable of oxidizing sodium butyrate without any inhibitory effect on the overall oxidative process. During all the other days studied it was observed that the muscle homogenate is incapable of oxidizing the added sodium butyrate under the same conditions.

The studies on the respiratory quotient of the atrophied muscle during the same days of atrophy, show a higher R.Q. throughout the experimental period. However, a slightly lower respiratory quotient was observed after 14 days. Oxygen uptake was found to be lower than that of the carbon dioxide output except for the slight difference observed after 14 days.

Results obtained on normal caged pigeons used in these experiments as controls show that the muscle homogenate is capable of oxidizing fatty acid (sodium butyrate). At the same time the experiments conducted on the respiratory quotients of the normal muscle show an average R.Q. of 0.996 ± 0.094 ie. about 0.88 - 1.10 indicating metabolism of carbohydrates.

During atrophy, a slight increase in the oxygen uptake was observed after the early days. In some of the birds studied a high rate of oxidation of malate and corres-

TABLE III

Oxygen consumption and the oxidative capacity of the breast muscle of pigeons that gave abnormal results under disuse atrophy

No. of Days	µl O_2 uptake due to the oxidation of malate	µl O_2 uptake due to the oxidation of malate and butyrate	Difference due to the oxidation of malate(negative difference due to butyrate)	µlO_2 uptake per mg. protein/30 minutes
	µl O_2 uptake / mg. protein / 30 minutes			
3 Days	10.26	8.925	1.336	0.5835
"	13.14	12.451	0.091	0.9175
30 Days	12.45	11.700	0.750	0.5411
"	16.260	15.590	0.691	0.6972
"	9.672	8.992	0.680	0.5972

ponding increase in oxygen uptake and carbon dioxide output were observed. The results of these observations are not taken into account in the average of the readings presented in Tables 1 and 2 but are placed separately in Table 3 as representing the abnormal cases observed.

Discussion

The importance of fat as a major source of energy and its role during sustained activity has been extensively studied by George and his associates (George and Jyoti, 1955, 1957; George and Talesara, 1962; George and Vallyathan, 1964). Relevant literature on the subject has been recently reviewed by Drummond and Black (1960). It was shown that about 77% of the energy expended in a pigeon during sustained muscular activity is derived from fat (George and Jyoti,1957). Further studies by George and Vallyathan (1964) have shown that during sustained muscular activity fat is being continuously supplied to the muscle from the adipose tissue and liver in the form of free fatty acids. Andres et al. (1956) have also shown evidence to the fact that direct oxidation of fat may be an important source of energy for the skeletal muscle.

Weinhouse et al. (1950) have shown that the pigeon breast muscle is capable of oxidizing fatty acids. In the normal birds used as controls in the present experiments, it was seen that the fatty acids are oxidized by the muscle. However, it should be mentioned that the control birds used were caged ones and hence proper comparison cannot be made

with the results of a normal active bird. The pigeon breast muscle when electrically stimulated was shown to have a high oxidative metabolism (Vallyathan and George, 1964) and a respiratory quotient of 0.72 indicating fat catabolism (Pishawiker,1962).

The present study on the capacity for fatty acid oxidation by the muscle homogenate show that during atrophy the muscle is incapable of oxidizing fatty acid (sodium butyrate) under in vitro conditions. Gould and Coleman (1961) studied the formation and accumulation of acetoacetate in muscle ~~in muscle~~ and liver of dystrophic mice. They showed that the muscle of dystrophic mice progressively accumulated acetoacetate as the disease progressed. However, from the present investigation it cannot be concluded that the results obtained are indicative of the level of fat metabolism because an enzyme which could prevent the accumulation of acetoacetate is lacking in the dystrophic muscle. These studies,therefore show, that during atrophy, muscle metabolism is changed from that of fat to carbohydrate. The early studies also give evidence to the fact that during atrophy there is a shift in the metabolism of pigeon breast muscle(Chapters1 and 2). It was shown that there is an increase in glycogen and phosphorylase in the narrow red fibres with a corresponding decrease in the succinic dehydrogenase during disuse atrophy in pigeon. From these observations it is clear that this inability of the muscle to utilize fat may be considered as one of the reasons for the large accumulation of lipid in the atrophied muscle, which would ultimately

replace muscle protoplasm.

The observations on the respiratory quotient of the muscle during varying periods of atrophy show a value of above 1 indicating a high conversion rate of carbohydrates into fat. In this context, the presence of bicarbonate in the system which might be expected to influence the rate of oxygen uptake and carbondioxide production, may be considered ineffective. It is known in such conversion of carbohydrates to fat, that the respiratory quotients in animals may rise above unity, to as high as 1.3 or 1.4 because of a substance rich in oxygen is converted into one which is relatively poor in oxygen (Lusk, 1928). Recent studies conducted on rat denervated skeletal muscle and mice with hereditary muscular dystrophy by Rossi et al. (1963) Zatti et al. (1962) and McCaman (1963) suggest that there is increased activity of the enzymes involved in pentose phosphate pathway. It is well known that the TPN required for the synthesis of fatty acids is supplied as TPNH through the oxidative pathway of hexose monophosphate(Siperstein, 1958). Rossi et al. (1963) further provided evidence of an increased rate of incorporation of labelled acetate into lipid in denervated muscles. These studies suggest the correlation between the increased respiratory quotient and the lowered fatty acid oxidation observed in the present study.

It is well known that increased carbohydrate metabolism is associated with increased fatty acid synthesis. Histochemical observations reported earlier (Chapter 1)

suggest that during atrophy carbohydrate metabolism is increased as indicated by the greater glycogen and phosphorylase content in the narrow red fibres. It is of interest to note that a similar metabolic pattern of having a low capacity for the fatty acid oxidation and increased glycogen content and high phosphorylase levels was observed in the breast muscle of a migratory starling during the pre-migratory period when large amounts of fat accumulate in its body (George and Vallyathan, 1964). It is also known that during the pre-migratory period the R.Q. of migratory birds rises above 1 indicating fat synthesis (Merkel, 1958). The observations reported in the present investigation give evidence to the fact that during atrophy there is an increased synthesis of fat in the muscle, which is not utilized and therefore gets accumulated in the muscle.