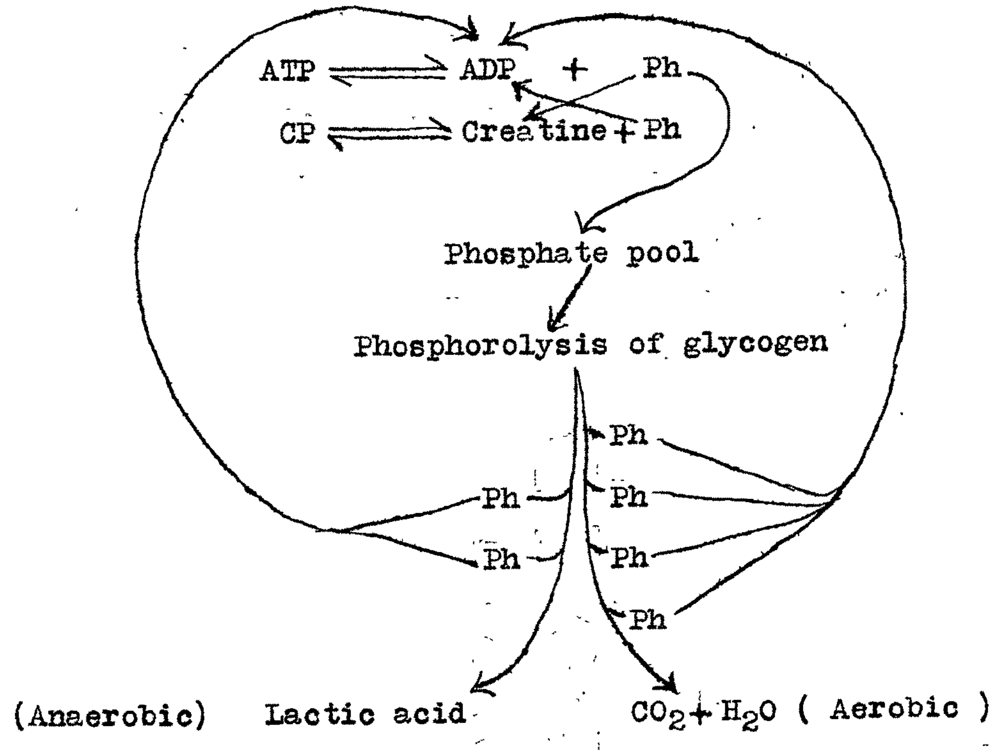
CHAPTER 10

AN EVALUATION OF THE METABOLIC EFFICIENCY OF THE RED AND WHITE FIBRES IN VERTEBRATE SKELETAL MUSCLES WITH PARTICULAR REFERENCE TO THE BREAST MUSCLE OF THE PIGEON AND ITS RELATION TO THE ENERGETICS OF MUSCULAR CONTRACTION

The recent histochemical studies in our laboratories on the voluntary muscle fibres in different animals in relation to their activity have revealed many interesting facts. In pigeon it was observed that the broad white fibres in the pectoralis major muscle contain only glycogen as the fuel store and the narrow red fibres contain both glycogen and fat, the latter predominating (George and Naik, 1958a). The latter fibres contain mitochondria in large numbers, while the former have very few or none (George and Naik, 1958b). Lipase activity is confined to the narrow fibres (Chapter 3). Oxidative enzymes are all concentrated in the red fibres, while the white fibres do not show the presence of these enzymes, not even lactic dehydrogenase in any significant amount (Chapter 8). The sarcoplasm of the broad white fibres do not show any alkaline phosphatase activity, while that of the narrow fibres shows a strong positive reaction due to this enzyme (Chapter 9). Blood supply to the broad fibres is scanty, while the narrow fibres are profusely supplied with blood (Naik, Ph.D. thesis). Despite these differences between the two types of fibres, the narrow white ones show a greater amount of CP and ATPase activity than the red ones and when fatigued a greater amount of inorganic phosphate (George and Pishawikar, unpublished). The breast muscle of the dove a closely related member of the same family is almost similar to the pigeon breast muscle with regard to the above features (Chapters 3 and 8). A remarkable feature of the breast muscle of these birds is that it does not contain fibres intermediate between the broad white variety and the narrow red variety with regard to their colour,size and enzyme concentration as is the case with the breast muscle of the bat and the leg muscle of the fowl (Chapters 8 and 9) and rat (Nachmias and Padykula,1958), where the concentration of the oxidizing enzymes vary from fibre to fibre depending on their diameter.

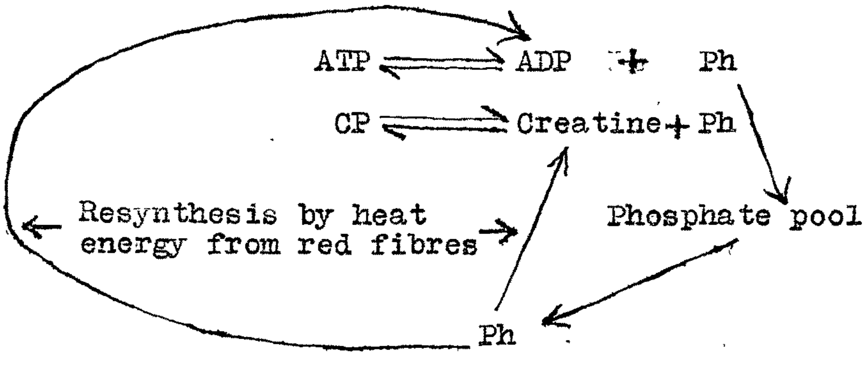
The question now arises as to what is the source of energy for contraction of the white fibres in the breast muscle of these birds and what special function, if any, is performed by them. Denny-Brown (1954) noted that the contrast between the light and dark fibres in the <u>pectoralis major</u> muscle of the pigeon is very great and the same nerve fibre can provide motor endings for both the types of fibres. He also observed that the dark and light fibres contract with the same speed. Hill (1958) has shown that when a muscle is stimulated a large amount of heat is produced first and contraction follows much later. This heat which he calls the heat of activation is probably produced by the breakdown of ATP. If the contractile process is directly brought about by the molecular changes in the structural protein of the muscle fibre during this reaction, as postulated by Szent Gyorgyi (1951), the heat production should be accompanied by contraction of the muscle, which however does not happen.

Most probably the heat energy from the energy rich phosphate bonds of ATP brings about contraction by some other unknown mechanism. It has been demonstrated recently that under experimental conditions muscle can be made to contract independently of chemical reactions (Guttman, Dawling and Ross, 1957). All the same, as far as our present knowledge goes, what is normally required for muscular contraction is ATP. ATP, once it is broken down can be resynthesized by the energy derived from the breakdown of CP or the oxidation of metabolites and CP is resynthesized via ATP by the energy derived from glycolysis and oxidation of lactic acid in a manner schematized below, by a process supposed to be coupled with electron transport.



The white muscle in general is known to derive energy for contraction solely by glycolysis and the oxidation of lactic acid. This scheme explains why in such muscles which are poor in oxidative enzymes there is a greater amount of CP (Lipmann,1941) and an increase in the amount of inorganic phosphate and creatine when fatigued. On further glycolysis the inorganic phosphate is converted to ATP and CP. All the available energy is thus gathered and stored in the energy rich phosphate bonds of ATP and CP for ready use. By this mechanism the efficiency of the muscle increases and according to Baldwin (1954), more than 60% of the total energy is captured and stored. But in long and sustained muscular activity it cannot happen because the cycle cannot operate for a long time unless the reduced Co 1 and the lactic acid formed are readily oxidized.

In the pigeon breast muscle the white fibres do not contain any significant amount of oxidative enzymes or even lactic dehydrogenase necessary for glycolysis for the production of energy required for contraction at a rate at which the red fibres which have a very high concentration of these enzymes contract. At the same time it is difficult to believe that the white fibres do not contract while the red ones are still active. If the white fibres contract in unison with the red fibres, the energy for their contraction should come from extraneous sources, since they themselves are not capable of generating the required energy because of the lack of the necessary enzymes. Since the enzymic splitting of the phosphate bond of ATP and CP liberates heat energy, it should also be possible to rebuild the phosphate bond by the same enzymic mechanism under normal physiological conditions , if heat energy is made available. I believe that the heat energy for the synthesis of ATP and CP in the white fibres is obtained from the red fibres. The red fibres can efficiently oxidize both carbohydrates and fat and since the mechanical efficiency of the individual fibre is low, large amount of heat energy is produced besides its own requirement. This energy is trapped by the white fibres and made use of in the synthesis of ATP and CP besides perhaps utilizing directly for contraction. It is possible that the energy of certain steps of respiratory chain is normally utilized in this way and not via phosphorylation. The measurement of the yield of phosphorylation in respiration might not be a complete measure of the efficiency of utilization of energy potentially available from respiration(Slater,1953). The following alternative scheme into which many observed facts regarding the red and white fibres in other vertebrate skeletal muscles also very easily fit,is therefore postulated.



This is only a theory. At present there is no direct quantitative data from thermodyanamics to support it and may be rejected if any is not forthcoming. But this in the absence of a better, more acceptable theory,is perhaps the only way in which the energetics of the white fibres in the pigeon breast muscle could be explained. However, it may be pointed out that the recent investigations of Lehninger et al(1958) have shown that when the digitonin fragments of mitochondria are incubated aerobically with ATP and inorganic phosphate labelled with P^{32} in the absence of oxidizable substrate or net electron transport, P^{32} is incorporated into the terminal phosphate of ATP in a reaction which is inhibited by dinitrophenol. Similarly Low et al (1958) observed that a virtually complete reduction of the respiratory chain is of relatively little significance for the rate of Pi-ATP exchange and the same is valid in the case of a virtually complete oxidation in the cytochrome region. According to the above scheme the efficiency of the muscle as a whole will be very high, because heat energy which otherwise is wasted can be captured and stored as ATP and CP without any expenditure of fuel. Such an economy of fuel should be of great advantage to those muscles such as pigeon breast muscle which indulge in long and sustained activity. The occurrence of relatively larger quantities of CP in white muscles which are very poor oxidative systems also becomes accountable. This also affords a mechanism for preventing the rapid rise in temperature of the muscle during activity by channeling the excess heat produced during contraction. Another possible function that can be attributed to the white fibres of the pigeon breast muscle is that they can store large amount of glycogen. It is known that fat is the chief fuel for energy for muscular contraction during long and sustained activity (Weis-Fogh, 1952; Bing, 1956; George and Jyoti, 1957). The oxidation of fatty acid has to go hand in hand with the breakdown of carbohydrates, the latter substance functioning as the sparker. Thus to put it in the words of Green (1955) " to keep the fatty acid oxidation pot always on the boil, a not inconsiderable amount of sparker must be present at all times". It can be said with a fair amount of certainty that the oxidation of fatty acids in the pigeon breast muscle takes place in the narrow red fibres. Hence the necessity for a provision for a lavish supply of carbohydrates to the narrow fibres need not be emphasized. Even if carbohydrate is not necessary for the oxidation of fatty acids (see chapter 7) the white fibres in this muscle can be considered as a store house of glycogen readily accessible to the narrow fibres, where it can be utilized with greater economy.

The functional significance of the occurrence of red and white fibres with intermediate types in muscles like the leg muscle of the fowl and rat and the breast muscle of the bat remains unexplained. It is shown that the concentration of oxidative enzymes in these fibres is related to their diameter and myoglobin content. This is significant because, for an adequate supply of oxygen the middle of the cell should not be too far from the nearest blood capillary(Hill, 1956). Oxygen is required more where oxidative enzymes are more. Hence the diameter of the red fibres has to be less and the blood supply and concentration of oxidative enzymes more. This is what is seen in these muscles. Even though it is shown that given a particular stimulus, white muscles contract quickly for a short time and that red muscles contract slowly for a long time, it is doubtful whether the different fibres in the above muscles can contract at different rates <u>in vivo</u>. Most probably it is that they all contract at the same rate. The energy expended by each fibre may vary according to the resistance offered by them depending on their structure and inclusions. The production of energy by oxidative processes in these muscles will naturally vary from fibre to fibre and the deficiency in some will be compensated by the others in a manner postulated above, with all its advantages as somewhat elicited in the old maxim " from each according to his ability and to each according to his needs".

In living systems the correlation between structure and function is well recognised without undue stress on the chronology of their origin. The chemical events taking place in living cells is part of a procedure. A procedure implies purpose and design and to approach the understanding of cell procedures their purposefulness has to be taken into consideration without losing sight of the structure and design of all the individual cells which constitute a functional system and the general design and organisation of the system as a whole. Such an approach has so far not been seriously made in understanding the physiology of the skeletal muscle . Chemical analysis of isolated muscle or the observations made on the working of the muscles under experimental conditions tell us very little about the normal functioning of the muscle. For example, the frog's sartorius is a quick muscle composed of broad white fibres having low oxidative system. Inferences drawn from the study of this muscle cannot hold good for white fibres in all muscles. The white fibres in the breast muscle of the pigeon are certainly not chemically analogous to the white fibres of the frog's sartorii, nor are the white fibres in the breast muscle of the bat to those of the pigeon. Similarly the gastrocnemius muscle of the rat which is considered to be a red muscle, in reality, is not a true red muscle, being composed of fibres which vary from red to white. The thermodyanamics of such muscles where there is a heterogeneous assemblage of fibres will certainly be different from that of a homogeneous muscle like the frog's sartorius. Even the breast muscle of the fowl which is white and more or less homogeneous is not comparable to the white muscle of the frog because of its higher enzyme concentration. Since the muscle is a living mechanical system driven by physico - chemical forces, a new approach has to be made in understanding the mechanical efficiency of the various muscles in different animals taking into account the morphology, structure, nature of the fuel store, myoglobin content, concentration of the different enzymes and such other related aspects. Even in the absence of quantitative data it is possible to visualise that such an approach to the problem would show that the mechanical efficiency of different muscles would be much higher than what it is thought to be at present.