CHAPTER 8

SOME GENERAL CONSIDERATIONS

In this chapter is attempted a general treatment of the histophysiology of the adipose tissue within the compass of what has been studied and presented in the preceding chapters which perhaps might otherwise appear as water-tight compartments. In doing so, it should, however, be made clear, that no claim of any considerable success in acquiring a comprehensive understanding of this complex tissue, is made, but on the other hand, I should consider my efforts worth their while if certain areas of uncertainty in our present state of knowledge regarding this tissue, are brought to light.

For the proper understanding and accurate evaluation of the functions of any tissue or organ, an intimate study of its structure is very necessary. The histological structure of the adipose tissue in the different vertebrate groups shows some interesting features. Certain mammals, possess two types of adipose tissue, namely, the brown and the yellow. Of the former, that occurring at the interscapular region is the most important and well developed and the yellow variety also occurs at various sites in the body. In other vertebrates, only the yellow type appears to be present. The histological structure of these two types of adipose tissue are different. The cells in the yellow adipose tissue is much bigger than that of its counterpart. The cytoplasm in the former is confined to the cell periphery and so also the nucleus. The inside of the cell is filled with a single fat droplet. The nuclei of the brown adipose tissue are eccentric and the fat droplets in the individual cells do not become confluent, with the result that the cytoplasm has a vacuolated appearence. The brown and yellow adipose tissue are named 'multilocular' and 'unilocular' respectively with referênce to the number and size of the lipid droplets present in the individual cells. The amount of cytoplasm is many times more in the brown adipose tissue than the yellow. The structure of the yellow adipose tissue in all the vertebrates studied is found to be the same, irrespective of the evolutionary status of the animal. This similarity in structure does not necessarily mean functional identity also. Nevertheless, the differences in the characteristics of the yellow adipose tissue in the different vertebrates appear to be only of degree and not of kind. The major component of the cytoplasm of the adipose tissue cells appears to be collagen. It should be of interest to note that the structure of the locust fat body is almost like the yellow adipose tissue of vertebrates, with the difference that along with fat cells in the locust, there are certain cells called oenocytes (Coupland,1957).

The histogenesis of the adipose tissue is not well understood. However, it is now more or less accepted that the two types originate at definite predestined sites in the body and keep up their individuality throughout the life span of the animal. Sidman (1956) has reported that brown adipose tissue does not differ fundamentally from the yellow with regard to development and also contended that intergrades between them also occur.

The main, if not the sole, constituent of the adipose tissue appears to be, lipids. The major part of the total lipids of the adipose tissue is in the form of neutral fat - glycerides. Phospholipids and cholesterol are present only in minor quantities, especially, in the yellow adipose tissue. The glycerides occurring in the brown and yellow adipose tissue are reported to be different, the former containing more saturated fats and the latter, unsaturated (Fawcett,1952). Unsaturated fats are more readily utilized than the saturated. Thus the yellow adipose tissue appears to be metabolically more important as supplier of fuel for energy. Lipids stored in the adipose tissue are not static reserves but are constantly being drawn out and restored (Schoenheimer,1946). The glycerides in the adipose tissue are either synthesized there itself or transported from extraneous sites of synthesis or both. The synthesis and breakdown of the glycerides, requires the enzyme, lipase. Lipase was detected in the adipose tissue of all the vertebrates studied and also in the fat body of an invertebrate, the desert locust. The question whether the enzyme under consideration is a 'true lipase' is now fairly settled by the fact that in the histochemical study, "Tween 80" used as the substrate, is specifically attacked by 'true lipase' (Gomori,1953). But all the same the possible occurrence of esterases also is not ruled out. However, the ascertion of certain workers that the fat-splitting enzyme of the adipose tissue is an esterase and of certain others that it is a lipoprotein lipase seems to be illfounded. The quantitative study of lipase by manometric method confirmed the occurrence of a 'true lipase' in the adipose tissue. The substrate in this case was tributyrin and the enzyme that could split tributyrin -'tributyrinase' - is now generally agreed to be a 'true lipase' and not an esterase (Chapter 6). The adipose tissue in the different vertebrates contain varying quantities of lipase. It appears that the amount of lipase present in this tissue can be regarded as an index of the capacity of the tissue to synthesize as well as split up glycerides. Large quantities of the lipase were found to be present in this tissue of the lizard, pigeon, rosy pastor and locust. In all the other animals studied the concentration of the enzyme was found to be comparatively low. These results show that lipase activity is less in the adipose tissue of those animals in which the fat is gradually built up and gradually used up, but on the other hand, it is very high in those in which fat is built up rapidly for a large scale utilization in a shorter period. The high figure in the lizard which may be true for other reptiles as well, is perhaps due to the fact that they are constantly threatened with starvation during some seasons and also they lay large, yolked eggs. Moreover, for a terrestrial reptile more fat synthesized and stored means more water conserved and more fat utilized means more metabolic water made available to meet acute conditions of draught. Seasonal variations in the lipase content of the adipose tissue of the frog was noted. During midsummer the enzyme activity was found to be lower than that during the rains. It should be pointed out that the breeding season of the frog is during the rains and for the manufacture of eggs and sperms fat is essential. The amounts of lipase present in the brown and yellow types of adipose tissue in the bat were found to be different, that in the latter being more. Neutral fat is more in the yellow adipose tissue and it is reported that fat in this tissue is mobilized in greater concentration at a faster rate than in the brown adipose tissue (Remillard,1958). This could be accounted for by the higher concentration of lipase in the yellow adipose tissue. Liver is the organ well-known to synthesize large amounts of fat. Adipose tissue can synthesize fatty acids at no lesser rate than liver, when reduced to cytoplasmic unit, namely, fat-free tissue or total nitrogen content (Feller,1954; Hausberger et al,1954). The esterification of the fatty acids thus synthesized, to glycerides, must be brought about by the lipase present. As stated earlier the capacity to synthesize fatty acids and further fats by the adipose tissue appears to be different in different animals. Thus in the case of the fowl, there is comparatively only a small quantity of lipase in the adipose tissue, it is reported that most of the synthesized fat in this tissue has its origin in other tissues (Bumgardner,1957). It is fairly safe to believe that the capacity to synthesize fat will vary proportionate to the lipase concentration. Nevertheless, more quantitative data are needed to establish this contention irrevocably.

Flying animals like birds, bat and insects (locust) have been shown to utilize chiefly fat for muscular energy during sustained exercise (George and Jyoti,1955 and 1957; Weis-Fogh, 1952). The adipose tissue with its large store of fat could supply this material to the muscle, as the muscle fat gets depleted and the intramuscular fat store may not be sufficient to meet the great demand for fuel during prolonged exercise such as migratory flights. Now the question that arises, is, in what form the fat is transported to the muscle from the adipose tissue. A definite answer to this is not yet available. Investigations in that direction are in progress in our laboratories.

Of the three birds studied, pigeon and rosy pastor are very good fliers, the former indulging many a time in sustained flight and the latter is a migrant, flying as far as southern Europe during the months of April-May. In sharp contrast, the domestic fowl is known to be a very poor flier. The lipase activity in the adipose tissue of the first two are very high and that of the fowl is low. The locust fat body also contains large quantities of lipase, and this animal is also known to be a good flier. A low lipase activity was recorded for the flying mammal (bat). This could be expected since, even though bats are able to fly and they may utilize fat for muscular energy, they seldom indulge in long range sustained flights like birds. On the other hand adipose tissue fat in bat acts as an energy store for slow and gradual utilization during hibernation or aestivation, as the case may be.

The study of the biochemical properties of the adipose tissue lipase of the pigeon by manometric method, revealed the following. An aqueous extract of the ether-defatted adipose tissue is active by itself and is not dependent on any added substances like cations. It does not seem to contain any metallic component in its enzyme protein and does not require metal ions for its activity. The enzyme appears to be an -SH enzyme or one requiring -SH or -S-S- for activity. This, however, is not in complete agreement with conclusions reached by Scaria (1958) and George and Scaria (1959) in their studies on the lipases of the pigeon pancreas and breast muscle. According to them, the pancreatic and breast muscle lipases are both metalloproteins or enzymes requiring metallic ions and reactive NH_2 groups for activity and are devoid of -SH or -S-S- groups. Apart from these differences there are also points of similarities betwеen the lipases obtained from different sources in the pigeon.

Alkaline and acid phosphatases were found in the adipose tissue of all the animals tested. There are a number of functions attributed to the former. But in the adipose tissue it appears to be specially connected with lipid metabolism. In skeletal muscle also this appears to be the case. Thus in the pigeon breast muscle it is reported to be present only in the fat-loaded narrow fibres and not in the glycogen-loaded broad ones (George, Nair and Scaria, 1958). In the adipose tissue the concentration of this enzyme, almost follows the pattern of distribution of lipase. Thus in the adipose tissue of the pigeon and rosy pastor it is comparatively more than that of the fowl. The brown and yellow adipose tissue of the bat do not differ much in their alkaline phosphatase content. This is more or less true of lipase also. Acid phosphatase is believed to be important in protein synthesis mechanism (Kivalo et al, 1958). ATPase was studied in the pigeon adipose tissue and the brown and yellow adipose tissue of the bat. The exact role of the considerable quantities of this enzyme present in the adipose tissue cannot be suggested in the present state of our knowledge. The concentrations of the different phosphate compounds in the adipose tissue are not known with respect to these animals. Zimny and Gregory (1958) investigating the composition of the brown adipose tissue of the 13-striped ground squirrel, has reported the concentrations of the following phosphate compounds in a decreasing gradation: inorganic phosphate, creatine phosphate and adenosine triphosphate (ATP).

The presence of Krebs cycle enzymes in the adipose tissue shows that tricarboxylic acid cycle operates in this tissue. The oxidative enzymes were found to be occurring in more or less equal concentrations in the yellow adipose tissue of the pigeon, fowl, rosy pastor and bat. Only the brown adipose tissue differed in this respect, having large quantities of these. The results of the in vitro oxygen consumption studies substantiate these findings. The oxygen consumption of the brown adipose tissue of the bat was very high while the values obtained for the yellow adipose tissue of the bat and the adipose tissue of the pigeon were comparatively low and these last two being more or less the same. One obvious point emerging from these results is that, the yellow adipose tissue of different animals differ mainly with respect to their lipid metabolism, while the oxidative metabolism is more or less the same. The opposite appears to be true when the brown and yellow adipose tissue are compared. Here the main difference seems to be in the oxidative metabolism. Sulphydryl groups were found to be much more in the brown adipose tissue than in its counterpart. This may be due to the higher dehydrogenase content since dehydrogenases are known to contain -SH groups in their protein.

The adipose tissue of the pigeon was found to be lacking in histochemically demonstrable glycogen. According to Fawcett (1952) the brown adipose tissue contains large quantities of glycogen and the yellow adipose tissue only negligible amounts of it. It appears that carbohydrate metabolism is of minor importance, if at all, with respect to yellow adipose tissue. Even the pathway of carbohydrate metabolism has been shown to be different.

The enzyme, phosphoglucomutase catalizing the reaction

$$\text{glucose-1-phosphate} \rightleftharpoons \text{glucose-6-phosphate},$$

is reported to be absent in the yellow adipose tissue of the rat and present in the brown type (Mirski,1942). Amylase is said to be operating in its place in the yellow adipose tissue. However, the adipose tissue is capable of actively synthesizing fatty acids from glucose (Hausberger et al,1954). The protein content of the fresh brown and yellow adipose tissue was found to differ much, the former containing more total proteins per unit weight.

In conclusion it may be said that the yellow adipose tissue plays a major role in the lipid metabolism of the different animals. Other metabolic activities are only of minor importance. The exact function of the brown adipose tissue is rather a controversial subject. Some of the earlier workers (as reviewed by Fawcett,1952; Menschik,1953 and Remillard,1958) called it 'hibernating gland' and 'cholesterol gland'. Both these names are discarded now, due to lack of adequate evidences to justify their retention. Recently Rothbard (1958) attributed a dual function to the brown adipose tissue in the metabolism of the mouse. He considers that it is needed in milk production and as energy source in the process of parturition. The function of this tissue in the male remains unexplained. My present studies have shown that the brown adipose tissue also should play an active part in the different metabolic activities along with the other tissues of the body, though with regard to lipid metabolism it is inferior to the yellow adipose tissue.