CHAPTER 3

HISTOCHEMISTRY OF THE ADIPOSE TISSUE OF A FLYING MAMMAL (BAT)

The occurrence of two types of adipose tissue, brown and yellow, in certain mammals is well-known and they have been the subject of detailed studies in recent years (Mirski,1942; Fawcett,1952; Menschik,1953; Sidman,1956; Rothbard,1958; Remillard, 1958). They are different in their histological structure. Sidman (1956) has reported that brown adipose tissue does not differ fundamentally from the yellow with regard to development and also contented that intergrades between them occur. In a few cases like that of rabbit, cat and man, much of the brown adipose tissue which is distinctly multilocular in the embryonic stages tends to become unilocular and to resemble the yellow type, later. But in the majority of other mammals (rodents and many hibernators) possessing brown adipose tissue in earlier stages retain the same condition in adult life also (Selye and Timiras,1949; Sidman, 1956). The brown and yellow adipose tissue are named 'multilocular' and 'unilocular' respectively, with reference to the number and size of the lipid droplets present in the individual cells. The physiological differences appear, however, to be mainly quantitative rather than qualitative. Fawcett (1952), using histochemical methods, suggested that there are quantitative differences in enzymic activities between brown and yellow adipose tissue of rat and claimed that brown adipose tissue is more active. Qualitative differences were not detected. On the other hand, Mirski (1942) had reported one instance of qualitative difference between brown

and yellow adipose tissue of rat, namely, the absence of the enzyme phosphoglucomutase in the latter.

In bats also these two types of adipose tissue are present. A histochemical and microchemical study of the lipids of the interscapular brown adipose tissue of the vespertilionid bat Myotis lucifugus lucifugus, was made by Remillard (1958). So far no data are available on the relative activities of these two types of adipose tissue of the bat. In the present chapter the results are reported of a comparative study of the histochemical reactions of lipase, alkaline phosphatase, acid phosphatase, ATPase, succinic dehydrogenase, lactic dehydrogenase, phospholipids, cholesterol, sulphydryl groups and water-insoluble aldehydes and ketones of the brown and yellow adipose tissue of the bat (Hipposideros speoris).

Material and Methods

The material used were the subcutaneous lobes, of interscapular brown and lateral abdominal yellow adipose tissue of the bat (H. speoris). The animals were decapitated and the tissues taken after most of the blood had been drained off. Fresh frozen sections were used for the study of enzymes and also for certain other histochemical observations.

Lipase activity was studied employing the "Tween" method of Gomori (Pearse,1954). "Tween 80" was used as substrate. Alkaline phosphatase was studied according to the revised method of Gomori, sodium glycerophosphate being used as the substrate. The revised method of Gomori was successfully employed on fresh frozen sections of adipose tissue to demonstrate acid phosphatase activity

(Glick,1949). ATPase was detected by the procedure of Pearse and Reis (Pearse,1954). Succinic dehydrogenase activity was studied by the method of Straus et al (Pearse,1954), 2:3:5 triphenyl tetrazolium chloride (TTC) being used as the hydrogen acceptor. The same method was extended to the study of lactic dehydrogenase also. Phospholipids were demonstrated by the acid haematein method of Baker (Pearse,1954). For the study of cholesterol, the Schultz method (Pearse,1954) was employed. Sulphydryl groups were detected by Bourne's nitroprusside test (Glick,1949), and water-insoluble aldehydes and ketones by the Albert and Leblond reaction (Glick,1949). The procedures followed in these studies were the same as adopted for the pigeon adipose tissue (Chapter 2, I).

Results

Lipase: Sections treated for lipase activity showed the precipitate more or less equally abundant in both the tissues (Figures 1 and 2). The brown adipose tissue with its larger amount of cytoplasm did not show any appreciable difference in this respect. Lipase activity was represented by the usual brownish precipitate.

Alkaline phosphatase: The intensity of the brown colour developed was almost the same or perhaps slightly more in the case of the brown adipose tissue.

Acid phosphatase: The activity of this enzyme in the two types of adipose tissue appeared to be almost the same. Figures 3 and 4 show brown and yellow adipose tissue treated for acid phosphatase activity. The nucleus as well as cytoplasm showed the presence of this enzyme, but the reaction in the former was stronger.

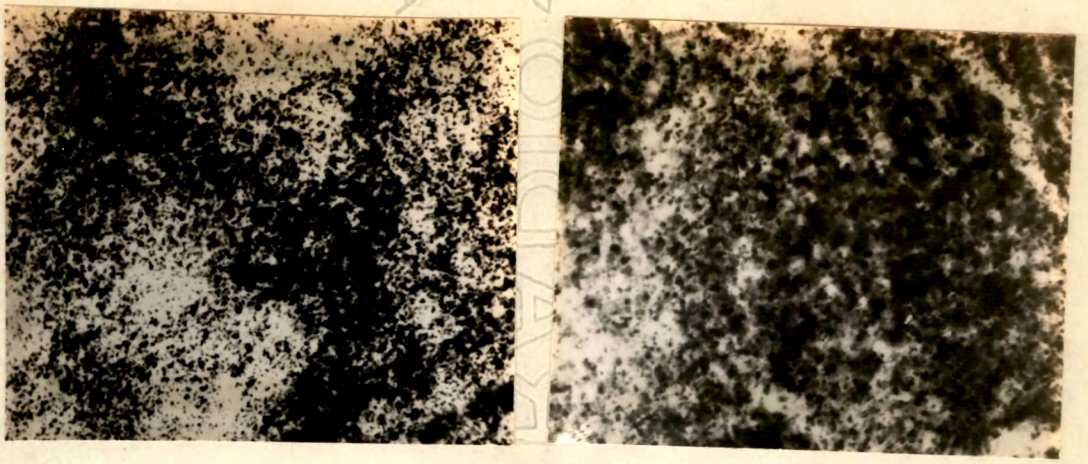

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Fig. 1 Fig. 2

Fig. 1 and 2 - Sections of the brown and yellow adipose tissue respectively, treated for lipase activity. The individual cells are obscured by the precipitate.

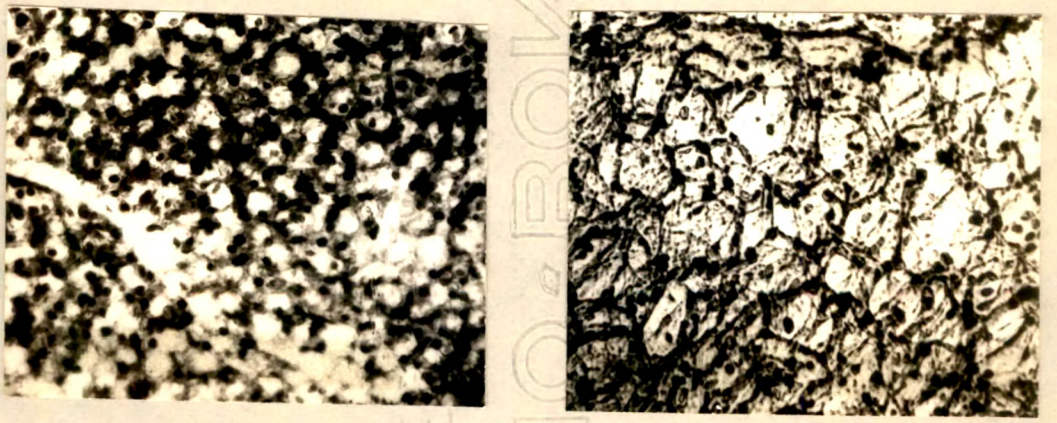

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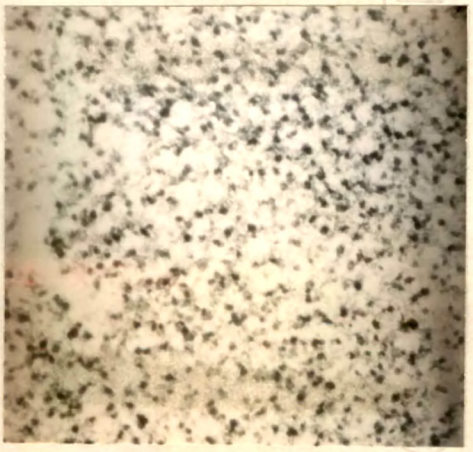
Fig. 3 Fig. 4

Fig. 3 and 4 - The brown and yellow adipose tissue respectively, treated for acid phosphatase activity.

ATPase: Figures 5 and 6 show sections of the brown and yellow adipose tissue treated for ATPase activity. The intensity of the

brown colour developed after the final treatment was found to be almost the same in both the types of adipose tissue. Both the nucleus and the cytoplasm gave a positive reaction, that in the former being stronger.



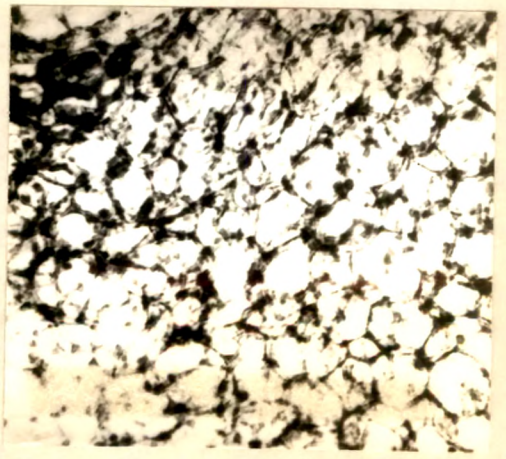


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Fig. 5 Fig. 6

Fig. 5 and 6 - The brown and yellow adipose tissue respectively, demonstrating ATPase activity.

Dehydrogenases: Succinic dehydrogenase and lactic dehydrogenase activities in these two types of adipose tissue showed a sharp difference. The brown adipose tissue showed a much higher activity. Section of the brown adipose tissue after a few minutes of incubation developed a deep red colour in contrast to a light pink in the case of the yellow adipose tissue.

Phospholipids: The brown adipose tissue revealed the presence of a higher concentration of phospholipids (Figures 7 and 8).

Cholesterol: Both the brown and yellow adipose tissue after treatment gave a very faint green coloration, indicating a positive reaction. The intensity of the colour developed was

slightly greater in the brown adipose tissue.

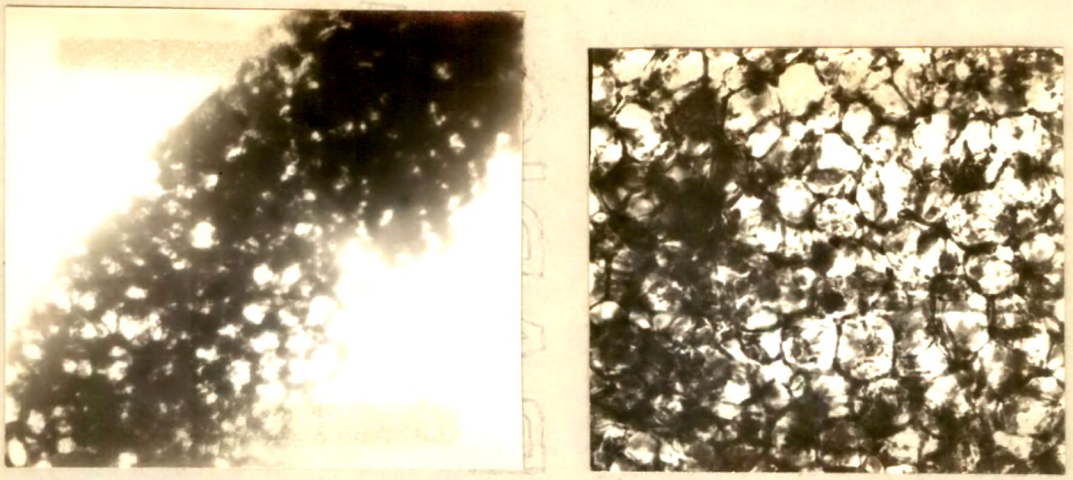

Fig. 7 Fig. 8

Fig. 7 and 8 - The brown and yellow adipose tissue respectively treated to show phospholipids.

Sulphydryl groups: The brown adipose tissue showed the presence of higher concentration of sulphydryl groups than the yellow.

Water-insoluble aldehydes and ketones: The brown and yellow adipose tissue gave almost the same reaction when tested for water-insoluble aldehydes and ketones.

Discussion

Histological differences between the two types of adipose tissue are quite distinct. The amount of cytoplasm present is very much greater in the brown adipose tissue than in its counterpart (Chapter 1). In structure both the brown and yellow adipose tissue of this bat more or less resembles the corresponding tissues of the rat, as discussed by Fawcett (1952).

The development of an abundant deposit of precipitate in

sections treated for lipase activity indicates that both these types have appreciable concentrations of lipase in them. Fawcett (1952) has shown by histochemical methods that the brown adipose tissue of the rat has a higher concentration of lipase than the yellow adipose tissue. However, he expressed doubt as to the presence of a 'true lipase' in the adipose tissue of the rat, since he used "Tween 40" and "Tween 60" as substrates. It may be repeated here that "Tween 80" was found to be attacked specifically by 'true lipase' and not esterase (Gomori,1953) and since in my studies I have used "Tween 80" as the substrate, it is concluded that the enzyme under consideration is a 'true lipase'.

The distribution of alkaline phosphatase, acid phosphatase and ATPase was found to be almost the same in the two types of adipose tissue. Fawcett (1952) reported higher concentration of alkaline phosphatase in the brown adipose tissue than in the yellow, in the rat. Both nucleus and cytoplasm gave a positive result. In a number of sections examined, the cytoplasm of the yellow adipose tissue appeared to be more active than that of the brown adipose tissue, and conversely in the case of the nuclei. This cannot be explained within the limits of our present state of knowledge.

The presence of greater concentration of succinic and lactic dehydrogenases in the brown adipose tissue is indicative of its higher oxidative activity. It is likely that the other oxidative enzymes are also more abundant in the brown adipose tissue. This is supported by the findings of various workers which have been reviewed by Remillard (1958). Fawcett (1952) reported higher succinic dehydrogenase activity in the brown adipose

tissue of the rat while Menschik (1953) found no difference between brown and yellow adipose tissue of the guinea-pig. However, the latter found a higher concentration of amine oxidase, ∝ naphthol oxidase and cytochrome oxidase in the brown adipose tissue.

Fawcett (1952) found higher concentration of phospholipids in the brown adipose tissue of the rat and Menschik (1953) in that of the guinea-pig. I too have obtained a similar result in the case of the bat. Fawcett (1952), however, failed to get a positive result for cholesterol in both types of adipose tissue in the rat, whereas Menschik (1953) reported the presence of more cholesterol and its esters in the brown adipose tissue of the guinea-pig. In the bat I got a faint reaction in both the types of adipose tissue.

The brown and yellow adipose tissue showed a striking contrast when tested for sulphydryl groups, the former showing an abundance. In the guinea-pig Menschik (1953) found no difference in reaction in the two types of adipose tissue.

The question arises whether these two types of adipose tissue are functionally the same or not. A definite answer to this is not yet available. Fawcett (1952) suggested that fat in the brown and yellow adipose tissue is different in chemical nature. The former is said to contain more saturated fat and phospholipids while the latter, more neutral fats. There appear to be seasonal variations also with regard to the amount and nature of the fat present. Remillard (1958) has stated that fat in the yellow adipose tissue is mobilized in a greater amount, faster than in the brown adipose tissue. Rothbard (1958) attributes 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.

Reviewing the work of various authors Fawcett (1952) has stated, "While no explanation for these several observations can be given at present, the fact that the two types of adipose tissue do differ markedly in their response to hormones, infectious agents and vitamin deficiency serves to emphasize the possibility of a significant difference in their function". My present study also shows that the two types of adipose tissue of this bat, are different in their histological structure (Chapter 1) as well as many of the physiological activities.

The occurrence of brown adipose tissue in the bat, a flying mammal, is certainly not an acquisition for the flying habit because it is absent in birds. It has often been stressed that this tissue has some important role in hibernation. But animals such as dog and rat which possess brown adipose tissue do not hibernate. It has been shown that extracts of this tissue from the ground squirrel and hedgehog injected into rats caused a lowering of metabolism (Hook,1940; Wendt,1937). Witschke and Maier (1932) showed that extracts of lymphatic tissue also caused a drop in metabolic rate. The crux of the problem therefore, seems to be that we have only a very imperfect understanding of the functions of this tissue. In conclusion, the brown adipose tissue appears to be a specialized adipose tissue in histological structure as well as physiological properties and may have some significant function in hibernators which depend on stored fat for energy.