ENTOMOLOGY

'Catalysomes', or Enzyme Caps on Lipid Droplets: an Intracellular Organelle

WHILE studying the distribution of acetylcholine and other esterases in the insect *Rhodnius*[1], it was observed that every droplet of triglyceride in the cells of the fat body has a small disk-like area or 'cap', 1μ or less in diameter, which is the site of an enzyme that will hydrolyse 5-bromoindoxyl acetate (method of Holt[2]) and naphthol AS acetate (method of Pearse[3]). This esterase is not inhibited by eserine or other inhibitors of the cholinesterase group; it was believed to be a lipase and it was concluded that the active disk or cap constitutes an organelle responsible for the liberation and perhaps the uptake of fatty acids in the droplets of stored lipid[1].

More recently it has been shown by Chino and Gilbert[4] that fatty acids in insects are transported in the form of diglycerides incorporated into lipoprotein; and that liberation of the stored lipid is an energy-demanding process which involves the simultaneous oxidation of carbohydrate[5].

In the course of an investigation of the histology and histochemistry of the fat body in *Rhodnius*, which is now being written up for publication, it was observed that nicotinamide–adenine dinucleotide diaphorase ($NADH_2$ as substrate, with nitro-BT as tetrazolium compound[6]), besides its expected localization in the mitochondria, is intensely active in the lipase-containing caps on the droplets of fat (Fig. 1, *a*). In the absence of added substrate endogenous activity gives a barely detectable reaction. Of the enzymes investigated so far, the 'caps' contain also the dehydrogenases of isocitrate (+nicotinamide adenine dinucleotide phosphate, NADP, as co-enzyme), α-ketoglutarate (+NAD), succinate (without added co-enzyme), malate (+NAD), glutamate (+NAD), β-hydroxybutyrate (+NAD) and a very weak lactate. The dehydrogenases of the pentose cycle acting on glucose-6-phosphate and 6-phosphogluconate (+NADP) are virtually absent. In the G-'Nadi' reaction indophenol blue is sharply localized in the muscle mitochondria[7]. In the fat body cells the dye rapidly accumulates in solution in the droplets of lipid, but the site of its formation cannot be localized.

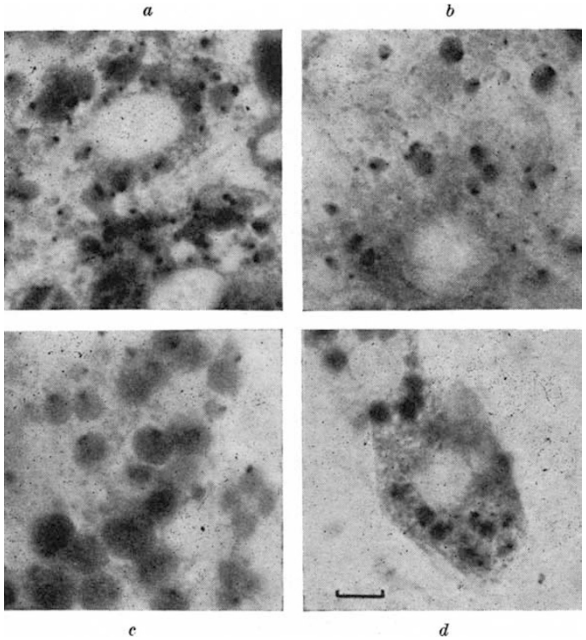

Fig. 1. Whole cells containing lipid droplets. Fixation: glutaraldehyde at *p*H 7·4. Fat staining: sudan 4. Light filter: greenish yellow. Scale (shown in *d*) equals 5μ. *a*, Fat body cells of *Rhodnius*: NAD-diaphorase. Mitochondria around nuclei, catalysomes on lipid droplets. *b*, The same, showing some catalysomes with invisibly small lipid droplets among the mitochondria. *c*, Fat cells in mesentery of mouse: 5-bromoindoxyl acetate method for esterase. Catalysomes in focus on many of the fat drops. *d*, The same: NAD-diaphorase. Mitochondria and lipid droplets with catalysomes

It appears likely, therefore, that these enzyme sites contain all the enzymes of the Krebs cycle and presumably the cytochromes. They are probably responsible for the synthesis of stored lipid (which is readily formed in the fat body cells from sugars and amino-acids[8]) as well as for its preparation for distribution in the circulating blood.

For convenience these organelles, which may be appropriately termed 'catalysomes', have been chiefly studied in the fat body of *Rhodnius*. But their presence is readily confirmed in the developing fat cells in the mesentery of the young mouse. Here they have the same appearance and contain both esterase and NAD-diaphorase (Fig. 1, *c* and *d*). In both insect and mammal they arise as intensely reactive granules in the cytoplasm between the mitochondria (Fig. 1, *b*). A minute droplet of lipid then appears in contact with the granule and as this droplet enlarges the granule becomes a crescent and finally a sharply localized and slightly raised spot on the surface of the oil drop. The fine structure of this organelle is being studied in collaboration by A. V. Grimstone.

Catalysomes must often have been seen before, but apart from their esterase activity[1], their significant association with lipid droplets has not been recognized. It was reported by Chino[9] that much of the cytochrome oxidase in the developing egg of the silkworm was contained in particles that were carried up with the lipid layer during the centrifugation of homogenates. These particles were probably catalysomes.

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[1] Wigglesworth, V. B., *Quart. J. Micros. Sci.*, **99**, 441 (1958).
[2] Holt, S. J., *Proc. Roy. Soc.*, B, **142**, 160 (1954).
[3] Pearse, A. G. E., *Intern. Rev. Cytol.*, **3**, 329 (1954).
[4] Chino, H., and Gilbert, L. I., *Science*, **143**, 359 (1964).
[5] Chino, H., and Gilbert, L. I., *Biochim. Biophys. Acta*, **98**, 94 (1965).
[6] Pearse, A. G. E., *Histochemistry* (London, Churchill, 1960).
[7] Wigglesworth, V. B., *Quart. J. Micros. Sci.*, **97**, 465 (1956).
[8] Wigglesworth, V. B., *J. Exp. Biol.*, **19**, 56 (1942).
[9] Chino, H., *Arch. Biochem. Biophys.*, **102**, 400 (1963).

Pertinacity of Host-seeking Behaviour of *Aedes aegypti*

MOSQUITOES on the hunt are persistent pests. To the best of our knowledge, this persistence has not been quantitated. Use of mosquitoes in lengthy biting or probing experiments requires that the drive to bite or probe does not fatigue during the minutes or hours of the experiment, so that the experimental variable can be reliably quantified. The following observations were made to record the pertinacity (and lack of fatigue) of *Aedes aegypti* females.

Adult female *Aedes aegypti*, var. *queenslandensis*, were reared in cages, 1 ft.^{3}, in the presence of males. They were fed 5 per cent sucrose solution until the experiment began. 5–9-day-old females were placed in small cages, 5 × 5 × 1·5 cm, made of a wire frame and covered with nylon net. In probing experiments these cages were suspended 1 cm above the inner surface of the forearm of a volunteer. The mosquitoes were observed and any attempts to probe were recorded.

Five mosquitoes in a small cage were observed for 30 min. The number probing towards the arm was recorded continuously. Ten replicates, using different mosquitoes but the same subject, were performed.

The 30-min experimental period was divided into three successive 10-min periods for purposes of comparison.