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Stability of Clearing-Factor Lipase in Rat Adipose Tissue

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The stability at 42°C of clearing-factor lipase in adipose tissue, and in intact fat-cells isolated from it, was investigated. That portion of the total activity of the tissue which is associated with the fat-cell is stable under such conditions. This stability is markedly diminished when the fat-cell is disrupted.

The main function of the enzyme clearing-factor lipase is believed to be the regulation of triglyceride removal from the blood by the extra-hepatic tissues (see Robinson, 1970), and the fact that the activity of the enzyme in adipose tissue from rats in the fed state is severalfold higher than it is in starved animals (Hollenberg, 1959; Cherkes & Gordon, 1959; Robinson, 1960) is consistent with this role, since the uptake of plasma triglycerides by adipose tissue declines markedly on starvation (Bragdon & Gordon, 1958).

Clearing-factor lipase probably hydrolyses the plasma triglycerides through its action at the luminal surfaces of the capillary endothelial cells of tissues that contain the enzyme (Robinson, 1970). However, in adipose tissue, some of the clearing-factor lipase activity is due to enzyme which is not at this site but which is present in association with the fat-cell component of the tissue (Rodbell, 1964). In animals in the fed state, the fat-cell enzyme accounts for only a small proportion of the total tissue activity, but in starved animals most of the much lower activity is due to enzyme which is associated with the fat-cells (Cunningham & Robinson, 1969), and it has been suggested that this fraction could be the precursor of the functional enzyme at the endothelial cell surface (Robinson & Wing, 1970).

Previous work has shown that when epididymal adipose tissue from rats in the fed state is incubated in an albumin-based medium at 37°C between 80 and 90% of the original activity declines with a half-life of about 1h, the remainder being essentially stable. However, in adipose tissue from starved rats, only some 10–20% of the activity is similarly unstable under these conditions at 37°C (Wing & Robinson, 1968; Cunningham & Robinson, 1969). This evidence that clearing-factor lipase exists in adipose tissue in stable and unstable states suggests that the former, predominating in tissue from starved animals, may be associated with the fat-cell, whereas the latter, present only in the tissue in animals in the fed state, may be outside the fat-cell at the endothelial cell surface. It does not, however, show whether there are, in fact, two distinct forms of the enzyme in adipose tissue, one inside and one outside the fat-cell, which differ in their stability, or whether only a single form of the enzyme exists and the fat-cell enzyme is stable simply by virtue of its state of association with the fat-cell. In the present study experiments are described to confirm the stability of the fat-cell enzyme and to determine which of the alternative explanations for this stability is correct. The materials and methods used are as previously reported (Cunningham & Robinson, 1969), stability of the enzyme being measured at 42°C and pH7.4 in an albumin-based medium similar to that used in the fat-cell isolation procedure. The enzyme is assayed in homogenates of acetone–diethyl ether-dried tissue preparations by its ability to hydrolyse chylomicron triglycerides and 1 unit of activity is defined as 1 μmol of free fatty acid released/h at 37°C. It is known that the enzyme is stable both to acetone and diethyl ether treatment and during the assay procedure itself (Robinson, 1963). In all the preparations used, enzyme activity was inhibited by more than 90% in the presence of 0.5M-NaCl solution and was not expressed against artificial triglyceride emulsions unless these were activated by preincubation with serum. It therefore had the characteristics of clearing-factor lipase (Robinson, 1963).

The results in Fig. 1 show that the clearing-factor lipase of fat-cells isolated from epididymal adipose tissue is indeed essentially stable to incubation at 42°C and that this is so whether the fat-cells are isolated from tissue from fed or from 24h-starved rats. Therefore, as Fig. 1 also shows, the enzyme associated with the fat-cells resembles in this respect that in intact tissue from starved rats and is distinct from that in intact tissue from fed rats, where some 80% of the activity has a half-life of about 30min under these conditions. No enzyme activity was detected in the medium in any of the incubations.

Clearing-factor lipase was measured in the above experiments in homogenates of acetone–ether-dried preparations made from the intact tissue or the isolated fat-cells after their incubation at 42°C. To determine whether or not the greater stability of the fat-cell enzyme was due to its association with the cell,

similar preparations were made from fat-cells immediately after they were isolated from tissue taken from fed and starved animals and the stability of homogenates of these to incubation at 42°C was compared with that of homogenates of acetone–ether-dried preparations of the intact tissue. The results in Fig. 2 show that in all such homogenates at least 90% of the enzyme activity is unstable, with a half-life similar to that of most of the activity of intact fat-bodies from fed rats. The low stable activity evident in Fig. 2 after long periods of incubation has been consistently found in all the acetone–ether-dried preparations used in these studies. It has the characteristic properties of clearing-factor lipase but its significance is at present unknown.

Experiments additional to those described above have been carried out in which the medium for the incubation of intact fat-bodies at 42°C was 25mM-NH_3, pH8.1, rather than Krebs–Ringer bicarbonate buffer, pH7.4, and in which acetone–ether-dried preparations of fat-bodies and fat-cells were homogenized in 25mM-NH_3, pH8.1, before being incubated at 42°C. In all these experiments the results were essentially as those reported in detail here. It seems clear, therefore, that although two forms of

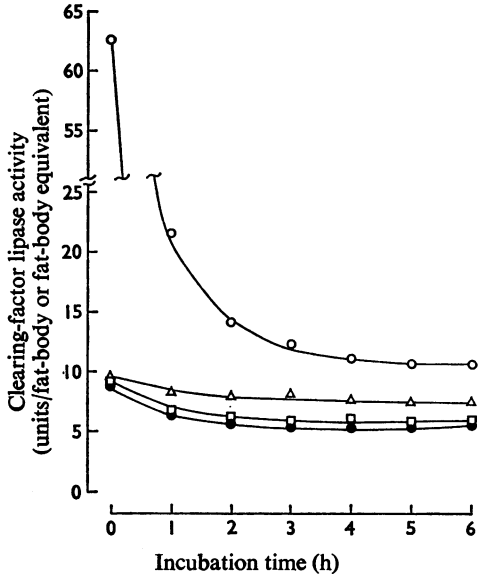

Fig. 1. *Loss of fat-body and fat-cell clearing-factor lipase activity at 42°C*

Intact epididymal fat-bodies, and fat-cells isolated from them, were incubated at 42°C in Krebs–Ringer bicarbonate buffer, pH7.4, containing 4% (w/v) bovine serum albumin (Cunningham & Robinson, 1969). The intact fat-bodies were from either fed (○) or 24h-starved rats (△) and the fat-cells were also isolated from either fed (□) or 24h-starved (●) rats. In each experiment with fat-bodies, 28 fat-bodies were incubated in 42ml of medium and four fat-bodies and 6ml of medium were removed at intervals Acetone–ether-dried preparations were then made from the fat-bodies and the medium was combined and clearing-factor lipase activity was assayed in homogenates of these made in 25mM-NH_3, pH8.1, at a preparation concentration of 10mg/ml (see Cunningham & Robinson, 1969). In the fat-cell experiments, the cells were isolated from 16 fat-bodies, suspended in 32ml of the medium and incubated. Homogeneous samples of the cells and medium mixture, each of 4ml and therefore containing cells derived from the equivalent of two fat-bodies, were taken at intervals and clearing-factor lipase activity was again measured in homogenates of acetone–ether-dried preparations of the cells and medium combined.

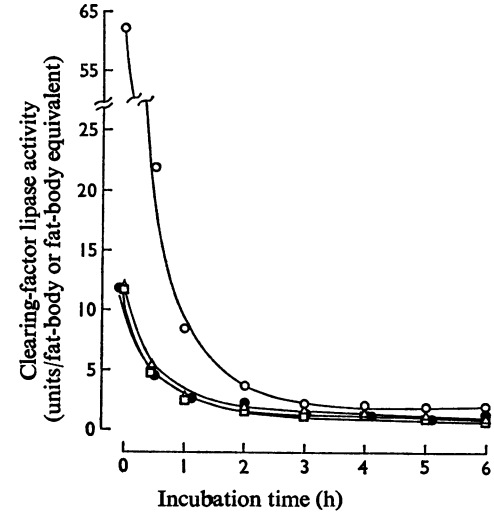

Fig. 2. *Loss of clearing-factor lipase activity of acetone–ether-dried preparations of fat-bodies and fat-cells at 42°C*

The acetone–ether-dried preparations were made from 16 fat-bodies from fed (○) or 24h-starved (△) rats and from fat-cells isolated from 16 fat-bodies from fed (□) or 24h-starved (●) rats. In each case the fat-bodies or the fat-cells were suspended in 24ml of the albumin-based medium described in the legend to Fig. 1 before the preparations were made. Homogenates of the preparations were then made in Krebs–Ringer bicarbonate buffer, pH7.4, at a concentration of 10mg/ml and incubated at 42°C and samples of the incubation mixture were taken at intervals for direct assay of clearing-factor lipase activity.

clearing-factor lipase may exist in adipose tissue, as other evidence indicates (Stewart & Schotz, 1971; Garfinkel & Schotz, 1972, 1973), the differences in stability of the enzyme in intact adipose tissue from fed and starved rats are primarily due to differences in the location of the enzyme in the tissue.

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