reticulum might suggest that addition of N-acetylglucosamine to the asparagine residue occurs while the nascent protein is associated with polyribosomes. But final conclusions must await information concerning the substrate specificity of UDP-N-acetylglucosamine-asparagine sequon N-acetyl-beta-D-glucosaminyltransferase and its mechanism of action.

The enzyme in the rough endoplasmic reticulum, being membrane-bound, is activated by Triton-X-100 over the concentration range 0.04-1.0%. Sonication leads to considerable losses of activity if continued for more than about 30s (Fig. 2a). Treatment of the membrane with phospholipase A₂ (EC 3.1.1.4) leads to an extensive loss in activity of the sugar transferase (Fig. 2a). It seems likely that the structural integrity of the membrane is important for the activity of the sugar transferase.

The enzyme in rabbit liver rough endoplasmic reticulum is activated to a small extent by CDP-choline (Fig. 2b). The effects of the nucleotide might be due to inhibition of a pyrophosphatase. On the other hand, CDP-choline might function to stimulate incorporation of N-acetylglucosamine into one or more lipid-linked intermediates (Mookerjea et al., 1972) which might participate in the reaction. N-Acetylglucosamine at concentrations up to 20mM does not appear to inhibit the enzyme.

The mechanism of incorporation in vivo of asparagine-linked carbohydrate moieties of glycoproteins is generally unresolved. Further studies of the N-acetylglucosaminyltransferase are likely to help in understanding this important biosynthetic pathway.

The work was supported by the Cancer Research Campaign and the British Council. Z. K. is a British Council Scholar on leave of absence from Tehran University Nuclear Centre, Tehran, Iran.

Melchers, F. (1973) *Biochemistry* 12, 1471–1475

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**Stability of Lipoprotein Lipase (Clearing-Factor Lipase) in Rat Cardiac Muscle**

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Current evidence suggests that the uptake of fatty acids from lipoprotein triglyceride by the myocardium is dependent on the lipoprotein lipase (clearing-factor lipase) activity present (Enser et al., 1967). In the heart, as in adipose tissue, it has been suggested that the enzyme exists in a functional and a non-functional form (Borensztajn & Robinson, 1970). Also, the functional lipoprotein lipase, which is considered to be directly involved in the hydrolysis of plasma lipoprotein triglyceride, is believed to be located on the surface of the endothelial cells of capillaries (Robinson, 1970). The functional enzyme in heart has to date been distinguished from the non-functional lipoprotein lipase by its rapid release from the tissue by heparin.
When fed rats are deprived of food the total lipoprotein lipase activity in the heart increases progressively, and most of the increase occurs in the heparin-releasable (i.e. proposed functional) fraction (Borensztajn et al., 1970). In contrast, the enzyme activity in adipose tissue, and particularly the functional component, decreases markedly under the same conditions (Cunningham & Robinson, 1969). The uptake of triglyceride fatty acids by adipose tissue from 24h-starved animals is virtually zero (Cryer et al., 1976), whereas the lower activity of the enzyme in the hearts of fed, as compared with similarly starved animals, is still associated with triglyceride fatty acid utilization. Fed animals show about one-third of the maximum rates observed with the hearts from starved animals (Borensztajn & Robinson, 1970). Also, when heparin has been perfused through hearts taken from fed animals, a certain proportion of the total lipoprotein lipase activity present can still be readily released. To investigate further the location of the pools of lipoprotein lipase activity in cardiac muscle, experiments similar to those of Cunningham & Robinson (1969) and Davies & Robinson (1973), which involved study of the stability of the enzyme in a variety of tissue

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**Fig. 1. Loss of lipoprotein lipase activity when heart slices were incubated at 42°C**

Slices (1 mm) from the hearts of either fed (●) or 24h-starved (○) rats were suspended in Krebs-Ringer bicarbonate buffer, pH 7.4, containing 4% (w/v) dialysed bovine serum albumin (either fraction V or essentially fatty acid-free) (Cunningham & Robinson, 1969). Each slice was suspended in 1 ml of medium. The slices were incubated at 42°C for the times specified and in such a way that slices from each heart studied were taken for enzyme assay at various times. The points indicate mean activities calculated from enzyme-activity measurements on between 2 and 13 slices from different hearts, each of which was assayed in duplicate. The bars indicate ±S.E.M. The enzyme activities were measured in aqueous homogenates of the slices. No activity could be detected in the medium.
preparations, have been carried out with heart muscle from both fed and starved rats. In adipose tissue, differences in the stability of the enzyme in preparations from fed and starved rats were primarily attributed to differences in the location of the enzyme in the tissue. The experiments on the stability of lipoprotein lipase present in heart muscle reported here, have led to preliminary studies of the enzyme activity present in isolated cardiocytes.

Fig. 1 shows the decline in lipoprotein lipase activity when 1 mm slices of ventricular tissue from either fed or 24 h starved rats were incubated at 42°C in the previously described medium (Davies & Robinson, 1973). The enzyme activity was measured under the standard assay conditions of Riley & Robinson (1974), and the activity expressed as μmol of free fatty acid released/h per g fresh wt. of tissue. The lipase activity was characterized as lipoprotein lipase in all the preparations by the degree of inhibition (>85%) observed in the presence of 0.6 M-NaCl. In the intact tissue (Fig. 1), the high enzyme activity characteristic of the starved state declined rapidly at first with a half-life of approx. 1 h. During the subsequent incubation period (2–7 h) the remaining enzyme activity

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**Fig. 2. Loss of lipoprotein lipase activity when homogenates of heart tissue and homogenates of acetone/ether-dried preparations of heart were incubated at 42°C**

Homogenates of heart tissue and acetone/ether-dried preparations of heart tissue taken from either fed or 24 h-starved rats were prepared in the medium described in the legend to Fig. 1 at a tissue concentration in both cases of 40 mg of fresh tissue/ml and incubated at 42°C. Samples of each homogenate were removed at the times indicated and the enzyme activity measured. The points (●, tissue homogenate, fed animals; ▲, acetone/ether-dried preparation homogenate, fed animals; ○, tissue homogenate, 24 h-starved animals; △, acetone/ether-dried preparation homogenate, 24 h-starved animals) indicate the means of duplicate determinations on at least two different homogenates of each type. The bars indicate ±S.E.M. when more than two homogenates were studied.
exhibited a considerable degree of stability. Thus for tissue taken from starved animals, 25% of the original activity remained at 2h and 18% still remained after 7h of incubation. This pattern showed considerable similarity to that observed by Davies & Robinson (1973) when adipose tissue from fed rats was incubated over the same time period. In comparison, when cardiac-muscle slices from fed animals were incubated (Fig. 1), the relatively low activity present declined to approximately the same value as that observed in the case of the tissue from starved animals within 2h. Although the absolute activity remaining after incubation was very similar for both the tissues from fed and starved animals, the proportion of the original activity remaining in the case of the tissue from fed animals was higher (approximately 35%). This contrasts with the situation described for adipose tissue from starved rats by Davies & Robinson (1973) in which about 90% of the originally low enzyme activity present was stable to incubation. Fig. 2 illustrates the decline in lipoprotein lipase activity observed when disrupted tissue preparations from either fed or starved animals were studied. In both cases tissues were either homogenized such that no intact cells were visible microscopically or acetone/ether-dried powders of the tissues were prepared without the addition of carrier protein as described previously (Davies et al., 1974). The acetone/ether-dried preparations were rehomogenized in the incubation buffer to study the pattern of enzyme-activity decline. In each of these disrupted tissue preparations the decline in enzyme activity was more rapid and more complete (Fig. 2) than previously observed with intact tissue preparations (Fig. 1). The approximate half-lives for both types of homogenate of hearts from fed and starved animals was of the order of 30min. This rapid, virtually complete loss of activity was similar to that observed previously for acetone/ether-dried preparations of adipose tissue (Davies & Robinson, 1973). By analogy with the adipose-tissue situation it is possible therefore that the relatively low, stable activity of the enzyme associated with intact tissue might be because of the enzyme's association with intact cells and as such might represent that proportion of the total tissue activity which is present in the cardiocytes. To test this possibility, enzyme activity has been measured in isolated cardiocytes, prepared essentially by the method of Powell & Twist (1976) and preliminary results are consistent with the possibility outlined above.


Inhibition of Phosphatidylcholine–Cholesterol Acyltransferase Activity by Mercaptoethanol in Mouse Plasma

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The studies of Glomset (1972) established the importance of the plasma enzyme phosphatidylcholine–cholesterol acyltransferase (EC 2.3.1.43; hereafter called 'acyl-

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