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Lipoprotein Lipase Activity of Rat Cardiac Muscle

THE INTRACELLULAR DISTRIBUTION OF THE ENZYME BETWEEN FRACTIONS PREPARED FROM CARDIAC MUSCLE AND CELLS ISOLATED FROM THE HEARTS OF FED AND STARVED ANIMALS

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1. Subcellular fractions, characterized by using morphological, compositional and enzymic markers, were prepared from rat heart tissue and cells isolated from the hearts of fed and 24h-starved rats. 2. The lipoprotein lipase activity of fractions from whole tissue and isolated cells was determined in either fresh fractions or in acetone/diethyl ether powders of the fractions. 3. Lipoprotein lipase activity was present in all the fractions from tissue and cells, but was found to be of highest relative specific activity in the microsomal (P) fractions. 4. In fractions prepared from the isolated cells of hearts from starved rats the proportion of the total lipoprotein lipase present and its relative specific activity in the microsomal fraction were greater than in the equivalent fractions from fed animals. 5. The enhancement of lipoprotein lipase activity as a result of the acetone/diethyl ether powder preparation of fractions was most extensive in the microsomal fractions. 6. Investigation of the microsomal fraction showed that the lipoprotein lipase activity present was in two pools, one of which was within endoplasmic-reticulum vesicles. 7. The observations were consistent with the possibility that the cardiac-muscle cell could be the origin of the lipoprotein lipase activity functional in triacylglycerol uptake by the heart.

Lipoprotein lipase (clearing-factor lipase; EC 3.1.1.34) regulates the removal of lipoprotein triacylglycerol fatty acids from the plasma by the extrahepatic tissues (Robinson, 1970). There is now considerable evidence to suggest that the changes in the enzymic activity that occur in response to a variety of conditions correlate closely with changes in the triacylglycerol fatty acid uptake of tissues such as heart (Rogers & Robinson, 1974), adipose tissue (Cryer *et al.*, 1976), mammary gland (Scow *et al.*, 1976) and skeletal muscle (Linder *et al.*, 1976). The general nature of the control exerted on these changes in enzyme activity has been studied extensively, particularly in adipose tissue (Robinson & Wing, 1970; Garfinkel & Schotz, 1973; Davies *et al.*, 1974; Cryer *et al.*, 1975*a*; Robinson *et al.*, 1975) and heart (Borensztajn & Robinson, 1970; Borensztajn *et al.*, 1973, 1975; Rogers & Robinson, 1974; Rault *et al.*, 1974). It is generally accepted that although the enzyme exerts its action at the luminal surface of the endothelial cell the source of the enzyme is either the adipocyte in adipose tissue (Robinson & Wing, 1971; Cryer *et al.*, 1975*b*; Nilsson-Ehle *et al.*, 1976) or the non-endothelial cells of the cardiac tissue (Borensztajn *et al.*, 1975; Chajek *et al.*, 1975, 1978*a*,*b*; Henson *et al.*, 1977; Chohan & Cryer, 1978) respectively. Although work with isolated-cell systems has revealed some aspects of the enzyme secretion from these cell types (Cryer *et al.*, 1975*b*; Chajek *et al.*, 1975, 1978*a*), little information is available concerning the intracellular events involved. As a preliminary step towards the elucidation of such events in adipose tissue the subcellular localization of fat-cell lipoprotein lipase activity has now been studied (Wolf *et al.*, 1975; Vanhove *et al.*, 1978). However, despite early interest (Alousi & Mallov, 1964; Gartner & Vahouny, 1966) a paucity of information concerning the subcellular distribution of the enzyme in preparations from heart tissue still exists. Our recent characterization of isolated-cell preparations from rat heart that are rich in both muscle cells and intracellular lipoprotein lipase activity (Chohan & Cryer, 1978) now affords the opportunity to study the subcellular localization of the enzyme in both fractions from cardiac tissue and in fractions from cells isolated from adult rat hearts.

Materials and Methods

Animals and tissues

Male rats of the MRC Hooded strain, from the colony of this Department, were used throughout.

The rats weighed 190–210g in the fed state and were maintained on modified diet 41B (Pilsbury and Co., Birmingham B5 7UG, U.K.). All the animals used in either the fed state or after 24h of starvation were killed by cervical dislocation, between 08:00h and 09:30h. Immediately after the death of the rats, hearts were rapidly excised and either used in the subcellular-fractionation studies or used for the preparation of isolated cells to be used in further fractionation studies.

Subcellular fractionation

(*a*) *Homogenization of heart tissue.* The hearts of either fed or 24h-starved rats were excised completely and the thin-walled auricular tissue and any remaining blood vessels were removed. The ventricular chambers were opened and the blood they contained washed away with 154mM-NaCl solution. The pooled tissue from up to eight hearts was then blotted dry and weighed. The tissue was minced exhaustively with scissors and suspended at a concentration of 30% (w/v) in 0.25M-sucrose solution containing 25mM-Tris/HCl buffer, pH7.4, containing 2.5mM-EGTA and 2.5mM-$MgCl_2$ at 4°C. This initial (30%, w/v) suspension was homogenized in a glass/Teflon Potter–Elvehjem homogenizer of 15ml capacity and an overall clearance of 0.3mm. The homogenization step entailed a single stroke of the pestle, which was rotated at motor setting 5 of a Tri-R stirrer (Tri-R Instruments Inc., New York, NY, U.S.A.). The suspension was then filtered through nylon mesh (0.1mm diameter). The residue on the mesh was removed and resuspended in the original volume of buffer. The homogenization step and the filtration were then repeated. The filtrates were pooled and the residue was treated again. The final concentration of the filtered homogenate prepared by this procedure was 10% (w/v).

(*b*) *Preparation and homogenization of cells isolated from hearts.* Cells were prepared from the hearts of either fed or 24h-starved rats by the modified procedure of Powell & Twist (1976) described by Chohan & Cryer (1978). The washed cells obtained from eight hearts were suspended in the buffered sucrose solution described above (5×10^6–6×10^6 cells/ml). The suspension was homogenized in an all-glass Dounce homogenizer (Kontes Glass Co., Vineland, NJ, U.S.A.) with the B pestle. The initial suspension was subjected to a single pass of the pestle. The material was then centrifuged at 100*g* for 2min to sediment unbroken cells. The supernatant was removed and set aside in an ice bath and the pellet washed by resuspension and recentrifugation. The supernatant from the wash was pooled with the original supernatant. The washed pellet was resuspended and the homogenization procedure repeated. The procedure was repeated seven times in all and the supernatants were pooled.

(*c*) *Centrifugal separation of fractions from homogenates of heart tissue and isolated cells.* Like the homogenizations, all the steps in the centrifugal procedure were carried out at 4°C. Subcellular fractions were obtained from the homogenates of both heart tissue and isolated cells by using the differential-centrifugation procedure described by Wang *et al.* (1977).

Before this procedure intact cells and fibrous material present in the crude homogenate were removed by centrifugation ($120g_{av.}$ for 5min) to give a preparation (homogenate 2) with which the isolated fractions were compared. Homogenate 2 contained more than 80% of the marker-enzyme activities, more than 75% of the lipoprotein lipase activity and more than 65% of the protein present in the crude homogenate.

The fractions obtained were designated nuclear (N), mitochondrial (M), lysosomal (L), microsomal (P) and soluble (cytosol; S). The fractions were characterized by light and electron microscopy and from the distribution of marker-enzyme activities. When sufficient material was available the pelleted fractions were washed by resuspension in the original volume of homogenization medium and subjected to re-centrifugation under the conditions appropriate for the recovery of the fraction in question. The nuclei and mitochondria of both tissue and cells and the lysosomes of tissue were washed, but insufficient material was available for the other fractions to be treated in this way.

Assay of markers for subcellular fractions

(*a*) *Protein.* Protein was determined by the method of Lowry *et al.* (1951), with dry bovine serum albumin [Sigma (London) Chemical Co., Kingston upon Thames, Surrey KT2 7BH, U.K.] as standard.

(*b*) *DNA.* DNA was determined by the method of Burton (1956) with a highly polymerized standard obtained from Sigma.

(*c*) *Marker-enzyme assays.* Enzymes were assayed by using the following methods: uridine diphosphatase (EC 3.6.1.6), Plaut (1963); glucose 6-phosphatase (EC 3.1.3.9), Baginski *et al.* (1974). The phosphate released in these enzymic reactions was determined by the method of Parvin & Smith (1969). Lactate dehydrogenase (EC 1.1.1.27) was assayed by the method of Bergmeyer & Bernt (1974), 5′-nucleotidase (5′-ribonucleotide phosphohydrolase; EC 3.1.3.5) by the method of Michell & Hawthorne (1965), succinate–cytochrome *c* oxidoreductase by the method of Polakis *et al.* (1965), NADPH–cytochrome *c* oxidoreductase (EC 1.6.99.1) by the method of Sottocasa *et al.* (1967) and cathepsin D (EC 3.4.23.5) by the method of Richards & Wusteman (1974).

Preparation of fractions, tissue and cells for lipoprotein lipase assay

The lipoprotein lipase activity of fractions, tissue and cells was determined in two types of preparation. The first was an aqueous homogenate of the material prepared in 50mM-NH_3/HCl buffer, pH8.1. The second was a homogenate of acetone/diethyl ether-dried powders of the material, prepared, with casein as carrier, as described by Cunningham & Robinson (1969) and Cryer & Jones (1978). The supernatant fractions were freeze-dried before acetone/diethyl ether-powder preparation.

Measurement of lipoprotein lipase activity

Total lipoprotein lipase activities were determined as the rate of non-esterified fatty acid release from an apolipoprotein C-II-activated triacylglycerol emulsion (Intralipid; Vitrum, Stockholm, Sweden) at 30°C as described by Cryer & Jones (1978). The enzyme was characterized in all the preparations by the degree of inhibition (>90% in most cases) observed when 0.6M-NaCl was present in the assay and by the obligatory requirement for serum in the assay. Activities and distributions are expressed as means ± S.E.M. with the numbers of observations in parentheses. Student's *t* test (Fisher & Yates, 1957) was used to assess the significance of the differences between means.

Further fractionation and incubation procedures

In some experiments membrane contamination of the nuclear, mitochondrial and lysosomal fractions (Widnell & Tata, 1964) was removed centrifugally. The membrane contamination of the nuclear fraction was removed by using the discontinuous-density-gradient system of Widnell & Tata (1964). In these experiments the washed nuclear pellet suspended in the buffered 0.25M-sucrose described above was layered on a similarly buffered 2.4M-sucrose solution and centrifuged for 1h at 4°C and 50000$g_{av.}$ by using an SW41 rotor in a Beckman L-2 65B centrifuge. The membranes collected near the interface and the nuclei formed a pellet. A similar procedure was used to remove membranous material from the mitochondrial and lysosomal fractions, but with these fractions the concentration of the underlying sucrose solution was 0.32M and the head was operated for 10min at 4°C and 10000$g_{av.}$ (Maggio *et al.*, 1963). The trypsin (EC 3.4.4.4; diphenylcarbamoyl chloride-treated; 9000 units as defined by the manufacturer/mg) and sodium deoxycholate used in the incubation experiments with microsomal fractions (see below) were obtained from Sigma.

Results and Discussion

Characterization of fractions obtained by differential centrifugation from homogenates of rat heart and cells isolated from heart tissue

By using the homogenization and centrifugation programmes described it was possible to prepare consistent subcellular fractions from rat hearts and isolated cells.

The distribution of the markers investigated between the fractions prepared from homogenates of both whole cardiac tissue and isolated cardiac muscle cells are shown in Table 1. To permit comparison between one marker and another the distributions of their relative specific activities among the fractions are shown in Fig. 1.

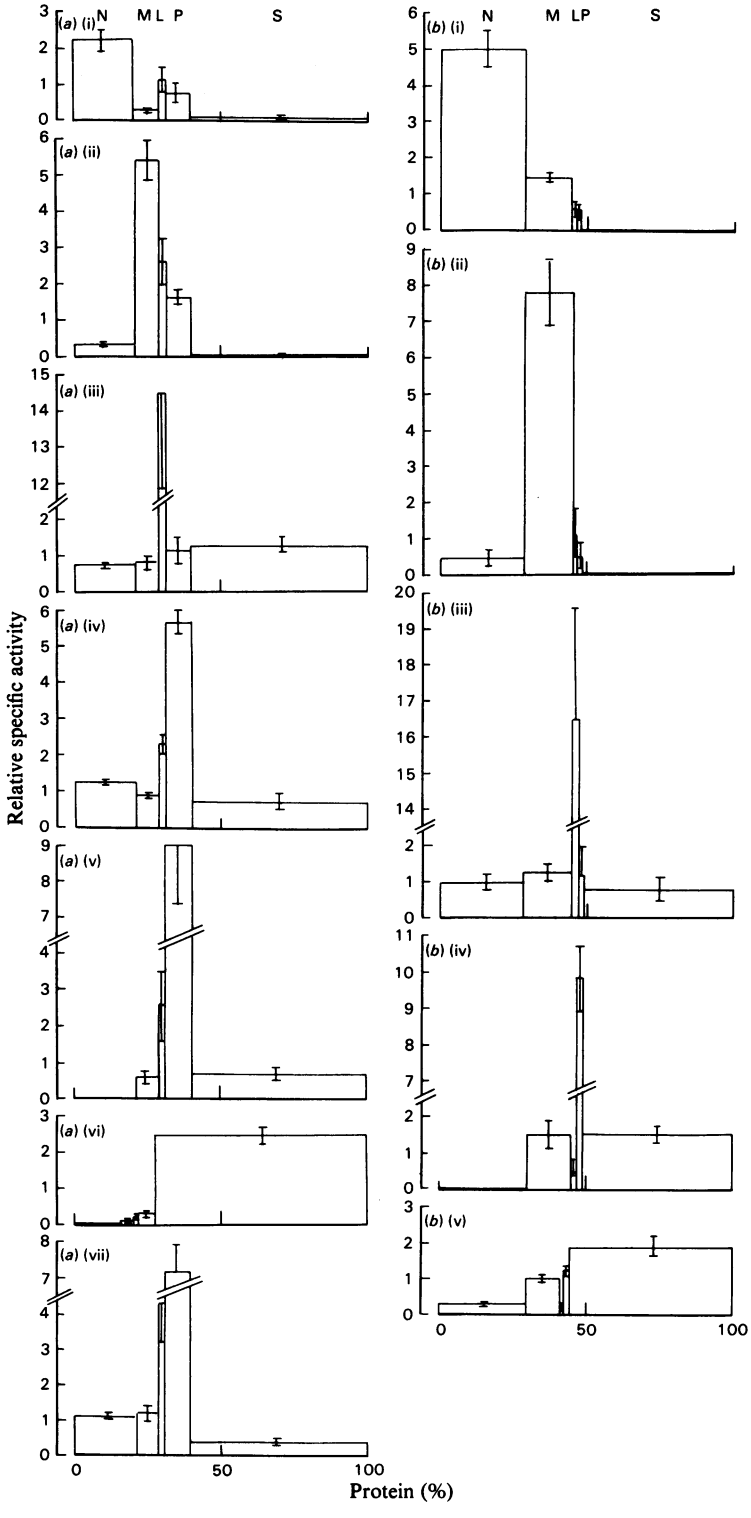
Considering the fractions in turn. The nuclear fractions were characterized by high recovery and increased relative specific concentrations of DNA. Both enzymic and electron-micrographic observations of this fraction indicated the relative absence of mitochondrial and microsomal material. The markers in the mitochondrial fraction showed a distinct distribution and this fraction contained most of the recovered succinate–cytochrome *c* oxidoreductase. The mitochondria isolated from tissue and cells showed, both from enzymic and electron-microscopic observations, a substantial freedom from specific contamination with other organelles.

The presence of lysosomes in rat heart tissue has been reported previously (Franson *et al.*, 1972; Wang *et al.*, 1977) and it is clear that under the conditions of the present experiments lysosomal particles at least as well characterized as those previously described have been prepared from both rat heart tissue and the isolated heart-muscle cells. The distribution of the microsomal markers uridine diphosphatase and NADPH–cytochrome *c* oxidoreductase indicates the relative purity of the microsomal fractions obtained.

The distribution of 5′-nucleotidase, a known plasma-membrane marker for the heart (Tada *et al.*, 1972; Saccomani *et al.*, 1974), which is shown in Table 1 and Fig. 1, was typical of previously reported studies that used whole cardiac tissue as starting material (Wang *et al.*, 1977; Bloomfield *et al.*, 1977), with the microsomal fraction containing the highest proportion of the activity. Electron-micrographic observation of the microsomal fractions from both tissue and cells showed them to be rich in smooth vesicles.

Distribution of lipoprotein lipase activity in subcellular fractions prepared from homogenates of cardiac tissue and cardiac-muscle cells obtained from fed or 24h-starved rats

Table 2 shows the distribution of lipoprotein lipase


(a) (i)
N
M
L
P
S
(a) (ii)
(a) (iii)
(a) (iv)
(a) (v)
(a) (vi)
(a) (vii)
(b) (i)
(b) (ii)
(b) (iii)
(b) (iv)
(b) (v)
Relative specific activity
Protein (%)
0
50
100

activity between fractions isolated from cardiac tissue obtained from fed and 24h-starved rats. The difference, already described by Borensztajn *et al.* (1970), between total tissue activity in fed and starved animals was also discernible in the absolute enzyme activities recovered in the subcellular fractions studied in the present paper. The enhancement in the measured activity in the fractions from both fed and starved cases as a result of acetone/diethyl ether treatment was obvious in all the fractions, with the marked exception of the cytosol (S) fraction, and was greatest in the microsomal (P) fraction. With fresh fractions of heart tissue assayed for lipoprotein lipase activity directly after preparation, the percentage distribution of activity between the fractions was unaffected by nutritional status with approx. 20% of the total activity being found in the microsomal fraction. This contrasts with the 10% of total lipoprotein lipase activity found in this fraction prepared from the epididymal adipose tissue of fed and starved animals by Vanhove *et al.* (1978). The identical pattern of distributions in the fresh fractions of the fed and starved cases was altered by acetone/diethyl ether powder preparation before enzyme assay. There was an apparent enrichment in the activity of lipoprotein lipase found in the particulate fractions, particularly the mitochondrial (M) and microsomal fractions (P) of both homogenate types at the expense of the cytosol (S) fraction.

Table 3 shows the results collected during similar fractionation studies with isolated cardiac-muscle cells. The overall distribution of protein between the fractions isolated from cell homogenates (Table 3) was distinct from that observed for whole-tissue fractions (Table 2). Particularly, less protein was recovered in the lysosomal and microsomal fractions from cells and proportionately more appeared in the cytosol (S) fraction of these homogenates when compared with those prepared from whole tissue.

As previously noted (Chohan & Cryer, 1978) the total lipoprotein lipase activity recovered in suspensions of cells prepared from adult rat hearts was unaffected by the nutritional state of the animals used. Table 3 also reaffirms that the enhancement in measured activity observed after acetone/diethyl ether-powder preparation was even more pronounced in the case of cells than with whole tissue (see also Chohan & Cryer, 1978). The distribution of lipoprotein lipase among the fractions prepared from homogenates of isolated cells was affected both by the nutritional status of the animal from which the hearts for cell preparation were obtained and by the type of preparation used in the assay of the enzyme. The microsomal fraction of cells isolated from the hearts of 24h-starved animals contained a significantly greater ($P<0.05$) proportion of the total homogenate activity than those from fed animals when fresh fractions were assayed. The difference in enzyme activity between the microsomal fractions from these two homogenate types was more pronounced ($P<0.001$) when acetone/diethyl ether powders of the fractions were used for the assay of lipoprotein lipase activity. Fig. 2 shows that the relative specific activity of lipoprotein lipase was generally highest in the microsomal fractions prepared from homogenates of both tissue and cells. Although the relative specific activity of the enzyme in the microsomal fraction prepared from whole tissue was unaffected by nutritional status in similar fractions from isolated cells, the relative specific activity was higher in preparations from starved compared with fed animals. This difference in the activity of cell fractions from fed and starved animals was accentuated in acetone/diethyl ether-powder preparations of the fractions.

Effects of NaCl on the lipoprotein lipase activity of homogenates and fractions

Although all the preparations were assayed under identical conditions and the lipoprotein lipase activity of homogenates and fractions prepared from whole tissue were inhibited by between 86 to 98% by 0.6M-NaCl, some of the cell fractions showed less clear responses. Thus although a high degree of inhibition was found in assays of cell homogenate enzyme activities and those of microsomal fractions, the percentage inhibition produced by NaCl in the lysosomal and mitochondrial fractions of cells was only between 52 ± 18 (mean$\pm$S.D.; $n=4$) and 76 ± 6 (mean$\pm$S.D.; $n=5$) % for both starved and fed cases. The activity of lipoprotein lipase in the cytosol (S) fractions from cells was inhibited by

Fig. 1. *Distribution of markers in subcellular fractions from rat heart tissue and isolated cardiac-muscle cells*
Each of the tissue or cell fractions from six separate fractionation experiments of each were assayed for the range of enzyme activities shown, and also DNA and protein. The results obtained for fractions from heart tissue homogenates are shown in (*a*) and those for cells isolated from heart tissue in (*b*). N, M, L, P and S represent the nuclear, mitochondrial, lysosomal, microsomal and cytoplasmic (soluble) fractions respectively. For (*a*) and (*b*), (i) shows DNA content, (ii) shows succinate–cytochrome *c* oxidoreductase activity and (iii) shows cathepsin D activity. (*a*) (iv) shows uridine diphosphatase activity, (*a*) (v) shows NADPH–cytochrome *c* oxidoreductase activity, (*a*) (vi) shows lactate dehydrogenase activity, and (*a*) (vii) shows 5′-nucleotidase activity. In (*b*), (iv) shows NADPH–cytochrome *c* oxidoreductase activity, and (v) shows lactate dehydrogenase activity. Relative specific activity is the percentage of enzyme activity in the fraction/the percentage of protein in the fraction. Bars indicate S.E.M.

Table 1. *Fractionation of homogenates of rat heart tissue and isolated cells from rat hearts by differential centrifugation*

The homogenates of tissue or cells and subcellular fractions from them prepared as described in the Materials and Methods section were assayed for DNA, protein and appropriate enzyme markers as described in the text. The distribution of each marker in the various fractions is presented as a percentage relative to the total recovered activity, which was in all cases greater then 75% of the homogenate 2 activity. The values are given as means ± S.E.M. for six independent experiments with whole cardiac tissue and seven independent experiments with isolated cells. There was no difference in the analysis of the fractions when material from either fed or 24h-starved rats were used.

Fraction	Source	DNA (%)	Protein (%)	Succinate–cytochrome *c* oxidoreductase (%)	Cathepsin D (%)	Uridine diphosphatase (%)	NADPH–cytochrome *c* oxidoreductase (%)	Lactate dehydrogenase (%)	5′-Nucleotidase (%)
Nuclei (N)	Tissue	78±6	20±4	9±2	17±2	30±3	0.2±0.2	1±0.1	27±2
	Cells	74±3	28±4	6±3	20±3	—	0	5±0.6	—
Mitochondria (M)	Tissue	78±1	9±1	68±2	10±3	11±2	18±5	1±0.1	13±2
	Cells	26±2	16±1	91±4	18±4	—	18±4	14±2	—
Lysosomes (L)	Tissue	3±0.9	4±0.2	10±2	24±6	8±2	5±1	0.4±0.03	11±1
	Cells	0.7±0.3	1±0.1	1.7±1	12±2	—	0.3±0.3	0.7±0.4	—
Microsomal fraction (P)	Tissue	4±1	6±0.6	15±2	5±2	28±4	49±6	2±0.2	34±2
	Cells	0.7±0.2	1±0.1	0.6±0.3	1±1	—	8±2	1±0.3	—
Cytosol (S)	Tissue	4±2	59±4	4±1	44±7	24±7	28±8	96±1	14±2
	Cells	0.0	53±4	0.0	44±12	—	67±5	78±3	—

Table 2. *Distribution of lipoprotein lipase in fractions from homogenates prepared from the cardiac tissue of fed and 24h-starved rats*

The tissue homogenates (homogenate 2) and subcellular fractions isolated as described in the text were assayed for lipoprotein lipase activity. The enzyme assay was carried out directly on the fractions or homogenates, or on homogenates of acetone/diethyl ether powders prepared from the fractions. In some experiments assays of lipoprotein lipase activity on single fractions were carried out with both types of preparation. The values are given as means ± S.E.M. for results collected from either four or five independent fractionations. In most of the experiments the marker-enzyme activities described in the text were measured to check the integrity of the fractions. These results have not been included since they reiterate those in Table 1 and Fig. 1(*a*). Lipoprotein lipase activity is expressed as μmol of non-esterified fatty acid released/fraction. The percentage of total protein recovered in the fractions was the same in all cases and represented: N, 30±1.8(18); M, 13.4±1.3(18); L, 4.1±0.4(18); P, 6.75±0.7(18); S, 48.0±2.0(18), the results being means ± S.E.M. for the numbers of independent observations shown in parentheses.

	Lipoprotein lipase activity							
Nutritional state ...	Fed				24h-starved			
Assay preparation ...	Fresh		Acetone/diethyl ether powder		Fresh		Acetone/diethyl ether powder	
Fraction	(units/fraction)	(%)	(units/fraction)	(%)	(units/fraction)	(%)	(units/fraction)	(%)
Homogenate	59±3	—	130±3	—	125±15	—	196±30	—
Nuclei (N)	11±2	20±3	28±8	26±4	21±4	23±3	37±8	25±2
Mitochondria (M)	6±1	12±2	21±3	22±2	12±1	14±2	29±5	21±3
Lysosomes (L)	5±0.5	12±3	10±2	9±0.5	9±2	11±1	9±2	7±2
Microsomal fraction (P)	11±1	22±1	32±4	32±2	15±2	17±1	48±11	32±4
Cytosol (S)	20±2	37±2	10±1	12±2	29±4	35±2	24±6	16±2
Recovery	89.5		78.4		68		75	

Table 3. *Distribution of lipoprotein lipase in fractions from homogenates of isolated cardiac-muscle cells from the hearts of fed and 24h-starved rats*

The cells and subcellular fractions of cells prepared as described in the text were assayed for lipoprotein lipase activity. The conditions of the study were described in the text and the legend to Table 2. The values represent cumulative data from either three or four independent experiments. Other details are as described in the legend to Table 2. The percentage of total protein recovered in the fractions was the same in all cases and represented: N, 22±2 (15); M, 17.3±1.4 (15); L, 1.5±0.15 (15); P, 1.47±0.16 (15); S, 58±1.3 (15), the results being means±S.E.M. for the numbers of independent observations shown in parentheses.

Nutritional state ...	Lipoprotein lipase activity							
	Fed				24h-starved			
Assay preparation ...	Fresh		Acetone/diethyl ether powder		Fresh		Acetone/diethyl ether powder	
Fraction	(units/fraction)	(%)	(units/fraction)	(%)	(units/fraction)	(%)	(units/fraction)	(%)
Homogenate	9±1	—	23±2	—	7±1	—	23±3	—
Nuclei (N)	2±0.2	39±1	4±1	40±7	1±0.3	40±5	3±1	34±8
Mitochondria (M)	1±0	21±1	1.5±0.4	16±4	1±0.1	23±3	1±0.2	13±2
Lysosomes (L)	0.1±0.01	2.5±0.3	1.2±0.4	10±3	0.06±0.02	2.1±0.6	0.7±0.1	8±1
Microsomal fraction (P)	0.4±0.0	9.7±0.1	1.5±0.5	17±6	0.4±0.1	13±4	3±0.3	31±4
Cytosol (S)	1.3±0.05	28±1	1.6±0.1	17±2	0.7±0.4	23±12	1.2±0.5	15±5
Recovery	52		43		44		40	

only 28±2 (mean±S.D.) and 36±23 (mean±S.D.) % in preparations from fed and starved animals respectively. Although corrections for this salt-resistant lipase activity present in these fractions have not been applied to the data in Table 3 and Fig. 2, the result of doing so would be to cause a relative enhancement of the proportional and relative specific activities of the enzyme in the microsomal fraction. The pattern of activity distribution between the fractions was not altered by this correction procedure.

Subfractionations, washing and incubation treatments applied to isolated fractions from homogenates of cardiac tissue

When loosely bound membranous material was removed by the discontinuous-density-gradient methods described in the Materials and Methods section the specific activity (units of enzyme activity/mg of protein) of lipoprotein lipase associated with the nuclear, mitochondrial and lysosomal fractions was unaffected. The activity lost from these fractions was found to be present in the separated membranes, where it was present at high specific activity together with a high specific activity of 5′-nucleotidase. The lipoprotein lipase activity associated with membrane-material-free nuclei could not be further localized in the organelle, since conditions specific for the removal of the outer nuclear membrane (Gurr *et al.*, 1963) lead to the irreversible inactivation of the enzyme. The exposure of nuclei to 1 or 5 units (as defined by the manufacturer) of heparin (Pularin; Evans Medical, Speke, Liverpool, U.K.) in the absence of phosphate, when no disruption of the organelle should occur (Bornens, 1973), was able to release only 15% of the lipoprotein lipase from the particulate material. Similarly when fractions from homogenates of either whole tissue or cells were exposed to 0.5M-NaCl solution only 10, 5, 26 and 10% of the lipoprotein lipase activity present in membrane-free washed nuclei, mitochondria, lysosomes and microsomal fraction were released from the organelles respectively. However, this small extent of release seemed not to be specific, since the specific activity of the enzyme in the supernatants from the 0.5M-NaCl solution treatment was lower than in the fractions. Also the specific activity of the enzyme in the fractions was unaffected by the treatment. However, the lipoprotein lipase activity of the nuclear, mitochondrial and lysosomal fractions seems to be significant in terms of the total distribution of enzyme activity and was quite firmly associated with the fractions. The significance of these observations is as yet unknown, but the possibility that artifactual post-homogenization association of the enzyme with these fractions occurred still remains.

A much increased relative specific activity of the enzyme was present in the microsomal fraction and

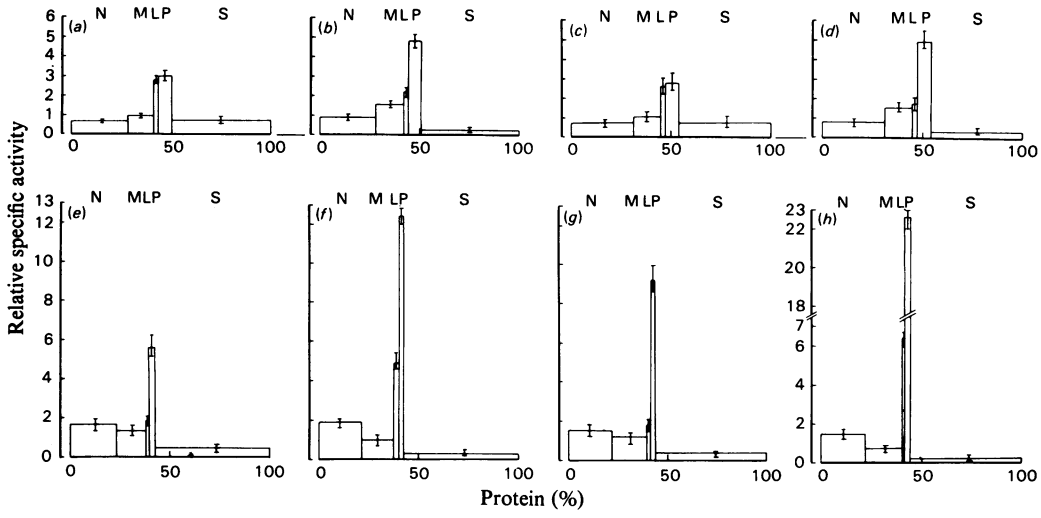


Fig. 2. *Distribution of lipoprotein lipase activity between fractions prepared from cardiac tissue and cells isolated from the hearts of fed and 24h-starved rats*

The lipoprotein lipase activity of subcellular fractions prepared from the hearts or isolated cells from the hearts of fed or 24h-starved rats was assayed either in fresh fractions or in acetone/diethyl ether powders of the fractions (see the text and the legend to Table 2). The diagrams represent (*a*) fresh fractions obtained from heart tissue of fed animals, (*b*) acetone/diethyl ether powders of fractions obtained from heart tissue of fed animals, (*c*) fresh fractions obtained from heart tissue of 24h-starved animals, (*d*) acetone/diethyl ether powders from the hearts of fed animals, (*e*) fresh fractions obtained from cells isolated from the hearts of fed animals, (*f*) acetone/diethyl ether powders of fractions obtained from cells isolated from the hearts of fed animals, (*g*) fresh fractions obtained from cells isolated from the hearts of 24h-starved animals and (*h*) acetone/diethyl ether powders of fractions obtained from cells isolated from the hearts of 24h-starved animals. Bars indicate S.E.M.

made this fraction of special interest. Particularly the difference between the lipoprotein lipase activity measured in fresh and acetone/diethyl ether-powder preparations was investigated further. First, the possibility of there being an inhibitor in the fraction, which was solubilized and removed by organic solvent treatment, was considered. Thus in two separate experiments the solvents used during acetone/diethyl ether-powder preparations were evaporated to dryness and the dried extract was reconstituted with the powder at the original concentration by either homogenization or sonication treatments. Neither means of reconstitution affected the activity of lipoprotein lipase measured in the acetone/diethyl ether powders of microsomal fractions, thus excluding the possibility of an acetone/diethyl ether-soluble inhibitor.

The enhancement in the microsomal lipoprotein lipase activity by acetone/diethyl ether powder preparation might therefore be considered to reflect the location of the enzyme within vesicular structures, where it may be unavailable for assay in the fresh fractions. Thus experiments that used conditions previously shown to release the contents of rat liver microsomal fractions (Kreibich *et al.*, 1973) were carried out with the rat heart microsomal fractions. In these experiments microsomal fractions from rat cardiac tissue were incubated at 0°C for 30min with seven different concentrations of deoxycholate ranging from 0.05 to 0.35% (w/v). These concentrations were not inhibitory to soluble lipoprotein lipase activity from the cytosol fractions and were found to have no effect on the activity of lipoprotein lipase detectable in assays of fresh microsomal fractions. Modified conditions for microsomal incubations were then developed. In these latter experiments microsomal fractions were incubated for 10min at 30°C in the absence or presence of 0.35% (w/v) deoxycholate and/or 50μg of trypsin/ml. The re-sedimented microsomal fractions and the supernatant fractions from these incubations were assayed for lipoprotein lipase activity directly or in some cases after acetone/diethyl ether-powder preparation. The results of these incubation experiments are summarized in Table 4. Although a decrease in total activity in the system incubated for 10min at 30°C occurred (to between 66 and 71% of original activity) (preparation B) a proportion of the microsomal activity, previously wholly particulate (preparation A), was released into the supernatant fraction. However, the particulate material after this incubation (the pellet of preparation B) responded to acetone/diethyl ether-powder treatment in the same manner as the

Table 4. *Effect of incubation with deoxycholate and trypsin on rat heart microsomal lipoprotein lipase activity*
Microsomal fractions prepared from cardiac tissue from either fed or 24h-starved rats were pooled and re-suspended in 10ml of buffered-sucrose medium. The lipoprotein lipase activity of the microsomal suspensions was assayed immediately, either directly or after acetone/diethyl ether-powder preparation, when no effects were seen, or after incubation for 10min at 30°C in the absence or presence of 0.35% (w/v) deoxycholate and/or 50μg of trypsin/ml. After incubation the suspensions were exposed to the gravitational field used to sediment microsomal fractions and the pellets and supernatants were assayed for lipoprotein lipase. The supernatant fractions were assayed immediately and the pellets were assayed either immediately or after acetone/diethyl ether-powder preparation. The activities measured in the various fractions from two separate and representative experiments are shown expressed in all cases as μmol of non-esterified fatty acid released/h per total fraction.

		Lipoprotein lipase activity (μmol of non-esterified fatty acid/h per fraction)			
		Fresh material		Acetone/diethyl ether powders	
Preparation	Expt. no. ...	1	2	1	2
(A) Unincubated microsomal fraction; suspension		29.7	23.3	102.9	83.7
(B) Microsomal fraction incubated for 10min at 30°C	Pellet	13.6	12.4	66.4	47.3
	Supernatant	6.1	4.2	—	—
(C) Microsomal fraction incubated for 10min at 30°C with deoxycholate	Pellet	20.2	—	31.1	—
	Supernatant	31.7	—	—	—
(D) Microsomal fraction incubated for 10min at 30°C with trypsin	Pellet	2.1	0.0	69.1	37.0
	Supernatant	1.5	1.0	—	—
(E) Microsomal fractions incubated for 10min at 30°C with deoxycholate and trypsin	Pellet	0.5	1.2	3.3	3.3
	Supernatant	1.4	1.9	—	—

unincubated microsomal fractions, showing a similar degree of lipoprotein lipase activity enhancement to the original material. When microsomal fractions were incubated with deoxycholate under these conditions considerable amounts of lipoprotein lipase activity were released into the supernatant, and the degree of enzyme activity enhancement observed after acetone/diethyl ether-powder preparation declined (preparation C) when compared with the previous cases (preparations A and B). The total measured enzyme activity after deoxycholate treatment was approximately twice that determined in the unincubated and control incubated cases. In preparation D when microsomal fractions were incubated for 10min with trypsin (50μg/ml) at 30°C very low amounts of activity were recovered in both pellet and supernatant, subsequent to incubation, although the equivalent concentration of trypsin included in the lipoprotein lipase assay incubation alone was without effect. If the pelleted material from this treatment, containing little or no lipoprotein lipase activity, was subjected to acetone/diethyl ether-powder treatment an almost complete recovery of activity equivalent to those seen for this fraction in preparation B was achieved. This situation was not, however, repeated when deoxycholate was included together with trypsin in preparation E. These observations together with the known distribution of enzymic and morphological markers in the heart microsomal fraction may be reconciled by the following interpretation. Although some of the lipoprotein lipase activity of the microsomal fraction was available for assay in fresh fractions a further location of the enzyme could be within vesicular structures where it was unavailable for assay except after membrane disruption by acetone/diethyl ether-powder preparation or deoxycholate treatment. Although the readily assayed lipoprotein lipase activity of fresh fractions was susceptible to trypsin, provided the vesicular structures were not disrupted the other latent lipoprotein lipase activity was protected and could be subsequently released and its activity expressed. This protected proportion of the lipoprotein lipase activity of the total fraction could be further proposed to be normally present in the lumen of the endoplasmic reticulum, which on tissue disruption is known to form closed vesicles, with the same inside–outside orientation as *in vivo* (Palade & Siekevitz, 1956). It might be further speculated that the lipoprotein lipase available for assay in fresh untreated fractions may be enzyme associated with the outside of the cell plasma membrane. This may be considered consistent since, first, heart plasma membranes form vesicles (Kidwai *et al.*, 1971; Barr *et al.*, 1974) that, from fat-cell (Jarett & Smith, 1974) and hepatocyte (Wisher & Evans, 1975, 1977)

observations, would be expected to have retained their functional polarity *in vivo*, and, secondly, in our experiments the microsomal fraction, under normal circumstances, had a lipoprotein lipase activity that was exclusively particle-bound, a high relative specific activity of 5′-nucleotidase (known to be oriented to the outside of plasma membrane vesicles; Widnell, 1972), a morphology rich in smooth vesicles and also had a fraction of total lipoprotein lipase activity [which, as a glycoprotein (Chung & Scanu, 1977), might be expected to orient towards the outside of the plasma membrane (Steck & Dawson, 1974; Bretscher & Raff, 1977)] that was susceptible to the action of trypsin.

Although the microsomal fraction of the cells from starved animals had a higher lipoprotein lipase activity than that from fed animals it is not possible to indicate in which of the two putative pools the principal enhancement occurred, but both sites may well be involved in the enhanced rate of enzyme secretion that would be necessary to maintain the increased activity of functional (heparin-releasable) enzyme activity reported to be at the endothelial cell surface during periods of starvation (Chajek *et al.*, 1975; Borensztajn *et al.*, 1975).

However, in general, the present results indicate that the distribution of lipoprotein lipase within the heart cell is consistent with the enzyme being secreted from the cell. The situation in heart tissue and cells reported in the present paper complements that already reported for fat-tissue and adipocyte lipoprotein lipase distributions and the alterations induced by nutritional changes (Vanhove *et al.*, 1978). The control of these changes in both adipose and heart tissue deserves further investigation.

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