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# Differences in the Metabolism of Very-Low-Density Lipoproteins by Isolated Beating-Heart Cells and the Isolated Perfused Rat Heart

## EVIDENCE FOR COLLAGENASE-RELEASED EXTRACELLULAR LIPOPROTEIN LIPASE

Otthakal V. RAJARAM, Michael G. CLARK and Philip J. BARTER
*Clinical Biochemistry Unit, School of Medicine, The Flinders University of South Australia, Bedford Park, South Australia* 5042, *Australia*

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1. The metabolism of VLD lipoproteins (very-low-density lipoproteins) was studied in intact isolated beating-heart cells and isolated perfused rat heart from starved animals by using [$^{14}$C]triacylglycerol fatty acid-labelled VLD lipoprotein prepared from rats previously injected with [1-$^{14}$C]palmitate. 2. $^{14}$C-labelled VLD lipoprotein was metabolized by the isolated perfused heart, but was only minimally metabolized by the heart cells unless an exogenous source of lipoprotein lipase was added. 3. Measurements of lipoprotein lipase at pH 7.4 with the natural substrate $^{14}$C-labelled VLD lipoprotein indicated that during collagenase perfusion of the heart the enzyme was released into the perfusate, the activity released being proportional to the concentration of collagenase used. Lipoprotein lipase activity in homogenates of hearts that had been perfused with collagenase showed a corresponding loss of activity. 4. At high perfusate concentrations of collagenase, inactivation of the released lipoprotein lipase occurred. 5. Lipoprotein lipase activity was largely undetectable in the homogenate of the isolated heart cells. 6. It is concluded that the lipoprotein lipase responsible for the hydrolysis of VLD lipoprotein triacylglycerol is predominantly located externally to the heart muscle cells and that its release can be facilitated by perfusion of the heart with bacterial collagenase.

There have been a variety of approaches directed towards elucidating the mechanism by which the heart hydrolyses plasma triacylglycerol, which is known to be an important source of energy for heart muscle cells (Olson & Hoeschen, 1967; Crass *et al.*, 1971; Crass, 1977). Studies performed *in vivo* (Eisenberg & Levy, 1975), in isolated perfused hearts (Delcher *et al.*, 1965; Enser *et al.*, 1967; Fielding & Higgins, 1974; Higgins & Fielding, 1975) and *in vitro* (Eisenberg & Rachmilewitz, 1975) have all implicated an involvement of lipoprotein lipase (EC 3.1.1.3) in the hydrolysis of the triacylglycerol-rich plasma lipoproteins. It has been suggested that this hydrolysis may occur at a site close to the endothelial cells lining the blood capillaries (Robinson, 1963; Blanchette-Mackie & Scow, 1971), where the lipoprotein lipase involved is believed to be located (Robinson, 1970). It has been reported in studies of heart cells grown in tissue culture (Henson *et al.*, 1977; Chajek *et al.*, 1977) and for isolated heart-cell suspensions (Bagby *et al.*, 1977; Chohan & Cryer, 1978) that lipoprotein lipase activity is associated with the heart muscle cells. Since in all four of these latter reports lipoprotein lipase activity was assayed at pH 8.0–8.1 by using synthetic substrates [either triolein emulsion (Henson *et al.*, 1977; Chajek *et al.*, 1977; Bagby *et al.*, 1977) or an apolipoprotein CII-activated triacylglycerol emulsion (Chohan & Cryer, 1978)], it is not known whether this intracellular lipoprotein lipase hydrolyses lipoprotein triacylglycerol. In the present study both the metabolism of VLD lipoprotein by the isolated heart muscle cells and the perfused heart and the activity of lipoprotein lipase have been assessed at pH 7.4 by using [$^{14}$C]triacylglycerol-labelled VLD lipoprotein isolated from rats that had been previously injected with [1-$^{14}$C]palmitate. We provide evidence to support the concept that the lipoprotein lipase directly involved in the hydrolysis of lipoprotein triacylglycerol is predominantly located externally to heart muscle cells.

Abbreviation used: VLD lipoprotein, very-low-density lipoprotein.



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## Experimental

*Preparation of $^{14}$C-labelled VLD lipoproteins*

Male Porton Wistar rats (Institute of Medical and Veterinary Science, Adelaide, Australia) weighing 200–230g and fed on commercial pellets *ad libitum*, were used for all experiments; unless otherwise specified they were starved for 18h before the experiments began. [1-$^{14}$C]Palmitic acid complexed to albumin was prepared for injection as described previously (Nestel & Barter, 1971), and injected intravenously (10$\mu$Ci/100g body wt.) into large male rats (weighing over 300g) which had been given free access to food and 10% (w/v) sucrose solution for the previous 18h. After 30min the animals were anaesthetized with ether and exsanguinated by syringe from the inferior vena cava, and blood was collected in 0.1% EDTA solution (pH 7.4). Plasma was separated at 4°C, and after the chylomicron fraction had been removed, the VLD lipoproteins were isolated at a density of 1.006g/ml (Havel *et al.*, 1955). The isolated VLD-lipoprotein fraction was dialysed at 4°C against three changes of 0.9% NaCl/0.01% EDTA (pH 7.4) to remove any [$^{14}$C]-bicarbonate. Subsequent extraction with chloroform/methanol (2:1, v/v) and t.l.c. on silicic acid with hexane/diethyl ether/methanol/acetic acid (90:20:3:2, by vol.) as solvent revealed that over 97% of the VLD-lipoprotein radioactivity resided in the triacylglycerol moiety.

*Preparation of post-heparin plasma*

Post-heparin plasma was obtained from intact rats 10min after intravenous administration of 250 i.u. of sodium heparin dissolved in 0.9% NaCl.

*Perfusion procedure*

The method of heart perfusion used in the present study was essentially the same as described by Scheuer & Berry (1967). Heparin was not administered to the animal before perfusion. The rat was anaesthetized with ether, and the abdomen and thorax were opened. After cannulation of the aorta and making an incision in the right atrium, the heart was removed and attached to the perfusion apparatus. The perfusion pressure was 60cm of water and the temperature of the perfusion fluid at entry into the heart was 37°C. The perfusion fluid was Krebs–Henseleit bicarbonate buffer (Dawson *et al.*, 1959) containing 1.3mM-$Ca^{2+}$ and was continuously equilibrated with $O_2/CO_2$ (19:1). The heart was pre-perfused for 10min without recirculation and then with recirculation using 70ml of perfusate containing $^{14}$C-labelled VLD lipoprotein ($11.45 \times 10^5$d.p.m.; triacylglycerol specific radioactivity $31.7 \times 10^3$d.p.m./$\mu$mol) for 30min. The effluent gas was bubbled through a $CO_2$ trap containing phenethylamine. In control runs in which the labelled perfusate was circulated through the apparatus with no heart present, approx. 20% of the labelled VLD lipoprotein was lost from the perfusate, but there was no $^{14}CO_2$ production. In subsequent experiments appropriate corrections were made for this loss.

*Isolation of heart cells*

Heart cells were isolated by the collagenase/hyaluronidase perfusion procedure (37°C) described by Clark *et al.* (1978), but with heparin omitted. The heart muscle cells were separated from non-muscle cells by centrifugation at 50*g* for 1min and washed once with Krebs–Henseleit bicarbonate buffer (pH 7.4) containing 1mM-EDTA and 10% (v/v) dimethyl sulphoxide. Finally, the heart muscle cells, representing 40–50% of the wet wt. of the heart, were suspended in this medium, kept at 0–4°C and used for the experiments within 1h. The average population of heart cells isolated at this stage contained 90–95% rod-like muscle cells (of which 95% excluded Trypan Blue) and 5–10% rounded non-intact muscle cells. Incubations of the cells were conducted for 30min at 37°C and pH 7.4 with 1.3mM-free $Ca^{2+}$. Examination of the cells at the end of this period indicated that at least 75–80% of the total cell population were rod-like and excluded Trypan Blue.

*Uptake of $^{14}$C-labelled VLD lipoprotein by the isolated heart muscle cells.*

Uptake of $^{14}$C-labelled VLD lipoprotein by the isolated heart cells was measured by incubating heart cells at 37°C in a standard bath assembly (which forms part of the YSI model 53 Biological Oxygen Monitor; Yellow Springs Instruments Co., OH, U.S.A). The standard bath assembly provides relatively air-tight sample chambers, which are stirred magnetically (480rev./min) and are temperature-controlled. The incubation medium consisted of a 2ml suspension of heart cells (22mg wet wt. of cells/ml), 1ml of $^{14}$C-labelled VLD lipoprotein ($2.2 \times 10^5$d.p.m.; triacylglycerol specific radioactivity $31.7 \times 10^3$d.p.m./$\mu$mol), 0.5ml of $Ca^{2+}$ (final concentration of free $Ca^{2+}$ in the incubation mixture was 1.3mM) and Krebs–Henseleit bicarbonate buffer, pH 7.4, containing 1mM-EDTA and 10% (v/v) dimethyl sulphoxide, to make a final volume of 5ml. Post-heparin plasma (0.5ml) was added as indicated. Samples of the incubation mixture (1ml) were withdrawn every 10min; 0.5ml was used for $^{14}CO_2$ determination and 0.5ml for re-isolation of the cells. The cells were washed once with Krebs–Henseleit bicarbonate buffer (pH 7.4), and the radioactivity incorporated was measured.

*Lipolytic activity*

Lipolytic activities of the homogenate of the whole heart, of isolated intact heart cells and of the homo-

genate of the isolated heart cells were assayed essentially as described by Nilsson-Ehle & Schotz (1976). The assays were carried out at pH 7.4 with the natural substrate ([$^{14}$C]triacylglycerol-labelled VLD lipoprotein), prepared as described above. The choice of the natural substrate avoided the need to add plasma activators and allowed direct comparisons between tissue or cell utilization rates and cell-free homogenate utilization rates all conducted at pH 7.4. In all cases tested, except the intact heart cells, the lipoprotein lipase activity determined by this procedure was activated by heparin and inhibited by 1 M-NaCl (e.g. see Table 4). The incubations were carried out at 37°C for 30 min in disposable glass tubes. The reaction was started by addition of the enzyme source and terminated by the addition of 3.25 ml of methanol/chloroform/heptane (141:125:100, by vol.) followed by 1.05 ml of 0.1 M-$K_2CO_3$/borate buffer (pH 10.5). After vigorous mixing, the tubes were centrifuged and the radioactivity in a 1 ml portion of methanol/water upper phase was measured. One unit of enzymic activity is defined as the release of 1 $\mu$mol of fatty acid/min at 37°C.

*Radioactivity determinations*

Radioactivity in the VLD lipoprotein, the perfusates, the heart cells, and the methanol/water upper phase in the lipolytic assay were determined as aqueous emulsions by adding 3 ml of scintillation fluid [4 g of 2,5-diphenyloxazole and 100 mg of 1,4-bis-(5-phenyloxazol-2-yl)benzene/litre of toluene/Triton X-100 (2:1, v/v)] to 0.2 ml of the aqueous sample. Radioactivity was measured for 4 min in a Packard Tri-Carb model 3255 liquid-scintillation spectrometer at an efficiency of 50%. Quenching was uniform and was monitored by the external-standard method by using samples of the same volume as the test samples. The values were subsequently converted into d.p.m.

$^{14}CO_2$ produced during the incubation of isolated heart cells with $^{14}$C-labelled VLD lipoprotein was measured as follows: 3 ml of 2 M-HCl was injected into a capped vial containing 0.5 ml of incubation mixture, and the liberated $^{14}CO_2$ was trapped in a central removable vial containing 0.5 ml of phenethylamine. After 2 h incubation at 37°C the central vial was removed, and, after the addition of 2 ml of scintillation fluid [4 g of 2,5-diphenyloxazole and 100 mg of 1,4-bis-(5-phenyloxazol-2-yl)benzene/litre of toluene/ethanol (2:1, v/v)], the radioactivity was measured at 64% efficiency in a Packard Tri-Carb liquid-scintillation spectrometer. Quenching, as determined by the external-standard method, was uniform and all values were subsequently converted into d.p.m. $^{14}CO_2$ produced during perfusion of $^{14}$C-labelled VLD lipoprotein through the heart was collected in a trap containing 10 ml of phenethylamine. At the end of the perfusion, a portion of the perfusate was acidified with 2 M-HCl, and the liberated $^{14}CO_2$ was trapped in phenethylamine. To 0.5 ml of each of these solutions of phenethylamine/$H^{14}CO_3$ was added 2 ml of scintillation fluid and the radioactivity was determined as described above.

Radioactivity in the total lipid extract of the heart was determined after evaporating the solvent. Scintillation fluid (10 ml) was added and the radioactivity was measured at 85% efficiency as described above. The values were converted to d.p.m.

*Analytical procedures*

Lipids were extracted with chloroform/methanol (2:1, v/v) and washed as described by Folch *et al.* (1957). Triacylglycerol mass was measured in a Technicon Autoanalyzer (Kessler & Lederer, 1965). Protein was determined by the method of Lowry *et al.* (1951) with bovine serum albumin as standard. Lactate dehydrogenase was determined by the method of Shonk & Boxer (1964).

*Chemicals*

[1-$^{14}$C]Palmitic acid (58 mCi/mmol) was from The Radiochemical Centre, Amersham, Bucks., U.K. Collagenases (type I, 144 units/mg; and type CLSPA, 650 units/mg; 1 unit liberates amino acids from collagen equivalent to ninhydrin colour of 1.0 $\mu$mol of L-leucine in 18 h at pH 7.4 and 37°C) were obtained from Worthington Biochemical Corp., Freehold, NJ, U.S.A. Hyaluronidase (type I), albumin (bovine serum), pyruvic acid (type II, sodium salt) and NADH (grade III, disodium salt) were obtained from Sigma Chemical Co., St. Louis, MO, U.S.A. Sodium heparin (5000 i.u./ml) was supplied by Allen and Hanburys, Glaxo-Australia. All other reagents were of analytical grade.

## Results

*Metabolism of albumin-bound [1-$^{14}$C]palmitate by isolated heart muscle cells*

When the heart muscle cells were incubated with albumin-bound [1-$^{14}$C]palmitate there was both uptake and metabolism (Table 1). During the 30 min incubation about 17% of the [$^{14}$C]palmitate was recovered within the cells or as released $CO_2$. The rate of oxidation of [$^{14}$C]palmitate by the heart cells was found to be approx. 3.6 $\mu$mol/30 min per g of heart cells, which was comparable with that reported for the perfused heart (Evans *et al.*, 1963).

*Uptake and metabolism of $^{14}$C-labelled VLD lipoprotein by isolated heart cells and the perfused heart*

Having established above that the intact isolated beating-heart cells had the capacity to oxidize albumin-bound palmitate, the next experiments were conducted to assess whether they could similarly

Table 1. *Metabolism of albumin-bound [1-$^{14}$C]palmitate by isolated heart muscle cells*

Heart cells were isolated from rats starved overnight. A heart-cell suspension (5mg wet wt. of cells/ml of incubation mixture) in Krebs–Henseleit bicarbonate buffer containing 1.3mM-free $Ca^{2+}$ was incubated at 37°C with albumin-bound [1-$^{14}$C]palmitate (264606d.p.m./ml of incubation mixture, containing 0.5% bovine serum albumin and 0.5mM-palmitate) in a total volume of 5ml. At the time intervals indicated, a sample of the incubation mixture was withdrawn to measure the radioactivity in the heart cells and in the medium. $^{14}CO_2$ produced in the reaction was measured after acidifying a portion of the incubation mixture with 2M-HCl and collecting the liberated $^{14}CO_2$ in 0.5ml of phenethylamine. Other details are as described in the text in the Experimental section. Values are means ± S.E.M. for three heart-cell preparations. Values for $^{14}CO_2$ production at 0min are from nine experiments.

| Time of incubation (min) | Metabolism of [1-$^{14}$C]palmitate (μmol) | | |
|---|---|---|---|
| | Uptake by cells | Recovery in medium | Oxidized to $^{14}CO_2$ |
| 0 | 0.21 ± 0.025 | 1.9 ± 0.045 | 0.024 ± 0.0045 |
| 10 | 0.24 ± 0.010 | 1.8 ± 0.045 | 0.045 ± 0.015 |
| 20 | 0.28 ± 0.020 | 1.8 ± 0.035 | 0.075 ± 0.025 |
| 30 | 0.26 ± 0.005 | 1.7 ± 0.045 | 0.090 ± 0.030 |

Table 2. *Metabolism of $^{14}$C-labelled VLD lipoprotein by isolated heart muscle cells*

A heart-cell suspension (22mg wet wt. of cells/ml) in Krebs–Henseleit bicarbonate buffer (pH 7.4) containing 1.3mM-free $Ca^{2+}$ was incubated at 37°C with $^{14}$C-labelled VLD lipoprotein ($221.3 \times 10^3$d.p.m./ml; triacylglycerol fatty acid specific radioactivity $31.7 \times 10^3$d.p.m./μmol) in a total volume of 5ml. At the time intervals indicated portions of the incubation mixture were withdrawn and the radioactivity in the cells was measured. $^{14}CO_2$ produced in the reaction was measured after acidification of a portion of the incubation mixture with 2M-HCl and collection of the liberated $^{14}CO_2$. Results are expressed as μmol of triacylglycerol fatty acid recovered in the respective fractions and were calculated by dividing total radioactivity by triacylglycerol fatty acid specific radioactivity. The assumption is made that all the radioactivity in these fractions was derived from triacylglycerol fatty acid. Values are means ± S.E.M. and the numbers of heart-cell preparations are indicated in the parentheses.

| Time of incubation (min) | Addition ... | Metabolism of $^{14}$C-labelled VLD lipoprotein (μmol) | | | |
|---|---|---|---|---|---|
| | | Uptake by cells | | Oxidized to $^{14}CO_2$ | |
| | | None (16) | Post-heparin plasma (8) | None (16) | Post-heparin plasma (8) |
| 0 | | 0.14 ± 0.02 | 0.69 ± 0.12 | 0.010 ± 0.0005 | 0.045 ± 0.003 |
| 10 | | 0.22 ± 0.035 | 3.87 ± 0.73 | 0.025 ± 0.003 | 0.395 ± 0.06 |
| 20 | | 0.29 ± 0.045 | 5.79 ± 0.97 | 0.030 ± 0.0025 | 1.07 ± 0.10 |
| 30 | | 0.43 ± 0.08 | 5.93 ± 1.18 | 0.040 ± 0.003 | 1.10 ± 0.075 |

oxidize [$^{14}$C]palmitate in the triacylglycerol VLD lipoprotein that had been previously prepared from the plasma of other animals. Table 2 shows that the uptake and metabolism of VLD-lipoprotein triacylglycerol fatty acid by the heart cells was very slow, equal to approx. one-tenth the rate of palmitate oxidation (Table 1). To check that the low rate resulted from low activity of the lipoprotein lipase, a source of this enzyme was added. The rate of metabolism of VLD-lipoprotein triacylglycerol fatty acid increased from 0.36 to 10.04μmol/30min per g wet wt. of heart cells after the addition of post-heparin plasma (Table 2).

In contrast with the isolated heart cells, the perfused heart was able to metabolize the $^{14}$C-labelled VLD lipoprotein without the addition of post-heparin plasma (Table 3). After 30min perfusion there was a net loss of about 12% of the $^{14}$C from the perfusate with a concomitant production of $^{14}CO_2$. The rate of oxidation of VLD-lipoprotein triacylglycerol fatty acid by the perfused heart was 1μmol/30min per g wet wt. of heart.

*Lipolysis of VLD lipoproteins by rat heart preparations*

In order to assess whether the apparent loss of lipoprotein lipase from the isolated heart cells resulted from perfusion of the heart with buffer or buffer containing collagenase, homogenates of hearts from each approach were assayed for lipoprotein lipase activity. In the homogenate of the whole heart, which had been perfused with buffer only, there was a significant lipolytic activity which was activated 1.5-fold by heparin and almost completely (90%) inhibited by 1M-NaCl (in the absence of heparin) (Table 4). On the assumption that the lipoprotein lipase is the rate-limiting step in the oxidation of VLD-lipoprotein triacylglycerol fatty

Table 3. *Metabolism of $^{14}$C-labelled VLD lipoprotein by the perfused rat heart*

Hearts were perfused for 30min with $^{14}$C-labelled VLD lipoprotein ($11.45 \times 10^5$d.p.m.; triacylglycerol fatty acid specific radioactivity $31.7 \times 10^3$d.p.m./μmol). Other details of perfusion conditions and $^{14}CO_2$ measurements are as given in the Experimental section. Results are expressed as μmol of the added triacylglycerol fatty acid recovered in the respective fractions. Values are means ± S.E.M. for four perfusions. Average weight of heart was 0.89g and volume of perfusion medium 70ml.

| | $^{14}$C-labelled VLD lipoprotein | | |
|---|---|---|---|
| Time of perfusion (min) | Perfusate concn. (μmol/70ml) | In total lipid fraction of the heart (μmol/g wet wt.) | Oxidized to $^{14}CO_2$ (μmol/g wet wt.) |
| 0 | 36.12 ± 0.28 | 0 | 0 |
| 30 | 31.79 ± 1.89 | 0.628 ± 0.036 | 1.022 ± 0.086 |

Table 4. *Lipolysis of VLD lipoproteins by rat heart preparations*

Details for perfusion and isolation of heart cells were as described in the text. Homogenates were prepared in 0.25M-sucrose containing 10mM-Tris/HCl (pH 7.4) and 1mM-EDTA. Lipolytic activity was measured at pH 7.4 using [$^{14}$C]triacylglycerol-labelled VLD lipoprotein as substrate. Incubations were conducted at 37°C for 30min in a medium containing 70mM-Tris/HCl (pH 7.4), 1% (w/v) bovine serum albumin, 232nmol of VLD-lipoprotein triacylglycerol (specific radioactivity of triacylglycerol 95.1d.p.m./nmol). The reaction was stopped by addition of methanol/chloroform/heptane (141:125:100, by vol.). Other details of enzyme assay are as described in the Experimental section. Results are means ± S.E.M. for four perfusions.

| | Lipolytic activity (nmol/min per mg of protein) | | | |
|---|---|---|---|---|
| Additions | Whole heart homogenate after buffer perfusion | Whole heart homogenate after collagenase perfusion | Isolated intact heart cells | Isolated heart cell homogenate |
| None | 4.66 ± 0.61 | 0.38 ± 0.17 | 0.24 ± 0.22 | 0.067 ± 0.02 |
| Heparin (5i.u./ml) | 7.05 ± 0.61 | 1.55 ± 0.23 | 0.22 ± 0.14 | 0.16 ± 0.09 |
| NaCl (1M) | 0.51 ± 0.10 | 0.26 ± 0.12 | 0.28 ± 0.17 | 0.09 ± 0.05 |

acid by the heart, the activity of 4.66nmol of fatty acid released/min represented approx. 73% of the activity of the perfused heart (Table 3). This suggested that a small but significant loss of lipoprotein lipase occurred when the heart was perfused with buffer alone. In the homogenates of the collagenase-perfused hearts, however, the loss was much greater; approx. 10% of the activity remained and this activity was activated and inhibited by heparin and 1M-NaCl respectively. Isolated intact heart cells and homogenates of the cells showed negligible lipolytic activity (not statistically different from each other) and the little activity that was observed was unresponsive to either heparin or 1M-NaCl (Table 4).

*Release and inactivation of lipoprotein lipase from rat hearts perfused with collagenase*

Since collagenase perfusion led to substantial loss of lipoprotein lipase activity from the heart, as assayed in the homogenate (Table 4), it was considered relevant to monitor the perfusate for lipoprotein lipase activity during collagenase perfusion. Table 5 shows the effect of various collagenase concentrations on the release and inactivation of heart lipoprotein lipase. Perfusion of the heart with buffer alone at 37°C caused no loss of lipoprotein lipase activity. However, with increasing amounts of crude collagenase (type I) up to 0.2mg/ml there was a corresponding increase in lipoprotein lipase released into the perfusate and a decrease in the amount of lipoprotein lipase retained by the heart. Also with increasing amounts of collagenase there was an apparent increased total loss of lipoprotein lipase activity from the system, activity that could not be detected in either the heart or the perfusate. For even higher concentrations of collagenase (e.g. 0.5mg of type I/ml), almost 92% of the lipoprotein lipase activity was lost from the heart and only 13.6% was recovered in the perfusate. For a more-pure form of the collagenase at a low concentration (e.g. 0.023mg of type CLSPA/ml), release from the heart was noted, but inactivation did not occur, as the recovery in the two fractions was equal to that in the heart originally. There was no evidence to suggest that collagenase perfusion caused a loss of cytosolic enzymes from the heart into the perfusate; lactate dehydrogenase activity of the perfusate did not exceed 5% of the heart content even at the highest concentration of collagenase (Table 5). Fig. 1(*a*) provides additional evidence suggesting that inactivation of the heart lipoprotein lipase occurs when exposed to collagenase. When the homogenate of the heart, which had been perfused with buffer, was incubated at 37°C with collagenase (0.5mg of type

Table 5. *Effect of collagenase perfusion on the release and inactivation of heart lipoprotein lipase*

Hearts were perfused in a recirculatory system with perfusion medium containing the specified concentration of collagenases type I (144 units/mg) or type CLSPA (650 units/mg). After a 30 min perfusion lipoprotein lipase and lactate dehydrogenase activities of the heart and of the perfusion medium were measured. [$^{14}$C]Triacylglycerol-labelled VLD lipoprotein was used as the substrate for lipoprotein lipase activity measurements. Details for the assay as well as other details are given in the text. Each value is that obtained for a single heart perfusion. Where appropriate the mean ± S.E.M. or individual values are given. The number of hearts perfused for each concentration of collagenase is shown in parentheses. Total values for the heart and perfusion medium are shown (n.d., not determined). Average weight of heart 0.89 g and volume of perfusion medium 70 ml.

| Collagenase concentration (mg/ml of perfusion medium) | | Lipoprotein lipase activity (nmol/min) | | Lactate dehydrogenase activity (μmol/min) | |
|---|---|---|---|---|---|
| | | Heart | Perfusion medium | Heart | Perfusion medium |
| Type I | 0 | 494 ± 26.3 (17) | 0 (1) | 227 (1) | 0 (1) |
| | 0.05 | 525, 263 (2) | 68.9 (1) | n.d. | n.d. |
| | 0.1 | 152, 157 (2) | 114, 88 (2) | 220 (1) | 5.25 (1) |
| | 0.2 | 51, 87 (2) | 151 (1) | n.d. | n.d. |
| | 0.5 | 40.8 (1) | 70 (1) | 264 (1) | 13.2 (1) |
| Type CLSPA | 0.023 | 361, 435 (2) | 190, 201 (2) | n.d. | n.d. |

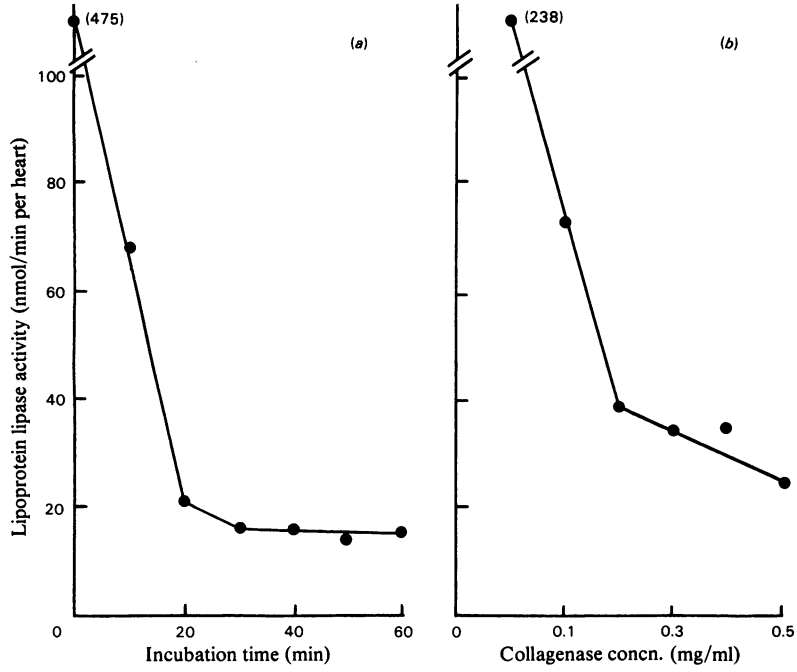


Fig. 1. *Inactivation of heart lipoprotein lipase by collagenase*

Homogenate was prepared from the heart perfused with buffer described in the text and in the legend to Table 4. (*a*) Heart homogenate was incubated at 37°C with collagenase type I (0.5 mg/ml) for specified periods. (*b*) Heart homogenate was incubated at 37°C for 30 min with various concentrations of type-I collagenase. After incubation, a portion of the sample was assayed for lipoprotein lipase activity, with VLD lipoprotein labelled with $^{14}$C in the triacylglycerol moiety as the substrate. Initial values are shown in parentheses.

I/ml) for various time intervals and then assayed for lipoprotein lipase activity there was rapid inactivation of lipase activity; approx. 85% of the activity was lost in 10 min.

In Fig. 1(*b*) the effect of collagenase concentration on the lipoprotein lipase activity is shown. At a low concentration of collagenase (0.05 mg of type I/ml) the lipoprotein lipase activity was inactivated only partially, and at a higher concentration lipoprotein lipase was inactivated by over 85%.

## Discussion

Since the isolation of lipoprotein lipase from the defatted preparation of heart tissues by Korn (1955*a*,*b*), there have been numerous reports on the occurrence as well as purification of lipoprotein lipase from rat heart (Aktin & Meng, 1972; Twu *et al.*, 1975), cultured myocardial cells (Henson *et al.*, 1977; Chajek *et al.*, 1977) and isolated adult rat myocytes (Bagby *et al.*, 1977; Chohan & Cryer, 1978). Lipoprotein lipase has been demonstrated to be multiphasic in rat heart (Aktin & Meng, 1972). It has been postulated that in the heart the lipoprotein lipase exists in functional and non-functional form and that the functional lipoprotein lipase is formed in sites that are not accessible to heparin and then transported to the site of action (Chajek *et al.*, 1975; Borensztajn *et al.*, 1975). The results of the published studies on the lipoprotein lipase activity of the rat heart are difficult to compare. Three types of preparations have been used: (i) homogenates of defatted powders of heart (Robinson, 1963; Robinson & Jennings, 1965; Chohan & Cryer, 1978), (ii) homogenates of fresh tissues (Mallov & Alousi, 1967; Mallov & Cerra, 1967; Chohan & Cryer, 1978) and (iii) 12000*g* supernatants (Bagby *et al.*, 1977). A variety of homogenizing media has been used, and the methods and conditions of assay have differed. In the present study we have measured lipolytic activity at physiological pH with the use of the natural substrate, VLD lipoprotein. This substrate allowed direct comparison between the activity of the perfused organ, isolated cells and homogenates, and avoided the need to include plasma activators. In addition, the characteristics of the activity determined with VLD lipoprotein were consistent with those described by Korn (1955*a*,*b*), being inhibited by 1M-NaCl and activated by heparin. In experiments with VLD lipoprotein as the substrate lipolytic activity was detected in homogenates of heart that had been perfused with buffer only. Thus our results agree with those of others with respect to the occurrence of lipoprotein lipase in rat heart. On collagenase perfusion, however, a significant amount of the enzyme was lost. This loss was a result of release of the enzyme in perfusion medium. Such a release of lipoprotein lipase in the perfusion medium after heparin perfusion of the heart has been reported by Enser *et al.* (1967). It is therefore likely that the lipoprotein lipase lost during collagenase perfusion represents the heparin-releasable extracellular enzyme, since under similar conditions it was found that lactate dehydrogenase was not released into the perfusate. This view is also supported by our finding that the rate of oxidation of [$^{14}$C]triacylglycerol-labelled VLD lipoprotein by the isolated heart cells was low compared with the perfused rat heart or when post-heparin plasma was present in the incubation. The minimal oxidation of [$^{14}$C]triacylglycerol-labelled VLD lipoprotein by the heart cells that was observed could be due to residual amounts of lipolytic activity present on the heart muscle cell surface. However, our results indicate that this residual lipolytic activity is insensitive to heparin or NaCl (Table 4) and may be analogous to the hepatic triacylglycerol lipase. It is known that hepatic triacylglycerol lipase is not inhibited by NaCl and also hydrolyses the triacylglycerol-rich lipoproteins, although less efficiently (Olivecrona *et al.*, 1977; Augustin *et al.*, 1976).

The mechanism by which collagenase leads to release of lipoprotein lipase from the perfused heart remains uncertain, although collagenase-mediated degranulation of cardiac mast cells [indirect evidence for mast cells in rat heart was obtained by Clark *et al.* (1979)] is a possibility, particularly since mast-cell granules contain heparin (Lagunoff & Pritzl, 1976).

In the present investigation, with VLD lipoprotein as the substrate, there was no evidence to support the existence of active lipoprotein lipase within the isolated heart cells. These data are at direct variance with reports by Bagby *et al.* (1977) and Chohan & Cryer (1978), who reported that isolated adult rat myocytes from starved animals contained lipoprotein lipase activities of 21.4 and 13.2$\mu$mol/h per g of cells respectively. Chohan & Cryer (1978) indicated that this represented approx. 15.7% of the total activity of the whole tissue when assayed as a homogenate. Alternatively, assays of homogenates of acetone/diethyl ether-dried powders indicated a recovery of 47% in the heart cells (Chohan & Cryer, 1978). Apart from major differences in the intactness of the cells between the reports of Bagby *et al.* (1977) (40–50% intactness of cells) and Chohan & Cryer (1978) (60% intactness) and our own (90% intact and stabilized against $Ca^{2+}$-ion-mediated damage), it appears likely that the difference in findings arise principally from the different lipoprotein lipase assays employed. In both previous reports (Bagby *et al.*, 1977; Chohan & Cryer, 1978) and for cultured heart cells (Henson *et al.*, 1977; Chajek *et al.*, 1977) the lipoprotein lipase activity was measured with [$^{3}$H]triolein emulsion as substrate. Indeed, in most of the studies reported the enzyme assay has been carried out at pH 8.1 with synthetic triacylglycerol emulsion and serum or specific serum lipoprotein cofactors, on the assumption that under conditions of alkaline pH, the possible interference of other lipases (which also act on triacylglycerol and have pH optima of 6.5 and 7.5) present in the heart are minimal (Borensztajn *et al.*, 1970). By using the natural substrate as others have done (Fielding & Higgins, 1974; Glangeaud *et al.*, 1977), this assumption has not been necessary. Thus it is not known whether this intracellular lipoprotein lipase (Bagby *et al.*, 1977; Chohan & Cryer, 1978) can also hydrolyse

lipoprotein triacylglycerol. Alternatively it is possible that the intracellular lipoprotein lipase represents a non-functional enzyme and only when it is transported to the site of action (extracellular) does it assume a role in the hydrolysis of VLD-lipoprotein triacylglycerol.

The major observation of the present work is that VLD-lipoprotein triacylglycerol-hydrolysing activity is released from the rat heart when perfused with collagenase preparations. This observation may account for the apparent loss of lipoprotein lipase indicated between isolated heart cells and whole tissue (Chohan & Cryer, 1978) and lend support to the earlier view that VLD lipoprotein is not taken into the cells but rather than hydrolysis occurs externally to the cells (Enser *et al.*, 1967; Mjos *et al.*, 1975).

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