# Release of clearing factor lipase by the perfused rat heart

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SUMMARY Rat hearts perfused with heparin in McEwen-albumin solution by the Langendorff technique released clearing factor lipase into the perfusate. Clearing factor lipase activity in the perfusate was high in the first minute of perfusion with heparin. With hearts from fed animals it remained at a high level for 10–15 min before falling gradually. With hearts from fasted animals it fell more rapidly and reached a low level by 10–20 min after starting perfusion. Total activity in the perfusate was greater with hearts from fed, than with those from fasted rats. If heparin was withdrawn from the perfusion fluid no sudden change occurred in the pattern of enzyme release.

After one hour's perfusion both fed and fasted hearts retained a considerable proportion of their original clearing factor lipase activity although enzyme in the perfusate had fallen to a low level by this time.

The enzyme content of unperfused hearts was higher in fed than in fasted rats.

KEY WORDS clearing factor lipase · rat · heart · perfusion · release · distribution · heparin · differences · fed · fasted

CLEARING factor lipase (lipoprotein lipase) is believed to play an important role in facilitating and regulating the passage of triglyceride fatty acids from the chylomicra and low density lipoproteins of the blood to the extrahepatic tissues. It is widely distributed in these tissues and may normally act close to the capillary endothelial cell surface (1, 2). The enzyme is liberated extremely rapidly into the blood following the injection of heparin intravenously in the whole animal (3, 4), but no detailed studies on the pattern of its release by heparin from particular tissues have been reported. The present work presents some observations on this point made on isolated perfused rat hearts.

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## MATERIALS AND METHODS

Wistar strain female rats weighing 200 g were either fed on their usual diet (Oxoid diet 41) until they were killed for experiment or were starved for 48 hr before use.

### *Perfusion Technique*

Rat hearts were perfused by the Langendorff method (5). As described previously (6), perfusion fluid from a reservoir was passed by gravity through a column enclosed in a water jacket and entered the coronary vessels from a cannula placed in the aorta. The perfusion pressure was 100 cm of water and the temperature of the fluid at entry into the heart was 36–37°. The fluid used was that of McEwen (7), containing salts, glucose (200 mg/100 ml), and sucrose (450 mg/100 ml), with added bovine albumin (Fraction V, Armour; 250 mg/100 ml). Heparin (Pularin-Evans; Evans Medical Ltd., Liverpool, England) at a concentration of 2 units/ml was present or absent as described. The pH of the fluid was adjusted in the reservoir by saturation with oxygen containing 5% carbon dioxide, and at entry into the heart was 7.7. The perfusion fluid was not recirculated but passed through the heart once only.

The hearts were excised under ether anesthesia and were cleaned and tied on to the cannula in chilled McEwen's solution. Perfusion, which was begun within 4 min of excision, was started with McEwen–albumin solution without heparin. When the heart had stabilized for a few minutes, the perfusion fluid was changed to McEwen–albumin solution containing heparin by switching to another reservoir. Experiments were timed from this point. The dead space in the perfusion system, from the tap controlling the reservoirs to the tip of the cannula, was 13 ml. Although the initial perfusion rate was only of the order of 6 ml/min, heparin may be presumed to have reached the heart within a minute of switching over because of the mixing that occurs in the column, which could readily be observed if colored

fluids were perfused. All perfusions were continued for 1 hr.

The contraction and perfusion rates of the hearts were not affected by the presence of heparin. The hearts beat well throughout the hour, though progressive falls in both rate of beat (from 210–270 beats/min at the start to 180–200 beats/min at the end) and perfusion volume (from 5–8.2 ml/min to 1.6–5 ml/min) occurred. From 130 to 340 ml of perfusate was collected in the hour in different experiments.

There were no consistent differences between hearts from fed and starved animals in contraction or perfusion rates.

### *Assay of Clearing Factor Lipase*

(*a*) *In rat heart acetone–ether powders.* Tissue clearing factor lipase levels can be determined satisfactorily in acetone–ether powders prepared from the fresh tissue. A suitable preparative technique involving homogenization of the tissue in acetone, followed by filtration and exhaustive washing of the tissue residue with acetone and ether and final drying of the powder, has been described recently (2).

In the present study acetone–ether powders were prepared from (i) hearts perfused with heparin in Mc-Ewen–albumin solution for 1 hr as described above. Five milliliters of fresh perfusion fluid containing heparin was added before the homogenization in acetone; (ii) control hearts perfused with heparin in McEwen–albumin solution for 2 min and subsequently incubated in their 2 min perfusates for 1 hr at 37°. The 2-min perfusate was included in the initial homogenization mixture in acetone; and (iii) hearts removed from the animal, rinsed in 0.9% (w/v) sodium chloride solution and homogenized immediately with 5 ml of McEwen–albumin solution containing heparin.

After the "powders" had been prepared they were stored in vacuo at 4° overnight. Despite their conventional name they contained connective tissue fragments and had, therefore, to be coarsely minced with scissors before weighing and homogenizing. Samples were homogenized at 4° in 0.025 N ammonia solution at pH 8.5 employing, as a standard procedure, seven full strokes of a single Potter-Elvehjem type homogenizer. Assay of the clearing factor lipase in the homogenate by measurement of the free fatty acids (FFA) released from a suitable triglyceride substrate was carried out by a technique which has been described previously (2). The triglyceride substrate used was a chyle–serum mixture prepared from 2 volumes of serum obtained from platelet-free citrated plasma by recalcification, and 1 volume of chyle collected from the thoracic ducts of rats which had been fed olive oil (8). The chyle contained between 150 and 200 μmoles of esterified fatty acid per milliliter.

FFA determinations were carried out on samples taken in triplicate from the assay mixture by a modification of Dole's technique (9) described by Salaman and Robinson (10). Not more than 20% of the added chyle triglyceride was hydrolyzed during the assay, and no appreciable decline in the rate of FFA release occurred with time during the longest incubation periods (120 min) used.

(*b*) *In rat heart perfusate samples.* The clearing factor lipase activities of 1- or 2-min samples of the perfusate were determined within 4 hr of the end of perfusion. Samples were taken frequently in the early part of each perfusion and less often in the later part and were stored at 4° until assayed.

Assays were carried out by measuring the FFA release from added chyle triglycerides at 37° in an incubation mixture containing 3 ml of perfusate, 1 ml of 20% (w/v) albumin (Fraction V, Armour) in 0.9% (w/v) sodium chloride solution at pH 8.1, and 1 ml of the chyle–serum mixture described above. Single samples (1 ml) of the incubation mixture were removed at 0 min and triplicate samples at 180 min. Not more than 10% of the chyle triglyceride was hydrolyzed and the release of FFA was shown to be linear over this period under the conditions described. FFA were estimated as described above.

Control assays were carried out with either a sample of the perfusing fluid before its passage through the heart or a pre-heparin sample of perfusate. The post-heparin perfusate activities have been corrected for the FFA release in these control assays, which was always very low.

## RESULTS

### *Clearing Factor Lipase Activity of Hearts from Fed and Fasting Rats*

As a preliminary to the perfusion studies the clearing factor lipase activity of hearts from rats fed their normal diet was compared with that of hearts from rats fasted for 48 hr. The mean value for five fed animals (163 ± 16 μmoles of FFA released by each heart per hr) was significantly greater ($P < 0.01$) than that for five fasted animals (124 ± 8 μmoles).

### *Release of Clearing Factor Lipase from Perfused Hearts by Heparin*

(*a*) *From rats fasted for 48 hr.* Four experiments were carried out with hearts from rats that had been fasted for 48 hr (Fig. 1). Clearing factor lipase activity was maximal in the first sample of perfusate (that is, the outflow during the first minute after the introduction of heparin into the perfusion system) and fell off in successive samples, at first rapidly to a low level, reached

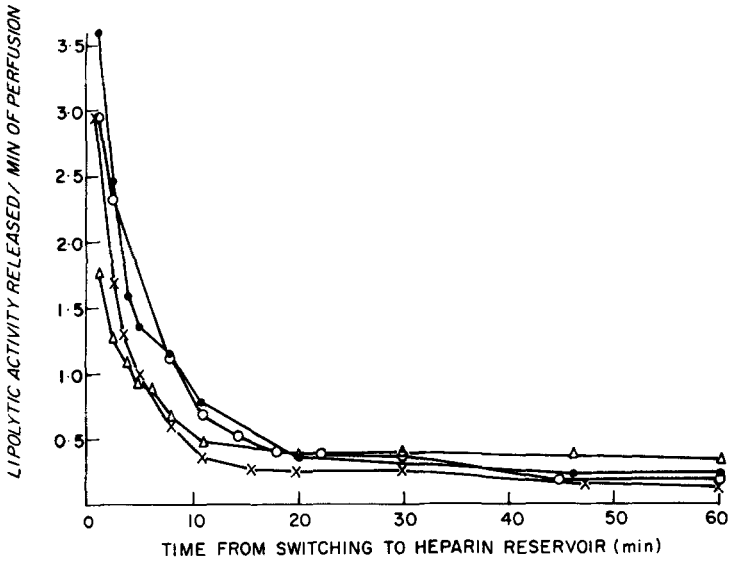


FIG. 1. The release by heparin of clearing factor lipase from the perfused hearts of fasting rats. Each curve represents an experiment on a single rat heart and shows the lipolytic activity[1] of 1- or 2-min perfusate samples collected at different times after the introduction of heparin into the perfusing fluid.

between 10 and 20 min after the introduction of heparin, and then more slowly.

The high level of enzyme activity in the first perfusate sample is noteworthy. It is evident that release of the enzyme by heparin is extremely rapid and that, since the first sample contained a considerable admixture of heparin-free perfusion fluid (see Perfusion technique), the initial figure obtained is lower than the actual initial output of enzyme.

At the end of the perfusion period the clearing factor lipase activity remaining in the hearts was determined and in Fig. 2 the values are compared with the total amount of enzyme released into the perfusate. It is evident that the heart tissue at the end of the perfusion still contains roughly twice the amount of enzyme in the perfusate. The failure of heparin to release appreciable amounts of enzyme in the later stages of the perfusion cannot, therefore, have been due to exhaustion of the enzyme in the tissue.

The sum of the lipolytic activity in the perfusate and the activity remaining in the perfused heart has a mean value of 99 ± 15 μmoles of FFA released per hr per heart for the four experiments. This figure may be compared with a mean total lipolytic activity of 82 ± 12 μmoles of FFA released per hr per heart for four hearts from fasted rats which were perfused for 2 min with the perfusion fluid containing heparin and then incubated intact for 1 hr at 37° in their 2-min perfusate.

[1] Lipolytic activity is defined here as micromoles of free fatty acid released per hr.

(*b*) *From fed rats.* Experiments similar to those described with the fasting animals were carried out with hearts from four rats which had been fed their normal diets (Fig. 3). Enzyme activity was considerable in the first samples collected after the introduction of heparin into the perfusion system though not so great as that from fasting animals and, instead of falling rapidly in succeeding samples, it either rose further or stayed at a plateau level for 10–15 min before falling gradually over the rest of the hour. At the end of the perfusion period the activity of the perfusate was about double that of perfusates from hearts from fasting animals.

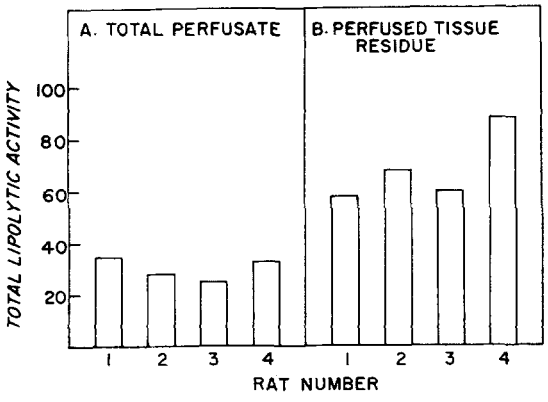


FIG. 2. The clearing factor lipase activity of the whole 1 hr perfusate (A) and of the heart at the end of the perfusion (B). The hearts were obtained from four fasting rats. The total lipolytic activity[1] of the perfusates is obtained from the areas under the curves in Fig. 1.

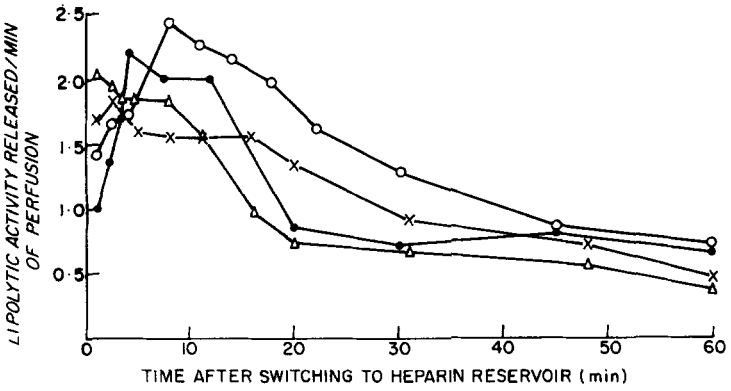


FIG. 3. The release by heparin of clearing factor lipase from the perfused hearts of rats fed their normal diet. Each curve represents an experiment on a single rat heart and shows the lipolytic activity[1] of 1- or 2-min perfusate samples collected at different times after the introduction of heparin into the perfusing fluid.

Figure 4 shows that in these rats also the decline in perfusate activity was not due to exhaustion of the enzyme in the tissue. Although the total amount of enzyme released into the perfusate by hearts from fed animals was substantially greater than from fasted animals, the amounts remaining in the tissue in the two nutritional states were similar. The higher clearing factor lipase content of unperfused hearts from fed animals seems to be accounted for by the presence of more enzyme capable of being released on perfusion with heparin.

The sum of the total perfusate lipolytic activity and the residual tissue activity gives a mean value for the four experiments of 151 $\mu$moles of FFA released per hr per heart. This figure may be compared with a mean total lipolytic activity of 120 $\mu$moles of FFA released per hr per heart for four control hearts from fed rats which were perfused for 2 min with heparin and then incubated intact for 1 hr at 37° in their 2-min perfusate.

As with the hearts from the fasted animals, the mean value for the sum of perfusate and residual tissue lipolytic activities is less than that for the mean activity of hearts immediately after their removal from the rat but greater than that for the control hearts perfused for 2 min and then incubated intact for 60 min at 37°. This may be accounted for by the slow destruction of clearing factor lipase at 37° which is known to occur in the absence of its triglyceride substrate. Such destruction would be minimal in the hearts treated immediately and might be expected to be greater in the intact hearts than in the perfused ones since in the latter the perfusate samples were stored at 0° until they were assayed.

The possibility that the lipolytic activity in the perfusate or in the acetone–ether powders might have been due to some lipase other than the clearing factor lipase was tested by assaying representative samples in the presence of 0.5 M NaCl solution, which abolishes clearing factor lipase action towards chylomicron triglycerides (2). Under these conditions perfusate and acetone–ether powder activities were both reduced to less than 5% of their original value.

### *Effect of Heparin Withdrawal on Release of Clearing Factor Lipase by Perfused Hearts*

Hearts from fed animals were perfused for 10 min with fluid containing heparin, then for either 20 or 30 min without heparin and, finally, again with heparin (Fig. 5).

Withdrawal of heparin did not cause any sudden drop in the level of enzyme in the perfusate, and the subsequent release pattern was similar to that shown in Fig. 3 for hearts perfused with heparin throughout. Although the possibility of passive retention of heparin within the blood vessels cannot be entirely excluded, this finding suggests that, once enzyme release is stimulated, it may

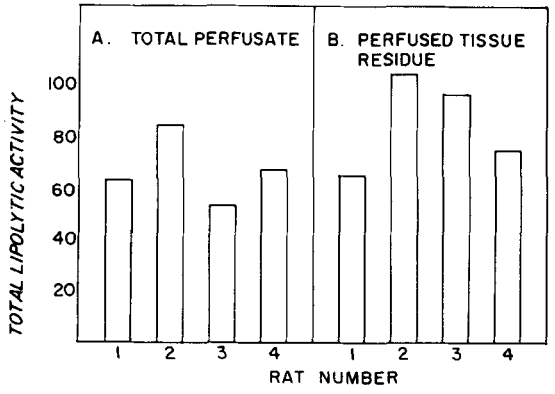


FIG. 4. The clearing factor lipase activity of the whole 1-hr perfusate (A) and of the heart at the end of the perfusion (B). The hearts were obtained from four fed rats. Determinations as for Fig. 2.

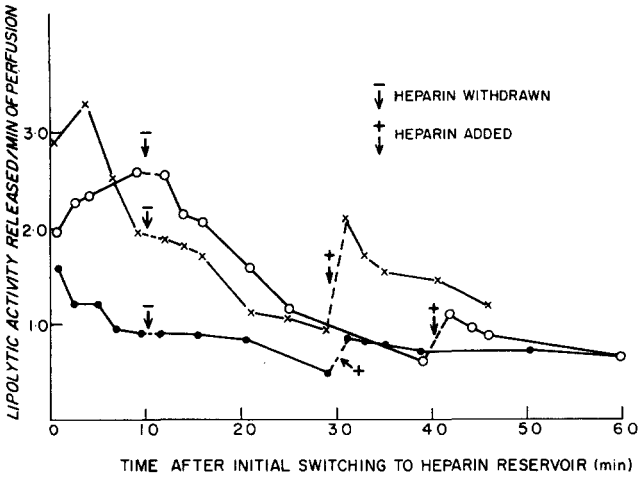


FIG. 5. The effect of heparin withdrawal and reintroduction on the release of clearing factor lipase from the perfused hearts of fed rats. Three hearts were perfused and the times of heparin withdrawal and reintroduction are indicated by arrows.

continue for some time in the absence of additional amounts of exogenous heparin.

Reintroduction of heparin caused an increase in the activity of the perfusate, though the new level reached was considerably lower than the initial level.

### *Distribution of Clearing Factor Lipase in Homogenates of Acetone–Ether Powders of Rat Hearts*

Studies with acetone–ether powders of rat adipose tissue have shown that only part of the enzyme in aqueous homogenates of such powders is in solution (10). This is true also for rat heart. The percentage of soluble enzyme in the homogenate rises as the tissue concentration is reduced (Table 1) and is increased, at a particular tissue concentration, by repeated reextraction with fresh medium (Table 2). Under the present assay conditions, such changes in the proportion of the total enzyme in solution do not alter the activity of the enzyme per milligram of tissue powder.

Since perfusion of hearts from both fed and fasting rats results in the release into the perfusate of a considerable proportion of the total clearing factor lipase content of the tissue, it seemed possible that the extent of extraction of the enzyme from acetone–ether powders prepared from the hearts after their perfusion might be considerably reduced. As shown in Table 3, however, no marked reduction in the percentage extraction was in fact observed and, as with unperfused hearts, most of the enzyme was brought into solution by repeated extraction.

TABLE 1 DISTRIBUTION OF CLEARING FACTOR LIPASE IN HOMOGENATES OF ACETONE–ETHER POWDERS OF RAT HEART

| Expt. | Powder Concentration in Extracting Solution (mg/ml) | Homogenate Lipase Activity in | |
|---|---|---|---|
| | | Supernatant Fraction | Precipitate |
| | | % | % |
| 1 | 7.5 | 43 | 57 |
| | 2.5 | 56 | 44 |
| 2 | 10.0 | 35 | 65 |
| | 5.0 | 51 | 49 |

In each experiment an acetone–ether powder was prepared as described under Materials and Methods from the combined hearts of three fed and three fasted rats. Homogenates, at powder concentrations of 10.0, 7.5, 5.0, or 2.5 mg/ml, were incubated for 10 min at 37°, and then part of each was centrifuged for 1 hr at 115,000 × *g* and 4° while the remainder was stored at 4°. The supernatant solutions were recovered and the precipitates redispersed in 0.025 N ammonia solution (pH 8.5) at the powder concentrations of the original homogenates.

## DISCUSSION

Although the clearing factor lipase activities of rat heart tissue found in the present work are considerably higher than those reported by Hollenberg (11) this could well be accounted for by improvements made in assay technique since the earlier study. Hollenberg found a progressive increase in the enzyme activity of the heart with increasing duration of starvation; this conflicts with the present finding of a decrease in activity after a 48 hr fast, and cannot so far be explained.

In the present investigation of acetone–ether powders of rat heart, only part of the clearing factor lipase activity was soluble in dilute ammonia under the extraction conditions described by Korn (12). The same is true

TABLE 2 EXTRACTION OF CLEARING FACTOR LIPASE FROM RAT HEART ACETONE–ETHER POWDERS

| | Clearing Factor Lipase Activity | |
|---|---|---|
| | FFA Released per mg of Tissue Powder | % of Homogenate Activity |
| | *μmole/hr* | |
| Homogenate | 0.55 | 100 |
| Supernatant 1 | 0.18 | 33 |
| Supernatant 2 | 0.11 | 20 |
| Supernatant 3 | 0.08 | 15 |
| Precipitate | 0.20 | 36 |

An aqueous homogenate of an acetone–ether powder of the combined hearts of three fed and three fasted rats was prepared as described under Materials and Methods at a powder concentration of 10 mg/ml. After incubation for 10 min at 37°, part of the homogenate was centrifuged for 1 hr at 115,000 × *g* and 4° while the remainder was stored at 4°. The supernatant solution was recovered and stored, while the precipitate was dispersed in 0.025 M ammonia solution (pH 8.5) at the original powder concentration. The centrifuging and redispersion procedures were repeated twice more.

TABLE 3 EXTRACTION OF CLEARING FACTOR LIPASE FROM RAT HEART ACETONE–ETHER POWDERS

| Expt. | | Clearing Factor Lipase Activity (μmole FFA released/mg powder per hr) | | |
|---|---|---|---|---|
| | | | Perfused Hearts | |
| | | Unperfused Hearts | Fed Rats | Fasted Rats (48 hr) |
| 1 | Homogenate | 0.54 (100) | 0.28 (100) | 0.21 (100) |
| | Supernatant | 0.25 (46) | 0.10 (36) | 0.06 (30) |
| | Precipitate | 0.29 (54) | 0.18 (64) | 0.15 (70) |
| 2 | Homogenate | 0.46 (100) | 0.25 (100) | 0.14 (100) |
| | Supernatant 1 | 0.24 (52) | 0.12 (48) | 0.06 (42) |
| | Supernatant 2 | 0.07 (15) | 0.04 (16) | 0.02 (14) |
| | Supernatant 3 | 0.04 (9) | 0.02 (8) | 0.01 (7) |
| | Supernatant 4 | 0.03 (7) | 0.01 (4) | 0.01 (7) |
| | Precipitate | 0.07 (15) | 0.05 (20) | 0.04 (28) |

Acetone–ether powders were prepared as described under Methods from rat hearts that had been perfused for 1 hr with heparin in McEwen–albumin solution. An additional powder was prepared from the combined hearts of three fed and three fasted rats. Homogenates of the powders in 0.025 M ammonia solution (pH 8.5) at a powder concentration of 7.5 mg/ml (Experiment 1) or 5.0 mg/ml (Experiment 2) were treated either as described in Table 2 (Experiment 1) or as described in Table 3 (Experiment 2), except that in the latter four reextractions were carried out. The figures in parentheses show the percentage of the homogenate activity present in each fraction.

of the enzyme in fresh heart[2] and in acetone–ether powders of adipose tissue (10). It seems advisable, therefore, always to use whole homogenates of tissues and of tissue powders rather than tissue extracts in studies of the enzyme content of different tissues. The association of part of the enzyme with the residue fraction does not appear to interfere with assay of the enzyme under the conditions described here.

The immediate appearance of clearing factor lipase when heparin is perfused through the vessels of the rat heart is consistent with earlier studies, which showed rapid release of the enzyme into the blood stream after the intravenous injection of heparin. It supports the postulate that in the extrahepatic tissues at least part of the enzyme may be associated with the surface of the capillary endothelial cells (1). However, the finding in both fed and fasting animals that considerable amounts of clearing factor lipase remain in the heart at a time when heparin is releasing very little additional enzyme into the perfusate raises the possibility that the clearing factor lipase may exist at more than one site in the tissue. The observations on extraction of enzyme from defatted tissue powders would be consistent with this. Thus, after perfusion of the hearts with heparin for 1 hr the percentage of the total enzyme that could be extracted with dilute ammonia solution from acetone–ether powders, though less than in unperfused hearts, was still considerable and, on repeated extraction, between 70 and 80% of the total activity was soluble. Similar findings have been obtained with aqueous homogenates of fresh heart tissue.[2] This appearance of more enzyme in solution in the perfused hearts after disintegration of the tissue structure could be explained if part of the enzyme in the intact heart was inaccessible to heparin in the perfusing fluid.

If the clearing factor lipase does play an important role in the tissues in facilitating and regulating the uptake of triglyceride fatty acids from the blood (see Introduction) it seems probable that this function will be carried out by that fraction of the total tissue enzyme which may be situated at the capillary cell surface and which, it is suggested, is released by perfusion of the heart with heparin. Since it has been shown that the amount of this fraction is reduced by about 50% in the fasting animal, it seems possible that the activity of the heart in the uptake of triglyceride fatty acids may be considerably reduced under these conditions even though its total clearing factor lipase content is only slightly changed.

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[2] D. S. Robinson and M. A. Jennings, unpublished observations.

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