The effect of fasting on the utilization of chylomicron triglyceride fatty acids in relation to clearing factor lipase (lipoprotein lipase) releasable by heparin in the perfused rat heart

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ABSTRACT Hearts from rats that have been starved for 10 or 24 hr oxidize ^{14}C-labeled chylomicron triglyceride fatty acids perfused through them at a higher rate than do hearts from rats in the fed state. Starvation for such periods increases the total clearing factor lipase activity of the heart. It is suggested that most of this increase may be accounted for by a rise in that portion of the total enzyme activity of the tissue that is released on perfusion with heparin. In rats starved for 48 hr, removal of this portion by heparin preperfusion reduces the capacity of the heart to oxidize ^{14}C-labeled chylomicron triglyceride fatty acids perfused subsequently by more than 80%.

It is concluded that correlations between triglyceride fatty acid utilization and clearing factor lipase activity in the heart should be sought only with that portion of the total enzyme activity which is released from the intact organ by heparin.

SUPPLEMENTARY KEY WORDS oxidation

PERFUSION OF HEPARIN through the capillary bed of the isolated rat heart causes the release of the enzyme, clearing factor lipase, into the perfusate (1, 2). Release occurs extremely rapidly so that, in a nonrecirculatory perfusion system, the highest levels of enzyme activity are found in samples of perfusate collected within the first minute after the introduction of heparin (2, 3). Enzyme activity in successive perfusate samples falls to a low level, despite the continuing presence of heparin. Nevertheless, even after periods of perfusion of up to 1 hr, a considerable proportion of the initial clearing factor lipase activity of the heart remains associated with the organ. These findings lead to the conclusion that only part of the total enzyme activity of the heart is at a site accessible to heparin in the perfusing fluid. It has been suggested that this portion of the enzyme activity can be identified with that which is believed to be associated with the capillary endothelial cells in vivo and to be active in the uptake of TGFA from the blood (2, 4).

Supporting evidence for a specific role of the heparin-releasable enzyme in TGFA utilization by the heart is provided by studies in which the organ is first perfused, in a nonrecirculatory system, with heparin and then with ^{14}C-labeled chylomicrons in which the bulk of the radioactivity is present in TGFA (3). Removal of the heparin-releasable enzyme markedly reduces the rate at which the hearts can oxidize the radioactive lipid.

In an accompanying paper it has been shown that the total clearing factor lipase activity of the rat heart rises markedly when the animals are starved for periods of 10–24 hr (5). The present study was initiated to investigate whether this rise in total heart enzyme activity was accompanied by an increase in the activity of the enzyme releasable by heparin in the perfused organ and, if so, whether there was a corresponding increase in the utilization of chylomicron TGFA by the heart.

MATERIALS AND METHODS

Female rats of the Wistar strain, weighing 180–200 g and maintained on Oxoid pasteurized breeding diet, contain-

Abbreviations: FFA, free fatty acids; TGFA, triglyceride fatty acids.

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ing approximately 50% carbohydrate, 20% protein, 5% fat, and 25% moisture, minerals and undigestible matter, were used. Fed animals are defined as rats which were maintained on this normal diet and which were killed between 8 a.m.–10 a.m. Starved animals are defined as rats which, after being maintained on the normal diet for several weeks, were starved for varying periods from 8 a.m. In some experiments rats with access to food were killed between 10 a.m. and noon. Such animals are described as rats on the normal diet.

The sources of materials and the methods used to determine FFA and TGFA were as described previously (3, 5), except where indicated.

Perfusion Procedure

Hearts were removed from rats under ether anesthesia and perfused in the nonrecirculatory system previously described (3). Not more than 30 sec elapsed from the moment of removal of the heart to the beginning of the perfusion. No heparin was used at this stage. The perfusion pressure was 100 cm of water, and the temperature of the perfusing fluid at entry into the heart was 37°C. Mechanical performance was monitored visually, and hearts which did not have an initial beat rate of 180–220 beats/min and a coronary flow rate of 6–8 ml/min were discarded. A progressive decrease in both beat rate and coronary flow rate usually occurred during the perfusions so that, after 30 min, they were, respectively, 100–120 beats/min and 4–5 ml/min. No effect of such declines in beat rate and flow rate on $^{14}CO_2$ production from ^{14}C-labeled substrates in the perfusion fluid was observed.

The perfusion fluid was Krebs–Henseleit bicarbonate buffer solution at pH 7.4 (6), gassed continuously with $O_2 + CO_2$ (95:5), to which rat serum (5%, v/v) and heparin (5 IU/ml), or ^{14}C-labeled chylomicrons, or albumin (1%, w/v) containing palmitate-1-^{14}C were added as indicated.

Techniques for the measurement of $^{14}CO_2$ evolved from the perfused heart and for the measurement of incorporation of ^{14}C-labeled materials in the perfusing fluid into the heart lipids were essentially as previously described (3), except that for the measurement of $^{14}CO_2$, the perfusate from the heart was collected directly in 10 N NaOH (1 ml) in graduated tubes (50 ml), into which was fitted the stopper containing the aortic cannula. Control experiments showed that all the $^{14}CO_2$ produced by the heart was present in the perfusate and was trapped in the alkali.

The probability (P) of the significance of a difference between means was tested by applying Behren's modification of Student's "t" test (7).

^{14}C-Labeled Lipid Preparations

Glycerol tripalmitate-1-^{14}C (25–30 mCi/mmole) and palmitate-1-^{14}C (50–60 mCi/mmole) were obtained from the Radiochemical Centre (Amersham, England). Radiochemical purity was checked by thin-layer chromatography.

^{14}C-labeled chyle was collected after 100 μCi of glycerol tripalmitate-1-^{14}C in 1 ml of olive oil was fed to rats with thoracic duct fistulas (3). The chyle, containing between 100–120 μeq of TGFA per ml, was washed with albumin solution in order to reduce the content of ^{14}C-labeled FFA. The method described by Ontko and Zilversmit (8) was used in a slightly modified form. The chyle was mixed at room temperature with an equal volume of 30% (w/v) albumin in 0.85% NaCl solution. After 30 min, 2 ml portions of the mixture were layered under 4 ml of 0.85% NaCl solution in Lusteroid (6.5 ml capacity) centrifuge tubes which were then centrifuged for 20 min at 30,000 g in the Spinco (Model L) preparative ultracentrifuge (No. 40.3 rotor). The surface layer of chylomicrons was separated from the albumin solution by puncturing the tubes at the base and allowing the albumin, as well as most of the salt solution, to escape. The chylomicrons were then recovered and dispersed by forcing them, several times, under pressure, through a hypodermic needle (27 gauge). Finally, they were filtered through a MF-Millipore filter (0.65 μ mean pore size). The amount of ^{14}C-labeled FFA present in the chyle, measured as previously described (3), was reduced by this procedure from approximately 0.8% to between 0.1 and 0.3% of the total lipid radioactivity. Just before use the chyle was diluted in the perfusion fluid to a concentration of 0.9 μeq of TGFA per ml. This concentration of TGFA was chosen on the basis of results of previous studies (3). The chyle was used within 4 days of its collection and within 2 days of the washing with albumin. It was stored in the undiluted state at 4°C.

Palmitate-1-^{14}C was complexed to serum albumin as described previously (9). The complex was filtered through a Millipore filter (0.65 μ mean pore size) and diluted in the perfusion fluid, immediately before use, to a concentration of 0.2 mM of palmitate in 1% (w/v) albumin.

Radioactivity Determinations

All radioactivity measurements were carried out by liquid scintillation counting using a Beckman scintillation spectrometer. At least 5000 counts were recorded for each sample, and relative efficiencies were established by internal standardization. The scintillation fluid was a 0.6% (w/v) solution of Butyl-PBD (2-(4-*tert*-butylphenyl) - 5 - (4 - biphenylyl) - 1,3,4 - oxadiazole, CIBA, (A.R.L.) Ltd., Duxford, Cambridge, England) in sulfur-free toluene containing 5% (v/v) methanol.

Clearing Factor Lipase Assay

Clearing factor lipase activity is expressed in terms of the quantity of FFA released at 37°C from a chylomicron triglyceride substrate. 1 unit of activity is defined as that which releases 1 μmole of FFA during incubation for 1 hr, and activities are expressed as units/g of tissue (fresh weight). Expression as units of activity per heart does not affect the significance of the findings.

The method of assay of the enzyme in homogenates of the fresh tissue, and in homogenates of acetone–ether powders prepared from homogenates of the fresh tissue, has been described (5). When the enzyme was assayed in samples of the heart perfusion fluid a similar method was used. The perfusate samples were collected in tubes at 0°C, and assays of aliquots (2.5 ml) of the samples were carried out within 30 min of the end of the perfusions. Variations in the concentration of heparin and of serum in the assays, resulting from the presence of these components in the perfusate samples, did not affect the activity of the enzyme (5).

RESULTS

The Release of Clearing Factor Lipase by Heparin from the Perfused Rat Heart

It has been shown that when the isolated rat heart is perfused with heparin in a nonrecirculatory system, the total clearing factor lipase activity released into the perfusate roughly corresponds in amount to that which is lost from the heart (2). This finding was based on the results of enzyme assays carried out on homogenates of acetone–ether powders prepared from the hearts before and after their perfusion with heparin. In an accompanying paper (5), assay of the enzyme in such defatted preparations was compared with its assay in homogenates of fresh heart tissue. It was shown that the activities of the two preparations were not related to each other in any simple fashion. In view of this, and because it was hoped that homogenates of fresh heart tissue could be used routinely to assay the enzyme, an experiment was carried out to see whether perfusion of the rat heart with heparin caused the enzyme activities of both types of tissue preparation to fall by similar amounts. Hearts from rats maintained on the normal diet were perfused with heparin for either 3 or 60 min and, at the end of these times, clearing factor lipase was assayed in homogenates of the fresh tissue and in homogenates of acetone–ether powders, as well as in samples of the heart perfusates. The enzyme was also assayed in corresponding tissue preparations from hearts which had not been perfused. The results in Table 1 show that a similar fall occurs in the enzyme activity of both types of preparations after the hearts have been perfused with heparin. After perfusion for either 3 or 60 min, the activity appearing in the perfusate is somewhat greater than the fall in the activity in either type of tissue preparation. This has been a consistent finding in several experiments of this type in which the hearts were perfused for different periods of time.

After perfusion of hearts for 3 min with perfusion fluid which contains heparin, the clearing factor lipase activity in the perfusate is approximately one-half of that which can be assayed in fresh tissue homogenates prepared from the hearts before perfusion (Table 1). Part of the activity in such fresh tissue homogenates is already in solution (5), and it was of interest to see whether the activity released by perfusion with heparin corresponded in any way to this soluble fraction. Accordingly, a group of hearts was perfused for 3 min with perfusion fluid which contained heparin while a control group of hearts was perfused without heparin. Fresh tissue homogenates were prepared at the end of the perfusion in each case, and supernatant and residue fractions were separated from the homogenates by centrifugation. The clearing factor lipase activities of the homogenates and of the supernatant and residue fractions were then determined. The results in Table 2 show that the distribution of enzyme activity between the supernatant and residue fractions is not significantly altered after perfusion with heparin. Similar findings have been obtained in experiments where the time of perfusion with heparin was 60 min. In an earlier study, perfusion of hearts with heparin was shown not to alter significantly the proportion of the total clearing factor lipase activity which was in solution in homogenates of acetone–ether powders prepared from the hearts (2).

TABLE 1 THE CLEARING FACTOR LIPASE ACTIVITIES OF HOMOGENATES OF FRESH AND OF DEFATTED HEART TISSUE AFTER PERFUSION OF THE HEARTS WITH HEPARIN

Perfusion Period	Perfusate	Fresh Tissue Homogenate	Defatted Tissue Homogenate
min	*units of lipase activity/g of tissue**		
0		127	242
3	60	75	186
60	101	38	157

Group of four hearts from rats on the normal diet were perfused for either 3 or 60 min with Krebs–Henseleit bicarbonate buffer (pH 7.4) containing heparin (5 IU/ml) and 5% (v/v) serum. All the hearts were finally perfused for 1 min with the buffer solution alone. The perfusates from the hearts in each group were combined, and the clearing factor lipase activities of duplicate samples were assayed. The ventricles of the hearts of each group were also combined and minced. Portions of each mince were homogenized in serum (30 mg/ml), and duplicate samples of the homogenate were taken for the direct assay of clearing factor lipase while the remainder was used for the preparation of acetone–ether powders. Duplicate portions of each powder were then homogenized and assayed. The enzyme was also assayed in serum homogenates, and in homogenates of acetone–ether powders, prepared from a group of four control hearts that had not been perfused.

* Fresh weight.

The Effect of Clearing Factor Lipase Release by Heparin on the Capacity of the Perfused Rat Heart to Oxidize Chylomicron TGFA

It was shown in a previous study (3) that the release of clearing factor lipase by heparin from the perfused rat heart substantially reduced the capacity of the heart to oxidize ^{14}C-labeled chylomicron lipid which was subsequently perfused. In those experiments, although the bulk of the radioactive lipid in the chylomicrons was ^{14}C-labeled TGFA, the amount of ^{14}C-labeled FFA present, though small, was sufficient to contribute a significant proportion of the residual $^{14}CO_2$ production by the hearts that had been preperfused with heparin.

The experiments have now been repeated with ^{14}C-labeled chylomicrons pretreated to reduce their content of ^{14}C-labeled FFA from 0.8% to between 0.1–0.3% of the total radioactivity. Hearts from rats that had been starved for 48 hr were used as in the earlier study. The results (Table 3) show that, with such chylomicrons, $^{14}CO_2$ production by hearts which have been preperfused with heparin for 15 min is less than 20% of that found with hearts which have been preperfused for the same time, but without heparin.

The residual $^{14}CO_2$ production by the hearts preperfused with heparin could be accounted for by oxidation of the small percentage of ^{14}C-labeled FFA still remaining in the chylomicrons. Thus, at an average coronary flow rate of 5 ml/min through hearts weighing approximately 600 mg, 225 μeq of TGFA are perfused per g of heart in 30 min. If only 0.1% of the total radioactivity is in the form of ^{14}C-labeled FFA, oxidation of this fraction could account for the 0.2 μeq of fatty acids oxidized per 30 min/g of heart by the hearts preperfused with heparin (Table 3).

In this experiment clearing factor lipase was assayed in the pooled preperfusate solutions. Activity equivalent to 69 units/g of tissue (fresh weight) was released into the perfusate in the hearts preperfused with heparin, while less than 5 units/g of tissue (fresh weight) was released in the hearts preperfused without heparin.

The reduction in oxidation of chylomicron TGFA following perfusion with heparin might be due to an effect of heparin on the capacity of the hearts to oxidize FFA produced from the triglycerides. This possibility was investigated using ^{14}C-labeled palmitic acid. The results in Table 4 show that the rate of oxidation of ^{14}C-labeled FFA is not altered by preperfusion of the hearts with heparin.

TABLE 2 THE EFFECT OF HEPARIN PERFUSION ON THE DISTRIBUTION OF CLEARING FACTOR LIPASE ACTIVITY IN HOMOGENATES OF FRESH HEART TISSUE

Perfusion Fluid	Perfusate	Homogenate	Supernatant Fraction	Residue Fraction
		*units of lipase activity/g of tissue**		
Without heparin	4	111	42 (38)	82 (74)
With heparin	62	64	27 (42)	41 (64)

Groups of four hearts, from rats on the normal diet, were perfused for 3 min with 5% (v/v) serum in Krebs–Henseleit bicarbonate buffer (pH 7.4), with or without heparin (5 IU/ml). The hearts were then perfused for 1 min with the buffer solution alone. The perfusates from the group of hearts perfused with and without heparin were combined, and the clearing factor lipase activity of duplicate samples was assayed. The ventricles of the hearts in each group were also combined and homogenized in 0.025 N NH_3–NH_4Cl at pH 8.1 (30 mg/ml). Portions of each homogenate were centrifuged for 30 min at 105,000 *g* at 0°C, and after removing the supernatant solution, the tissue residue was redispersed in the NH_3–NH_4Cl buffer at a concentration equivalent to that in the original homogenate. Enzyme was then assayed in duplicate samples of the homogenate, supernatant solution, and redispersed residue. Each result represents the mean activity of the duplicate assays. The figures in parentheses show the percentage of the homogenate activity found in the supernatant and residue fractions.

* Fresh weight.

The Effect of Fasting on the Release of Clearing Factor Lipase by Heparin from the Perfused Rat Heart

The total clearing factor lipase activity of the rat heart rises markedly in animals that have been starved for between 10 and 24 hr (5). To investigate the effect of such a period of starvation on the clearing factor lipase activity released by heparin from the perfused organ, hearts from rats in the fed state and from rats that had been starved

TABLE 3 THE EFFECT OF HEPARIN PREPERFUSION ON THE OXIDATION OF ^{14}C-LABELED CHYLOMICRON LIPID BY THE PERFUSED RAT HEART

Preperfusion Fluid	Perfusion Period (min) 0–5	5–10	10–15	15–20	20–25	25–30	Total
	*μeq of TGFA oxidized/g of tissue**						
Without heparin	0.06 ± 0.01	0.17 ± 0.05	0.22 ± 0.07	0.23 ± 0.08	0.23 ± 0.06	0.22 ± 0.08	1.13 ± 0.36
With heparin	0.013 ± 0.001	0.030 ± 0.006	0.04 ± 0.01	0.04 ± 0.01	0.05 ± 0.01	0.04 ± 0.01	0.20 ± 0.05

Two groups of six hearts from rats that had been starved for 48 hr were perfused for 15 min with 5% (v/v) serum in Krebs–Henseleit bicarbonate buffer solution (pH 7.4), with and without heparin (5 IU/ml), and then for 5 min with the buffer solution alone. The hearts were then perfused for 30 min with Krebs–Henseleit buffer containing albumin-washed ^{14}C-labeled chylomicrons (0.9 μeq of TGFA/ml). The perfusates during this period were collected over 5-min intervals, and their $^{14}CO_2$ content was determined. The results are expressed as the means ± SD.

* Fresh weight.

for 10 hr were perfused for 15 min in a nonrecirculatory system with perfusion fluid that contained heparin. The clearing factor lipase activities of sequential samples of the perfusates, and of aqueous homogenates of the hearts at the end of the perfusion, are shown in Table 5. Clearly, the increase in the total lipase activity of the heart as a result of fasting is accounted for, in large part, by an increase in the enzyme activity which is released within 30 sec of the introduction of heparin into the perfusing fluid. Thus, in the first 30 sec, the perfusate from the hearts of the fed rats contained activity equivalent to 16 units/g (fresh weight), while that from the hearts of the starved rats contained activity equivalent to 140 units/g (fresh weight). It is noteworthy, however, that the enzyme activity remaining in the hearts at the end of the perfusion was also higher in the starved rats.

The Effect of Fasting on the Capacity of the Perfused Rat Heart to Oxidize Chylomicron Triglyceride Fatty Acids

To study the effect on the oxidation of TGFA by the perfused rat heart of a period of fasting that produces a marked rise in the clearing factor lipase activity releasable by heparin, ^{14}C-labeled chylomicrons, pretreated to reduce their content of ^{14}C-labeled FFA, were perfused for 30 min through hearts from fed rats and from rats that had been starved for 10 hr. The pattern of $^{14}CO_2$ production was similar in each group of hearts. However, the rate of $^{14}CO_2$ production was markedly higher ($P < 0.01$) in the starved group (Table 6). Though the pattern of incorporation of ^{14}C into the heart lipids was not studied in detail (3), total incorporation at the end of the perfusion was also much higher in the starved group. Clearing factor lipase activities, measured at the end of the perfusions in homogenates of portions of the heart ventricles in 0.025 N NH_3–NH_4Cl (pH 8.1; 30 mg/ml), were, respectively, 85.5 and 263 units/g of tissue (fresh weight) in the fed and starved groups.

On the assumption that approximately 225 μeq of TGFA are perfused per g of heart during the 30 min (the perfusion period of these experiments, see above), the hearts from the fed and starved animals oxidized respectively 0.6 and 1.2% of the TGFA passing through their coronary circulations. In the starved animals, a further 1.3% was recovered in the heart lipids. Part of this could represent TGFA retained at the capillary wall (3).

The hearts of the starved animals probably derived a large part of their energy requirements from oxidation of

TABLE 4 Lack of Effect of Preperfusion with Heparin on the Oxidation of Palmitate-1-^{14}C by the Perfused Rat Heart

Preperfusion Fluid	Perfusion Period (min) 0–5	5–10	10–15	15–20	20–25	Total
	*μmoles of palmitic acid oxidized/g of tissue**					
Without heparin	0.06 ± 0.05	0.13 ± 0.07	0.15 ± 0.06	0.16 ± 0.05	0.18 ± 0.07	0.68 ± 0.28
With heparin	0.06 ± 0.05	0.13 ± 0.06	0.15 ± 0.07	0.16 ± 0.07	0.19 ± 0.08	0.69 ± 0.29

Two groups of four hearts from rats that had been starved for 48 hr were perfused for 15 min with 5% (v/v) serum in Krebs–Henseleit bicarbonate buffer solution (pH 7.4), with and without heparin (5 IU/ml), and then for 5 min with the buffer solution alone. The hearts were then perfused for 25 min with Krebs–Henseleit buffer containing palmitate-1-^{14}C (0.2 mM) complexed to 1% (w/v) albumin. The perfusates during this period were collected over 5-min intervals, and their $^{14}CO_2$ content was determined. The results are expressed as the means ±SD.

* Fresh weight.

TABLE 5 The Effect of Fasting on the Release of Clearing Factor Lipase from the Perfused Rat Heart by Heparin

Nutritional State of Animals	Perfusate: Perfusion Period (min) 0–0.5	0.5–1.0	1–3	3–5	5–15	0–15	Heart at End of Perfusion
	*units of lipase activity/g of tissue**						
Fed	16	6	5	2	9	38	47
Starved 10 hr	140	13	19	9	34	215	99

Groups of five hearts, taken either from rats on the normal diets which were killed between 8 a.m. and 9 a.m. (fed rats) or from rats that had been starved for 10 hr from 8 a.m. on the day of the experiment, were perfused with 5% (v/v) serum in Krebs–Henseleit bicarbonate buffer (pH 7.4) containing 5 IU heparin/ml. All the perfusate from each heart was collected in a series of samples over the time intervals shown. The perfusate samples at each time interval from each group of hearts were combined, and the clearing factor lipase activity was measured in duplicate. At the end of the perfusion, the ventricles of the hearts of each group were also combined and homogenized in 0.025 N NH_3–NH_4Cl at pH 8.1 (30 mg/ml). Duplicate samples of each homogenate were assayed for clearing factor lipase. Mean values of the assays are reported.

* Fresh weight.

TABLE 6 THE EFFECT OF FASTING ON THE OXIDATION OF ^{14}C-LABELED CHYLOMICRON LIPID BY THE PERFUSED RAT HEART

Nutritional State of Animals	Perfusion Period (min) 0–5	5–10	10–15	15–20	20–25	25–30	Total	^{14}C Incorporated into Heart Lipids
	*μeq of TGFA oxidized/g of tissue**							*μeq of TGFA/g of tissue**
Fed	0.10 ± 0.02	0.20 ± 0.10	0.23 ± 0.12	0.25 ± 0.14	0.24 ± 0.12	0.23 ± 0.13	1.25 ± 0.60	0.42 ± 0.20
Starved 10 hr	0.14 ± 0.02	0.43 ± 0.10	0.54 ± 0.10	0.53 ± 0.12	0.52 ± 0.14	0.50 ± 0.17	2.66 ± 0.40	3.03 ± 1.70

Groups of six hearts, taken either from rats on their normal diets which were killed between 8 a.m.–10 a.m. (fed rats) or from rats that had been starved for 10 hr from 8 a.m. on the day of the experiments, were perfused for 2 min with Krebs–Henseleit bicarbonate buffer (pH 7.4) and then for 30 min with buffer containing albumin-washed ^{14}C-labeled chylomicrons (0.9 μeq TGFA/ml). The perfusates during the 30 min-perfusion were collected over 5-min intervals, and their $^{14}CO_2$ content was determined. At the end of each perfusion, the heart was perfused again for 2 min with the buffer alone. Portions of the heart ventricles were then taken for measurement of ^{14}C incorporated into the tissue lipids. Results are expressed as the means ± SD.

* Fresh weight.

the perfusate chylomicron TGFA in these experiments. The maximum rate of $^{14}CO_2$ output, during the 15–20 min perfusion period, represents the oxidation of 6.4 μeq of TGFA per hr/g of tissue (fresh weight). On the assumption that the amount of O_2 taken up was approximately 240 μmoles/hr per g of tissue (fresh weight) (3), approximately two-thirds of this uptake would be required for the complete oxidation of the chylomicron TGFA. The maximum rate of TGFA oxidation observed with the hearts from fed rats was 3 μeq/hr per g of tissue (fresh weight). This would account for about one-third of the concomitant O_2 uptake.

Similar experiments to those reported in detail in Table 6 have been carried out with hearts from rats that were starved for 24 hr. In a group of eight hearts, the total $^{14}CO_2$ production over a 25 min perfusion period was equivalent to the oxidation of 2.50 ± 0.37 μeq of TGFA/g of tissue (fresh weight) and incorporation into the heart lipids at the end of the perfusion was equivalent to 1.71 ± 0.30 μeq of TGFA/g of tissue (fresh weight). In a control group of hearts from fed animals, the corresponding values for $^{14}CO_2$ production and tissue incorporation were 1.46 ± 0.50 and 0.74 ± 0.27, respectively.

The Effect of Fasting on the Oxidation of ^{14}C-Labeled Palmitic Acid by the Perfused Rat Heart

The increased oxidation of chylomicron TGFA by hearts from rats starved for 10 and 24 hr might have been due to an increase in the capacity of the hearts to oxidize FFA produced from the triglycerides. This possibility was studied by perfusing hearts from fed and 24-hr–starved rats with ^{14}C-labeled palmitic acid. The results in Table 7 show that $^{14}CO_2$ production was actually reduced ($P < 0.001$) with the hearts from starved animals. Similar findings have been reported by Vahouny, Katzen, and Entenman (10), though Opie, Evans, and Shipp (11) found no significant differences in FFA utilization by hearts from fed and starved animals.

TABLE 7 THE EFFECT OF FASTING ON THE OXIDATION OF PALMITATE-1-^{14}C BY THE PERFUSED RAT HEART

Nutritional State of Animals	μmoles of Palmitic Acid Oxidized/g of Tissue*	^{14}C Incorporated into Heart Lipids μmoles of Palmitic Acid/g of Tissue*
Fed	0.63 ± 0.12	0.35 ± 0.06
Starved 24 hr	0.39 ± 0.12	0.26 ± 0.11

Groups of 12 hearts, taken either from rats which were on the normal diets and which were killed between 8 a.m.–10 a.m., or from rats that had been starved for 24 hr before being killed between 8 a.m.–10 a.m., were perfused for 2 min with Krebs–Henseleit bicarbonate buffer (pH 7.4) and then for 15 min with buffer containing palmitate-1-^{14}C (0.2 mM) complexed to 1% (w/v) albumin. $^{14}CO_2$ production and ^{14}C incorporation into heart lipids were determined as described in Table 6, incorporation into heart lipids being measured in only seven hearts from each group. Results are expressed as the means ± SD.

* Fresh weight.

DISCUSSION

The role of clearing factor lipase in facilitating and regulating the uptake of TGFA from the blood by the extrahepatic tissues has received considerable emphasis in recent years (see references 4, 12). The enzyme is believed to hydrolyze chylomicron and very low density lipoprotein triglycerides at, or close to, the surface of the endothelial cells of the blood capillaries to produce FFA. These are readily able to enter the tissue cells either to be oxidized, particularly by muscle, or to be reesterified and stored as triglycerides, especially by adipose tissue. In the lactating animal, high clearing factor lipase activity in the mammary gland is thought to result in the uptake of a considerable proportion of the circulating TGFA by this organ, prior to their secretion as milk triglycerides.

Evidence that the extent of uptake of TGFA by particular extrahepatic tissues depends on the activity of the enzyme in these tissues is available for adipose tissue and for the mammary gland (13). However, correlations between TGFA uptake and clearing factor lipase activity

are not so clear in the case of muscle. In particular, though the activity of the enzyme has been reported to be altered in heart muscle in different physiological and pathological conditions, and after a variety of experimental treatments, the results of different studies are not always in agreement, and the changes observed sometimes fail to correlate with reported changes in the utilization of TGFA by the heart (13). The findings reported in an accompanying paper offer some possible explanations for the discrepancies between the published reports on heart clearing factor lipase activity (5). Those in the present study suggest that correlations between TGFA uptake and clearing factor lipase activity should only be sought with that portion of the total tissue activity which is capable of being released from the intact organ by heparin and which is, by implication, present at the capillary wall site. Thus, the results showing that the capacity of the heart to utilize TGFA is dramatically reduced following perfusion with heparin, suggest that only the heparin-releasable enzyme is concerned in TGFA uptake. Again, the increase in TGFA utilization by hearts from rats which have been starved for 10 hr is associated with a marked increase in the total clearing factor lipase activity of the heart that is largely accounted for by an increase in the enzyme activity which is releasable by heparin.

The precise relationship between clearing factor lipase which can be released by heparin in the perfused heart and that which cannot remains to be clarified. However, there is some evidence that, in adipose tissue, the enzyme exists in at least two sites (14). A small proportion of the total enzyme activity of this tissue in the fed animal is present in association with the fat cell, but the major part is situated elsewhere. It is this latter fraction which appears to be functional in TGFA uptake in the fed state and which may well be localized at the capillary wall surface. Some evidence has been obtained that the enzyme associated with the fat cell is a precursor of the functional enzyme. It is not yet known whether a similar situation exists for the enzyme in heart muscle.

The present observations clearly cannot be directly extrapolated to conditions in vivo. Nevertheless, the finding that, in equivalent perfusions, oxidation of TGFA by hearts from rats starved for up to 24 hr is much more efficient than by hearts from fed rats, does suggest that the efficiency of utilization of plasma TGFA by heart muscle in vivo may also be increased under such fasting conditions. The concentration of TGFA in the plasma of rats starved for 24 hr is between 0.5–0.9 μeq/ml[1], and, in the present study, oxidation of TGFA perfused through the heart at a concentration of 0.9 μeq/ml was able to provide about two-thirds of the total energy requirements. Although energy requirements are likely to be considerably higher in the working heart (3) and alternative substrates are available to the heart in vivo, the contribution made by the plasma TGFA could still be appreciable under such conditions. The finding that the activity of clearing factor lipase in diaphragm muscle is also increased markedly in rats starved for up to 24 hr (5) raises, moreover, the further possibility of increased efficiency of TGFA utilization by muscle in general.

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[1] Unpublished experiments.