Metabolism of Triglyceride Fatty Acid by the Perfused Rat Heart

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1. Chyle lipids, labelled with ^{14}C , are taken up and oxidized by the isolated perfused rat heart. 2. In recirculatory perfusions, when chyle lipids are the sole exogenous energy source, about 24% of the total oxygen uptake is accounted for by their oxidation. This proportion is not changed by starvation of the rats for 48 hr. and falls when an external work load is imposed on the left ventricle. 3. With albumin in the perfusion medium, the rate of $^{14}\text{CO}_2$ output is reduced by half and there is a rise in the proportion of ^{14}C -labelled free fatty acids in the medium. 4. Clearing-factor lipase appears in the perfusion medium when chyle lipids are perfused through the heart. In the absence of albumin, the activity of the medium enzyme is low and only a small proportion of the $^{14}\text{CO}_2$ output can be accounted for by the oxidation of free fatty acids released by it. In the presence of albumin, the enzyme is more active in the medium. 5. When a substantial proportion of the total clearing-factor lipase is removed from the heart by a prior perfusion with heparin, ^{14}C -labelled chyle lipid perfused subsequently is oxidized at only half the normal rate.

TGFA§ are taken up from the blood at a rapid rate by a variety of extrahepatic tissues (Bragdon & Gordon, 1958; Olivecrona, 1962; Bezman-Tarcher, Otway & Robinson, 1965) and they could provide these tissues with an important source of metabolic energy. Much circumstantial evidence suggests that this uptake of TGFA depends upon the hydrolysis of the triglycerides to FFA by the enzyme clearing-factor lipase (or lipoprotein lipase), and that this hydrolysis occurs at a site in the tissues which may be close to the capillary wall (Robinson & French, 1960; Robinson, 1963).

In several recent investigations the oxidation to $^{14}\text{CO}_2$ of ^{14}C -labelled TGFA recirculated through the isolated rat heart has been reported (Delcher, Fried & Shipp, 1965; Kreisberg, 1966a; Crass & Meng, 1966). The results of the present work suggest that clearing-factor lipase is released from the heart into the recirculation medium under such

circumstances and that it may act there on the perfusate triglycerides to produce FFA. At least part of the $^{14}\text{CO}_2$ production found could therefore have been derived from the oxidation of ^{14}C -labelled FFA produced in the perfusion medium (Shipp, Opie & Challoner, 1961; Evans, Opie & Shipp, 1963) rather than from TGFA taken up and oxidized by the heart.

In two of the studies in which ^{14}C -labelled chyle lipids were perfused (Kreisberg, 1966a; Crass & Meng, 1966), the perfusion medium contained albumin as such or as a serum component. This would act as an acceptor of ^{14}C -labelled FFA, either present initially as a minor component of the chyle lipids or produced from them during the course of the perfusion. In the study of Crass & Meng (1966), furthermore, the serum albumin contained unlabelled FFA which could have contributed to the energy requirements of the heart. The presence of albumin therefore seems likely to have complicated further the interpretation of these studies.

In view of these considerations, in the present study ^{14}C -labelled chyle lipid was perfused through the rat heart in the absence of albumin or other serum proteins, and when the extent of clearing-factor lipase action in the perfusion medium was minimal and known. Evidence for the uptake and oxidation of the chyle TGFA, without prior release

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§ Abbreviations: TGFA, triglyceride fatty acid(s); FFA, free fatty acid(s).

of FFA into the perfusion medium, has been obtained.

Crass & Meng (1964) and Robinson & Jennings (1965) have shown that perfusion of the rat heart with solutions containing heparin leads to the release of a substantial proportion of the total clearing-factor lipase of the heart into the perfusate. In view of the suggested role of this enzyme in the uptake of TGFA from the blood, the effect of pre-perfusion with heparin on the capacity of hearts to utilize ^{14}C -labelled chyle lipid has also been studied.

MATERIALS AND METHODS

Perfusion procedures

Recirculation and once-through systems. In the majority of experiments, hearts from female albino rats (200–240 g.), fed *ad libitum* on a normal laboratory stock diet and anaesthetized with intraperitoneal Nembutal (12 mg.), were perfused at 35° in the recirculation system of Morgan, Henderson, Regen & Park (1961). No heparin was used.

Immediately after their removal from the animals, the hearts were tied on to the perfusion cannulae and pre-perfused for 15 min. at a pressure of about 60 mm. Hg in a non-recirculatory system with Krebs–Henseleit bicarbonate buffer, pH 7.4 (Krebs & Henseleit, 1932), gassed continuously with $\text{O}_2 + \text{CO}_2$ (95:5). When plasma, serum or heparin was added to the pre-perfusion fluid a further non-recirculatory perfusion with plain Krebs–Henseleit bicarbonate buffer for 5 min. followed the pre-perfusion.

The hearts were then usually transferred to a recirculation perfusion system. This contained 20 ml. of ^{14}C -labelled chyle in Krebs–Henseleit buffer which was gassed continuously with $\text{O}_2 + \text{CO}_2$ (95:5). The effluent gas was bubbled through a column of 3N-NaOH soln. to trap $^{14}\text{CO}_2$ present in the gas phase. Heart rate, coronary flow rate and perfusion pressure were monitored at 5 min. intervals (Opie, 1965). The mean perfusion pressure was 80 mm. Hg, but in individual hearts this was varied from 60 to 120 mm. Hg, to keep the coronary flow rate constant at about 8 ml./min. (Opie, 1965).

In some experiments the hearts were transferred from the pre-perfusion system to a once-through perfusion system and perfused with about 200 ml. of the gassed ^{14}C -labelled chyle perfusate. The effluent from the heart, instead of being returned through the sintered-glass filter of the heart chamber (see Fig. 1 of Morgan *et al.* 1961), was collected into graduated tubes (50 ml.) into which the stopper containing the aortic cannula fitted. As the effluent accumulated, air forced out of the collecting tube was passed through 3N-NaOH.

Perfusions with left atrial cannulation. To study the effect of increased muscular work on the metabolism of ^{14}C -labelled chyle a working rat heart system (Morgan, Neely, Brineaux & Park, 1965a; Morgan *et al.* 1965b) was used with the following modifications. Hearts were taken from male albino rats (320–450 g.) fed *ad libitum* and anaesthetized with intraperitoneal Nembutal. No heparin was used. The isolated heart was arrested in ice-cold buffer, mounted on the aortic cannula and promptly washed through with gassed Krebs–Henseleit bicarbonate buffer containing glucose (4 mM). The left atrium was immediately tied to its

cannula and, within 2 min. of removing the heart from the rat, the left heart was allowed to work at an atrial input pressure of 10 cm. H_2O and against an aortic pressure of 90 cm. H_2O . Between 80 and 90 ml. of ^{14}C -labelled chyle in Krebs–Henseleit bicarbonate buffer gassed with $\text{O}_2 + \text{CO}_2$ (95:5) was then recirculated at 35°.

At the end of all perfusions the hearts were flushed through with 20 ml. of the Krebs–Henseleit bicarbonate buffer at 4°, removed from the cannulae and cut in two pieces. These were lightly blotted and weighed on a torsion balance. Usually one piece was used for lipid analysis and the other for clearing-factor lipase assay. Except in the perfusions with left atrial cannulation, the final wet weights of the hearts ranged from 0.7 to 1.0 g.

Oxygen uptake. This was measured in a variety of perfusion conditions with the oxygen electrode of Bishop (1960), obtained from Electronic Instruments Ltd., Richmond, Surrey, directly coupled to an amplifying recorder (Kipp micrograph BD2 of Kipp, Delft, The Netherlands). The electrode was fitted into a small glass chamber (1 ml. capacity) and samples of the perfusion media were diverted through this.

Additions to pre-perfusion media. Serum or plasma was sometimes present in the pre-perfusion media at final concentrations of 1% or 5% (v/v), the medium being adjusted, after gassing, to pH 7.4 with NaOH. When heparin was also present it was usually added to final concentration 5 i.u./ml.

To obtain serum and plasma, blood was collected from male rats weighing 300–400 g. as described by French, Robinson & Florey (1953). Serum was prepared by allowing the blood to clot for 30 min. at 37° and then centrifuging for 15 min. at 2500 g at room temperature. Plasma was prepared by collecting the blood into a solution of heparin (1000 i.u./ml.) to a final heparin concentration in the plasma of 5 i.u./ml., which prevented clotting during the course of the experiments. The plasma was separated by centrifuging the blood for 15 min. at 2500 g at room temperature.

Preparation of ^{14}C -labelled chyle. The collection and washing of ^{14}C -labelled chyle from rats with thoracic-duct fistulae was carried out as previously described (Bezman-Tarcher *et al.* 1965), except that 100 μC of glycerol tri[1- ^{14}C]-palmitate in 1 ml. of olive oil was fed to each rat. The washed chyle was added to gassed Krebs–Henseleit bicarbonate buffer and passed through a sintered-glass filter (porosity grade 2) before use. The perfusate TGFA concentration was varied in some experiments but was usually 0.7 $\mu\text{equiv.}$ /ml., as estimated from the glyceride glycerol content (Van Handel & Zilversmit, 1957).

Analytical procedures

Disappearance of radioactivity from the perfusate. This was determined by taking samples (0.1 ml.) in triplicate from the perfusate which accumulated above the sintered-glass filter (see Fig. 1 of Morgan *et al.* 1961) immediately before and after the perfusion period. Each sample was evaporated to dryness in a vacuum desiccator over P_2O_5 and counted in 7 ml. of phosphor solution of the following composition: 2,2'-*p*-phenylene-bis-5-phenyloxazole (0.2 g./l.) and *p*-terphenyl (4 g./l.) in toluene diluted with methanol (5 parts of terphenyl-2,2'-*p*-phenylene-bis-5-phenyloxazole in toluene and 2 parts of methanol). Radioactivity was assayed on a Tritomat 6020 (Isotopes Development Ltd., Beenham, Berks.) or a Beckman Scintillation Spectrometer (Beckman

Instruments Ltd., Glenrothes, Fife, Scotland), at least 5000 counts being recorded for each sample. Relative efficiencies were established by internal standardization.

¹⁴CO₂ present in the perfusate and the sodium hydroxide column. This was measured by acidification of appropriate samples in a Warburg-type flask (Snyder & Godfrey, 1961) and collection of the liberated CO₂ in 0.4 ml. of *m*-Hyamine in methanol, the radioactivity of which was then counted in 7 ml. of the phosphor solution described above. The main compartment contained about 50% methanol (v/v) to prevent distillation of the methanol from the centre well; otherwise drying of the Hyamine occurred, and this resulted in imperfect suspension of the Hyamine in the phosphor solution.

Fractionation of heart ¹⁴C-labelled lipid. A portion of each heart was homogenized for 1 min. at top speed in 5 ml. of methanol in an MSE top-drive homogenizer. Chloroform (10 ml.) was added and, after shaking, the mixture was allowed to stand overnight at room temperature. The procedure of Folch, Lees & Sloane-Stanley (1957) was then used to purify the extracted lipids, which were finally dissolved and stored at 4° in 10 ml. of chloroform.

Separation of phospholipids, cholesteryl esters and mono-, di- and tri-glycerides was carried out by thin-layer chromatography on a portion (2–3 ml.) of the chloroform extract, to which a mixture of 25 µg. of monostearin and 40 µg. of palmitic acid was added as carrier lipid, by using a modification of the method of Bezman-Tarcher *et al.* (1965). Before chromatography the plate was developed for a distance of 9 cm. in 2% (v/v) acetic acid in diethyl ether and then dried in air for 15 min. The lipid extract was reduced in volume to 0.1–0.2 ml. and added in a series of spots across the plate to form a thin band at the origin. The developing solvent was hexane–diethyl ether–acetic acid–methanol (100:20:2:1.8, by vol.) and the separated lipid bands were detected with iodine. Each lipid band was scraped off the chromatogram and the lipid was eluted on a small column with 15 ml. of methanol. After evaporation to dryness, the lipids were redissolved in 1 ml. of methanol and 7 ml. of phosphor solution was added before the radioactivity was measured as described above. Recovery of the ¹⁴C-labelled lipid applied to the thin-layer plates ranged between 98% and 102%.

In preliminary studies all the radioactivity associated with the separated mono- and di-glyceride and with the cholesteryl ester and phosphatide bands was shown to remain with these components on rechromatography. However, whereas FFA were localized visually as a distinct band between the triglyceride and diglyceride bands, a small amount of radioactive material was also spread over the area between the tri- and di-glyceride bands. When the eluted lipid from this whole area was rechromatographed, the radioactivity was concentrated in two distinct bands, corresponding to the positions occupied by FFA and diglycerides. Standard diglyceride samples showed no tendency to spread into the area occupied by FFA.

These findings seem best explained by assuming that some breakdown of the triglyceride of the lipid extract to FFA and diglyceride occurs during the chromatographic separations. Although less than 1% of the total triglyceride radioactivity is involved, the true radioactivity of the FFA fraction cannot be determined in such circumstances. For this reason, FFA radioactivity levels were determined by fractionation of the lipid extract on columns of Florisil (Carroll, 1961). An appropriate sample (1–3 ml.) of the

unfractionated extract in chloroform was evaporated to dryness, redissolved in diethyl ether and applied to a column of Florisil (3 g.; 100–200 mesh) that had been activated at 110° and which was not hydrated. Neutral lipids were eluted with 100 ml. of 2% (v/v) methanol in diethyl ether and FFA with 100 ml. of 4% (v/v) acetic acid in diethyl ether. Phospholipids were not eluted. A portion of the FFA eluate was reduced in volume and, after acidifying with H₂SO₄, the FFA was extracted into hexane. Samples of the hexane extract were then evaporated to dryness and the lipid was dissolved in 7 ml. of the phosphor solution for radioactive assay.

Fractionation of medium ¹⁴C-labelled lipid. This was achieved by a combination of chromatography on thin-layer plates and on Florisil columns as described for the fractionation of heart ¹⁴C-labelled lipid. Samples of the perfusate (5 ml.) were extracted in 100 ml. of chloroform–methanol (2:1, v/v), purified by the Folch technique and stored in 10 ml. of chloroform.

Assay of clearing-factor lipase. Enzyme activities are expressed in terms of the amount of FFA released by the enzyme during the hydrolysis of chyle triglycerides (Robinson, 1963). The assay systems of Robinson & Jennings (1965) were used to measure the enzyme activity of samples of perfusion medium and of heart tissue. Heparin was present at a final concentration of 3 i.u./ml. in all the assays of medium enzyme activity and all such determinations were carried out within 3 hr. of the collection of the media, which had been kept at 4°. Assays of heart tissue enzyme were carried out on acetone–ether-dried powders that were prepared immediately after the perfusion was completed and which were then stored *in vacuo* at 4° overnight. Homogenates were prepared in an ammonia buffer medium (0.025 *N*, pH 8) containing 1 i.u. of heparin/ml., and were present in the assay system at a concentration of about 1 mg. of powder dry wt./ml. The pH of the assays was 8.1.

To test whether appreciable clearing-factor lipase action could occur in the perfusion medium during the perfusions (see the Discussion section), assay of the perfusate enzyme was also carried out at pH 7.4 in the absence and in the presence of albumin. When a heart was perfused for 10 min. in a non-recirculatory system with heparin (5 i.u./ml.) in Krebs–Henseleit bicarbonate buffer, the activity of the perfusate enzyme at pH 7.4 in the absence and presence of albumin was less than 5% and 33% respectively of that measured under the usual assay conditions in the presence of albumin at pH 8.1.

Chemicals. Florisil (Floridin Co., Tallahassee, Fla., U.S.A.) was obtained from British Drug Houses Ltd., Poole, Dorset, and silical gel G from E. Merck A.-G., Darmstadt, Germany. Hyamine, *p*-terphenyl and 2,2'-*p*-phenylene-bis-5-phenyloxazole were obtained from Nuclear Enterprises Ltd., Edinburgh, Scotland. Monostearin was obtained from Calbiochem, Los Angeles, Calif., U.S.A., and palmitic acid from Koch–Light Laboratories Ltd., Colnbrook, Bucks. Tripalmitin and cholesteryl stearate, used as standards in the chromatographic fractionations, were obtained from the Hormel Institute, University of Minnesota, U.S.A. Diolein was a purified commercial product. Glycerol tri[1-¹⁴C]palmitate was obtained from The Radiochemical Centre, Amersham, Bucks., and heparin (Pularin) from Evans Medical Supplies Ltd., Liverpool. Albumin (bovine serum albumin, fraction V) was obtained from Armour Pharmaceuticals Co. Ltd., Eastbourne,

Table 1. *Effects on the metabolism of ^{14}C -labelled chyle by the perfused rat heart of perfusate glyceride concentration, duration of perfusion, starvation and presence of albumin*

Perfusion conditions were as described in the text unless otherwise indicated. Results are expressed as means $\pm$ s.e.m. with the numbers of hearts in parentheses. Percentage recovery of ^{14}C is percentage of ^{14}C disappearance from perfusate accounted for by sum of apparatus uptake, incorporation into heart lipid and $^{14}\text{CO}_2$ formation. Albumin: dialysed bovine serum albumin at a concentration of 3 g./100 ml. of perfusate.

Expt. no.	Concn. of perfusate glyceride fatty acid (μ equiv./ml.)	Perfusion period (min.)	Special conditions	Utilization of perfusate ^{14}C -labelled glyceride fatty acid (μ equiv./g. of heart final wet wt.)				
				Disappearance from perfusion medium	Uptake by apparatus	Uptake into heart lipid	Appearance as $^{14}\text{CO}_2$	Percentage recovery of ^{14}C
1	0.7	0-5		0.63 ± 0.24 (5)	0.33 (1)	0.36 ± 0.06 (3)	0.09 ± 0.012 (5)	124
2	0.7	0-10		1.53 ± 0.33 (10)	0.51 ± 0.15 (6)	0.39 ± 0.06 (3)	0.21 ± 0.03 (11)	73
3	0.7	0-20		3.09 ± 0.42 (9)	1.17 ± 0.21 (4)	0.81 ± 0.12 (4)	0.57 ± 0.06 (10)	83
4	0.7	0-30		2.43 ± 0.21 (13)	1.11 ± 0.27 (5)	0.93 ± 0.54 (3)	0.75 ± 0.09 (13)	115
5	1.4	0-30		—	—	—	0.96 ± 0.15 (3)	—
6	2.8	0-30		—	—	—	0.36 ± 0.15 (3)	—
7	0.7	0-10	Rats starved for 48 hr.	1.23 ± 0.09 (5)	—	—	0.27 ± 0.03 (5)	—
8	0.7	0-20	Rats starved for 48 hr.*	3.09 ± 0.06 (5)	—	—	0.72 ± 0.12 (5)	—
9	0.7	0-30	Rats starved for 48 hr.	2.25 ± 0.21 (5)	—	—	0.87 ± 0.02 (5)	—
10	0.9	0-20	Albumin present	2.10 ± 0.27 (5)	—	—	0.33 ± 0.06 (5)	—

* Pre-perfused for 25 min. instead of 15 min. (see the Materials and Methods section).

Sussex. Other chemicals were obtained from British Drug Houses Ltd. and were of AnalaR grade.

Expression of results. Approx. 98% of the radioactivity in the ^{14}C -labelled chyle lipids was present in mono-, di- or tri-glycerides, over 92% being present in the triglyceride component (Table 3). The possibility that minor non-glyceride components of the chyle might be selectively utilized by the heart appears unlikely for the reasons outlined in the Discussion section. Consequently it has seemed legitimate to use the specific activities of the chyle glyceride fatty acids, which were measured in each experiment, to express the amounts of radioactivity found as $^{14}\text{CO}_2$ and in heart lipids, as well as radioactivity disappearing from the perfusate, in terms of their equivalents as $\mu\text{equiv.}$ of perfusate glyceride fatty acid.

Since the donor rats, from which the ^{14}C -labelled chyle was obtained, were fed with olive oil containing a trace amount of glycerol tri[1- ^{14}C]palmitate, the chyle glycerides contained predominantly oleic acid, though the ^{14}C label was mainly in glyceride palmitic acid. Though differences in the metabolism of oleic acid and palmitic acid by the perfused rat heart have been reported (Evans, 1964), these are not of significance in the present study. It has been shown that fatty acids labelled only in the carboxyl group are appropriate for the study of fatty acid oxidation by the rat heart (Evans *et al.* 1963).

The results are expressed as mean values $\pm$ S.E.M. and the difference between two means is held to be significant when P , derived from Student's t test, is < 0.05 (Snedecor, 1946).

RESULTS

Utilization of ^{14}C -labelled chyle by rat hearts perfused in a recirculatory system

The results of studies in which hearts were perfused for 30 min. at three different concentrations of ^{14}C -labelled chyle are shown in Table 1 (Expts. 4–6). Although the perfusion media contained no albumin or other added serum proteins, oxidation of the chyle lipids occurred. The overall rates of $^{14}\text{CO}_2$ formation were similar at concentrations of 0.7

and 1.4 $\mu\text{equiv.}$ of TGFA/ml. and less at the higher concentration of 2.8 $\mu\text{equiv.}/\text{ml.}$

At all concentrations studied and throughout the perfusion, the heart rates, coronary flow rates and perfusion pressures remained relatively constant. At a concentration of 0.7 $\mu\text{equiv.}$ of TGFA/ml. the mean heart rate was 170 beats/min., the mean coronary flow rate was 8.1 ml./g. of heart wet wt./min. and the mean perfusion pressure was 88 mm. Hg. However, the force of contraction, as gauged visually, which was well maintained in 12 out of 14 hearts at a concentration of 0.7 $\mu\text{equiv.}$ of TGFA/ml., deteriorated during one of three perfusions at 1.4 $\mu\text{equiv.}/\text{ml.}$ and in four of ten perfusions at 2.8 $\mu\text{equiv.}/\text{ml.}$ Many of the hearts with a poor contractile force also developed arrhythmias and, in an experiment in which two hearts were perfused at 8.5 $\mu\text{equiv.}/\text{ml.}$ and two hearts at 17 $\mu\text{equiv.}/\text{ml.}$, arrhythmias developed after 5 min. and the force of cardiac contraction became very feeble after about 10 min.

Because of the mechanical deterioration at high concentrations of chyle, all subsequent experiments were carried out at concentrations of either 0.7 or 0.9 $\mu\text{equiv.}$ of chyle TGFA/ml. of perfusion medium, hearts with a poor contractile force at any stage during a perfusion being rejected.

The effect of the duration of the perfusion on the uptake of ^{14}C from the perfusate and on the incorporation of ^{14}C into CO_2 and heart lipid is also shown in Table 1 (Expts. 1–4). The rate of $^{14}\text{CO}_2$ formation was low initially (0–5 min. period) but then increased to a maximum, between 10 and 20 min., of 0.36 $\mu\text{equiv.}$ of TGFA/g. of heart wet wt./10 min., before declining to a lower rate between 20 and 30 min. Similar lag periods in $^{14}\text{CO}_2$ output have been described by Crass & Meng (1966) and by Kreisberg (1966a).

The amount of radioactivity which appeared in

Table 2. *Effects of time of perfusion on the percentage distribution of ^{14}C in the lipids of the rat heart perfused with ^{14}C -labelled chyle*

Perfusion conditions were as described in the text. The concn. of the perfusate glyceride fatty acid was 0.7 $\mu\text{equiv.}/\text{ml.}$ Results are expressed as means $\pm$ S.E.M. with the numbers of observations in parentheses. FFA, free fatty acids; TG, triglyceride; DG, diglyceride; MG, monoglyceride; PL, phospholipid; CE, cholesteryl ester.

Time of sampling
from beginning of
perfusion (min.)

	FFA	TG	DG	MG	PL	CE
5	11.6 $\pm$ 1.1 (3)	71.0 $\pm$ 3.1 (3)	5.9 $\pm$ 0.6 (3)	3.5 $\pm$ 0.2 (3)	4.8 $\pm$ 1.5 (3)	1.4 $\pm$ 0.3 (3)
10	10.1 $\pm$ 2.1 (3)	74.8 $\pm$ 0.6 (3)	5.6 $\pm$ 0.1 (3)	2.6 $\pm$ 0.1 (3)	6.8 $\pm$ 0.4 (3)	0.9 $\pm$ 0.5 (3)
20	4.4 $\pm$ 0.5 (4)	83.4 (2)	5.1 $\pm$ 0.1 (4)	1.7 $\pm$ 0.2 (4)	4.9 $\pm$ 0.5 (4)	0.6 (2)
30	2.7 $\pm$ 0.1 (3)	84.9 (2)	4.7 $\pm$ 0.2 (3)	0.9 $\pm$ 0.1 (3)	5.3 $\pm$ 1.0 (3)	0.3 (2)

the heart lipids also increased with the time of perfusion. Although the percentage of the total radioactivity that was present as FFA and as monoglyceride fell significantly as the perfusion progressed (Table 2), the increase in the total heart lipid radioactivity which accompanied this change resulted in similar absolute radioactivity values in FFA and monoglycerides at 5 min. and at 20 min. The major absolute increase in heart lipid radioactivity with increased time of perfusion occurred in the triglyceride fraction.

The overall distribution of radioactivity in the heart lipids is similar to that reported by Delcher *et al.* (1965), after the perfusion of ^{14}C -labelled lipoproteins of $d < 1.006$, except that a lower percentage of the total radioactivity is present in phospholipids. Kreisberg (1966a) and Crass & Meng (1966), who perfused ^{14}C -labelled chyle, reported even higher percentages of total tissue radioactivity as phospholipid. They, however, used chromatography on silicic acid columns to separate their phospholipids and it has been our experience with the latter technique that the phospholipid fraction contains labelled glyceride impurities. Scheuer & Olson (1967) in a recent study also perfused ^{14}C -labelled chyle and found, at similar perfusate TGFA concentrations, about 7% of the tissue radioactivity in phospholipids. They chromatographed the cardiac phospholipids twice on silica gel plates to avoid contamination with neutral glycerides.

During control studies in which ^{14}C -labelled chyle was recirculated through the perfusion apparatus without a heart being present, a considerable, and somewhat variable, disappearance of radioactivity from the medium was observed. No $^{14}\text{CO}_2$ appeared in the medium or in the gas phase during such control perfusions and there was no change in the distribution of ^{14}C in the different lipid fractions of the medium. Similar losses of radioactivity from the medium during control perfusions have been reported by Delcher *et al.* (1965) and Scheuer & Olson (1967), and it appears that radioactive lipid is sequestered on the perfusion apparatus. The low recoveries of radioactivity evident from the data of Crass & Meng (1966) and the 20% loss of radioactivity reported by Kreisberg (1966a) could be accounted for by such sequestration during perfusions with the heart present.

In Table 1 the extent of uptake of radioactivity by the apparatus was assessed from the results of control perfusions carried out in the absence of a heart. At least one-third of the radioactivity which disappeared from the medium was accounted for in this way. The apparent return of radioactivity to the medium at the later stages of a 30 min. perfusion may be due to the release of such sequestered radioactivity into the medium and the rather variable

recoveries of ^{14}C to the uncertainty of the extent of such uptake in any particular experiment. Nevertheless, the major fates of ^{14}C were determined (Table 1). Incorporation of radioactivity into non-lipid heart intermediates was not measured and this may account for about 5% of the disappearance of ^{14}C (Kreisberg, 1966a).

Loss of ^{14}C from the medium also occurred while the perfusate was being gassed and recirculated during the preliminary equilibration of the perfusion system. In 28 perfusions, 19% of the initial radioactivity was lost. The medium concentrations of chyle TGFA given in the Tables have been corrected to allow for this.

Effect of starvation. Starvation of the donor rats for 48 hr. caused no significant change in either ^{14}C disappearance from the perfusate or in $^{14}\text{CO}_2$ formation after 10, 20 or 30 min. recirculation perfusions of the hearts, although the mean $^{14}\text{CO}_2$ production was higher than with hearts from rats fed *ad libitum* (Table 1, Expts. 7-9).

Effect of albumin. The presence of albumin in the perfusion medium reduced the amount of ^{14}C disappearing from the medium and approximately halved the rate of $^{14}\text{CO}_2$ formation (Table 1, Expt. 10). The distribution of ^{14}C amongst the medium lipids altered markedly during the course of a 20 min. perfusion in the presence of albumin, whereas it did not change in its absence (Table 3). In its presence, the percentage of the total radioactivity associated respectively with FFA and monoglycerides in the medium rose approximately ten- and four-fold. Marked increases in ^{14}C -labelled FFA as a percentage of the total medium radioactivity were also reported to occur during perfusions of ^{14}C -labelled chyle in the presence of albumin, by Crass & Meng (1966) and by Kreisberg (1966a).

In the presence of albumin there was a much smaller loss of radioactivity by absorption on to the perfusion apparatus during the preliminary equilibration periods and during control perfusions carried out in the absence of a heart.

Effect of increased muscular work. The external work of hearts cannulated via the left atrium and perfused with ^{14}C -labelled chyle was assessed by measuring the total left ventricular output (aortic output, 15-30 ml./min., plus coronary flow, 15-20 ml./min.). The total output of 30-50 ml./min. was ejected against a pressure head of 90 cm. H_2O and the left ventricle was therefore performing external work of a magnitude similar to that occurring *in vivo*. The preparation was metabolically stable, as shown by oxygen uptakes (μmoles of oxygen/g. wet wt./min.): 9.5 ± 1.2 , 9.9 ± 1.3 , 8.9 ± 1.1 and 8.5 ± 0.9 after 5, 10, 15 and 20 min. perfusion (four hearts per group). The mean rate of $^{14}\text{CO}_2$ production from ^{14}C -labelled chyle at a perfusate

concentration of $0.7 \mu\text{equiv.}$ of TFGA/ml. was, in a group of seven hearts, $0.48 \pm 0.09 \mu\text{equiv.}$ of ^{14}C -labelled glyceride fatty acid/g. of heart wet wt./20 min.

Effect of pre-perfusion with heparin on clearing-factor lipase release and ^{14}C -labelled chyle utilization by the perfused rat heart

Clearing-factor lipase release. Crass & Meng (1964) reported that heparin in 5% (v/v) serum released more clearing-factor lipase from the rat heart in a recirculatory perfusion system than did heparin in salt solution. Preliminary studies were carried out to investigate this effect in a non-recirculatory perfusion system. Hearts from starved rats were used because the pattern of clearing-factor release by heparin was known to be more suited to the studies to be carried out than that found with hearts from fed animals (Robinson & Jennings, 1965).

The mean total clearing-factor lipase activity of a group of 12 unperfused hearts from rats starved for 48 hr. was $107 \mu\text{moles}$ of FFA/hr./heart. After perfusion of such hearts in a once-through system with heparin (5 i.u./ml. of perfusion medium) for 12 min., approx. 30% of this activity appeared in the perfusate; most of the enzyme was released in the first few minutes of the perfusion (Table 4). In the presence of 5% (v/v) serum or plasma, the amount of enzyme released increased to about 40%. Though these findings suggest that the effect of serum or plasma on enzyme release is not very marked, the hearts were perfused with heparin immediately after being tied to the perfusion cannulae. Serum from the blood contained in the heart was therefore present in the initial perfusate samples in all cases and this may have had an effect on the release of enzyme. Thus, when this blood was first removed from the hearts, less enzyme release occurred in the first 3 min. of the perfusion with the heparin and plasma (Table 4). Similar effects were observed when serum was used instead of plasma.

Plasma in the perfusion medium caused some depression of cardiac contractility. Serum (5%) was therefore used in all the pre-perfusion media in subsequent studies and there was no preliminary wash-out of contained blood.

Experiments were carried out in which hearts from rats that had been starved for 48 hr. were perfused in a non-recirculatory system with 5% serum in Krebs-Henseleit bicarbonate buffer containing 5 i.u. of heparin/ml. for 15 min., followed by Krebs-Henseleit bicarbonate buffer for 5 min. The hearts were then transferred to a recirculatory system and perfused with ^{14}C -labelled chyle in the buffer solution. Control perfusions without heparin in the pre-perfusion system were also carried out. The results in Table 5 show the levels of clearing-

Table 3. *Effects on the percentage distribution of ^{14}C in the perfusate lipids of various perfusion conditions*

Concn. of perfusate glyceride fatty acid ($\mu\text{equiv./ml.}$)	Perfusion conditions	Time of sampling from beginning of perfusion (min.)	Percentage distribution of radioactivity among perfusate lipids					
			FFA	TG	DG	MG	PL	CE
0.7	Unperfused	—	0.8 ± 0.2 (6)	92.5 (2)	4.8 (2)	0.5 (2)	0.5 (2)	0.7 (2)
	Control recirculation without heart	20	0.8 (2)	93.8 (2)	3.9 (2)	0.8 (2)	0.5 (2)	0.6 (1)
	Recirculation with heart	10	0.8 ± 0.07 (3)	92.0 (2)	4.8 ± 0.3 (3)	0.5 ± 0.04 (3)	0.3 ± 0.03 (3)	0.3 (3)
0.7	Recirculation with heart; pre-perfusion with heparin Recirculation with heart	20	1.3 ± 0.1 (12)	91.9 ± 0.4 (12)	4.5 ± 0.2 (3)	0.8 ± 0.07 (12)	0.5 ± 0.09 (12)	0.5 ± 0.07 (12)
		30	0.9 ± 0.15 (5)	92.0 ± 0.2 (3)	4.8 ± 0.3 (3)	0.6 ± 0.11 (3)	0.4 ± 0.05 (3)	0.5 ± 0.14 (3)
		20	2.5 ± 0.4 (5)	89.1 ± 1.3 (5)	5.5 ± 0.6 (5)	1.4 ± 0.3 (5)	0.6 ± 0.14 (5)	0.6 ± 0.09 (5)
0.9	Recirculation with heart	20	9.7 ± 2.2 (3)	82.9 ± 2.6 (3)	3.7 ± 0.3 (3)	2.3 ± 0.2 (3)	0.6 ± 0.04 (3)	0.8 ± 0.06 (3)

Perfusion conditions were as described in the text and as indicated in this Table and in Table 6. Results are expressed as means $\pm$ s.e.m. with the numbers of hearts in parentheses. Albumin, dialysed bovine serum albumin at a concn. of 3 g./100 ml. of perfusate. FFA, free fatty acids; TG, triglyceride; DG, diglyceride; MG, monoglyceride; PL, phospholipid; CE, cholesteryl ester.

Table 4. Release of clearing-factor lipase from hearts perfused with heparin in a non-recirculatory system

All hearts were from rats that had been starved for 48 hr. Conditions of perfusion were as described in the text. Serum, plasma and heparin were present in Krebs-Henseleit bicarbonate buffer as indicated. A, Hearts perfused immediately with the solution indicated and effluent collected from the first contraction after mounting; B, hearts perfused for 5 min. with Krebs-Henseleit bicarbonate solution containing no heparin, before being perfused with the solution indicated. Times show the periods of collection of effluent from the first contraction after mounting, except as under B. Values are expressed as means $\pm$ S.E.M. with the numbers of hearts in parentheses.

Time of collection (min.)	Clearing-factor lipase activity (μ moles of FFA/hr./total perfusate)					
	Heparin		Serum + heparin (5 i.u./ml.)		Plasma (5%) + heparin (5 i.u./ml.)	
	2 i.u./ml.	5 i.u./ml.	1% serum	5% serum	A	B
0-3	19 (2)	24 $\pm$ 3 (4)	—	—	37 $\pm$ 4 (6)	24 $\pm$ 3 (4)
3-6	3 (2)	5 $\pm$ 1 (4)	—	—	4 $\pm$ 2 (6)	7 $\pm$ 1 (4)
6-12	5 (2)	5 $\pm$ 1 (4)	—	—	4 $\pm$ 1 (6)	6 $\pm$ 1 (4)
0-12	27 (2)	34 $\pm$ 5 (4)	35 $\pm$ 5 (5)	45 $\pm$ 2 (12)	45 $\pm$ 4 (10)	37 $\pm$ 5 (4)

Table 5. Clearing-factor lipase distribution during heart perfusions with heparin and with 14 C-labelled chyle lipids

All hearts were from rats that had been starved for 48 hr. Conditions of perfusion were as described in the text. All the effluent was collected during the 15 min. non-recirculatory pre-perfusion. The hearts were washed through with Krebs-Henseleit bicarbonate for 5 min. before being transferred to the recirculatory system containing 14 C-labelled chyle lipid (0.9 μ equiv. of TGFA/ml.). Enzyme activities are expressed as means $\pm$ S.E.M. with the numbers of hearts in parentheses.

	Clearing-factor lipase activity (μ moles of FFA released/hr./heart or total perfusate)	
	Pre-perfusion without heparin	Pre-perfusion with heparin
Total medium activity during non-recirculatory pre-perfusion	4 $\pm$ 1 (18)	42 $\pm$ 2 (20)
Total medium activity during recirculatory perfusion with chyle lipids:		
at 10 min.	1 $\pm$ 0.2 (10)	7 $\pm$ 2 (5)
at 20 min.	3 $\pm$ 0.3 (5)	9 $\pm$ 2 (5)
Activity in heart at end of perfusion	96 $\pm$ 8 (15)	78 $\pm$ 7 (15)

factor lipase activity found in the various perfusion media and in the hearts after the perfusion. No significant activity was found in the perfusate during the 5 min. perfusion with buffer alone.

In the absence of heparin, a definite, though low,

activity was detected in the perfusate during the non-recirculatory perfusion and during the recirculatory perfusion with 14 C-labelled chyle lipid. In the presence of heparin, about 40% of the initial heart activity appeared in the perfusate during the non-recirculatory perfusion, and during the recirculatory perfusion with 14 C-labelled chyle lipid the activity was higher than with hearts which had not been pre-perfused with heparin. The perfusate contained a higher proportion of radioactivity in FFA and monoglycerides after a heparin pre-perfusion followed by a recirculatory perfusion with 14 C-labelled chyle lipid (Table 3), indicating that the enzyme released into the medium was acting on the chyle triglycerides.

In the experiments with hearts pre-perfused with heparin, the sum of the final enzyme activities present in all the perfusates and that left in the hearts (129 μ moles of FFA/hr./heart) is 20% greater than that found in the hearts initially (107 μ moles of FFA/hr./heart). This may be accounted for by differences in the systems used to assay medium and heart tissue activities. Part of the enzyme in homogenates of acetone-dried preparations of the heart tissue is associated with the tissue residue (Robinson & Jennings, 1965) and may not be fully active.

The activity of perfusate clearing-factor lipase was also measured when the recirculatory perfusion medium contained albumin (3%, w/v) as well as 14 C-labelled chyle. In studies with and without heparin in the pre-perfusion fluid, the mean perfusate activities after 10 min. recirculatory perfusion with the 14 C-labelled chyle were approximately double those reported in Table 5, though the differences between the means were not significant.

Utilization of 14 C-labelled chyle. The effect of

Table 6. *Effect of heparin pre-perfusion on the subsequent utilization of ^{14}C -labelled chyle lipids by the perfused rat heart*

All hearts were from rats that had been starved for 48 hr. The hearts were pre-perfused in a non-recirculatory system for 15 min. with 5% (v/v) serum in Krebs–Henseleit bicarbonate buffer, pH 7.4, with and without heparin (5 i.u./ml.) and then for 5 min. with the buffer solution alone. They were then perfused with ^{14}C -labelled chyle lipids in Krebs–Henseleit bicarbonate buffer (0.9 $\mu\text{equiv.}$ of TGFA/ml.) in either a recirculatory (20 min. perfusion) or a non-recirculatory system (15 min. perfusion). Results are expressed as means $\pm$ s.e.m. with the numbers of hearts in parentheses.

Experimental system	Utilization of perfusate ^{14}C -labelled glyceride fatty acid ($\mu\text{equiv.}$ /g. of heart final wet wt.)		Percentage of heart lipid radioactivity present as FFA
	Appearance as $^{14}\text{CO}_2$	Uptake into heart lipid	
Recirculatory			
No heparin	0.72 ± 0.12 (5)	—	—
Heparin	0.36 ± 0.12 (5)	—	—
Non-recirculatory			
No heparin	0.48 ± 0.05 (9)	1.13 ± 0.13 (9)	6.1 ± 1.0 (9)
Heparin	0.21 ± 0.04 (9)	1.01 ± 0.11 (8)	3.7 ± 0.3 (7)

releasing part of the heart clearing-factor lipase by a prior perfusion with heparin on the subsequent utilization of ^{14}C -labelled chyle was studied in two series of experiments. In the first, perfusion of the ^{14}C -labelled chyle was carried out for 20 min. in a recirculatory system and the $^{14}\text{CO}_2$ production was halved in hearts that had been pre-perfused with heparin (Table 6). These findings are difficult to interpret because more clearing-factor lipase accumulated in the medium during the recirculatory perfusion after the hearts were pre-perfused with solutions containing heparin (Table 5), so that more hydrolysis of the medium triglyceride with greater production of FFA occurred (Table 3). Consequently a second series of experiments was carried out in which the hearts were perfused with ^{14}C -labelled chyle for 15 min. in a non-recirculatory system. The oxidation of the ^{14}C -labelled chylomicron lipid was again reduced by half ($P < 0.01$) in hearts that had been pre-perfused with heparin (Table 6). Incorporation of ^{14}C into the heart lipids was not significantly affected although the percentage of the total radioactivity present as FFA in the heart fell ($P < 0.025$). There were no significant changes in the percentage of the perfusate radioactivity present as FFA.

The uptake of oxygen (μmoles of oxygen/g. wet wt./min.) during the pre-perfusion period was 4.7 ± 0.2 (9) in hearts perfused without heparin, and 5.1 ± 0.3 (7) in hearts perfused with heparin. During the subsequent perfusion with ^{14}C -labelled chyle lipids, the mean uptake of oxygen was 3.9 ± 0.3 (7) in hearts pre-perfused without heparin, and 3.9 ± 0.4 (8) in hearts pre-perfused with heparin.

DISCUSSION

Recirculatory systems. The results of the present study confirm the findings of others that ^{14}C -labelled chyle lipids are oxidized by rat hearts perfused in

recirculatory systems. The appearance of clearing-factor lipase in the perfusion medium, however, raises the possibility that at least part of the $^{14}\text{CO}_2$ produced is the result of the uptake and oxidation of ^{14}C -labelled FFA formed by hydrolysis of the chyle TGFA in the medium. In the present study, clearing-factor lipase activity in the perfusion medium, even in the absence of albumin and when there was no pre-perfusion with heparin (Table 5), was still significant when the enzyme was assayed under optimum conditions at pH 8.1 in a system containing albumin as an acceptor of the released FFA. However, at pH 7.4 and in the absence of albumin (that is, under the conditions of perfusion) the activity of the medium clearing-factor lipase is reduced at least 20-fold (see the Materials and Methods section). At the 20 min. activity value (Table 5) 1 μmole of ^{14}C -labelled FFA would be produced therefore under optimum assay conditions during the 20 min. perfusion period, but only 0.05 μmole under the conditions of the perfusion itself. Thus, even if it be assumed that such FFA is preferentially oxidized by the heart, only a small proportion of the actual $^{14}\text{CO}_2$ output (see Table 1) is attributable to it.

With albumin (3%, w/v) in the perfusate, clearing-factor lipase activity measured at pH 7.4 rises to one-third of that found at pH 8.1 (Materials and Methods section). Part of the rise in the percentage of ^{14}C -labelled FFA in the medium which occurs during perfusions with albumin present (Kreisberg, 1966a; Crass & Meng, 1966; Table 3 of this paper) could therefore be due to the activity of the enzyme in the perfusion medium and, under such circumstances, an appreciable proportion of the total $^{14}\text{CO}_2$ production could be due to the oxidation of medium ^{14}C -labelled FFA. These possibilities also exist in the studies that have been carried out with ^{14}C -labelled lipoprotein lipid since

lipoproteins may also act as acceptors of FFA (Goodman & Shafir, 1959), and there was, in this work also, a marked rise in the percentage of the total radioactivity present as ^{14}C -labelled FFA in the medium during the perfusion (Delcher *et al.* 1965).

The action of albumin as an acceptor of FFA raises a further problem. Some ^{14}C -labelled FFA, produced by hydrolysis after the uptake of chyle lipid by the heart, may return to the perfusion medium, because the hydrolysis appears to occur at or close to the capillary wall (Robinson, 1963). Such ^{14}C -labelled FFA, when bound to albumin, may be less readily available for oxidation. Moreover, if the albumin present in the medium contains unlabelled FFA, as in the present study and in the work of Crass & Meng (1966), this will dilute any ^{14}C -labelled FFA present. The lower $^{14}\text{CO}_2$ output found in the experiments reported in Table 1 when albumin was present may be attributable to these causes.

Such uncertainties make it difficult to assess the significance of perfusion experiments with albumin present and experiments in its absence are required to find out whether the heart can take up and oxidize TGFA.

Because small percentages of the ^{14}C in the chyle are present in FFA, mono- and di-glycerides, cholesteryl esters and phospholipids (Table 3), the question arises whether part or all of the $^{14}\text{CO}_2$ could be derived from the selective oxidation of these components. The minor components of the chyle are not preferentially removed from the perfusate, however, as shown by the unchanged distribution of ^{14}C during the perfusion. Moreover, preferential oxidation of minor chyle components, after uptake of the chyle lipid by the heart, could not have accounted for a major part of the $^{14}\text{CO}_2$ output because much higher rates of uptake of ^{14}C -labelled chyle would have been necessary. Thus less than 1% of the ^{14}C in the chyle is present as ^{14}C -labelled FFA, phospholipids or cholesteryl esters. Only a very small proportion of the total $^{14}\text{CO}_2$ output could therefore be derived from the selective oxidation of these fractions after uptake of the chyle lipid. Phospholipid fatty acids were also excluded by Delcher *et al.* (1965) as a significant source of carbon dioxide in their studies with ^{14}C -labelled lipoprotein lipid. The oxidation of ^{14}C -labelled di- and mono-glycerides, the radioactivity of which in the chyle forms a higher proportion of the total, could well contribute a significant, though still minor, proportion of the total $^{14}\text{CO}_2$ output.

The percentage that is oxidized of the ^{14}C -labelled chyle taken up by the heart appears to increase progressively as the perfusion progresses. Thus, if the total uptake is taken as the sum of the recoveries of ^{14}C in $^{14}\text{CO}_2$ and in heart lipid, then the percentage oxidized is 20%, 35%, 41% and 45% at 5, 10, 20

and 30 min. respectively (Table 1). Possibly at the early time-intervals most of the ^{14}C -labelled lipid is retained at the capillary wall (see below). Alternatively there could be a progressively smaller usage of endogenous myocardial substrates, with their replacement by products of hydrolysis of the chylomicron TGFA. This would imply an alteration in the specific activities of the lipid pools in the heart because the amount of ^{14}C -labelled FFA does not change appreciably with increasing time of perfusion.

Effect of pre-perfusion with heparin. Only a proportion of the total clearing-factor lipase activity of the heart was released into the medium by heparin. The enzyme remaining in the heart had the properties of the clearing-factor lipase (Robinson & Jennings, 1965), although absolute identity of the released and retained enzyme remains to be established. The possibility that enzyme released by heparin is located at or close to the capillary wall and that the remainder is in some less accessible site has already been discussed (Robinson & Jennings, 1965).

Release of about 40% of the heart clearing-factor lipase by heparin led to a reduction by half in the capacity of the tissue to oxidize ^{14}C -labelled chyle lipid. Though the possibility that pre-perfusion with heparin may have altered the size of intracellular lipid pools and, for this reason, have led to a fall in the $^{14}\text{CO}_2$ output cannot be entirely excluded, the reduction is consistent with other evidence for the involvement of clearing-factor lipase in the uptake and oxidation of TGFA by extrahepatic tissues (Robinson, 1963).

The residual $^{14}\text{CO}_2$ output may be explained in several ways. Clearing-factor lipase which remains in the tissue may be able to hydrolyse the ^{14}C -labelled chyle TGFA. The appearance of clearing-factor lipase in the chyle perfusion media when the hearts had been pre-perfused with heparin suggests that some enzyme is still accessible to the chyle lipids. Alternatively, as has already been mentioned ^{14}C -labelled FFA and mono- and di-glycerides in the chyle may be oxidized even though hydrolysis of TGFA by clearing-factor lipase is prevented. A further possibility is that some TGFA may be taken up by the perfused heart without the action of clearing-factor lipase. Chylomicrons could enter the tissue cells intact and their triglyceride could then be hydrolysed by other lipases. Though studies with the electron microscope suggest that chylomicrons *in vivo* are bound at the surface of the endothelial cells and do not cross the capillary walls as such (French, 1963), the fact that albumin molecules leave the perfusion medium in the perfused rat heart (Sutherland & Young, 1966) indicates that the permeability of the vessel walls may be greater in this preparation.

Effect of starvation. The percentage of ^{14}C recovered in the heart after the injection of ^{14}C -labelled chyle lipids is three times as great in starved rats as in carbohydrate-fed animals (Bragdon & Gordon, 1958). Moreover, carbohydrate can spare the oxidation of fatty acids by a variety of tissues (Fritz, 1961). For such reasons, nutritional conditions might be expected to influence the rate of TGFA uptake and oxidation by the perfused heart. Kreisberg (1966a), however, could find no significant effect of starvation for 48 hr. on the uptake or oxidation of ^{14}C -labelled chylomicron lipid and these findings were confirmed in the present investigation. Gousios, Felts & Havel (1963) have reported higher percentage uptakes of perfusate triglyceride by the rabbit heart after starvation for 3 days but the absolute rates did not differ.

Rate of $^{14}\text{CO}_2$ output. The maximum rate of $^{14}\text{CO}_2$ production reported here was observed during the 10–20 min. perfusion period and was equivalent to the complete oxidation of 2.16 μmoles of fatty acid/g. wet wt./hr. (Expts. 2 and 3, Table 1), which would require between 50 and 60 μmoles of oxygen. The mean myocardial oxygen uptake during these perfusions was 234 μmoles of oxygen/g. wet wt./hr., so that about one-quarter of this would be accounted for by the complete oxidation of the chylomicron triglyceride fatty acid at its maximum rate. On the assumption that the oxygen uptake in other work with perfused rat hearts was similar to that in this study, the proportion of the total uptake accounted for by oxidation of perfusate lipid has varied at corresponding lipid concentrations between 18% and 40% (Delcher *et al.* 1965; Crass & Meng, 1966; Kreisberg, 1966a; Scheuer & Olson, 1967). A substantial increase in the rate of oxidation has been found at higher perfusate lipid concentrations by Kreisberg (1966a) and Scheuer & Olson (1967) but this was not observed in the present study.

When the oxygen uptake of the heart was doubled by allowing the left ventricle to perform work, the rate of ^{14}C -labelled chyle oxidation did not increase. This suggests that, as during increased contractility after adrenaline stimulation (Kreisberg, 1966b), alternative endogenous substrates are used in preference to chyle lipids. The possibility that the rate of utilization of chyle triglyceride is limited by its rate of hydrolysis by clearing-factor lipase cannot be excluded.

The results of the present study show that the uptake and oxidation of chyle TGFA can provide a substantial proportion of the energy needs of the perfused rat heart and suggest that heart clearing-factor lipase may be involved in these processes. The possibility that chyle TGFA are still utilized to a significant extent in the presence of other exogenous myocardial substrates such as glucose,

lactic acid, ketone bodies and FFA remains to be established. In studies *in vivo* of TGFA uptake by the heart in man no convincing arteriovenous differences have been demonstrated (Carlsten, Hallgren, Jegenburg, Svanborg & Werkö, 1961; Willebrands, 1964).

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