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METABOLISM OF FATTY ACIDS IN THE ISOLATED PERFUSED RAT HEART

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SUMMARY

1. Rat hearts were perfused with a medium containing two different radioactive fatty acids, labeled with ^{14}C and ^{3}H, respectively.

2. No preference was found for the extraction from the medium of the following acids tested: palmitic, stearic, oleic and linoleic acid. However, the distribution of these acids in the heart lipids differed.

3. In the neutral lipids of the heart, over 96% of the fatty acids incorporated were found in the triglyceride fraction. In this fraction the incorporation of palmitic acid exceeded that of its competitors: stearic, oleic and linoleic acids.

4. In the phospholipids of the heart, the lecithin fraction contained over 70% of the fatty acids incorporated. Here, stearic and linoleic acid incorporation exceeded that of palmitic acid, while oleic acid was incorporated to a similar extent.

5. In the lecithin molecule labeled linoleic acid was confined practically to the β-position, while labeled palmitic acid was distributed evenly between the α- and β-positions.

6. A faster turnover of linoleic acid than of palmitic acid in the lecithin molecule was suggested by results obtained from perfusing the prelabeled heart without radioactive substrates. The presence of enzyme systems in the myocardium which remove fatty acids from the β-position of lecithin (*i.e.*, primarily linoleic acid) was demonstrated.

7. The rate of fatty acid incorporation into lipids was independent of the heart beat.

8. Lowering of the surrounding temperature decreased the esterification of palmitic acid into neutral glycerides to a greater extent than into phospholipids.

INTRODUCTION

Three main methods have been utilized in the study of the free fatty acid metabolism by the mammalian heart. These are the assay of arterio-coronary sinus differences of free fatty acids[1-6], the perfusion of the isolated heart[7-10] and the incubation of heart slices[11]. The advantages and limitations of the first approach have been discussed by BING[12]. The use of heart slices provides information about the presence

of various enzyme systems in this tissue. However, this method is not optimal for the study of metabolic processes in an organ which under physiological conditions is continuously at work. The perfusion of the isolated heart provides a system in which substrates can be introduced through the coronary circulation and their metabolism can be studied in the working heart. The limitations of this approach are those of any model system and the conclusions derived should be applied to conditions *in vivo*, with appropriate reservations.

The first direct demonstration of the extraction of free fatty acids by the human heart was that of GORDON *et al.*[1,2]. ROTHLIN AND BING[4] analyzed the free fatty acid composition of arterial and coronary sinus blood in man and dog and found that oleic acid was extracted by the myocardium to a greater extent than palmitic, stearic and linoleic acids. Similar findings in the human were reported by CARLSTEN *et al.*[5]. These authors, however, found also a high rate of extraction of stearic acid and almost none of linoleic acid. On the other hand MILLER *et al.*[6] pointed out that in the dog the extraction rate of the free fatty acids by the heart was linoleic > oleic > stearic > palmitic.

It is generally accepted that the fatty acids extracted are oxidized to CO_2 and might also be stored in heart lipids[13]. The fate of the fatty acids extracted by the myocardium was first demonstrated by SHIPP *et al.*[8] in the isolated perfused rat heart. They found about half of the extracted [^{14}C]palmitate as CO_2 and about 25% in tissue lipids. These findings were extended by OLSON[9], who demonstrated that the [^{14}C]palmitic acid incorporated into heart lipids was evenly distributed between neutral lipids and phospholipids

In the present study both aspects of the free fatty acid metabolism, namely the extraction of labeled free fatty acids and their distribution in intracellular lipids were investigated using the perfused rat heart.

MATERIALS AND METHODS

Preparation of perfusion medium

The perfusion medium consisted of Krebs–Henseleit carbonate buffer (pH 7.4), 5 mM glucose, 0.14 mM bovine serum albumin and unless otherwise stated 0.14 mM labeled fatty acid. The bovine serum albumin (Pentex Inc.) dialyzed twice against 0.9% NaCl at 4°, contained 0.2 μmole of fatty acid per μmole of albumin. The labeled fatty acids were complexed to albumin as described before[4]. Radioactive fatty acids were checked for purity by gas–liquid chromatography. [1-^{14}C]Palmitic acid, was found to be 98% palmitic and 2% pentadecanoic acid; [1-^{14}C]linoleic acid contained more than 99% linoleic acid. [1-^{14}C]Stearic acid contained 96% stearic acid, 1% heptadecanoic and 3% palmitic acid. All fatty acids were obtained from Radiochemical Center, Amersham, Great Britain. [1-^{14}C]Oleic acid (California Corp. for Biochemical Research) contained 97% oleic acid, 2% palmitic acid and 1% stearic acid. [9,10-$^{3}H_2$]Palmitic acid from New England Nuclear Corp. was purified by thin-layer chromatography on silicic acid plates using a solvent system of ethyl ether–light petroleum (30°–40°)–acetic acid (25:75:2, v/v). The purified fraction contained 98% palmitic acid, 1% pentadecanoic and 1% myristic acid. Radiochemical purity of the labeled fatty acids was determined by gas–liquid chromatography, trapping the column effluent on anthracene carridges as described by KARMEN *et al.*[15]. With

[1-^{14}C]palmitic acid more than 98%, with [9,10-$^{3}H_2$]palmitic acid 97% of the effluent radioactivity was recovered in the palmitic acid peak. With [1-^{14}C]linoleic, stearic and oleic acids, 98.5%, 96% and 96%, respectively, of the radioactivity was recovered in the fatty acid peaks. Non-radioactive palmitic, stearic, oleic and linoleic acids were obtained from the Hormel Institute, Austin, Minn., U.S.A.

The perfusion apparatus and technique

The perfusion apparatus used was essentially that described by MORGAN *et al.*[16] for recirculation experiments. The pump was a Sigmamotor model; all tubing was made of white silicon rubber (Esco Rubber Co. London, Great Britain), boiled for 20 min in two changes of distilled water and rinsed extensively in ethanol. The stopper closing the upper chamber was made of teflon. These precautions were found to be mandatory since surgical tubing was found to release into the perfusate containing serum albumin, materials, which gave titratable acidity and after methylation showed up as several distinct peaks on gas–liquid chromatography.

Male albino rats of the Hebrew University strain were used, weighing 200–250 g and fed *ad libitum* a Purina Laboratory chow diet. The animals were anaesthetized with diethyl ether and the heart was removed rapidly and immersed in ice cold 0.9% NaCl. No anticoagulant was used. After the heart beat stopped a 16-gauge cannula was inserted into the aorta and tied in place with a silk ligature. 5 ml of ice-cold saline were introduced through the cannula in order to remove the blood from the coronary circulation. The heart was then perfused with Krebs–Henseleit carbonate medium without substrate at 38° to remove any remaining blood and restore the beat. The first 8 ml were discarded and then the heart was introduced into the perfusion chamber and the fatty acid–albumin complex was added to the upper chamber. The preparation was considered satisfactory when the heart beat was regular, ranging 180–220 contractions/min, and the coronary flow was constant and ranged between 4 and 7 ml per min. The perfusion pressure was maintained at 35–50 mm Hg, the duration of perfusion was 20 min, during which time the perfusion fluid, which collected on the scintered glass filter, was exposed to a mixture of 95% O_2–5% CO_2. At the end of the perfusion 5 ml of Krebs–Henseleit buffer were passed through the cannula from a syringe in order to remove any labeled material which remained in the blood vessels of the heart. The heart was chilled, the chambers were opened and blotted dry. The heart was then homogenized in 25 ml ethanol–ethyl ether (3:1, v/v) in a conical all-glass homogenizer. The perfusion fluid was collected and made up to 25 ml with Krebs–Henseleit buffer which was used to rinse the heart and the perfusion apparatus.

Chromatographic and analytical procedures

The perfusate was extracted according to DOLE[17] in a separating funnel. The heptane was evaporated under reduced pressure and redissolved quantitatively in 10 ml heptane in a glass-stoppered centrifuge tube and vigorously shaken for 5 min with an equal volume of 0.05% aq. H_2SO_4 according to TROUT *et al.*[18], in order to free the heptane layer from lactic acid. Aliquots of the heptane phase were taken for fatty acid titration, radioactivity determinations and gas–liquid chromatography. They were kept under N_2 at 0° and were analyzed within 3 h. Free fatty acids derived from the heptane layer were converted to methyl esters with diazomethane in ether,

and chromatographed on a Barber Colman gas chromatograph Model-10, with a β-ionization detector. Ethylene glycol succinate or adipate polyester was used as stationary phase at 175°. The methyl ester peaks were identified by comparison of their retention times with those of known standard fatty acids. Linearity of the detector was checked by using a reference mixture obtained from the National Heart Institute, Bethesda, Md., U.S.A. Peak areas were measured by triangulation, and the per cent composition by weight was transformed to mole %.

The specific activity of a given labeled fatty acid in the perfusate was calculated using the formula

$$\text{specific activity of labeled fatty acid} = \frac{\text{counts/min}}{\mu\text{moles total free fatty acid} \times \mu\text{mole \% labeled free fatty acid}}$$

Zero-time values of the free fatty acid concentration and composition of the perfusate, as well as the specific activity of the labeled fatty acids was determined on aliquots of the perfusion mixture taken at the onset of the experiment.

The ethanol–ethyl ether extract of the heart homogenate was fractionated into neutral lipids and phospholipids by batch elution from silicic acid as described before[19]. The extent of esterification of the radioactive fatty acid in the neutral lipid fraction was determined by trapping the unesterified fatty acid on MgO–celite column[20]. Separation of the neutral lipids into cholesterol ester, tri-, di- and monoglycerides was performed on Florisil columns[21]. Phospholipids were separated on thin-layer silicic acid plates, using the solvent system of chloroform–methanol–water (65:25:4, v/v). The preparation of samples for radioactivity determination was carried out as described previously[19].

Neutral lipid and phospholipid fatty acid esters were determined according to the method of STERN AND SHAPIRO[22]. Protein was estimated turbidimetrically, using bovine serum albumin as standard[23].

Radioactivity determination

The radioactivity determination was performed with a Tricarb liquid-scintillation spectrometer Model-314 EX, with toluene containing 0.4% diphenyloxazole and 0.01% *p*-bis[2-(5-phenyloxazolyl)]benzene as the scintillating fluid. Samples containing the radioisotopes ^{3}H and ^{14}C were counted simultaneously at 1030 V. One scaler was set at 100 % gain and a 90–800 V window, while the second scaler was set at 5 % gain and a 100–1000 V window. Under these conditions the efficiency of the first scaler was 23 % and 25 % for ^{14}C and ^{3}H, respectively. The second scaler counted only ^{14}C with an efficiency of 46 %. Using the 314 EX-model the simultaneous equation method of OKITA *et al.*[24] gave results comparable to those obtained by a modified screening method.

Assay of phospholipase activity

Preparation of substrate. [1-^{14}C]Palmitoyllecithin, prepared biosynthetically from rat liver after injection of [1-^{14}C]palmitic acid, and α-acyl-[1-^{14}C]palmitoyllysolecithin, derived from the labeled lecithin after enzymic hydrolysis with *Crotalus adamanteus* venom[25] were used as substrates. Both substrates were purified by rechromatography on thin-layer silicic acid plates as described previously[26].

Preparation of heart homogenates. The hearts were removed and washed as described above, minced and homogenized with 5 vol. of ice-cold KCl–Tris buffer (0.154 M KCl–0.5 M Tris (pH 7.4), 19:1, v/v), in an all-glass conical homogenizer. The homogenate was recentrifuged at 20000 × *g* for 20 min. All the operations were carried out at 0–5°. The 20000 × *g* supernatant, with a protein concentration of 5–7 mg/ml served as the source of the enzyme. Incubation was carried out in 25-ml volumetric flasks at 37° with shaking, for 4 h. The reaction was stopped by the addition of ethanol–ethyl ether (3:1, v/v) which was brought to boiling. The ethanol–ether extract was centrifuged, evaporated to dryness, and dissolved in 0.5 ml of methanol. Aliquots were chromatographed on thin-layer silicic acid plates and the ratioactivity of the lysolecithin and the liberated fatty acid was determined as described before[26].

RESULTS

Incorporation of [1-^{14}C]palmitic acid and [1-^{14}C]linoleic acid

Rat heart perfused *in vitro* was shown to take up labeled palmitic acid from the perfusing fluid. The fatty acid was incorporated into heart lipids, about 70% being found in the neutral lipid fraction. The specific activity of this fraction was higher than that of the phospholipid fraction. Similarly [1-^{14}C]linoleic acid could be demonstrated to be taken up from the perfusing fluid and was recovered in both lipid fractions (Table I). The percentage of incorporation of linoleic acid into the phospholipid fraction seemed to exceed that of the palmitic acid. However, on statistical analysis this difference was not found to be significant.

TABLE I

INCORPORATION OF 1-^{14}C-LABELED FATTY ACIDS INTO LIPIDS OF THE PERFUSED RAT HEART

Conditions of perfusion: perfusion medium, volume 14 ml, consisted of Krebs–Henseleit carbonate buffer (pH 7.4), 5 mM glucose, 0.14 mM either [1-^{14}C]palmitic or [1-^{14}C]linoleic acid, complexed to 0.14 mM bovine serum albumin. Perfusion time: 20 min at 37° with 95% O_2–5% CO_2.

Fatty acid incorporated into lipids (mμmoles/heart)	*% Distribution of labeled fatty acid*		*Counts/min/μequiv ester*	
	Neutral lipids	*Phospholipids*	*Neutral lipids*	*Phospholipids*
[1-^{14}C]*Palmitic acid*				
275	72	28	11 700	2350
232	76	24	8 630	1465
200	69	31	5 850	1555
192	68	32	5 400	1650
296	77	23	8 600	1630
Mean ± *S.E.* 239.0 ± 14.7	72.4	27.6		
[1-^{14}C]*Linoleic acid*				
297	75	25	7 400	1580
202	72	28	3 680	840
176	70	30	6 600	1840
208	62	38	3 700	1500
174	63	37	3 800	1170
Mean ± *S.E.* 211.4 ± 22.4	68.4	31.6		

Comparative uptake of ^{3}H- and ^{14}C-labeled fatty acids

In order to compare the uptake and incorporation of different fatty acids in the same preparation, use was made of ^{3}H- and ^{14}C-labeled fatty acids introduced together into the perfusing fluid. Table II presents results obtained by perfusing the heart with a mixture of [9,10-$^{3}H_2$]palmitic and [1-^{14}C]palmitic acid–albumin complex.

TABLE II

COMPARISON OF SIMULTANEOUS INCORPORATION OF [9,10-$^{3}H_2$]PALMITIC ACID AND [1-^{14}C]PALMITIC ACID INTO NEUTRAL LIPIDS AND PHOSPHOLIPIDS OF THE PERFUSED RAT HEART

Conditions of perfusion as in Table I.

Experiment No.	Fatty acid incorporated as % of each labeled fatty acid in perfusate					
	Neutral lipids			Phospholipids		
	^{3}H	^{14}C	^{3}H—^{14}C	^{3}H	^{14}C	^{3}H—^{14}C
1	6.7	7.0	—0.3	5.6	6.6	—1.0
2	7.3	8.1	—0.8	1.8	2.2	—0.4
3	5.8	6.0	—0.2	2.4	3.6	—1.2
4	9.6	10.4	—0.8	5.6	6.8	—1.2
5	11.3	11.8	—0.5	3.6	4.1	—0.5
6	10.6	11.0	—0.4	2.6	3.9	—1.3
7	11.9	11.4	+0.5	3.3	4.5	—1.2
8	8.8	9.1	—0.3	3.3	4.4	—1.1
		Mean	—0.362		*Mean*	—0.987
		$t =$	2.66		$t =$	8.6
		$P <$	0.05		$P <$	0.001

The purpose of this experiment was to test whether the heart will discriminate between a ^{3}H- and a ^{14}C-labeled fatty acid. It can be seen that in the neutral lipid fraction a small (3.8%) difference between the ^{3}H- and ^{14}C-labeled palmitic acid was observed. In the phospholipids this difference between ^{3}H and ^{14}C was much more pronounced (22 %). The reason for this different behaviour between ^{3}H- and ^{14}C-labeled palmitic acid was further investigated. It could not be accounted for by preferential quenching of ^{3}H. In order to rule out preferential adsorption of ^{3}H on silicic acid during the fractionation procedure (see METHODS) the following control experiment was performed. To a homogenate of heart in ethanol–ethyl ether a mixture of [^{3}H]- and [^{14}C]palmitic acid was added. The lipid extract was evaporated and dissolved in light petroleum (40–60°). The latter was adsorbed on silicic acid and extracted with chloroform or with methanol. The $^{3}H/^{14}C$ ratio in the chloroform or methanol extract was found to be the same as that of the original mixture added, indicating that no loss of ^{3}H occurred during the extraction and adsorption procedures. The loss of ^{3}H was rather constant, hence in the subsequent experiments the amount of [^{3}H]palmitic acid recovered in the neutral lipid fraction was corrected by 3.8 % whereas that in the phospholipid fraction was corrected by 22 %.

The comparison of incorporation of [^{3}H]palmitic acid to that of either [^{14}C]-stearic, oleic or linoleic acid by cardiac muscle, exposed to a pair of fatty acids during 20 min of perfusion, is summarized in Table III. At comparable fatty acid concentrations and fatty acid to albumin ratio of 1:1, all pairs of fatty acids were

incorporated to a similar extent. At a fatty acid to albumin ratio of 3:1 significantly more linoleic and palmitic acid was incorporated into cardiac lipids than at 1:1 ratio. The distribution of the labeled fatty acids between the two lipid fractions varied with each fatty acid. In the neutral lipids, palmitic acid was incorporated to a greater

TABLE III

COMPARATIVE INCORPORATION OF PAIRS OF ^{3}H- AND ^{14}C-LABELED FATTY ACIDS INTO LIPIDS OF PERFUSED RAT HEART

Conditions of perfusion as in Table I. The results in the 4th column are given as mean ± S.E. of the mean. B *vs.* C: $t = 3.32$; $P < 0.02$. B *vs.* A: $t = 0.82$; $P > 0.1$.

Pair of labeled fatty acids in perfusate	*Labeled fatty acids in perfusate (μmoles)*	*Number of expts.*	*Labeled fatty acids incorporated into total lipids (mμmoles/heart)*
[9,10-$^{3}H_2$]Palmitic acid [1-^{14}C]Palmitic acid	2.00	8	215 ± 69
[9,10-$^{3}H_2$]Palmitic acid [1-^{14}C]Stearic acid	2.15	6	231 ± 27 (A)
[9,10-$^{3}H_2$]Palmitic acid [1-^{14}C]Oleic acid	2.04	6	210 ± 68
[9,10-$^{3}H_2$]Palmitic acid [1-^{14}C]Linoleic acid	1.70	5	186 ± 48 (B)
[9,10-$^{3}H_2$]Palmitic acid [1-^{14}C]Linoleic acid	3.60*	4	387 ± 38 (C)

* Fatty acid to albumin ratio 3:1.

extent than either stearic, oleic or linoleic acid. In the phospholipid fraction significantly more stearic and linoleic acid was recovered than palmitic acid. No significant difference in the incorporation of oleic and palmitic acid was found in the phospholipids (Table IV).

Fractionation of neutral lipids and phospholipids

Fractionation of neutral lipids disclosed that 95–98% of the radioactive neutral lipids were recovered in the triglyceride fraction. The phospholipids were separated into 5 main components. 64–81% of the total radioactivity was recovered in the lecithin fraction (Table V).

Disappearance of labeled fatty acids from the perfusing fluid

The disappearance of the pairs of labeled fatty acids from the perfusing fluid was studied in all the experiments described above. It can be seen in Table VI that during 20 min of perfusion between 0.73 and 0.80 μmole of labeled fatty acids was removed from the perfusing fluid. At the end of perfusion the ratio $^{3}H/^{14}C$ in the fatty acids of the perfusing fluid was found to be the same as the initial $^{3}H/^{14}C$ ratio. In the same experiments it was possible to determine whether release of fatty acids from the heart takes place during recirculation perfusion. Since release of fatty acids would cause a fall in the specific activity of the labeled fatty acid in the perfusing fluid this parameter was determined. As seen in Table VI no significant alteration in the specific activity of either palmitic, oleic, stearic, or linoleic acid occurred.

TABLE IV

COMPARISON OF SIMULTANEOUS INCORPORATION OF PAIRS OF ^{3}H- AND ^{14}C-LABELED FATTY ACIDS INTO NEUTRAL LIPIDS AND PHOSPHOLIPIDS OF PERFUSED RAT HEART

Conditions of perfusion as in Table I. The perfusion medium contained in Expts. 1–6: 0.077 mM, in 7–12: 0.073 mM, in 13–16: 0.042 mM of each fatty acid; in Expts. 17–21: 0.078 mM [9,10-$^{3}H_2$]-palmitic acid and 0.042 mM [1-^{14}C]linoleic acid.

Experiment No.	*Fatty acid incorporated as % of each labeled fatty acid in perfusate*					
	Neutral lipids			*Phospholipids*		
	^{3}H	^{14}C	^{3}H—^{14}C	^{3}H	^{14}C	^{3}H—^{14}C
			[^{3}H]Palmitic–[^{14}C]stearic acid			
1	5.3	5.3	0	2.3	3.5	−1.2
2	12.0	10.7	+1.3	3.4	4.8	−1.4
3	9.4	8.8	+0.6	3.5	6.5	−2.8
4	4.9	4.1	+0.8	3.5	6.0	−2.5
5	8.6	7.6	+1.0	2.5	4.5	−2.0
6	4.2	3.3	+0.9	3.7	6.1	−2.4
			Mean +0.766			*Mean* −2.05
			t = 4.25			t = 7.82
			$P <$ 0.01			$P <$ 0.001
			[^{3}H]Palmitic–[^{14}C]oleic acid			
7	8.6	7.0	+1.6	3.8	4.7	−0.9
8	3.4	3.1	+0.3	2.6	2.8	−0.2
9	6.8	5.9	+0.9	5.3	4.7	+0.6
10	7.7	6.6	+1.1	4.1	4.0	+0.1
11	6.5	5.7	+0.8	9.7	8.3	+1.4
12	6.9	6.9	0	2.2	2.4	−0.2
			Mean +0.783			*Mean* −0.333
			t = 3.36			t = 0.41
			$P =$ 0.02			$P >$ 0.1
13	15.3	13.2	+2.1	5.5	5.3	+0.2
14	17.1	14.4	+2.7	5.2	5.5	−0.3
15	14.4	11.3	+3.1	5.6	5.1	+0.5
16	13.5	12.2	+1.3	5.5	4.1	+1.4
			Mean +2.3			*Mean* +0.45
			t = 5.85			t = 1.26
			$P =$ 0.01			$P >$ 0.1
			[^{3}H]Palmitic–[^{14}C]linoleic acid			
17	8.8	5.7	+3.1	3.7	6.8	−3.1
18	9.1	6.0	+3.1	3.7	5.9	−2.2
19	4.3	2.6	+1.7	3.5	5.0	−1.5
20	6.0	3.6	+2.4	3.5	5.0	−1.5
21	7.1	4.4	+2.7	3.5	5.0	−1.5
			Mean +2.6			*Mean* −1.96
			t = 10.0			t = 6.2
			$P <$ 0.001			$P <$ 0.01

Metabolic and positional asymmetry of the lecithin molecule

To examine further the fate of the labeled fatty acids in heart glycerides and phospholipids the following experiments were performed. The hearts were perfused for 20 min with [^{3}H]palmitic acid and [^{14}C]linoleic acid. They were then perfused with

TABLE V

SEPARATION OF NEUTRAL LIPIDS AND PHOSPHOLIPIDS SYNTHESIZED IN THE ISOLATED RAT HEART, PERFUSED WITH ^{14}C-LABELED FATTY ACIDS

Labeled fatty acid in perfusate	*Neutral lipids % distribution of labeled fatty acids*				*Phospholipids % distribution of labeled fatty acids*				
	Cholesterol	*Triglycerides*	*Diglycerides*	*Monoglycerides*	*Phosphatidyl-serine*	*Lysolecithin*	*Sphingomyelin*	*Lecithin*	*Phosphatidyl ethanolamine*
[^{14}C]Palmitic acid	0	98	2	0	2	1	6	75	16
	0	98	2	0	6	2	9	72	11
[^{14}C]Stearic acid	0	98	2	0	4	1	12	65	18
	0	95	5	0	4	1	13	64	18
[^{14}C]Oleic acid	0	95	5	0	7	4	7	67	15
	0	95	5	0	6	9	3	68	14
[^{14}C]Linoleic acid	0	95	5	0	3	0	1	81	15
	0	96	4	0	5	0	2	79	14

TABLE VI

DISAPPEARANCE OF PAIRS OF ^{3}H- AND ^{14}C-LABELED FATTY ACIDS FROM THE PERFUSION MEDIUM DURING 20 min OF PERFUSION OF RAT HEARTS

The number of experiments is given between parentheses. The results are given as mean ± S.E. of the mean.

Labeled fatty acids in medium	*Total fatty acids in medium (μmoles)*	*Fatty acid removed from medium (μmoles)*	*A**	*A* — 1.000*	*Specific activity of fatty acids (counts/min/μequiv × 10^3)*			
					at zero time		*at 20 min*	
					^{3}H	*^{14}C*	*^{3}H*	*^{14}C*
[9,10-^{3}H$_2$] C 16:0	2.38	0.80	1.007	+0.07	157	130	145	124
[1-^{14}C] C 16:0	(5)	±0.118	±0.043	t = 0.33			±2.0	±6.3
				P > 0.1				
]9,10-^{3}H$_2$] C 16:0	2.30	0.74	0.975	−0.025	720	378	718	354
[1-^{14}C] C 18:0	(6)	±0.075	±0.021	t = 1.53			±14.1	±13.7
				P > 0.1				
[9,10-^{3}H$_2$] C 16:0	2.10	0.73	0.987	−0.013	980	542	912	534
[1-^{14}C] C 18:1	(6)	±0.088	±0.017	t = 1.2			±58.2	±12.2
				P > 0.1				
[9,10-^{3}H$_2$] C 16:0	2.13	0.80	0.968	−0.032	1170	875	1138	874
[1-^{14}C] C 18:2	(5)	±0.065	±0.020	t = 2.6			±31.5	±29.6
				P > 0.05				

* $A = \frac{^{3}\text{H}/^{14}\text{C ratio in medium at 20 min}}{^{3}\text{H}/^{14}\text{C ratio in medium at zero time}}$.

10 ml of Krebs–Henseleit carbonate buffer in order to remove any trapped unesterified labeled fatty acids from the blood vessels. The heart was then transferred to a second perfusion apparatus and perfused for additional 20 min with Krebs–Henseleit carbonate–glucose medium (A). In a second series of experiments the period of labeling was followed by non-recirculation perfusion, the perfusing fluid containing

TABLE VII

CHANGES IN $^{3}H/^{14}C$ RATIO IN HEART GLYCERIDES AND PHOSPHOLIPIDS PERFUSED WITH [^{3}H]PALMITIC AND [^{14}C]LINOLEIC ACID AND THEN REPERFUSED WITH UNLABELED MEDIUM

A, the perfusion medium during the last 20 min consisted of Krebs–Henseleit carbonate buffer, with 5 mM glucose. B, during the last 20 min the hearts were perfused without recirculation with a perfusion medium consisting of Krebs–Henseleit carbonate buffer, 5 mM glucose and 0.5% bovine albumin. I *vs.* II: $P > 0.1$; III *vs.* IV: $P < 0.001$.

Conditions and duration of perfusion (min)		*No. of expts.*	*$^{3}H/^{14}C$ in heart glycerides*	*$^{3}H/^{14}C$ in heart phospholipids*
in presence of [^{3}H]- and [^{14}C]fatty acids	*without [^{3}H]- and [^{14}C]fatty acids*		*$^{3}H/^{14}C$ in medium*	*$^{3}H/^{14}C$ in medium*
			(Mean ± S.E. of mean)	
20	0	9	1.52 ± 0.074 (I)	0.62 ± 0.029 (III)
20	20 (A)	5	1.70 ± 0.074 (II)	1.37 ± 0.117 (IV)
20	20 (B)	5	1.56 ± 0.034	1.46 ± 0.022

also 0.5% bovine serum albumin (B). During the entire span of the experiment the heart rate remained unaltered. The results presented in Table VII are expressed as $^{3}H/^{14}C$ ratio in either glycerides or phospholipids which have been normalized to the $^{3}H/^{14}C$ ratio of the original perfusing fluid. It can be seen that in the phospholipid fraction there is a marked change in $^{3}H/^{14}C$ ratio in both series of experiments, A and B as compared to the control in which the perfusion has not been continued. This change in $^{3}H/^{14}C$ ratio indicates that during the subsequent perfusion a preferential dissimilation of [^{14}C]linoleic acid from the phospholipids has occurred. Since two-thirds or more of all radioactivity found in the phospholipids has been recovered in lecithin (Table V) it seemed plausible, that the removal of the [^{14}C]linoleic acid might be due to a phospholipase A activity. This enzyme is known to hydrolyze the β-ester bond of lecithin[27], into which linoleic acid is preferentially incorporated as seen in

TABLE VIII

DISTRIBUTION OF [1-^{14}C]PALMITIC ACID AND [1-^{14}C]LINOLEIC ACID IN THE α- AND β-POSITION OF LECITHIN DERIVED FROM HEART AND LIVER

Isolation of lecithin and degradation procedure as in METHODS.

Labeled substrate	*Origin of [^{14}C]lecithin*	*Radioactivity (%) of lecithin (after lysis with venom)*			
		Lysolecithin		*Fatty acid*	
		I	*II*	*I*	*II*
[^{14}C]linoleyl*-lecithin	Heart	5	6	95	94
[^{14}C]Palmitoyl*-lecithin	Heart	54	50	46	50
[^{14}C]Palmitoyl**-lecithin	Liver	12	15	88	85

* Derived biosynthetically by perfusing the heart with [1-^{14}C]linoleic acid or [1-^{14}C]palmitic acid.
** Derived from the liver of a rat injected with [1-^{14}C]palmitic acid.

Table VIII. The presence of phospholipase activity in the myocardium was demonstrated using the 20 000 × *g* supernatant of rat-heart homogenate. In this preparation two enzymes were present. One, which is heat stable, was shown to split the β-bond of the lecithin molecule; the other, heat labile, hydrolyzed α-acyl-lysolecithin (Table IX).

TABLE IX

LECITHINASE AND LYSOLECITHINASE ACTIVITY OF RAT-HEART HOMOGENATES

Conditions of incubation: the incubation medium contained 3 ml of 20000 × *g* supernatant of a 20% heart homogenate in 0.154 M KCl–0.5 M Tris buffer (pH 7.4) (19:1, v/v) (protein content 5 mg/ml) and 1 μmole of labeled substrate. Incubated in air at 37°, with shaking for 4 h.

Labeled substrate	*Treatment of homogenate*	*% radioactivity recovered in*	
		Lysolecithin	*Fatty acid*
[^{14}C]Palmitoyllecithin of liver*	None	35	65
	10 min at 70°	90	10
[^{14}C]Palmitoyllysolecithin of liver	None	5	95
	10 min at 70°	90	10

* In the preparation used 88% of the labeled fatty acid was in the α- and 12% in β-position (see Table VIII).

Rate of esterification at low temperature and in a non-contracting heart

A possible relation between the rate of esterification of fatty acids into heart lipids and the contractions of heart muscle was investigated. As seen in Table X, the rate of incorporation of [^{14}C]palmitic acid and the rate of cardiac contractions fell progressively when the temperature of the perfusion medium was lowered. In order to separate the effect of temperature from that of the rate of contraction, perfusions were carried out in presence of increased potassium concentration, which caused cardiac standstill. The rate of flow through the non-contracting heart was kept at 5–7 ml/min, by raising the perfusion pressure to 70 mm Hg. The data presented demonstrate the independence of the rate of incorporation and the distribution of the labeled fatty acids between neutral lipids and phospholipids, of cardiac contraction.

TABLE X

EFFECT OF TEMPERATURE AND CARDIAC STANDSTILL ON THE INCORPORATION OF [1-^{14}C]PALMITIC ACID INTO LIPIDS BY THE PERFUSED RAT HEART

Conditions of perfusion as in Table I. A *vs.* B: $P < 0.001$; A *vs.* C: $P < 0.001$; A *vs.* D: $P > 0.1$.

Temperature of the perfusate (°C)	*Heart rate per min*	*No. of expts.*	*Fatty acid incorporated into total lipids (mμmoles)*	*% distribution of labeled fatty acids*	
				Neutral lipids	*Phospholipids*
37	180–220	5	239 ± 14.7 (A)	72.4	27.6
27	80–100	4	98 ± 14.0 (B)	70.0	30.0
23	50–70	6	55 ± 2.0 (C)	57.0	43.0
37*	0	6	235 ± 18.8 (D)	75.0	25.0

* Plus 34 mM KCl in perfusate.

DISCUSSION

In the present study the comparative uptake and distribution of isotopically labeled free fatty acids in the perfused rat heart were investigated. When the fatty acids complexed to serum albumin were introduced into the perfusate in pairs, equal extractions with the palmitic–oleic, palmitic–stearic and palmitic–linoleic acid pairs was found, indicating that under the experimental conditions employed the heart did not discriminate between the different fatty acids studied. These results differ from those of other investigators[4–6] who concluded from measurements of the arteriovenous differences in free fatty acid composition that oleic acid was extracted preferentially by the myocardium. However, this observation was difficult to explain in view of the demonstration of an equal extraction of oleic and palmitic acid by the rat diaphragm[28]. Hence ROTHLIN AND BING[4] suggested that the change in the free fatty acid composition of the coronary sinus blood might not be due to a greater extraction of oleic acid, but rather to a change in the free fatty acid fraction caused by an exchange between glyceride fatty acids and the free fatty acids mediated by the heart lipoprotein lipase (glycerol ester hydrolase, EC 3.1.1.3)[29]. Since in the present investigation the perfusate was devoid of any source of esterified fatty acids, such a reaction could not have taken place, unless the esterified lipids of the myocardium served as substrate. It was possible to show, however, that during the 20 min of perfusion no release of unlabeled free fatty acids from the heart into the perfusate had occurred.

In contrast to the indiscriminate uptake of the different fatty acids from the perfusion medium their intracellular distribution between neutral lipid and phospholipids varied quite markedly, in accord with the fatty acid composition of both lipid fractions in the heart. In analogy, NEPTUNE *et al.*[28] reported an equal uptake of oleic and palmitic acid by the rat diaphragm and a different distribution of these fatty acids between neutral lipids and phospholipids. The rat epididymal fat pad incubated *in vivo*[30], with two differently labeled fatty acids, was shown to incorporate palmitic acid slightly faster than oleic and linoleic acid and considerably more rapidly than stearic acid. This somewhat different pattern of behaviour with regard to stearic acid seems to indicate that a relation exists between the rate of uptake of a given fatty acid and its subsequent intracellular utilization. The finding that the stearic acid was incorporated to a large extent in the phospholipid fraction of the heart lipids and the fact that in adipose tissue the phospholipids form only a minor component of the total lipid could explain the different behaviour of the adipose tissue cell and the cardiac muscle cell toward this fatty acid.

With all fatty acids used in the present investigation more radioactive label was recovered in the triglycerides than in the phospholipids. This distribution of the labeled fatty acid varies from that reported by OLSON[9] who perfused rat hearts for 75 min and obtained an equal distribution of [1-^{14}C]palmitic acid between the neutral lipids and phospholipids. Since the perfusate used by OLSON was devoid of glucose it is possible that during this time glycogen was depleted[8] and α-glycerophosphate became limiting, which would interfere with the continued synthesis of the neutral glycerides. On the other hand phospholipid synthesis might have proceeded unimpeded by the lack of α-glycerophosphate, through the acylation of lysolecithin, as described for lung, liver[31,32], and aortic homogenates[26].

The rise in the $^{3}H/^{14}C$ ratio in the phospholipid fraction in experiments with

[^{3}H]palmitic and [^{14}C]linoleic acid, when the perfusion was continued in the absence of labeled substrate (Table VI), could be due to either an enrichment of the phospholipids with [^{3}H]palmitic acid, derived from the neutral glycerides, or by a preferential loss of [^{14}C]linoleic acid. Since this change in the ^{3}H/^{14}C ratio in the phospholipid fraction was not accompanied by a concurrent fall in the glycerides, the first alternative could be excluded. In addition the demonstration of lecithinase A (phosphatide acyl-hydrolase, EC 3.1.1.4) in the myocardium, in the present and other studies[33], would corroborate the possibility that preferential loss of linoleic acid from the β-position of lecithin occurred. The change in the ^{3}H/^{14}C ratio in the phospholipids seems also to imply that following the action of lecithinase A, the acyl transferase[31,32] competed successfully with lysolecithinase (lysolecithin acyl-hydrolase, EC 3.1.1.5) for the newly formed substrate, resulting in a lecithin molecule with a higher ^{3}H/^{14}C ratio.

The positional and metabolic asymmetry of the lecithin molecule derived from mammalian liver has been emphasized by HANAHAN AND BLOMSTRAND[34]. More recently LANDS AND MERKL[35], using liver microsomes and α- and β-acyl-lysolecithin, have shown a preferential incorporation of linoleic acid into the β-position and of stearic acid into the α-position of lecithin. From the present study the following conclusions can be drawn regarding the asymmetry of the heart lecithin molecule. Firstly, linoleic acid is incorporated preferentially to palmitic acid into lecithin, when both are presented simultaneously. Secondly, linoleic acid is recovered practically only in the β-position of the lecithin molecule, while palmitic acid distributes evenly between the two positions. Thirdly, the linoleic acid is lost more rapidly from the lecithin molecule, thus displaying a faster turnover.

Similar observations have been carried out on the lecithin of erythrocytes by two groups of investigators, who noted preferential incorporation of linoleic acid over that of oleic and palmitic acid[36] and the affinity of the linoleic acid for the β-bond of lecithin[37]. In addition a rather fast turnover rate of the linoleic acid of erythrocyte lecithin was indicated by a rapid enrichment of this fraction in linoleic acid following corn-oil feeding to rabbits[37].

The finding of loss of ^{3}H in the experiments with [9,10-$^3H_2$]palmitic acid and [1-^{14}C]palmitic acid remains unexplained so far. One plausible explanation could be that of a 9–10 desaturation of palmitic acid by the heart, which is still being investigated.

It seems of interest that at 27° the rate of esterification of the fatty acids in the mammalian heart has decreased by only 60% and this enzymic activity could be detected even at 23°. The change in the usual distribution of the labeled palmitic acid between neutral lipids and phospholipids suggests that the fall in temperature does have different effects on various enzymic pathways. This information might be of value in relation to the use of hypothermia in cardiac surgery.

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