Utilization of Endogenous Lipid by the Isolated Perfused Rat Heart

By ROBERT E. OLSON* AND ROBERT J. HOESCHEN†

*Department of Biochemistry and Nutrition, Graduate School of Public Health,
University of Pittsburgh, Pittsburgh, Pa., U.S.A., and
Department of Biochemistry, University of Oxford*

(Received 23 September 1966)

1. The lipids of the rat heart have been studied with regard to amount, classes present and fatty acid composition of free fatty acids, triglycerides and phospholipids. Myocardial lipid contained 300 μ moles of total fatty acid/g. dry wt. of which only 2–4 μ moles were free; the remainder was esterified, chiefly as phospholipid. Neutral esters, of which triglyceride was the principal form, made up 15% of the total fatty acids. 2. When normal hearts were perfused with a nutrient-free medium until exhaustion, the triglyceride concentration declined from 43 to 13 μ moles/g. dry wt. The content of phospholipids, partial glycerides and cholesterol esters did not change. When the lipids of the rat heart were labelled with [$1\text{-}^{14}\text{C}$]palmitate before perfusion with non-nutrient medium, radioactivity disappeared from the triglyceride, diglyceride and free fatty acid fractions, but not from the phospholipid or other ester classes. 3. These experiments support the view that only a small fraction of the total cardiac lipid, principally triglycerides and to a smaller extent diglycerides, is available as a source of fuel in the absence of exogenous substrate.

It is well established that endogenous substrates provide respiratory fuel in the isolated perfused rat heart. This is borne out by the fact that perfused hearts will contract vigorously for 1–2 hr. in the absence of substrates in the perfusing medium (Fisher & Williamson, 1961*a,b*; Zachariah, 1961). Even in the presence of a variety of exogenous substrates, the endogenous stores contribute to total tissue respiration (Fisher & Williamson, 1961*b*; Williamson & Krebs, 1961; Williamson, 1962; Evans, Opie & Shipp, 1963; Shipp, Matos, Knizely & Crevasse, 1964*a*), although high concentrations of pyruvate do effectively suppress the oxidation of endogenous substrates (Olson, 1962; Evans, Opie & Renold, 1963).

The source of endogenous fuel may be the tissue amino acids, glycogen or lipids. Fisher & Williamson (1961*a*) calculated that in the absence of exogenous substrate the glycogen of the perfused rat heart would be used in 14–19 min., and Shipp *et al.* (1964*a*) demonstrated that perfused hearts from fed rats were depleted of glycogen in 10 min. Several investigators (Hicks & Kerly, 1960;

Williamson & Krebs, 1961; Shipp *et al.* 1964*a*) have demonstrated that amino acids normally contribute very little to the energy metabolism of the heart, and Williamson & Krebs (1961) excluded amino acids as endogenous substrates for the perfused rat heart because they could not demonstrate the accumulation of ammonia, urea, glutamate or aspartate. The conclusion therefore seems inescapable that the tissue lipids play a key role in sustaining the contractility of the perfused heart beyond that time when carbohydrate stores are exhausted.

At the time when the perfused heart eventually fails, analyses of the myocardium reveal the presence of large amounts of lipid. It follows therefore that only a portion of the endogenous lipid of the heart is available for fuel. Cruickshank & Kosterlitz (1941) found in the isolated rat heart–lung preparation that neutral-lipid fatty acids were mobilized during perfusion whereas the amount of tissue phospholipids remained constant. On the other hand Shipp, Thomas & Crevasse (1964*b*) suggested, on the basis of studies with perfused rat hearts prelabelled with [$1\text{-}^{14}\text{C}$]palmitate *in vivo*, that phospholipids, and to a smaller extent triglycerides, were the major lipid sources of fuel for oxidation. Olson (1962) found that the extent of incorporation of [$1\text{-}^{14}\text{C}$]palmitate into triglycerides fluctuated widely in the perfused

* Present address: Department of Biochemistry, St Louis University School of Medicine, St Louis, Mo. 63104, U.S.A.

† Present address: Department of Medicine, University of Manitoba, Winnipeg 3, Manitoba, Canada.

rat heart, depending on the availability of competing fuels for respiration, whereas the incorporation of label from [1-¹⁴C]palmitate into phospholipids was singularly unaffected. This result suggested that heart triglyceride, or a portion thereof, was used for respiration whereas phospholipids were principally structural and not easily mobilized for use as fuel. This possibility is examined more directly in the present paper by measurement of the amounts of the principal classes of lipid that are present in the isolated rat heart at various times after perfusion with substrate-free medium. Further experiments in which perfused hearts were prelabelled with [1-¹⁴C]-palmitate are also reported.

MATERIALS AND METHODS

Animals. Male albino rats of both the Wistar and Sprague-Dawley (Holtzman) strains were fed on a stock diet *ad libitum* before use. They were killed either by decapitation or thoracotomy after ether anaesthesia. The method of killing had no effect on the results.

Perfusion media. The usual perfusion solution was Krebs-Henseleit bicarbonate medium (Krebs & Henseleit, 1932) containing half the original amount of CaCl_2 (1.25 mM instead of 2.50 mM). In some experiments [1-¹⁴C]palmitate (New England Nuclear Corp., Boston, Mass., U.S.A.) as an ethanolic soapy solution was added dropwise to bovine serum albumin in Krebs-Henseleit bicarbonate medium to provide a perfusion solution containing 0.85 mM-palmitate (0.5 $\mu\text{C}/\text{m-mole}$) in the form of a complex with 0.5% serum albumin. This represents a palmitate/albumin molar ratio 10:1.

Perfusion procedure. The perfusion apparatus was that of Bleehe & Fisher (1954). The gas mixture of $\text{O}_2 + \text{CO}_2$ (95:5) was saturated with water vapour at 38° before being introduced into the gas lift. The perfusate volume was 25–35 ml. and the perfusion pressure 60 cm. H_2O . The bath temperature was 38°. Hearts were quickly removed from the rats, chilled in Krebs-Henseleit bicarbonate buffer at 4°, mounted on the aortic cannula, and perfused for 1–2 min. with the buffer at room temperature in an open non-recirculating system to remove blood and restore the beat. The cannulated heart was then detached from the wash-out system and mounted in the chamber of the perfusion apparatus. The time between opening the thorax and the beginning of the perfusion at 38° was less than 3 min. At the end of the perfusion, the heart was removed from the cannula, chilled, blotted dry, weighed and subjected to lipid extraction and analysis. A small sample was used for determination of dry weight.

Analytical methods. The oxygen consumption was measured polarographically with either an open (Fisher & Williamson, 1961a) or closed oxygen electrode by monitoring the $p\text{O}_2$ of the effluent perfusate and measuring the coronary flow with a bubble flow-meter.

Lipids were extracted from individual hearts, or pools of three or four hearts, with 20 vol. of chloroform-methanol (2:1, v/v) by homogenization in a VirTis 45 high-speed rotary blade or by grinding in washed sea sand. The crude extract, containing 30–120 mg. of lipid, was washed accord-

ing to the method of Folch, Lees & Sloane-Stanley (1957). The chloroform phase was dried *in vacuo* with a rotary evaporator and the total lipids were weighed. A portion of the total lipid (30–100 mg.) was chromatographed on a column (internal diam. 1 cm.) of 3–5 g. of silicic acid (Unisil, 100–200 mesh; Clarkson Chemical Co., Williamsport, Pa., U.S.A.) by the method of Borgström (1952), with 100 ml. of chloroform to elute the neutral lipids and 100 ml. of methanol to elute the phospholipids. The neutral lipids were further separated by thin-layer chromatography with silica gel G as the absorbent and a solvent system of hexane-diethyl ether-acetic acid (160:40:3, by vol.) (Mangold & Malins, 1960). The lipids were located by exposure to I_2 vapour or 0.005% Rhodamine 6G in ethanol, the latter being preferred if gas-liquid chromatography were done (Nichaman, Sweeley, Oldham & Olson, 1963). The following approximate *R* values were observed with standard lipid preparations: monoglycerides, 0.03; diglycerides, 0.13; free cholesterol, 0.17; free fatty acids, 0.27; ubiquinone (coenzyme Q), 0.37; triglycerides, 0.57; cholesteryl esters, 0.77; hydrocarbons, 0.85. The ubiquinone spot was remarkably prominent with I_2 vapour, presumably because of its large number of double bonds. Its concentration in heart is about 200 $\mu\text{g}/\text{g}$. fresh wt. (Gold & Olson, 1966). The lipid zones were scraped from the thin-layer plate and the lipids quantitatively extracted with chloroform for further analysis (Nichaman *et al.* 1963).

Acyl esters were measured in the total lipid extract, the total phospholipid fraction and in the cholesteryl ester and the triglyceride and partial glyceride fractions by the method of Rapport & Alonzo (1955). A portion of the neutral-lipid fraction eluted from the silicic acid with chloroform was used for measurement of the free fatty acids by micro-titration (Olson, Kneebone & McCaffrie, 1967). Lipid phosphorus was measured by the method of King (1932) or Berenblum & Chain (1938).

Gas-liquid chromatography was employed to determine the fatty acid distribution in the various lipid fractions. At Oxford, Pye argon chromatographs (4 ft. linear columns) were used. One was packed with 20% Apiezon L on Embacel (May and Baker Ltd., Dagenham, Essex) sieved to 90–100 mesh. Argon flow was 50 ml./min. and the temperature was 200°. The other was packed with 15% polyethylene glycol adipate ester on 100–120-mesh acid-washed Celite and maintained at 180°. At Pittsburgh, a Barber-Coleman model 10 instrument, equipped with a 6 ft. $\times$ $\frac{1}{8}$ in. U-tube column packed with 14% polyethylene glycol adipate on 80–100-mesh Chromosorb W was used. The temperature of the column and the argon inlet pressure were maintained so that the retention time of methyl palmitate was 5–6 min.

The fatty acids were analysed as their methyl esters. Free fatty acids, recovered from the soapy solution remaining after titration by acidification and extraction with light petroleum, were dried and methylated with diazomethane (Biran & Bartley, 1961). Fatty acids of the triglyceride fraction were generally converted into methyl esters by transmethylation overnight at 76° in Teflon-lined screw-cap vials (15 ml.) with 6 ml. of benzene-methanol-conc. H_2SO_4 (123:62:1, by vol.). The methyl esters were extracted from the reaction mixture with hexane and dried. Phospholipid fatty acid methyl esters were obtained by mild alkaline hydrolysis with 0.5 N-NaOH in methanol for 15 min. The methyl esters were extracted from the reaction mixture

with chloroform and dried. Sphingomyelin, which is a minor constituent of heart lipids, is not hydrolysed by this procedure. In some instances the neutral-lipid and phospholipid fractions were hydrolysed with 2.0N-KOH in 50% (v/v) methanol at 30° for 72 hr. in the presence of 0.1% of quinol. The fatty acids were recovered, dried and methylated with diazomethane. Analyses of these fractions did not differ significantly from those of fractions obtained by the transmethylation procedures. Fatty acid peaks were identified by the use of known standards and the response was linear.

Radioactivity of [^{14}C]palmitate in the perfusate and in the various lipid fractions of the myocardium was measured in a Packard Tri-Carb liquid-scintillation spectrometer. Corrections were made for efficiency and quenching by the use of external standards. The phosphor was 2,5-diphenyl-oxazole (0.4%) and 1,4-bis-(5-phenyloxazol-2-yl)benzene (0.01%) in toluene. All reagents employed in the analytical methods were of analytical grade.

RESULTS

Lipid composition of normal rat heart. The lipid composition of rat hearts that had been washed free of coronary blood and extracted immediately is shown in Table 1. The total lipid of the rat heart made up 13.5–14.1% of the dry wt. or 3.0–3.2% of the fresh wt. The hearts contained about 300 μ -moles of fatty acid/g. dry wt., accounting for about 66% of the total lipid present. Only 2–3 μ -moles of this fatty acid were free, the remainder being esterified, chiefly as phospholipid. The molar ratio 1.8 for phospholipid phosphorus/fatty acid is satisfactory considering the fact that 15–30 μ -moles of plasmalogen/g. dry wt. is present in the heart (Rapport & Lerner, 1959). Triglycerides accounted for most of the neutral fatty acid esters, the amounts of partial glycerides and cholesteryl esters being small. Total neutral esters made up only 15% of the total fatty acids in rat heart.

The distribution of fatty acids in the free fatty acid, triglyceride and phospholipid lipid classes in the heart is shown in Table 2. All three fractions are distinctive in their fatty acid profile and more or less similar to these fractions in other tissues (Getz,

Bartley, Stirpe, Notton & Renshaw, 1961). Somewhat surprising is the low content of linoleic acid and the relatively high content of myristic acid in the free fatty acid fraction. A study of the free fatty acids of rat plasma showed that they were significantly different from those in the heart. The linoleic acid concentration was twice as high in plasma as in heart, the palmitoleic acid concentration was higher and myristic acid was present in plasma only in traces.

Effect of perfusion of rat hearts to exhaustion on the lipid composition. Hearts were perfused with Krebs–Henseleit bicarbonate solution for various times from 60 min. to exhaustion, the latter state being attained in from 70–125 min. Noticeable dilation of the heart, decrease in contractile power and slowing of the beat were noted terminally. The oxygen consumption of the heart over the period of the perfusion decreased from Q_{O_2} (μ l. of oxygen/mg. dry wt./hr.) 40 in the first 15 min. to approx. 35 over the next hour and then to about 25 before exhaustion. This is in excellent agreement with the observations by Fisher & Williamson (1961a).

The changes in the lipid content of the heart during such perfusions are shown in Table 3. Concentrations are expressed in terms of tissue dry weight because of the increased hydration of the heart that occurs with perfusion. Whereas initially the dry weight as a percentage of the fresh weight was 22–25%, the values for exhausted perfused hearts ranged from 16 to 20%. Phospholipid fatty acids did not decrease significantly during ex-

Table 2. *Distribution of fatty acids in three classes of lipids in normal rat heart*

The fatty acids were determined by gas-liquid chromatography. All acids listed were positively identified. Amounts of individual fatty acids present are expressed in terms of the area of each peak, determined as the product of peak height and width at half height, as a percentage of the total peak area. The fatty acids are designated in terms of chain length and unsaturation, e.g. palmitic acid is $\text{C}_{16:0}$. Smaller amounts of fatty acids of odd carbon number and shorter chain length were detected in the chromatograms. Standard deviations on repetitive measurements averaged 5%. Values represent averages of six to ten denominations.

Fatty acid	Free fatty acids	Triglycerides	Phospholipids
$\text{C}_{14:0}$	5.5	1.7	0.1
$\text{C}_{16:0}$	31.0	24.5	15.1
$\text{C}_{16:1}$	4.2	5.1	2.1
$\text{C}_{18:0}$	24.0	8.5	19.1
$\text{C}_{18:1}$	20.1	30.1	10.2
$\text{C}_{18:2}$	7.1	18.5	27.5
$\text{C}_{20:4}$	2.4	3.1	15.6
$\text{C}_{22:6}$	—	—	2.1
$\text{C}_{22:6}$	—	—	9.5

Table 1. *Lipid composition of the normal rat heart*

Twenty rat hearts were analysed in six batches. The variance is presented as s.e.m.

Lipid	Concn. (μ moles of fatty acid or μ g. atoms of P/g. dry wt.)
Cholesteryl esters	1.5 $\pm$ 1.0
Free fatty acids	2.5 $\pm$ 1.0
Partial glycerides	4.0 $\pm$ 0.6
Triglycerides	40.1 $\pm$ 2.0
Phospholipids	250.0 $\pm$ 5
Lipid phosphorus	140.0 $\pm$ 6
Total lipid	298.0 $\pm$ 10

Table 3. *Acyl ester content of rat hearts before and after perfusion with non-nutrient medium*

Group 1 was a control group of hearts that were not perfused; group 2 hearts were perfused for 60 min.; group 3 hearts were perfused until exhaustion and failure, the times ranging from 71 to 125 min. with an average of 95 min.; group 4 hearts were perfused with [^{14}C]palmitate for 30 min. and analysed as controls for group 5 in which non-nutrient medium was substituted at 30 min. and the perfusion continued until exhaustion. The time of perfusion after change of medium ranged from 50 to 75 min., providing a total perfusion time of from 80 to 105 min. (mean 92 min.). Concentrations are expressed as μmoles of esterified fatty acid/g. dry wt. of heart $\pm$ S.E.M.

Group	No. of hearts	Time of perfusion (min.)	Total lipid	Phospholipid	Triglyceride	Monoglyceride	Diglyceride	Cholesteryl esters
1	10	0	305.1 $\pm$ 6.9	255.7 $\pm$ 8.3	42.5 $\pm$ 6.5	1.6 $\pm$ 0.2	3.1 $\pm$ 0.5	1.5 $\pm$ 0.3
2	4	60	260.8 $\pm$ 2.8	235.5 $\pm$ 6.4	23.0 $\pm$ 1.0	1.2 $\pm$ 0.6	3.8 $\pm$ 2.0	2.6 $\pm$ 2.0
3	8	95	279.1 $\pm$ 8.1	258.7 $\pm$ 8.5	13.6 $\pm$ 2.0	2.0 $\pm$ 0.1	2.4 $\pm$ 0.2	1.2 $\pm$ 0.4
4	4	30	296.8 $\pm$ 12.8	250.8 $\pm$ 5.3	40.8 $\pm$ 2.0	1.8 $\pm$ 0.3	2.4 $\pm$ 0.2	2.4 $\pm$ 1.6
5	4	92	274.8 $\pm$ 4.3	260.8 $\pm$ 6.6	17.1 $\pm$ 1.8	1.4 $\pm$ 0.3	1.4 $\pm$ 0.2	0.9 $\pm$ 0.3

Table 4. *Changes during perfusion in endogenous heart lipids labelled with [^{14}C]palmitate*

Heart groups 4 and 5 were those of Table 3. Group 4 hearts were perfused with [^{14}C]palmitate for 30 min. and were analysed as controls for group 5 in which non-nutrient medium was substituted at 30 min. and the perfusion was continued until exhaustion. The results are expressed as μmoles of [^{14}C]palmitate in each lipid class and are obtained by dividing the disintegrations/min. in each fraction by the specific activity of the [^{14}C]palmitate in the perfusate. Values are given as means $\pm$ S.E.M.

Group	Total time of perfusion (min.)	Total lipid	Phospholipid	Triglyceride	Monoglyceride	Diglyceride	Cholesteryl esters	Free fatty acids
4	30	15.44 $\pm$ 1.82	1.80 $\pm$ 0.27	10.28 $\pm$ 1.48	0.17 $\pm$ 0.01	1.33 $\pm$ 0.29	0.09 $\pm$ 0.02	1.17 $\pm$ 0.58
5	92	5.77 $\pm$ 1.12	1.84 $\pm$ 0.20	2.78 $\pm$ 0.92	0.17 $\pm$ 0.13	0.38 $\pm$ 0.11	0.09 $\pm$ 0.02	0.33 $\pm$ 0.04

haustive perfusion and values for lipid phosphorus also remained constant ($150 \pm 5 \mu\text{moles/g. dry wt.}$). Likewise, the concentrations of cholesteryl esters and partial glycerides were essentially constant. The amount of triglyceride, on the other hand, declined steadily with the time of perfusion from 42.5 to $13.6 \mu\text{moles/g. dry wt.}$

Metabolism of ^{14}C -labelled fatty acids in the myocardium during perfusion with nutrient-free medium. The heart lipids were first labelled with [^{14}C]palmitate by perfusion with [^{14}C]palmitate in the form of a complex with bovine serum albumin for 30 min. At this time four hearts (group 4) were perfused with cold 0.15M-potassium chloride to arrest the beat and remove radioactive substrate from the coronary vasculature. Four other hearts (group 5) were transferred to a second perfusion vessel and perfused with Krebs-Henseleit bicarbonate solution until exhaustion. These were also then 'washed out' with 0.15M-potassium chloride. Both groups of hearts were analysed for lipids and radioactivity in each lipid class. The results in

Table 4 show the levels of radioactivity, expressed as μmoles of [^{14}C]palmitate, in each class of lipids at the time of analysis. There was a highly significant ($P < 0.01$) loss of radioactivity from the total heart lipids during the perfusion with the Krebs-Henseleit bicarbonate solution. This was accounted for by a decline in the radioactivity of only three lipid classes, triglycerides, diglycerides and free fatty acids. This result is consistent with the decrease in the myocardial content of triglyceride that occurred during the perfusions. Thus, as shown in Table 3, the decrease in triglyceride fatty acid content was approx. $24 \mu\text{moles/g. dry wt.}$ ($P < 0.01$). The change in triglyceride palmitate content, as calculated from the radioactivity loss, was $7.5 \mu\text{moles/g.}$ and palmitate makes up about 30% of the total triglyceride fatty acid under these conditions. Diglyceride radioactivity showed a smaller fall, significant at the 5% level. The decrease in the radioactivity of the free fatty acid was expected in view of its primary role as a mitochondrial fuel.

DISCUSSION

The lipid composition of the normal rat heart is similar to that reported for ox heart (Kochen, Marinetti & Stotz, 1959) and features a high content of phospholipid and a relatively low content (15%) of neutral fatty acid esters, of which triglycerides form 85%. The free fatty acid contents were in the range 1.5–4.0 μ moles/g. dry wt. or 300–800 μ moles/g. fresh wt. This is much lower than that reported by Neptune, Sudduth & Foreman (1959) in rat diaphragm (800–2500 μ equiv./g. fresh wt.), but higher than that found by Garland & Randle (1963) in diaphragm (157 μ equiv./g. fresh wt.) and in hearts perfused for 15 min. with nutrient-free medium (63 μ equiv./g. fresh wt.). Cardiac free fatty acids are not in equilibrium with the free fatty acids of plasma, nor are they similar in their distribution to any endogenous or exogenous class of esterified lipid, being richer in palmitate and myristate and poorer in linoleate. The particular distribution may reflect the relative rates at which individual fatty acids enter and leave the intracellular pool, via transport, hydrolysis of intracellular lipid, esterification or oxidation.

Our results clearly indicate that triglycerides, and to a smaller extent diglycerides, are the principal fatty acid esters mobilized for fuel in hearts perfused without exogenous substrate. During perfusions to exhaustion, 70% of the triglyceride originally present in the heart disappeared whereas the myocardial phospholipid content remained constant, suggesting that a small amount of triglyceride and virtually all of the phospholipid were essential for the structural integrity of the cell membrane systems.

While this work was in progress, Denton & Randle (1965) made similar observations incidental to a study of the hormonal control of lipid concentrations in rat heart and gastrocnemius. The findings of Shipp *et al.* (1964b) are paradoxical, since they found in similar experiments that the concentration of heart phospholipids declined by 15% on a wet-weight basis with exhaustive perfusion (an amount consistent with increased hydration of the heart) whereas the amount of cardiac triglycerides remained constant. They concluded that phospholipids were the primary source of fatty acids for oxidation, a conclusion at variance with our findings. Neptune, Sudduth, Foreman & Fash (1960) found appreciably greater uptake of 14 C-labelled fatty acids into the triglycerides of isolated rat diaphragms than into the phospholipids during 2 hr. of incubation *in vitro*. This was followed by a greater decrease in the amount and specific activity of the muscle triglycerides than of the phospholipids after a further 2 hr. of incubation. These findings are in accord with our study of loss of radioactivity from pre-

labelled lipid fractions during perfusion of hearts with non-nutrient medium.

The present findings do not mean that the cardiac phospholipid pool is stagnant. There are several mechanisms by which phospholipids are synthesized and degraded and these include the removal and addition of acyl groups with no alteration in the remainder of the molecule (Lands, 1965). Acyl hydrolases for phosphatide have been demonstrated in many tissues, including the rat heart (Robertson & Lands, 1962; Stein & Stein, 1963), but transferases appear to be more prominent and active in heart muscle (Erbland & Marinetti, 1965a,b). Biosynthesis of phospholipids from fatty acids occurs in the heart, but the rate of turnover is much lower and less variable under given environmental conditions than that of triglycerides. Olson (1962) showed that when [14 C]palmitate oxidation by the perfused rat heart was inhibited by the presence of preferred substrates (pyruvate, acetoacetate) in high concentration, the intracellular free fatty acid and triglyceride contents rose, and triglyceride synthesis was enhanced five- to six-fold. Under these conditions, however, there was no change in the modest incorporation of [14 C]palmitate into phospholipids. Under the conditions of the experiments reported here, with exogenous substrate absent, triglycerides were mobilized, but again phospholipid concentrations and turnover were not affected.

These findings are consistent with the behaviour of isolated mitochondria in which phospholipids are intrinsic structural elements of the membrane and do not supply substrate for oxidation (Rossiter & Strickland, 1960; Ansell & Hawthorne, 1964). They also re-emphasize the importance of a supply of exogenous substrate in the metabolism of the heart *in situ*.

We thank Professor Sir Hans Krebs, F.R.S., for his interest in this work and helpful suggestions. We are indebted to Dr John Gilbertson and Dr Charles Sweeley for stimulating discussions and to Mr Chris Rogers and Mrs Irma McCaffrey for able technical assistance. This work was aided by Grants HE-5276 and HE-7468 from the U.S. Public Health Service. R.E.O. thanks the Guggenheim Foundation, New York, for a fellowship and the U.S. State Department for a Fulbright travel grant during his sabbatical year (1961–62) at Oxford University.

REFERENCES

- Ansell, G. B. & Hawthorne, J. N. (1964). *Biochim. biophys. Acta Library* vol. 3: *Phospholipids: Chemistry, Metabolism and Function*, p. 244. New York: Elsevier Publishing Co.
- Berenblum, I. & Chain, E. (1938). *Biochem. J.* **32**, 295.
- Biran, L. A. & Bartley, W. (1961). *Biochem. J.* **79**, 159.
- Bleehen, N. M. & Fisher, R. B. 1954. *J. Physiol.* **123**, 260.
- Borgström, B. (1952). *Acta physiol. scand.* **25**, 101.
- Cruickshank, E. W. H. & Kosterlitz, H. W. (1941). *J. Physiol.* **99**, 208.

- Denton, R. M. & Randle, P. J. (1965). *Nature, Lond.*, **208**, 488.
- Erbland, J. F. & Marinetti, G. V. (1965a). *Biochim. biophys. Acta*, **106**, 128.
- Erbland, J. F. & Marinetti, G. V. (1965b). *Biochim. biophys. Acta*, **106**, 144.
- Evans, J. R., Opie, L. H. & Renold, A. E. (1963). *Amer. J. Physiol.* **205**, 971.
- Evans, J. R., Opie, L. H. & Shipp, J. C. (1963). *Amer. J. Physiol.* **205**, 766.
- Fisher, R. B. & Williamson, J. R. (1961a). *J. Physiol.* **158**, 86.
- Fisher, R. B. & Williamson, J. R. (1961b). *J. Physiol.* **158**, 102.
- Folch, J., Lees, M. & Sloane-Stanley, G. H. (1957). *J. biol. Chem.* **266**, 497.
- Garland, P. B. & Randle, P. J. (1963). *Nature, Lond.*, **199**, 381.
- Getz, G. A., Bartley, W., Stirpe, F., Notton, B. M. & Renshaw, A. (1961). *Biochem. J.* **80**, 176.
- Gold, P. H. & Olson, R. E. (1966). *J. biol. Chem.* **241**, 3507.
- Hicks, R. M. & Kerly, M. (1960). *J. Physiol.* **150**, 621.
- King, E. J. (1932). *Biochem. J.* **26**, 292.
- Kochen, J., Marinetti, G. V. & Stotz, E. (1959). *J. Lipid Res.* **1**, 147.
- Krebs, H. A. & Henseleit, K. (1932). *Hoppe-Seyl. Z.* **210**, 33.
- Lands, W. E. M. (1965). *Annu. Rev. Biochem.* **34**, 313.
- Mangold, H. K. & Malins, D. C. (1960). *J. Amer. Oil Chem. Soc.* **37**, 383.
- Neptune, E. M., jun., Sudduth, H. C. & Foreman, D. R. (1959). *J. biol. Chem.* **234**, 1659.
- Neptune, E. M., jun., Sudduth, H. C., Foreman, D. R. & Fash, F. J. (1960). *J. Lipid Res.* **1**, 229.
- Nichaman, M. Z., Sweeley, C. C., Oldham, N. M. & Olson, R. E. (1963). *J. Lipid Res.* **4**, 484.
- Olson, R. E. (1962). *Nature, Lond.*, **195**, 597.
- Olson, R. E., Kneebone, G. & McCaffrie, I. (1967). *Analyt. Biochem.* (in the Press).
- Rapport, M. M. & Alonzo, N. (1955). *J. biol. Chem.* **217**, 193.
- Rapport, M. M. & Lerner, B. (1959). *Biochim. biophys. Acta*, **33**, 319.
- Robertson, A. F. & Lands, W. E. M. (1962). *Biochemistry*, **1**, 804.
- Rossiter, R. J. & Strickland, K. P. (1960). In *Lipid Metabolism*, p. 69. Ed. by Bloch, K. New York: John Wiley and Sons Inc.
- Shipp, J. C., Matos, O. E., Knizely, H. & Crevasse, L. E. (1964a). *Amer. J. Physiol.* **207**, 1231.
- Shipp, J. C., Thomas, J. M. & Crevasse, L. (1964b). *Science*, **143**, 371.
- Stein, O. & Stein, Y. (1963). *Biochim. biophys. Acta*, **70**, 517.
- Williamson, J. R. (1962). *Biochem. J.* **83**, 377.
- Williamson, J. R. & Krebs, H. A. (1961). *Biochem. J.* **80**, 540.
- Zachariah, P. (1961). *J. Physiol.* **158**, 59.