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The Role of the Plasma Membrane in Fatty Acid Uptake by Rat Liver Parenchymal Cells

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1. Suspensions of isolated rat liver parenchymal cells incorporate [^{14}C]palmitic acid into glycerides at about 40% of the rate obtained with liver slices. 2. At short time-intervals most of the incorporation is into phosphatidylcholine and this is recovered mainly in the plasma-membrane fraction. 3. At later times (5 min to 2 h) the [^{14}C]palmitic acid is mainly found in triglyceride, but this is not recovered in the plasma-membrane fraction. 4. Addition of lysophosphatidylcholine increases incorporation of palmitic acid into both phosphatidylcholine and triglyceride, with maximum effect at about 0.1 mM. 5. *In vivo*, 1 min after injection of [^{14}C]palmitic acid, radioactive phosphatidylcholine is concentrated in the plasma-membrane fraction, but the proportion present in this fraction declines rapidly. 6. The phosphatidylcholine of the plasma-membrane fraction has, at 1 min after injection, a specific radioactivity 30-fold greater than that of the whole tissue. 7. This phosphatidylcholine reaches its maximum specific radioactivity before the tissue phosphatidic acid or diglyceride. 8. The phosphatidylcholine of the plasma-membrane fraction has a very rapid turnover. 9. It is proposed that the rapid formation of phospholipids in the plasma membrane is by acylation of their lyso-derivatives and the role of this process in fatty acid uptake is discussed.

Non-esterified fatty acids are very efficiently removed from plasma by liver and as incorporation into glycerides is rapid, little is present as non-esterified fatty acid in the cells (Göransson & Olivecrona, 1964; Higgins & Green, 1966). The way in which fatty acids cross the liver plasma membrane is not known. They may diffuse across the membrane as non-esterified fatty acids or they may be converted by the plasma membrane into their CoA derivatives (Pande & Mead, 1968) or the smaller *S*-acylpantetheine derivatives (Trams, Fales & Gal, 1968). Both of these should cross the lipid membrane more easily than the parent compounds.

Earlier work (Higgins & Green, 1967) suggested that plasma-membrane phospholipids could also be involved in uptake of non-esterified fatty acids. Incorporation of radioactive non-esterified fatty acids into liver phospholipids occurs before incorporation into triglycerides (Baker & Schotz, 1967; Laudat, Koenig & Laudat, 1969) and it has been reported that the phospholipids of the plasma membrane have the highest specific radioactivity of all cell fractions (Stahl & Trams, 1968). Liver readily takes up lysophosphatidylcholine and incorporates it into phosphatidylcholine (Stein & Stein, 1966) and this acylation process has been shown to occur *in vivo* and *in vitro* in the plasma membrane as well as in crude mitochondrial and microsomal fractions (Elovson, 1965; Scherphof & van Deenen, 1966; Reshef & Shapiro, 1966; van den Bosch & van Deenen, 1965; Stahl & Trams, 1968; Nachbaur, Colbeau & Vignais, 1969; Stein, Widnell & Stein, 1968; Åkesson, Elovson & Arvidson, 1970). Since the first demonstration by Lands (1958) of lysophosphatidylcholine–phosphatidylcholine cycles, their operation in many different tissues and cells has been established and this made it of interest to carry out a more detailed study of uptake of non-esterified fatty acids by liver. Some of the results have been reported in a preliminary form (Wright & Green, 1969).

MATERIALS AND METHODS

[1-^{14}C]Palmitic acid was obtained from The Radiochemical Centre, Amersham, Bucks., U.K., collagenase (EC 3.4.4.19) from Calbiochem Ltd., London W.1, U.K., and hyaluronidase (EC 4.2.99.1) from Sigma (London) Chemical Co. Ltd., London S.W.6, U.K. Lysophosphatidylcholine and crystalline bovine serum albumin were from Koch–Light Laboratories Ltd., Colnbrook, Bucks., U.K., and the latter was freed of fatty acids by the method of Chen (1967). [^{14}C]Palmitic acid–albumin complexes were prepared as described by Shohet, Nathan & Karnovsky (1968) and stored aseptically. They contained 1 μmol of palmitic acid (about 5×10^6 c.p.m.)/μmol of albumin. Silica gel G was supplied by E. Merck A.G.,

Darmstadt, Germany. Hooded rats of either sex weighing about 200g, were used throughout.

Hepatic parenchymal cell suspensions. The method of Howard & Pesch (1968) was used. Each preparation was examined under the microscope at the conclusion of the experiment to confirm that most of the cells still excluded the stain Trypan Blue.

The preparation of isolated liver cells in good condition can be a rather uncertain process and the ability to recognize the state of a preparation from a microscopic examination takes time to acquire. The enzymes used vary greatly and it is necessary to test samples, particularly of collagenase, before use, since some preparations are damaging to the cells. The suspensions must be handled very gently at all stages and only allowed to contact silicone-treated glass surfaces. Yields are very small, about 5% of the total number of parenchymal cells being recovered.

Elsbach & Rizack (1970) suggested that collagenase preparations contained phospholipase C (EC 3.1.4.3) activity. However, under the conditions used for cell isolation it was found that only about 0.9% of the phosphatidylcholine in the cells could have been hydrolysed.

Standard incubation conditions. Unless otherwise stated, isolated cells, containing about 5mg of protein, were incubated with 0.1μmol of palmitic acid (as the albumin complex) in 3ml of glucose-free Hanks solution (Hanks & Wallace, 1949). The incubations were carried out at 37°C with shaking under O_2+CO_2 (95:5).

After the appropriate time, the cells were separated by centrifuging at 50*g* for 45s and washed twice with ice-cold glucose-free Hanks solution.

Injection of palmitic acid–albumin complex. All injections were made under Nembutal anaesthesia into the exposed femoral vein over a period of about 30s. Palmitic acid (0.1μmol) in 0.5ml of glucose-free Hanks solution was injected. After the appropriate time, the liver was removed and homogenized in ice-cold 0.3M-sucrose, adjusted to pH7.4 with $NaHCO_3$. Part of this homogenate was extracted immediately with chloroform–methanol and the rest used for preparation of the plasma-membrane fraction.

Preparation of plasma-membrane fractions. For whole liver, the method of Coleman, Michell, Finean & Hawthorne (1967) was used. For isolated cells the 'acetic acid method' of Warren, Glick & Nass (1966), which has been applied to a number of different types of cells, was used. This was adopted because the yield obtained by the previous method (Graham, Higgins & Green, 1968) was very low. The method of Warren *et al.* (1966) gave a membrane preparation that, under phase-contrast microscopy, appeared to consist of empty sacs, as with the other types of cell to which it has been applied. This preparation contained about 12% of the total cell protein and the specific activities of L-leucyl-β-naphthylamidase (EC 3.4.1.1) and alkaline phosphatase (EC 3.1.3.1) were five- to six-fold greater than in the whole cells. These enzymes are concentrated in the plasma membrane of liver cells (Graham *et al.* 1968) and although the degree of enrichment is not as great as in other preparations, it must be remembered that here the starting material was isolated hepatic cells, not a whole liver.

Extraction and separation of lipids. Lipids were extracted by the method of Albrink (1960) or, if sucrose was present, by that of Hanson & Olley (1963). The major lipid classes were isolated by t.l.c. as described by Boberg (1966). Monoglycerides were separated from the phospholipids by extraction with diethyl ether. No significant amount of ^{14}C was found in this fraction. The phospholipids were then extracted from the adsorbent (Biezenski, 1967) and rechromatographed on thin layers of silica gel G with a solvent system of chloroform–methanol–NH_3–water (26:14:1:1, by vol.). Pure reference compounds were run with each lipid sample. As ^{14}C was found only rarely and in small amounts in cholesterol esters and the sphingomyelin/phosphatidylserine fraction, these have not been included in the results.

Measurement of radioactivity. Samples were prepared as described by Higgins & Green (1966) and the radioactivity was counted in a Beckman LS 200 liquid-scintillation spectrometer. Corrections for quenching were not found to be necessary.

Other determinations. Protein was determined by the method of Lowry, Rosebrough, Farr & Randall (1951) and phospholipids were estimated from the lipid phosphorus content (Fiske & Subbarow, 1925). Non-esterified fatty acids were determined by the method of Duncombe (1963).

RESULTS

Incorporation of [^{14}C]palmitic acid into liver-cell glycerides in vitro. In most experiments low concentrations of palmitic acid (0.03mM) were used. Under these conditions, total incorporation into cell glycerides was about 0.13–0.2nmol/h per mg of cell protein. At palmitic acid concentrations of 0.5–1.5mM, incorporation into cell glycerides was about 4–7nmol/h per mg of cell protein, as compared with 9–14nmol/h per mg of cell protein for liver slices under the same conditions. However, although not as efficient as slices, the cells showed the metabolic changes observed in the intact liver after starvation (Wright & Green, 1969) and so seem to be representative of the tissue.

Distribution of [^{14}C]palmitic acid in cell lipids. Over a wide range of palmitic acid concentrations incorporation was mainly (53–82%) into neutral glycerides as *in vivo* and, in all but one case, triglycerides contained most ^{14}C (Fig. 1). Maximum incorporation was obtained at about 0.5mM-palmitic acid, which is above the normal physiological concentration of non-esterified fatty acids.

The values given in Table 1 show that the pattern of palmitic acid incorporation changes with time. After 2min, most of the glyceride ^{14}C was in the phosphatidylcholine and phosphatidylethanolamine fractions but as time went on the proportion present in these fractions decreased whereas that in the neutral glycerides increased, to become after 15min the predominant fraction. These results confirm those obtained in other experiments in which cells were incubated in the presence of cofactors (Wright & Green, 1969) although in this case incorporation at later times was into phos-

phatidic acid rather than into neutral glycerides. The experiments performed over 2min employed a much higher concentration of palmitic acid than the others and so total incorporation was greater than would otherwise be the case. This was done to check that the rapid incorporation of palmitic acid into phosphatidylcholine and phosphatidylethanolamine seen in the earlier experiments (Wright & Green, 1969) did not merely reflect the low concentration of palmitic acid used. As can be seen, this still occurs when physiological concentrations of the acid are employed. The experiment performed over 60min resulted in a much lower incorporation of palmitic acid into glycerides than expected. Results from other experiments performed under the same conditions gave an incorporation of about 0.25nmol per mg of protein. The reason for this low incorporation is not known but

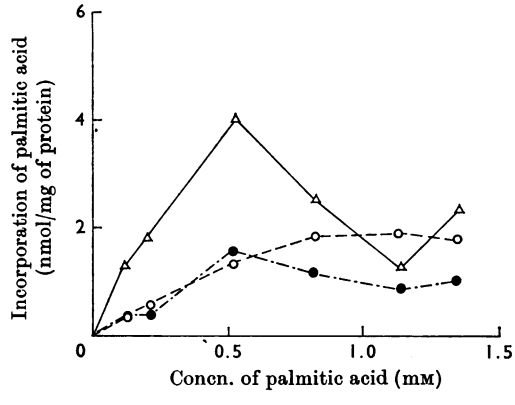

Fig. 1. Incorporation of [^{14}C]palmitic acid into the lipids of isolated liver cells *in vitro*. The cells were incubated for 1h under standard conditions except that the concentration of palmitic acid was varied. Each point is the mean of two experiments. ○, Phospholipid; △, triglyceride; ●, diglyceride.

the distribution of the ^{14}C among the lipid classes agrees with those obtained at 15 and 120min.

Location of ^{14}C-labelled phospholipids in the liver cell. A plasma-membrane fraction was separated, as described in the Materials and Methods section, from the cells used in some of the above experiments. The proportion of the total ^{14}C-labelled phospholipid present in this fraction was determined (Table 2). It is obvious that after short incubation periods, when most of the incorporated ^{14}C is present as phosphatidylcholine and phosphatidylethanolamine, this is mainly recovered in the plasma-membrane fraction. As time goes on and ^{14}C begins to accumulate in neutral glycerides these are not recovered in this fraction. (At most 15.7% of the tissue ^{14}C-labelled neutral glycerides were recovered in the plasma-membrane fraction.) Because of the very small amounts of material involved, specific radioactivities were not determined, but the amount of protein in this fraction is on average only 12.2% of the total cell protein. Thus the initial incorporation of fatty acids appears to be into the plasma-membrane phosphatidylcholine and phosphatidylethanolamine, and this must contain a small proportion of the total cell phospholipid of high specific radioactivity.

Effect of lysophosphatidylcholine. The above results suggested that the rapid incorporation into membrane phospholipids was via direct acylation of the lyso-compounds. Confirmation of this was obtained by determining the effect of adding lysophosphatidylcholine to the system (Table 3). This increased incorporation into glycerides at all concentrations used, with a maximum effect at 0.1mM. The increased incorporation of [^{14}C]-palmitic acid is not just into phospholipids but into di- and tri-glycerides as well. Maximum stimulation of incorporation into phospholipids is about sixfold and into neutral glycerides 16-fold. These results demonstrate that radioactive phosphatidylcholine (and probably phosphatidylethanolamine)

Table 1. *Distribution of ^{14}C among lipid classes after incubation of isolated liver cells with [^{14}C]palmitic acid*

The incubations were carried out under standard conditions. Each result is the mean of two or three experiments except that for 60min which is from one experiment.

		Incorporation of [^{14}C]palmitic acid				
Time (min)	Concn. of [^{14}C]palmitic acid (mM)	Total glycerides (nmol/mg of protein)	Neutral glycerides (%)	Phosphatidylcholine (%)	Phosphatidylethanolamine (%)	Phosphatidic acid (%)
2	1.33	0.15	7.0	53.1	23.4	12.6
15	0.03	0.075	64.1	28.8	3.2	2.5
60	0.03	0.085	78.8	8.0	5.1	7.2
120	0.03	0.62	83.3	15.5		1.6

Table 2. *Distribution of* ^{14}C*-labelled phospholipid within the cell after incubation* in vitro *with* [^{14}C]*palmitic acid*

The experiments from which the results were obtained are described in Table 1.

Time (min)	^{14}C in phospholipids (c.p.m.)		Protein content (mg)	
	Whole cells	Plasma-membrane fraction	Whole cells	Plasma-membrane fraction
2	880	680	5.4	0.75
60	450	310	5.2	0.95

Table 3. *Effect of lysophosphatidylcholine on the incorporation of* [^{14}C]*palmitic acid into liver-cell glycerides*

Isolated liver cells were incubated for 1h under standard conditions with [^{14}C]palmitic acid (1.05mM). Lysophosphatidylcholine was added to give the required final concentration.

Concn. of lysophosphatidylcholine (mM)	Incorporation of [^{14}C]palmitic acid (nmol/mg of protein)	
	Neutral glycerides	Phospholipids
0	2.3	2.0
0.03	1.8	3.3
0.07	6.5	4.2
0.10	38.4	12.6
0.15	17.8	11.8
0.20	2.4	8.4

is formed by acylation of the corresponding lyso-compound. As the major effect is to increase incorporation into triglyceride (in only one experiment did this contain less than 60% of the neutral glyceride ^{14}C), it appears that addition of lysophosphatidylcholine greatly facilitates passage of fatty acids into the interior of the liver cell. Damage to the cells seems unlikely at the concentrations used since they are below the value (0.3mM) found in plasma *in vivo*.

Studies in vivo. To assess the validity of the use of isolated cells, experiments complementary to those described above were carried out *in vivo*. Because of the larger quantities of tissue involved, specific radioactivities could be determined in these investigations. The uptake and incorporation into liver lipids of injected [^{14}C]palmitic acid is shown in Table 4. As expected from earlier studies (Göransson & Olivecrona, 1964; Higgins & Green, 1966) uptake was very rapid, reaching a maximum after 2min. At this time, nearly 50% of the injected fatty acid was present in the liver, rather more than is usually found in fed animals (30–40%). This may be because only a small amount of labelled palmitic acid was injected. After 1min, about 25% of the lipid ^{14}C was present in non-esterified fatty acids but by 2min the value was only 11% and by 5min, 6% of the total, showing the very rapid rate of incorporation into glycerides. The values then start to rise again, to 14% after 10min and 23% after 20min, although no more uptake was occurring. This suggests that after a while some of the newly formed glycerides are hydrolysed inside the liver cell.

The incorporation into whole-tissue glycerides is similar to that observed previously (Higgins & Green, 1966) with 47–61% incorporated into phospholipids and 30–48% into triglycerides throughout the 20min period studied. There are no consistent changes in the relative proportions of ^{14}C in the major lipid classes but there is a steadily decreasing proportion of the phospholipid ^{14}C in phosphatidic acid and phosphatidylethanolamine and an increase in the proportion present in phosphatidylcholine in the whole tissue.

In the plasma-membrane fraction most of the ^{14}C was present in phospholipid at all times. Radioactivity was detected in the neutral lipids but it seemed very variable. As there is only a small amount of neutral lipid in the membrane, contamination is a serious problem but a small amount of ^{14}C present could indicate a high specific radioactivity for this material, which merits a more detailed investigation. The [^{14}C]palmitic acid in non-esterified fatty acids reached a maximum at 2min and then declined, although that present in the whole tissue was increasing at later times. The steady increase in the proportion of phospholipid ^{14}C present as phosphatidic acid and phosphatidylethanolamine as the total amount of radioactivity decreased, was also in contrast with the pattern in whole liver. Even after 10min phosphatidylethanolamine was the major labelled species.

Fig. 2 presents a comparison of the specific radioactivities of whole-tissue phosphatidylcholine and triglyceride with plasma-membrane phosphatidylcholine. At the earliest time investigated (1min), the specific radioactivity of the plasma-membrane-fraction phosphatidylcholine was 31-fold greater than that of the whole-tissue lipids, but

Table 4. *Distribution of* ^{14}C *in rat liver after injection of* [^{14}C]*palmitic acid* in vivo

[^{14}C]Palmitic acid (0.1 μmol) was injected into the femoral vein of each rat and the liver removed after the appropriate time-interval. The liver was immediately homogenized and one portion used to prepare the plasma-membrane fraction while the rest was extracted. The protein weights were multiplied by the factor (wt. of homogenate/wt. of sample taken), so that all refer to the original wt. of liver. Results are the means of two or three experiments except that for 20 min which is from one experiment.

			Incorporation of [^{14}C]palmitic acid						
			(c.p.m./mg of protein)				% of total in phospholipids		
	Time after injection (min)	Protein content (mg)	Non-esterified fatty acids	Phospho-lipid	Di-glyceride	Tri-glyceride	Phospha-tidyl-choline	Phospha-tidyl-ethanol-amine	Phospha-tidic acid
Whole liver	1	1100	5	7	2	6	57.5	31.5	11.0
	2	900	6	22	6	19	70.5	22.0	8.0
	5	1180	2	15	2	9	74.5	18.0	7.5
	10	1200	5	14	1	15	73.0	19.5	7.0
	20	1090	9	18	1	12	83.0	12.0	5.0
Plasma-membrane fraction	1	4.0	127	160			80.3	15.0	4.8
	2	3.2	136	218			63.5	32.0	4.5
	5	2.6	83	144			68.5	20.0	11.5
	10	7.1	25	97			31.5	48.0	20.5
	20	2.9	*	18			*	(100)	*

* Below limit of measurement.

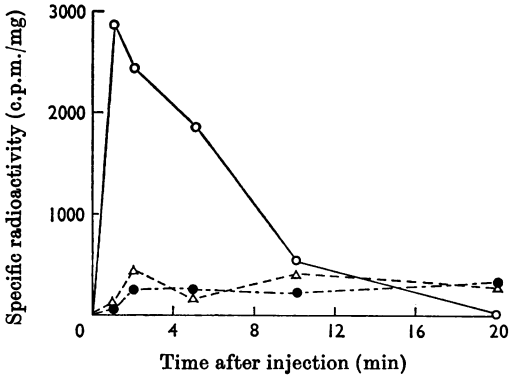

Fig. 2. Comparison of the specific-radioactivity changes in liver plasma-membrane-fraction phosphatidylcholine and whole-liver phosphatidylcholine and triglycerides after intravenous injection of [^{14}C]palmitic acid. The experiments from which the results were obtained are described in Table 4. ○, Plasma-membrane-fraction phosphatidylcholine; ●, total liver phosphatidylcholine; △, total liver triglyceride.

within 10 min they have reached similar values. Although no ^{14}C was detected in the plasma-membrane-fraction phosphatidylcholine at 20 min this is probably because the amount present was too small to measure rather than that none was present. The maximum specific radioactivity of the plasma-membrane-fraction phosphatidylcholine occurred before that of the whole-tissue phosphatidic acid or diglyceride (Table 4), assuming that the amount of these compounds stays constant.

DISCUSSION

Isolated liver cells have several advantages over other systems used for studying cellular uptake and metabolism. In homogenates, structural relationships are lost and cell contents are diluted. With slices there are the problems that the exposed surfaces consist mainly of broken cells and that diffusion into the middle of the tissue can be restricted. The former becomes especially acute when trace amounts of radioactive starting material are employed. There are several reports that cells in very good condition can be obtained by the method employed here, or slight modifications of it, but in view of the difficulties mentioned in the Results section, it is not surprising that others have not found the method satisfactory (Jungalwala & Dawson, 1970). In this laboratory it has proved to give much better results than all the other methods investigated.

The results obtained from studies performed both *in vitro* and *in vivo* confirm earlier findings (Higgins & Green, 1967) and emphasize the importance of the liver plasma membrane in the uptake and metabolism of non-esterified fatty acids. The total contribution of this fraction is difficult to assess

because plasma membranes cannot be quantitatively recovered from the tissue. On the other hand, all subcellular fractions prepared by standard techniques contain pieces of the plasma membrane; the microsomal fraction, with which many studies of lipid metabolism are made, contains about half of the total (Hinton, Dobrota, Fitzsimons & Reid, 1970). Thus the metabolic properties of cell fractions do not necessarily reflect intracellular events. The findings of this study also illustrate how misleading measurements of the specific radioactivity of lipid fractions of the whole tissue can be.

The results obtained with isolated cells in this and earlier work (Wright & Green, 1969) are clear-cut. The palmitic acid rapidly incorporated into phosphatidylcholine and phosphatidylethanolamine is mainly in the plasma-membrane fraction. Only estimates can be made of the situation *in vivo*. From the recovery of plasma-membrane marker enzymes, it can be calculated that about 7.5% of the total plasma membranes of the tissue are actually isolated in the 'plasma-membrane fraction'. Coleman *et al.* (1967) obtained a value of about 15% but as they obtained about twice as much protein as was recovered here (Table 4), the value of 7.5% seems reasonable. On this basis, an estimation of the proportion of ^{14}C-labelled phospholipid and non-esterified fatty acids present in the plasma membranes can be made (Table 5). It appears that, as *in vitro*, most of the radioactive phospholipid in the cells 1 min after injection could be present in the plasma membranes. As uptake proceeds over the next minute, the proportion present in the plasma membrane falls and continues to fall as time goes on. The value for 10 min seems anomalous but the plasma-membrane preparation in that experiment contained a lot of protein (Table 4) and could well have been contaminated.

The high concentration of labelled non-esterified fatty acids estimated to be present in the plasma membranes (Table 5) appears to rule out the possibility that the labelled phospholipid recovered in the plasma-membrane fraction is formed during the lengthy preparation procedure from bound non-esterified fatty acids. Uptake of non-esterified fatty acids involves an initial binding to the plasma membrane *in vivo* and *in vitro*. Incorporation into glycerides must occur as the fatty acid enters the cell or immediately afterwards since there is little if any labelled non-esterified fatty acid inside the cell during the period of uptake. The results also suggest that the rate-limiting step is not binding to the outside of the membrane but transfer across it. As metabolism proceeds, cell glycerides are hydrolysed, since at 10 and 20 min the amount of [^{14}C]palmitic acid in liver non-esterified fatty acids increases, but this is not located in the plasma membrane. Winand, Furnelle & Christophe (1969) showed the existence of two separate pools of non-esterified fatty acids and experiments performed *in vitro* with liver cells indicate that one pool is located on the outside of the plasma membrane and one is inside the cell (J. A. Higgins & C. Green, unpublished work).

The rate of decrease in the specific radioactivity of the plasma-membrane phosphatidylcholine with time (Fig. 2) indicates that this has a very rapid turnover. It seems unlikely that the material is simply exchanging with unlabelled plasma phospholipids, since the rate of exchange is much greater than other measured exchanges (Reed, 1968; Jungalwala & Dawson, 1970). Further, Shohet (1970) showed that in erythrocytes the phosphatidylcholine formed from circulating non-esterified fatty acids did not exchange, whereas other phosphatidylcholine molecules did. It seems then that the plasma-membrane phospholipids transfer their labelled fatty acid inside the cell.

It can be calculated that the rat liver takes up about 1 mg of non-esterified fatty acid per min (Boberg, 1969) and if this all entered via the

Table 5. *Calculation of the proportion of total liver* ^{14}C-*labelled non-esterified fatty acids and phospholipid present in the plasma membranes after injection of* [^{14}C]*palmitic acid* in vivo

The calculations are made from the results given in Table 4 assuming that only 7.5% of the total plasma membranes of the tissue was recovered in the 'plasma-membrane fraction'.

Time after injection (min)	^{14}C-labelled non-esterified fatty acids (% of total liver ^{14}C)	^{14}C-labelled non-esterified fatty acids (Calculated present in plasma membranes) (%)	^{14}C-labelled phospholipid (Calculated present in plasma membranes) (%)
1	25.3	110.6	103.4
2	11.1	116.0	46.0
5	7.7	104.6	33.4
10	14.3	46.8	56.2
15	22.4	0	3.3

plasma-membrane phosphatidylcholine, this would have a half-life of about 2.5min. The observed value of about 5min (Fig. 2) is close to this and indicates that an appreciable amount of fatty acid taken up by liver could pass through the plasma-membrane phospholipids. This also agrees with the findings of Baker & Schotz (1967) and Laudat *et al.* (1969).

The rapid incorporation of [^{14}C]palmitic acid into these phospholipids and the effect of lysophosphatidylcholine (Table 4) support the idea that direct acylation of the lyso-derivatives occurs (Elovson, 1965; Åkesson, 1970; Åkesson *et al.* 1970). Isolated rat liver plasma-membrane fractions readily incorporate non-esterified fatty acids and lysophosphatidylcholine into phospholipids (Higgins & Green, 1967; Stein *et al.* 1968) but there is some dispute over the location of the enzymes involved (Pande & Mead, 1968; Portman, Alexander & Osuga, 1969; Lippel, Robinson & Trams, 1970; Eibl, Hill & Lands, 1969). However, two quite different methods of preparing plasma-membrane fractions were used in the present studies and gave similar results.

Åkesson *et al.* (1970) injected [^{3}H]palmitic acid into rat portal veins over 1s and followed its metabolism over short time-intervals. About 25% of palmitic acid incorporation into liver phospholipids was by acylation of the lyso-derivatives. Their results show a rapid incorporation into phosphatidylcholine before the much greater incorporation into phosphatidic acid. Although most of the [^{3}H]palmitic acid was incorporated into the former at position 1, the liver produces and acylates both 1- and 2-acyl isomers of lysophosphoglycerides (Lands & Merkl, 1963; Merkl & Lands, 1963; van den Bosch & van Deenen, 1965; Scherphof, Waite & van Deenen, 1966). Apparently during fatty acid uptake, phospholipids are formed both by *de novo* synthesis and by acylation of their lyso-derivatives, the proportion of each probably depending on the nature and concentration of the fatty acid (Elovson, 1965; Laudat *et al.* 1969; Åkesson, 1970; Åkesson *et al.* 1970).

Transfer of the fatty acid from the plasma membrane to the cell interior could involve operation of a phosphatidylcholine/lysophosphatidylcholine cycle in the membrane but as lysophosphatidylcholine is readily available from the plasma and the cell interior, removal of phosphatidylcholine molecules as such or after conversion into other di-acyl glycerides, could occur. Acylation of membrane lysophosphoglycerides may also serve functions other than transport (van den Bosch & van Deenen, 1965; Stein *et al.* 1968).

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