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Lipid Metabolism in the Perfused Chicken Liver

THE UPTAKE AND METABOLISM OF OLEIC ACID, ELAIDIC ACID, *CIS*-VACCENIC ACID, *TRANS*-VACCENIC ACID AND STEARIC ACID

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Comparative studies were made of the uptake and metabolism of *cis*- and *trans*-octadecenoic acids by the perfused chicken liver. No differences were observed in the rates of uptake of the isomers. There was considerable incorporation of radioactivity into triglycerides and phospholipids, and some release of labelled lipid into the perfusate was observed. The *cis*-fatty acids were more readily incorporated into triglycerides than phospholipids, the reverse being true of the *trans*-fatty acids. Examination of the intramolecular distribution of fatty acids in triglycerides showed that the *trans*-fatty acid and stearate mainly occupied the 1- and 3-positions, and *cis*-fatty acids the 2-position. In the phospholipids phosphatidylcholine and phosphatidylethanolamine the *trans*-fatty acids again behaved like stearic acid and favoured the 1-position. No evidence was obtained of atypical patterns of uptake or metabolism of the *trans*-fatty acids.

Fatty acids of *trans* configuration are formed by the partial hydrogenation of polyenoic acids by bacteria, or by the use of a catalyst as in the industrial process of hardening fats. Rumen microorganisms show a considerable capacity for biohydrogenation and the depot fats of cattle may contain up to 10% *trans*-fatty acids, which are mainly *trans*-Δ^9-, *trans*-Δ^{10}- and *trans*-Δ^{11}-octadecenoic acids (Swern, Knight & Eddy, 1952). Fats of non-ruminants are usually free of *trans*-fatty acids, but pigs on a diet with a high *trans*-fatty acid content were found to have relatively large quantities deposited in the adipose tissue of various organs (Johnston, Johnson & Kummerow, 1957*a*). In contrast, Kaufmann & Bandyopadhyay (1965) could not detect *trans*-fatty acids in the brain tissue of animals fed on diets rich in these acids and concluded that there are certain membrane barriers that *trans*-fatty acids fail to penetrate. Johnston, Johnson & Kummerow (1957*b*) postulated a similar barrier in rat placental tissue, although McConnell & Sinclair (1937) and Ono & Frederickson (1964) have obtained results to the contrary with rat placental tissue. G. Billek & F. Strauch (unpublished work) have shown the presence of *trans*-fatty acids in the brain and foetal tissue of the rat.

Certain differences in the metabolism of *cis*- and *trans*-fatty acids are well recognized. There are marked differences in the intramolecular distribution of *cis*- and *trans*-fatty acids in triglycerides. The *cis*-fatty acids predominate at the 2-position and the *trans*-fatty acids behave like saturated fatty acids and occur mainly at the 1- and 3-positions (Duncan & Garton, 1967, 1969).

In the present investigation the uptake of ^{14}C- and ^{3}H-labelled *cis*-Δ^9-, *trans*-Δ^9-, *cis*-Δ^{11}- and *trans*-Δ^{11}-octadecenoic acids and octadecanoic acid by the perfused chicken liver was examined. The pattern of incorporation into liver and plasma lipids, and the intramolecular distribution of the fatty acids in the liver triglycerides and phospholipids were also determined.

MATERIALS AND METHODS

Liver perfusion. The livers from fed birds were perfused as described by Bickerstaffe, West & Annison (1970) except that the perfusion medium was supplemented with albumin-bound ^{14}C- or ^{3}H-labelled fatty acids. Glycogenolysis makes good the glucose utilized in the system, and fortuitously maintains the glucose concentration in the perfusate at 300–450 mg/100 ml, which is within the physiological range. This inevitably results in unphysiological contents of liver glycogen, but systems that maintain unchanged glycogen contents in perfused preparations have not yet been described. Since the naturally occurring fatty acids oleic acid and stearic acid were always present in the perfusion medium, the corresponding radioactive fatty acids were added without carrier. Labelled *trans*-octadecenoic acids and *cis*-vaccenic acid were added to the perfusion fluid with sufficient carrier to raise their initial concentration two- to three-fold in excess of that of oleic acid.

The added fatty acids, after saponification with 50% excess of KOH, were bound to bovine serum albumin by

the procedure of Laurell (1957) so that the fatty acid/albumin ratio did not exceed 2:1 (w/w). The final mixture (20ml) of fatty acids was added to the perfusate (300ml) as a single injection 30min after the portal catheter had been connected to the perfusion apparatus. The perfusion was continued for a further 150min.

The criteria used to assess the efficiency of the perfused liver preparation were defined by Bickerstaffe *et al.* (1970).

Extraction of plasma and liver lipids. Lipids were extracted from plasma (8ml) and liver (25g) by the method of Folch, Lees & Sloane-Stanley (1957) but with 0.1M-potassium phosphate buffer, pH6.0, instead of water. Internal markers, heptadecanoic acid (0.015mg/ml of plasma, 0.05mg/g of liver) and glycerol triheptadecanoate (0.04mg/ml of plasma, 0.4mg/g of liver), were added to the plasma and liver extracts. Liver lipids, which contain substantial quantities of cholesterol esters and triglycerides, were satisfactorily separated on thin layers (0.4mm thick) of silica gel G, activated for 60min at 105°C on long glass plates (20cm × 34cm) with the solvent system diethyl ether–toluene–ethanol–acetic acid (200:250:10:1, by vol.), air-dried and redeveloped in the system diethyl ether–light petroleum (b.p. 40–60°C) (3:47, by vol.). Plasma lipids were separated to the same extent, but in a shorter time, on normal thin layer plates (20cm × 20cm) coated with silica gel G, activated as above, and developed with the single solvent system diethyl ether–light petroleum (b.p. 40–60°C)–acetic acid (15:35:1, by vol.). In both instances, the plates were subsequently sprayed with an aqueous solution (0.1%, w/v) of Ultraphor (Badische Anilin und Soda Fabrik, Ludwigshufen-am-Rhein, Germany) and examined under u.v. light. Lipid fractions were normally eluted with diethyl ether–light petroleum (b.p. 40–60°C)–formic acid (50:50:1, by vol.) except for the phospholipid band, which was eluted with chloroform–methanol–water (200:100:1, by vol.). The extracted liver and plasma phospholipids, triglycerides and fatty acid fractions were transmethylated as outlined below.

Transmethylation. Samples were transferred to test tubes, evaporated to dryness under N_2 and 4ml of methylating mixture (methanol–benzene–conc. H_2SO_4, 20:10:1, by vol.) was added to each. The mixture was heated under reflux at 80°C for 90min and then cooled to room temperature, and 10% Na_2CO_3(5ml) and light petroleum (5ml) were added. After shaking, the lower phase was removed by aspiration and the light petroleum phase was washed twice with 10% Na_2CO_3 (5ml) and once with water (5ml). After the last wash, the light petroleum was dried with anhydrous Na_2SO_4 and transferred to a screw-capped vial.

Gas–liquid chromatography. The proportion of the individual long-chain fatty acid methyl esters was determined by using a Pye Model 104 gas chromatograph operated at 180°C and fitted with a flame ionization detector and a coiled glass column (120cm long, 4mm int. diam.). The column support was kieselguhr Chromosorb W (60–80 mesh) coated with 10% polyethylene glycol adipate. The areas of peaks on the chromatograms were calculated by triangulation and used to determine the concentration of plasma free fatty acids, triglyceride and phospholipid as described by West & Rowbotham (1967). The radioactivities of known portions of the fatty acids were also counted in toluene (15ml) containing 2,5-diphenyloxazole (4g/l) and the specific radioactivities of the substances were calculated.

Separation of cis- *and* trans-*fatty acids.* Silica gel G (22.5g) was thoroughly mixed with 50ml of water containing 2.25g of $AgNO_3$, and applied (0.25mm thick) to plates (20cm × 20cm) and activated for 30min at 105°C.

Samples were dissolved in the minimum amount of light petroleum (b.p. 40–60°C) and transferred to the prepared plates, which had marker lanes with methyl esters of stearic acid, oleic acid, elaidic acid, linoleic acid and linolenic acid. The plates were pre-run in ether to just above the origin and then developed twice in diethyl ether–light petroleum (b.p. 40–60°C) (1:19, v/v). The plates were air-dried, sprayed with 0.2% dichlorofluorescein in ethanol and examined under u.v. light. The fractions were transferred to sintered-glass funnels and eluted with 20ml of diethyl ether–light petroleum (b.p. 40–60°C) (1:1, v/v). The *cis*- and *trans*-monoene methyl ester fractions were examined by g.l.c., together with a known amount of methyl heptadecanoate added as internal standard, and the ratio of *cis*-Δ^9- to *trans*-Δ^9-octadecenoic acid was determined. The radioactivities of portions of the separated isomers were also counted and the specific radioactivities calculated.

Determination of double-bond position in fatty acids. Monoenoic acid (0.5mg) was shaken in a stoppered tube with 1ml of solution A [K_2CO_3 (51.2mg) in 100ml of 2-methylpropan-2-ol–water (3:2, v/v)] and 1ml of solution B [$KMnO_4$ (16mg) and $NaIO_4$ (854mg) in 100ml of water] for 2h at room temperature according to the modified method of Davidoff & Korn (1963). The reaction was stopped by the addition of solid sodium metabisulphite until a colourless solution was obtained. It was then acidified with 5M-HCl. After the addition of water, monocarboxylic acids were extracted with 5ml of ice-cold light petroleum (b.p. 40–60°C) and dicarboxylic acids with equal volumes of ether. The extracts were washed, dried with anhydrous Na_2SO_4 and, after methylation with diazomethane, separated and identified by g.l.c.

Analysis of liver triglycerides. The fatty acid compositions at the 1-, 2- and 3-positions of the liver triglycerides were determined by using pancreatic lipase as described by Lands, Pienngер, Slakey & Zschocke (1966). Triglyceride (2–5mg) was dissolved in diethyl ether–light petroleum–formic acid (50:50:1, by vol.), transferred to a small test tube and evaporated to dryness under N_2. Then 1.0M-NaCl (0.15ml), 1.0M-tris–HCl buffer, pH8.05 (0.10ml), and lipase [0.05ml from 25mg of ether-extracted steapsin (Nutritional Biochemicals Co., Cleveland, Ohio, U.S.A.) in 0.5ml of water] were added to the triglyceride, and the reaction mixture was shaken on a Vortex mixer, at room temperature, for approx. 3min. After the addition of 0.2ml of 5M-HCl the lipids were extracted with chloroform–methanol (2:1, v/v) and then with chloroform. The combined extracts were evaporated to dryness under N_2, taken up in a minimal amount of chloroform–methanol (2:1, v/v) and separated by t.l.c. with the solvent system diethyl ether–light petroleum (b.p. 40–60°C)–acetic acid (15:35:1, by vol.). Fatty acid, triglyceride and monoglyceride bands were eluted with diethyl ether–light petroleum–formic acid (50:50:1, by vol.) and methylated, and the methyl esters were separated by g.l.c.

Phospholipid analysis. Liver phospholipids were separated into classes by t.l.c. with the solvent system chloroform–methanol–acetic acid–water (170:30:20:7, by vol.). After location with iodine vapour, the origin,

lysophosphatidylcholine, sphingomyelin, phosphatidylcholine and phosphatidylethanolamine fractions were eluted with chloroform–methanol–water (1000:500:3, by vol.). The phosphatidylcholine and phosphatidylethanolamine were subjected to enzymic hydrolysis. The samples were evaporated to dryness under N_2, and 0.5ml of diethyl ether, 2.3mg of snake-venom enzyme (from *Crotalus adamanteus*; Koch–Light Laboratories Ltd., Colnbrook, Bucks., U.K.) and 1.5ml of sodium borate buffer, pH7.0, were added. The reaction mixture was shaken for 2h at 37°C, after which 5ml of chloroform–methanol (2:1, v/v) was added and the lipids were extracted. The extract was separated by t.l.c. with the above solvent system, and unchanged phospholipid, free fatty acid and lysophospholipid fractions were extracted, their radioactivities counted and finally methylated to obtain the methyl esters and fatty acid composition. The specificity of phospholipase A was checked against glycerophosphorylethanolamine 2-isolaurate 1-oleate.

Materials. Stearic acid, oleic acid and elaidic acid were obtained from Sigma (London) Chemical Co. Ltd., London S.W.6, U.K., and were at least 98% pure. The *cis*- and *trans*-isomers of vaccenic acid were isolated from crude vaccenic acid (Koch–Light Laboratories Ltd.) by preparative t.l.c. on chromatoplates of silica gel G impregnated with $AgNO_3$ with the solvent system described above.

[1-^{14}C]Oleic acid (57.2mCi/mmol), [1-^{14}C]stearic acid (37.1mCi/mmol), [9,10-$^3H_2(n)$]oleic acid (733mCi/mmol) and [9,10-$^3H_2(n)$]stearic acid (457mCi/mmol) were purchased from The Radiochemical Centre, Amersham, Bucks., U.K., and purified by t.l.c. on $AgNO_3$-impregnated silica gel. [1-^{14}C]Elaidic acid (57.2mCi/mmol) and *trans*-[U-^{14}C]vaccenic acid (6.2mCi/mmol) were prepared by isomerization of [1-^{14}C]oleic acid and *cis*-[U-^{14}C]-vaccenic acid respectively in 6M-HNO_3 and 2M-$NaNO_2$ in dimethylCellosolve for 1h at 65°C (Litchfield, Henlow, Isbell & Reiser, 1965). The isomers were separated by t.l.c. on $AgNO_3$-impregnated silica gel G as described above. *cis*-[U-^{14}C]Vaccenic acid was prepared from the lipid extract of a culture (100ml) of *Rhodopseudomonas palustris* inoculated with 1mCi of [^{14}C]acetate (Wood, Nichols & James, 1965). Glycerophosphorylethanolamine 2-isolaurate 1-oleate was kindly synthesized by Dr M. I. Gurr (Unilever Research Laboratory, Colworth House).

RESULTS AND DISCUSSION

The perfused chicken liver proved to be a suitable preparation with which to study the uptake and metabolism of pairs of fatty acids of *cis* and *trans* configuration, labelled with ^3H or ^{14}C. The choice of fatty acids for these comparative studies was partly dictated by the availability of labelled materials, since elaidic acid and *cis*- and *trans*-vaccenic acid were available labelled only with ^{14}C. Direct comparison of the metabolism of the isomers of vaccenic acid, for example, was not possible.

The concentrations of the *trans*-fatty acids and of *cis*-vaccenic acid in the perfusion fluid quickly declined to values too low for detection. Oleic acid and stearic acid concentrations fell initially, and

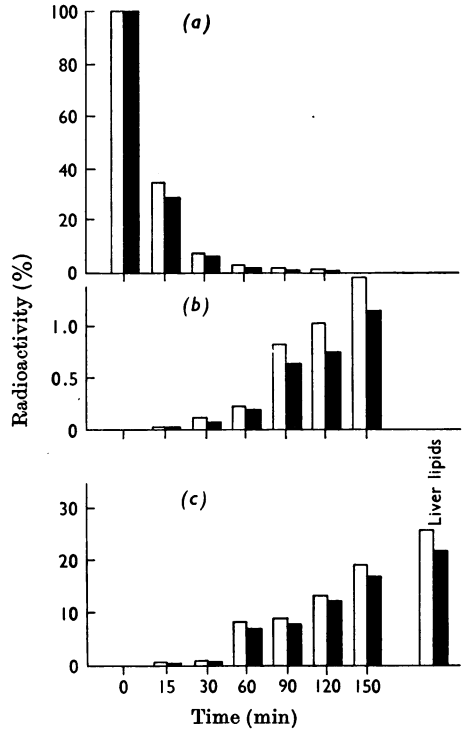

Fig. 1. Distribution of radioactivity in plasma lipids, at various time-intervals, and liver lipids, after the perfusion of a chicken liver with [1-^{14}C]elaidic acid and [9,10-$^3H_2(n)$] oleic acid. □, ^{14}C radioactivity; ■, ^3H radioactivity. (*a*) Plasma free fatty acids; (*b*) plasma phospholipids; (*c*) plasma triglycerides.

then remained roughly constant at about half of the initial value, reflecting continuous uptake and production from liver lipids and possibly blood lipids since the radioactive fatty acids were largely incorporated into liver lipids within 30min (Figs. 1–4).

The proportions of fatty acids taken up by the perfused liver in 30min were independent of the initial concentration, which agrees with results obtained by other workers (Richards, Ruderman & Herrera, 1968; Mayes & Felts, 1967; Hillyard, Cornelius & Chaikoff, 1959). In agreement with the results obtained with other tissues (G. Billek & F. Strauch, unpublished work; Coots, 1964) showing the absence of mechanisms adversely influencing the transport of *trans*-fatty acids, no restrictions on the uptake of *trans*-fatty acids were observed in the perfused chicken liver. Other workers have postulated a membrane barrier in brain tissue (Kaufmann & Bandyopadhyay, 1965) and placental tissue (Johnston *et al.* 1957*b*) that prevents the

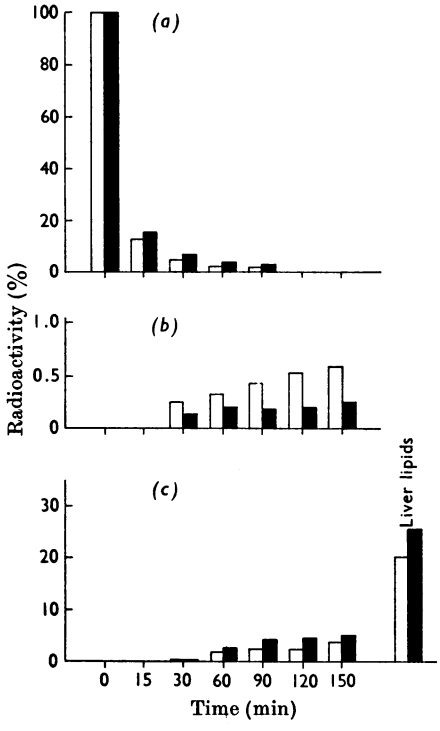

Fig. 2. Distribution of radioactivity in plasma lipids, at various time-intervals, and liver lipids, after the perfusion of a chicken liver with *cis*-[1-^{14}C]vaccenic acid and [9,10-$^{3}H_2(n)$]oleic acid. □, ^{14}C radioactivity; ■, ^{3}H radioactivity. (*a*) Plasma free fatty acids; (*b*) plasma phospholipids; (*c*) plasma triglycerides.

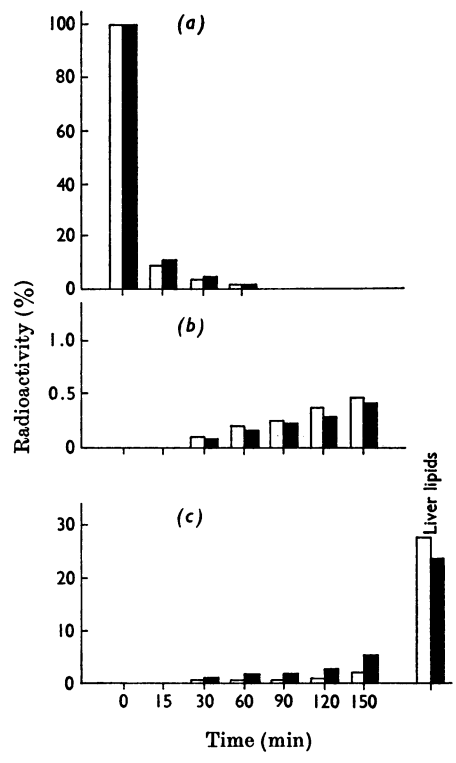

Fig. 3. Distribution of radioactivity in plasma lipids, at various time-intervals, and liver lipids, after the perfusion of a chicken liver with *trans*-[1-^{14}C]vaccenic acid and [9,10-$^{3}H_2(n)$]oleic acid. □, ^{14}C radioactivity; ■, ^{3}H radioactivity. (*a*) Plasma free fatty acids; (*b*) plasma phospholipids; (*c*) plasma triglycerides.

absorption of *trans*-fatty acids. The incorporation of the labelled fatty acids into liver triglycerides and phospholipids and the release of labelled lipid into the perfusate are shown in Table 1. It was not possible to obtain samples of liver tissue before or during the perfusion, since even slight tissue damage resulted in extensive loss of perfusion fluid ('haemorrhage') from the tissue. Since the rate of release of labelled lipids into the perfusate varied widely in different experiments the liver and perfusate lipids were considered as a single pool when calculating the incorporation of fatty acids into the various lipid classes. The amounts of triglyceride and phospholipid in the perfusate were substantially greater at the end of the perfusion than at the start, whereas the reverse was true for the free fatty acid concentrations, demonstrating the uptake of free fatty acids and the concomitant synthesis and release of phospholipid and triglycerides by the perfused chicken liver.

The recovery of radioactivity from the total lipid pools varied from 25 to 74% in the different experiments, but in any particular experiment the recoveries of the individual acids of any particular pair were comparable. These results show that there were no significant differences in the incorporation of oleic acid, elaidic acid and *trans*-vaccenic acid into the various lipids, but stearic acid and *cis*-vaccenic acid were less readily utilized for overall lipid synthesis. Stearic acid was included as a typical saturated fatty acid, but as a result of the well-established desaturase activity of chicken liver (Allen, Johnson, Fogerty, Pearson & Shenstone, 1967) a substantial proportion of the added material appeared in the triglyceride and phospholipid fractions as oleic acid (Table 1).

Examination of the extent of incorporation of the positional isomers of octadecenoic acids into triglycerides and phospholipids (Table 2) showed that, although the Δ^{9}-octadecenoic acids were incorporated to a greater extent than the Δ^{11}-isomers into triglycerides, the reverse was true when the phospholipids were examined. The

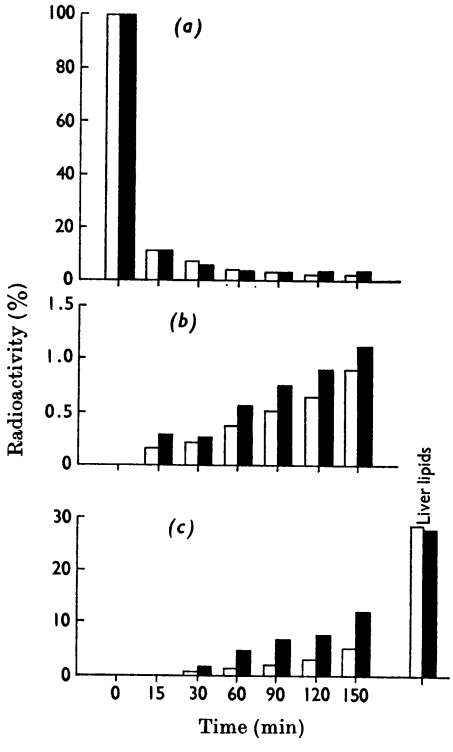

Fig. 4. Distribution of radioactivity in plasma lipids, at various time-intervals, and liver lipids, after the perfusion of a chicken liver with [1-^{14}C]stearic acid and [9,10-$^{3}H_2(n)$]oleic acid. □, ^{14}C radioactivity; ■, ^{3}H radioactivity. (*a*) Plasma free fatty acids; (*b*) plasma phospholipids; (*c*) plasma triglycerides.

distribution of geometrical isomers between the phospholipid and triglyceride fractions revealed a relationship between the *cis*- and *trans*-isomers of Δ^9-octadecenoic acid and Δ^{11}-octadecenoic acid (Table 3). Since the relative rates of incorporation of the geometrical isomers are so similar for these acids, the double bond in the Δ^9- or Δ^{11}-position has little effect on the extent of fatty acid incorporation into triglycerides and phospholipids. Any metabolic difference that was observed between the isomers was due entirely to the spatial configuration at the Δ^9- or Δ^{11}-position.

Several workers (Nestel & Steinberg, 1963; Richards *et al.* 1968) have shown that raising the concentration of free fatty acids in the perfusate (from 0.2 to 4.0m-equiv./l) increased the proportion of fatty acids incorporated into triglycerides relative to phospholipids. In the present experiments, in which the concentrations of oleate and stearate were always in the range 0.35–0.45m-equiv./l in the initial perfusate, the distribution of labelled fatty acids between phospholipids and triglycerides was markedly correlated with the overall recovery of radioactivity, as shown by the values for stearic acid and oleic acid (Table 4). This finding is consistent with either preferential oxidation of fatty acids (before or after incorporation into triglyceride) since the added radioactivity not recovered in lipids must have reflected the extent of fatty acid oxidation. No other component in the system showed appreciable radioactivity.

Detailed comparison of the relative incorporation of *cis*- and *trans*-fatty acids into complex lipids was confined to three experiments in which radioactivity recoveries were similar. The mean ratios of the distribution of radioactivity between phospholipids and triglycerides (liver and perfusate) expressed as elaidic acid/oleic acid and *trans*-vaccenic acid/*cis*-vaccenic acid were in reasonably close agreement (0.75 and 0.69 respectively), and indicated once again that the spatial configuration at the double bond was more important than the position of the double bond. The preference of elaidic acid and stearic acid for phospholipids was also noted by Coots (1964) after feeding rats with elaidic acid. Conversely, Richards *et al.* (1968) found that triglycerides contained a higher percentage of oleic acid than did the phospholipids when rat livers were perfused with oleic acid. This difference in the distribution of fatty acids between phospholipids and triglycerides has been noted by other workers (Dustin, Fredrickson, Landat & Ono, 1961; Stein & Shapiro, 1959; Nestel & Steinberg, 1963) and was probably due to enzyme specificity in the acylation of glycerol 3-phosphate (Hill, Husbands & Lands, 1968), although transacylation reactions cannot be ruled out (Lands, Blank, Nutter & Privett, 1966).

Analysis of the fatty acids of the labelled triglyceride and phospholipid fractions isolated from the liver at the end of the perfusion showed that 90–95% of the radioactivity was present in the fatty acid added to the perfusion system, i.e. there was little interconversion of fatty acids, except with stearic acid, which in each case was partly desaturated to oleic acid (Table 1). There was no evidence of the accumulation of *trans*-5-tetradecenoic acid or *trans*-5-dodecenoic acid in liver tissue, as found by Willebrands & Van der Veen (1966) when rat hearts were perfused with *trans*-vaccenic acid and elaidic acid, suggesting that the β-oxidation of *trans*-fatty acids was not inhibited in the present experiments. Although a double bond of *cis* configuration was introduced into the Δ^9-position of stearic acid, no double bond was introduced into *cis*-vaccenic acid or *trans*-vaccenic acid even though the Δ^9-position was vacant, suggesting that the desaturase enzyme in chicken liver is specific for saturated acids. Previous workers (Holloway, Peluffo & Wakil, 1963) have shown that oleoyl-CoA is desaturated

Table 1. *Incorporation of oleic acid, elaidic acid,* trans-*vaccenic acid,* cis-*vaccenic acid and stearic acid into the lipids of the perfused liver and perfusate*

Livers were perfused for 170min; radioactive substrates were added 30min after the start of the perfusion. The total volume of the perfusate was 300ml. Abbreviations: Ol, oleic acid; El, elaidic acid; *t*V, *trans*-vaccenic acid; *c*V, *cis*-vaccenic acid; St, stearic acid.

Pairs of fatty acids added to perfusate in separate experiments ...	El (^{14}C)	Ol (^{3}H)	El (^{14}C)	Ol (^{3}H)	El (^{14}C)	Ol (^{3}H)	*c*V (^{14}C)	Ol (^{3}H)	*t*V (^{14}C)	Ol (^{3}H)	St (^{14}C)	Ol (^{3}H)	*t*V (^{14}C)	St (^{3}H)	*c*V (^{14}C)	St (^{3}H)
Liver and perfusate lipid pools (μmol)																
Liver (final)																
Triglyceride	153		111		185		174		166		289		252		190	
Phospholipid	483		410		490		451		585		495		248		400	
Free fatty acid	25		29		17		24		25		14		10		10	
Perfusate																
Triglyceride, initial	8		43		25		38		49		24		39		49	
final	19		66		128		60		53		45		117		95	
Phospholipid, initial	29		48		42		43		41		21		31		36	
final	37		60		56		74		64		30		46		49	
Free fatty acid, initial	30		40		81		46		44		37		61		37	
final	19		7		13		11		18		9		40		18	
Recovery of radioactivity (%) in liver and perfusate lipids																
Total lipids	71.8	74.1	44.7	38.3	47.7	48.0	25.4	30.6	30.0	29.6	40.7	45.7	49.3	46.2	54.9	60.6
Liver triglyceride	50.1	53.5	18.0	18.2	19.0	20.8	14.0	20.9	12.5	17.9	8.2	21.8	19.3	11.9	23.8	19.6
Liver phospholipid	13.8	11.1	5.9	2.1	8.6	6.3	4.7	3.3	13.3	5.2	9.5	4.2	7.3	6.1	6.0	7.8
Perfusate triglyceride	4.1	0.7	14.9	12.5	17.8	16.8	3.7	4.9	1.7	4.9	4.8	11.7	15.5	13.9	17.8	16.6
Perfusate phospholipid	0.8	0.6	1.4	1.1	0.5	0.6	0.6	0.2	0.5	0.4	0.8	1.1	1.9	1.0	1.5	1.1
Radioactivity in triglycerides / Radioactivity in phospholipids ratio	3.7	4.6	4.5	9.7	4.0	5.3	3.3	7.4	1.0	4.1	1.3	6.3	3.8	3.6	5.5	4.1
Desaturation of stearic acid (%) in																
Total triglycerides	—	—	—	—	—	—	—	—	—	—	38.8	—	—	11.5	—	15.2
Total phospholipids	—	—	—	—	—	—	—	—	—	—	8.5	—	—	7.9	—	24.9

Table 2. *Extent of incorporation of* cis- *and* trans-*octadecenoic acids into the phospholipid and triglyceride fractions of liver and perfusate*

	Incorporation (relative to that of oleic acid)			
Fatty acid	Total phospholipid (liver+ perfusate)	Total triglyceride (liver+ perfusate)	Liver phospholipid	Liver triglyceride
Elaidic acid	1.62	1.02	1.80	0.95
Oleic acid	(1.00)	(1.00)	(1.00)	(1.00)
trans-Vaccenic acid	2.48	0.67	2.64	0.68
cis-Vaccenic acid	1.56	0.56	1.58	0.58
Stearic acid	1.94	0.39	2.26	0.38

Table 3. *Relative rates of incorporation of* cis- *and* trans-*octadecenoic acids into the phospholipid and triglyceride fractions of liver and perfusate*

	Relative rate of incorporation (*trans*-fatty acid/*cis*-fatty acid ratio)			
Fatty acid ratio	Total phospholipid	Total triglyceride	Liver phospholipid	Liver triglyceride
$\frac{\text{Elaidic acid}}{\text{Oleic acid}}$ ratio	1.62	1.02	1.80	0.95
$\frac{\textit{trans}\text{-Vaccenic acid}}{\textit{cis}\text{-Vaccenic acid}}$ ratio	1.59	1.18	1.67	1.18

Table 4. *Relationship between the amount of added radioactive stearic acid and oleic acid accounted for as lipid (in liver and perfusate) at the end of the perfusion, and the distribution of the fatty acids between the total triglyceride and total phospholipid pools*

Fatty acid	Expt. no.	Recovery of fatty acid (%)	Incorporation into triglyceride relative to that into phospholipid
Stearic acid	1	45	4.1
	2	33	3.6
	3	23	1.3
Oleic acid	4	34	9.7
	5	29	7.4
	6	28	4.1

to octadeca-6,9-dienoyl-CoA by rat liver microsomes, but no comparable conversion was observed in the present experiments.

The distribution of oleic acid and stearic acid on the glycerol molecule of liver triglycerides was determined by comparing the fatty acid composition of the triglyceride with that of the monoglyceride isolated after hydrolysis with pancreatic lipase (Table 5). As expected, the unsaturated fatty acids were concentrated at the 2-position. This method could not be used for the elaidic acid and vaccenic acid isomers because the acids were present in amounts too small for quantitative analysis. Further information was provided, however, by comparing the distribution of radioactivity in the triglyceride with that in the monoglyceride produced by hydrolysis with pancreatic lipase (Table 6). If we bear in mind that oleic acid largely occupies the 2-position of glycerol, then the distribution of ^{14}C and ^{3}H showed that *cis*-vaccenic acid behaved like oleic acid, and that elaidic acid and stearic acid favoured the 1- and 3-positions. The results with *trans*-vaccenic acid suggested that a greater proportion of this acid occupied the 2-position than was the case with

Table 5. *Positional distribution of palmitic acid, stearic acid, oleic acid and linoleic acid in chicken liver triglycerides*

Fatty acid	Fatty acid composition (mol/100 mol)		Positional distribution of fatty acid (%)	
	Triglyceride	Monoglyceride	Position 2	Positions 1 and 3
Palmitic acid	31.8	11.7	12.4	87.6
Stearic acid	16.4	7.2	14.6	85.4
Oleic acid	35.6	53.8	50.3	49.7
Linoleic acid	8.4	18.2	72.3	27.7

Table 6. *Positional distribution of* cis- *and* trans-*monoenoic acids in chicken liver triglycerides determined by the* $^{14}C/^{3}H$ *ratios in the intact triglycerides, and in the* 2-*monoglycerides produced by the action of pancreatic lipase*

Fatty acids perfused through liver	Positional distribution relative to that of oleic acid (=100)	
	Triglyceride	2-Monoglyceride
cis-[^{14}C]Vaccenic acid + [^{3}H]oleic acid	14.3	12.6
trans-[^{14}C]Vaccenic acid + [^{3}H]oleic acid	12.5	9.5
[^{14}C]Elaidic acid + [^{3}H]oleic acid	23.1	5.4
[^{14}C]Stearic acid + [^{3}H]oleic acid	22.4	13.1

Table 7. *Distribution of fatty acids in phospholipids isolated from chicken liver*

The results are expressed as the percentage distribution of radioactivity on silicic acid plates developed in chloroform–methanol–acetic acid–water (170:30:20:7, by vol.).

Fatty acid perfused through chicken liver ... / Phospholipid	Distribution (%)				
	Oleic acid	Elaidic acid	*cis*-Vaccenic acid	*trans*-Vaccenic acid	Stearic acid
Phosphatidylcholine	48	26	45	29	25
Phosphatidylethanolamine	33	49	38	55	54
Sphingomyelin	19	25	17	16	21

elaidic acid and stearic acid. The similar distribution of *trans*-fatty acids and stearic acid was also noted in triglycerides isolated from various tissues (Duncan & Garton, 1967) and in the milk fat of ruminants (Woodrow & de Man, 1968).

Differences were found in the metabolism of *cis*- and *trans*-fatty acids when the distribution of radioactivity was examined in phosphatidylcholine, phosphatidylethanolamine and sphingomyelin (Table 7). The *trans*-fatty acids and stearic acid were located in phosphatidylethanolamine rather than phosphatidylcholine, in which oleic acid and *cis*-vaccenic acid were concentrated. Examination of the positional distribution of fatty acids in phosphatidylcholine and phosphatidylethanolamine by using the phospholipase A technique showed that stearic acid was located at the 1-position and oleic acid at the 2-position of the phospholipids (Table 8). The distributions of *cis*-vaccenic acid, *trans*-vaccenic acid and elaidic acid in phosphatidylethanolamine and phosphatidylcholine were also determined by the $^{14}C/^{3}H$ ratio in the intact phospholipids and in the corresponding lyso derivatives prepared by the action of phospholipase A (Table 9). The results showed that *cis*-vaccenic acid and oleic acid largely occupied the 2-position, and *trans*-fatty acids and stearic acid the 1-position. Since the distribution of the fatty acids is similar to that obtained with triglycerides it is likely that the specificity of the enzymes is operative at the esterification of glycerol 3-phosphate, although the possible existence of a transacylase should not be ignored.

In the present studies the effect of the position of

Table 8. *Positional distribution of fatty acids in chicken liver phospholipids*

Fatty acid	Fatty acid composition (mol/100mol) Phosphatidylethanolamine	Lysophosphatidylethanolamine	Calc. percentage of fatty acid at position 1	Fatty acid composition (mol/100mol) Phosphatidylcholine	Lysophosphatidylcholine	Calc. percentage of fatty acid at position 1
$C_{16:0}$ acid	16.9	32.9	97.2	27.3	37.5	68.7
$C_{18:0}$ acid	30.4	55.0	90.6	21.7	38.3	89.0
$C_{18:1}$ acid	5.8	4.9	44.2	17.8	8.5	22.8
$C_{18:2}$ acid	12.6	2.9	11.6	16.6	8.6	24.6
$C_{20:4}$ acid	13.0	0.6	2.4	4.1	0.0	0.0
$C_{22:6}$ acid	15.0	0.5	1.5	4.5	0.0	0.0

Table 9. *Positional distribution of* cis- *and* trans-*monoenoic acids in phosphatidylethanolamine and phosphatidylcholine determined by the* $^{14}C/^{3}H$ *ratios in the intact phospholipids and the fatty acid released from the secondary position in glycerol by the action of phospholipase C*

Fatty acids perfused through liver	$^{14}C/^{3}H$ ratio relative to that of oleic acid (=100) Phosphatidylethanolamine	Fatty acid	Phosphatidylcholine	Fatty acid
cis-[^{14}C]Vaccenic acid+[^{3}H]oleic acid	24	20	28	26
trans-[^{14}C]Vaccenic acid+[^{3}H]oleic acid	118	24	38	19
[^{14}C]Elaidic acid+[^{3}H]oleic acid	147	21	54	24
[^{14}C]Stearic acid+[^{3}H]oleic acid	153	11	44	11

the double bond and the spatial configuration at the double bond has been examined in perfused chicken livers with isomers of octadecenoic acids. The Δ^9 isomers were more readily incorporated into liver lipids than the corresponding Δ^{11} isomers, but the differences between the positional isomers were small. The main metabolic differences were seen with the *cis* and *trans* isomers. The *trans*-fatty acids with the double bond at the Δ^9- or Δ^{11}-position had similar metabolic properties to stearic acid, so the specificities of the enzymes in chicken liver are governed by the spatial configuration of the fatty acids rather than the position or absence of double bonds. The spatial configuration of *trans*-fatty acids is in fact a straight cylindrical configuration like that of stearic acid, whereas unsaturated fatty acids such as *cis*-vaccenic acid and oleic acid are characterized by an L-shaped configuration (Vandenheuvel, 1963).

Partially hydrogenated fats containing fatty acids of *trans* configuration are frequently included in poultry diets. Although slight differences in their metabolism relative to the corresponding *cis*-fatty acids have been demonstrated, no evidence was obtained to suggest that *trans*-fatty acids are in any way detrimental to hepatic metabolism.

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