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The effects of administering *N*-(2-benzoyloxyethyl) norfenfluramine to rats on the hepatic synthesis of glycerolipids

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N-(2-Benzoyloxyethyl) norfenfluramine (S-780) was administered to rats by stomach tube at a dose of 50 mg kg^{-1} of body weight. Livers of the rats which were given an acute dose of the drug synthesized more triacylglycerol, phosphatidylcholine and phosphatidylethanolamine from [1,3-^{3}H]glycerol and [^{14}C]palmitate than did those of control rats. The measurements were made by injecting a mixture of the radioactive precursors into the portal veins of anaesthetized rats and freeze clamping a portion of the liver 1 min later. Different results were obtained after treating rats daily with S-780 for 5 days. Liver slices from these rats synthesized less triacylglycerol and relatively more phosphatidylinositol plus phosphatidylserine from [^{3}H]glycerol than did those of control rats. S-780 treatment depressed the hepatic synthesis of phosphatidylcholine and phosphatidylethanolamine as measured *in vivo* after intraportal injection of [^{14}C]palmitate and [^{3}H]glycerol. Chronic treatment with S-780 also depressed food intake and lowered liver weight and body weight of rats fed the 41B diet. The results are discussed in relation to the effects of S-780 on the synthesis of glycerolipids.

Fenfluramine is an antiobesity agent and its derivative *N*-(2-benzoyloxyethyl)norfenfluramine (S-780) exhibits hypolipidaemic properties (Duhault & Boulanger, 1965; Duhault & Malen, 1970; Kaye, Tomlin & Galton, 1975; Riveline, 1975). These compounds are able to inhibit the synthesis of glycerolipids *in vitro* by a variety of tissues including preparations of rat and human liver (Marsh & Bizzi, 1972; Bowley, Manning & Brindley, 1973), rat intestine (Dannenburg, Kardian & Norrell, 1973) and human adipose tissue (Wilson & Galton, 1971). The decrease in the incorporation of glycerol phosphate into lipids observed in these experiments appears to arise through a fairly specific inhibition of phosphatidate phosphohydrolase (EC 3.1.3.4) (Brindley & Bowley, 1975a, b). This enzyme is also inhibited by a wide variety of amphiphilic amines including chlorpromazine, desmethylimipramine, local anaesthetics and mepyramine (Brindley & Bowley, 1975a). The inhibition of phosphatidate phosphohydrolase leads to a redirection of glycerolipid synthesis away from triacylglycerol, phosphatidylcholine and phosphatidylethanolamine and towards the synthesis of acidic phospholipids such as phosphatidylinositol, cardiolipin and CDP-diglyceride (Eichberg & Hauser, 1974; Brindley & Bowley, 1975a, b; Allan & Michell, 1975; Abdel-Latif, Lakshmanan & Smith, 1976). It is thought that the interaction of the cationic drugs with acidic phospholipids, or their effects on the metabolism of these lipids, could explain many of their therapeutic effects or side effects (Brindley, Allan & Michell, 1975; Michell, Allan & others, 1976).

Fenfluramine and related compounds exhibit two main therapeutic effects. One is an anorectic action in the hypothalmus and it is possible that an altered metabolism of acidic phospholipids could be involved in this phenomenon (Brindley & Bowley, 1975c). The present work is concerned with the second effect which is an interference with the metabolism of carbohydrates and fats. In particular, experiments were performed to investigate whether S-780 interferes with the hepatic synthesis of glycerolipids when administered *in vivo*.

MATERIALS AND METHODS

Animals

Male Wistar rats were obtained from the Nottingham University Joint Animal Breeding Unit, Sutton Bonington, Leics, U.K.

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Materials

The source of most of the materials has already been described (Sánchez, Nicholls & Brindley, 1973; Brindley & Bowley, 1975a). Gum tragacanth (Grade A) was obtained from Bush, Boake & Allan, Ash Grove, Hackney, London, U.K.; the 41B diet and the small animal vitamin-salt mixture were from Pilsbury's Ltd, Birmingham, U.K. and the casein (light, white, soluble) from Fisons Scientific Apparatus, Loughborough, Leics., U.K. Cellulose was a gift from Bayer Chemicals Ltd, Richmond, Surrey, U.K. Corn oil, corn starch and sucrose were ordinary domestic products.

Diets

Unless otherwise stated rats were fed 41B pelleted diet which according to the manufacturer contained by weight 45% of digestible carbohydrate, 14% of digestible crude protein, 6% crude fibre, 2% of digestible crude oil plus the required vitamins and minerals. Synthetic diets were fed in a powder form. The high starch diet contained (by weight) 60% of corn starch, 22% of casein, 10% of cellulose, 3% of vitamin-salt mixture and 5% of corn oil. The composition of the high sucrose diet is that described by Duhault, Boulanger & others (1976). Animals on all diets were allowed free access to food. In those experiments where the food intake was measured rats were transferred separately to grid-bottomed cages six days before administering S-780.

Administration of S-780

Rats were fed between 10 am–12 noon by stomach tube with S-780 50 mg kg^{-1} body weight. The S-780 was suspended immediately before use in 0·5% (w/v) gum tragacanth at 10 mg of S-780 ml^{-1}. This treatment was continued for the number of days indicated in the different experiments. In all experiments the control rats were intubated with the appropriate volume of gum tragacanth suspension. All measurements of the rates of glycerolipid synthesis were made 3 h after the final intubation with S-780 or with gum tragacanth. During this 3 h period the rats were deprived of food but not water.

Measurement of glycerolipid synthesis in rat liver homogenates and slices

Lipid synthesis by the homogenates and slices were determined using *sn*-[1,3-^{3}H]glycerol 3-phosphate and [1,3-^{3}H]glycerol respectively as described by Brindley & Bowley (1975a).

Measurement of glycerolipid synthesis in rat liver in vivo

The method is based upon that originally described by Åkesson, Elovson & Arvidson (1970). Rats were lightly anaesthetized with diethyl ether and their abdomens opened. An injection (0·25 ml) was made into the portal vein and consisted of 212 μM-potassium [^{14}C]palmitate (58 μCi μmol^{-1}) and 188 μM [1,3-^{3}H]glycerol (1·8 Ci $mmol^{-1}$) in 0·9% (w/v) NaCl containing 1·6 mg ml^{-1} of fatty acid-poor bovine serum albumin. These concentrations of precursors are within the normal physiological range (Robinson & Newsholme, 1969; Duhault & Malen, 1970). The injection was delivered into the vein in approximately 1 s. At a predetermined time a portion of the right side of the median lobe (approximately 1 g wet weight) was cut off and clamped between two aluminium blocks which had been cooled in liquid nitrogen. The liver sample was frozen solid within 3 s of removal from the rat. This sample was weighed in a tube cooled in an ice-salt bath and 3·75 ml of ice-cold chloroform–methanol (1:2; v/v) was added g^{-1} wet weight of liver. Lipids were then extracted by the method of Hajra, Seguin & Agranoff (1968). The bottom phase containing lipid was washed twice with synthetic top phase (Brindley & Bowley, 1975a).

Samples of the water-soluble and of the lipid-soluble extracts from the livers were taken for the determination of ^{14}C and ^{3}H by liquid scintillation counting. In addition, 100 μl samples of the lipid extract were analysed by t.l.c. on plates of Kieselgel 60/Kieselgur F254 according to Brindley & Bowley (1975a). Areas of the plates corresponding to various lipid classes were scraped into counting vials and 1·5 ml of water was added, followed by 15 ml of Triton X-100-xylene (1:2; v/v) containing 5·5 g of 2,5-diphenyloxazole $litre^{-1}$ and 0·1 g of 1,4-bis- (5-phenyloxazol-2-yl) benzene $litre^{-1}$. The vials were shaken vigorously and left for about 16 h in the dark. They were then shaken again and the radioactivity was determined by liquid scintillation counting in a Packard 3375 Tri-Carb Spectrometer (Manning & Brindley, 1972). The scintillator mixture eluted all of the radioactive lipid from the silica gel, which formed a thin layer at the bottom of the vials. This made it possible to use an external standard ratio as a means of quench correction. However, this technique may be inappropriate if a scintillation counter is used which introduces the external standard pellet underneath the layer of silica gel. The results for lipid analysis were accepted only if the recovery of both isotopes from the t.l.c. plates was within the range

90–110 %. Total lipid samples were also degraded by vigorous alkaline hydrolysis (Manning & Brindley, 1972) and the results indicated that neither radioactive precursor had undergone extensive modification before being incorporated into lipid.

The mean uptake of [^{14}C] palmitate into the liver reached a maximum 30 s after injection and this represented 44–60 % of the administered [^{14}C] palmitate. During the period from 30 s to 5 min the [^{14}C] palmitate present in the liver was incorporated into glycerolipids (Fig. 1). After 5 min an average of 43 % of the injected [^{14}C] palmitate was present in hepatic glycerolipids. Only 2–3 % of the hepatic ^{14}C was isolated in the non-lipid fraction. The activity of ^{14}C in the diacylglycerol pool was in a virtually steady state for 3 min, whereas that of phosphatidate was declining at 30 s. During the 5 min period triacylglycerol, phosphatidylcholine, and phosphatidyl-

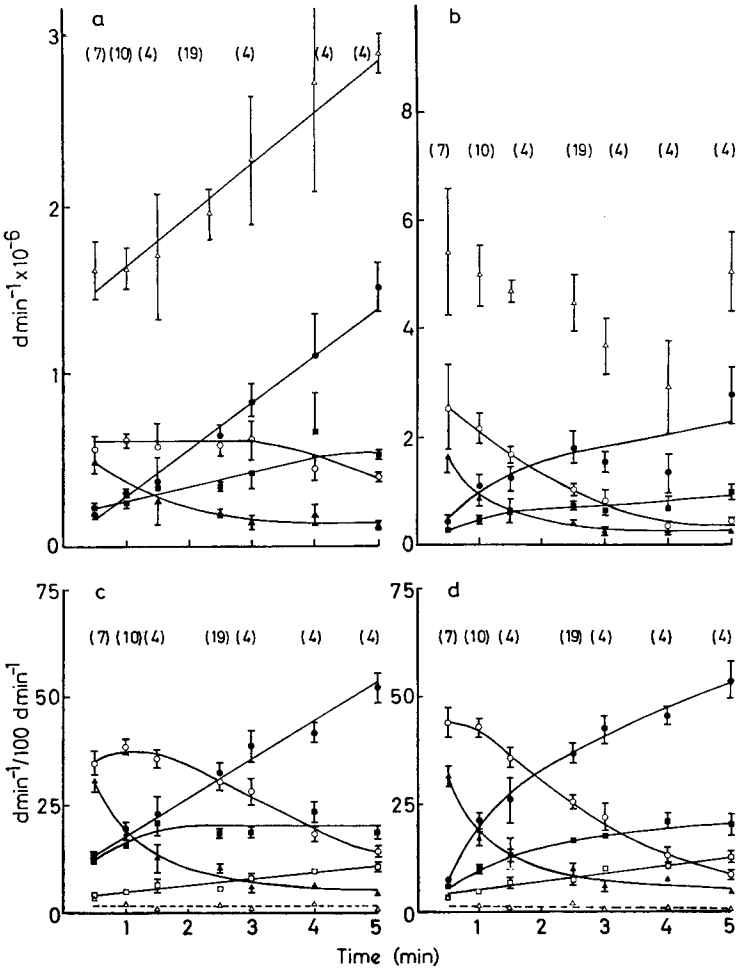

FIG. 1. Time courses of incorporation of [^{14}C]palmitate and of [^{3}H]glycerol into hepatic glycerolipids in rat. Rats (controls) were intubated for 5 days with 5 ml of 0·5% (w/v) gum tragacanth kg^{-1} body wt. A solution of [^{14}C]palmitate and [^{3}H]glycerol was injected into the portal veins of these rats (Materials and Methods section). The incorporation of [^{14}C]palmitate (Fig. 1a, c) and [^{3}H]glycerol (Fig. 1b, d) into total lipids (△), phosphatidylserine plus phosphatidylinositol (△ – – – △), phosphatidylcholine (■), phosphatidylethanolamine (□), phosphatidate (▲), diacylglycerol (○) and triacylglycerol (●) are shown as mean incorporations ±1 s.e. For clarity, the absolute incorporations into phosphatidylethanolamine and into phosphatidylserine plus phosphatidylinositol have been omitted from Fig. 1a and c but are included in Fig. 1b and d as relative incorporations. The number of rats used at each time period is indicated in parentheses. y axes a—[^{14}C]palmitate incorporated into glycerolipids per liver, b—[^{3}H]glycerol incorporated into glycerolipids per liver, c—relative incorporation of [^{14}C]palmitate into glycerolipids, d—relative incorporation of [^{3}H]glycerol into glycerolipids.

ethanolamine were synthesized at about constant rates from [^{14}C] phosphatidate and [^{14}C] diacylglycerol (Fig. 1a, c).

The mean recovery of ^{3}H in the liver was maximum at 30 s and represented 20–44% of the injected dose. Of the total [^{3}H] glycerol administered about 2–3% was recovered in hepatic lipids and this did not increase significantly from 30 s to 5 min after injection. Because of this the ^{3}H-activity in phosphatidate and diacylglycerol declined quicker than the ^{14}C-activity. The rates of synthesis of triacylglycerol, phosphatidylcholine and phosphatidylethanolamine remain constant for about 90 s (Fig. 1b and d). The results in Fig. 1d resemble those of Åkesson & others (1970) who used about 10 times the concentration of glycerol in their injections as that used in the present work. The time period chosen for studying the effects of S-780 on lipid metabolism was 1 min after injection when triacylglycerol, phosphatidylcholine and phosphatidylethanolamine were synthesized at approximately constant rates.

RESULTS

Effects of S-780 on the body weight, liver weight and food intake of rats fed the 41B diet

The S-780 treated rats ate less food and lost more weight over the 5 day period than did the control rats and the greatest differences occurred within the first 24 h of treatment (Table 1). Over the 5 day period food intake was depressed by approx. 44%. The liver weights of the S-780-treated rats were significantly lower than those of the control rats (Table 1).

Table 1. *Body weights and liver weights of control and S-780-treated rats.* Rats were treated for 5 days with S-780 or with gum tragacanth (control rats) as described in the Materials and Methods section. Values are expressed as means with 1 s.d., and the number of rats is shown in parentheses. The significance of the difference between control and S-780-treated rats is indicated as follows: $**P < 0.01$; † $P < 0.005$; †† $P < 0.001$

	(h) Time*	Control	S-780-treated
Body weight (g)	0	214 ± 12 (59)	213 ± 9 (30)
	96	217 ± 10 (59)††	200 ± 12 (30)
Body weight change (g)	0–24	1 ± 5 (59)††	−11 ± 7 (30)
	24–48	1 ± 4 (59)	2 ± 5 (30)
	48–72	1 ± 4 (59)†	−2 ± 5 (30)
	72–96	0 ± 6 (59)	−1 ± 5 (30)
	0–96	4 ± 7 (59)††	−13 ± 7 (30)
Food consumption (g)	0–24	21 ± 6 (47)††	6 ± 3 (30)
	24–48	22 ± 4 (47)††	12 ± 5 (30)
	48–72	19 ± 4 (47)††	14 ± 4 (30)
	72–96	22 ± 5 (47)††	15 ± 6 (30)
	0–96	84 ± 13 (47)††	47 ± 9 (30)
Liver wt (g)	96	8·13 ± 0·9 (68)††	7·18 ± 0·8 (29)
$\frac{\text{Liver wt}}{\text{Body wt}} \times 100$	96	3·77 ± 0·3 (61)**	3·58 ± 0·3 (29)

* Time after treatment began.

Effect of acute administration of S-780 on the synthesis of glycerolipids in vivo

Rats were intubated with a single dose of 50 mg of S-780 kg^{-1} of body weight and the rate of hepatic lipid synthesis was measured 3 h later. The S-780 treated rats took up more of the [^{14}C] palmitate into the liver and synthesized more triacylglycerol from this precursor than did the control rats (Table 2). Conversely, the accumulation of [^{14}C] palmitate in diacylglycerol, in phosphatidate and in phosphatidylinositol plus phosphatidylserine was lower in the S-780-treated rats. These differences were also reflected in the relative distribution (d. min^{-1}/100d. min^{-1}) of [^{14}C] palmitate among the various lipid classes. Expression of the results in this form compensates for some of the variations in hepatic uptake and in the rates of lipid synthesis in different rats.

The incorporation of [^{3}H]glycerol into phosphatidylcholine, phosphatidylethanolamine, triacylglycerol and total lipid was greater in the S-780-treated rats than in the controls (Table 2). In percentage terms the synthesis of phosphatidylinositol plus phosphatidylserine, phosphatidate, and diacylglycerol was less in the S-780-treated rats whereas the relative synthesis of triacylglycerol was greater.

Similar results to those in Table 2 were obtained when rats were fed for 4 weeks on a diet containing 60 % (w/w) sucrose before the experiment. This diet was used to enhance the synthesis of triacylglycerol in the liver (Lamb & Fallon, 1974).

The effect of a five day treatment with S-780 on the hepatic synthesis of glycerolipids

There were no significant differences between the rates at which liver homogenates of S-780-treated rats (7 animals) and control rats (7 animals) synthesized phosphatidate and acyl-glycerols. The ability of liver slices to synthesize glycerolipids was also tested (Table 3). The percentage of triacylglycerol synthesized by the S-780 treated rats was significantly less than that produced by the control rats after 7, 10 and 13 min of incubation. Conversely, the percentage of the lipid glycerol which was isolated in the fraction containing phosphatidylinositol plus phosphatidylserine was significantly higher in the S-780-treated rats.

The incorporation of [^{3}H]glycerol (d. min^{-1}) into total lipids and into diacylglycerol was significantly greater in the rats treated with S-780 (Table 4). The S-780-treated rats synthesized relatively less phosphatidylcholine and relatively more diacylglycerol from both precursors than did the control rats (Table 4). The relative rate of incorporation of

Table 2. *The effect of acute treatment with S-780 on the synthesis of glycerolipids in rat liver* in vivo. 12 rats were treated with a single dose of S-780 and 11 control rats were treated with gum tragacanth. 3 h later they were injected intraportally with [^{14}C]palmitate and [^{3}H] glycerol and the rates of hepatic glycerolipid synthesis were measured (Materials and Methods section). All rats were fed the 41B diet and the results are expressed as means with 1 s.d. The flux from phosphatidate to diacylglycerol is calculated by summing the incorporations into diacylglycerol, triacylglycerol, phosphatidylcholine and phosphatidylethanolamine.

	Incorporation of [^{14}C]palmitate				Incorporation of [^{3}H]glycerol			
	d. min^{-1} × 10^{-3} min^{-1} g^{-1} liver		d. min^{-1}/100 d. min^{-1}		d. min^{-1} × 10^{-3} min^{-1} g^{-1} liver		d. min^{-1}/100 d. min^{-1}	
	Control	S-780 treated	Control	S-780 treated	Control	S-780 treated	Control	S-780 treated
Phosphatidylinositol + phosphatidylserine	59 ± 23 P <0·01	35 ± 15	3 ± 1 P <0·01	2 ± 1	20 ± 8	16 ± 8	3 ± 1 P <0·001	1 ± 1
Phosphatidylcholine	249 ± 57	224 ± 67	12 ± 2	12 ± 1	64 ± 24 P <0·01	88 ± 16	8 ± 2	7 ± 1
Phosphatidylethanolamine	85 ± 22	80 ± 28	4 ± 1	4 ± 1	36 ± 12 P <0·005	48 ± 8	4 ± 1	4 ± 1
Phosphatidate	315 ± 104 P <0·02	207 ± 99	16 ± 5 P <0·01	10 ± 4	128 ± 56	112 ± 64	16 ± 7 P <0·01	9 ± 4
Diacylglycerol	762 ± 152 P <0·005	563 ± 152	38 ± 2 P <0·001	29 ± 4	340 ± 112	320 ± 80	41 ± 4 P <0·001	26 ± 5
Triacylglycerol	524 ± 191 P <0·001	831 ± 184	26 ± 7 P <0·001	43 ± 6	240 ± 152 P <0·001	639 ± 128	27 ± 11 P <0·001	51 ± 7
Flux from phosphatidate to diacylglycerol	1504 ± 515	1666 ± 381	81 ± 6 P <0·01	88 ± 4	607 ± 320 P <0·001	1083 ± 172	80 ± 8 P <0·005	88 ± 5
Total glycerolipid	1752 ± 618	1929 ± 456	—	—	747 ± 356 P <0·001	3246 ± 184	—	—
Total [^{14}C]palmitate in liver	3811 ± 1392 P <0·02	5474 ± 1674	—	—	—	—	—	—
Non-lipid ^{3}H in liver	—	—	—	—	4651 ± 1590	5202 ± 1291	—	—

[^{14}C]palmitate into phosphatidylethanolamine was also lower in the S-780-treated rats.

This experiment was repeated with rats fed for 10 days on a diet containing 60% (w/w) of corn-starch, before being treated for 5 days with S-780 while being maintained on the same diet. After 5 days the S-780-treated rats (16 animals) lost an average 3 g more ($P < 0·05$) body weight than the control rats (15 animals). The patterns of the incorporations were similar to those in Table 4. S-780-treated rats synthesized more total lipid ($P < 0·05$) and diacylglycerol ($P < 0·005$) from [^{3}H]glycerol. However the only significant difference in the percentage distribution of the radioactivity was a decreased proportion of [^{14}C]palmitate in phosphatidylcholine ($P < 0·005$).

DISCUSSION

A method is described for measuring the kinetics of hepatic glycerolipid synthesis from [^{14}C]palmitate and [^{3}H]glycerol *in vivo*. These precursors would have been diluted with endogenous substrates to

Table 3. *The incorporation of [1,3-^{3}H]glycerol into various lipid classes by liver slices obtained from control and S-780-treated rats.* Rats were treated for 5 days with S-780 or with gum tragacanth (control rats) as described in the Materials and Methods section. The values are quoted with 1 s.d. and the numbers in parentheses are the numbers of rats used. Significant differences between control and S-780 treated rats are indicated as follows: *P <0·05; **P <0·01.

Group	(min)	% glycerol incorporated into the various lipid products: I	II	III	IV	V	VI
(1) Control	4	1 ± 0·4 (5)	2 ± 0·4 (5)	3 ± 1 (5)	16 ± 7 (5)	63 ± 8 (5)	13 ± 3 (5)
	7	1 ± 1 (5)	4 ± 1 (5)	4 ± 0·5 (5)	12 ± 4 (5)	54 ± 3 (5)	24 ± 1 (5)
	10	2 ± 0·6 (7)	5 ± 1 (7)	6 ± 0·4 (7)	9 ± 2 (7)	44 ± 3 (7)	32 ± 3 (7)
	13	2 ± 0·5 (5)	7 ± 1 (5)	7 ± 0·4 (5)	8 ± 2 (5)	37 ± 3 (5)	37 ± 5 (5)
(2) S-780 treated	4	*2 ± 1 (6)	4 ± 1 (6)	4 ± 1 (6)	16 ± 7 (6)	63 ± 9 (6)	11 ± 2·5 (6)
	7	*3 ± 1 (6)	6 ± 2 (6)	6 ± 2 (6)	11 ± 4 (6)	57 ± 5 (6)	*18 ± 4 (6)
	10	**3 ± 1 (7)	7 ± 2 (7)	6 ± 2 (7)	8 ± 4 (7)	49 ± 4 (7)	*26 ± 4 (7)
	13	3 ± 1·5 (6)	9 ± 3 (6)	8 ± 3 (6)	8 ± 4 (6)	42 ± 5 (6)	*29 ± 5 (6)

I Phosphatidyl serine + phosphatidyl inositol. II Phosphatidylcholine. III Phosphatidylethanolamine. IV Phosphatidate. V Diacylglycerol. VI Triacylglycerol.

Table 4. *The effect of chronic treatment with S-780 on the synthesis of glycerolipids in rat liver* in vivo. Rats were treated daily for 5 days with S-780, 50 mg kg^{-1} body wt (8 rats) or with gum tragacanth (10 control rats) and 3 h later injected intraportally with [^{3}H]glycerol and [^{14}C]palmitate (Materials and Methods section). All rats were fed the 41B diet. The rates of glycerolipid synthesis are quoted as means with 1 s.d. The flux from phosphatidate to diacylglycerol is calculated by summing the incorporations into diacylglycerol, triacylglycerol, phosphatidylcholine and phosphatidylethanolamine.

	Incorporation of [^{14}C]palmitate				Incorporation of [^{3}H]glycerol			
	d. $min^{-1} \times 10^{-3}$ min^{-1} per liver		d. min^{-1}/100 d. min^{-1}		d. $min^{-1} \times 10^{-4}$ min^{-1} per liver		d. min^{-1}/100 d. min^{-1}	
	Control	S-780 treated	Control	S-780 treated	Control	S-780 treated	Control	S-780 treated
Phosphatidylinositol + phosphatidylserine	39 ± 39	26 ± 13	2 ± 2	1 ± 1	80 ± 6	7 ± 5	2 ± 2	1 ± 1
Phosphatidylcholine	257 ± 64	206 ± 90	16 ± 1 P <0·01	12 ± 4	46 ± 14	51 ± 18	10 ± 3 P <0·02	7 ± 1
Phosphatidylethanolamine	77 ± 26	52 ± 26	5 ± 1 P <0·02	3 ± 1	23 ± 10	27 ± 13	5 ± 2	4 ± 2
Phosphatidate	322 ± 155	283 ± 219	19 ± 5	15 ± 7	83 ± 32	105 ± 78	17 ± 5	14 ± 8
Diacylglycerol	631 ± 129	940 ± 476	39 ± 5 P <0·001	50 ± 6	276 ± 88 P <0·005	360 ± 70	43 ± 7 P <0·01	52 ± 7
Triacylglycerol	309 ± 64	348 ± 180	20 ± 4	19 ± 7	110 ± 64	128 ± 73	21 ± 6	18 ± 8
Flux from phosphatidate to diacylglycerol	1275 ± 245	1558 ± 682	79 ± 7	84 ± 7	396 ± 157	471 ± 209	79 ± 7	82 ± 8
Total glycerolipid	1648 ± 399	1867 ± 837	—	—	496 ± 177 P <0·025	700 ± 162	—	—
^{14}C in whole liver	3399 ± 863	3837 ± 734	—	—	—	—	—	—
Non-lipid ^{3}H in whole liver	—	—	—	—	5851 ± 4186	11089 ± 5960	—	—

varying degrees in different rats. It was impractical to compensate for this by measuring, as a function of time, the specific radioactivities of glycerol and palmitate in the blood, and of glycerol phosphate, dihydroxyacetone phosphate, palmitate and the different molecular species of phosphatidate and diacylglycerol in the liver. Even had this been done, it is not known in detail how differences in the availability of endogenous fatty acids affect the metabolism of palmitate and glycerol; nor is it known to what extent there are different metabolic pools of diacylglycerol and phosphatidate. Therefore the interpretation which can be put upon the information is limited. The results simply indicate the fate of exogenously administered [^{3}H]glycerol and [^{14}C] palmitate in hepatic glycerolipid synthesis. The results do not explain the reasons for any of the observed changes which may have resulted from isotope dilution, substrate availability or changes in enzyme activity.

Acute treatment with S-780 probably promotes the mobilization of adipose tissue triacylglycerol which results in increased concentrations of plasma glycerol and unesterified fatty acids (Duhault & Malen, 1970). This is not observed after prolonged treatment. The acute administration of S-780 is accompanied by an increased flux of phosphatidate to diacylglycerol (Table 2) and an increase in hepatic triacylglycerol (Marsh & Bizzi, 1972). The isotope dilution of [^{14}C]palmitate and [^{3}H]glycerol should have been greater in the S-780-treated rats than in the controls, yet an increased lipid synthesis was observed (Table 2). The liver is known to increase its capacity to synthesize triacylglycerol when the supply of glycerol and fatty acids is elevated: this is observed in starvation (Vavrečka, Mitchell & Hübscher, 1969; Mangiapane, Lloyd-Davies & Brindley, 1973). These adaptations appear to involve an increase in the activity of phosphatidate phosphohydrolase and the results in Table 3 are compatible with this.

Treatment of rats with S-780 produced an anorectic effect and a loss of body weight. After five days the daily changes in body weight were not significantly different from those of the control rats (Table 1). These results agree with those of Duhault & Malen (1970). S-780 decreased the weight of the liver, probably due to a reduction in triacylglycerol content (Duhault & others, 1976) and it also lowered the rate of triacylglycerol synthesis by liver slices (Table 2). The rates of triacylglycerol synthesis *in vivo* were not significantly depressed by S-780 treatment (Table 4). This difference may be because the predominant end product of lipid synthesis in the liver slices is triacylglycerol (Table 2), whereas *in vivo* phosphatidylcholine and phosphatidylethanolamine are synthesized to a relatively greater extent (Tables 3 and 4). The effect of S-780 on lipid synthesis *in vivo* was to decrease the rate of synthesis of phosphatidylcholine and phosphatidylethanolamine (Table 4). These lipids, like triacylglycerol, are derived directly from diacylglycerol, and yet a decrease in their synthesis was observed when the accumulation of radioactive precursors in diacylglycerol was increased (Table 4). This may imply that the flux of substrates through

choline and ethanolamine phosphotransferase reactions was reduced. Similar results were obtained with the rats fed the high carbohydrate diet and in these experiments the weight change over the five day period was less than 2 % lower in the S-780-treated rats than in the controls. Duhault & Malen (1970) have already concluded that S-780 has effects on carbohydrate and lipid metabolism other than those which can be related to anorexia.

The results demonstrate the differences between the effects of acute and chronic administration of S-780 on lipid metabolism. The inhibition of synthesis of triacylglycerol, phosphatidylcholine and phosphatidylethanolamine in the livers of rats treated for five days with S-780 was predicted from work *in vitro* (Brindley & Bowley, 1975 a, b). However, direct evidence for the inhibition of phosphatidate phosphohydrolase, which was postulated to produce these effects, was not obtained. The only indication for this having happened was the increased incorporation of glycerol into the acidic lipid fraction containing phosphatidylinositol plus phosphatidylserine (Table 2).

The effects of chronic administration of S-780 in decreasing the synthesis of triacylglycerol, phosphatidylcholine and phosphatidylethanolamine could partly explain the reduced synthesis and secretion of very low density lipoproteins which has been observed with this drug (Marsh & Bizzi, 1972; Kaye & others, 1975; Duhault & others, 1976). However the mechanism by which this hypotriglyceridaemic effect is achieved requires further investigation.

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