

Studies on the Lipid Composition of the Rat Liver Endoplasmic Reticulum after Induction with Phenobarbitone and 20-Methylcholanthrene

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1. The cholesterol content, proportions of different phospholipids and fatty acid components of phosphatidylcholine and phosphatidylethanolamine were studied in rat liver endoplasmic-reticulum membrane, after a single injection of 20-methylcholanthrene or injections of phenobarbitone for 5 days. 2. A marked decrease in the proportion of cholesterol occurred 5 days after injection of 20-methylcholanthrene or phenobarbitone. 3. The proportion of phosphatidylcholine was increased by injection of phenobarbitone and minor changes occurred in other phospholipids. 4. Phenobarbitone caused the proportion of linoleic acid in phosphatidylcholine and phosphatidylethanolamine to increase to 120–125% of the control and the proportion of oleic acid, arachidonic acid and docosahexaenoic acid to decrease. 5. 20-Methylcholanthrene caused an increase in the proportion of oleic acid in phosphatidylcholine and ethanolamine to 125–140% of the control, 1 day after injection. 6. The increased proportion of linoleic acid in phosphatidylcholine after phenobarbitone injection occurs simultaneously with the increase of cytochrome *P*-450 concentration, the rate of oxidative demethylation of aminopyrine and the rate of hydroxylation of biphenyl. It is therefore considered that distinct species of phosphatidylcholine or phosphatidylethanolamine containing linoleic acid in the β position are essential in the endoplasmic-reticulum membrane for optimal activity of oxidative demethylation.

Administration of phenobarbitone to animals induces the proliferation of the smooth endoplasmic reticulum of liver parenchymal cells (Remmer & Merker, 1963). The drug-hydroxylating enzymes, NADPH-cytochrome *c* reductase and cytochrome *P*-450 are induced to 3–5 times control activities (Orrenius *et al.*, 1965) and the rate of protein synthesis in the membrane is increased (Arias *et al.*, 1969). The administration of phenobarbitone for 5 days causes an increase in the total quantity of lipid, but no change in the proportions of lipids in the liver microsomal fraction (Glaumann & Dallner, 1968). It is also reported, however, that the proportion of phosphatidylcholine in the membrane increases significantly after injection of phenobarbitone for 3 days (Young *et al.*, 1971).

The important role that phospholipids play in microsomal drug hydroxylation has been demonstrated by preparation of solubilized components that will not function in a reconstituted system unless phospholipid is added (Lu *et al.*, 1969; Strobel *et al.*, 1970). Further, treatment of microsomal suspensions with phospholipase causes a significant decrease of activity of *N*-demethylation of benzphetamine or aminopyrine, but aniline hydroxylation is unaffected (Chaplin & Mannering, 1970; Eling & DiAugustine, 1971). Unsaturated fatty acids of the phospholipids of the endoplasmic reticulum may be destroyed by

induction of peroxide formation, and this also causes a marked decrease in the rate of oxidative demethylation (Wills, 1971).

In view of the dependence of hydroxylation on membrane phospholipids, the nature and composition of the phospholipids have been studied during induction by phenobarbitone and 20-methylcholanthrene. These two inducers have been compared because there are well-established differences between the inductive effects of polycyclic hydrocarbons and phenobarbitone on cytochrome *P*-450 and on the proliferation of the membranes of the endoplasmic reticulum (Fouts & Rogers, 1965; Conney, 1967).

Materials and Methods

Materials

Cytochrome *c*, NADP⁺, ATP, ADP and CTP were obtained from Boehringer Corp. (London) Ltd., London W.5, U.K. DL-Isocitrate, isocitrate dehydrogenase and fatty acid methyl esters were from Sigma (London) Chemical Co., Kingston-upon-Thames, Surrey, U.K., and aminopyrine and sodium phenobarbitone were from John Bell and Croyden, London W.1, U.K.

Silica gels H and G for t.l.c. were supplied by Camag, Mittenz, Switzerland, and 10% polyethylene

glycol on Chromosorb W (80–100 mesh), for g.l.c., by Phase Separation Ltd., Queensferry, Flintshire, U.K.

Organic solvents were obtained from May and Baker, Dagenham, Essex, U.K., or from BDH Chemicals Ltd., Poole, Dorset, U.K., and were distilled twice from an all-glass apparatus before use. Synthetically pure phospholipid standards and 20-methylcholanthrene were supplied by Koch–Light Ltd., Colnbrook, Bucks., U.K. All other chemicals were AnalaR grade and obtained from BDH.

Animals

Male Wistar rats aged 4–6 weeks and weighing 120–140g were used in groups of four. One group received, by intraperitoneal injection, a 20mg/kg dose of 20-methylcholanthrene dissolved in arachis oil (5ml/kg) and another group received 100mg/kg. A third, the control group, received arachis oil alone and the fourth group was injected intraperitoneally with phenobarbitone dissolved in 0.9% (w/v) NaCl (100mg/kg per day) for 5 days. All the animals received an equal amount of arachis oil to obviate any effect the oil might have on the lipid composition of the microsomal membranes.

Methods

Preparation of microsomal fraction. The livers were excised and homogenized in 3vol. of 1.15% (w/v) KCl at 4°C. The post-mitochondrial supernatant prepared by centrifuging the homogenate for 20min at 9000g_{av.} was centrifuged for 60min at 100000g_{av.} and the pellet, after washing, was used as the source of liver endoplasmic reticulum.

Cytochrome P-450. This was measured by the method of Omura & Sato (1964).

NADPH-cytochrome c reductase. This activity was measured by the method of Williams & Kamin (1962).

Aminopyrine N-demethylase. This enzyme was assayed by the method described by Wills (1969), with some modifications. The incubation mixture contained 0.02M-sodium phosphate buffer, pH7.4, 0.01M-MgCl₂, 0.01M-aminopyrine, 0.0625M-KCl, 125μM-NADPH and 4mg of protein/ml in a final volume of 5ml. Samples of volume 2ml were removed at 0 and 7min.

Biphenyl hydroxylase. The hydroxylation of biphenyl was measured by the method of Creaven *et al.* (1965).

Protein determination. Protein was measured by the method of Lowry *et al.* (1951). Bovine plasma albumin was used as a standard.

Extraction and separation of lipids. Lipids were extracted from the microsomal pellet by the method of Bligh & Dyer (1959). 2,6-Di-t-butyl-4-methylphenol

(5mg/100ml) was added to all solvents to prevent autoxidation of the lipids. Neutral lipids were separated by t.l.c. on Silica gel G by the method of Malins & Mangold (1960). Phospholipids were separated by t.l.c. on 'basic' silica-gel H plates by the method of Skipski *et al.* (1964). Lipids were detected by exposing the dry plates to iodine vapour for 10s.

Cholesterol determination. Cholesterol was measured by the method of Mann (1961) after separation from the cholesterol esters and 2,6-di-t-butyl-4-methylphenol by t.l.c. (Dodge & Phillips, 1966). The cholesterol spots (10–100μg) were scraped off the t.l.c. plates into centrifuge tubes and dissolved in 0.5ml of acetic acid, containing 0.05% FeCl₃. They were warmed to 80°C in a water bath. The reagent [3.5ml, containing 0.05% FeCl₃ in acetic acid mixed with conc. H₂SO₄ in the ratio of 4:3 (v/v), just before use] was warmed to 80°C, added to each tube and mixed thoroughly. After centrifugation to remove the silica gel, the absorbance of the supernatant was measured at 560nm and compared with 20, 50 and 100μg standards of cholesterol that had been treated in the same way.

Determination of phospholipid phosphorus. The method of Rouser *et al.* (1966) was used.

Preparation of fatty acid methyl esters from phospholipids. Phospholipids for fatty acid analysis were applied as a streak on 0.5mm-thick silica gel H t.l.c. plates (20cm×20cm) and separated by using the solvent system described by Skipski *et al.* (1964), but to which 2,6-di-t-butyl-4-methylphenol (5mg/100ml) had been added. The plates were developed at 4°C, dried under a stream of N₂ and the edges of the separated phospholipid were detected by using an iodine pencil. The remainder of the phospholipid streaks were protected from the iodine vapour and were eluted from the silica gel with chloroform-methanol-water (10:10:3, by vol.). Silica gel was removed by filtration through a sintered-glass filter and washed once with chloroform-methanol (1:1, v/v). Methanol and water were removed from the filtrate after adjusting the water content and separating the phases by centrifugation at 0°C (Bligh & Dyer, 1959) and the chloroform layer was evaporated under a reduced pressure of N₂. The fatty acid methyl esters were prepared by the method of Morrison & Smith (1964) by using borosilicate glass tubes housed inside brass reaction tubes.

Separation and determination of the fatty acid methyl esters

A Pye model 104 gas-liquid chromatograph with Vitatron recorder and a hydrogen flame ionization detector was used. The column (156cm×3mm) was packed with 10% polyethylene glycol adipate on Chromosorb W (80–100 mesh). The column temperature was 180°C and flow rate of N₂ carrier

was 60ml/min. Loads of 0.5–5.0 μ g of fatty acid methyl ester mixtures were injected into the top of the column and were identified by comparison with authentic standards. The linearity of the detector response to the weight of the sample was checked and peak areas were calculated as the product of the peak height and the peak width at half peak height.

Results

The ratio of cholesterol/phospholipid in the endoplasmic-reticulum membranes, the proportions of different phospholipids and the fatty acids esterified in the membrane phosphatidylcholine and phosphatidylethanolamine were measured at intervals up to 13 days after the first injection of phenobarbitone or methylcholanthrene.

Cholesterol/phospholipid ratios

Injection of phenobarbitone (100mg/kg per day) for 5 days caused the proportion of cholesterol in the microsomal membrane to fall to 73% of the control value (Table 1), but 9 days after phenobarbitone administration was stopped the cholesterol/

phospholipid ratio was restored to control values. A single injection of 20mg of 20-methylcholanthrene/kg caused a small decrease in the cholesterol/phospholipid ratio in the endoplasmic reticulum 5 days later. Cholesterol in the membrane increased 12h after injection of 100mg of 20-methylcholanthrene/kg, but fell after a further 12h, and 13 days later the proportion of cholesterol in the membrane was less than in the controls (Table 1).

Phospholipid composition of the endoplasmic reticulum

The changes in each phospholipid class, expressed as a molar percentage of the total phospholipids, caused by the two drugs were small. Proportions of minor components (phosphatidylinositol and phosphatidylserine measured together, and sphingomyelin) were decreased and the proportion of phosphatidylcholine was increased. Maximum increase of phosphatidylcholine occurred 5 days after the cessation of phenobarbitone administration. The proportions of the phospholipids returned to control values 9 days after phenobarbitone administration was stopped (Table 2).

Table 1. Effect of phenobarbitone and 20-methylcholanthrene on the ratio of cholesterol to total phospholipid in the endoplasmic-reticulum membrane

Phenobarbitone (100mg/kg per day) was administered for 5 days. 20-Methylcholanthrene (20 or 100mg/kg) was injected in a single dose. For other details see the text. Control values are means (\pm S.E.M) of 16 animals and 8 animals were used for each determination after injection. *P* values greater than 0.05 are shown as NS (not significant).

Time after first injection	Molar ratio of cholesterol/total phospholipid			
	Control	Phenobarbitone	20-Methylcholanthrene	
			(20mg/kg)	(100mg/kg)
12h	0.111 \pm 0.007	0.098 \pm 0.003 (NS)	—	0.144 \pm 0.004 (<i>P</i> < 0.01)
24h	0.111 \pm 0.007	0.117 \pm 0.004 (NS)	—	0.093 \pm 0.009 (NS)
5 days	0.106 \pm 0.003	0.077 \pm 0.003 (<i>P</i> < 0.001)	0.086 \pm 0.003 (<i>P</i> < 0.001)	0.099 \pm 0.002 (NS)
13 days	0.106 \pm 0.003	0.112 \pm 0.005 (NS)	0.108 \pm 0.006 (NS)	0.086 \pm 0.006 (<i>P</i> < 0.01)

Table 2. Composition of the phospholipids prepared from the endoplasmic reticulum of the livers of rats after treatment with phenobarbitone

Phenobarbitone (100mg/kg per day) was administered for 5 days. Control values are means (\pm S.E.M.) of determinations on 6 animals, and 8 animals were used for each determination after injection. *P* values greater than 0.05 are shown as NS (not significant).

Time after first injection (days)	Content (% of total phospholipid)				Recovery (%)
	Phosphatidylethanolamine	Phosphatidylinositol + phosphatidylserine	Phosphatidylcholine	Sphingomyelin	
0 (control)	20.2 \pm 0.7	14.1 \pm 0.5	53.0 \pm 1.1	3.7 \pm 0.7	89.9 \pm 1.6
1	21.8 \pm 1.4 (NS)	13.1 \pm 0.5 (NS)	53.8 \pm 0.7 (NS)	3.9 \pm 0.1 (NS)	92.6 \pm 2.4
3	22.8 \pm 0.4 (<i>P</i> < 0.001)	11.4 \pm 0.3 (<i>P</i> < 0.001)	55.7 \pm 0.6 (<i>P</i> < 0.02)	1.7 \pm 0.1 (<i>P</i> < 0.01)	91.5 \pm 0.7
5	22.7 \pm 0.3 (<i>P</i> < 0.001)	12.1 \pm 0.2 (<i>P</i> < 0.001)	54.9 \pm 0.7 (NS)	2.1 \pm 0.3 (<i>P</i> < 0.05)	91.8 \pm 0.9
7	20.7 \pm 0.4 (NS)	12.4 \pm 0.2 (<i>P</i> < 0.01)	56.3 \pm 0.95 (<i>P</i> < 0.05)	1.6 \pm 0.4 (<i>P</i> < 0.02)	90.6 \pm 0.8
9	19.9 \pm 0.7 (NS)	11.4 \pm 0.3 (<i>P</i> < 0.001)	58.9 \pm 1.4 (<i>P</i> < 0.01)	1.6 \pm 0.1 (<i>P</i> < 0.01)	91.4 \pm 1.7
13	19.4 \pm 0.2 (NS)	14.4 \pm 0.4 (NS)	53.9 \pm 1.0 (NS)	2.6 \pm 0.2 (NS)	90.2 \pm 0.8

On day 9 after injection of 20-methylcholanthrene (100mg/kg) changes in the phospholipid composition were similar to those observed after phenobarbitone treatment (Table 2). The percentage of phosphatidylinositol and phosphatidylserine measured together

Table 3. Fatty acid composition of phosphatidylcholine and phosphatidylethanolamine prepared from the endoplasmic reticulum of untreated rats

Determinations were made on 8 animals and s.e.m. values are shown. For experimental details see the text.

Fatty acid species	Percentage of total fatty acids	
	Phosphatidylcholine	Phosphatidylethanolamine
C _{16:0}	22.9 ± 0.4	19.4 ± 0.3
C _{18:0}	19.3 ± 0.4	23.2 ± 0.3
C _{18:1}	9.9 ± 0.3	5.5 ± 0.2
C _{18:2}	19.2 ± 0.2	9.1 ± 0.3
C _{20:4}	14.4 ± 0.3	19.4 ± 0.4
C _{22:6}	7.4 ± 0.2	17.2 ± 0.4

decreased from 14.1 ± 0.5% to 11.3 ± 0.4% and that of sphingomyelin decreased from 3.7 ± 0.7% to 1.3 ± 0.3%. Phosphatidylcholine increased from 53.0 ± 1.1% to 58.0 ± 1.5%. On days 1, 3, 5, 7 and 13 after injection of methylcholanthrene changes in phospholipid composition were similar to those observed after phenobarbitone treatment, but were not always significant. Changes in phospholipid composition caused by injection of a smaller dose of methylcholanthrene (20mg/kg) were qualitatively the same as those caused by the larger dose.

Fatty acid composition of the phospholipids

Fatty acid methyl esters were prepared from the whole phospholipid mixture extracted from the liver microsomal fraction of untreated rats or from those that had been injected 12 and 24h previously. After reaction with BF₃ complex at 100°C the sphingomyelin and vinyl ether bonds of plasmalogens remain intact (Morrison & Smith, 1964), but the ester bonds are split so that only the fatty acids in ester linkage

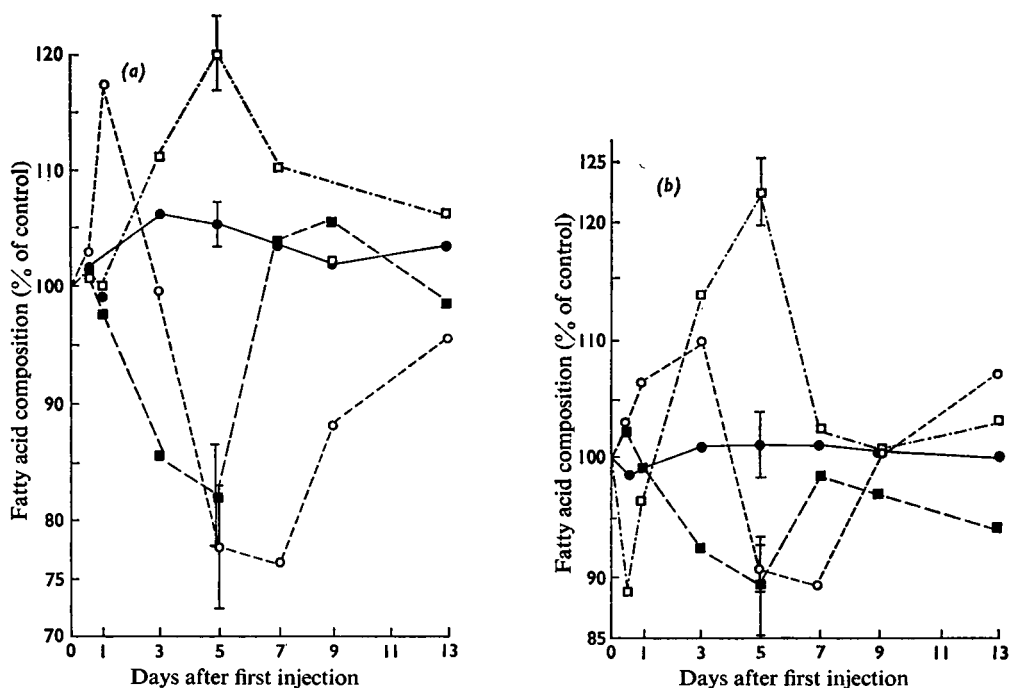


Fig. 1. Fatty acid composition of phosphatidylcholine (a) and phosphatidylethanolamine (b) prepared from the endoplasmic reticulum of rats after treatment with phenobarbitone

Phenobarbitone (100mg/kg) was injected daily for 5 days. Values are expressed as a percentage of those determined for untreated rats (Table 4) and s.e.m. values (vertical bars) are shown for determinations on day 5 of each experiment. Control values were determined with 30 rats, and 8 rats were used on each day after the first injection. ○, Oleic acid; ●, palmitic acid and stearic acid; □, linoleic acid; ■, arachidonic acid and docosahexaenoic acid.

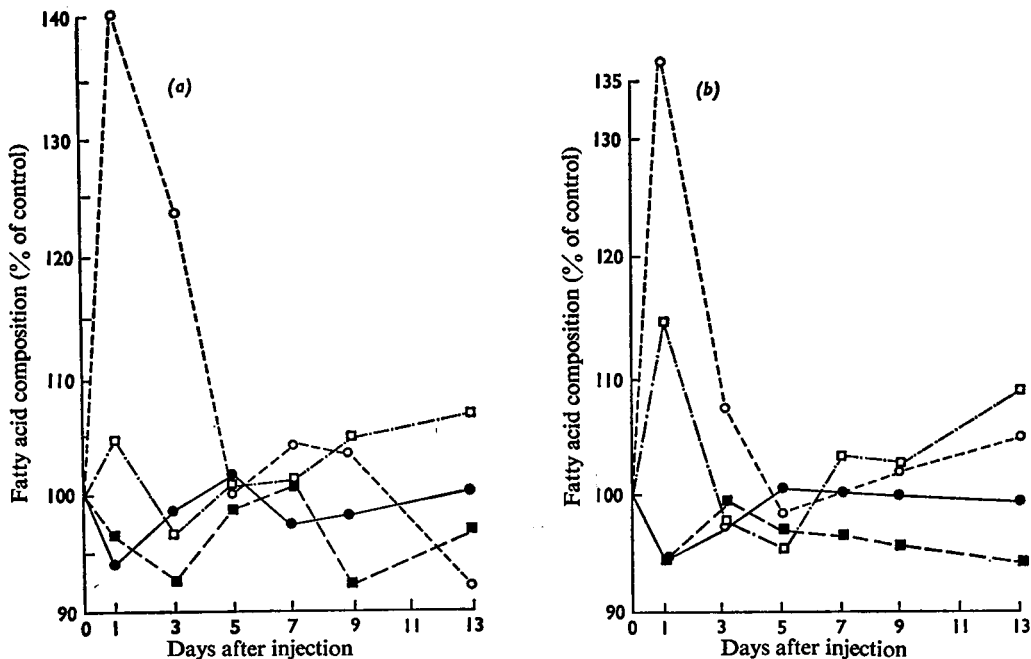


Fig. 2. Fatty acid composition of phosphatidylcholine (a) and phosphatidylethanolamine (b) prepared from the endoplasmic reticulum of rats after treatment with 20-methylcholanthrene

A single injection of 20-methylcholanthrene (20mg/kg) was given to each rat. Other details and symbols as in Fig. 1. Control values were determined with 30 rats, and 8 rats were used on each day after injection. S.E.M. values for day 5 after injection have been omitted for clarity. For phosphatidylcholine the values were: oleic acid $104 \pm 2.9\%$, palmitic acid and stearic acid $104 \pm 2.9\%$, linoleic acid $101 \pm 3.7\%$, and arachidonic acid + docosahexaenoic acid $98 \pm 7.8\%$, and for phosphatidylethanolamine: oleic acid $99 \pm 7.3\%$, palmitic acid and stearic acid $101 \pm 3.3\%$, linoleic acid $96 \pm 6.6\%$, and arachidonic acid + docosahexaenoic acid $97 \pm 4.3\%$.

Table 4. Effect of the administration of 20-methylcholanthrene on cytochrome P-450, NADPH-cytochrome c reductase, aminopyrine N-demethylase and biphenyl hydroxylase in the liver microsomal fraction

20-Methylcholanthrene was injected (20mg/kg). For determinations of protein, cytochrome P-450, NADPH-cytochrome c reductase and aminopyrine demethylase control values are means (\pm S.E.M.) of determinations on 20 animals, and 8 animals were used for each determination after injection. *P* values greater than 0.05 are shown as not significant (NS). For biphenyl 4-hydroxylase and biphenyl 2-hydroxylase 9 animals were used for control determinations, and 3 animals for each determination after injection.

Time after injection (days)	Yield of microsomal protein (mg/g of liver)	Cytochrome P-450 ($E_{450-490}$ /g of liver) (difference spectrum)	NADPH-cytochrome c reductase activity (mmol/min per g of liver)	Aminopyrine N-demethylase activity (nmol/min per g of liver)	Biphenyl 4-hydroxylase activity (μ g/min per g of liver)	Biphenyl 2-hydroxylase activity (μ g/min per g of liver)
0 (control)	20.1 ± 0.7	1.19 ± 0.07	0.45 ± 0.002	272 ± 4	6.66 ± 0.82	0.735 ± 0.127
1	19.1 ± 0.4 (NS)	1.88 ± 0.17 ($P < 0.001$)	0.47 ± 0.04 (NS)	252 ± 18 (NS)	25.0 ± 3.1	8.50 ± 0.61
3	21.0 ± 1.4 (NS)	2.56 ± 0.3 ($P < 0.001$)	0.61 ± 0.15 (NS)	228 ± 11 ($P < 0.001$)	13.3	8.58
5	20.6 ± 1.3 (NS)	2.28 ± 0.15 ($P < 0.001$)	0.67 ± 0.05 ($P < 0.001$)	272 ± 16 (NS)	15.0	9.30
7	20.7 ± 1.3 (NS)	2.35 ± 0.22 ($P < 0.001$)	0.54 ± 0.06 ($P < 0.05$)	209 ± 10 ($P < 0.001$)	16.65	8.38
9	—	2.4	0.48 ± 0.08 (NS)	212 ± 10 ($P < 0.001$)	14.32	9.56
13	21.1	2.05 ± 0.36 ($P < 0.001$)	0.65 ± 0.03 ($P < 0.001$)	—	—	—

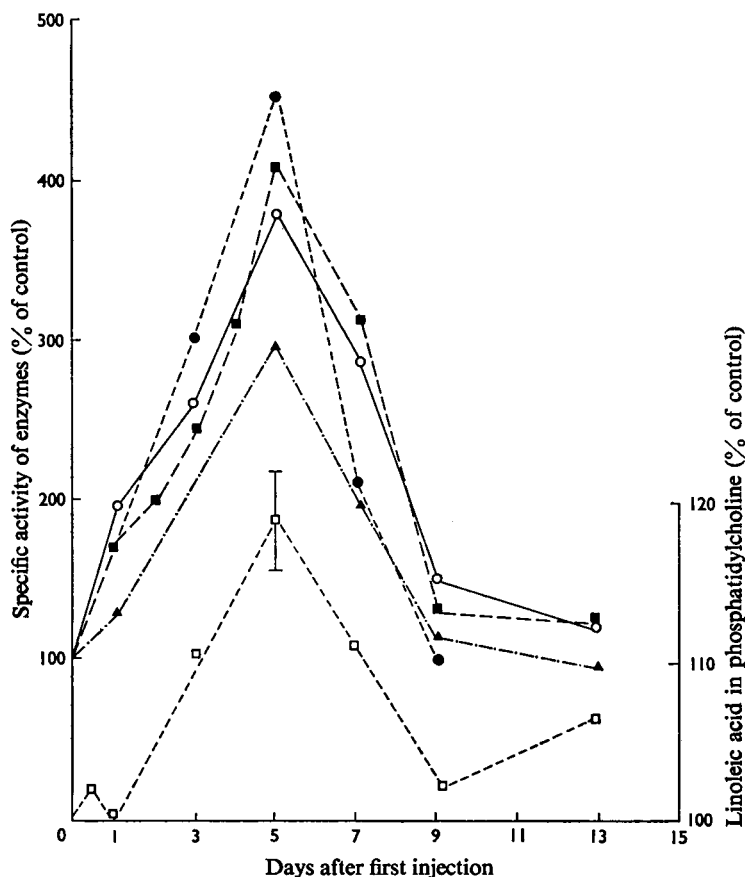


Fig. 3. Comparison of cytochrome P-450 concentration, NADPH-cytochrome c reductase, aminopyrine N-demethylase and biphenyl hydroxylase activities with the proportion of linoleic acid in phosphatidylcholine after administration of phenobarbitone

All values are expressed as percentages of those obtained for untreated rats. Phenobarbitone (100mg/kg) was administered daily for 5 days. Values were calculated from determinations on not less than 8 rats. S.E.M. values are shown for linoleic acid in phosphatidylcholine. ■, Cytochrome P-450; ○, NADPH-cytochrome c reductase; ▲, aminopyrine demethylase; ●, biphenyl 2- and 4-hydroxylase; □, linoleic acid in phosphatidylcholine.

with the glycoposphatides were measured. In the eight experiments $96.4 \pm 1.1\%$ (S.E.M.) of the fatty acids of phosphatidylcholine and $91.5 \pm 1.3\%$ (S.E.M.) of the fatty acids of phosphatidylethanolamine were transmethylated. Only the main fatty acid esters were determined and individual esters were expressed as percentage by weight of the total of the main fatty acids. Values for the control animals are tabulated in Table 3.

There were changes in the pattern of fatty acids recovered from the microsomal phospholipids 24h after phenobarbitone and 20-methylcholanthrene injection, but although these changes were small they were significant. The average amount of oleic acid

recovered 24h after injection of 20-methylcholanthrene into 4 animals increased from $8.1 \pm 0.3\%$ to $9.5 \pm 0.6\%$ and the proportion of stearic acid was decreased from $25.2 \pm 0.5\%$ to $22.7 \pm 0.4\%$. At 24h after phenobarbitone injection the proportion of linoleic acid was increased from $15.2 \pm 0.5\%$ to $16.5 \pm 0.4\%$ and arachidonic acid and docosahexaenoic acid were decreased from $20.3 \pm 0.3\%$ to $18.7 \pm 0.5\%$ and from $8.9 \pm 0.4\%$ to $8.0 \pm 0.2\%$ respectively. In both series of experiments the percentage of palmitic acid remained constant at $22.2 \pm 0.4\%$.

The fatty acid composition of phosphatidylcholine and phosphatidylethanolamine of the control animals

did not change after intraperitoneal injection of arachis oil (5ml/kg) during the 13-day period of the experiment. Variation from the control values of the major fatty acids caused by injection of either phenobarbitone or 20-methylcholanthrene during 13 days after the start of the experiment is shown in Figs. 1 and 2 respectively. Injection of phenobarbitone (100mg/kg per day) for 5 days caused a progressive increase in the linoleic acid content of phosphatidylcholine and phosphatidylethanolamine and a decrease in the arachidonic acid and docosahexaenoic acid in both phospholipids (Fig. 1). After an initial increase in the first 24h the proportion of oleic acid also decreased. After phenobarbitone administration was stopped the proportion of polyunsaturated acids returned immediately to control values, but linoleic acid and oleic acid returned to control values more slowly.

Injection of 20-methylcholanthrene into rats caused changes in the fatty acid composition of phosphatidylcholine and phosphatidylethanolamine that were quite different from those observed after injection of phenobarbitone. The proportion of oleic acid was markedly increased within the first 24h after injection of 20-methylcholanthrene (20mg/kg), but returned nearly to control values between 3–5 days later (Fig. 2). Linoleic acid increased to a small extent after 1 day, but the proportion of the other fatty acids in both phosphatidylcholine and phosphatidylethanolamine remained constant. A much greater dose of 20-methylcholanthrene (100mg/kg) injected into rats had qualitatively the same effect as the smaller dose on the proportion of oleic acid in phosphatidylcholine and phosphatidylethanolamine, but the effect was more prolonged, and normal values were not observed until 9–13 days after the injection. In addition, the proportion of linoleic acid in the phosphatidylcholine and phosphatidylethanolamine was decreased during the same period. Changes were more marked in phosphatidylcholine than in phosphatidylethanolamine and, during the first 24h, the proportions of arachidonic acid and docosahexaenoic acid were increased in phosphatidylcholine, but not in phosphatidylethanolamine.

Effect of injection of phenobarbitone and 20-methylcholanthrene on oxidative demethylation of aminopyrine and hydroxylation of biphenyl

Samples of microsomal suspensions were taken from control animals and from animals injected with phenobarbitone or 20-methylcholanthrene for determination of the rates of oxidative demethylation of aminopyrine, hydroxylation of biphenyl, activity of NADPH-cytochrome *c* reductase and cytochrome *P*-450 concentration. These determinations were made at the same times at which samples were taken for lipid analysis and enabled direct comparisons

to be made between enzyme activity and lipid composition of the membrane (Table 4 and Fig. 3). A very clear correlation was observed between the increased rate of oxidative demethylation of aminopyrine and increased linoleic acid in phosphatidylcholine after phenobarbitone injection (Fig. 3).

Discussion

During induction by phenobarbitone and 20-methylcholanthrene changes have been demonstrated in the cholesterol content of the membrane (Table 1), the phospholipid composition (Table 2) and the fatty acid composition (Figs. 1, 2 and 3).

Phenobarbitone is known to cause proliferation of the smooth endoplasmic reticulum (Remmer & Merker, 1963), and since the smooth endoplasmic reticulum is richer in cholesterol than the rough endoplasmic reticulum (Colbeau *et al.*, 1971) the effect of phenobarbitone injection might be expected to increase the proportion of cholesterol in the endoplasmic reticulum, but concentrations of cholesterol decreased (Table 1). Phenobarbitone stimulates the synthesis of several membrane-bound enzymes such as UDP-glucuronyltransferase and NADPH-cytochrome *c* reductase and cytochrome *P*-450 and it is possible that membrane that is essential for the structure or activity of these enzymes is synthesized with a smaller cholesterol content than the whole natural membrane.

The proportion of phosphatidylcholine in the endoplasmic membrane increased slightly after the injection of phenobarbitone but the maximum was reached several days after the phenobarbitone injections were stopped (Table 2). These results are in agreement with those of Young *et al.* (1971), but not with results reported by Glaumann & Dallner (1968). 20-Methylcholanthrene had a similar effect on the membrane. Increased synthesis or decreased breakdown of phosphatidylcholine may account for these changes, but as the maximum effect is observed much later than the induced enzyme activity (Table 4, Fig. 3), there appears to be no correlation between the induced enzyme activity and the phosphatidylcholine content of the membrane.

Of much greater importance is, however, the marked increase of the proportion of linoleic acid in phosphatidylcholine and phosphatidylethanolamine of the endoplasmic reticulum caused by phenobarbitone injection (Fig. 1). It is particularly significant that the time-course of the increased incorporation of linoleic acid corresponds almost exactly to that of increase of concentration of components and activity of the drug-hydroxylation system (Fig. 3). It appears likely that a selected species of phosphatidylcholine or phosphatidylethanolamine containing linoleic acid in the β position is essential for the formation of the membrane and associated proteins during the

induction process. Phosphatidylcholine rather than phosphatidylethanolamine may be primarily involved because it has been shown to be much more efficient in activating a solubilized preparation of NADPH-cytochrome *c* reductase and cytochrome *P*-450 than is phosphatidylethanolamine (Strobel *et al.*, 1970).

The increased proportion of linoleic acid in the phosphatidylcholine and phosphatidylethanolamine after phenobarbitone injection (Fig. 1) may be explained in either one of two ways. The pool of linoleic acid available for diglyceride synthesis could be increased as a result of the inhibition of the synthesis of arachidonic acid and docosahexaenoic acid, and as a consequence the quantities of arachidonic acid and docosahexaenoic acid in the liver would decrease. This was in fact observed after phenobarbitone injection (Fig. 1). Alternatively, there may be no alteration in the rates of synthetic pathways utilizing linoleic acid, and phosphatidylcholine and phosphatidylethanolamine containing linoleic acid may be sequestered preferentially in membrane lipoproteins and thus be withdrawn from the more mobile phospholipid pool (Pasternak & Bergeron, 1970).

The early changes that occur after the administration of 20-methylcholanthrene (Table 4 and Fig. 2) and the subsequent recovery to control values are less easy to explain. After the administration of 20-methylcholanthrene the proportion of linoleic acid did not increase in either phosphatidylcholine or phosphatidylethanolamine after either 20 or 100mg of 20-methylcholanthrene/kg. If the β -linoleoyl phosphatidylcholine is necessary for the electron transfer from NADPH to cytochrome *P*-450, the failure to stimulate the incorporation of linoleic acid may explain why 20-methylcholanthrene does not generally cause an increased rate of side-chain oxidation, e.g. aminopyrine *N*-demethylation (Conney, 1967).

These findings indicate that the fatty acid composition of the phospholipids may regulate the structure of the endoplasmic membrane by influencing hydrophobic bonds between membrane proteins and phospholipids. In addition phospholipids of specific structure may play an essential functional role in oxidative demethylation in the endoplasmic reticulum.

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