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THE PROLIFERATION OF HEPATOCYTES AND THE LIPID COMPOSITION OF THE ENDOPLASMIC RETICULUM AFTER INDUCTION OF DRUG-METABOLIZING ENZYMES WITH *trans*-STILBENE OXIDE

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Summary

Three aspects of the induction of drug-metabolizing enzymes brought about by *trans*-stilbene oxide have been investigated. (1) The liver hypertrophy in rats treated with *trans*-stilbene oxide was found to result solely from an increase in the number of cells in this organ, without any increase in the size of each individual cell. (2) Administration of *trans*-stilbene oxide also produces a 27% increase in the phospholipid content of the hepatic endoplasmic reticulum, i.e., a limited proliferation of this organelle occurs. (3) Furthermore, induction causes changes in the lipid composition of the endoplasmic reticulum. The cholesterol content is decreased, the relative content of sphingomyelin is also lowered, and a number of changes in the fatty-acid composition occur as well. All of these effects would tend to increase the fluidity of the phospholipid bilayer of the endoplasmic-reticulum membrane and may thus affect drug metabolism.

Introduction

Recently, it has been demonstrated [1—3] that *trans*-stilbene oxide induces drug-metabolizing enzymes in a pattern much different to those observed with the classical inducers, phenobarbital and methylcholanthrene. Phenobarbital and methylcholanthrene induce the cytochrome *P*-450 system to a greater

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extent than other detoxication enzymes. In contrast, *trans*-stilbene oxide causes epoxide hydratase and glutathione S-transferase activities to increase to 700 and 300—400%, respectively, of the control values, wherease cytochrome *P*-450 and NADPH-cytochrome *c* reductase are only induced to about 220% of the control levels.

Administration of *trans*-stilbene oxide to rats also results in dramatic liver hypertrophy. It is of interest to know whether this hypertrophy reflects an increase in the number of hepatocytes and/or hypertrophy of the individual cells. In addition, since three of the enzymes induced are localized on the endoplasmic reticulum, the question arises as to whether *trans*-stilbene oxide also affects the phospholipid bilayer of this organelle, for instance, by causing hypertrophy of the bilayer or by altering its composition. These aspects of the induction brought about by *trans*-stilbene oxide have been investigated and are described here.

Materials and Methods

Chemicals. *trans*-Stilbene oxide was purchase from EGA-Chemie, Steinheim/Albuch, F.R.G., and was at least 97% pure as determined by its melting point and by NMR and infrared spectroscopy. Diazomethane was prepared from *p*-tolylsulfonylmethylnitrosamide (Merck) according to the method of DeBoer and Backer [4]. Cholesterol, phospholipid (phosphatidylcholine, P5388, P0763, P1013, P7763, P6263; phosphatidylethanolamine, P4513, P5138, P6386; phosphatidylserine, P6641; phosphatidylinositol, P0639; sphingomyelin. S7004), and fatty-acid standards were purchased from Sigma Chemical Co. and found to be essentially free from contamination by thin-layer chromatography and gas chromatography, respectively. All other chemicals were obtained from common commercial sources and were of reagent grade.

Animals and microsomes. Male Sprague-Dawley rats weighing 150—180 g were used in all experiments. For induction, the animals received 400 mg *trans*-stilbene oxide/kg body weight in corn oil intraperitoneally once daily for 5 days before killing. All rats were starved overnight in order to reduce liver glycogen before decapitation and preparation of liver microsomes according to the method of Ernster et al. [5].

Enzyme assays. NADPH-cytochrome *c* reductase was assayed according to the method of Dallner [6], epoxide hydratase activity was determined by a modification [7] of the method of Oesch et al. [8], and glutathione S-transferase activity was quantitated spectrophotometrically at 30°C using 1,2-dichloro-4-nitrobenzene as the second substrate [9,10]. Cytochrome *c* oxidase [11], AMPase [12], and acid phosphatase [13] were also assayed using reported procedures. Homogenates were sonicated twice for 30 s while immersed in an ice-water bath in order to obtain maximal enzyme activities.

Chemical determinations. DNA was measured according to the method of Burton [14]. Phospholipids were extracted using chloroform/methanol (2 : 1), according to the method of Folch et al. [15] and were separated by thin-layer chromatography on silica gel using a solvent system composed of chloroform/methanol/acetic acid/water (25 : 15 : 4 : 2, v/v) according to the method of Skipski et al. [16]. The phospholipids were visualized with iodine vapor and

identification was made on the basis of standards run concomitantly. The spots were outlined with a pencil and wetted slightly to facilitate transfer of the silica gel to a centrifuge tube using a spatula. Areas containing no spot were also removed as controls. The samples were eluted four times with chloroform/methanol (2 : 1), the combined eluates transferred to a volumetric flask, and an appropriate sample taken for analysis. The sample was evaporated under N_2 and its phosphate content assayed by using the method of Bartlett [17]. The recovery of lipid phosphorus from the thin-layer plates was consistently 90—100% for samples from both control and induced animals.

Cholesterol was determined by using the method of Rudel and Morris [18] as follows: 0.1 ml of the microsomal suspension was mixed with 0.3 ml of 33% KOH and 3 ml of 95% ethanol. The tube was then stoppered and placed in a water bath at 60° C for 15 min. After cooling, 5 ml of petroleum ether and 3 ml of water were added. The tube was capped and shaken for 1 min to extract cholesterol. 1 ml of the petroleum-ether phase was pipetted into another tube and the solvent evaporated under N_2. Subsequently, 2 ml of *o*-phthalaldehyde solution (50 mg/100 ml glacial acetic acid) were added, followed 10 min later by 1 ml of conc. H_2SO_4. The absorbance at 550 nm was determined 10—20 min after the addition of H_2SO_4. A blank and cholesterol standard were saponified and extracted in the same manner.

For fatty acid analyses, phospholipid extracts were applied as 1.0—1.5 cm streaks on silica-gel plates, separated as described above, and visualized by spraying with distilled water. Each phospholipid band was then extracted and saponified with 1 N NaOH in 95% ethanol for 1 h. Free fatty acids were converted into methyl esters using diazomethane in the presence of a catalytic amount of methanol as described by Schenk and Gellerman [19]. Fatty-acid methyl esters were subsequently applied to a column packed with 15% diethylene glycol succinate polyester on 80—100 mesh Chromosorb. The column was operated isothermally at 190° C in a Packard Model 409 gas chromatograph equipped with a flame-ionization detector.

Results

Liver hypertrophy caused by trans-stilbene oxide

As can be clearly seen from Table I, the livers of rats treated with *trans*-stilbene oxide increase dramatically in size. At the same time, the treated

TABLE I

LIVER HYPERTROPHY CAUSED BY ADMINISTRATION OF *trans*-STILBENE OXIDE

Rats were induced and DNA was measured as described in Materials and Methods. The values given are the means ± S.D. for the number of animals given in parentheses.

Parameter	Control rats	Induced rats	Induced/control
Body weight (g)	185 ± 4 (7)	157 ± 8 (18)	0.85
Liver weight			
g	7.7 ± 0.7 (7)	10.1 ± 0.7 (18)	1.31
% of body weight	4.2 ± 0.4 (7)	6.5 ± 0.3 (18)	1.55
DNA (mg/g liver)	2.55 ± 0.13 (7)	2.41 ± 0.36 (11)	0.95

animals have a poor appetite and do not grow at the same rate as the controls. This effect on appetite is the only 'toxic' effect of *trans*-stilbene oxide which we observed. Expressed as a percentage of body weight, the induced livers are 55% larger than the controls.

Making the assumption that *trans*-stilbene oxide does not significantly affect the degree of hyperploidy in the liver, the DNA content can be used to compare the number of cells per g of induced and control liver. As also shown in Table I, induction has essentially no effect on the amount of DNA per g liver. Thus, the liver hypertrophy caused by treatment of rats with *trans*-stilben oxide appears to be almost entirely the result of a proliferation in the number of hepatocytes.

Phospholipid content of the endoplasmic reticulum after administration of trans-stilbene oxide

In order to determine whether *trans*-stilbene oxide causes hypertrophy of the endoplasmic reticulum, the phospholipid content of liver microsomes from control and induced animals was measured. In addition, epoxide hydratase and glutathione S-transferase were assayed and found to be induced as usual, i.e., to 750 and 400% of control values, respectively (Table II). These increases, together with the expected increase in liver weight (Table I), assured us that maximal induction had been achieved.

We wished to draw conclusions about the phospholipid content of the endoplasmic reticulum from the phospholipid content of the microsomal fraction. In order to do this, it is necessary to know how much of the endo-

TABLE II

EFFECTS OF *trans*-STILBENE OXIDE ON EPOXIDE HYDRATASE, ON GLUTATHIONE *S*-TRANSFERASE, AND ON THE RECOVERY AND CONTAMINATION OF THE MICROSOMAL FRACTION FROM RAT LIVER

The values presented are the means ± S.D. of the number of animals given in parentheses.

Enzyme	Control rats	Induced rats	Induced/control
Epoxide hydratase [a]	3.22 ± 0.31 (7)	24.1 ± 4.0 (11)	7.48 [h]
Glutathione *S*-transferase [b]	24.5 ± 3.8 (7)	100 ± 8.0 (11)	4.08 [h]
NADPH-cytochrome *c* reductase [c]			
microsomal	1.64 ± 0.28 (7)	3.81 ± 0.69 (11)	2.32 [h]
total	3.69 ± 0.57 (7)	7.93 ± 1.19 (11)	2.15 [h]
recovery [d]	44.4%	48.0%	1.08
Microsomal cytochrome *c* oxidase [e]	1.22 ± 0.17 (7)	0.66 ± 0.08 (11)	0.54 [h]
Microsomal AMPase [f]	2.23 ± 0.11 (7)	1.20 ± 0.15 (11)	0.54 [h]
Microsomal acid phosphatase [f]	1.23 ± 0.26 (7)	0.99 ± 0.09 (11)	0.80 [g]

[a] nmol styrene glycol formed/min per mg microsomal protein.
[b] nmol 1,2-dichloro-4-nitrobenzene conjugated/min per mg supernatant protein.
[c] μmol cytochrome *c* reduced/min per g liver (total activity was determined on the homogenate used to prepare microsomes after a short sonication).
[d] (Microsomal activity/total activity) × 100.
[e] μmol cytochrome *c* oxidized/min per g liver.
[f] μmol P_i liberated/min per g liver.
[g] Difference significant at the $P < 0.02$ level, as determined by Student's *t*-test.
[h] Difference significant at the $P < 0.001$ level, as determined by Student's *t*-test.

plasmic reticulum is recovered in the microsomes and how much contamination by other organelles is present. Using NADPH-cytochrome *c* reductase as marker, a 45% recovery was found in liver microsomes from control animals and a 48% recovery in induced microsomes (Table II). In addition, it is clear that NADPH-cytochrome *c* reductase activity is induced to about 220% of the control level by *trans*-stilbene oxide. This agrees well with the extent to which cytochrome *P*-450 is increased [3] and suggests that this inducer affects both protein components of the cytochrome *P*-450 system similarly.

Surprisingly, liver microsomes prepared from rats treated with *trans*-stilbene oxide are significantly less contaminated by mitochondria (as indicated by the levels of cytochrome *c* oxidase), by fragments of the plasma membrane (AMPase), and by lysosomes (acid phosphatase) than are control microsomes (Table II). Intact mitochondria, plasma-membrane fragments and lysosomes comprise roughly 6, 8 and 1%, respectively, of the control microsomal phospholipid [20]. Consequently, the decreased contamination would be expected to decrease the phospholipid content of induced microsomes by approx. 6%.

Nonetheless, the phospholipid content of induced microsomes is slightly, but significantly higher than that of control microsomes (Table III). When these values are converted into phospholipid content of the endoplasmic reticulum (see legend to Table III), taking both recovery and contamination into consideration, *trans*-stilbene oxide is found to cause about a 25% hypertrophy in the phospholipid bilayer of this organelle. Such a small change would not be easily detected by electron microscopy; and, indeed, it has been

TABLE III

EFFECT OF *trans*-STILBENE OXIDE TREATMENT ON THE PHOSPHOLIPID AND CHOLESTEROL CONTENTS OF RAT LIVER MICROSOMES

The values presented are the means ± S.D. for the number between control and induced rats of animals given in parentheses. All differences between control and induced rats are significant at the $P < 0.001$ level, as determined by Student's *t*-test.

	Control rats	Induced rats	Induced/control
Phospholipid [a]			
microsomal	8.27 ± 0.68 (7)	10.6 ± 1.1 (11)	1.28
endoplasmic reticulum [b]	14.8 ± 1.3 (7)	18.8 ± 2.0 (11)	1.27
Cholesterol [c]			
microsomal	813 ± 76 (7)	587 ± 49 (11)	0.72
endoplasmic reticulum [d]	1830 ± 171 (7)	1322 ± 110 (11)	0.72
Molar ratio of cholesterol: phospholipid in microsomes	0.25	0.14	0.58

[a] μmol lipid phosphorus/g liver.

[b] Calculated as follows:

$$\frac{\text{microsomal phospholipid}}{\text{\% recovery of NADPH-cytochrome } c \text{ reductase in the microsomes (see Table II)}} \times \text{\% of microsomal phospholipid which originates from the endoplasmic reticulum (approx. 80\% for control and 85\% for induced; see text).}$$

[c] μg/g liver wet weight.

[d] Calculated as follows:

$$\frac{\text{microsomal cholesterol}}{\text{\% recovery of NADPH-cytochrome } c \text{ reductase in the microsomes}}$$

reported on the basis of electron micrographs that *trans*-stilbene oxide does not cause hypertrophy of the endoplasmic reticulum [2].

Cholesterol content of the endoplasmic reticulum after administration of trans-stilbene oxide

It can be seen from Table III that administration of *trans*-stilbene oxide also decreases the cholesterol content of rat liver microsomes. Since the cholesterol content decreases while the phospholipid content increases, the ratio between these two lipid components is lowered more than 40% (Table III).

The plasma membrane is rich in cholesterol and part of the decrease in this component after induction may result from the reduced contamination of the microsomal fraction by fragments of the plasma membrane. Colbeau et al. [21] have reported that the molar ratio of cholesterol-to-phospholipid in the rat liver plasma membrane is between 0.46 and 0.76. Using the highest of these figures together with the value of 8% for contamination of microsomes by plasma membrane (see above), it can be calculated that roughly 25% of the cholesterol in the control microsomal fraction is present in plasma-membrane fragments. Consequently, a 46% decrease in this contamination would result in a 12% decrease in the cholesterol content of the microsomal fraction. This constitutes less than half of the 28% decrease which is seen after induction with *trans*-stilbene oxide. It should be remembered that this is an estimation of the greatest possible extent to which changes in contamination by plasma membrane could affect the microsomal cholesterol content after induction.

Phospholipid composition of the endoplasmic reticulum after administration of trans-stilbene oxide

Table IV shows the phospholipid compositions of the same microsomes used for the measurements presented in Tables II and III. No significant effect on the glycerophospholipids was observed, but the percentage content of sphingomyelin was decreased 42% as a result of the induction.

15—30% of the total phospholipids of the plasma-membrane fraction from rat liver is sphingomyelin [21—23]; and plasma membranes are thus particularly rich in this phospholipid. Since contamination of induced microsomes by plasma-membrane fragments is less than that of control microsomes, calculations were made to determine whether the decreased sphingomyelin content

TABLE IV

THE EFFECT OF INDUCTION WITH *trans*-STILBENE OXIDE ON THE PHOSPHOLIPID COMPOSITION OF RAT LIVER MICROSOMES

The values given are percentages of the total phospholipid and are the means ± S.D. for five animals.

Phospholipid	Control microsomes	Induced microsomes
Phosphatidylcholine	58.1 ± 2.2	60.6 ± 1.7
Phosphatidylethanolamine	23.0 ± 3.6	24.1 ± 2.5
Phosphatidylserine + phosphatidylinositol	12.6 ± 1.2	11.8 ± 1.0
Sphingomyelin	6.2 ± 0.4	3.6 ± 0.5 [a]

[a] Difference significant at the $P < 0.001$ level, as determined by Student's *t*-test.

TABLE V

THE EFFECT OF INDUCTION WITH *trans*-STILBENE OXIDE ON THE FATTY-ACID COMPOSITION OF THE TOTAL LIPID FRACTION OF RAT LIVER MICROSOMES

Rats were induced, microsomes prepared, and fatty-acid composition of the total lipid fraction determined as described in Materials and Methods. The values given are percentages of the total fatty acids, as determined by the area under the peaks on the gas chromatogram. These values are the means ± S.D. for six animals.

Fatty acids	Control microsomes	Induced microsomes
16:0	22.1 ± 1.8	22.2 ± 1.8
16:1	0.8 ± 0.3	0.5 ± 0.1
18:0	15.7 ± 1.0	17.8 ± 1.4 [b]
18:1	8.7 ± 1.1	7.2 ± 0.6 [b]
18:2	19.1 ± 2.5	16.1 ± 1.3 [a]
20:3	0.9 ± 0.2	0.9 ± 0.2
20:4	20.6 ± 1.6	23.2 ± 1.1 [c]
20:5	1.2 ± 0.2	1.0 ± 0.2
22:5	1.3 ± 0.5	1.4 ± 0.4
22:6	8.9 ± 1.2	9.9 ± 0.5
Unsaturated	61.5	60.2
Saturated / Unsaturated	0.614	0.664
Monoenoic	9.5	7.9
Polyunsaturated	52.0	52.5
18:2/20:4	0.927	0.694

[a] Difference significant at the $P < 0.05$ level, as determined by Student's *t*-test.
[b] Difference significant at the $P < 0.02$ level, as determined by Student's *t*-test.
[c] Difference significant at the $P < 0.01$ level, as determined by Student's *t*-test.

TABLE VI

THE EFFECT OF INDUCTION WITH *trans*-STILBENE OXIDE ON THE FATTY-ACID COMPOSITION OF THE PHOSPHATIDYLCHOLINE OF RAT LIVER MICROSOMES

Rats were induced, microsomes prepared, phospholipids separated, and the fatty-acid composition of the phosphatidylcholine determined as described in Materials and Methods. The values given are percentages of the total fatty acids, as determined by the area under the peaks on the gas chromatogram. These values are the means ± S.D. for five animals.

Fatty acids	Control microsomes	Induced microsomes
16:0	27.8 ± 2.8	29.1 ± 3.5
16:1	0.9 ± 0.5	0.8 ± 0.3
18:0	18.8 ± 0.4	17.5 ± 1.3
18:1	5.7 ± 0.6	5.1 ± 0.9
18:2	14.7 ± 1.4	15.4 ± 2.3
20:3	0.6 ± 0.2	0.4 ± 0.1
20:4	22.5 ± 1.9	24.6 ± 2.0
20:5	1.3 ± 0.5	1.2 ± 0.4
22:6	7.9 ± 0.9	5.8 ± 1.0
Unsaturated	53.6	53.3
Saturated / Unsaturated	0.869	0.874
Monoenoic	6.6	5.9
Polyunsaturated	47.0	47.4
18:2/20:4	0.653	0.626

TABLE VII

THE EFFECT OF INDUCTION WITH *trans*-STILBENE OXIDE ON THE FATTY-ACID COMPOSITION OF THE PHOSPHATIDYLETHANOLAMINE OF RAT LIVER MICROSOMES

Rats were induced, microsomes prepared, phospholipid separated, and the fatty-acid composition of the phosphatidylethanolamine determined as described in Materials and Methods. The values given are percentages of the total fatty acids, as determined by the area under the peaks on the gas chromatogram. These values are the means ± S.D. for five animals.

Fatty acids	Control microsomes	Induced microsomes
16:0	27.5 ± 1.8	24.3 ± 2.8
16:1	2.6 ± 0.7	1.7 ± 0.8
18:0	23.0 ± 1.8	24.2 ± 1.2
18:1	4.9 ± 1.0	4.3 ± 0.9
18:2	8.9 ± 1.0	8.2 ± 1.4
20:4	19.5 ± 2.4	21.8 ± 1.3
22:6	13.6 ± 0.9	15.6 ± 3.7
Unsaturated	49.5	51.6
$\frac{\text{Saturated}}{\text{Unsaturated}}$	1.02	0.94
Monoenoic	7.2	6.0
Polyunsaturated	42.0	45.6
18:2/20:4	0.456	0.376

could be accounted for by the decreased contamination. Assuming that plasma-membrane phospholipids are 30% sphingomyelin and that 8% of the total phospholipids of control microsomes originates from the plasma membrane (see above), then 2.4% of the control microsomal phospholipids is plasma-membrane sphingomyelin and the corresponding value for induced microsomes is 1.3%. The difference of 1.1% is considerably less than the difference of 2.6% shown in Table III. Thus, at the very most, 40% of the decreased sphingomyelin content after induction results from a decreased contamination of the microsomes by fragments of the plasma membrane.

Fatty acid composition of the endoplasmic reticulum after administration of trans-stilbene oxide

Table V presents the fatty-acid composition of the total lipid fraction isolated from the same microsomes used for the measurements shown in Tables II—IV. As can be seen, the fatty-acid composition is changed by induction with *trans*-stilbene oxide. The percentage contents of stearic acid and arachidonic acid increase, whilst those of palmitic acid and linoleic acid decrease. Accordingly, the ratio of linoleic-to-arachidonic acid decreases to the greatest extent, from 0.927 to 0.694. Meanwhile, the ratio of saturated-to-unsaturated fatty acids does not change significantly.

Tables VI and VII show the fatty-acid composition of phosphatidylcholine and phosphatidylethanolamine, respectively, isolated from the same microsomes. Because of large standard deviations, the differences between control and induced animals were not significant in these cases, but the ratio of C18:2-to-C20:4 has a tendency to decrease, as it did in the case of total lipid.

Discussion

Cell proliferation

The livers of rats treated with *trans*-stilbene oxide increase by about 55% in weight (as expressed as a percentage of body weight). A similar increase in liver weight is observed in connection with induction by phenobarbital [24], but methylcholanthrene does not cause liver hypertrophy [25]. In the case of phenobarbital, each individual hepatocyte appears to increase initially in size and only later in the induction process is the total number of hepatocytes increased [26,27]. *trans*-Stilbene oxide seems to increase the number of hepatocytes without affecting their size.

Hypertrophy of the endoplasmic reticulum

At the same time, phenobarbital treatment causes more than a doubling of the phospholipid content of the liver endoplasmic reticulum [24]. *trans*-Stilbene oxide causes this phospholipid content to increase by only about 25% and methylcholanthrene does not affect it at all [25]. The increase in total microsomal phospholipid caused by phenobarbital has been suggested to be a result of increased phospholipid biosynthesis [28] and/or decreased breakdown [29]; while the increase caused by polychlorobiphenyl seems to result from inhibition of phospholipid catabolism [30]. Which of these processes occurs after administration of *trans*-stilbene oxide remains to be established.

It is tempting to speculate that an increased bilayer area might be required to accommodate the increased amounts of microsomal drug-metabolizing enzymes that result from induction. Judging from a comparison between the specific activity of the homogeneous enzymes and the original microsomes, epoxide hydratase and cytochrome *P*-450 constitute approx. 2 and 3—5%, respectively, of the total protein of the endoplasmic reticulum. Thus, induction of these enzymes by *trans*-stilbene oxide should increase the protein content of the endoplasmic reticulum by approx. 17—19%. Indeed, induced microsomes do contain 26% more protein per g liver than do control microsomes [3]. These figures are in approximate agreement with the observed 27% increase in the phospholipid content of the endoplasmic reticulum.

Cholesterol content of the endoplasmic reticulum

In agreement with our findings for *trans*-stilbene oxide, Davison and Wills [31] have reported that induction with phenobarbital decreases the proportion of cholesterol in the liver microsomal fraction to 73% of the control value. It is well known that smooth microsomes are richer in cholesterol than rough microsomes [21,32] and that phenobarbital causes proliferation of the smooth portion of the endoplasmic reticulum [24]. Therefore, Davison and Wills regarded their findings as unexpected.

Many studies suggest that cholesterol interacts with membrane phospholipids and renders the bilayer more condensed, rigid, and resistant to temperature changes (Refs. 33—35; see also references in Ref. 35). Cholesterol appears to interact with neutral phospholipids in the following order of affinity: sphingomyelin > phosphatidylcholine > phosphatidylethanolamine [36]. This preferential interaction of cholesterol with sphingomyelin is very interesting

in connection with the observation that *trans*-stilbene oxide treatment lowers the contents of both sphingomyelin and cholesterol in the endoplasmic reticulum, without having any effect on the content of other phospholipids (see below).

Phospholipid composition of the endoplasmic reticulum

In agreement with our finding for *trans*-stilbene oxide, it has been reported that treatment of animals with phenobarbital [30,31,37—40], methylcholanthrene [30,40], or polychlorobiphenyl [30] has only a very small effect on the phospholipid composition of liver microsomes. The maintenance of the characteristic phospholipid composition of the endoplasmic reticulum may be indispensable to the performance of its important functions. The one consistent change in phospholipid composition caused by *trans*-stilbene oxide is a decrease in the percentage content of sphingomyelin; this is also the case for phenobarbital [3,38] and polychlorobiphenyl [30].

Methylcholanthrene and polychlorobiphenyl inhibit the synthesis of choline-containing phospholipids at the site of CDP-choline formation [41,42]. However, phosphatidylcholine may also be formed via the *N*-adenosylmethionine-dependent methylation of phosphatidylethanolamine. There are some reports that this methylation activity is increased in the induced state and that the content of phosphatidylcholine is controlled by this reaction [41,43,44]. It seems likely that the increased activity of the methylation pathway can compensate for the decreased synthesis of phosphatidylcholine via CDP-choline; so that the only net result of diminished CDP-choline formation is a reduction in the relative content of sphingomyelin. It remains to be seen whether similar processes explain this reduction in the case of *trans*-stilbene oxide.

Fatty-acid composition of the endoplasmic reticulum

The properties of phospholipids are also greatly influenced by the nature of the fatty-acyl groups they contain. Relative to other subcellular fractions, microsomes are rich in essential fatty acids (i.e., the total contents of linoleic acid and polyenoic acids containing 20 or 22 carbon atoms). The polyenoic-acid content of phospholipids is of special interest in several respects.

Administration of *trans*-stilbene oxide results in a decrease in the microsomal content of linoleic acid and an increase in the microsomal content of arachidonic acid. There are several discrepancies in the literature concerning the effects of other inducers on the fatty-acid composition of microsomes. Davison and Wills [31] found that phenobarbital causes a progressive increase in the linoleic acid content of phosphatidylcholine and phosphatidylethanolamine and a decrease in their contents of arachidonic and docosahexaenoic acid. However, other researchers have failed to detect changes in the fatty-acid composition of different microsomal phospholipids after phenobarbital induction [45—47].

The fatty-acid composition of microsomes can also be altered by giving animals a diet deficient in essential fatty acids. Kaschnitz [48] reported that such a diet caused a 43% decrease in the drug-hydroxylating activity of microsomes and a 27% decrease in the content of cytochrome *P*-450. A marked decrease in the microsomal content of arachidonic acid was also observed

and concluded to be at least partly responsible for the changes in enzyme content and activity. Other observations, e.g., that the fatty-acid composition of microsomal phospholipids does not change after the injection of arachis oil [31] and that essential fatty acids are preferentially esterified to position 2 of phosphoglycerides, also suggest that arachidonic acid may be essential to the functioning of membranes.

There is considerable evidence that the highly specific fatty-acid structures of phospholipids are produced by rearrangement of the acyl groups subsequent to the de novo synthesis of the molecule [49]. In particular, arachidonic acid is known to be introduced into phospholipids by this deacylation-reacylation mechanism [50]. However, treatment with phenobarbital had no effect on the specific activities of monoacylphosphoglyceride acyltransferase and of acyl-CoA hydrolase [47]. It was concluded that the changes in fatty-acid composition caused by this drug probably result from changes in the availability of precursors for acyl-CoA synthesis.

In addition, both *trans*-stilbene oxide and phenobarbital [46,47] cause a decrease in the percentage content of monenoic fatty acid in total microsomal lipid. It has been established that linoleate is converted to arachidonate in microsomes through the process of chain elongation and desaturation. Thus, the increase in arachidonic-acid content caused by *trans*-stilbene oxide may be intimately related to the decrease in the content of monoenoic acid.

Membrane fluidity and biological activity

Three of the most important determinants of membrane fluidity are the molar ratio of cholesterol : phospholipid, the degree of unsaturation of the acyl chains in the membrane phospholipids, and the ratio between the contents of sphingomyelin and of other phospholipids, especially phosphatidylcholine (see Ref. 51 and references contained therein). Treatment of rats with *trans*-stilbene oxide decreases the cholesterol content of the hepatic endoplasmic reticulum, increases the relative content of polyunsaturated fatty acids in the lipids of this organelle, and decreases the relative sphingomyelin content. All three of these changes should lead to an increase in the fluidity of the phospholipid bilayer of the endoplasmic reticulum.

There are indications from a number of different systems that membrane fluidity may be of importance in controlling different membrane functions (see Ref. 51 and references contained therein). The increased activities of the cytochrome *P*-450 system and of epoxide hydratase after *trans*-stilbene oxide induction appear simply to result from increases in the amounts of the proteins involved [1—3]. However, more subtle features of drug metabolism, for example, interactions between NADPH-cytochrome *P*-450 reductase, cytochrome *P*-450, epoxide hydratase, cytochrome b_5, UDPglucuronosyl transferase, etc., in the membrane of the endoplasmic reticulum, might well be affected by the fluidity of the bilayer.

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