Biochimica et Biophysica Acta, 689 (1982) 539–547
Elsevier Biomedical Press

BBA 71287

PHOSPHOLIPID AND ENZYME ARRANGEMENTS OF RAT LIVER ROUGH MICROSOMAL SUBFRACTIONS FROM CONTROL AND METHYLCHOLANTHRENE-TREATED ANIMALS

C. VALTERSSON [a], U. BRUNK [c] and G. DALLNER [a,b]

[a] *Department of Biochemistry, Arrhenius Laboratory, University of Stockholm,* [b] *Department of Pathology at Huddinge Hospital, Karolinska Institutet, Stockholm and* [c] *Department of Pathology, University of Linköping, Linköping (Sweden)*

(Received October 22nd, 1981)
(Revised manuscript received April 26th, 1982)

Key words: Microsomal subfraction; Enzyme heterogeneity; Cytochrome P-450; Phosphatidylethanolamine; Cross-linking; (Rough microsome)

Rough microsomes from rat liver of both control and methylcholanthrene-treated animals were subfractionated on a discontinuous sucrose gradient into three fractions according to their sedimentation velocity. The slowly sedimenting vesicles were enriched in electron transport enzymes, while those in the pellet showed higher phosphatase and ATPase activities. Methylcholanthrene treatment introduced typical changes in enzyme composition, mainly an increase of the cytochrome *P*-448. The individual phospholipids exhibited an identical distribution pattern in the three subfractions and no change occurred after induction with methylcholanthrene treatment. Nearest neighbour analysis of phosphatidylethanolamine with dinitrodifluorobenzene revealed a similar pattern in the enzymatically different subfraction, that is, no cross-linking with phosphatidylserine occurred. One-third of the phosphatidylethanolamine was in monomer and dimer form and about two-thirds was protein linked. When membrane and enzyme synthesis was induced, cross-linking to proteins were substantially decreased. The experiments indicate that the phospholipids are distributed in a homogenous fashion in the lateral plane of the rough microsomal membrane and do not support the possibility that phosphatidylethanolamine is specifically associated with cytochrome *P*-450.

Introduction

The rough endoplasmic reticulum of rat liver is known to exhibit a high turnover rate which may be attributed to the fact that this system produces a number of macromolecules for other intracellular membranes. In this way, the rough endoplasmic reticulum synthesizes proteins and lipids for its own membrane, for other organelles and for secretion [1]. It appears that the individual enzymes or enzyme systems are located in distinct patches in the membrane [2]. This specialization is indicated by the heterogeneous distribution of functions in vesicles after subfractionation of both rough and smooth microsomes [3–6] and this heterogeneity is further accentuated after breakdown of the vesicles into smaller units [7,8]. On the other hand, the lateral distribution of phospholipids in the endoplasmic reticulum membranes has not been studied and it is not known whether these lipids in some way follow the heterogeneous distribution of various established enzymes. It is indicated that induction of the hydroxylating system results in a change of the phospholipid pattern of the microsomes which would propose that the newly appearing enzymes have a specificity in lipid requirement and the lipids are heterogeneously distributed in the lateral plane [9–11]. It has been suggested that the phosphatidylethanolamine has a preferential association with

0005-2736/82/0000–0000/$02.75

cytochrome *P*-450 [12,13]. Phospholipid heterogeneity can be studied in subfractions where heterogenous distribution of enzymes occurs. In spite of the possibility that lipids are mobile in membranes, it is probable that at least a part of individual phospholipids are specifically associated with enzyme proteins [14,15]. Consequently, accumulation of individual enzymic proteins in specific subfractions should result in an enrichment of individual phospholipids and this heterogeneity could be revealed by lipid analysis.

In this study a discontinuous sucrose gradient was used to isolate rough microsomal vesicles from liver of control and methylcholanthrene-treated rats in order to isolate vesicles enriched in enzymes of the hydroxylating system. These subfractions were utilized for phospholipid analysis and parallel measurements of enzyme activities and amounts.

Materials and Methods

Male rats weighing about 90 g were used. The animals were starved for 20 h before killing. Methylcholanthrene was injected intraperitoneally (2 mg/100 g body weight) daily during 5 days and phenobarbital intraperitonally (8 mg/100 g) daily during 3 days. In experiments with microsomes of newborn rats, the liver was removed from 2-day-old rats.

Total rough microsomes were prepared as described previously by prolonged centrifugation on a discontinuous sucrose gradient [16]. The pellet, together with the fluffy layer just above it, was supplemented with 0.05 M sucrose and suspended by hand homogenization. 4 ml of this suspension containing rough microsomes from 2.7 g liver was layered over a discontinuous sucrose gradient consisting of 17 ml 0.9 M and 17 ml 0.6 M sucrose. Centrifugation was performed in an SW 27 rotor (Beckman) at $80000 \times g$ for 1 h. At the end of centrifugation the fat layer on the top was discarded. The upper part, containing about 4/5 of the 0.6 M sucrose (called top fraction) and the lower part, consisting of about 1/5 of the 0.6 M sucrose together with the 0.9 M sucrose (called the middle fraction), were removed and the particulate components were pelleted without dilution by centrifugation at $105000 \times g$ for 1 h. These two fractions together with the pellet obtained from the subfractionation on the discontinuous gradient were suspended in 0.15 M Tris-HCl, pH 8.0 and recentrifuged at $105000 \times g$ for 45 min. The pellets were resuspended in 1 ml 0.25 M sucrose and used for the analysis. The fractionation procedure is schematically illustrated in Fig. 1.

Protein was determined according to Lowry et al. [17] with bovine serum albumin as a standard. RNA was determined by the orcinol method [18]. Enzyme activities and amounts were determined by previously described methods [19,20].

Total phospholipids were extracted, partitioned and analysed as described previously [21]. Separation of individual phospholipids were made by two-dimensional thin-layer chromatography on silica gel-coated plates (Merck).

The spots were visualized by iodine vapor, scrapped off and hydrolysed overnight on a sand bath in the presence of 1 ml perchloric acid. Aliquots were evaporated by heating. After addition of 50 μl 2.5 M H_2SO_4 and 0.2 ml H_2O, the tubes were placed in a boiling water bath for 10 min. The mixture was supplemented with 0.5 ml H_2O, 50 μl 2.5% ammonium molybdate and 50 μl 10% ascorbic acid. After heating at 45°C for 20 min, the absorbance was measured at 820 nm.

The procedure of the cross-linking studies of microsomes with difluorodinitrobenzene (DFDNB)will be described elsewhere [22]. The interaction of this probe with membrane components of erythrocytes was studied previously in detail and found to give reliable and reproducible results [23]. Shortly, the incubation medium contained 40 mM NaCl, 120 mM $NaHCO_3$, pH 8.5, 8 mM EDTA, 0.25 M sucrose, 11.5 mg microsomal protein and an amount of DFDNB which is given in the individual experiments in a final volume of 4 ml. After incubation for 17 h at 20°C the medium was diluted with 5 ml cold 0.25 M sucrose and centrifuged at $105000 \times g$ for 45 min. The pellet was extracted with chloroform/methanol (2:1, v/v) and the individual dinitrobenzene derivatives were isolated by thin-layer chromatography.

For electron microscopy the fractions were pelleted and fixed in 1% OsO_4 in 0.15 M sodium cacodylate-HCl buffer, pH 7.4, for 60 min at 4°C. The fixed pellets were dehydrated and embedded in epoxy resin.

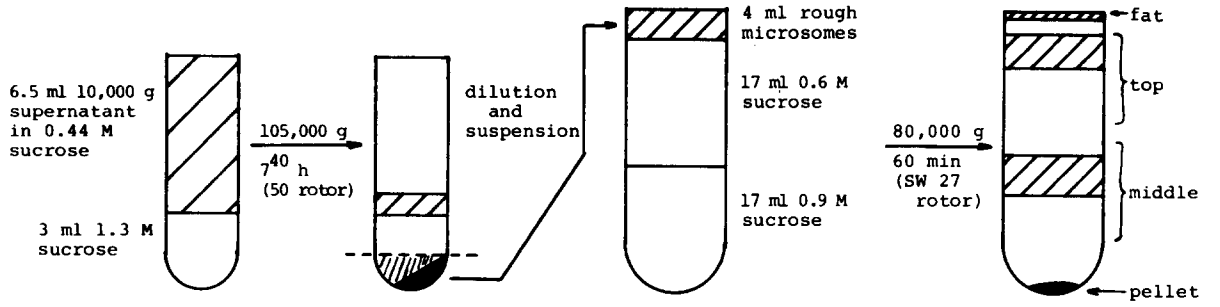

Fig. 1. Schematic representation of the fractionation procedure used to prepare rough microsomal subfractions.

Results

Rough microsomal vesicles have a high equilibrium density ranging between 1.18 and 1.25. The separation system we have used here is a discontinuous gradient in the low-density range, and therefore the separation of vesicles takes place almost exclusively on the basis of size. Previous experiments, using rate differential centrifugation, employed continuous sucrose gradients in the range used here [3]. The discontinuous gradient applied was arbitrarily chosen in order to separate rough microsomes into low and high sedimentation velocity particle fractions. In this way it was possible to rapidly and simply obtain vesicles with different physicochemical and enzymic properties in amounts which allow chemical measurement such as separation of individual phospholipids. The two layers of sucrose and the centrifugation conditions were selected after a series of experiments in which distribution of various enzymes were followed.

Electron microscopical investigation demonstrated intact vesicles with ribosomes in all three fractions. The upper fraction contains vesicles with relatively homogeneous size and most vesicles have attached ribosomes with a varying number (Fig. 2). There are a few vesicles without ribosomes which are either smooth contaminants or are from the Golgi system. The vesicles in the middle fraction are somewhat larger than those in the upper fraction (Fig. 3). Most of the vesicles are non-broken, many have a content and most of them have attached ribosomes. In the pellet, the vesicle size variation is pronounced but in general these are considerably larger than the vesicles in the former two fractions (Fig. 4). Most vesicles are intact, have bound ribosomes and some of them are heavily coated with ribosomes. The subfractions prepared from the liver of the methylcholanthrene-treated rats were similar in appearance to those of the control rats in Fig. 2–4. Fig. 5 shows the pellet obtained after subfractionation of rough microsomes from methylcholanthrene-treated rats. The size distribution of the vesicles and their ribosome coating is similar to that of the control, indicating the centrifugation procedure employed may be used also for subfractionation of rough microsomes from treated rats.

The chemical composition of the isolated fractions appears in Table I. There are two main separated fractions on protein and phospholipid basis. These were recovered at the top and in the pellet, but a somewhat smaller amount of protein is in the intermediate position. The protein/phospholipid ratio is not very different in the fractions which were washed with alkaline buffer in order to remove adsorbed proteins. Relatively high amount of RNA is present in all fractions. The RNA/phospholipid ratio is somewhat higher in the upper two fractions in comparison with the pellet. In some experiments the three subfractions were placed on a continuous sucrose gradient in which the vesicles are sedimented but the ribosomes retained on the gradient [24]. After this centrifugation the amount of RNA decreased in the upper two fractions and the RNA/phospholipid ratio was similar to that in the pellet. The main reason for differences in RNA content among subfractions can therefore be explained by the presence of free ribosomes. After methylcholanthrene treatment there is a moderate increase of the number of

TABLE I

CHEMICAL COMPOSITION OF ROUGH MICROSOMAL SUBFRACTIONS

Fractions were prepared as described in Materials and Methods. The values are the means ± S.E. of seven experiments expressed in mg/g liver.

Fractions	Protein	Phospholipid	RNA	Protein: phospholipid (w/w)	RNA: phospholipid (w/w)
Control					
Total	6.3±0.51	2.7±0.11	1.02±0.13	2.3	0.38
Top	2.8±0.29	1.0±0.13	0.52±0.07	2.8	0.52
Middle	0.8±0.10	0.3±0.03	0.14±0.02	2.7	0.46
Pellet	2.0±0.11	0.8±0.11	0.30±0.04	2.5	0.38
Methylcholanthrene-induced					
Total	10.6±1.51	3.8±0.47	1.52±0.10	2.8	0.40
Top	4.4±0.48	1.5±0.15	0.74±0.06	2.9	0.42
Middle	2.1±0.16	1.0±0.08	0.34±0.03	2.1	0.34
Pellet	2.5±0.15	1.0±0.12	0.34±0.04	2.4	0.34

vesicles mainly in the top and in the middle fractions. The protein/phospholipid and the RNA/phospholipid ratios are unchanged in comparison with the control indicating the lack of significant chemical changes during the induction.

The enzymes studied are distributed unevenly among the subfractions (Table II). NADH- and NADPH-cytochrome *c* reductase and glucose-6-phosphatase activities are higher in the top and in some extent also in the middle fractions than in the pellet. Similarly, the specific amount of cytochrome b_5 and *P*-450 in the top fraction is 2–3-times higher. On the contrary, both phosphatase and ATPase activities showed highest activity in the pellet. Methylcholanthrene treatment causes the specific activities of NADH- and NADPH-cytochrome *c* reductase, ATPase and phosphatase to decrease. This decrease is not

TABLE II

ENZYME COMPOSITION OF ROUGH MICROSOMAL SUBFRACTIONS

The values are the means ± S.E. of six experiments. Cyt, cytochrome; G6Pase, glucose-6-phosphatase.

Fractions	NADH-cyt *c* reductase [a]	NADPH-cyt *c* reductase [a]	Cyt b_5 [b]	Cyt *P*-450 [b]	ATPase [c]	Phosphatase	G6Pase [c]
Control							
Total	1.9±0.13	0.50±0.04	0.7±0.06	1.2±0.11	5.6±0.51	2.5±0.25	42.0±5.1
Top	2.2±0.20	0.72±0.06	1.1±0.16	1.7±0.13	4.4±0.50	1.6±0.22	56.0±4.8
Middle	1.5±0.21	0.55±0.06	0.5±0.05	0.9±0.10	5.8±0.76	2.5±0.23	42.0±3.2
Pellet	1.5±0.22	0.33±0.03	0.5±0.07	0.6±0.09	6.8±0.66	3.3±0.20	38.0±2.8
Methylcholanthrene-induced							
Total	1.5±0.15	0.34±0.04	1.3±0.13	4.1±0.42	2.2±0.20	1.0±0.11	42.0±4.2
Top	1.6±0.19	0.44±0.06	1.5±0.10	6.4±0.51	2.0±0.22	0.4±0.05	44.0±3.7
Middle	1.2±0.15	0.21±0.02	1.1±0.12	3.7±0.33	2.0±0.17	0.9±0.07	40.0±4.3
Pellet	1.1±0.09	0.21±0.03	1.2±0.12	3.0±0.34	3.4±0.34	1.3±0.02	34.0±3.9

[a] μmol cyt *c* reduced per min per mg phospholipid.
[b] nmol/mg phospholipid.
[c] μmol P_i/20 min per mg phospholipid.

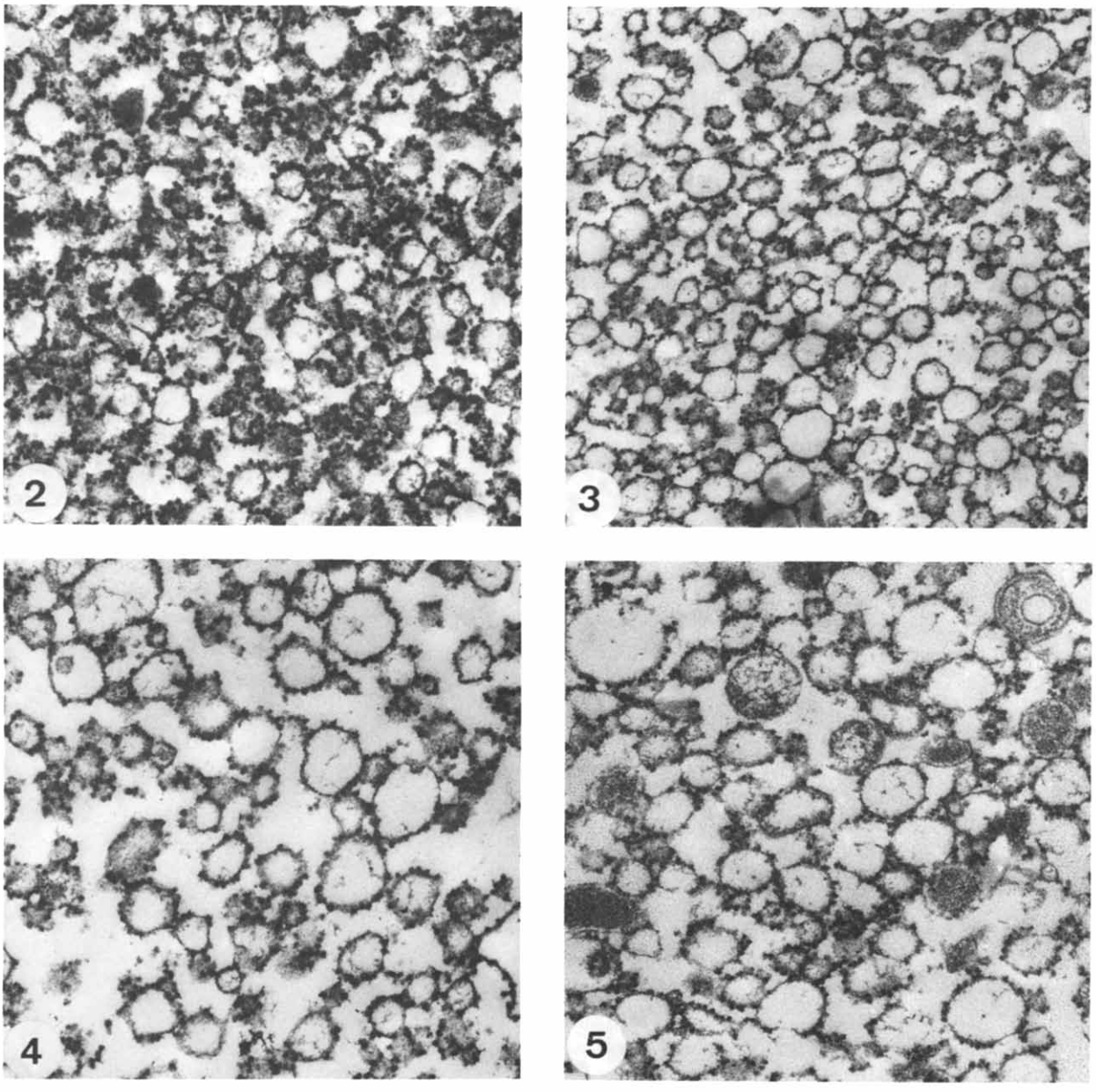

Fig. 2. The top fraction after subfractionation from control rat. Vesicles with attached ribosomes. A number of free ribosomes and a few contaminating smooth vesicles. ×40000.

Fig. 3. The middle fraction after subfractionation from control rat. Intact vesicles, most of them are heavily coated with ribosomes. ×40000.

Fig. 4. The pellet fraction after subfractionation from control rat. Mostly vesicles with a larger size than in the other two fractions. No smooth vesicles are visible. ×40000.

Fig. 5. The pellet fraction after subfractionation from methylcholanthrene-treated rat. The appearance of the vesicles is similar to that of the vesicles from control rat. A few contaminating lysosomes. ×40000.

limited to one type of membrane since it is observed in all subfractions. The amount of both cytochromes is significantly increased in all subfractions of rough microsomes. Cytochrome *P*-450 (measured as *P*-448) increases 4–5-times in the fractions and the specific amount in the top fraction is more than twice as much as in the pellet after induction.

Rough microsomal membranes are rich in phosphatidylcholine and phosphatidylethanolamine (PE) which make up 55% and 28% of the total phospholipids, respectively (Table III). Phosphatidylinositol, particularly phosphatidylserine, and sphingomyelin are present only in small amounts. Induction results in an increase of all the individual lipids in the total rough microsomes, thus maintaining the percent distribution of the lipids. The phospholipid pattern in the membranes of the three subfractions is identical both qualitatively and quantitatively (Table III). After induction there is a sizeable increase in the amount of lipids both in the top and in the middle fractions while the amount is practically unchanged in the pellet. The distribution of the individual lipids in all subfractions is identical indicating that the induction resulted in a change in membrane amount and enzyme content but not in membrane phospholipid composition.

In order to obtain information about the intramembranous arrangement of PE in the subfractions, experiments with the bifunctional reagent DFDNB were performed and the interaction of PE was analyzed (Table IV). In total microsomes about 2/3 of the lipid is cross-linked to protein and the major part of the remaining PE is in monomer form when 0.75 mM DFDNB is employed. This concentration of reagent was found to give, after titration, maximal cross-linking of PE to protein. The results were very similar when the three rough microsomal subfractions were incubated. The majority of PE could be cross-linked to protein, and the amount of dimers, PE-DNP-PE and PE-DNP-PS, represented only minor fractions.

The enzyme composition of microsomal membranes is greatly changed by methylcholanthrene induction as described in Table V. It is well established that in newborn rat several microsomal enzymes are synthesized in accelerated extent after birth while others increase slowly [25]. Also, after phenobarbital induction the enzymes of the hydroxylating system are trebled in the first 3 days [26]. As indicated in Table V, none of these processes of increased enzyme synthesis change the PE content in the membrane. However, the cross-linking pattern after incubation with DFDNB displays a considerable change. In all three cases, characterized by enzyme and also by membrane induction, the interaction of PE with proteins decreases and the amount of monomers and dimers increases.

TABLE III

PHOSPHOLIPID COMPOSITION OF ROUGH MICROSOMAL SUBFRACTIONS

The lipids were separated by two-dimensional thin-layer chromatography, developed with chloroform/methanol/acetic acid/water (106:50:12:6, v/v) in the first direction and with chloroform/acetone/methanol/acetic acid/water (50:20:10:10:5, v/v) in the second direction. The individual spots were used for phosphate measurements. The results are the means of five experiments. PC, phosphatidylcholine; PE, phosphatidylethanolamine; PI, phosphatidylinositol; PS, phosphatidylserine; Sph, sphingomyelin.

Fractions	Phospholipid	Lipid P			
		Control		Methylcholanthrene-induced	
		µg/g liver	% of total	µg/g liver	% of total
Total	PC	47.5	55	60.8	50
	PE	24.2	28	36.5	30
	PI	9.5	11	14.6	12
	PS	3.4	4	6.1	5
	Sph	2.6	3	3.6	3
Top	PC	18.6	58	28.3	59
	PE	9.6	30	13.9	29
	PI	2.3	7	4.3	9
	PS	1.3	4	1.5	3
	Sph	0.7	2	0.5	1
Middle	PC	5.6	56	18.2	57
	PE	3.1	31	9.6	30
	PI	1.0	10	3.5	11
	PS	0.2	2	1.0	3
	Sph	0.2	2	0.3	1
Pellet	PC	14.6	57	17.9	56
	PE	7.2	28	8.6	27
	PI	2.0	8	2.9	9
	PS	1.3	5	1.6	5
	Sph	0.5	2	1.0	3

TABLE IV

CROSS-LINKING OF PHOSPHATIDYLETHANOLAMINE IN ROUGH MICROSOMAL SUBFRACTIONS WITH 1,5-DIFLUORO-2,4-DINITROBENZENE (DFDNB)

The individual fractions were incubated with 0.75 mM DFDNB and after lipid extraction the individual lipid derivatives were separated on two-dimensional thin-layer chromatography on silica gel plates (Merck). The chromatogram was developed in the first direction with chloroform/methanol/water (130:45:8, v/v) and in the second direction with chloroform/acetone/methanol/acetic acid/water (50:20:10:10:5, v/v). The values are the means of four experiments. PE, phosphatidylethanolamine; PS, phosphatidylserine; FDNP, fluorodinitrophenyl; DNP, dinitrophenyl.

Fractions	Percentage of total PE			
	Monomer PE-FDNP	Dimer PE-DNP-PE	Dimer PE-DNP-PS	Protein PE-DNP-protein
Total microsomes	26	9	2	63
Rough microsomes				
Top	25	10	2	63
Middle	24	12	2	64
Pellet	27	8	3	65

TABLE V

INTERACTION OF 1,5-DIFLUORO-2,4-DINITROBENZENE WITH MICROSOMAL PHOSPHATIDYLETHANOLAMINE AFTER VARIOUS TYPES OF INDUCTION

Total microsomes were prepared from the control, newborn and drug-treated rat livers and incubated with the DFDNB concentration that gave a maximal cross-linking of PE to protein. The probe concentration in the control microsomes was 0.75 mM and for newborn, methylcholanthrene- and phenobarbital-induced microsomes 0.5 mM DFDNB was used. The values are the means of six experiments. PE, phosphatidylethanolamine; PS, phosphatidylserine; PL, phospholipid; FDNP, fluorodinitrophenyl; DNP, dinitrophenyl.

Rat	Treatment	PE % of total PL	Percentage of total PE			
			Monomer PE-FDNP	Dimer PE-DNP-PE	Dimer PE-DNP-PS	Protein PE-DNP-protein
Adult	None	28	26	9	2	63
Newborn (2-day-old)	None	27	33	22	3	42
Adult	Methylcholanthrene	30	32	17	2	48
Adult	Phenobarbital	28	32	17	2	39

Discussion

A simple procedure for the isolation of three rough microsomal subfractions from rat liver is described. The method showed three fractions to have an identical phospholipid but a heterogenous enzyme distribution. Both total and rough microsomes were previously subfractionated with different types of gradient centrifugation [2]. It was established that both microsomal fractions contain vesicles with heterogenous enzymic composition. The results of our experiments are in agreement with those of previous ones and demonstrate that heterogeneity is a relative concept. No single subfractions of microsomes contain only one set of enzymes because of the limited size of individual

enzyme patches within the vesicle membrane structure. The individual subfractions in these experiments were enriched 2–3-times in respect to enzyme activity or content in comparison with an other subfraction which is in agreement with the heterogeneity obtained in other investigations [3,5]. An arbitrary discontinuous gradient is naturally less suitable for the analytical study of enzyme distribution pattern, but such a system is a necessity in studies involving chemical measurement. Some phospholipids are present in microsomes only as 2–3% of the total and their quantitative analysis is limited by the amount or nature of the microsomal material. The procedure described in this paper represents a simple way to separate the main subfractions of rough microsomes.

In contrast to the enzymic heterogeneity, the isolated subfractions exhibit a homogenous distribution of the different types of phospholipids. The conclusion is, therefore, that the microsomal membrane in the lateral plane does not display a heterogeneous distribution of phospholipids which is in sharp contrast to the protein pattern. One may argue that submicrosomal particles can display some lipid heterogeneity since enzyme heterogeneity increases when microsomal vesicles are fragmented and the submicrosomal particles are subfractionated. However, there is no reason to believe that enzyme and lipid heterogeneity is a parallel phenomenon.

One of the main functions of phospholipids in membranes is regulation of the activities of integral membrane enzymes [27]. If a large amount of lipids is associated functionally with enzymes and these enzymes possess a specificity for a type of phospholipid, one would expect compartmentalization and probably also heterogeneous distribution in the lateral plane. In fact, it has been proposed, based on experiments with reconstituted model membranes, that the main function of phosphatidylethanolamine is the association with and activation of cytochrome *P*-450 [12,13]. This cytochrome represents as much as 4–5% of the microsomal protein and after induction its amount increases 3–4-fold [28]. The enzyme should interact with the major part of the PE if this lipid is the specific component of the lipid *P*-450-protein complex. Since we do not know the number of lipid molecules associated with *P*-450 in the membrane, it is not possible to calculate the amount of PE which is utilized for activation of the hydroxylation system, but this portion of PE would probably represent a major part of the total. If this is the case one would expect some heterogeneity concerning distribution of PE in the rough microsomal subfractions where *P*-450 distribution is highly heterogeneous particularly after methylcholanthrene treatment. In our analysis we were unable to detect a distribution pattern of PE which indicates that *P*-450-rich subfractions are preferentially assocaited with the amino lipid. Also, in other types of *P*-450 induction such as phenobarbital treatment and induction after birth, the PE amount in the membrane is identical with that of the control.

Nearest neighbour analysis of membrane aminophospholipids with bifunctional reagents gave valuable information on the lipid arrangements within erythrocyte membrane [29] and the use of DFDNB gave interesting results concerning microsomal membranes. In direct opposition to the erythrocyte studies, the microsome studies, including total microsomes, rough microsomes and rough microsomal subfractions, all showed that the majority of PE can be cross-linked to proteins and both PE dimers and PE interaction with PS were negligable. On the other hand, induction occurring after birth or xenobiotica change PE arrangement as indicated by the considerably decreased interaction of PE with protein. Subtle changes in the membrane make-up during membrane synthesis can be detected by application of this probe.

The experimental data in this paper are in contradiction with the idea that PE is functionally and structurally associated with cytochrome *P*-450. In all the induction processes studied here, there is a rapid increase in the amount of *P*-450 and at the same time, the PE-DNP-protein complex exhibits a sizeable reduction. The experiments with the cross-linking reagent DFDNB indicate a distribution pattern of PE which differs considerably from that of the erythrocyte membrane. A compartmentalization of phosphatidylinositol and -choline was previously suggested in microsomal membranes [30,31] and this type of arrangement is probably also valid for the microsomal phosphatidylethanolamine.

Acknowledgements

This work was supported by grants from the Swedish Medical Research Council and the Swedish Society of Medical Sciences.

References

1 Palade, G.E. (1975) Science 189, 347–358
2 DePierre, J.W. and Dallner, G. (1975) Biochim. Biophys. Acta 415, 411–472
3 Dallner, G., Bergstrand, A. and Nilsson, R. (1962) J. Cell Biol. 38, 257–276
4 Glaumann, H. and Dallner, G. (1968) J. Lipid Res. 9, 720–729
5 Beaufay, H., Amar-Costesec, A., Thinès-Sempoux, D., Wibo, M., Robbi, M. and Berthet, J. (1974) J. Cell Biol. 61, 213–231
6 Morimoto, T., Matsuura, S., Sasaki, S., Tashiro, Y. and Omura, T. (1976) J. Cell Biol. 68, 189–201
7 Dallman, P.R., Dallner, G., Bergstrand, A. and Ernster, L. (1969) J. Cell Biol. 41, 357–377
8 Winquist, L. and Dallner, G. (1976) Biochim. Biophys. Acta 436, 399–412
9 Feuer, G., Miller, R., Cooper, S.D., De la Iglesia, F.A. and Lumb, G. (1973) Int. J. Clin. Pharmacol. Ther. Toxicol. 7, 13–25
10 Ishidate, K. and Nakazawa, Y. (1976) Biochem. Pharmacol. 25, 1255–1260
11 Suzuki, Y., DePierre, J.W. and Ernster, L. (1980) Biochim. Biophys. Acta 601, 532–543
12 Stier, A., Böstering, B. and Finch, S.A.E. (1978) FEBS Lett. 91, 109–112
13 Stier, A., Kühnle, W., Bösterling, B. and Finch, S.A.E. (1979) in The Induction of Drug Metabolism (Estabrook, R.W. and Lindenlaub, E., eds.), pp. 226–240, F.K. Schattauer Verlag, Stuttgart-New York
14 Fleischer, B., Casu, A. and Fleischer, S. (1966) Biochem. Biophys. Res. Commun. 24, 189–194
15 Grover, A.K., Slotboom, A.J., DeHaas, G.H. and Hammes, G.G. (1975) J. Biol. Chem. 250, 31–38
16 Dallner, G. (1974) Methods Enzymol. 31, 181–201
17 Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265–275
18 Ceriotti, G. (1955) J. Biol. Chem. 214, 59–70
19 Eriksson, L.C. (1973) Acta Pathol. Microbiol. Scand. Suppl. 239, 1–72
20 Beaufay, H., Amar-Costesec, A., Feytmans, E., Thinès-Sempoux, D., Wibo, M., Robbi, M. and Berthet, J. (1974) J. Cell Biol. 61, 188–200
21 Eggens, I., Valtersson, C., Dallner, G. and Ernster, L. (1979) Biochem. Biophys. Res. Commun. 91, 709–714
22 Valtersson, C. and Dallner, G. (1982) J. Lipid Res. 23, 868–876
23 Marinetti, G.-V. and Love, R. (1974) Biochem. Biophys. Res. Commun. 61, 30–37
24 Dallner, G. and Nilsson, R. (1966) J. Cell Biol. 31, 181–193
25 Dallner, G., Siekevitz, P. and Palade, G.E. (1966) J. Cell Biol. 30, 97–117
26 Orrenius, S., Ericsson, J.L.E. and Ernster, L. (1965) J. Cell Biol. 25, 627–639
27 Coleman, R. (1973) Biochem. Biophys. Acta 300, 1–30
28 Werringloer, J. and Estabrook, R.W. (1979) in The Induction of Drug Metabolism (Estabrook, R.W. and Lindenlaub, E., eds.), pp. 269–305, F.K. Schattauer Verlag, Stuttgart-New York
29 Marinetti, G.V. and Crain, R.C. (1978) J. Supramol. Struct. 8, 191–213
30 Brophy, P.J., Burbach, P., Nelemans, S.A., Westerman, J., Wirtz, K.W.A. and Van Deenen, L.L.M. (1978) Biochem. J. 174, 413–420
31 Higgins, J.A. (1981) Biochim. Biophys. Acta 640, 1–15