Biochimica et Biophysica Acta, 729 (1983) 9–16
Elsevier Biomedical Press

BBA 71567

EFFECT OF FATTY ACID DEFICIENCY ON MICROSOMAL MEMBRANE FLUIDITY AND COOPERATIVITY OF THE UDP-GLUCURONYLTRANSFERASE

CELINA E. CASTUMA and RODOLFO R. BRENNER

Instituto de Investigaciones Bioquímicas de La Plata, UNLP-CONICET, Facultad de Ciencias Médicas, UNLP, Calle 60 y 120, 1900 La Plata (Argentina)

(Received May 3rd, 1982)
(Revised manuscript received August 3rd, 1982)

Key words: Fat deprivation; Hill coefficient; Membrane fluidity; UDPglucuronyltransferase; Fatty acid; (Guinea pig liver microsome)

The effect of fat deprivation on microsomal membrane fluidity of guinea-pig livers and the kinetic cooperativity of UDP-glucuronyl transferase towards its natural substrate, the UDP-glucuronic acid, were studied. Fat deprivation in the diet of weanling guinea-pigs evoked a typical essential fatty-acid-deficient pattern in the composition of the microsomal membrane. The unsaturated:saturated fatty acid ratio progressively declined in the membrane during the 21-day period tested. This decline determined a gradual increase in the fluorescence anisotropy (r_s) of the membrane labeled with diphenylhexatriene and the apparent microviscosity of the lipid bilayer calculated from these values increased from 1.1 to 1.8 poise. In addition, when the infinitely slow decaying fluorescence anisotropy (r_∞), which is proportional to the square of the lipid order parameter, was calculated from r_s data, a significant increase in these parameters was also obtained. Furthermore, this decrease of the double bond index:saturated acid ratio of the membrane was associated with a parallel increase in Hill coefficients of the UDP-glucuronyl transferase that gradually lost the negative homotropic effect and cooperativity of UDP-glucuronic acid. The Hill coefficient varied from 0.39 to 0.98 during the 21-day period studied. Our observations indicate on one side that changes in the fat composition of the diet are accompanied by modifications in the lipid composition and fluidity of the microsomal membrane, and the apparent cooperativity of the enzyme. On the other side, they suggest that the evaluation of Hill coefficients of UDP-glucuronyl transferase might be used as a sensitive test to investigate conformational changes in the microsomal membrane of the liver.

Introduction

Consistent evidence has been gathered showing that lipid bilayer fluidity and liquid-crystal gel phase transitions play an important role in modifying the properties of many membrane bound enzymes [1–4]. Phospholipids are predominant constituents of membranes and their fluidity may depend on the fatty acid composition. The unsaturated:saturated fatty acid ratio is a very important factor that determines fluidity changes, and it is known that this ratio is modified by many physiological and pathological agents [5–7].

Many of the changes in membrane conformation and protein-lipid interactions are so subtle that they may not be detected by physical methods. However, Farías et al. [3,8,9] have demonstrated that the evaluation of changes in Hill coefficients of membrane-bound allosteric enzymes is a useful and very sensitive test for detecting and recording membrane conformation modifications and protein-lipid interactions. This method has been applied to erythrocytes and *E. coli* membranes [10,11] but not yet to the microsomal mem-

brane that is our main objective.

UDP-glucuronyl transferase (EC 2.4.1.17) is an hepatic microsomal enzyme which catalyzes the transfer of glucuronic acid from UDP-glucuronic acid to a variety of hydroxyl, carboxyl and amino acceptors [12–14]. The enzyme activity depends on membrane phospholipids [15–17] and in addition, Vessey et al. [18] demonstrated that it shows negative homotropic cooperativity towards its natural substrate, the UDP-glucuronic acid. Therefore, we considered that this enzyme might fulfill our needs and the determination of Hill coefficients for the acid probably served to record modifications in the microsomal membrane. For this purpose, a modification of guinea-pig microsomal membrane of the liver was evoked by a progressive essential fatty acid deficiency and the activity and Hill coefficients of the UDP-glucuronyl transferase were measured. The results were correlated to the fatty acid composition and microviscosity of the membrane measured by a physical method. They showed significant changes in Hill coefficient.

Experimental procedures

Materials. UDP-glucuronic acid (ammonium salt) and *p*-nitrophenol were purchased from Sigma Chemical Company. *p*-Nitrophenol was purified by recrystallization in ethanol/water. 1,6-Diphenyl-1,3,5-hexatriene was purchased from Aldrich Chemical Company.

Animals and diets. Male guinea pigs weighing 100–200 g were divided into two groups after weaning (17 days old). One group was fed a semi-synthetic diet according to Reid and Briggs [19] and the other the same purified diet where sucrose substituted corn oil. The animals were pair-fed and given water ad libitum. They were killed by decapitation on day 7, 14 or 21 after the administration of the diets. The different diets produced no significant effect on the weights of either the animals or livers.

Preparation of liver microsomes. After decapitation, livers were rapidly excised, washed and homogenized with 0.25 M sucrose (1 : 3, w/v). The crude homogenate was centrifuged at $12000 \times g$ for 10 min at 0°C to remove cell debris and mitochondria. The supernatant was filtered through cheesecloth and spun at $105000 \times g$ for 60 min in a Spinco model L2 ultracentrifuge. The microsomal fraction was suspended once with 0.25 M sucrose and centrifuged again. The final pellet was resuspended in the same homogenizing solution (1 : 2, v/v). Protein was determined by the method of Lowry et al. [20]. Microsomes were stored at −70°C and used within 2 weeks. Microsomal aliquots of some of the animals were separated and further purified by the method of Glaumann et al. [21] that includes osmotic shock by suspension in water and gently sonication. This preparation was used to check the membrane purity and absence of trapped non-membrane constituents. Lipid composition and fluorescence anisotropy of this highly purified membrane was not significantly different from those of the washed microsomes. Therefore, washed microsomes were normally used throughout all the experiments.

Enzyme assay. Initial rates of UDP-glucuronyl transferase towards *p*-nitrophenol was determined according to Zakim and Vessey [22]. The activity of the microsomal nucleotide pyrophosphatase was measured in preliminary studies and found to be extremely low in the microsomal preparations used. The incubation mixture contained 50 mM Tris HCl (pH 7.4), 0.4 mM *p*-nitrophenol, 0.2–25 mM UDP-glucuronic acid and 1–1.5 mg microsomal protein. The final volume was 0.5 ml. All assays were run in duplicate.

Fluorescence anisotropy. Fluorescence anisotropy (r_s) was determined according to the fluorescence polarization technique of Shinitzky and Barenholz [23] using the lipid-soluble fluorescent probe, 1.6 diphenyl-1,3,5-hexatriene. All measurements were recorded at 355 nm in an Aminco-Bowman spectrofluorometer equipped with a glass prism. Light scattering was less than 5% and fluorescence values were corrected in accordance. The phospholipid:diphenylhexatriene ratio was always maintained at more than 200:1 (mol:mol) in order to minimize probe-probe interactions and perturbations of the membrane bilayer. Apparent microviscosity values were calculated from the approximate expression

$$\bar{\eta} = \frac{2.4 r_s}{0.362 - r_s}$$

derived from Perrin equation [23], where r_s mea-

sures anisotropy defined as

$$r_s = \frac{I_{\parallel} - I_{\perp}}{I_{\parallel} + 2I_{\perp}}$$

The steady-state fluorescence anisotropy (r_s) was resolved into a quickly decaying component r_f and an infinitely slow decaying component by the procedure of Van Blitterswijk et al. [24] using the empirical curves of these authors. The order parameter (S_{DPH}) that refers to the mean position of the diphenylhexatriene along the lipid acyl chains [24,25] was calculated by the equation $S^2 = r_\infty / r_0$ [24] where r_0 is the limiting anisotropy of the probe considered equal to 0.362.

Lipid analysis. Aliquots of the suspended microsomes were extracted by the procedure of Folch et al. [26]. Fatty acid composition was determined by gas-liquid chromatography of the methyl esters in a Hewlett-Packard apparatus model 5840 A. A 6-mm column filled with 10% SP-2330 on 100-200 Chromosorb WAW was used. The temperature was programmed for a linear increase of 2 K per min from 140°C to 220°C.

Cholesterol was determined by the method of Huang et al. [27] and phospholipid phosphorus by the procedure of Chen et al. [28].

Results

Fatty acid composition of microsomal membrane

Table I shows the fatty acid composition of liver microsomes of guinea pigs fed a fat-free diet after weaning. The comparison of these animals with the controls indicates a progressive development of an essential fatty acid deficiency. Data evidence a gradual decrease of linoleic and arachidonic acids and a gradual increase of oleic and palmitoleic acids that are typical symptoms of early essential fatty acid deficiency. However, this effect is produced without an important increase of 20:3 (ω:9) acid during the period of deficiency investigated. The data of Table I also demonstrate a progressive decrease on the double bond index:saturated fatty acid ratio. This ratio has been used as a chemical measure of membrane fluidity [3]. This assumption is valid when other parameters such as cholesterol:phospholipid ratio remain constant and the fatty acid double bonds

TABLE I

FATTY ACID COMPOSITION OF MICROSOMAL MEMBRANES OF GUINEA PIG LIVERS EXPRESSED IN PERCENT WEIGHT

Minor components make for 100%. Results obtained from a pool of five animals.

Fatty acids	Period of fat deficiency in days			
	0	7	14	21
14:0	0.5	0.6	0.8	1.0
16:0	13.4	14.7	16.2	18.7
16:1	0.7	1.3	2.5	3.4
18:0	24.6	24.4	24.1	24.0
18:1	8.9	12.3	14.4	16.7
18:2	31.5	26.7	21.0	15.6
20:3 (ω:9)	0.8	0.9	1.2	1.3
20:4 (ω:6)	8.9	8.1	7.6	7.0
22:5 (ω:3)	0.6	0.5	0.3	0.2
22:6 (ω:3)	1.6	1.2	0.8	0.7
DBI/SFA [a]	3.00	2.64	2.26	1.89
Phospholipid/ Cholesterol	3.09	3.10	3.10	3.10

[a] $\frac{\text{Double bond index}}{\text{Saturated fatty acid}} = \frac{\Sigma(\text{number unsaturated mol} \times \text{number double bonds})}{\Sigma \text{ number saturated mol}}$.

of compared membranes have the same *cis* structure. In our experiments the cholesterol:phospholipid ratio of both treated and control animals remain fairly constant (Table I) and the diets tested do not contain *trans*-acids. Therefore, the decrease of the double bond index:saturated acid ratio found might indicate a decrease in the lipid bilayer fluidity of the microsomal membrane evoked by the fat-free diet.

Effect of essential fatty acid deficiency on UDP-glucuronyl transferase activity and Hill coefficients

Initial rates of UDP-glucuronyl transferase activity were determined in the microsomes at different periods of fat deficiency. They were measured as a function of the concentration of UDP-glucuronic acid at a fixed concentration of *p*-nitrophenol as aglycone. In normal animals, the Lineweaver-Burk plot presented in Fig. 1 shows a curve that, in accordance with Vessey et al. [18], would indicate a negative homotropic cooperativ-

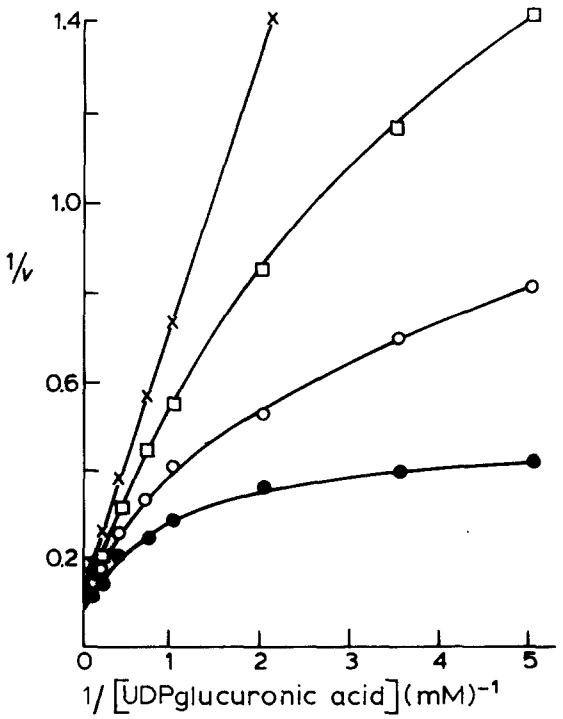

Fig. 1. Double-reciprocal plots for UDP-glucuronyl transferase in hepatic microsomes at different periods of fat deficiency: zero time, ●———●; 7 days, ○———○; 14 days, □———□; and 21 days, ×———×. Incubation mixture contained 0.4 mM *p*-nitrophenol and 1 mg microsomal protein in a final volume of 0.5 ml. The temperature of incubation was 37°C. Aliquots were removed at 4, 8 and 12 min. Remaining nitrophenol was determined at 400 nm. v is expressed as nmol of *p*-nitrophenol conjugated per min per mg microsomal protein. Small differences in the periods of fat free diet feeding using older animals evoked similar curves.

ity at low concentration of substrate in the sequential binding of UDP-glucuronic acid to UDP-glucuronyl transferase. Fat deficiency in the animal diet evoked a change in the curve and progressive elimination of the cooperativity that depended on the period of deficiency. In addition, data of enzyme activity – calculated from the double-reciprocal plot with 0.4 mM *p*-nitrophenol – show a small but progressive decrease from 11.8 in normal microsomes to 10.0, 8.9, 8.0 nmol/min per mg protein in microsomes of animals of 7, 14 and 21 days of deficiency, respectively. Equivalent results were found by Spector et al. [29].

With the kinetic data obtained, Hill coefficients for UDP-glucuronic acid were calculated [30] using the following equation:

$$\log \frac{v}{V - v} = n \log S - \log K_m$$

where: v is the initial rate for each substrate concentration, V, maximal rate; S, substrate concentration; n = Hill coefficient. Hill coefficients so calculated, at different periods of fat deprivation, can be observed in Fig. 2. This parameter changed from 0.39 in control microsomes to 0.98 in microsomes of animals deprived of fat during 21 days. The Hill coefficient correlated ($R = 0.98$) the double bond index:saturated fatty acid ratio of the microsomes during the period studied (Fig. 3).

Fluorescence anisotropy of microsomal membrane

To corroborate that the decrease of double bond index:saturated acid ratio produced by the fat deficiency would correlate a real decline of the fluidity of the membrane, the fluorescence anisotropy (r_s) of the membrane was measured by a physicochemical method. From these data ob-

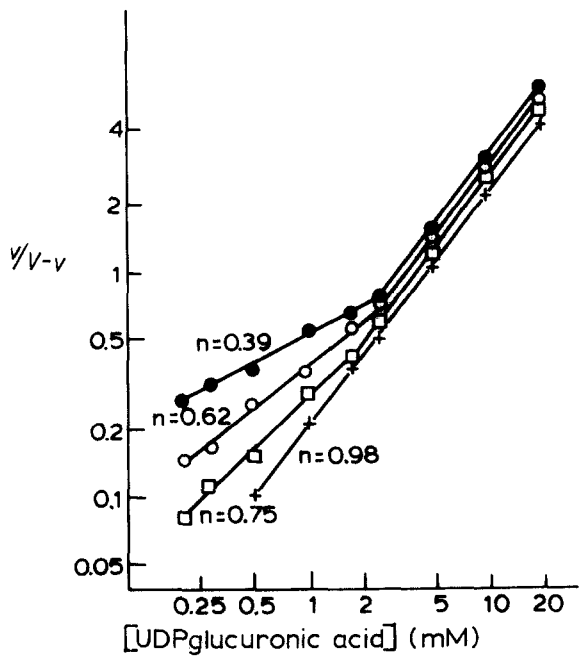

Fig. 2. Hill plots of UDP-glucuronyl transferase at different periods of fat deficiency. Experimental conditions and symbols as in Fig. 1. Under slightly different experimental conditions, similar curves and Hill coefficients of 0.38, 0.63 and 0.90 were found after 14 and 28 days of fat free diet.

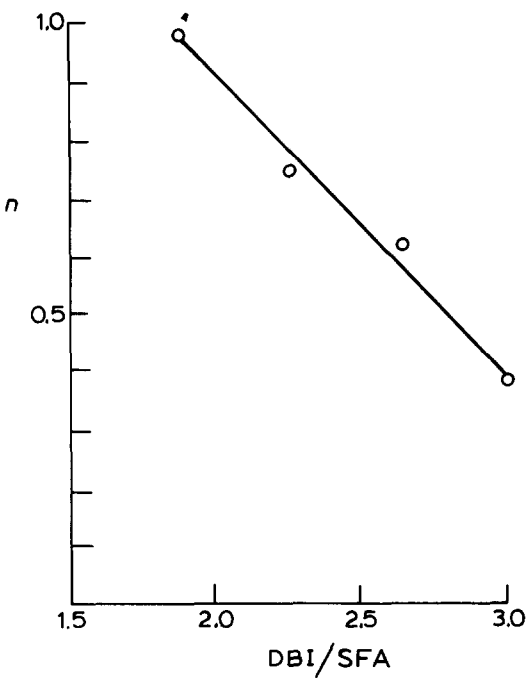

Fig. 3. Correlation of Hill coefficients of UDP-glucuronyl transferase to the double bond index:saturated acid ratio (DBI/SFA) of microsomal membrane. Correlation coefficient $R = 0.98$. Regression coefficients $a = 1.95$, $b = -0.52$. Similar correlations were found in slightly different experiments.

tained by polarized fluorescence using the method of Shinistzky and Barenholz [23], apparent microviscosities were calculated. They are illustrated in Table III and show a progressive increase of the apparent microviscosity with the period of fat deficiency. When r_∞ and S_{DPH} were calculated instead of microviscosities, as indicated in the experimental part, an increase of these parameters was also observed (Table II). These parameters are considered to define the reciprocal of the 'lipid fluidity' [24]. Therefore, whichever is the interpretation of fluorescence anisotrophy data, they would corroborate that the fluidity of the microsomal membrane was decreased by effect of the fat-free diet.

TABLE II

FLUORESCENCE ANISOTROPY AND MICROVISCOSITY OF LIVER MICROSOMES AT 37°C

Fluorescence anisotropy (r_s) was determined by fluorescent polarization as described in experimental part. Microviscosities (η) as well as r_f, r_∞ and S_{DPH} were calculated by the methods of Shinitsky and Barenholz [23] and Van Blitterswijk et al. [24], respectively. Experiments carried out with older animals and small differences in the periods of fatty acid deficiency showed similar results.

Period of fat deficiency (days)	r_s	η (poise)	r_f	r_∞	S_{DPH}
0	0.115	1.1	0.060	0.055	0.390
14	0.135	1.4	0.055	0.080	0.470
21	0.154	1.8	0.049	0.105	0.539

Discussion

It has been repeatedly shown that the lipid composition of the endoplasmic reticulum membrane is easily modified by physiological and non-physiological factors [31,32] and undergoes circadian changes [5]. Therefore, one of the most important problems concerning the endoplasmic reticulum biochemistry is to understand and explain the intimate mechanism of the membrane functions dependent on lipid-protein interactions. These interactions may evoke conformation changes in the proteins involved, thereby altering the kinetic properties of enzymes and transport units. The present study demonstrates that in guinea pig, dietary fat deprivation produces a marked and progressive alteration in the apparent negative cooperativity of microsomal UDP-glucuronyl transferase towards its natural substrate changing the Hill coefficient from 0.39 to 0.98 in 21 days. This change takes place together with a progressive development of an essential fatty deficient pattern in the fatty acid composition of the microsomes correlated to a decrease of the double bond index:saturated acid ratio and a modification of the physical properties of the membrane. This decrease of the unsaturated:saturated fatty acid ratio of the membrane was undoubtedly favoured in the guinea pig by the reduced compensatory biosynthesis of eicosa-5,8,11-trienoic acid (Table I).

The physical properties of the membranes were studied by steady-state fluorescence polarization [23] using 1,6-diphenyl-1,3,5-hexatriene probe. Fluorescence anisotropy data have been repeatedly utilized to assess the fluidity of biological membranes in terms of microviscosity (η), calculated

from Perrin equation. Although the use of a probe in fluorescent measurements measures physical properties only in the region where the dye is incorporated, the solubility and efficiency of the 1,6-diphenyl-1,3,5-hexatriene used has been considered excellent to determine the fluorescence anisotropy [23] of the bulk lipids of membranes. However, the meaning of the microviscosity data, so calculated, must be evaluated with certain qualifications. In first place, the microviscosity data so obtained do not represent absolute values. In second place, the application of Perrin equation assumes an isotropic medium and as Jähnig [33], Heyn [34] and Van Blitterswijk et al. [24] have indicated, membranes are anisotropic. Therefore, microviscosity data calculated by the procedure of Shinitzky and Barenholz [23] using the Perrin equation are not an exact analogue of viscosity. Notwithstanding, from a practical point of view they are useful to compare membrane fluidities, since they have been shown to correlate with acyl chain mobilities determined by other techniques [35] and, with this restriction, they have been used in the present work. Steady-state fluorescence anisotropy (r_s) data obtained in membranes using the diphenylhexatriene probe have been resolved [24,33,34] into a fast kinetic decaying component (r_f) related to rotational relaxation time and, therefore, a more realistic parameter for viscosity estimation, and a static part (r_∞) proportional to the square of the lipid order parameter (S_{DPH}) [33,34].

The calculation of r_∞ and S_{DPH} are possible from r_s data using the procedure of Van Blitterswijk et al. [24] and the empirical curve of r_f vs. r_s reported in their work. Table II illustrates the values of r_∞ and S_{DPH} calculated by this procedure. They indicate that the limiting long time fluorescence anisotropy r_∞ determined exclusively by the microsomal membrane structural order [24] would be the major contributor to the progressive increase of the steady-state fluorescence anisotropy (r_s) caused by the fat-free diet and the changes in the lipidic composition of microsomes. As Van Blitterswijk et al. [24] have stated, the structural order of membrane lipids is related to the degree of molecular packing, and thereby they suggest an inverse relationship to lateral diffusion of membrane lipids and proteins. Accordingly, these authors justify the use of the term fluidity as the reciprocal of the lipid order parameter, S.

In view of the above statements, we can conclude that the fluorescence anisotropy, r_s, data measured in the present experiments, converted to either apparent microviscosities or r_∞ and order parameters (S_{DPH}) shows a physical decrease in the fluidity of the microsomal lipids due to the treatment. These results are in accordance with the decrease of the double bond index:saturated acid ratio considered as an indication of a decrease of the lipid fluidity [3,32]. In consequence, the apparent variation of the Hill coefficient of the UDP-glucuronyl transferase evoked by the fat-free diet would be apparently produced by changes in the lipidic composition and fluidity of the microsomal membrane. More strictly using the Jähnig [33], Heyn [34] and Van Blitterswijk et al. [24] theoretical and empirical analyses of r_s values, these changes could be possibly attributed to a progressive modification in the molecular packing of the lipid bilayer.

These conclusions are supported by the properties of the enzyme already known: (a) the UDP-glucuronyl transferase is an integral protein of the membrane [17]; (b) the activity of the enzyme is constrained by the structure of the phospholipid environment in intact membranes [16], with an specific requirement for phosphatidylcholine [36]; (c) the constraint is relieved by phospholipase A treatment [16]; (d) the reactivation of lipid-depleted enzyme is influenced by the length and degree of unsaturation of the acyl chain of added choline phospholipids [37]; (e) the fluidity of microsomal phospholipid bilayer is involved in the modulation of UDP-glucuronyl transferase [36,38]. Therefore, the results of the present experiment would have two important implications: (1) they would indicate that the conformation and properties of the UDP-glucuronyl transferase, as measured by the Hill coefficients, are smoothly modulated by the fluidity of the lipid environment here evoked by the diet composition; (2) the apparent cooperativity and the determination of Hill coefficients of the liver UDP-glucuronyl transferase may be used with the corresponding qualification as a very sensitive test to detect possible changes in the lipid bilayer of the membrane.

Abundant literature dealing with other mem-

branes supports the application of the cooperative behaviour of membrane-bound enzymes to detect modifications at the membrane fluidity level produced by essential fatty acid deficiency [10,39,40], cholesterol administration [41] and hormonal treatment [42]. The sensitivity of the test has been analyzed by Siñeriz et al. [8]. As stated by Wyman [43], the free energy change involved in an allosteric transition is small, in the order of 1–3 kcal/mol, and Siñeriz et al. [8], using a greatly simplified calculation, estimated that a variation in the interaction energy as low as 700 cal/mol would be enough to cause a significant variation in the Hill coefficient.

The modulation of UDP-glucuronyl transferase by means of changes in the fluidity of the supporting lipid bilayer would have an outstanding physiological importance. The glucuronidation reaction is the phase II step of the biotransformation of drugs, xenobiotics and endogenous substrates which takes place in the endoplasmic reticulum of the cell. It follows the phase I reaction that is evoked by the cytochrome *P*-450-dependent hydroxylation of the substrates and both steps control the detoxication mechanism of the animal [44]. Therefore, dietary changes would be able to modulate this mechanism. Thus, it may be anticipated that agents modifying the fluidity of microsomes would be able to modify the cooperativity of the enzyme and detoxication mechanism. In order to confirm our results, further research is in progress.

Acknowledgments

Skilful technical assistance of Miss Ana Bernasconi is acknowledged. The research was supported by grants form CONICET and CIC of the Buenos Aires Province. R.R.B. is a member of the Carrera del Investigador Científico, CONICET, Argentina.

References

1 Raison, J. (1973) Energetics 4, 285–309
2 Strittmatter, P. and Rogers, M.J. (1975) Proc. Natl. Acad. Sci. U.S.A. 72, 2658–2661
3 Farias, R.N., Bloj, B., Morero, R.D., Siñeriz, F. and Trucco, R.E. (1975) Biochim. Biophys. Acta 415, 231–251
4 Sandermann, H., Jr. (1978) Biochim. Biophys. Acta 515, 209–237
5 Actis, Dato, S.M., Catalá, A. and Brenner, R.R. (1973) Lipids 8, 1–6
6 Peluffo, R.O., Nervi, A.M. and Brenner, R.R. (1976) Biochim. Biophys. Acta 441, 25–31
7 Holloway, C.T. and Garfield, S.A. (1981) Lipids 16, 525–532
8 Siñeriz, F., Farías, R.N. and Trucco, R.E. (1975) J. Theor. Biol. 52, 113–120
9 Farías, R.N. (1980) in Advances in Lipid Research (Paoletti, R. and Kritchevsky, D., ed.), Vol. 17, pp. 258–282, Academic Press, New York
10 Bloj, B., Morero, R.D. and Farías, R.N. (1974) J. Nutr. 104, 1265–1271
11 Morero, H., Siñeriz, F. and Farías, R.N. (1974) J. Biol. Chem. 249, 7701–7706
12 Dutton, G.J. and Storey, I.D.E. (1954) Biochem. J. 57, 275–280
13 Dutton, G.J. (1956) Biochem. J. 64, 693–701
14 Axelrod, J., Inscoe, J.K. and Tomkins, G.M. (1957) Nature 179, 538–539
15 Vessey, D.A. and Zakim, D. (1971) J. Biol. Chem. 246, 4649–4656
16 Zakim, D., Goldemberg, J. and Vessey, D.A. (1973) Eur. J. Biochem. 38, 59–63
17 Pechey, D.T., Graham, A.B. and Wood, G.C. (1978) Biochem. J. 175, 115–124
18 Vessey, D., Goldemberg, J. and Zakim, D. (1973) Biochim. Biophys. Acta 309, 58–66
19 Reid, M.E. and Briggs, G.M. (1953) J. Nutr. 51, 341–354
20 Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265–275
21 Glaumann, H., Bergstrand, R. and Ericsson, J.L.E. (1975) J. Cell. Biol. 64, 356–376
22 Zakim, D.A. and Vessey, D. (1971) J. Biol. Chem. 246, 4649–4656
23 Shinitzky, M. and Barenholz, Y. (1978) Biochim. Biophys. Acta 515, 367–394
24 Van Blitterswijk, W.J., Van Hoeven, R.P. and Van der Meer, B.W. (1981) Biochim. Biophys. Acta 644, 323–332
25 Andrich, M.P. and Vanderkooi, J.N. (1976) Biochemistry, 15, 1257–1261
26 Folch, J., Lees, M., Sloane-Stanley, G.H. (1957) J. Biol. Chem. 226, 497–509
27 Huang, T.C., Chen, C.P., Wefler, V. and Raftery, J. (1961) Anal. Chem. 33, 1405–1406
28 Chen, P.S., Toribara, T.Y. and Warner, H. (1956) Anal. Chem. 28, 1756–1758
29 Spector, A.A., Kaduce, T.L. and Pane, R.W. (1980) L. Lipid Res. 21, 169–179
30 Segel, I.H. (1975) Enzyme Kinetics, pp. 371, John Wiley & Sons, New York
31 Brenner, R.R., Garda, H., De Gómez Dumm, I.N.T. and Pezzano, H. (1981) in Essential Fatty Acids and Prostaglandins, Progress in Lipid Research (Holman, R.T., ed.), pp. 315–321, Pergamon Press, Oxford
32 Lippiello, P.M., Holloway, C.T., Garfield, S.A. and Holloway, P.W. (1979) J. Biol. Chem. 254, 2004–2009
33 Jähnig, F. (1979) Proc. Natl. Acad. Sci. U.S.A., 76, 6361–6365

34 Heyn, M.P. (1979) FEBS Lett. 108, 359–364
35 Suurkuusk, J., Lentz, B.R., Barenholz, Y., Biltonen, R.L. and Thompson, T.E. (1976) Biochemistry, 15, 1393–1401
36 Singh, O.M.P., Graham, A.B. and Wood, G.C. (1981) Eur. J. Biochem. 116, 311–316
37 Frikson, R.M., Zakim, D. and Vessey, D.A. (1978) Biochemistry, 17, 3706–3711
38 Cremmings, J., Graham, A.B. and Wood, G.C. (1980) Biochem. J. 185, 521–526
39 Bloj, B., Morero, R.D., Farías, R.N. and Trucco, R.E. (1973) Biochim. Biophys. Acta 311, 67–79
40 Goldemberg, A.L., Farías, R.N. and Trucco, R.E. (1972) J. Biol. Chem. 247, 4299–4304
41 Bloj, B., Morero, R.D. and Farías, R.N. (1973) FEBS Lett. 38, 101–105
42 Massa, E.M., Morero, R.D., Bloj, B. and Farías, R.N. (1975) Biochem. Biophys. Res. Commun. 66, 115–122
43 Wyman, J. (1963) Cold Spring Harb Symp. Quant. Biol. 28, 483–488
44 Stier, A. (1976) in Biochemical Pharmacology, Vol. 25, pp. 109–113, Pergamon Press, Oxford