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MEMBRANE FLUIDITY AND DRUG METABOLISM IN LIVER MICROSOMES OF LEAN, ob/ob AND db/db MICE.

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SUMMARY

The thermally-induced changes of a cytochrome P-450 dependent activity (ethoxycoumarin-O-deethylase) and of the fluorescence polarization of diphenylhexatriene were compared in microsomes from lean, ob/ob and db/db mice. In lean mice, biphasic plots were obtained with break points in the same range of temperature by both methods, whereas, in ob/ob and db/db mice, no discontinuities were observed. These results may be related to a modified fatty acid composition of microsomal membranes in mutant mice. They exemplify the influence of the lipid environment on the monooxygenase system as also shown by the modified binding constants of cytochrome P-450 towards type II substrates in db/db mice.

In 1968, Lu and Coon (1) resolved the liver monooxygenase system into three components : cytochrome P-450, NADPH-cytochrome P-450 reductase and a heat-stable factor which was further identified as phosphatidylcholine (2). The involvement of phospholipids has also been suggested by various studies on microsomes depleted in lipids by organic solvent extraction (3) or by phospholipases (4), and phospholipids are absolutely required for reconstitution of monooxygenase activity (5). In a previous work (6) we have reported some differences in the activity of the mixed-function oxidase (MFO) system in ob/ob and db/db mice as compared with their respective wild strain, and it was postulated that they might be due to a modified lipid environment of cytochrome P-450. In the present study, the lipid environment of liver microsomal cytochrome P-450 was investigated by comparing the thermally induced changes in a cytochrome P-450 dependent activity (ethoxycoumarin-O-deethylase) and in the fluidity of microsomal membranes assessed by polarization of the diphenylhexatriene probe. Results were tentatively correlated with the fatty acid composition of microsomal membranes.

METHODS

7-10 weeks old male db/db mice of the C57BL/Ks strain, ob/ob and +/+ mice of the C57BL/6 strain were obtained from the "Centre d'Elevage du

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CNRS" Orléans, France. Liver microsomal membranes were prepared as described by Degkwitz et al. (7). Ethoxycoumarin-O-deethylase activity was measured as described by Ullrich and Weber (8). Fluorescence polarization was measured as indicated by Shinitzky and Barenholz (9) with about 0.1 mg of microsomal protein per ml in 50 mM Tris-HCl buffer (pH 7.4), 150 mM KCl, 10 mM $MgCl_2$ to which DPH in tetrahydrofuran was added at 1 µM final concentration. After incubation for 45-60 min. at 20°C, the temperature was reduced and fluorescence polarization measured at about 2°C intervals in a Jobin-Yvon JY3D spectrofluorometer. Fluorescence intensities were determined with polarizers parallel and perpendicular to the vertically-polarized exciting beam (λ Exc. 366 nm, λ Em. 454 nm). The value of polarization "P" was calculated as described by Shinitzky and Barenholz (9). The fatty acid composition of microsomal phospholipids was determined according to the method of Pileire (10)

RESULTS AND DISCUSSION

The effect of temperature on the initial rate of 7-ethoxycoumarin-O-deethylase reaction is shown as an Arrhenius plot (figure 1). For microsomal preparations from lean mice of the C57BL/6 strain, the plot is bipha-

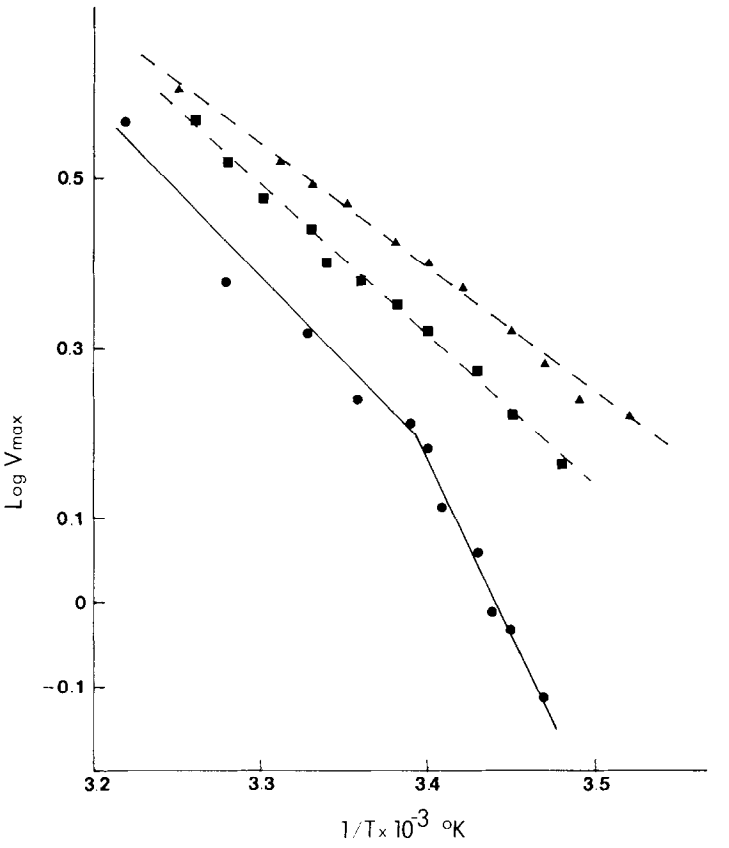

Figure 1 Arrhenius plot of 7-ethoxycoumarin-O-deethylase activity (expressed as nmol x min^{-1}x $nmol^{-1}$ cytochrome P-450) in liver microsomal membranes from lean (●), ob/ob (■) and db/db (▲) mice.

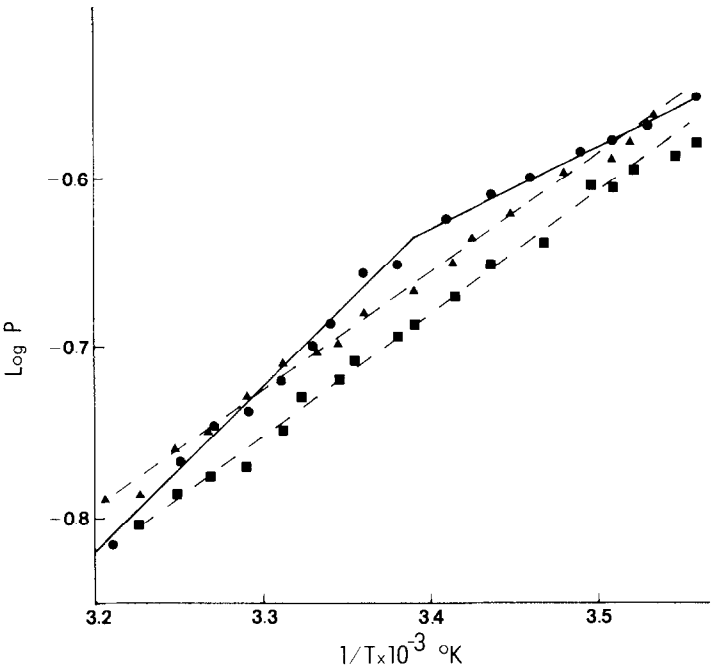

Figure 2 Arrhenius plot of fluorescence polarization P, of diphenylhexatriene in liver microsomal membranes from lean (●), ob/ob (■) and db/db (▲) mice.

sic and exhibits a break point at 20 ± 1°C (mean ± standard error of the mean for three determinations). Activation energies are respectively 69.2 ± 9.8 and 30.8 ± 1.4 kJ x mol^{-1} below and above the break. These results are consistent with those of Duppel and Ullrich (11). Data from ob/ob and db/db mice fail to show any discontinuity in the Arrhenius plots. Similarly, the fluorescence polarization plots are biphasic in microsomal membranes from lean mice (break point at 22.5 ± 1.0°C) and linear in membranes from ob/ob an db/db mice (figure 2). The discontinuity in the fluorescence polarization plot of DPH probe in membranes is considered as the transition of the physical state of membrane lipids from a solid or gel phase to a liquid crystalline phase (12). Such change in membrane fluidity is usually accompanied by modifications of the activity of membrane bound enzymes (13), as shown by the Arrhenius plot of ethoxycoumarin-O-deethylase activity (figure 1). The temperature of the phase transition mainly depends on the fatty acid composition of membrane phospholipids (14). Previous works reported an increased synthesis of fatty acids (15) and glycerolipids (16) in the liver of ob/ob mice, as well as a modified phospholipid composition of their microsomal membranes (17). Therefore, we have investigated the fatty acid pattern of microsomal membranes in the different strains of mice (Table 1). Several modifications were observed but the most prominent feature is a relative

TABLE I : Fatty acid composition of liver microsomes from lean, ob/ob and db/db mice

	C 16	C 16 : 1	C 18	C 18 : 1	C 18 : 2	C 20 : 3	C 20 : 4
lean mice of the C57BL/6 strain	31.7 ± 1.0	<1.8	22.5 ± 1.1	9.5 ± 0.6	16.7 ± 0.9	<1.8	13.0 ± 1.1
ob/ob mice of the C57BL/6 strain	27.4 ± 1.3*	<1.7	27.9 ± 1.5	17.8 ± 1.5**	7.1 ± 0.7***	3.5 ± 0.5	16.5 ± 1.3
db/db mice of the C57BL/Ks strain	26.4 ± 1.6*	2.8 ± 0.4	19.7 ± 1.3	26.9 ± 1.7***	9.6 ± 0.4**	2.2 ± 0.5	13.0 ± 3.1

Values are the means ± SEM of results from 3 to 4 animals and expressed as a percentage of total fatty acids.

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$, as compared with lean mice.

TABLE II : Binding affinities (Ks values) for aniline (type II) and benzphetamine (type I) in liver microsomes from lean, ob/ob and db/db mice.

	ANILINE	BENZPHETAMINE
lean mice of the C57BL/6 strain	263 ± 13	11.5 ± 1.9
ob/ob mice of the C57BL/6 strain	383 ± 70	17.8 ± 3.1
db/db mice of the C57BL/Ks strain	560 ± 93*	17.1 ± 1.5

Ks values (10^{-6} mol x l^{-1}) determined at 22-24°C, are the means ± SEM of results from 3 animals.

* $p < 0.05$, as compared with lean mice.

increase of oleic acid (C 18:1) and decrease of linoleic acid (C 18:2) in ob/ob and db/db mice as compared to the normal strain. The relative increase of oleic acid might be related to a higher insulin responsiveness of the Δ 9 insaturase as was shown in tissues of diabetic, insulin-treated rats (18) (ob/ob and db/db mice are hyperinsulinemic). In order to evaluate the possible effect of modified lipid composition on the accessibility of substrates to cytochrome P-450, binding affinities were measured towards a type I (benzphetamine) or type II (aniline) substrate (table II). The decreased affinity of cytochrome P-450 towards aniline in db/db mice appears to be the most significant difference. Such a result might show that the lipid environment of cytochrome P-450 modifies heme reactivity as well as the accessibility of substrates to the hydrophobic binding site. A previous result (6) might be similarly interpreted : in ob/ob mice, 2-hydroxylaminopropane exibits a decreased ability to form the heme-nitroso complex absorbing at 455 nm.

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