STRUCTURAL AND FUNCTIONAL CHANGES IN THE LIVER MICROSCOMES OF NZB MICE IN THE COURSE OF AUTOIMMUNE DISEASE

V. P. Kaznacheev, O. A. Gromova, V. A. Kozlov, I. B. Tsyrlov, and V. V. Lyakhovich

UDC 616-092:612.017.1-07:616.36-018.1-076.5

In the course of development of autoimmune disease in NZB mice damage to the liver is accompanied by changes in the structural component of the microsomal membrane and by an increase in the hydrophobic character of the space surrounding the heme moiety of cytochrome P-450. Changes in the spectral constants of binding of the substrates with cytochrome P-450 and a decrease in the activity of enzymes of the microsomal electron-transport chain were observed.

In mice of the NZB strain the liver, among other organs, is involved in the pathological process during the development of genetically determined autoimmune disease which follows a course similar to that of systemic lupus erythematosus in man [6]. Lesions of the liver of varied severity have also been observed in patients with lupus erythematosus. In some cases the liver lesion comes to dominate the clinical picture of the disease [1]. Accordingly, NZB mice were used as an original model of the chronic liver lesion arising as a result of the developing pathological process.

Activity of the enzymes of the main electron-transport chain located in membranes of the endoplasmic reticulum of the liver cells, a lesion of which is regarded as of primary importance in certain toxic manifestations, was therefore investigated.

The study of this problem is interesting in connection with the discovery of the general principles governing chronic lesions of the liver of varied character, and it may shed light on certain factors concerned with the pathogenesis of systemic lupus erythematosus in man.

EXPERIMENTAL METHOD

Mice of strain NZB of both sexes were used in the experiments at the age of 10 months, when marked signs of the disease are observed in 60% of the animals, and also at the age of 2 months, when symptoms of the disease are completely absent [6]. Mice of line C57BL acted as the control.

The microsomal fraction was isolated from the mouse liver by differential centrifugation [18]. Quantitative estimation of cytochrome P-450 was carried out spectrophotometrically [12]. The ethyl isocyanide differential spectra of the reduced microscomes [9] were determined at 2 pH values (8.7 and 6.0) at which the ethyl isocyanide components exist mainly in the "455" and "430" states. The spectral constants of interaction between the hydroxylation substrates and cytochrome P-450 (K_S) were studied [16].

Activity of NADP · H_2-cytochrome C reductase was determined [13]. The initial velocity of the NADP · H_2-cytochrome P-450 reductase reaction was calculated [5] assuming a coefficient of millimolar extinction of 91 for cytochrome P-450. The protein concentration was determined by Lowry's method [10].

Central Research Laboratory, Novosibirsk Medical Institute. Institute of Clinical and Experimental Medicine, Siberian Branch, Academy of Medical Sciences of the USSR. Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 76, No. 12, pp. 55-59, December, 1973. Original article submitted January 25, 1973.

TABLE 1. Spectral Constants of Binding of Substrates with Cytochrome P-450 and Activity of Enzymes of Electron-Transport Chain in Liver Microsomes of NZB Mice Aged 2 and 10 Months and of C57BL Mice

Line of mice	Cytochrome P-450 (in nmoles/mg protein)	K_s (in mmoles)		NADP·H_2-cytochrome C reductase (in nmoles reduced cytochrome c/mg protein per minute)	NADP·H_2-cytochrome P-450 reductase (in nmoles reduced cytochrome P-450/mg protein per minute)
		hexobarbital	aniline		
C57BL	0,52	—	1,3	147,3	2,63
NZB:					
2 months	0,55	—	1,8	154,2	2,93
10 months	0,52	0,25	3,2	92,5	1,92

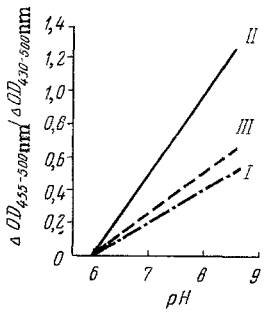

Fig. 1

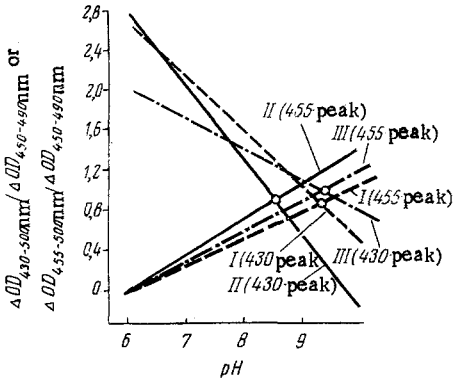

Fig. 2

Fig. 1. Ratio between intensities of peaks of ethyl isocyanide differential spectra of reduced cytochrome P-450 at 455 and 430 nm (455/430) during a change in pH in NZB mice aged 2 (I) and 10 (II) months and in C57BL mice (III).

Fig. 2. Intensities of 455- and 430-peaks of ethyl isocyanide differential spectra as a function of pH in NZB mice aged 2 (I) and 10 (II) months and in C57BL mice (III).

EXPERIMENTAL RESULTS

Microscopic investigation revealed diffuse degeneration of the hepatocytes of the NZB mice at the age of 10 months. The results of the tests for total proteins ruled out a protein character of the contents of the vacuoles and suggested lipid degeneration of the hepatocytes. The connective tissue of the liver of the mice aged 10 months contained more connective-tissue cells than the mice aged 2 months, and they formed small groups around the portal tracts and, occasionally, inside the hepatic lobules.

The content of cytochrome P-450 in the microsomes of all groups of mice investigated was about the same (Table 1).

It is now considered [7] that the spectral properties of cytochrome P-450 are due in many respects to its interaction with other components of the membrane, and they may be linked with the hydrophobic character of the space surrounding the heme moiety, or they may be determined by the conformation of the specific protein which is maintained by hydrophobic interactions. The work of Imai et al. [8, 9] has shown convincingly that the ratio between the two Cope peaks of the ethyl isocyanide spectra of reduced cytochrome P-450 is connected with the surroundings of the membrane and may be used as an indicator of the hydrophobic character of the heme surroundings.

It will be clear from Fig. 1 that this ratio in NZB mice aged 10 months at pH 7.4 is much higher than in mice of the same line aged 2 months and in C57BL mice. The results given in Fig. 2 show that at a pH

value giving equal absorption of both peaks the ratio between the intensities of each of them and the peak of absorption of the CO-difference spectrum of cytochrome reduced by dithionite is about equal. It is considered [9] that this may be evidence that the observed change in the ratio 455/430 is probably not due to a change in the molar extinction of the ethyl isocyanide components.

It will be noted in Fig. 2 that the equal absorption of the peaks at 455 and 430 nm in the liver microsomes of NZB mice aged 10 months was observed at a lower pH value. A similar phenomenon has been observed [9, 17] in the liver microsomes of rats induced by methylcholanthrene, for which the surroundings of the heme were shown to be more hydrophobic than in microsomes of uninduced animals [9].

The results suggest that in the course of development of the pathological process in NZB mice definite structural changes take place in the liver microsomes and are manifested as an increase in the hydrophobic properties of the membrane in the region of the heme moiety of the cytochrome P-450.

It is logical to suppose that a change in the hydrophobic interactions of the heme of the cytochrome P-450 with its surroundings in the membrane may lead to changes in its affinity for the substrate and, despite the equal quantity of hemoprotein in all groups of mice investigated, may lead to different levels of functional activity. This was confirmed experimentally by an investigation of the binding of substrates of the first and second types (hexobarbital and aniline, respectively) with cytochrome P-450. The spectral changes on the addition of increasing doses of hexobarbital to the suspension of microsomes were small in all groups of mice investigated. In NZB mice aged 2 months and in C57BL mice no clear spectral changes could be obtained under these conditions. However, in NZB mice aged 10 months binding was appreciable. More intensive spectral changes accompanied the binding of aniline. The value of K_S was greatest in NZB mice at the age of 10 months, suggesting lower affinity of cytochrome P-450 for aniline in this group of animals (Table 1).

The differences observed in the changes in binding substrates of the first and second types may be due to different mechanisms of binding substrates with the cytochrome P-450 molecule. Substrates of the first type are considered to bind with the hydrophobic protein site of the cytochrome [2, 14, 16], and during an increase in the hydrophobic properties of the surroundings of the hemoprotein an increase in the degree of affinity for substrates of the first type can be postulated. The second type of spectral changes are connected with the formation of ferrihemochrome [2, 16]. The increase in K_S for substrates of the second type when the surroundings of the heme are more strongly hydrophobic is not yet fully understood.

The microsomal polyenzyme system of the electron transport chain is known to be membrane-bound and to react precisely to changes in the native structure by a change in activity. Kinetic investigations of the activity of NADP · H_2-cytochrome c reductase – the initial site of the main microsomal electron transport chain – and also of NADP · H_2-dependent reduction of cytochrome P-450 showed a considerable decrease in activity in the liver microsomes of NZB mice at the age of 10 months (Table 1), possibly due to certain structural changes in the membrane. Taking into account the important catalytic role of the phospholipid component in the manifestation of NADP · H_2-cytochrome P-450 reductase activity [11, 15] it is logical to suggest a change in the quantitative or qualitative composition of the phospholipids in the microsomes of NZB mice in the course of autoimmune disease.

In the context of discussion of these results it is interesting to note that during regeneration of the liver after partial hepatectomy [4] and also in the course of chronic poisoning of rats with carbon tetrachloride, when for a long time the processes of degeneration and repair run parallel to each other, structural changes aimed at reducing the unsaturation of the fatty acids of the phospholipids playing an important role in the maintenance of the hydrophobic properties of the microsomal membrane [19], take place in the microsomes of the liver. These changes may evidently play some part in increasing the resistance of animals to a long and constantly acting toxic factor, and they may also contribute to the so-called paradoxical effect of the action of poisons [3].

It can be concluded from the analysis of these observations and data in the literature that the change in the structural organization of the microsomal membranes reflects adaptation of the cells at the molecular level during chronic exposure to a harmful factor; this analysis also emphasizes the regulatory role of natural membranes as dynamic, metabolically active formations.

LITERATURE CITED

1. Z. A. Bondar', Clinical Hepatology [in Russian], Moscow (1970), p. 269.
2. L. M. Raikhman, V. S. Belova, M. R. Borukaeva, et al., Biokhimiya, No. 4, 674 (1971).
3. D. S. Sarkisov, L. D. Krymskii, K. I. Dzarakhotov, et al., Byull. Éksperim. Biol. i Med., No. 7, 115 (1969).
4. G. Fex, Biochim. Biophys. Acta, 231, 161 (1971).
5. Y. Gosspelius, H. Thor, and S. Orenius, Chem.-Biol. Interactions, 1, 125 (1969-1970).
6. J. Howie and B. J. Helyor, Adv. Immunol., 9, 215 (1968).
7. Y. Imai and R. Sato, J. Biochem. (Tokyo), 62, 464 (1967).
8. Y. Imai and R. Sato, J. Biochem. (Tokyo), 63, 270 (1968).
9. Y. Imai and P. Siekevite, Arch. Biochem., 144, 143 (1971).
10. O. H. Lowry, N. J. Rosebrough, A. L. Farr, et al., J. Biol. Chem., 193, 265 (1951).
11. A. Y. Lu, H. W. Strobel, and M. J. Coon, Biochem. Biophys. Res. Commun., 36, 545 (1969).
12. T. Omura and R. Sato, J. Biol. Chem., 239, 2370 (1964).
13. A. H. Phillips and R. J. Langron, J. Biol. Chem., 237, 2652 (1962).
14. J. E. Schenkman, Hoppe-Seylers Z. Physiol. Chem., 349, 1624 (1968).
15. J. B. Schenkman, Chem.-Biol. Interactions, 3, 306 (1971).
16. J. B. Schenkman, H. Remmer, and R. W. Estabrook, Molec. Pharmacol., 3, 113 (1967).
17. N. E. Sladek and G. J. Mannering, Biochem. Biophys. Res. Commun., 24, 668 (1966).
18. J. R. Tata, in: Subcellular Components, London (1969), p. 83.
19. I. B. Tsyrlov and V. V. Lyachovich, Biochem. Pharmacol., 21, 2540 (1972).