EXPERIMENTAL RESULTS

At all times in the course of ETAE the plasma TBAP level and the UFA level in erythrocyte membranes were raised, whereas PRE was lowered (Table 1). These data, as well as data in the literature showing that PRE is directly dependent on the antioxidant supply of the membranes [6], suggest that activation of LPO is connected with inhibition of the antioxidant system. Considering that intensification of LPO in the brain is reflected in an increased lipoperoxide concentration in the peripheral blood [5], lysis of neurons observed in ETAE can be explained by activation of LPO. The absence of any direct dependence of the time course of the structural changes in the brain on values of the parameters of LPO intensity investigated at different stages of development of ETAE will be noted. Intensification of the neurohistological features of aggravation of the degenerative changes can be observed throughout a 60-day period of investigation [4]. The increase in the intensity of LPO takes place only during the first 30 days, and in the later stages a tendency is found toward normalization of the plasma TBAP, erythrocyte membrane UFA, and PRE levels. This divergence of the parameters of severity of the pathological process can probably be attributed to the inequality of the relative contribution of LPO activation to the pathochemistry of ETAE during the different periods of its course. In the early stages it probably occupies a determinant position, but later a more important role passes to other types of disturbances of brain metabolism.

LITERATURE CITED

1. V. N. Anders and D. D. Orlovskaya, Zh. Nevropatol. Psikhiatim S. S. Korsakova, No. 7, 111 (1982).
2. V. I. Benisovich and L. I. Idel'son, Vopr. Med. Khimii, No. 6, 596 (1973).
3. Yu. A. Vladimirov and A. I. Archakov, Lipid Peroxidation in Biological Membranes [in Russian], Moscow (1972).
4. V. M. Kovalenko and V. S. Tsivil'ko, "A model of encephalopathy," Author's Certificate 767820 dated June 6, 1980.
5. G. N. Kryzhanovskii, E. V. Nikushkin, V. A. Voronko, and V. E. Braslavskii, Byull. Éksp. Biol. Med., No. 11, 36 (1983).
6. A. A. Pokrovskii and A. A. Abramov, Vopr. Pitan., No. 6, 44 (1964).
7. L. L. Prilipko and R. R. Lideman, Vestn. Akad. Med. Nauk SSSR, No. 1, 33 (1982).
8. F. Morle, L. Morle, M. Aguercif, et al., C. R. Soc. Biol., 174, 598 (1980).
9. Satoh Kei, Clin. Chim. Acta, 90, 37 (1978).
10. A. Shiotani, J. Watanabe, L. Matsuoka, et al., J. Biochem., 88, 677 (1980).

ROLE OF THE MICROSOMAL ETHANOL-OXIDIZING SYSTEM IN REGULATION OF LINOLEYL-CoA-DESATURASE ACTIVITY AFTER LONG-TERM ALCOHOLIZATION

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Long-term exposure to ethanol is characterized by various disturbances of lipid metabolism, one of which is lowering of desaturase activity [2, 10]. The fatty acid desaturase is the terminal electron acceptor in the NADH-cytochrome b_5-dependent microsomal oxidation system, and for that reason the level of activity of this enzyme is closely linked with function of this electron transfer chain. Activity of the latter is linked, in turn, with the NADPH-cytochrome P-450-dependent microsomal oxidation system which, in the opinion of some workers [10], can be attributed to the absence of high specificity of these systems for oxidation

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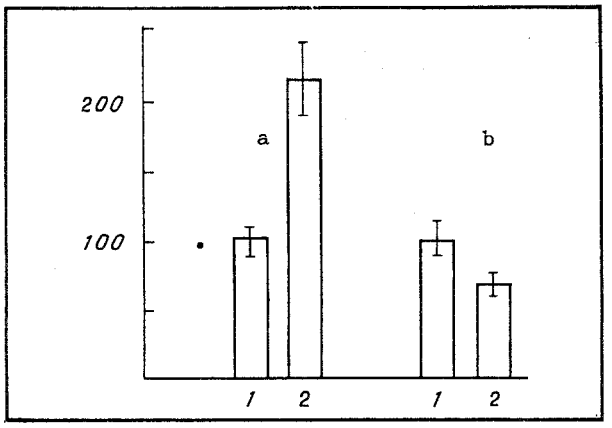

Fig. 1. Activity (in %) of microsomal ethanol-oxidizing system (a) and of linoleyl-CoA-desaturate (b) in liver of control (taken as 100%) animals (1) and during long-term ethanol loading (2).

of NADH and NADPH. An ethanol-oxidizing system (MEOS), involving NADPH-cytochrome C-reductase, cytochrome P-450, and phosphatidylcholine, also functions in the microsomes, and differs from microsomal NADPH-oxidase activity [7].

The aim of this investigation was to estimate linoleyl-CoA-desaturase activity in the rat liver in connection with functioning of the NADH- and NADPH-dependent microsomal electron transport chains in order to elucidate the mechanism of the changes in activity of this enzyme during long-term alcohol loading.

EXPERIMENTAL METHOD

Noninbred male albino rats weighing 200-220 g were used. The animals were kept on a semisynthetic balanced diet [8]. For 14 days the animals were given ethanol by the intragastric route in a dose of 7 g/kg daily. The rats were decapitated 12 h after the last injection. Concentrations of cytochrome b_5 and P-450, activity of NADPH- and NADH-oxidase and oxidoreductases, and activity of ethylmorphine demethylase and aniline hydroxylase in the microsomal fraction of rat liver were determined spectrophotometrically by the method described previously [3]. The rate of ethanol oxidation [6] and linoleyl-CoA-desaturate activity (using 1-^{14}C-linoleic acid, from Amersham International, England, as the substrate [5]), also were investigated in the microsomes. The results were subjected to statistical analysis by Student's t test.

EXPERIMENTAL RESULTS

Long-term ethanol administration caused a marked decrease in linoleyl-CoA-desaturase activity in the rat liver (Fig. 1). Other components of the NADH-dependent chain were activated at the same time: oxidation of NADH was increased, and activity of NADH-cytochrome b_5-reductase was increased appreciably. The concentration of cytochrome b_5, a supplier of electrons from flavoprotein to linoleyl-CoA-desaturase, was virtually unchanged under the experimental conditions used (Table 1). Investigation of the parameters of the NADPH-dependent electron transport chain revealed activation in this sector also: the cytochrome P-450 level was raised, and NADPH-oxidase and NADPH-cytochrome c-reductase were activated. Under these circumstances the rate of hydroxylation of type II substrates (aniline) remained unchanged, whereas that of type I substrates (ethylmorphine) was reduced (Table 1). At the same time the rate of ethanol oxidation in the endoplasmic reticulum was doubled (Fig. 1).

It is generally accepted that the dominant pathway of ethanol catabolism in the liver is the alcohol-dehydrogenase reaction, and that MEOS and systems involving catalase are alternative pathways. However, the level of ethanol oxidation by systems unconnected with alcohol dehydrogenase amounts to 20-25 to 50% or more of the total ethanol metabolism [12]. Among these systems MEOS occupies the leading position [7]. With an increase in the concentration of ethanol, its fraction metabolized by MEOS with the participation of cytochrome P-450 is increased, and this is expressed by an increase in MEOS activity during chronic ethanol consumption [6]. The results of evaluation of alcohol-dehydrogenase activity during long-term ethanol loading are contradictory [13], although this enzyme has the capacity to generate

TABLE 1. Activity and Content of Components of the Monooxygenase System of Rat Liver during Long-Term Ethanol Loading (all parameters calculated per kilogram protein)

Parameter	Control	Experiment
NADPH-oxidase, μmoles/sec	113.6 ± 8.0	142.6 ± 1.3*
NADPH-cytochrome P-450-reductase, mmole	0.59 ± 0.05	0.73 ± 0.08*
Cytochrome P-450, mmole	0.72 ± 0.04	0.89 ± 0.03*
NADH-oxidase, μmoles/sec	65.3 ± 8.0	81.3 ± 5.3*
NADH-cytochrome b_5-reductase, mmoles/sec	4.8 ± 1.08	8.26 ± 1.13*
Cytochrome b_5, mmole	0.52 ± 0.06	0.59 ± 0.02
Ethylmorphine-N-demethylase, μmoles HCHO/sec	237 ± 1.6	213 ± 3.3*
Aniline-hydroxylase, μmoles aminophenol/sec	25.5 ± 1.6	24.5 ± 1.0

Legend. *$p < 0.05$.

large quantities of NADH even in the absence of a visible increase of activity, measured under standard optimal conditions.

As the results show, activation of MEOS due to chronic exposure to ethanol is accompanied by elevation of the level of the terminal electron acceptor of the NADPH-dependent chain, namely cytochrome P-450, and some increase in NADPH and activity of NADPH-cytochrome P-450-reductase — the initial and middle components of the chain respectively (Table 1). Besides increased activity of MEOS, the velocity of cytochrome P-450-dependent oxidation of other substrates also is reduced, due, as we know, to competition for binding sites, reducing equivalents, and so on. Meanwhile an increase in the rate of NADH oxidation and in activity of NADH-cytochrome b_5-reductase is observed in the NADH-dependent system, while the cytochrome b_5 level remains unchanged and desaturase activity is depressed. The impression is obtained that under conditions of long-term ethanol loading, electrons are intensively transferred through NADH-cytochrome b_5-reductase from NADH to cytochrome P-450, and from thence to MEOS. The possibility of interaction of this kind between NADPH- and NADH-specific electron transport chains in microsomes has been described previously [1].

Under conditions of excessive ethanol intake, alcohol dehydrogenase is the main supplier of reducing equivalents in the liver [13]; moreover, activity of other NADH-generating enzymes is depressed during chronic alcohol poisoning (this includes lactate dehydrogenase and its isozymes, glyceraldehyde phosphate dehydrogenase, glutamate dehydrogenase, α-keto-acid dehydrogenases, etc.) [4]. It can be tentatively suggested that during ethanol loading definite interconnection exists between alcohol dehydrogenase and MEOS: alcohol dehydrogenase NADH, electrons from which are transferred through NADH-cytochrome b_5-reductase to cytochrome P-450.

Reoxidation of cytochrome b_5-reductase by cytoplasmic NADH and transfer of reducing equivalents to microsomal oxidases take place when the cytosol NADH level is elevated during long-term ethanol consumption [10]. These reducing equivalents are subsequently used for ethanol oxidation in the endoplasmic reticulum, whereas activation of MEOS in turn facilitates reoxidation of NADH, the rate of which is the limiting factor in ethanol oxidation by alcohol dehydrogenase [12]. Considering the facts described above, it can be accepted that under conditions when the main potential of the NADH- and NADPH-dependent chains is switched to oxidation of ethanol in the endoplasmic reticulum, an inadequate supply of electrons for the desaturase reaction will reduce the activity of this process. Such a situation is observed during induction of monooxygenases by phenobarbital, when the increase in the rate of oxidation of substrates involving cytochrome P-450 leads to a decrease in desaturase activity [9].

It is quite natural that the suggested mechanism of inhibition of linoleyl-CoA-desaturase activity during long-term ethanol consumption is not the only one possible. We know that long-term ethanol intake inhibits protein synthesis [11] and lowers the level of essential fatty acids in the liver [2], and consequently, output of enzyme protein may be reduced or insufficient substrate (linoleic acid) may be available for the desaturase reaction.

However, the data given in this paper are evidence of a possible fall in linoleyl-CoA-desaturase activity in the rat liver during long-term ethanol consumption as a result of diversion of the stream of electrons for ethanol oxidation in the microsomes.

LITERATURE CITED

1. A. I. Archakov, V. M. Devichenskii, and I. I. Koruzina, Biokhimiya, 39, No. 5, 903 (1974).
2. V. U. Buko, Vopr Med. Khim., No. 2, 89 (1986).
3. L. I. Sushko, G Z. Abakumov, P. I. Lukienko, and G. G. Voronov, Vopr. Med. Khim., No. 5, 108 (1985).
4. Yu. M. Ostrovskii (ed.), Ethanol and Metabolism [in Russian], Minsk (1983).
5. J. De Gomez Dumm and R. Brenner, Lipids, 10, No. 6, 315 (1975).
6. C. Lieber and L. De Carli, J. Biol. Chem., 245, No. 9, 2505 (1970).
7. C. Lieber, R. Teschke, G. Hasumura, and L. De Carli, Fed. Proc., 34, No. 11, 2062 (1975).
8. C. Lieber and L. De Carli, Alcoholism, 6, No. 4, 523 (1982).
9. N. Oshino, I. Imai, and R. Sato, J. Biochem. (Tokyo), 9, No. 1, 155 (1971).
10. J. Ozols, Ann. Clin. Res., 8, Suppl. 17, 182 (1976).
11. A. Smith-Kielland, L. Svendsen, A. Bessesen, and J. Morland, Acta Pharmacol. (Copenhagen), 51, Suppl. 1, Abst. 12 (1982).
12. H. Thieden, Acta Pharmacol. (Copenhagen), 36, 7 (1975).
13. G. Ugarte, Front. Gastroent. Res., 2, 150 (1976).

EFFECT OF HEMODYNAMIC DISTURBANCES ON THE RAT LIVER TRANSEPITHELIAL POTENTIAL IN VIVO

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Most pathological states of the liver are accompanied as a rule by disturbances of the blood flow through the organ, and these in turn may specifically aggravate the pathological process [1]. An important factor in the development of experimental and clinical hepatology is the establishment of simple, reliable methods of continuous monitoring of the state of the liver function in vivo in various hemodynamic disturbances.

The trabecular structure of the hepatic lobule provides a series of barriers between the sinusoidal space and the biliary capillary. Under these circumstances the leading role in the maintenance of the barrier function is played by membranes and intercellular junctions of hepatocytes bounding the biliary capillary [2]. By analogy with other barrier tissues, forming continuous epithelial layers bearing a transepithelial potential (TEP; the skin, the intestinal wall, the wall of the gallbladder, and so on) [4], the presence of a similar potential can also be postulated on the boundary between the intestinal space and the internal lumen of the hepatic biliary capillary.

Since there is no information on this question in the accessible literature, the investigation described below was undertaken with the aim of developing a method of recording TEP and studying its response to various changes in the hepatic blood flow.

EXPERIMENTAL METHOD

Experiments were carried out on male Wistar rats weighing 150-200 g. To record TEP laparotomy was performed, the common bile duct was dissected, and a thin polyethylene cannula, connected through an agar bridge to an Ag–AgCl electrode, was introduced into it. The drainage

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