5. U. K. Laemmli, Nature, 227, 680 (1970).
6. A. Y. H. Lu and W. Levin, Biochem. Biophys. Res. Commun., 46, 1334 (1972).
7. D. E. Ryan, P. E. Thomas, D. Korzeniovski, et al., J. Biol. Chem., 254, 1365 (1979).
8. D. E. Ryan, P. E. Thomas, and W. Levin, J. Biol. Chem., 255, 7941 (1980).
9. D. E. Ryan, S. Jida, A. W. Wood, et al., J. Biol. Chem., 259, 1239 (1984).
10. C. J. Serabjit-Singh, C. R. Wolf, and R. M. Philpot, J. Biol. Chem., 254, 9901 (1979).
11. D. J. Simmons, P. A. Lalley, and C. B. Kasper, J. Biol. Chem., 260, 515 (1985).
12. B. J. Song, T. Fujino, S. S. Park, et al., J. Biol. Chem., 259, 1395 (1984).
13. P. E. Thomas, L. M. Reik, D. E. Ryan, et al., J. Biol. Chem., 259, 3890 (1984).
14. H. Towbin, T. Staehelin, and J. Gordon, Proc. Natl. Acad. Sci. USA, 76, 4350 (1979).

STATE OF THE HEPATIC CYTOCHROME P-450 SYSTEM IN RATS VARIOUSLY PREDISPOSED TO EXPERIMENTAL ALCOHOLISM

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Chronic administration of ethanol to experimental animals is known to increase the intensity of metabolism not only of ethanol itself, but also of other xenobiotics of the microsomal fraction of the liver, in these animals [4]. However, information in the literature does not answer the question whether these changes in metabolism are the result of differences in the initial reactivity of the liver microsomes or whether they are secondary in character and are caused by chronic alcoholization.

It was accordingly decided to study activity of the cytochrome P-450-dependent mono-oxygenase system of the liver in animals selected on the basis of the strength of their initial alcohol motivation without contact with ethanol, and also the dynamics of cytochrome P-450 activity during voluntary alcoholization for 10 days.

EXPERIMENTAL METHOD

Experiments were carried out on 44 noninbred male rats weighing 250-300 g. Predisposition to the development of experimental alcoholism was estimated by measuring the total time of immobilization (TTI) in a compulsory swimming test, by the method suggested by the writers previously [2]. Animals with a TTI of under 140 sec were classified as not predisposed to develop experimental alcoholism, those with a TTI of over 300 sec as animals predisposed to develop this experimental pathology. Thus, for the experiments 22 rats predisposed (TTI 308.3 ± 5.4 sec) and 22 rats not predisposed (TTI 130.0 ± 10.8 sec) to develop experimental alcoholism were selected for the experiments. Activity of the mono-oxygenase system of the liver was studied in some animals (11 from each group) after contact for 10 days with alcohol in individual cages measuring 40 × 12 × 15 cm, equipped with graduated bowls containing water and 15% ethanol solution. Biochemical investigations on the remaining animals were carried out after they had been kept under similar conditions for 10 days, but without contact with alcohol. The animals received food ad lib.

The state of the cytochrome P-450-dependent mono-oxygenase system of the liver was determined as follows. The animals were killed 24 h after withdrawal of ethanol. The microsomal fraction was obtained from liver homogenate of the experimental animals by differential centrifugation [5]. The protein concentration of the microsomes was determined by a modified Lowry's method [6]. The state of the hydroxylating complex was assessed from the content of

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TABLE 1. Parameters of Activity of Hepatic Cytochrome P-450 System in Rats Differing in Predisposition to Development of Experimental Alcoholism (M ± m)

Group of animals and experimental conditions	Cytochrome, nmoles/mg protein		Enzymes, nmoles/min/mg protein		
	P-450	b_5	Aminopyrene N-demethylase	Aniline p-hydroxylase	NADPH-cytochrome c-reductase
Potentially alcoholic rats rats	1,16±0,16	0,77±0,7	10,23±1,35	0,48±0,18	56±14
Rats rejecting alcohol	0,85±0,17**	0,64±0,09*	6,88±1,20**	0,61±0,13*	38±10*
Potentially acoholic rats with access to it	1,13±0,15	0,76±0,10	9,67±1,03	0,78±0,18	98±25
Rats rejecting alcohol with access to it	0,88±0,12**	0,65±0,07*	6,30±1,75**	0,58±0,11*	53±8**

Legend. *P < 0.05, **P < 0.01 compared with first group of animals.

individual components and activity of cytochrome P-450-dependent enzymes. The concentrations of cytochromes P-450 and b_5 were determined [7] on a "Specord" spectrophotometer, and the rate of p-hydroxylation of aniline and of N-demethylation of aminopyrine and NADPH-cytochrome c-reductase activity also were determined [3].

The results were subjected to statistical analysis by the Fisher–Student parametric test. Differences were considered to be statistically significant at the P < 0.05 level.

EXPERIMENTAL RESULTS

As Table 1 shows, activity of the mono-oxygenase system was 36-48% higher in animals predisposed to experimental alcoholism than in animals not so predisposed.

The next series of experiments showed that "potentially alcoholic" rats consumed 42.5 ± 5.0 ml/kg of 15% ethanol solution per diem during contact with alcohol for 10 days. In rats not predisposed to develop experimental alcoholism, this figure was 6.4 ± 1.5 ml/kg. The study of the hepatic cytochrome P-450 system in these animals revealed no changes in the above parameters during voluntary consumption of alcohol for 10 days. The only exception was NADPH-cytochrome c-reductase, whose activity rose significantly in animals of both groups by 40-75%.

It can be concluded from the analysis of these results that consumption of alcohol for 10 days under conditions of free access to both water and 15% ethanol solution has no inducing action on the hepatic cytochrome P-450-dependent mono-oxygenase system independently of the animals' predisposition to develop experimental alcoholism. If previous data [1], showing intensification of the pharmacokinetics of a test dose of ethanol under the influence of voluntary alcohol consumption for 10 days, are examined in the light of the results now obtained it can thus be concluded that the growth of metabolic tolerance to ethanol observed under these conditions takes place not on account of the microsomal ethanol-oxidizing system, but evidently on account of induction of alcohol dehydrogenases.

LITERATURE CITED

1. Yu. V. Burov, N. V. Vlasova, A. B. Kampov-Polevoi, and A. P. Rodionov, Vopr. Med. Khimii, No. 6, 24 (1983).
2. A. B. Kampov-Polevoi, in: The Pharmacology of Experimental Alcoholism [in Russian], Moscow (1982), pp. 130-135.
3. I. I. Karuzina and V. I. Archakov, in: Modern Methods in Biochemistry [in Russian], Moscow (1977), pp. 49-62.
4. T. Ariyosshi, E. Takasbatake, and H. Remmer, Life Sci., 9, 361 (1970).
5. J. T. Ahokas, O. Pelconen, and N. T. Kaki, Cancer Res., 37, 3737 (1977).
6. E. F. Hartree, Anal. Biochem., 48, 422 (1972).
7. T. Omura and R. Sato, J. Biol. Chem., 239, 2379 (1964).