10. W. Sieghart and M. Karobath, Nature, 286, 285 (1980).
11. P. Skolnick, P. J. Marangos, F. K. Goodwin, et al., Life Sci., 23, 1473 (1978).
12. R. F. Squires and C. Braestrup, Nature, 266, 732 (1977).

# ENDOGENOUS ETHANOL LEVEL AND ALCOHOL MOTIVATION

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Predisposition to the development of experimental alcoholism in animals has been shown to depend on the rate of ethanol metabolism [1]. Meanwhile differences in the rate of elimination of ethanol by patients with chronic alcoholism and nonalcoholics have been reported [3-5]. However, the possibility of studying the kinetics of ethanol as a diagnostic and prognostic criterion reflecting the risk of development of an addiction for alcohol is limited by the fact that such investigations require administration of alcohol to the subjects and repeated taking of blood samples. The study of endogenous ethanol (EE), a natural substrate of ethanol-oxidizing systems, indirectly reflecting the level of their activity, could be of great practical value in this respect.

The object of this investigation was to study correlation between the intensity of alcohol motivation and the blood EE level in animals and man.

## EXPERIMENTAL METHOD

The EE level was determined in 57 men aged 28-42 years suffering from chronic alcoholism in stage II, on the 20th-25th day of their stay in the clinic after relief of withdrawal symptoms. They had received no drugs during the 4-5 days before the investigation. The control group consisted of 47 healthy male volunteers aged 24-42 years who had consumed no alcoholic drinks during the 7-10 days before the investigation. Blood (2-3 ml) was taken from the cubital vein always at the same time of day — 9:30-11:00 a.m., 1.5-2 h after breakfast. Each subject was tested once only.

Experiments were carried out on 25 noninbred male rats weighing 180-200 g, 10 C57BL mice weighing 30 g, and genetically predisposed toward alcohol consumption, and on 10 CBA mice weighing 30 g, with genetically determined aversion for alcohol. The intensity of the initial alcohol motivation of the rats was measured in a situation of minimal stress, namely being kept in groups of four in a plastic cage measuring 32 × 47 × 16 cm, equipped with a feeding bowl and two graduated receptacles — one with water, the other with 15% ethanol solution. Four groups of animals, each containing four rats, were selected: two groups were formed by rats with an EE level below 4 μg/ml (mean 3.75 μg/ml), the other two consisted of animals with EE above 7 μg/ml (mean 7.53 μg/ml). The testing period was 10 days. The intensity of alcohol motivation of the rats was determined by the standard method, by keeping them for 10 days in individual cages measuring 5 × 9 × 5 cm, equipped with two graduated receptacles — one containing water, the other a 10% solution of ethanol.

The EE level was determined by gas—liquid chromatography. Samples of 0.5 ml blood, taken from the subjects, were treated with 0.5 ml of 50% TCA and 0.5 ml of 1% isopropyl alcohol, which served as internal standard. Flasks were sealed and 0.25 ml of 30% $NaNO_2$ was injected into them from a syringe. The contents of the flask were shaken and the gaseous phase withdrawn by means of a syringe and introduced into the chromatograph.

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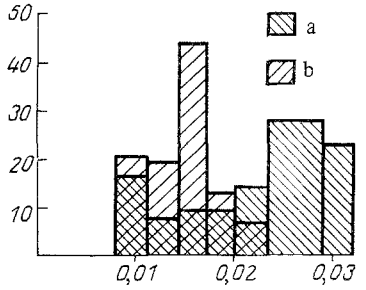


Fig. 1. Distribution of alcoholic subjects (in %) depending on EE level. Abscissa, EE level (in %). a) Normal subjects, b) alcoholic patients.

Samples of 0.2 ml blood from the animals were injected with a pipet into 15-$cm^3$ flasks containing 0.1 ml of 0.2% $NaNO_2$ solution, and 0.2 ml of 0.0026% aqueous solution of isopropyl alcohol was added. Samples for analysis on the Tsvet-110 standard gas–liquid chromatograph with flame-ionization detector were prepared by a modified method of paraphase concentration of impurities.

The results were analyzed by Student's test.

## EXPERIMENTAL RESULTS

It will be clear from Fig. 1 that the EE level both in alcoholics and in normal subjects varied within wide limits. However, the percentage distribution of the subjects by this parameter shows a marked predominance of individuals with a low EE level among the alcoholics compared with the control group.

In C57BL mice with genetically determined alcohol motivation (mean daily consumption of 10% ethanol solution over 150 ml/kg) a lower EE level was found (3.26 ± 0.70 μg/ml) than in CBA mice, with genetically determined aversion for alcohol (consumption under 1 ml/kg of 10% ethanol solution, EE level 6.31 ± 0.25 μg/ml).

During the first 3 days of the experiment noninbred rats with both low and high EE levels consumed a considerable volume of 15% ethanol solution (70.5 and 62.5 ml/kg, respectively). Later, however, rats with a high EE level completely ceased to consume alcohol. In rats with a low EE level the alcohol consumption after the 3rd day of the experiment fell to 14.3 ml/kg and thereafter was stabilized at that level.

The lower blood EE levels of the alcoholic patients than the control may be attributable, first, to induction of ethanol-oxidizing enzyme systems, developing during systematic alcohol abuse, and second, to initial metabolic differences.

The results of the experiments on both noninbred animals and inbred mice, the intensity of whose alcohol motivation is genetically determined, point to an undisputed connection between the initial EE level and the ease of formation of an alcohol motivation in the animals, in agreement with data in the literature [2]. There are therefore good grounds for considering correlation between a low EE level and a high risk of development of alcoholism, although it is possible that the compensatory induction of ethanol-oxidizing enzyme systems, taking place as a result of prolonged alcohol abuse, may lead to an even greater fall in the EE level.

## LITERATURE CITED

1. Yu. V. Burov, G. I. Absava, A. B. Kampov-Polevoi, et al., Farmakol. Toksikol., No. 1, 50 (1981).
2. Yu. M. Ostrovskii, in: The Biochemistry of Alcoholism [in Russian], Minsk (1980), p. 106.
3. F. L. Iber, N. Carulli, and R. M. H. Kater, Fed. Proc., 28, 625 (1969).
4. R. M. H. Kater, N. Carulli, and F. L. Iber, Am. J. Clin. Nutr., 22, 1608 (1969).
5. R. M. H. Kater, P. Zeive, and F. Tobon, Gastroenterology, 56, 412 (1969).