with XCM, the schedule of administration of the preparation should ensure the shortest duration of their contact with the blood cells.

It can be concluded from the results that XCM reversibly reduced the ability of red cells to undergo deformation and that this reduction is concentration-dependent and can be arranged in the following order: bilimin > 76% triombrast = iodamide-380 > 20% bilignost > 50% bilignost > metrizamide. The action of triombrast, iodamide, and metrizamide on red cell deformability is attributable to the osmotic activity of their solutions and is linked with an increase in viscosity of the internal centers of the red cells. For angiourography it is therefore preferable to use the new nonionic XCM of metrizamide type, with low osmotic activity.

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EFFECT OF ANTISERUM TO BRAIN γγ-ENOLASE (PROTEIN 14-3-2) ON ETHANOL CONSUMPTION BY RATS

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Much information has now been obtained on the disturbance of function of individual enzymes and enzyme systems of the brain during the formation of alcohol addiction [1, 4]. It is not yet clear, however, whether changes observed in enzyme activity in the brain are primary factors in the pathogenesis of alcoholism or the result of a general disturbance of metabolism caused by chronic ethanol poisoning. One possible experimental approach to the solution of these problems is by selective inhibition of activity of individual enzymes (or isozymes) in the brain with the aid of specific immune sera, followed by investigation of the dynamics of ethanol consumption by the animals.

The aim of this investigation was to study the role of isozymes of brain enolase in the mechanisms of formation of addiction to ethanol. The glycolytic enzyme enolase is represented in nerve tissue by three isozymes with a dimer structure (αα, αγ, and γγ), of which isozymes containing the γ-subunit are specific for nerve tissue [3]. The isozyme γγ-enolase is contained mainly in neurons, and it is generally considered to be a marker of neuronal cells [9].

EXPERIMENTAL METHOD

The γγ-isozyme of enolase was isolated from bovine brain by the method in [8] without modifications [2]. The sample of γγ-enolase thus obtained was electrophoretically homogeneous. It was later used to immunize rabbits [6]. The immune serum was kept in the freeze-dried form at 4°C and dissolved in sterile distilled water immediately before the experiments with intracisternal injections. Immune activity of the serum was verified in Ouchterlony's test

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TABLE 1. Inhibition of γγ-Enolase in Vitro by Specific Antiserum

Enolase isozyme	Enolase activity, ΔU/ml enolase solution		
	without addition of serum	control serum	antiserum to γγ-enolase
αα	1,80	0,80	0,40
γγ	0,64	0,64	0,16

TABLE 2. Relative Proportions of Enolase Isozymes in Rat Brain after Intracisternal Injection of Antiserum to γγ-Enolase

Experimental conditions	Enolase isozymes, %		
	αα	αγ	γγ
Intact rats	44,2	36,0	19,8
Injection of immune serum	43,9	36,1	20,0
Injection of γγ-antiserum	47,6	38,1	14,3

against purified γγ-enolase and the soluble fraction of rat brain cytoplasm. To study the ability of antiserum to γγ-enolase to inhibit γγ-enolase activity in vitro, 0.5 ml of control or immune rabbit serum was added to 0.5 ml of the protein solution. The results were read 16 h later as the decrease in enolase activity.

The reconstituted γγ-antiserum (10 μliters) was injected once into the cisterna magna of rats (n = 30) anesthetized with ether. Control animals (n = 20) were given an injection of 10 μliters of nonimmune serum. The rats were decapitated 36 h later and the distribution of the enolase isozymes (αα, αγ, and γγ) investigated in the whole brain [2, 8]. In a parallel series of experiments on rats (n = 40), kept for 7 months under conditions of enforced alcoholization (15% ethanol solution as the sole source of liquid for drinking), the effect of intracisternal injection of γγ-antiserum and of nonimmune serum (control) on consumption of 7.5% ethanol solution, given free choice between alcohol and water, was studied. Before injection of the antiserum, a group of rats with preference for ethanol consumption (PE), consuming 7.5% ethanol solution in an amount of more than 50% of all fluid drunk, and an intermediate group (IG), consuming ethanol solution in an amount equal to 15-40% of all fluid drunk, were isolated. After injection of the antiserum, ethanol and water consumption was recorded daily for 12 days. The results were subjected to statistical analysis by Student's test.

EXPERIMENTAL RESULTS

In experiments in vitro the control serum obtained from an intact rabbit nonspecifically inhibited αα-enolase activity but did not affect γγ-enolase (Table 1), in agreement with our previous data [6]. Antiserum to γγ-enolase had an inhibitory effect on both enolase isozymes, reducing activity of the αα- and γγ-isozymes by 2 and 4 times, respectively. Evidently the γγ-antiserum, as well as the intact (control) serum, has a nonspecific inhibitory section on the αα-isozyme, whereas the γγ-isozyme is inhibited only by the corresponding γγ-antiserum.

After intracisternal injection of 10 μliters of nonimmune serum (control) the isozyme spectrum of whole rat brain enolase was identical with the spectrum of intact animals (Table 2). The relative percentages of the αα, αγ, and γγ isozymes in the first case were 43.9: 36.1:20, and in the second case 44.2:36:19.8. Injection of γγ-antiserum caused redistribution of the isozymes toward a significant decrease in activity of the γγ-enolase and a relative increase in activity of αα- and αγ-isozymes. Hence it follows that the γγ-antiserum, injected into the cisterna magna, interacts with the corresponding antigen (γγ-enolase), causing a decrease in its activity.

In experiments to study the effect of the γγ-antiserum on consumption of 7.5% ethanol solution and water by rats kept for 7 months under conditions of enforced alcoholization, the quantity of the ethanol solution and water drunk was measured. The total volume of fluid drunk by PE and IG rats was similar. Injection of nonimmune rabbit serum did not change the ethanol consumption in either group. The total volume of liquid drunk in this case was increased due to an increase in water consumption (Table 3). Antiserum to γγ-enolase reduced ethanol consumption in both groups, more especially in IG (from 8.3 ± 1.3 to 4.8 ± 1.4 ml daily). Under these circumstances there was a compensatory increase in water consumption. so that the total volume of liquid drunk did not change significantly. The time course of consumption of ethanol solution and water by animals of the PE and IG categories, shown in Figs. 1 and 2, reveals that injection of γγ-antiserum into animals of both groups potentiated their mean daily fluctuation in the consumption of fluid, especially of water.

Intracisternal injection of immune serum to γγ-enolase thus led to reorganization of the isozyme spectrum of the brain enolase and to a decrease in the consumption of ethanol

TABLE 3. Effect of Intracisternal Injection of Antiserum to Protein 14-3-2 on Consumption of Ethanol and Water (in ml) during Chronic Poisoning of Rats (M ± m)

Experimental conditions	Fluid consumed	PE		IG	
		before injection	after injection	before injection	after injection
Control (nonimmune serum)	7.5% Ethanol	15,3±0,9	13,7±1,2	8,1±1,1	7,7±1,2
	Water	7,3±1,2	11,0±1,4	17,2±1,9	22,7±2,5
	Total . . .	22,6±1,5	24,7±1,6	25,3±2,7	30,4±2,8
Antiserum to protein 14-3-2	7:5%:Ethanol	15,9±1,1	12,1±1,4*	8,3±1,3	4,8±1,4*
	Water	8,7±0,4	16,7±2,3*	18,6±1,0	21,2±1,7
	Total . . .	24,9±1,2	28,8±3,1	26,9±2,0	26,0±2,0

Legend. *p < 0.05 compared with group before injection.

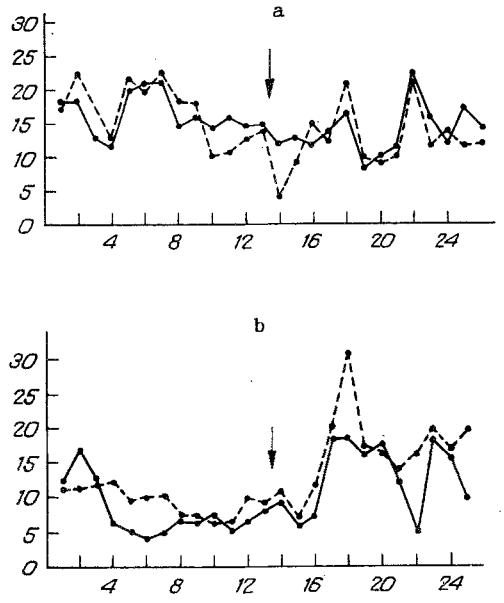

Fig. 1

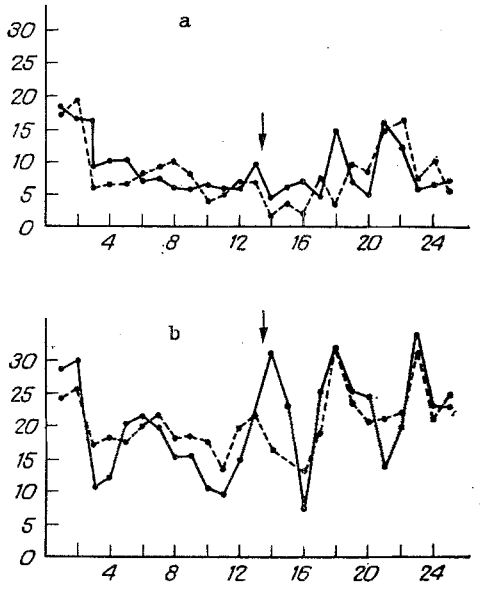

Fig. 2

Fig. 1. Effect of intracisternal injection of antiserum to γγ-enolase on time course of consumption of 7.5% ethanol solution (a) and water (b) by PE rats. Abscissa, days of experiment; ordinate, level of consumption of fluid (in ml). Continuous line, control; broken line, antiserum to γγ-enolase. Arrow indicates time of intracisternal injection.

Fig. 2. Effect of intracisternal injection of antiserum to γγ-enolase on time course of consumption of 7.5% ethanol solution (a) and water (b) by IG rats. Legend as to Fig. 1.

solution. This suggests that neurospecific enolase is involved in the mechanisms of formation of addition to ethanol. The question arises: how much of the enolase γγ-isozyme could be inhibited by injection of 10 μliters of immune serum? Measurement of enolase activity in the cytosol from whole rat brain showed that its total activity, expressed per gram of brain tissue, is 20 ΔU/g. The mean weight of the rat brain in the present experiments was 1.5 g. Consequently, total whole brain enolase activity was 30 ΔU. In experiments in vitro 50 μliters of γγ-antiserum reduced the enolase activity (0.64 U/g) fourfold (Table 1). Activity of γγ-enolase accounts for 20% of the total brain enolase activity (Table 2), i.e., its activity in whole rat brain is 6 ΔU/g. In experiments in vivo antiserum to γγ-enolase was injected in a volume of 10 μliters (5 times less than in the experiments in vitro). It is easy to calculate from the data given above that in this case the maximal reduction of

total whole brain γγ-enolase activity did not exceed 8.6% (which corresponds to inhibition of about 35-40% of all enolase activity in the neurons). However, the conventional nature of the assumption that antiserum is uniformly distributed throughout the mass of the brain will be evident. There is no doubt that local inhibition of γγ-enolase is much more marked. This hypothesis is in full agreement with the fact that ethanol consumption is reduced in animals receiving immune serum. The neuronal enolase isozyme evidently participates in the mechanisms of formation of addiction to ethanol. The molecular mechanism of the effect of γγ-antiserum on ethanol consumption is not clear. It can be tentatively suggested that inhibition of glycolytic neurospecific γγ-isozyme lowers the intensity of energy metabolism in neurons. It is evident that γγ-antiserum has no direct effect on the enzyme systems of glycolysis in the liver, in which up to 95% of ethanol entering the body is oxidized [5, 7]. The inhibitory action of γγ-antiserum on ethanol consumption is therefore evidently determined by the central effects caused by reduction of the energy supply to the brain neurons.

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CHARACTERISTICS OF MEMORY IN MRL/1 MICE AND EFFECT OF THYMIC PEPTIDES ON IT

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Much progress has been made in recent years in our understanding of the immunopathogenesis of rheumatic diseases [11]. Nevertheless their onset after stressful situations, the high frequency of placebo effects, and the absence of any parallel trend between clinical manifestations and immunologic parameters cannot be explained by the autoimmune theory of development. It is therefore important to study the physiological mechanisms maintaining the immune control of homeostasis, i.e., the suprasystemic mechanisms of regulation of immunity [5, 12], in particular, from the standpoint of N. P. Bekhtereva's concept of the stable pathological state of the brain on the matrix of long-term memory as its basis [2].

We shall examine one of the commonest forms of rheumatic disease, namely rheumatoid arthritis (RA), as a disease of adaptation with primary disturbance of the suprasystemic mechanisms of regulation of the immune control of homeostasis, realized clinically as RA by virtue of a genetic predisposition toward the development of an autoimmune lesion of connective tissue and, in particular, of joints [9]. With the foregoing facts in mind, it was

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