7. H. Bolander and G. Wahlström, Neuropharmacology, 23, 977 (1984).
8. W. Kelly, Annu. Rev. Pharmacol., 15, 327 (1975).
9. J. König and R. Klippel, The Rat Brain: a Stereotaxic Atlas of the Forebrain and Lower Parts of the Brain Stem, Baltimore (1963).
10. R. Myers, Methods in Psychobiology, Vol. 2, London (1972), pp.169-211.
11. J. Picard and P. Morelis, C. R. Acad. Sci. (Paris), 284D, 581 (1977).
12. R. Spector and A. Lorenzo, J. Pharmacol. Exp. Ther., 188, 55 (1974).

EFFECT OF A 3-HYDROXYPYRIDINE ANTIOXIDANT ON LEARNING IMPAIRMENT AND LIPOFUSCIN ACCUMULATION INDUCED BY ETHANOL

O. A. Kutepova, T. A. Voronina, and N. N. Zolotov

UDC 615.272.014.425.015.4:612.821.44].076.9

KEY WORDS: antioxidants; ethanol; learning; lipofuscin.

In the modern view the cell membrane is an important component of the primary process of aging [3], and activation of lipid peroxidation (LPO), observed during aging when antioxidant protection is weakend, is a universal mechanism of biomembrane modification and damage. Disturbance of structural and functional regulation of neuron membranes may lead to irreversible brain damage and to intracellular lipofuscin formation. Most investigators consider that the principal role in the genesis of lipofuscin is played by LPO processes, followed (or accompanied) by polymerization of low-molecular-weight aldehydes with primary amino groups of proteins. As a result of polymerization, compounds of the Schiff base type are formed, thanks to the presence of which lipofuscin possesses the property of autofluorescence [6]. On the other hand we know that under the influence of chronic ethanol consumption by rats and mice the content of saturated fatty acids is increased in their brain phospholipids; the cholesterol concentration also is increased, leading to a change in the structural organization of the membranes [7]. It has also been shown that prolonged alcoholization leads to persistent impairment of learning ability in mice [10]. The parameters of behavior under these circumstances approximate to those in old animals, and long-term alcoholization can thus be regarded as a model of rapid aging [11].

The ability of natural and synthetic antioxidants (tocopherol, ionol, etc.) to delay the development of aging processes is well known [4]. The aim of this investigation was to assess the protective action of the antioxidant 2-ethyl-6-methyl-3-hydroxypyridine (3-HP) on impairment of learning ability and lipofuscin accumulation in the brain tissues of animals induced by ethanol. It was shown previously that 3-HP can lengthen the survival of animals [4] and improve learning capacity in old rats [1].

EXPERIMENTAL METHOD

Experiments were carried out on noninbred female albino mice aged initially 3 months, and exposed to ethanol for 5 months by Freund's method [10] in our own modification. The animals were divided into three groups: 1) mice receiving 15% ethanol solution instead of drinking water for 5 months; 2) mice receiving 15% ethanol solution together with 3-HP instead of drinking water; 3) intact mice receiving water. Mice initially aged 15 months also were used. All the animals were kept on a standard diet. In the course of the experiments it was found that both control and experimental animals, weighing 32-37 g, consumed on average 4-5 ml per mouse per day throughout the experiment, but the volume of alcohol consumed by one mouse daily, calculated as absolute ethanol, was 0.56-0.75 ml, and the amount of 3-HP consumed simultaneously with ethanol was 0.60-0.75 mg/day per mouse (or 20-25 mg/kg body weight/day).

Research Institute of Pharmacology, Academy of Medical Sciences of the USSR, Moscow.
(Presented by Academician of the Academy of Medical Sciences of the USSR A. V. Val'dman.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 107, No. 3, pp. 314-317, March, 1989. Original article submitted April 5, 1988.

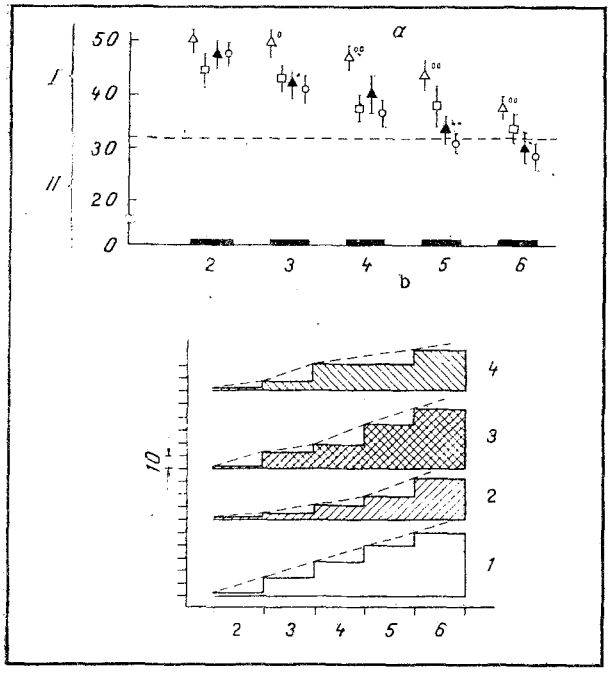

Fig. 1. Effect of 3-HP on defensive avoidance conditioning in alcoholized mice. a) Changes in latent period of reflex during its formation. Abscissa, time of learning (in days); ordinate, latent period of reflex (in conventional timer units). I) latent period of unconditioned reflex; II) latent period of conditioned reflex. Broken line indicates time of foot shock. Empty triangles – ethanol; filled triangles – ethanol + 3-HP; circles – control (3 months); squares – control (15 months); *$p < 0.05$, **$p < 0.01$ compared with mice receiving ethanol. $^{0}p < 0.05$; $^{00}p < 0.01$ compared with control; b) relative learning speed of animals. Abscissa, time of training (in days); ordinate, ratio of difference in latent period of reflex (in conventinal timer units) to latent period of reflex on previous day of training (in %). 1) Control (3 months), 2) ethanol; 3) ethanol + 3-HP, 4) control (15 months).

The animals' behavior was recorded 2 weeks after the end of alcoholization in a shuttle box ("Ugo Basile," Italy). A conditioned reflex was formed in the mice to simultaneous presentation of photic and acoustic stimuli (3 sec or 32 conventional timer units), under the influence of which the animal had to run into the same compartment of the box. Mice which did not run received a painful electric foot shock (5 sec or 46 conventional timer units). The interval between training exercises was 20 sec. Each mouse received 40 training exercises per session daily. The animals were trained for 6 days. the latent period of avoidance of foot shock by the animals from the time of presentation of the conditioned stimulus was recorded in conventional timer units.

The animals were decapitated 10 days after the end of training. The brain was removed, weighed, and homogenized, then extracted with a mixture of chloroform and ethanol (2:1) as described in [10]. The lipofuscin content was determined by the method in [8] on an "Opton" spectrofluorometer on the basis of the intensity of fluorescence of extracts from mouse brain homogenates at 436 nm, the wavelength of excitation being 360 nm. The experimental results were subjected to statistical analysis by the Student and Wilcoxon–Mann–Whitney method on an Apple-IIe computer using the "Biostatistics-III" program.

EXPERIMENTAL RESULTS

The study of the behavior of the alcoholized mice in the shuttle box 2 weeks after withdrawal of ethanol revealed marked delay in learning compared with control animals of the same sex and age (Fig. 1a). The difference was statistically significant on the 3rd, 4th, 5th, and 6th days of training. Mature mice aged 15 months also demonstrated reduced learning ability in a shuttle box and did not reach the fully trained level by the 6th day of testing. Addi-

TABLE 1. Effect of 3-HP on Intensity of Fluorescence of Mouse Brain Extracts after Chronic Alcoholization

Experimental conditions	Weight of brain, mg	Intensity of fluorescence, conventional units
Control – 3 months (13)	465,0±9,8	34,3±1,5
Control – 15 months (8)	462,1±13,5	121,5*±5,3
Ethanol (10)	470,8±8,3	114.8*±7,3
Ethanol + 3-HP (11)	469,9±11,1	47,5±4,5

Legend. *$p < 0.001$ compared with control. Number of animals given in parentheses.

tional administation of 3-HP preserved this learning ability in mice subjected to chronic alcoholization. This group of animals completed the learning process 3, 5, and 6 days earlier than mice receiving ethanol alone. The differnce between the different experimental groups of animals was seen even more demonstratively on analysis of the relative learning speed (Fig. 1b). In control animals, this was linear in character. Mice receiving ethanol alone had a slower learning speed than animals of the control group. Mice receiving 3-HP together with ethanol approximated in the character of their learning to control animals. Training mature (15 months) mice did not follow a uniform pattern, evidence of their reduced learning ability.

Investigation of extracts from brain homogenates revealed a higher intensity of fluorescence in material from mice aged 15 months than from the control animals (3 months). The weight of the brain was the same in the mice of all experimental groups. The intensity of fluorescence also was considerably increased in the chronically alcoholized mice. Meanwhile in mice taking 3-HP together with ethanol, fluorescence of the brain extracts was virtually identical in intensity throughout the period of alcoholization to that observed in control animals (Table 1).

The increase in the intensity of fluorescence in extracts of brain homogenates could reflect the more rapid formation of lipofuscin in the mouse brain tissues during chronic alcohol consumption. Thus 3-HP, when given together with ethanol, reduced lipofuscin accumulation in the brain during chronic alcoholization and also preserved the learning ability of the mice.

According to some investigators [2, 6, 12] accumulation of lipofuscin (or aging pigment) is regarded as the final stage of LPO processes in vivo and is used as an indicator of the free radical nature of cell damage during aging, and can be used as a parameter of chronological age. Our results showing increased fluorescence of extracts from mouse brain homogenates at the age of 15 months are in agreement with existing views according to which aging is accompanied by lipofuscin accumulation in the brain [12]. Long-term exposure to ethanol also leads to activation of LPO in animal tissue [9]. The absence of antioxidants, such as α-tocopherol, from the diet also leads to more rapid deposition of lipofuscin in the cells of various tissues [13] and on the other hand, supplementing of the diet with vitamin E and other antioxidants, such as meclofenoxate, inhibits lipofuscin accumulation in the mouse brain [10, 12]. However, there is also evidence that although supplementing the diet with vitamin E reduces lipofuscin accumulation, it has no significant effect on learning deficiency either in mice subjected to chronic alcoholization or in old animals [10]. Lipofuscin accumulation in the brain tissues by itself probably, therefore, has no direct relationship to impairment of learning in animals whether during natural aging or during chronic alcoholization.

The ability of 3-HP to reduce lipofuscin formation in the mouse brain during chronic alcholization may therefore be connected with the antioxidative activity of this compound [5], whereas preservation of ability to form conditioned reflexes in mice is probably largely realized through its ability to increase the resistance of the animal to the action of extremal factors.

LITERATURE CITED

1. T. A. Voronina, T. L. Garibova, and L. D. Smirnov, Byull. Eksp. Biol. Med., No. 9, 307 (1986).

2. J. F. Mead, in: Free Radicals in Biology, ed. by W.A. Pryor, Academic Press, New York (1976), Vol. 1, pp. 51-68.
3. I. Zh. Nad', Zh. Obshch. Biol., 43, No. 3, 335 (1982).
4. L. K. Obukhova, Usp. Khimii, 44, No. 10, 1914 (1975).
5. L. D. Smirnov and K. M. Dyumaev, Khim.-farm. Zh., No. 4, 28 (1982).
6. K. R. Brizzee and J. M. Ordy, Age Pigments, ed. by R. S. Sohal, Amsterdam (1981), pp. 102-154.
7. J. H. Chin and D. B. Goldstein, Membrane Fluidity in Biology, ed. by R. C. Aloia and J. M. Boggs, Vol. 3, New York (1985), pp. 1-38.
8. B. L. Fletcher, C. J. Dillard, and A. L. Tappel, Anal. Biochem., 52, 1 (1973).
9. S. W. French, The Biology of Alcoholism, Vol. 1, New York (1971), p. 437.
10. G. Freund, Life Sci., 24, No. 2, 145 (1979).
11. G. Freund, Alcoholism, 6, No.1, 13 (1982).
12. K. Nandy, Mech. Aging Dev., 8, 131 (1978).
13. A. L. Tappel, Ann. N.Y. Acad. Sci., 203, 12 (1972).

REDUCTION OF ACUTE ETHANOL TOXICITY BY ZINC SULFATE

A. B. Kampov-Polevoi and A. V. Skal'nyi

UDC 615.917.547.262].036.11.015.2:615.31:546.47

KEY WORDS: acute toxicity of ethanol; zinc sulfate; biotic doses; mice.

Chronic alcoholization of man and animals leads to the development of symptoms of zinc deficiency, associated with lowering of the Zn^{++} concentration in the blood, liver, and brain [5, 9, 10]. Experimental [5, 6] and clinical [5, 10] investigations have demonstrated positive results from the use of zinc salts to correct disorders induced by chronic alcohol intoxication. The therapeutic effect of zinc salts is usually associated with its effect on activity of certin enzymes (alcohol dehydrogenase, alkaline phosphatase, aminotransferases, etc.), on neurotransmitter metabolism, and on lipid peroxidation. The use of small (from 5 to 50 μg/kg) doses of zinc sulfate and chloride may also antagonize the acute toxic effects of ethanol and increase the survival rate of mice receiving lethal doses of alcohol [6, 8, 13].

The strongest protective effect against acute alcohol intoxication (AAI) has been observed after preliminary administration of fractional doses of zinc preparations [6, 8].

However, although the spectrum of action of zinc preparations includes a marked antialcoholic effect, the possibility that they may have a sobering effect has not hitherto been studied.

The aim of this investigation was to study the possibility of reducing the narcotic and acute toxic action of ethanol by administering zinc sulfate to animals with alcohol intoxication.

EXPERIMENTAL METHOD

Experiments were carried out on 260 noninbred male albino mice weighing 18-24 g, divided into groups with 17-32 animals in each group. The investigations were carried out in accordance with the technical recommendations of the Pharmacological Committee of the USSR [1]. The acute toxicity of ethanol (LD_{50}) was studied first and was shown to be 9.9 g/kg for this population of mice. In the experiments of series I, 30 min after intraperitoneal injection of 25% ethanol solution in a dose of 9.9 g/kg, a solution of analytically pure $ZnSO_4 \cdot 7H_2O$ in doses of 100, 50,

Laboratory for the Search for and Study of Agents for the Prevention and Treatment of Drug Addiction, Research Institute of Pharmacology, Academy of Medical Sciences of the USSR. Department of Rehabilitation of Children with Cerebral Paralysis, V. P. Serbskii All-Union Research Institute of General and Forensic Psychiatry, Ministry of Health of the USSR, Moscow.
(Presented by Academician of the Academy of Medical Sciences of the USSR A. V. Val'dman.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 107, No. 3, pp. 317-318, March, 1989. Original article submitted April 5, 1988.

 0007-4888/89/0003-0346$12.50