Pharmacology Biochemistry & Behavior, Vol. 17, pp. 1037–1041, 1982. Printed in the U.S.A.

Ethanol Intoxication and Withdrawal Among Three Age Groups of C57BL/6NNIA Mice

W. GIBSON WOOD,[1] H. JAMES ARMBRECHT AND RONALD W. WISE

Geriatric Research, Education, and Clinical Center, VA Medical Center, St. Louis, MO 63125
Departments of Medicine and Biochemistry
St. Louis University School of Medicine, St. Louis, MO 63125

Received 21 May 1982

WOOD, W. G., H. J. ARMBRECHT AND R. W. WISE. *Ethanol intoxication and withdrawal among three age groups of C57BL/6NNIA mice*. PHARMAC. BIOCHEM. BEHAV. 17(5) 1037–1041, 1982.—Data have not been forthcoming on the effects of chronic ethanol administration on intoxication and severity of withdrawal using animals representative of the life-span of a particular species. The purpose of this study was to examine ethanol intoxication and withdrawal among three age groups (3, 14, 25 months) of C57BL/6NNIA male mice. Ethanol was administered in a liquid diet for 14 days. Pair-fed control groups and laboratory chow groups were also employed. Blood ethanol levels, signs of intoxication and withdrawal, liquid diet consumption, and body weight were measured. Old mice were significantly more intoxicated than younger mice. However, young mice consumed more ethanol as compared to the older mice. Blood ethanol levels did not differ among the three age groups, although variability was high within each age group. The ethanol liquid diet groups did not show a decrease in body weight. Withdrawal was more severe for old animals than younger animals. The greater effects of ethanol observed in the old animals do not appear to be attributable to age differences in blood ethanol levels, amount of ethanol consumed, or body weight loss.

Aging Ethanol intoxication and withdrawal Age differences C57BL/6NNIA mice Ethanol liquid diet

THE effects of ethanol are related to the age of the organism. Using mice and rats representative of the life-span, age-related differences in response to ethanol have been reported for measures such as voluntary ethanol consumption and the effects of acute administration of ethanol on body temperature, motor activity, and loss and regaining of righting response (see [17] for a review). Generally, voluntary ethanol consumption declines with increasing age [15] although some studies have reported greater consumption for old animals as compared to younger animals [6]. The relationship between voluntary ethanol consumption and age is dependent on several factors, e.g., cross-sectional versus longitudinal design, ethanol concentration, gender, strain/stock [17].

Studies on acute administration of ethanol among different age groups have reported that old animals are more affected as compared to younger animals [1,11]. Ethanol-induced hypothermia has been found to be greater in 18 to 20 month old female Sprague-Dawley rats as compared 2 to 3 and 11 to 12 month animals [1]. It also was observed that the oldest group slept longer following an injection of ethanol (4.0 g/kg) as compared to the other two age groups. Old C57BL/6 mice (24 months) have been found to sleep longer following an injection of ethanol as compared to mice six months of age [11]. The oldest group lost and regained the righting response at lower brain ethanol levels as compared to the 6 month old animals.

Voluntary ethanol consumption and effects of acute administration of ethanol among different age groups have been examined in several studies. Whereas, data have been forthcoming on effects of acute administration of ethanol, very little data are available on the effects of chronic ethanol administration among aging animals. To our knowledge, there has not been a published study that has examined physical dependence to ethanol using aged animals. Abu Murad, Begg, Griffiths, and Littleton [2] have reported that "weanling" male mice (8–10 g) of the TO strain and adult mice (30–35 g) differed in severity of withdrawal signs and duration of withdrawal after exposure to ethanol vapor for 10 days. The adult mice showed more severe signs of withdrawal and the duration of withdrawal was longer. Blood ethanol levels did not differ between the two age groups during the 10 days of ethanol administration. Abu Murad *et al.* [2] demonstrated that ethanol withdrawal is more severe for older animals however, the ages of the two groups were not reported. In addition, because the weanling animals were not fully developed, e.g., sexually immature, it is possible that the observed differences are the result of maturation rather than changes occurring with aging in mature animals.

It is not known if mature animals representative of the life-span of the species differ in the effects (e.g., intoxication and withdrawal) of chronic ethanol administration. Therefore, the purpose of this study was to examine ethanol intox-

[1]Requests for reprints should be addressed to W. Gibson Wood, VA Medical Center, GRECC 111G-JB, St. Louis, MO 63125.

ication and withdrawal among three different age groups of C57BL/6NNIA male mice administered ethanol in a liquid diet.

METHOD

Subjects

C57BL/6NNIA male mice of three different ages (3 months, 14 months, and 25 months) were used. The mean life-span of the C57BL strain has been reported to be approximately 676 days [13]. Animals were purchased from the Special Aging Colony of the National Institute on Aging that is maintained by Charles River Laboratories, Wilmington, Massachusetts. Animals were allowed to acclimatize to the laboratory for four weeks prior to the beginning of the experiment. Laboratory chow and water were provided ad lib before the experiment began.

Liquid Diet Administration

Three diet groups were used: (1) ethanol-liquid diet; (2) a pair-fed control liquid diet; and (3) a laboratory chow control diet. Each diet group contained eight animals per age group. Ethanol (5% w/v) was administered in the Bio-Serv F711 liquid mouse diet. The liquid diet control groups were pair-fed the Bio-Serv liquid mouse control diet. The laboratory chow control groups received Purina chow and water ad lib. The liquid diets were administered in modified Richter tubes (Bio-Serv 41). Measurement of liquid diet consumption was made at 8:00 a.m. and 4:00 p.m. each day. Fresh diets were administered daily at 4:00 p.m. The amount of liquid diet administered to the pair-fed control animals was based on the amount consumed by the ethanol animals the previous day. Body weight was not reduced prior to administration of the diets.

Signs of intoxication were observed every two days at 8:00 a.m. Intoxication was measured using the scoring system developed by Freund [4]: No signs, 0; Stage 1, ataxic, rapid gait; Stage 2, impaired gait, falling to side; and Stage 3, coma. Observations were made by two individuals who were not aware of which treatment an animal was receiving. Body weight was recorded the day before diets were administered and every two days during administration of the diets.

Blood ethanol levels were measured at 8:00 a.m. on Day 4 and Day 12. Twenty μl of whole blood was withdrawn from the tail in heparinized capillary tubes from animals in each group. The whole blood was added to 60 μl of 6.25% (w/v) trichloroacetic acid and centrifuged for 5 minutes at 1000 $\times$ g. The supernatant was withdrawn and frozen immediately for analysis using the enzymatic method of Bucher and Redetzki [3].

Withdrawal

Ethanol was withdrawn from the liquid diet on Day 15 at 8:00 a.m. Animals in each liquid diet group continued to receive the liquid diet without ethanol during the withdrawal period. Signs of withdrawal were observed every two hours for the first eight hours and then at 12 and 24 hours following removal of ethanol. Withdrawal was measured using the signs developed by Goldstein and Pal [5] (e.g., hypoactivity, tremor, convulsions on handling, spontaneous convulsions). Blood ethanol levels were measured following withdrawal at 0 and 8 hours using the procedures described under diet administration.

Data for stages of intoxication and signs of withdrawal were analyzed using Mann-Whitney U-test [7] and Kruskal-Wallis analyses [7]. Analyses of variance and a priori t-tests for multiple comparisons were used for data analyses of blood ethanol levels, ethanol consumption, and body weight [9].

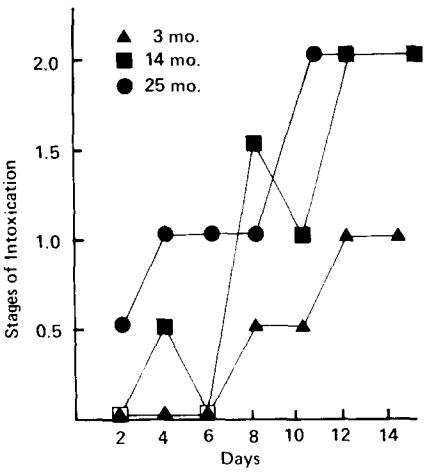

FIG. 1. Median stage of intoxication for each ethanol group. Each point represents the median of eight animals.

RESULTS

Age was related significantly to stages of intoxication (Kruskal-Wallis=16.47, $p<0.001$). Figure 1 shows median stages of intoxication across the 14 day period of ethanol administration for the three age groups. Overall, the level of stage of intoxication for the 25 month group was higher than the level for the other two age groups (Mann-Whitney U-tests, p's<0.03). Differences between the 14 and 25 month age groups were greater generally in the first 6 days of ethanol administration than for the remaining part of the experiment. Stage of intoxication was significantly higher for the 14 month age group as compared to the 3 month group (Mann-Whitney U-tests, $p<0.003$). The largest difference occurred between Days 8 and 12.

Severity of intoxication increased across the 14 days of ethanol admnistration (Fig. 1). The two older groups showed a greater increase in intoxication as compared to the 3 month group.

Ethanol consumption, expressed as g/kg of 95% ethanol differed significantly, $F(2,21)=6.97$, $p<0.005$ among the three age groups. It can be seen in Fig. 2 that the 3 month age group consumed more ethanol as compared to the other two age groups. Multiple comparison tests indicated that this difference was significant (t-test, $p<0.02$) when the 3 month group was compared to each of the older groups. Ethanol consumption was lowest for the 25 month age group. Mean differences between the 14 and 25 month age groups were not significant (t-test, $p>0.05$).

Table 1 lists the means and standard error of the means for body weight for each age and treatment group. Body weight was significantly related to age, $F(2,63)=107.52$, $p<0.001$. Young animals weighed less as compared to older animals. The treatment effect of diet was significant, $F(2,63)=4.77$, $p<0.01$. In addition, the interaction among

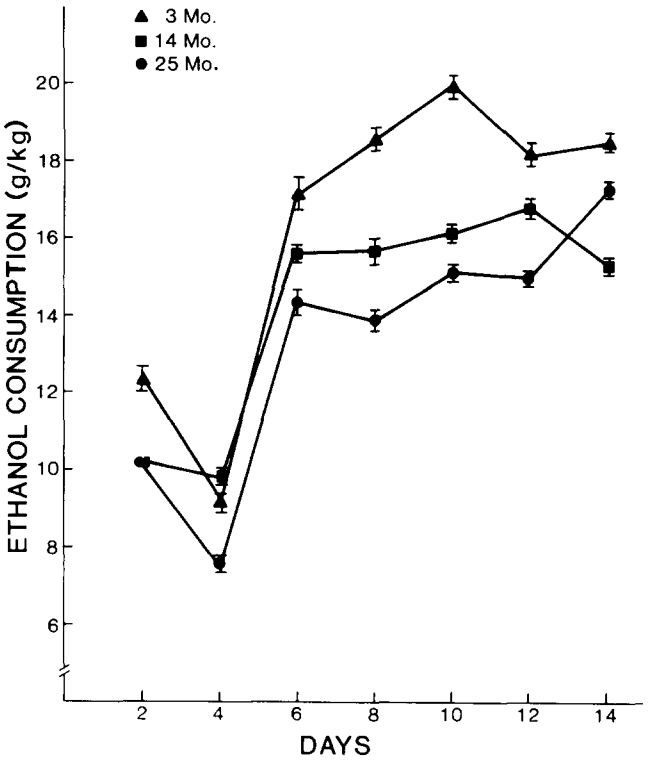

FIG. 2. Mean (±SEM) consumption of 95% ethanol (v/v) expressed as grams of ethanol per kilogram of body weight. Each point represents the mean for two days of ethanol consumption.

age, diet, and time of measurement was significant, $F(8,126)=2.54$, $p<0.02$. This interaction is attributable to the small decrease in body weight observed for the 14 and 25 month pair-fed control groups. The percent decrease from the first weight measurement to the last weight measurement was 8% and 5% for the 14 and 25 month age groups. It can be seen in Table 1 that there was a slight increase in body weight for the ethanol groups. The laboratory chow groups also showed an increased in weight over the 14 day period.

Table 2 lists the means and standard error of the means for blood ethanol level for each age group. Age differences were not significant, $F(2,21)=0.85$, $p=0.44$ although the mean blood ethanol level was higher at both measurement periods for the 3 month group as compared to the other two age groups. The absence of significant differences may have resulted from the variability within each age group. Blood ethanol levels increased significantly, $F(1,21)=13.51$, $p<0.001$ from Day 4 to Day 12. This increase occurred for each age group.

Age was related to severity of withdrawal when measured by general body tremor. Data are presented in Fig. 3 that describe the median degree of general body tremor following withdrawal. Overall, body tremor for the 25 month age group differed significantly from the tremor for the 3 month group (Mann-Whitney U-test, $p<0.05$). Body tremor across the 24 hour period for the 14 month age group was not significantly different from the other two age groups. However, it can be seen in Fig. 3 that general body tremor was greatest between 4 and 8 hours for both the 14 and 25 month age groups as compared to the 3 month age group.

TABLE 1

BODY WEIGHTS FOR EACH GROUP DURING ETHANOL ADMINISTRATION

Group	Day 0	2	4	6	8	10	12	14
Ethanol								
3 mo	25.27*	24.97	24.90	25.11	25.12	25.31	25.46	25.67
	0.31†	0.28	0.43	0.32	0.34	0.29	0.64	0.65
14 mo	31.91	31.82	31.43	31.90	32.26	32.16	32.66	32.70
	0.54	0.62	0.43	0.52	0.61	0.54	0.58	0.43
25 mo	33.40	32.92	33.06	32.88	33.13	33.48	33.32	33.43
	0.93	1.16	1.12	1.04	1.11	1.09	1.16	0.99
Pair-fed								
3 mo	25.00	25.41	24.83	24.63	24.42	24.77	25.12	25.05
	0.42	0.52	0.66	0.38	0.33	0.34	0.20	0.54
14 mo	31.57	30.41	30.77	29.85	31.08	30.08	29.37	29.05
	0.93	1.15	0.83	0.85	0.74	0.67	0.62	0.58
25 mo	33.52	33.56	31.85	31.40	31.28	31.82	32.53	31.96
	0.54	0.87	0.78	0.84	0.64	0.66	0.67	0.63
Lab Chow								
3 mo	25.66	25.86	25.76	26.15	26.65	27.01	27.61	27.36
	0.67	0.68	0.66	0.65	0.54	0.68	0.72	0.71
14 mo	31.34	31.55	31.71	31.82	32.30	31.75	32.31	31.78
	0.94	1.05	0.99	0.94	1.07	0.94	0.93	0.93
25 mo	33.86	33.83	33.57	34.57	34.66	34.28	35.21	34.61
	0.85	0.79	0.89	0.73	0.83	0.90	0.92	0.72

*Mean of 8 animals.
†Standard error of the mean.

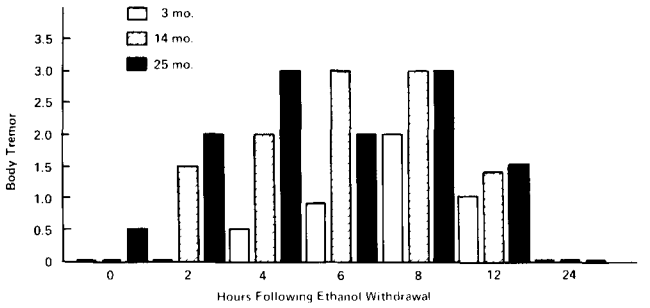

FIG. 3. Median score for body tremor observed during withdrawal from ethanol for each ethanol age group. Each bar represents the median of eight animals.

TABLE 2

BLOOD ETHANOL LEVELS (MG/DL) DURING ETHANOL ADMINISTRATION

Age Group	Day 4	Day 12
3 mo	91.38* ± 38.85†	235.74 ± 54.33
14 mo	78.63 ± 38.09	156.15 ± 43.97
25 mo	41.59 ± 13.14	168.33 ± 55.36

*Each data point represents the mean of 8 animals.
†Standard error of the mean.

TABLE 3

HYPOACTIVITY LEVELS DURING WITHDRAWAL FOR EACH ETHANOL GROUP

	Hours						
Age Groups	0	2	4	6	8	12	24
3 mo	0*	0	0	2.0	1.0	1.0	0
14 mo	0	0	1.0	2.0	2.0	1.0	0
25 mo	1.0	1.0	2.0	2.0	2.0	2.0	0

*Each data point represents the median of 8 animals.

Table 3 contains the medians for hypoactivity. Signs of hypoactivity increased over the withdrawal period. The largest increase was observed for the two older groups as compared to the youngest group but this difference was not significant. Spontaneous and induced convulsions were not observed among the three ethanol age groups.

Blood ethanol levels did not differ significantly among the three age groups ($p=0.85$). The means and standard error of the means for each age group when ethanol was removed at 8:00 a.m. were: 3 month 134.98±59.42; 14 month 102.69±42.81; 25 month 151.68±58.05. Ethanol was not detected in blood eight hours following withdrawal for any of the three age groups.

DISCUSSION

The effects of chronic administration of ethanol on intoxication and severity of withdrawal were greater for aged mice as compared to younger mice. These results are in agreement with studies that have examined the effects of acute administration of ethanol among different age groups of animals (e.g., [1,11]. Aged animals are more affected by both acute and chronic ethanol administration than younger animals.

The response to ethanol of the 14 month and 25 month age groups was generally similar. It might be hypothesized that the differences between the young and older mice are due to developmental changes occurring within the young animals as opposed to an aging process(es) per se. While the present study does not address this issue, the results do underscore the necessity of using more than two age groups when studying age differences.

The age differences reported here in severity of ethanol intoxication and ethanol withdrawal do not appear to be explained by age differences in ethanol consumption, blood ethanol levels, or ethanol-induced changes in body weight. The 3 month old group consumed significantly more ethanol (g/kg) as compared to the other two age groups. Similar results have been reported for voluntary ethanol consumption of different age groups of C57BL mice [15]. An explanation for the lower ethanol consumption of the older animals in the present study may have resulted from a decline in general activity. The old animals were more intoxicated than the younger animals which may have resulted in a reduction of activity and reduced caloric demand. Partial support for this explanation of reduced caloric demand is demonstrated by the absence of weight loss in the two oldest ethanol groups even though they consumed less of the ethanol-liquid diet. The two oldest pair-fed control groups showed a slight decrease in body weight.

Blood ethanol levels did not differ significantly among the three age groups during intoxication or during the withdrawal period. These data should be interpreted cautiously because of the large variability for blood ethanol levels within each age group. This variability may be due in part to animals consuming the liquid diet at different times over 24 hours. Blood ethanol was measured in the morning. It has been reported that blood ethanol levels of C57BL mice are higher in the evening than in the morning [8]. To our knowledge, there are no data on aging and diurnal rhythms of blood ethanol levels or the pattern of liquid diet consumption among different age groups of mice.

The age differences in intoxication and withdrawal also cannot be attributed to ethanol-induced decreases in body weight. There was a slight increase in body weight of each ethanol group over the 14 days of ethanol administration.

Age differences were observed for signs of withdrawal. However, withdrawal was generally mild for the three age groups. Spontaneous and induced convulsions were not observed. The absence of convulsions during withdrawal using the C57BL strain has been reported previously [8]. However, convulsions during withdrawal in the C57BL strain have been observed (e.g., [10]). It was reported that C57BL/6 male mice showed spontaneous convulsions and convulsions induced by handling. Body weight decreased approximately 12% during the ethanol administration period and when ethanol was withdrawn the mean blood ethanol level was 264±34 mg/dl in the above-mentioned study [10]. In the present study, a decrease in body weight was not observed for any of the age groups receiving the ethanol diet, the blood ethanol levels were lower, and the concentration of

the ethanol solution used was slightly less which might account for the absence of spontaneous convulsions and induced convulsions.

The greater effect of ethanol on aging mice as compared to younger mice is not the result of age differences in ethanol consumption, blood ethanol levels or ethanol-induced changes in body weight. There are several different mechanisms that might explain age-related differences in response to ethanol. The mechanisms include age differences in metabolism, percentage of body water to body weight, biochemical and biophysical changes in brain [16]. Old animals do metabolize ethanol at a slower rate as compared to younger animals. It has been reported that 6 month mice metabolize a 3 g/kg dose of ethanol at a rate of 0.46 μmol/min/g as compared to the rate of 0.37 μmol/min/g for 24 month old mice [11]. While the differences in metabolism were significant, it was concluded that these differences could not account for the greater effects of ethanol observed in old mice [11].

Another explanation that has been proposed is that age differences in response to ethanol may be the result of differences in the percentage of body water to body weight [18]. York [18] reported that when a dose of ethanol was based on body water as compared to body weight, age differences were small and non-significant. In the present study, body water differences alone do not explain the age diferences in response to ethanol. Aged mice consumed less ethanol and had lower blood ethanol levels but were more impaired as compared to younger animals.

Ethanol-induced changes in body temperature may contribute to the age-related effects of ethanol. Aging animals may have difficulty regulating body temperature in response to chronic ethanol administration and during withdrawal. Ethanol-induced hypothermia has been found to be greater in old rats following acute administration of ethanol than in younger rats [1]. It might be hypothesized that age-related differences in response to chronic administration of ethanol would be reduced if hypothermia was inhibited.

A fourth explanation for age-related differences in response to ethanol may be differences in the biochemical and biophysical activity of brain [14]. Aging animals lose the righting response at lower brain ethanol levels as compared to younger animals [11]. It has been shown that the activity of $(Na^+ + K^+)$-ATPase in synaptosomes from old C57BL/10 (26–29 months) mice was inhibited more following perturbation with ethanol than synaptosomes from 3 month old mice [14]. The activity of this enzyme has been associated with ethanol intoxication and withdrawal however, many questions remain as to the involvement of $(Na^+ + K^+)$-ATPase in ethanol intoxication and withdrawal [12].

Aged animals are affected more by both acute and chronic administration of ethanol as compared to younger animals. The causal mechanisms for these age-related differences remain to be elucidated. It may be that a variety of factors, e.g., metabolism, body water, brain sensitivity account for age differences in response to ethanol. The clinical implications of these results are that moderate alcohol consumption by aged individuals may have more severe pathological effects than similar amounts of alcohol consumed by younger individuals. Obviously, much more work is needed in the area of aging and the effects of alcohol because of the increasing number of aging individuals in the population.

ACKNOWLEDGEMENTS

A preliminary report of these data were presented at the 13th Annual Medical-Scientific Conference of the National Alcoholism Forum, April 1982. The research was supported by the Medical Research Service of the Veterans Administration. Appreciation is extended to Cheryl Duff, David Rocco and Judy Walsh for expert technical assistance.

REFERENCES

1. Abel, E. L. and J. L. York. Age-related differences in response to ethanol in the rat. *Physiol. Psychol.* **7**: 391–395, 1979.
2. Abu Murad, C., S. J. Begg, P. J. Griffiths and J. M. Littleton. Hepatic triglyceride accumulation and the ethanol physical withdrawal syndrome in mice. *Br. J. exp. Pathol.* **58**: 606–615, 1977.
3. Bucher, T. and H. Redetzki. Eine spezifische photometrische bestimung von athylalkohol auf fermentativen wege. *Klin. Wschr.* **29**: 615–621, 1951.
4. Freund, G. Alcohol withdrawal syndrome in mice. *Archs Neurol.* **21**: 315–320, 1969.
5. Goldstein, D. B. and N. Pal. Alcohol dependence produced in mice by inhalation of ethanol: Grading the withdrawal reaction. *Science* **172**: 288–290, 1971.
6. Goodrick, C. L. Alcohol preference of the male Sprague-Dawley albino rat as a function of age. *J. Geront.* **22**: 369–371, 1967. 1967.
7. Hays, W. L. *Statistics*. New York: Holt, Rinehart and Wilson, 1963.
8. Kakihana, R. Alcohol intoxication and withdrawal in inbred strains of mice: Behavioral and endocrine studies. *Behav. neural. Biol.* **26**: 97–105, 1979.
9. Kirk, R. E. *Experimental Design: Procedures for the Behavioral Sciences*. Belmont, CA: Brooks/Cole, 1968.
10. Rabin, R. A., B. B. Wolfe, M. D. Dibner, N. R. Zahniser, C. Melchior and P. B. Molinoff. Effects of ethanol administration and withdrawal on neurotransmitter receptor systems in C57 mice. *J. Pharmac. exp. Ther.* **213**: 491–496, 1980.
11. Ritzmann, R. F. and A. Springer. Age-differences in brain sensitivity and tolerance to ethanol in mice. *Age* **3**: 15–17, 1980.
12. Roach, M. K. Changes in the activity of Na^+ K^+-ATPase during acute and chronic administration of ethanol. In: *Biochemistry and Pharmacology of Ethanol*, vol. 2, edited by E. Majchrowicz and E. P. Noble New York: Plenum Press, 1979, pp. 67–80.
13. Storer, J. B. Longevity and gross pathology at death in 22 inbred mouse strains. *J. Geront.* **21**: 404–409, 1969.
14. Sun, A. Y. and T. Samorajski. Effect of age and alcohol on $(Na^+ + K^+)$-ATPase activity of whole homogenate and synaptosomes prepared from mouse and human brain. *J. Neurochem.* **24**: 161–164, 1975.
15. Wood, W. G. Ethanol preference in the C57BL/6 and BALB/c mice at three ages and eight ethanol concentrations. *Expl Aging Res.* **2**: 425–434, 1976.
16. Wood, W. G. Age-associated differences in response to alcohol in rats and mice: A biochemical and behavioral review. *Expl Aging Res.* **2**: 543–562, 1976.
17. Wood, W. G. and H. J. Armbrecht. Behavioral effects of ethanol in animals: Age differences and age changes. *Alcoholism: Clin. exp. Res.* **6**: 3–12, 1982.
18. York, J. L. The influence of age upon the physiological response to ethanol. In: *Alcoholism and Aging: Advances in Research*. edited by W. G. Wood and M. F. Elias. Boca Raton, FL: CRC Press, 1982, pp. 99–113.