The results with the compounds reported here are somewhat disappointing. Simple addition of an aromatic side chain to 2-PAM, with the increased opportunity for hydrophobic or π bonding to the enzyme, as in the monoquaternary compounds, reduces the reactivation rate. The 2-PAM "diquaternaries" VII and VIII are markedly superior to IX, yet they have been returned only to the activity of the parent 2-PAM. Still, we feel that the rationale on which the design of these compounds is based is reasonable and bears additional exploration. Perhaps our choice of compounds was too narrow. AChE is known to have extensive hydrophobic binding regions near the anionic subsite [16, 17] which should be exploitable. And the combination of the intrinsic reactivity of 2-PAM with the diquarternary multiplication factor observed for 3- and 4-PAMs, if it could be achieved, would give compounds with second-order reactivation rate constants of the magnitude of 10^5–10^6 M^{-1} min^{-1}. Such an objective is well worth additional effort.

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REFERENCES

1. G. B. Koelle (Sub Ed.), *Handbuch Exp. Pharmakol*, Vol. 15, p. 921. Springer, Berlin (1963).
2. E. Heilbronn and B. Tolagen, *Biochem. Pharmac.* **14,** 73 (1965).
3. E. Bay and G. M. Steinberg, *Evaluation of Chemotherapeutic Compounds in Nerve Agent Poisoning*, EATR 4716, June 1973, Edgewood Arsenal, MD National Technical Information Service, Dept. of Commerce, Springfield, Va. 22151.
4. N. K. Schaffer, S. C. May, Jr. and W. H. Summerson, *J. biol Chem.* **206,** 201 (1954).
5. J. Patocka, *Colln Czech. chem. Commun.* **37,** 899 (1972).
6. K. Schoene and E. M. Strake, *Biochem. Pharmac.* **20,** 1041 (1971).
7. K. Schoene, *Biochem. Pharmac.* **21,** 163 (1972).
8. T. A. Loomis and B. Salefsky, *Toxic. appl. Pharmac.* **5,** 685 (1963).
9. B. E. Hackley, Jr., L. M. Berkowitz and M. D. Pankau, *Design and Synthesis of Potential Chemotherapeutic Compounds for Treating Poisoning by Anticholinesterases. Preparation of 3-Benzyloxypicolinaldoximes*, EATR 4079; AD-809230L, March 1967. Defense Documentation Centre, Cameron Station, Alexandria, Va. 22314.
10. W. K. Berry and D. R. Davies, *Biochem. J.* **100,** 572 (1966).
11. R. J. Kitz, S. Ginsburg and I. B. Wilson, *Biochem. Pharmac.* **14,** 1471 (1965).
12. H. Lineweaver and D. Burk, *J. Am. chem. Soc.* **56,** 658 (1934).
13. E. Heilbronn, *Biochem. Pharmac.* **12,** 25 (1963).
14. J. Patocka, *Colln Czech. chem. Commun.* **38,** 2996 (1973).
15. E. I. C. Wang and P. E. Braid, *J. biol. Chem.* **242,** 2683 (1967).
16. G. M. Steinberg, M. L. Mednick, J. Maddox, R. Rice and J. Cramer, *J. med. Chem.* **18,** 1056 (1975).
17. M. I. Kabachnik, A. P. Brestkin, N. N. Godovikov, M. J. Michelson, E. V. Rozengart and V. I. Rozengart, *Pharmac. Rev.* **22,** 355 (1970).

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Effect of chronic ethanol administration on liver alcohol dehydrogenase activity in mice

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It has been established that about 90 per cent of ingested ethanol is metabolized in the liver [1] and that the rate of ethanol metabolism is markedly increased in humans and experimental animals after chronic ethanol consumption [2–5]. The enzyme alcohol dehydrogenase (ADH), located in hepatic parenchymal cells, has a primary function in the metabolism of ethanol by degrading it to acetaldehyde [6]. Studies designed to investigate the possibility of increased ADH activity to explain increased ethanol metabolism after chronic administration have produced a variety of results. Some investigators found an increase in ADH activity after chronic administration [2,7–9] and others found no change or a slight decrease [10]. Strains of mice which have a genetically determined preference for ethanol in a two-bottle test showed heightened activity of both ADH and acetaldehyde dehydrogenase prior to alcohol adminstration [11]. However, the relationship between liver ADH activity and ethanol metabolism is poorly correlated in the intact animal regardless of strain [12]. Furthermore, an increase in the rate of ethanol metabolism can occur without changes in ADH activity [3,4,13,14], and thus the overall correlation between ADH activity and increased ethanol metabolism is not strong. More recent investigations have been directed toward explaining the enhanced rates of ethanol metabolism via a microsomal ethanol-oxidizing system [15] or by the increased rate of nicotinamide dinucleotide regeneration by the mitochondria in a hypermetabolic state induced in the liver by chronic ethanol consumption [16].

The present study rather than attempting to elucidate the relationship between ADH activity and ethanol metabolism was designed to examine the induction effect of

chronic ethanol consumption on ADH activity by administration through drinking water. Male mice were allowed to consume a 15% ethanol solution as the sole source of liquid from age of weaning (21 days old) to an age of 73–74 days. At the termination of the experiment, animals were sacrificed and liver ADH activity was determined and compared to that of the controls.

Male Swiss–Webster mice (*Mus musculus*) obtained at 21 days of age from Simonsen Laboratories, Calif., were given a 15% ethanol solution (diluted from 95% by volume) as the sole source of liquid. In the control group, tap water and Purina rat chow were given *ad lib.* to day of sacrifice. All of the mice were tested for a preference to 15% ethanol by the three-tube method [17] at days 51–56. These mice were part of another study on aggression and alcohol preference.

Eight mice from the experimental group and twelve from the control group were randomly selected, weighed, and sacrificed by decapitation. The livers were excised, rinsed in double distilled water, blotted dry, and weighed. The homogenization of the entire liver was performed in 5 vol. (w/v) of 0.25 M sucrose in ice in a glass dounce by five to seven strokes. The resulting slurry was spun down at 20,000 *g* for 10 min in a refrigerated centrifuge and the supernatant removed. This was assayed immediately or frozen overnight before assaying. No loss of activity was detected from freezing.

The assay for ADH [11] was as follows. The reaction mix consisted of 8.35 ml of 0.1 M glycine adjusted to pH 9 with sodium hydroxide, 0.4 ml of 2% ethanol (diluted from 95%), 0.5 ml of 10 mg/ml of NAD (obtained from Sigma Chemical Co., MO; solution made fresh on day of assay), and 0.4 ml of supernatant. This mix was warmed in a 37 water bath prior to initiation of the reaction and kept warm during the measurements. The increase in absorbance created by the NADH liberation was read from 1-ml aliquots taken at 1-min intervals for 8 min at 340 nm on a Hitachi double beam spectrophotometer using a distilled water blank. Each sample was measured in duplicate and the average change in optical density per min was expressed per g wet weight of liver. Significance of the data obtained from the two groups was determined by means of Student's *t*-test.

The activity of ADH as measured by liberation of NADH for the control group was found to be 10.51 ± 0.659 (in thousandths of optical density units/min/wet weight of liver ± S. E.) with a range of 7.09. The ADH activity of the experimental group was 12.87 ± 0.663 with a range of 5.80. These data can also be expressed in terms of μmoles NADH/min/gram wet weight of liver by using the molar extinction coefficient of 6.22×10^3, and thus become 1.69 ± 0.16 for the control group and 2.08 ± 0.18 for the experimental. Using these data, $t = 2.411$, or $P = 0.015$.

From the preference test to 15% ethanol at days 51–56, it was found that two mice from the control group and one from the experimental group showed a preference.

In this study, a prolonged administration of a moderate concentration of ethanol produced a 20 per cent increase in hepatic ADH activity over controls. Although this increase is not as great at the highest level of induced activity (50 per cent) previously reported by others [2], it was statistically significant at a high level ($P = 0.015$) and points to the validity of the concept of alterations in ADH activity in response to chronic ethanol exposure at moderate dosages. Studies in which a decrease in ADH activity was found after prolonged ethanol administration suggest the possibility of some pathological effect on the hepatic parenchymal cells and a concomitant decrease in ADH activity due to high doses of ethanol [18]. In particular, there was an increase and then a decrease in ADH activity in rats given 20% ethanol as drinking fluid for 26 weeks. In another study [10], a correlation was found between decreased ADH activity and fatty infiltration of the liver in rats at high ethanol exposure (20% ethanol as drinking fluid, 6 g ethanol/kg of rat every day by stomach tube for 6 weeks). Although in the present study we did not determine the presence or absence of liver pathology, it is probable that the treatment did not produce the detrimental effects of the ethanol administrations found by others. In conclusion, chronic exposure to ethanol at a moderate concentration as drinking fluid is effective in inducing an increase in ADH activity.

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REFERENCES

1. E. Jacobsen, *Pharmac. Rev.* **4,** 107 (1952).
2. R. D. Hawkins, H. Kalant and U. M. Khana, *Can. J. Physiol. Pharmac.* **44,** 241 (1965).
3. C. S. Lieber and L. M. DeCarli, *J. biol. Chem.* **245,** 2505 (1970).
4. F. Tobon and E. Mezey, *J. Lab. clin. Med.* **77,** 110 (1971).
5. E. Mezey, *Biochem. Pharmac.* **21,** (1972).
6. E. K. Marshall and W. F. Fritz, *J. Pharmac. exp. Ther.* **109,** 431 (1953).
7. R. M. Dajani and J. M. Orten, *Fedn. Proc.* **19,** 47 (1960).
8. G. E. McClearn, E. L. Bennett, M. J. Herbert, R. Kakihana and K. Schlesinger, *Nature. Lond.* **203,** 793 (1964).
9. J. H. Mendelson, N. K. Mellow and C. Corbett, *J. Psychiatr. Res.* **3,** 133 (1965).
10. N. J. Greenberger, R. B. Cohen and K. J. Isselbacher, *Lab. Invest.* **14,** 264 (1965).
11. J. R. Sheppard, P. Albersheim and G. E. McClearn, *Biochem. Gen.* **2,** 205 (1968).
12. E. C. Wilson, in *Biochemical Factors of Alcoholism* (Ed. R. P. Maickel), pp. 115–24. Pergamon Press, New York (1967).
13. L. Videla and Y. Israel, *Biochem. J.* **118,** 275 (1970).
14. L. Videla, J. Bernstein and Y. Israel, *Biochem. J.* **134,** 507 (1973).
15. C. S. Lieber, E. Rubin and L. M. DeCarli, *Fedn Proc.* **23,** 2060 (1975).
16. Y. Israel, L. Videla and J. Bernstein, *Fedn Proc.* **34,** 2052 (1975).
17. R. D. Myers and R. B. Holm, *Psychom. Sci.* **6,** 235 (1966).
18. R. M. Dajani, J. Danielski and J. M. Orten, *J. Nutr.* **80,** 196 (1963).