I determined the barium content of seawater samples taken in the San Diego Trough off the Southern California coast by the isotope dilution method, using a 60° sector, single-focusing, solid-source mass spectrometer with a radius of curvature of 30 cm, equipped with an electron multiplier. An enriched ^{135}Ba spike, obtained from the Oak Ridge National Laboratory, was dissolved in 5 percent nitric acid to make up a standard solution containing 283 μg of barium per milliliter. The isotopic compositions of the common and spike barium were determined (Table 1).

About 60 g of unfiltered seawater was introduced into a 100-ml Teflon beaker, and 100 μl of the barium spike solution was added. The mixture was gently heated below boiling for 48 hours to ensure the equilibration of the spike and sample barium. About 10 μl of the solution (containing about 0.005 μg of barium) was placed on a tantalum filament that had been heated at 2.5 amperes to cleanse the surface. The details of the mass spectrometric operation have been described elsewhere (*4*). The filament current was slowly raised to about 1.8 amperes, corresponding to a temperature of 1000°C. At this temperature, the barium isotopic spectrum was observed. The changes in the $^{135}Ba/^{138}Ba$ ratio, precisely measured to ± 0.1 percent, were used to compute the barium concentrations of seawater samples (Table 2).

The barium content of seawater above the thermocline was 12 μg/kg, and that below the thermocline to a depth of 968 m increased slightly to between 15 and 22 μg/kg. The low barium content of the euphotic zone may be caused by the downward transport of barium by marine organisms. These barium concentrations are similar to those reported earlier for the pristine waters of the Pacific Ocean (*2, 3*).

The barium concentration in seawater is regulated by SO_4^{2-}. If we use as the negative logarithm of the solubility product constant of $BaSO_4$ (*2*) for the surface and 968-m waters values of 10.05 and 10.17, respectively, and take the SO_4^{2-} concentration in seawater as 28 m*M*, thermodynamic calculations show that the total *soluble* barium concentrations in the surface water could reach 46 μg/kg, and those of the 968-m water could reach 35 μg/kg. Thus, Southern California coastal waters are undersaturated with $BaSO_4$.

Sections of the Southern California outer continental shelf have been leased for offshore oil and gas exploration. Many potentially toxic chemical compounds will be unavoidably released into the ocean during the drilling operations. Any spillage of drilling mixture (in 1975, the United States used 1.4 million metric tons of barite in the drilling mixture), which will result in dissolution of the $BaSO_4$, can be readily detected in terms of a significant increase in Ba^{2+} in these waters. Therefore, the barium content of seawater may serve as an indicator to monitor drilling-related contamination.

Table 2. Barium content of Southern California coastal waters (32°44.4′N, 117°43.0′W) on 4 December 1975; maximum water depth, 1077 m.

Depth (m)	Temperature (°C)	Salinity (per mil)	Density	Barium (μg/kg)
1	14.62	33.62	1.02503	11
2	14.21	33.62	1.02511	12
196	8.84	34.09	1.02648	21
291	8.13	34.20	1.02667	17
488	6.42	34.30	1.02699	15
582	5.76	34.34	1.02711	18
683	5.09	34.39	1.02723	17
776	4.74	34.43	1.02730	15
963	4.06	34.49	1.02742	22
968	4.05	34.49	1.02742	22

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References and Notes

1. T. J. Chow, "Chemical composition of sea water," in *The Ocean Handbook*, R. Horne, Ed. (Dekker, New York, in press).
2. ——— and E. D. Goldberg, *Geochim. Cosmochim. Acta* **20**, 192 (1960).
3. M. P. Bacon and J. M. Edmond, *Earth Planet. Sci. Lett.* **16**, 66 (1972); M. Bender, T. Snead, L. H. Chan, M. P. Bacon, J. M. Edmond, *ibid.*, p. 81; M. Bernat, T. Church, C. J. Allegre, *ibid.* **17**, 75 (1972).
4. T. J. Chow and C. C. Patterson, *ibid.* **1**, 397 (1966); K. K. Turekian and D. G. Johnson, *Geochim. Cosmochim. Acta* **30**, 1153 (1966); L. H. Chan and J. M. Edmond, *Trans. Am. Geophys. Union* **56**, 1001 (1975).
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Ethanol-Induced Depletion of Cerebellar Guanosine 3′,5′-Cyclic Monophosphate

Abstract. *Single doses of ethanol induce a severe depletion (95 percent) of cerebellar guanosine 3′,5′-cyclic monophosphate (cyclic GMP) within 1 hour after administration. The degree of this depletion is dose-dependent and is a function of the amount of ethanol in the blood. Interactions between ethanol and cyclic GMP may account for some of the intoxicating properties of ethanol.*

The role of guanosine 3′,5′-cyclic monophosphate (cyclic GMP) in neuronal function is just beginning to be understood. Cyclic GMP may be a mediator of the actions of acetylcholine (ACh) in the superior cervical ganglion (*1*), cerebral cortex (*2*), and cerebellum (*3*) and may play an additional role as an antagonist of the actions of adenosine 3′,5′-cyclic monophosphate (cyclic AMP). The relation of cyclic nucleotides to neuroexcitability has prompted several investigations of the effects of ethanol on the cyclic AMP system (*4*), but so far there have been no reports of the effect of ethanol on cyclic GMP. Because ethanol induces ataxia which is probably cerebellar in origin (*5*) and cyclic GMP is involved in excitatory responses in the cerebellum (*6*), we undertook a study to determine whether ethanol has any effect on cyclic GMP concentration in the cerebellum.

Table 1. Cerebellar cyclic GMP and cyclic AMP 2 hours after a single dose of ethanol. Each value (mean ± standard error) was obtained from five to ten animals.

Dose (g/kg)	Cyclic GMP (pmole/mg of protein)	Cyclic AMP (pmole/mg of protein)	Blood ethanol (mg/dl)
	Control		
None	4.0 ± 0.14	3.4 ± 0.16	
	Ethanol-treated		
1	4.3 ± 0.58		31 ± 10
2	2.6 ± 0.42*		100 ± 6
3	1.4 ± 0.11*		183 ± 11
4	1.0 ± 0.16*		285 ± 24
6	0.5 ± 0.04*	3.2 ± 0.08	373 ± 9

*Denotes statistical significance ($P < .05$) as determined by Student's *t*-test.

Male Sprague-Dawley rats (200 to 300 g) that had been deprived of food overnight were given ethanol (2 to 6 g/kg) as a 20 percent (weight to volume) aqueous solution by intragastric intubation. At intervals after treatment the animals were killed by focused microwave irradiation (*7*), the brains were excised, and the cerebellums were removed for analysis. Cyclic GMP and cyclic AMP were isolated (*8*); cyclic AMP was quantitated by the competitive protein binding assay of Gilman (*9*), and cyclic GMP was measured

by the binding assay of Murad *et al.* (*10*) as modified by Dinnendahl (*11*). Concentrations of ethanol in the blood were determined enzymatically with the use of the Calbiochem ethanol Stat-Pack.

A single dose of ethanol at 6 g/kg decreased the cerebellar cyclic GMP by 80 percent at 2 hours after treatment; there was no alteration in cyclic AMP concentrations (Table 1). Depletion was observed to be dose-dependent. On closer examination, with doses of ethanol at 6 g/kg of body weight, cyclic GMP was depleted maximally by 95 percent 1 hour after administration (Fig. 1). Over a 30-hour period cyclic GMP slowly returned to control concentrations in parallel with the elimination of ethanol in the blood.

So far as we know, ethanol is one of the most effective compounds capable of depleting cyclic GMP in the cerebellum. It seems likely, however, that this is not a property specific to ethanol. Pentobarbital (*12*) and diazepam (*13*) both depress cerebellar cyclic GMP concentrations. On the other hand, this response does not appear to be a result of anesthesia since ether raises the concentration of cyclic GMP in the cerebellum (*12*). Cyclic GMP depletion is not confined to the cerebellum, but has been observed to occur all over the brain (*14*).

The potential significance of this finding depends on the role of cyclic GMP in the function of the cerebellum. Little is known about the location of cyclic GMP, but experiments with mutant mice deficient in different cell types suggest that cyclic GMP is localized in Purkinje cells (*15*). Purkinje cells play a role in controlling body musculature by providing inhibitory input to other areas of the brain and derive their excitatory input from climbing and mossy fibers projecting from the brainstem (*16*). Iontophoretic application of cyclic GMP to Purkinje cells suggests that it mediates excitatory influences (*6*).

The mechanism by which ethanol depletes cerebellar cyclic GMP is unknown. However, several possibilities exist. Ethanol could directly inhibit guanylate cyclase, the enzyme that converts guanosine triphosphate to cyclic GMP, or stimulate cyclic nucleotide phosphodiesterase, the enzyme that metabolizes cyclic GMP to guanosine monophosphate. Studies of the effects of ethanol on these enzymes in vitro have shown them to be unaffected (*4, 14*).

An alternative explanation of these results might involve an effect of ethanol on putative neurotransmitters thought to be interacting with the cyclic GMP system. Acetylcholine has been shown to

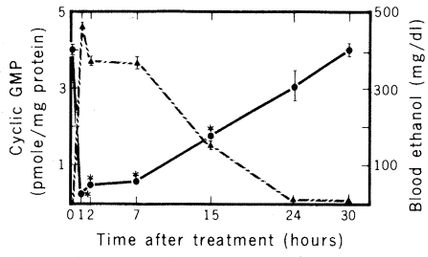

Fig. 1. Concentration of cyclic GMP (solid line) in the cerebellum as a function of time after treatment and the concentration of ethanol (dot and dashed line) in the blood. Each value represents the mean ± standard error and was obtained from five to ten animals. The asterisks denote statistical significance ($P < .05$) as determined by Student's t-test.

stimulate the formation of cyclic GMP in the superior cervical ganglion (*1*), cerebral cortex (*2*), and cerebellum (*3*), an effect that can be blocked by atropine, a cholinergic antagonist. This suggests that cyclic GMP concentrations may in part be regulated by cholinergic activity. Ethanol has been reported to inhibit ACh release in vivo as much as 60 percent in the cerebral cortex and reticular formation (*17*). If cerebellar ACh release is reduced, the resultant reduction of the interaction of ACh with its receptor might lead to a depression of cyclic GMP levels. Further support of this possibility is the ability of oxotremorine, a central cholinergic agonist, to elevate and of atropine to depress cyclic GMP in the cerebellum (*18*). On the other hand, iontophoretic application of acetylcholine to Purkinje cells is not consistently excitatory (*6*).

Other lines of evidence suggest that the amino acids γ-aminobutyric acid (GABA) and glutamate, in addition to their possible roles as neurotransmitters, may regulate cerebellar cyclic GMP. Changes in cyclic GMP concentrations have been reported to be positively correlated to glutamate concentrations and negatively correlated to GABA concentrations (*19*). Glutamate is excitatory and GABA is inhibitory to Purkinje cells (*6, 20*). A single dose of ethanol has no effect on cerebellar GABA, but a single dose reduces glutamate by 15 to 20 percent (*21*). The degree to which glutamate release may be inhibited cannot be deduced from these data. In any event, alterations in the actions of glutamate might explain in part the ethanol-induced depletion of cyclic GMP. Whether disturbances in the interaction between cyclic GMP and neurotransmitters is in any way responsible for some of the biochemical effects of ethanol will depend on further research into the nature of these interactions.

From our data it appears that when the concentration of ethanol in the blood reaches 100 to 150 mg/dl (as is encountered with moderate drinking), a significant reduction of cerebellar cyclic GMP occurs. How cyclic GMP is related to the intoxicating properties of ethanol is yet to be determined. A major neurological decrement of ethanol intake is lack of muscular coordination. Since insufficient excitatory input to Purkinje cells can lead to ataxia (*22*), disruption of the actions of cyclic GMP in its role as a possible mediator of excitatory influences might explain in part the ataxia observed after drinking alcoholic beverages.

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References and Notes

1. P. Greengard and J. W. Kebabian, *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **33**, 1059 (1974).
2. T. P. Lee, J. F. Kuo, P. Greengard, *Proc. Natl. Acad. Sci. U.S.A.* **69**, 3287 (1972); T. W. Stone, D. A. Taylor, R. E. Bloom, *Science* **187**, 845 (1975).
3. J. F. Kuo, T. P. Lee, P. L. Reyes, K. C. Walton, T. E. Donnelly, Jr., P. Greengard, *J. Biol. Chem.* **247**, 10 (1972).
4. M. A. Israel, H. Kimura, K. Kiriyama, *Experientia* **28**, 149 (1973); K. Kiriyama and M. A. Israel, *Biochem. Pharmacol.* **22**, 2919 (1973); L. Volicer and B. I. Gold, *Life Sci.* **13**, 269 (1973); S. W. French, D. S. Palmer, M. E. Narod, P. E. Reid, C. W. Rama, *J. Pharmacol. Exp. Ther.* **194**, 319 (1975); L. Volicer and B. I. Gold, in *Biochemical Pharmacology of Ethanol*, E. Majchrowicz, Ed. (Plenum, New York, 1975), p. 211.
5. E. Eidelberg, M. L. Bond, A. Kelter, *Arch. Int. Pharmacodyn. Ther.* **52**, 213 (1971).
6. S. R. Siggins, S. J. Henriksen, S. C. Landis, *Neurosci. Abstr.* **1**, 378 (1975).
7. Litton Menumaster 70/50, modified by Medical Engineering Consultants, Lexington, Mass. (1.3 kw, 3.5-second exposure time).
8. C. C. Mao and A. Guidotti, *Anal. Biochem.* **59**, 63 (1974).
9. A. G. Gilman, *Proc. Natl. Acad. Sci. U.S.A.* **67**, 305 (1970).
10. F. Murad, V. Mangarillo, M. Vaughan, *ibid.* **68**, 736 (1971).
11. V. Dinnendahl, *Naunyn-Schmiedebergs Arch. Pharmakol.* **284**, 55 (1974).
12. H. Kimura, E. Thomas, F. Murad, *Biochim. Biophys. Acta* **343**, 519 (1974).
13. C. C. Mao, A. Guidotti, E. Costa, *Brain Res.* **83**, 516 (1975).
14. W. A. Hunt, J. D. Redos, G. N. Catravas, T. K. Dalton, in preparation.
15. C. C. Mao, A. Guidotti, S. Landis, *Brain Res.* **90**, 335 (1975).
16. J. R. Bloedel, *Prog. Neurobiol. (Oxford)* **2**, 1 (1973).
17. C. K. Erickson and D. T. Graham, *J. Pharmacol. Exp. Ther.* **185**, 583 (1973).
18. J. A. Ferrendelli, A. L. Steiner, D. B. McDougal, Jr., D. M. Kionis, *Biochem. Biophys. Res. Commun.* **41**, 1061 (1970).
19. C. C. Mao, A. Guidotti, E. Costa, *Brain Res.* **79**, 510 (1974).
20. J. Chajo, Y. Yamada, C. Yamamota, *Exp. Brain Res.* **23**, 293 (1975).
21. I. A. Sytinsky, B. M. Guzikov, M. V. Gomanko, V. P. Ermin, N. N. Konovalova, *J. Neurochem.* **25**, 43 (1975).
22. J. M. Brookhart, in *Handbook of Physiology*, section 1, *Neurophysiology*, J. Field, Ed. (Waverly, Baltimore, 1960), vol. 2, p. 1245.

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