be closer to the value of P_1 derived here.

If CH_4 is being removed from the atmosphere at a rate of 1.45×10^{14} mole year^{-1} or 2.3×10^{15} g year^{-1}, then a source of CH_4 of this magnitude is required to maintain the steady state. Koyama (*22*) has estimated a yearly production of CH_4 of 2.7×10^{14} g, of which about two-thirds was from paddy fields. Ehhalt (*23*) has suggested that Koyama's estimate was too low, and Robinson and Robbins (*6*) revised Koyama's estimate by including swamp lands and hot, humid tropical areas as additional CH_4 sources. Their estimate of CH_4 production is 1.45×10^{15} g year^{-1}, in agreement with the value deduced here. The lifetime of CH_4 derived from our analysis is 1.5 years.

Another point is worth consideration with respect to the role of the OH radical as the major source and removal mechanism of CO in the troposphere. Bainbridge and Heidt (*18*) have found that the mixing ratio of CH_4 is nearly constant with increasing altitude up to the tropopause, and Junge *et al.* (*9*) report a similar constancy for the CO mixing ratio. These observations may be taken as further confirmation of the determining role of OH in the CO-CH_4 cycle in nature (*24*).

In summary, it has been shown that the residence time of CO in the troposphere is about 0.1 year and that the major mechanism for the removal of CO is oxidation by OH in the troposphere. Concomitantly, the major source of CO is the oxidation of CH_4 by OH in the troposphere, amounting to a production rate of 5×10^{15} g year^{-1}, some 25 times greater than the production rate of CO from the combustion of fossil fuels. The average OH concentration in the troposphere required to maintain this balance is 2.3×10^6 molecule cm^{-3}, or twice this value during sunlight. This model could be confirmed by a measurement of the concentration of the OH radical in remote areas. Such a determination would have added importance because the OH radical also appears to play a dominant role in the overall chemistry of the troposphere as indicated by Levy (*12*) and by McConnell *et al.* (*13*), who derive a similar magnitude for the OH concentration from photochemical considerations.

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8. R. E. Lingenfelter [*Rev. Geophys.* **1**, 35 (1963)] has calculated the rate of production of ^{14}C by cosmic-ray neutrons as a function of altitude, latitude, and time and obtained 2.5 ^{14}C atom cm^{-2} sec^{-1} as the global average. D. Lal and B. Peters [*Progr. Elem. Particle Cosmic Ray Phys.* **6**, 1 (1962)], taking the average height of the tropopause to be about 11 km, estimate that 1.1 ^{14}C atom cm^{-2} sec^{-1} are produced in the troposphere. The total production of ^{14}CO from cosmic-ray neutrons in the troposphere, P_3, is then 290 mole year^{-1}. The assumption is made that all of the ^{14}CO produced above the tropopause is converted to $^{14}CO_2$ before exchanging into the troposphere.
9. C. Junge, W. Seiler, and P. Warneck [*J. Geophys. Res.* **76**, 2866 (1971)] give 0.12 ppm as the average global concentration of CO in the troposphere. If we take the average height of the tropopause to be 11 km, the troposphere contains 1.4×10^{20} mole [L. Goldberg, in *The Earth as a Planet*, G. P. Kuiper, Ed. (Univ. of Chicago Press, Chicago 1954)]. The total CO content of the troposphere at a concentration of 0.12 ppm, (CO), is then 1.7×10^{13} mole.
10. Three measurements of the specific activity of CO in the atmosphere have been made by MacKay *et al.* (*3*) at Tonawanda, New York. The average specific activity from their measurements was 12.3 disintegration min^{-1} g^{-1}, and the average CO concentration was 0.3 ppm. The fraction of ^{14}CO in these CO samples is then 1.06×10^{-12}, and the corresponding atmospheric concentration of ^{14}CO is 3.3×10^{-13} ppm. If we assume that this concentration of ^{14}CO is uniform throughout the troposphere, the total ^{14}CO content of the troposphere, (^{14}CO), is 45 mole.
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3 December 1971

Synergy of Ethanol and Putative Neurotransmitters: Glycine and Serine

Abstract. *The putative neurotransmitters, glycine and serine, significantly enhanced the sleeping time (loss of the righting reflex) that was induced by ethanol in mice. The observed synergistic effect between ethanol and the amino acids is probably not related to an alteration of ethanol metabolism, but rather to an interaction of these compounds in the central nervous system.*

Glycine may act as an inhibitory transmitter in the brain and spinal cord areas in many animal species (*1*). It was reported recently that glycine is synthesized in brain almost exclusively by serine hydroxymethyltransferase (E.C. 2.1.2.1) (*2*). Serine, a less potent depressant amino acid than glycine, decreases the firing of spinal neurons that are spontaneous or are chemically evoked (*3*).

The inhibitory actions of ethanol in the central nervous system have been well documented (*4*). Synergistic effects on its duration of action have been observed between ethanol and other agents such as barbiturates, serotonin and its metabolites, and γ-hydroxybutyrate (*5*).

We report here on the interaction of ethanol with glycine or with serine, amino acids known to be depressants, and the demonstration that these agents enhance the soporific action of ethanol in mice.

The measured response, sleeping time, was defined as the length of time required for an animal to regain the

righting reflex (*6*). If a mouse did not awaken within 2½ hours after administration of ethanol, a score of 150 minutes was assigned as its sleeping time.

In the first experiment, drugs were administered to groups of mice as follows (millimoles per 100 g of body weight): group A, 0.9 mmole of glycine; group B, saline plus 10 mmole of ethanol; group C, either 0.45 or 0.9 mmole of glycine plus 10.0 mmole of ethanol. All compounds were given intraperitoneally; for the combined treatments, the agents were administered simultaneously. The ethanol solutions were prepared in saline (30 percent by volume), and glycine was dissolved in saline at a concentration of 60 mg/ml.

The effects of these treatments on sleep induced by ethanol are shown in Fig. 1. A significant synergistic effect was observed ($P < .001$) in mice treated with glycine and ethanol at only the 0.9 mmole/100 g dose of glycine. The effect was probably not an additive one, since the combination dose caused a duration of sleep of approximately ten times the sum of the sleeping time when either glycine or ethanol was given alone.

Glycine was administered to mice after ethanol treatment in order to further evaluate this augmentation. Five minutes after the onset of sleep induced by ethanol (12.5 mmole/100 g) glycine was administered to the animals (0.45 and 0.9 mmole/100 g) (*7*). As in the previous experiment, a significant ($P < .001$) increase in sleeping time was observed only in those mice treated with the higher dose of glycine. The combination of ethanol and glycine resulted in a duration of sleep that was 1.8 times the sum of the sleeping time when glycine or ethanol was administered separately.

Serine, at a concentration of 60 mg/ml, also was tested for its effect on sleep induced by ethanol and was administered in amounts equimolar to the glycine doses.

A less marked synergistic effect was observed with serine than with glycine. As measured by Student's *t*-test, the effect of the serine (0.9 mmole/100 g) and ethanol (10.0 mmole/100 g) combination differed significantly from the effect of serine or ethanol alone ($P < .05$). The loss of righting reflex in the mice receiving the combination (for the 0.45 mmole/100 g dose of serine) was two times the sum of the duration of sleep when individual doses were injected.

When serine was given to mice 5 minutes after the onset of sleep induced by ethanol, a significant increase in sleeping time was observed with both the 0.45 and the 0.9 mmole/100 g dose ($P < .001$). The effect of the combination dose was probably not an additive one; sleeping time was 1.6 and 1.4 times the sum of the sleeping time when serine or ethanol was injected individually.

For analysis of ethanol in the blood and the brain, other groups of mice were injected with saline (volume equivalent to 0.9 mmole per 100 g of either serine or glycine) and ethanol (10.0 mmole/100 g), glycine (0.9 mmole/100 g) and ethanol (10.0 mmole/100 g), and serine (0.9 mmole/100 g) and ethanol (10.0 mmole/100 g); blood and brain samples were taken from these groups at 15, 40, and 90 minutes after ethanol administration (Fig. 2). These time intervals were selected since they approximated the average duration of sleep for the three treatment groups. After the mice were killed, the blood and brain from each animal were analyzed for ethanol by a modification of the gas chromatographic method (*8*). At least six mice were used for each determination.

The concentration of ethanol in the blood or brain after administration of saline and ethanol did not differ significantly from those obtained after administration of glycine and ethanol or of serine and ethanol despite the fact that, with glycine or serine treatment, the animals slept longer than the controls did (Fig. 2). The mice treated with saline and ethanol generally were awake 15 minutes after treatment, whereas the mice treated with serine and ethanol were asleep at 40 minutes, and the mice treated with glycine and ethanol were asleep at 90 minutes.

Thus, glycine and serine, possible inhibitory transmitters in the mammalian central nervous system (*3, 9*), enhance the soporific action of ethanol in mice.

When serine was given simultane-

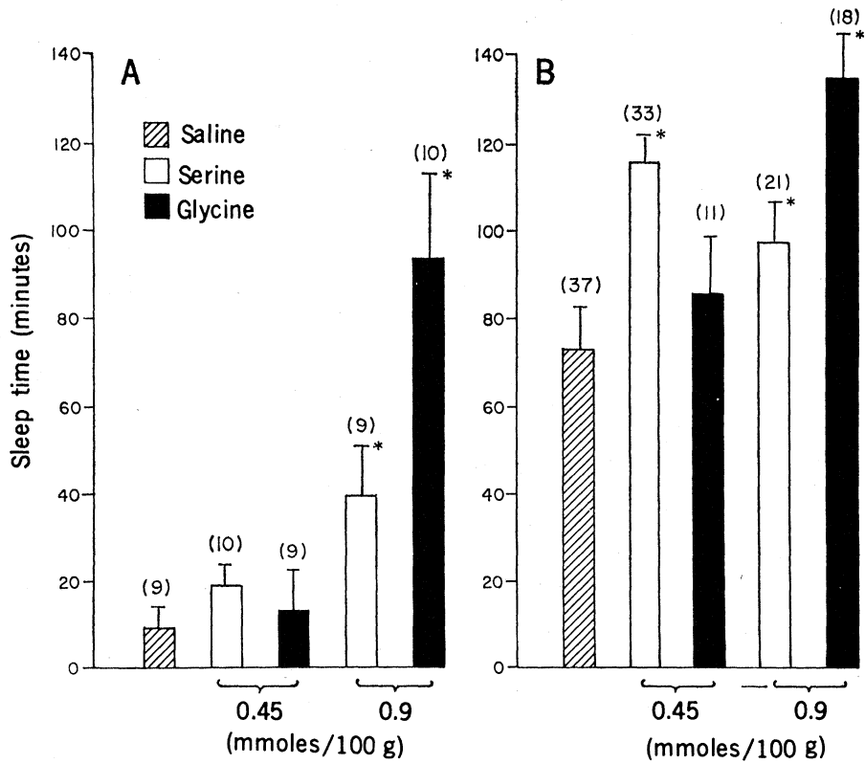

Fig. 1. Effects of saline, glycine, or serine on sleep induced by ethanol in mice. (A) Effects when agents were administered simultaneously with 10 mmole/100 g of ethanol. (B) Effects when agents were administered 5 minutes after 12.5 mmole/100 g of ethanol. Glycine and serine were without any observable effect at the 0.9 mmole/100 g dose. The vertical bars are the mean sleeping time ± the standard errors of the means (vertical brackets). The number of mice used in each experiment is shown in parentheses above each bar in the graph. Asterisk indicates that the results are significantly different from those obtained solely with saline treatment ($P < .001$, by Student's *t*-test).

ously with ethanol, it was less effective than glycine in enhancing sleeping time. A similar difference in potency was reported by Curtis and co-workers (*9*) who demonstrated that serine was much less potent than glycine in decreasing the firing of spinal horn interneurons. However, when a 0.45 mmole/100 g dose of serine or glycine was given after administration of ethanol, glycine did not significantly affect the action of ethanol, whereas serine enhanced ($P < .001$) the sleep induced by ethanol (*10*). We cannot at the present time offer any reasonable explanation for this result; it may reflect differences in cerebral uptake of amino acids after ethanol administration (*11*) or it may be due to differences in the amount of alcohol administered in our studies.

Since equivalent concentrations of ethanol in the blood and brain were obtained from mice treated with saline and ethanol, serine and ethanol, and glycine and ethanol, the synergistic effect induced by serine or glycine is probably not related to an alteration of ethanol metabolism. A more direct interaction of these depressant amino acids and ethanol may exist in the central nervous system. The enhancement by glycine or serine of sleep induced by ethanol may be the result of inhibition of similar sites in the brain (*12*).

Although glycine is not a direct product of glutamine metabolism, it is a very strong inhibitor of glutamine synthetase (*13*), suggesting that the steady state amounts of glycine in the cell are critically related to glutamine synthesis and metabolism. It is also known that γ-aminobutyric acid (GABA), a possible neurotransmitter at inhibitory synapses, is derived from glutamate and has been implicated in the depressant actions of ethanol in the central nervous system. The GABA content of the brain was found to be elevated in starved rats receiving large doses of ethanol (*14*). Since administration of glutamine to rats decreased the severity of alcoholic intoxication (*15*) and reduced the effect of ethanol on the concentration of GABA in brain (*14*), it is possible that the potentiative effect observed between glycine and ethanol in our study may be due to the inhibitory action of glycine or glutamine metabolism. As a result of this inhibition, more glutamate may become available

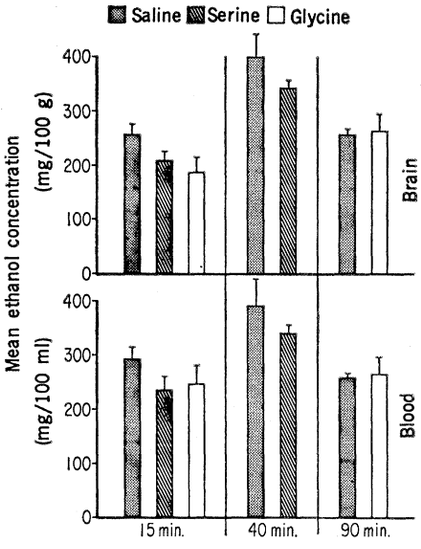

Fig. 2. Concentrations of ethanol in the blood and the brain after administration of saline and ethanol (10.0 mmole/100 g), glycine (0.9 mmole/100 g) and ethanol (10.0 mmole/100 g), or serine (0.9 mmole/100 g) and ethanol (10.0 mmole/100 g). Results are given as means ± standard error of the mean with six mice represented in each group. Analysis of ethanol concentration in blood were performed 15, 40, or 90 minutes after administration of ethanol.

for subsequent conversion to GABA; increased production of this neuroinhibitor may contribute to the depressant actions of ethanol.

Glycine is a constituent of protein foods such as whole egg, beef, gelatin, and so forth (*16*). The interactions between glycine and ethanol described here might occur in humans, but their existence or effects are yet to be determined; they could have a direct bearing on the nutritional factors associated with alcoholism. Ethanol can alter monoamine metabolism in the normal brain (*17*). This change might result in an imbalance of certain inhibitory monoamines, such as glycine (*11*), and thereby play a significant role in the development of alcoholism.

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15 November 1971; revised 10 January 1972