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Ethanol and Neuronal Metabolism

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MANDEL, P., M. LEDIG AND J.-R. M'PARIA. *Ethanol and neuronal metabolism.* PHARMAC. BIOCHEM. BEHAV. 13: Suppl. 1, 175–182, 1980.—The effect of ethanol on membrane enzymes (Na^+, K^+ and Mg^{2+}ATPases, 5'nucleotidase, adenylate cyclase) alcohol dehydrogenase, aldehyde dehydrogenase and superoxide dismutase were studied in nerve cells (established cell lines, primary cultures of chick and rat brain) cultured in the presence of 100 mM ethanol, and in total rat brain, following various ethanol treatments of the rats (20% ethanol as the sole liquid source, intraperitoneal injection). The results show a difference between neuronal and glial cells. Most of the observed changes in enzymatic activities returned rapidly to control values when ethanol was withdrawn from the culture medium or from the diet. Alcohol dehydrogenase was more stimulated by ethanol than aldehyde dehydrogenase; therefore acetaldehyde may be accumulated. The inhibition of superoxide dismutase activity may allow an accumulation of cytotoxic O_2^- radicals in nervous tissue and may explain the polymorphism of lesions brought about by alcohol intoxication.

Alcohol Nervous tissue Cell culture Enzymes

IT IS well established that alcohol abuse produces a great variety of alterations in the central nervous system. However, it is difficult to define the direct effect of alcohol on neuronal cells, in view of the multiple effects of alcohol on different organs (liver, kidney, endocrine glands), and the secondary effects which may occur as a result of the alterations of these other organs.

In order to understand the actions of ethanol at the cellular level more clearly, cultured neurons represent a convenient system, since they allow the investigation of direct effects on specific cell types in a well-defined medium. It is well-established that alcohol intoxication affects the structure and function of biological membranes [18], so we studied the effect of ethanol on specific membrane enzyme activities (Na^+, K^+ and Mg^{2+}ATPases, adenylate 5'nucleotidase, adenylatecyclase). Furthermore, we investigated the effect of ethanol on alcohol metabolizing enzymes (alcohol dehydrogenase, aldehyde dehydrogenase). Since we postulated that some damage of nervous tissue occurring during alcohol intoxication may result from an accumulation of cytotoxic oxygen radicals, we also examined ethanol's effects on superoxide dismutase, the enzyme which is involved in inactivation of oxygen radicals and which protects the tissues against the cytotoxic effect of oxygen and its metabolites [6,8].

METHOD

Two cell types can be used in tissue culture to investigate the effect of alcohol:

Primary cultures (embryo brain cells or brain cells from new-borns.) Pure neurons can be obtained starting from 8-day-old chick embryo brain [19]. Glial cells were obtained starting from 16-day-old chick embryo brain [2], or from new-born rat brain.

Tumoral and transformed cells(C6 glial astrocytoma ATTC [1]—NN hamster astroblasts [17]—MI neuroblastoma C 1300 isolated in our laboratory [4]—MT17 14-day-old embryo brain cells from balb/c mice transmformed by simian virus SV 40). Most of these cultures show a high rate of proliferation and large quantities of material can be made available. But it must be kept in mind that we are working with tumoral cells, and the results must be controlled with normal cultures, and by *in vivo* experiments. The cells were grown in the presence or absence of 100 mM ethanol using techniques described previously [4,22].

RESULTS

Morphological Effects

When the cells were grown in presence of 100 mM ethanol, no significant changes in growth rate or morphology, examined by optical microscopy, were observed. A slight increase in growth rate was observed in some cell lines, not in others [22].

Biochemical Effects

Membrane-bound Enzymes

Large changes in membrane enzyme activities were observed in most of the cell types we tested.

Na^+, K^+ and Mg^{2+}ATPases. The Na^+, K^+ATPase activity was decreased in chick neurons and in two transformed cell lines (MT17 and NN) grown in the presence of 100 mM ethanol. There was no effect in a tumoral cell line (C6), or in primary cultures of rat brain glial cells. In chick glial cells, a significant increase in Na^+, K^+ATPase activity was observed (Fig. 1).

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The Mg^{2+}ATPase activity did not change in the NN cell line and in chick glial cells, but there was a significant increase in the MT17 cells, the chick neurons and in primary cultures of rat brain glial cells. In C6 cells there was no change, as was found for the Na^+, K^+ATPase activity (Fig. 2). *In vivo*, whole rat brain Na^+, K^+ATPase activity decreased by about 50% when 2 g ethanol/kg were injected into the rats 10 min before decapitation (Control: 2.51 ± 0.40, ethanol-treated 1.02 ± 0.37, $p<0.01$). On the other hand, the Na^+, K^+ATPase activity increased by about 50% when 2 g of ethanol/kg was injected once daily for 3 days (Control: 2.51 ± 0.40, ethanol-treated 4.67 ± 1.39, $p<0.001$).

When 20% ethanol was given as sole liquid source, the Na^+, K^+ATPase activity increased progressively up to 6 weeks. If ethanol was withdrawn from the diet, the Na^+, K^+ATPase activity returned to the control levels within 3 days (Fig. 3). No significant changes in Mg^{2+}ATPase activity were found *in vivo*.

5'-Nucleotidase. 5'-Nucleotidase activity did not change either in ethanol-treated cell cultures, or when ethanol was injected into the rats. When the rats received 20% ethanol in drinking water, an increase in 5'-nucleotidase activity was observed during the first two weeks; later on, no significant change was observed (Fig. 4).

Adenylatecyclase. Adenylatecyclase activity decreased *in vivo* up to three weeks when the rats received 20% ethanol in drinking water; later on, no significant change was observed (Fig. 5).

Alcohol Metabolizing Enzymes

Low alcohol dehydrogenase and aldehyde dehydrogenase activity were measured in nerve cells. The values ranged from $3–10\times10^{-3}$ U/mg protein (one unit being the quantity of enzyme which metabolizes one μmole of substrate per minute).

In the presence of 100 mM ethanol, alcohol dehydrogenase activities were about twice the control values in C6 cells treated for 9 days, in chick neurons, chick glials cells and rat brain glial cells treated for 4 days, as well as *in vivo* in whole rat brain after the animals received 3 injections of 5 g of ethanol/kg each, or after 8 weeks of feeding with 20% ethanol as the sole liquid source (Fig. 6).

Aldehyde dehydrogenase activity also increased after ethanol treatment (Fig. 7), but in most cases (except for chick glial cells) to a lower extent than alcohol dehydrogenase activity, as shown in Fig. 8.

Superoxide Dismutase

Superoxide dismutase activity was inhibited in glial cells (C6, chick glial cells, rat brain glial cells) when the cells were grown in the presence of 100 mM ethanol. The superoxide dismutase activity of the neuronal cells (MI, MT17, chick neurons) was not changed, as shown in Fig. 9.

In vivo, the superoxide dismutase activity was inhibited by about 25% after one injection of 5 g of ethanol/kg (control: 15.95 ± 0.47; ethanol-treated: 12.03 ± 1.00, $p<0.05$) and by about 40% after 3 repeated injections of 2 g of ethanol/kg (control: 15.95 ± 0.47; ethanol-treated: 9.56 ± 2.70, $p<0.05$). A single dose of 2 g/kg had no effect.

When rats were drinking 20% ethanol, the whole brain superoxide dismutase activity decreased progressively, and reached a plateau after 6 weeks with about 35% inhibition of the activity, as compared to controls. Withdrawal of ethanol after 4 weeks produced a return to the control level within 48 hr (Fig. 10).

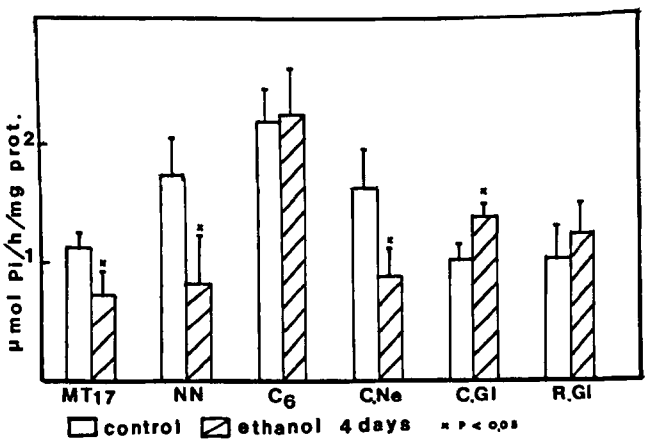

FIG. 1. Na^+, K^+ATPase activity (measured according to Ledig *et al.* [11]) of cultured nerve cells (MT17 (13 exp.); NN (6 exp.); C6 (6 exp.); C.Ne=chick neurons (9 exp.); C.Gl=chick glial cells (9 exp.); R.Gl=rat glial cells (9 exp.)) treated during 4 days with 100 mM ethanol.

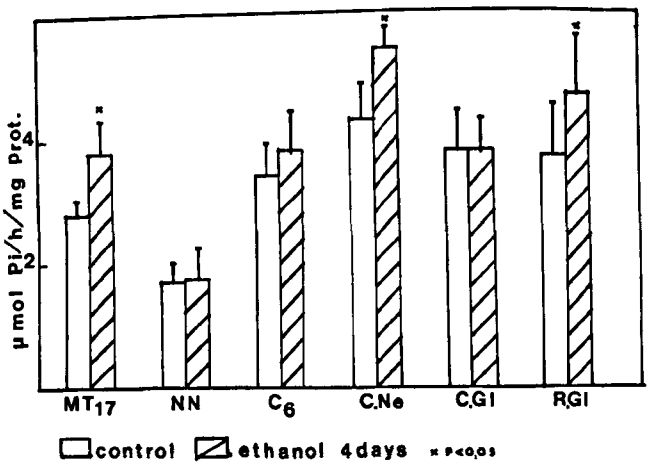

FIG. 2. Mg^{2+}ATPase activity (measured according to Ledig *et al.* [11]) of cultured nerve cells (MT17 (13 exp.); NN (6 exp.); C6 (6 exp.); C.Ne=chick neurons (9 exp.); C.Gl=chick glial cells (9 exp.); R.Gl=rat glial cells (9 exp.)) treated during 4 days with 100 mM ethanol.

DISCUSSION

Several direct effects of ethanol on the enzymatic activities we tested could be demonstrated in tissue culture. In some cases, differences were observed between ethanol effects on neurons and on glial cells. For example, we found a decrease in Na^+, K^+ATPase activity in neuronal cells, and various effects in glial cells. There was a decrease in activity in transformed cell lines, and an increase in activity in chick glial cells, while there was no change in the activity in either

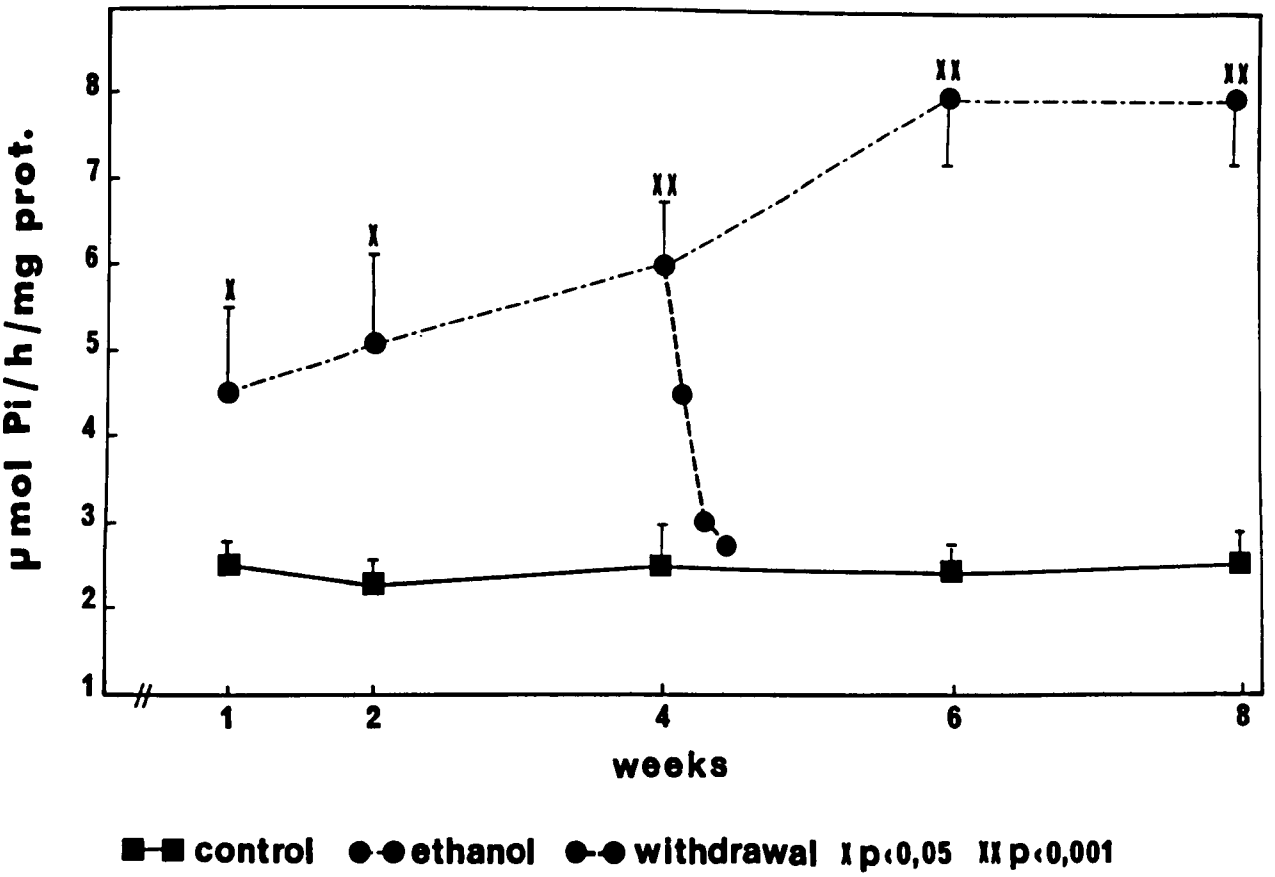

FIG. 3. Na^+, K^+ATPase activity (measured according to Ledig *et al.* [11]) of whole rat brain after the animals were given a 20% ethanol solution as the sole liquid source. The heavy dashed line (●– –●) indicates the return to normal of Na^+, K^+ATPase activity following withdrawal of the ethanol solution. Values represent means ± SD of 4 animals.

the tumoral C6 cell line or in the rat glial cells. The Mg^{2+}ATPase activity increased in chick neurons, rat glial cells and in one transformed cell line. No change was observed for the other cell lines and the chick glial cells. Thus, a striking difference in response of the two ATPase activities was shown. The most important finding seems to be the difference in the effect of ethanol on neurons and glial cells, which should be considered when a mixed cell population is examined, as is usually the case in *in vivo* studies.

Differences in ATPase activities were also observed by Syapin *et al.* [22]. Furthermore, *in vitro* inhibition by ethanol of Na^+, K^+ATPase activity in subcellular fractions of brain has been reported by Israel and Salazar [10] and by Sun and Samorajski [21]. Various effects of ethanol on brain Na^+, K^+ATPase activity have also been found *in vivo*, since a single injection of ethanol produced an inhibition of the Na^+, K^+ATPase activity, whereas repeated injections increased the activity. Chronic intake of ethanol progressively increased Na^+, K^+ATPase activity as shown by Roach *et al.* [16] and by Guerri *et al.* [9].

Inhibition of the Na^+, K^+ATPase activity apparently results from the lipophilic interaction of ethanol with the plasma membrane. Chin and Goldstein [3] showed recently that as little as 0.02 M ethanol increased the fluidity of synaptosomal and erythrocyte membranes *in vitro*. The increase in Na^+, K^+ATPase activity caused *in vivo* by chronic alcohol exposure seems rather to be an adaptive response, which could result from synthesis of protein components of the enzyme complex or alterations in membrane conformation, possibly brought about by changes in lipid configuration or composition.

Tolerance seemed to occur to the effects of ethanol on 5'-nucleotidase and adenylatecyclase activity, since both activities underwent changes during the earlier part of ethanol exposure, and activity returned to control levels at later periods.

Despite conflicting views about the presence of alcohol metabolizing enzymes in nervous tissues [13,14], we could measure low alcohol dehydrogenase and aldehyde dehydrogenase activities in cultured neurons and in rat brain. We found a stimulation of these activities when cells were grown in presence of alcohol, or when alcohol was given *in vivo*.

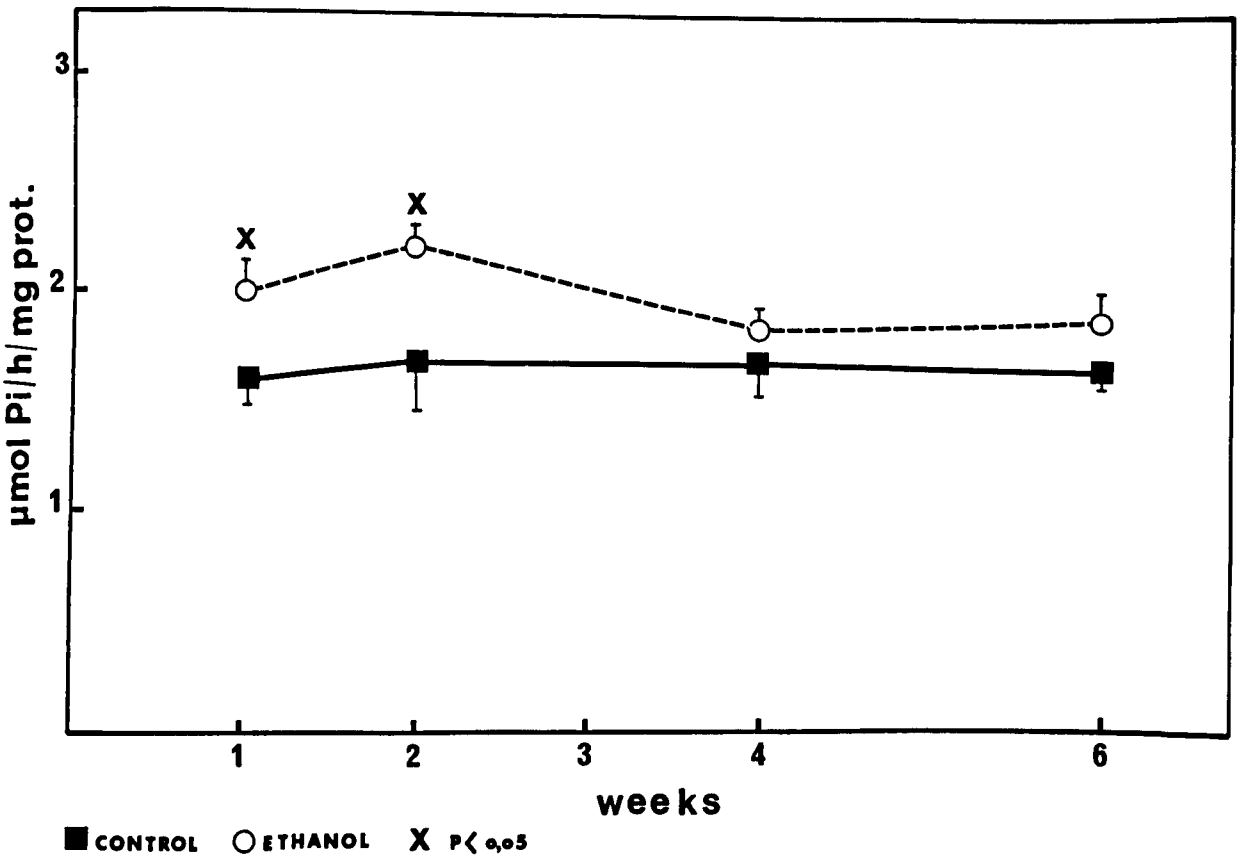

FIG. 4. 5'nucleotidase activity (measured according to Stefanovic, Mandel and Rosenberg [20]) of rat brain after the animals received a 20% ethanol solution as the sole liquid source for 6 weeks (means of 4 values ± SD).

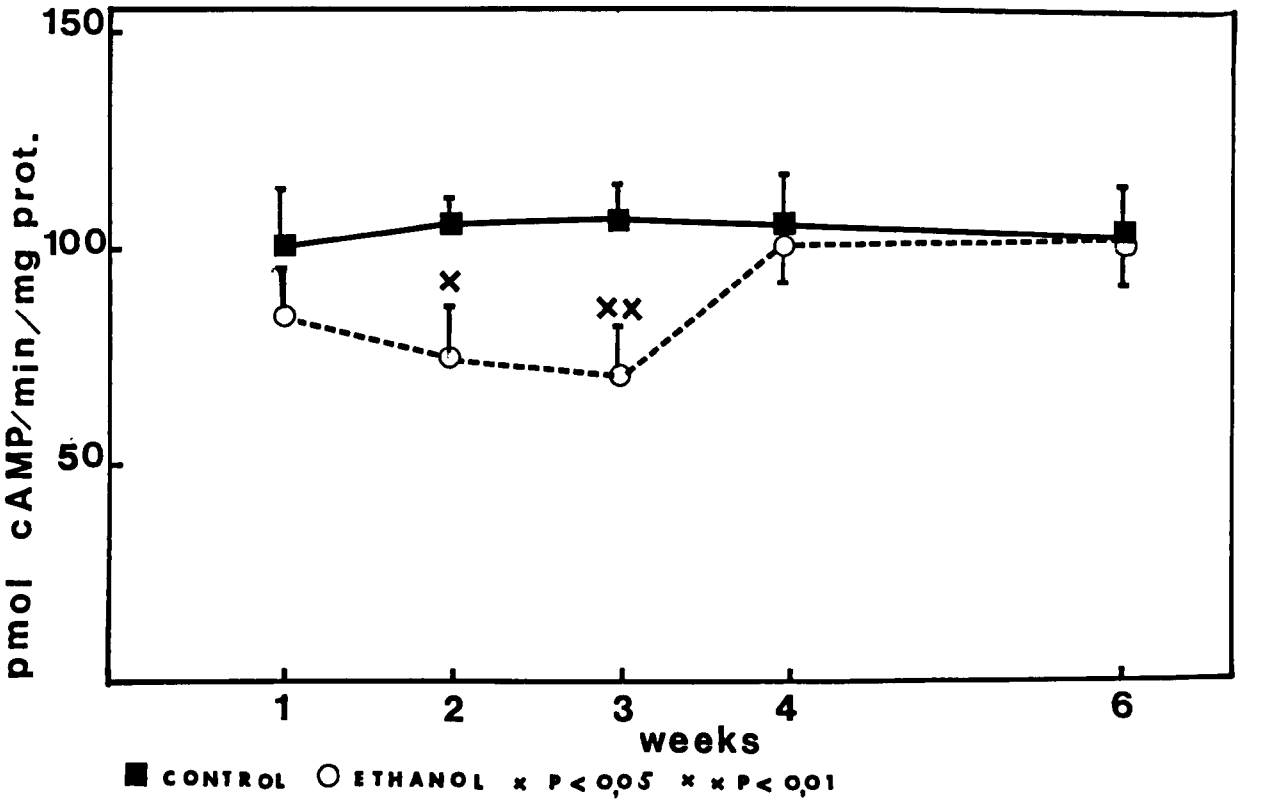

FIG. 5. Adenylatecyclase activity (measured according to Ramachandran and Lee [12]) of rat brain after animals received a 20% ethanol solution as the sole liquid source for 6 weeks (means of 4 values ± SD).

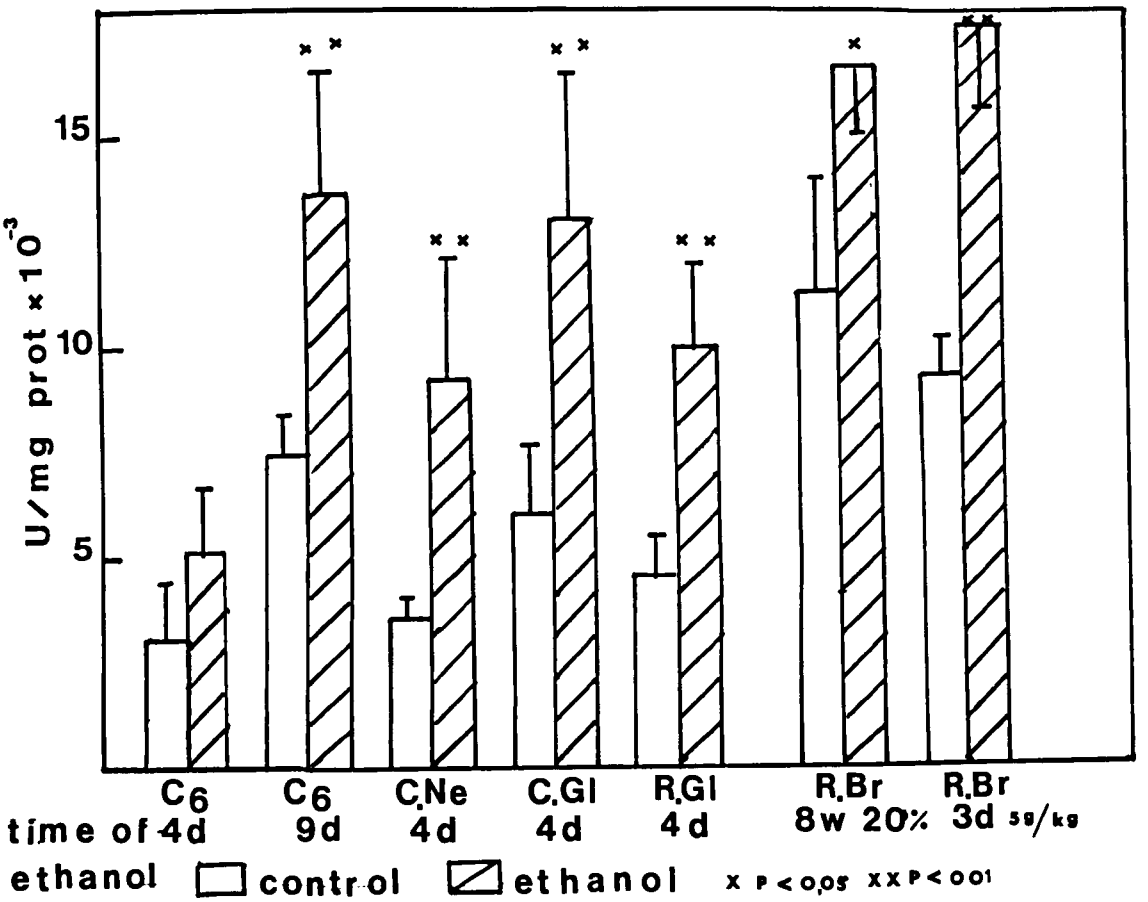

FIG. 6. Effect of ethanol on alcohol dehydrogenase (ADH) activity (measured according to Woronick [23]) of cultured nerve cells (C6 (5 exp.); C.Ne=chick neurons (5 exp.); C.Gl=chick glial cells (5 exp.); R.Gl=rat glial cells (4 exp.)) treated with 100 mM ethanol, and of rat brain (RBr 8w 20%=rats drinking a 20% ethanol solution as the sole liquid source for 8 weeks (5 exp.); RBr, 3d 5g/kg=rats receiving daily intraperitoneal injections fo 5 g of ethanol/kg body weight for 3 days (5 exp.)).

Our results show that in most cases, the stimulation of alcohol dehydrogenase activity was greater than the corresponding increase in aldehyde dehydrogenase activity. The expected accumulation of cytotoxic acetaldehyde could, in part, account for the disorders of neuronal function following alcohol abuse.

Our results showed a significant and rapid effect of ethanol on superoxide dismutase activity. As for the ethanol effect on Na^+, K^+ATPase activity, the response of superoxide dismutase to ethanol depended upon the cell type. Superoxide dismutase in glial cells was more sensitive to ethanol than the enzyme in neuronal cells. *In vivo*, inhibition occurred only after chronic treatment, since superoxide dismutase activity did not change when 2 g of ethanol/kg body weight was injected once, whereas the activity decreased when the same dose was injected 3 times. Chronic intake of alcohol also led to a progressive decrease in superoxide dismutase activity. Alterations of superoxide dismutase activity following alcohol exposure may be tissue-specific, since an increase in erythrocyte superoxide dismutase activity has recently been reported to occur in black alcoholics [5].

In nervous tissue, the inhibition of superoxide dismutase activity could allow an accumulation of cytotoxic O_2^- radicals, which could explain some effects of alcohol on the nervous system.

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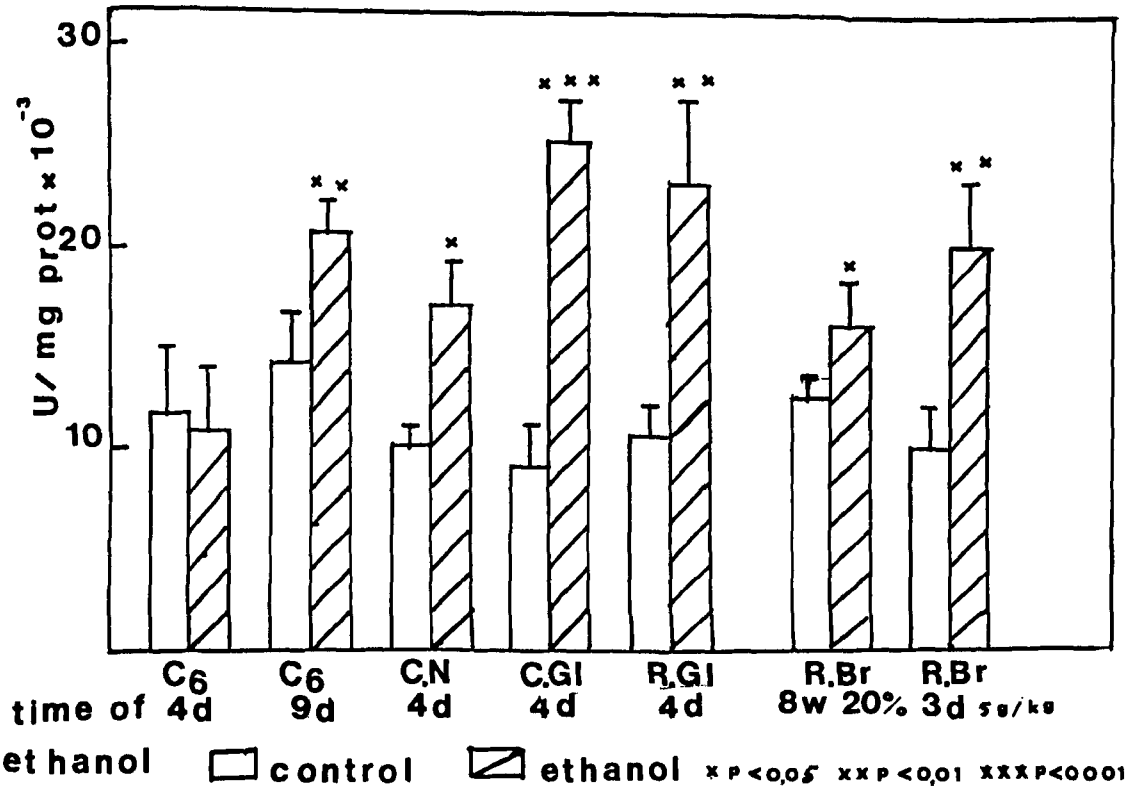

FIG. 7. Effects of ethanol on aldehyde dehydrogenase (AcDH) activity (measured according to Redmond and Cohen [15]) of cultured nerve cells (C6 (5 exp.); C.Ne=chick neurons (5 exp.); C.Gl=chick glial cells (5 exp.); R.Gl=rat glial cells (4 exp.)) treated with 100 mM ethanol, and of rat brain (RBr 8w 20%=rats drinking a 20% ethanol solution as the sole liquid source for 8 weeks (5 exp.); RBr, 3d 5 g/kg=rats receiving daily intraperitoneal injections of 5 g of ethanol/kg body weight for 3 days (5 exp.)).

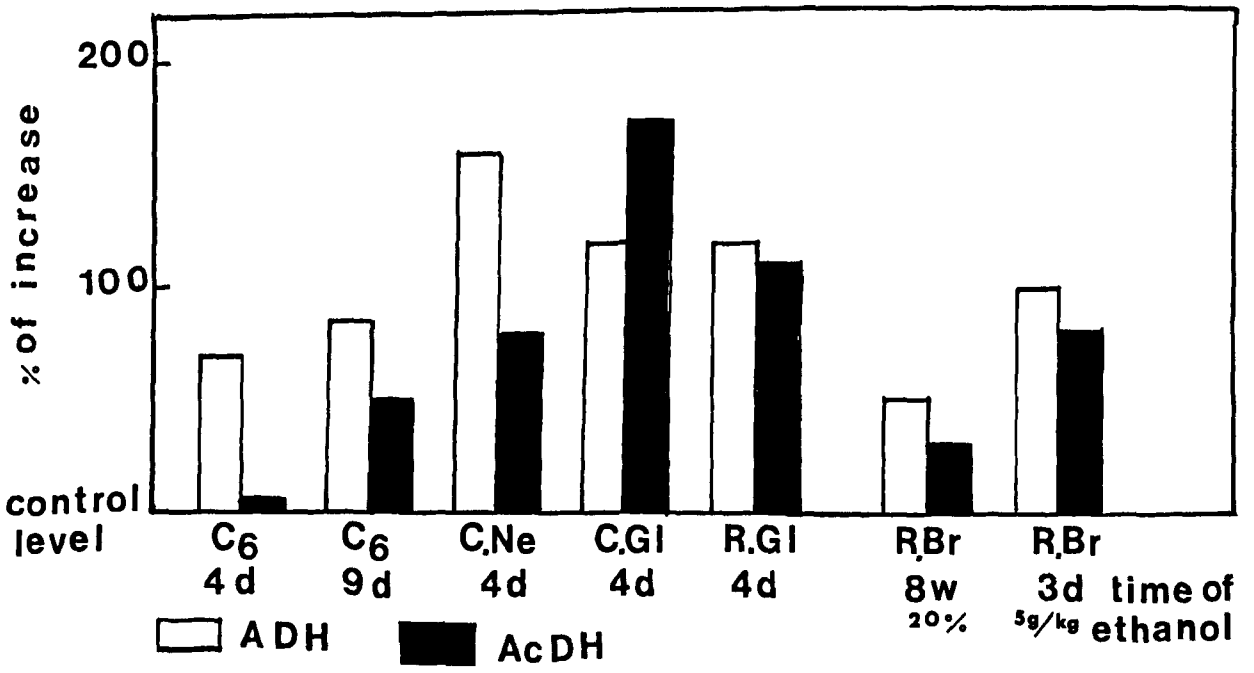

FIG. 8. Effect of ethanol on alcohol dehydrogenase (ADH) (measured according to Woronick [20]) and aldehyde dehydrogenase (AcDH) measured according to Redmond *et al.* [1588). Activity expressed as percent stimulation compared to control values of: cultured nerve cells (C6 (5 exp.); C.Ne=chick neuron neurons (5 exp.); C.Gl=chick glial cells (5 exp.); R.Gl=rat glial cells (4 exp.)) treated with 100 mM ethanol, and of rat brain (RBr 8w 20%=rats drinking a 20% ethanol solution as the sole liquid source for 8 weeks (5 exp.); RBr, 3d 5 g/kg=rats receiving daily intraperitoneal injections of 5 g of ethanol/kg body weight for 3 days (5 exp.)).

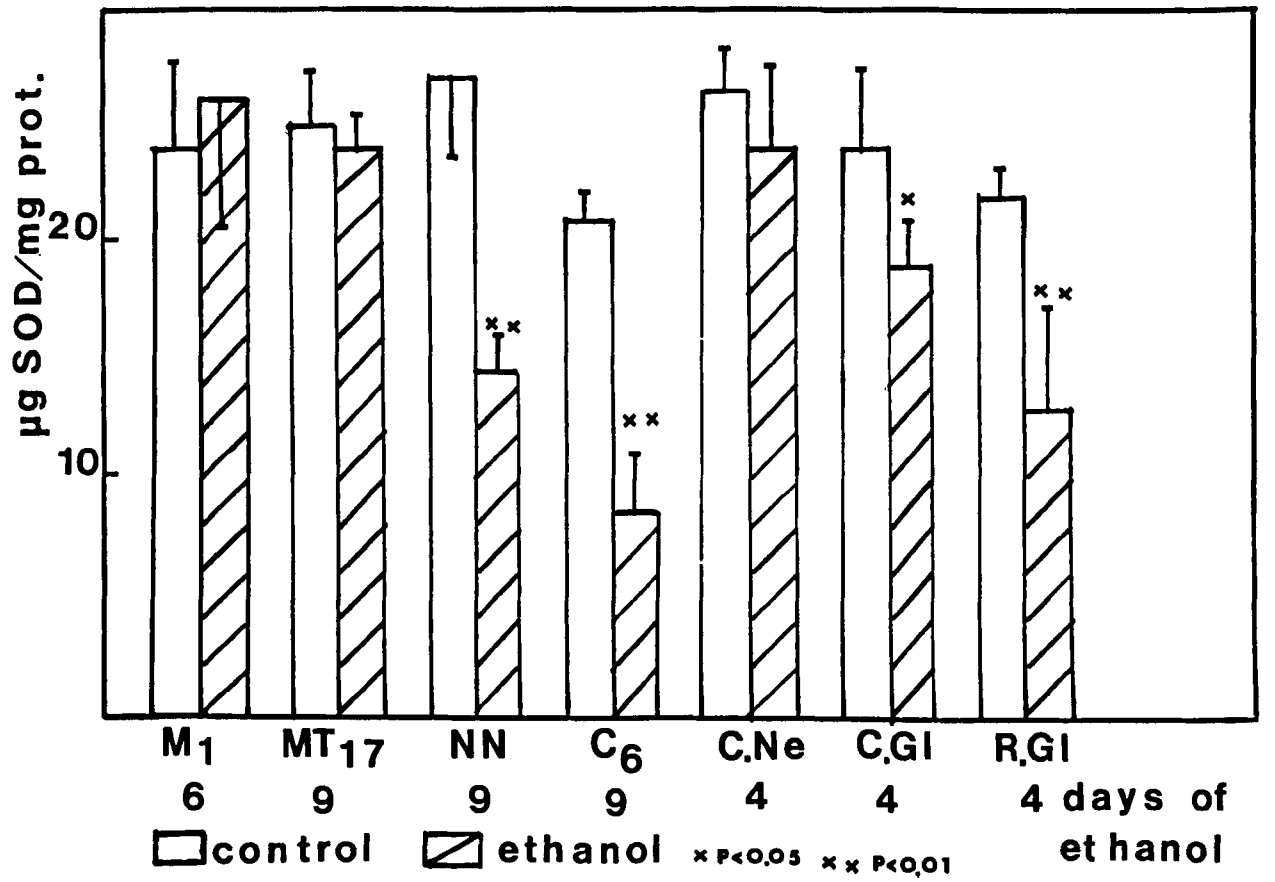

FIG. 9. Superoxide dismutase (SOD) activity (measured according to Fried [7]) of cultured nerve cells (MI (4 exp.); MT17 (7 exp.); NN (6 exp.); C6 (6 exp.); C.Ne=chick neurons (12 exp.); C.Gl=chick glial cells (12 exp.); R.Gl=rat glial cells (6 exp.)) treated for various times with 100 mM ethanol.

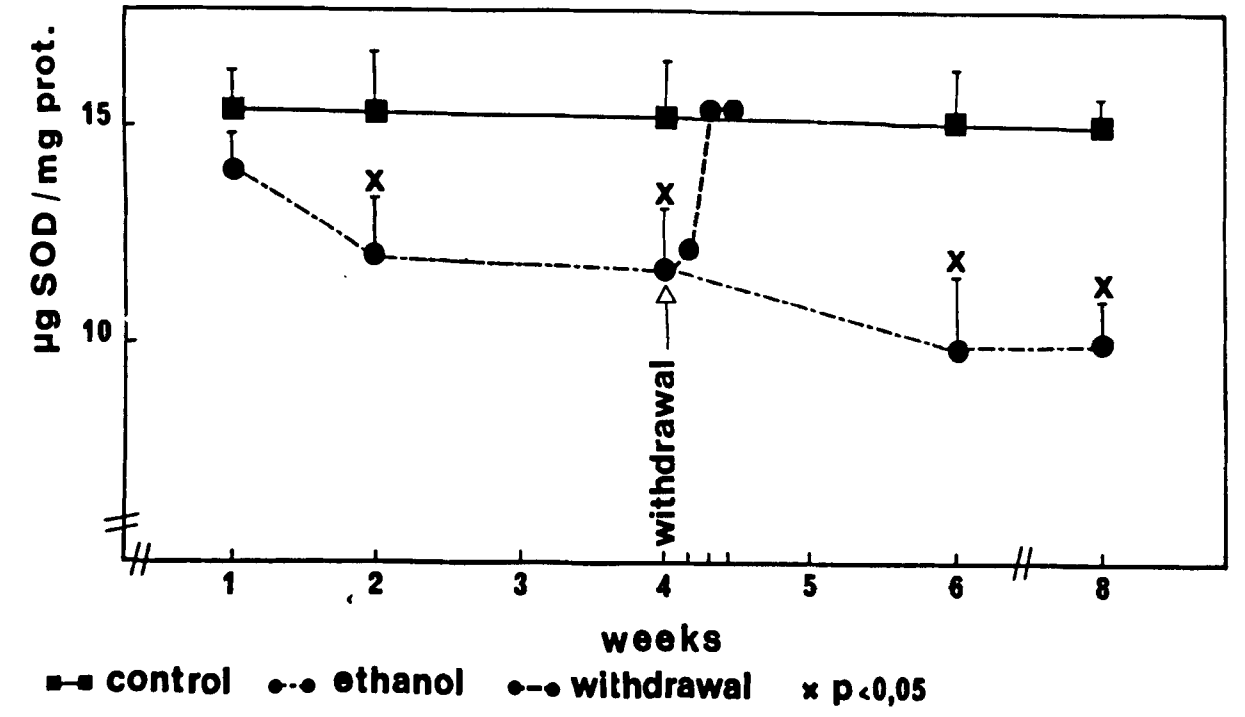

FIG. 10. Superoxide dismutase (SOD) activity (measured according to Fried [7]) of rat brain after the animals received a 20% ethanol solution as the sole liquid source. The heavy dashed line (●--●) indicates the return to normal of superoxide dismutase activity when the liquid diet was withdrawn after 4 weeks of exposure. Values represent mean ± SD from 4 animals.

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