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Reversal by methoxyethylmercury intoxication of NAD induced activation of glutamate dehydrogenase from rat liver

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SOME organic mercury compounds change *in vitro* the effect of allosteric ligands[1] on glutamate dehydrogenase (L-glutamate: NAD(P) oxidoreductase (deaminating), EC 1.4.1.3.).[2] NAD is a coenzyme for this enzyme, but may also act as an allosteric activator.[3–5] As we were interested in mercury effects on the subcellular level *in vivo*, we investigated the activating effect of NAD on the glutamate dehydrogenase acitvity in a liver homogenate from methoxyethylmercury*-intoxicated rats.

The rats were given daily subcutaneous injections of MeEHg, the last about 24 hr before being killed. Ultrasonically-treated suspensions of liver mitochondria in 0·05 M phosphate buffer pH 7·5, passed through a Sephadex G 25 column, were used as the enzyme. The mercury content of the suspensions was determined as mercury dizionate by a method developed at our institute.† The biuret method was used for protein determinations. The glutamate dehydrogenase activity was measured as change in extinction at 340 mμ. A Zeiss selfrecording spectrophotometer RPQ 20 was employed. The reaction mixtures appear in Fig. 1.

The NAD activation of the enzyme is reflected in the deviation from linearity in the Lineweaver–Burk plot.[3] As is seen from Fig. 1, there is no activation following MeEHg treatment of the enzyme *in vitro*, and a slight inhibition seems to be present. The same effects are found without adding mercury *in vitro* when an enzyme from a mercury intoxicated rat is tested.

We do not claim that the present findings explain the symptoms of MeEHg intoxication. Nevertheless they show that the inhibitory effect on the enzymes caused by a change in the sulfhydryl groups does not represent the only possible mechanism whereby mercury may alter the metabolic activity of the cells. The altered reaction to allosteric ligands may well be a common factor as regards

* The seed dressing agent methoxyethylmercury acetate (MeEHg) was a gift from A/B Casco, Stockholm, Sweden.

† Karl Wülfert, personal communication (1966).

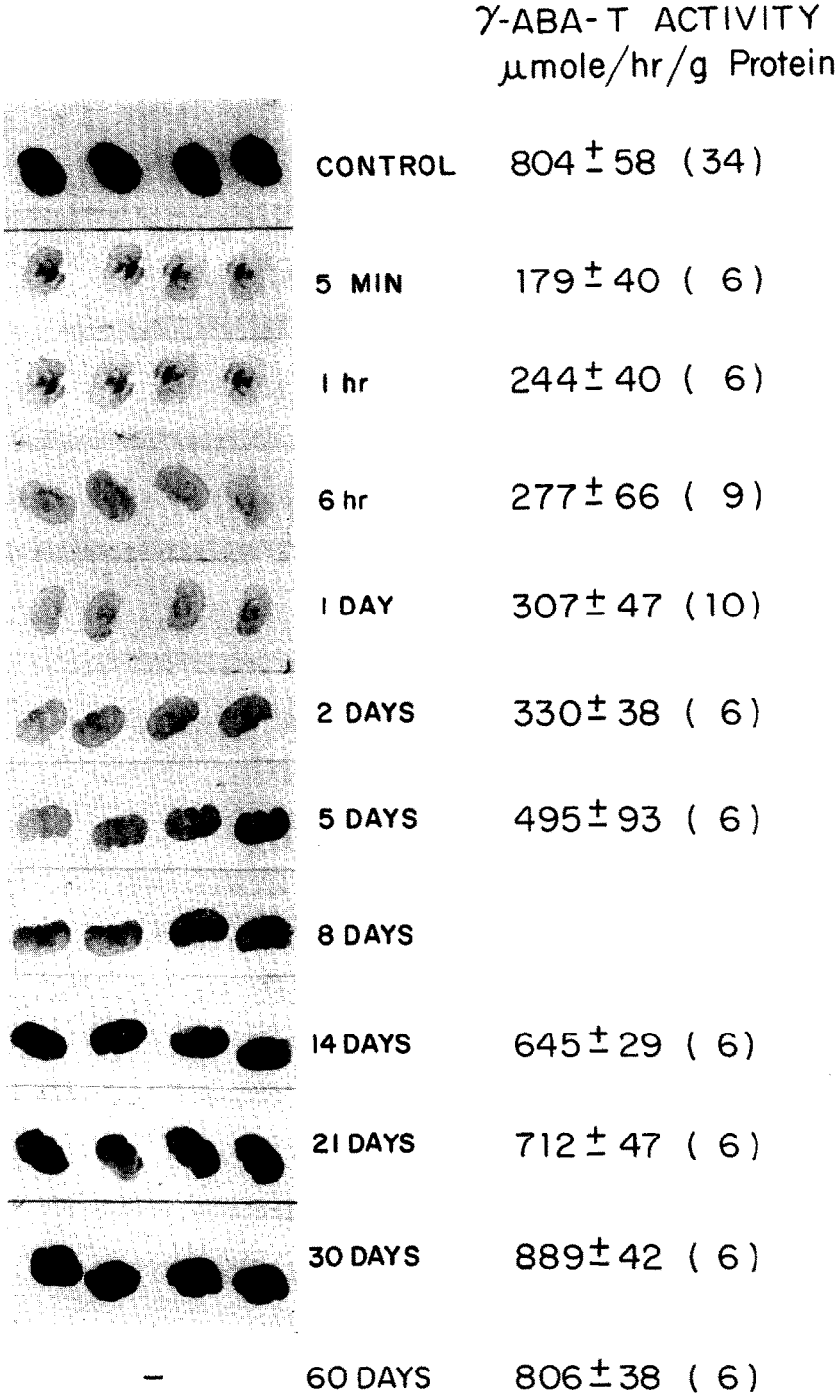

FIG. 1. Histochemically visualized γABA-T-S activity and chemically measured γABA-T activity in sections of brains of mice before and at various times after intraperitoneal administration of AOAA (25 mg/kg). The numbers in parentheses indicate the number of individual mice employed in the chemical studies.

[See Note by Morton K. Rubinstein and Eugene Roberts].

B.P. *facing page 1140*

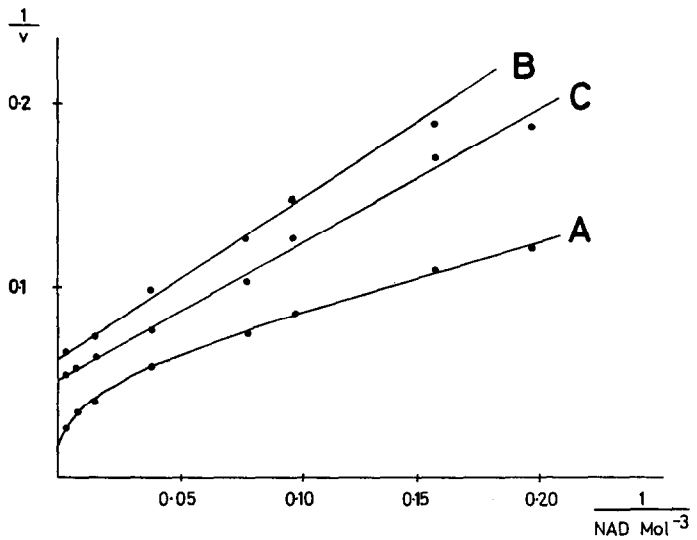

FIG. 1. Reciprocal plot of glutamate dehydrogenase activity in rat liver mitochondria from normal and methoxyethylmercury intoxicated rats. The reaction mixture contained 0·13 M L-glutamate, 0·03 M niacinamide, 0·0004 M KCN, and NAD as indicated, in 0·05 M phosphate buffer pH 7·5. The reactions were started by adding mitochondrial suspension corresponding to 1 mg of protein.

A indicates enzyme from control rat, B the same enzyme preincubated for 10 min at room temperature with 7·5 μg methoxyethylmercury acetate. C shows enzyme containing 0·125 μg mercury from a methoxyethylmercury intoxicated rat.

$v = E_{340\ m\mu} \times 10^2$/min.

the effect of some mercury compounds on cell metabolism. Corresponding reactions are found *in vitro* for other enzymes.[6, 7] As the allosteric mechanism appears to be a general principle in the regulation of cell metabolism, a variety of metabolic changes may be associated with mercury intoxication.

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