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In vitro Inactivation of Hepatic Alcohol Dehydrogenase and Aldehyde Dehydrogenases from Rats by Dithiocarbamates With or Without Metals

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Alcohol dehydrogenase (ADH, EC 1.1.1.1.) is localized mainly in the hepatic cytoplasm. ADH catalyses the degradation of alcohols, esters etc. Aldehyde dehydrogenases (ALDH, EC 1.2.1.3.) are bound predominantly to the hepatic mitochondrial and endoplasmic reticular membranes; a small part of their total activity is measured in the cytosol. ALDH catalyses the oxidation of acetaldehyde - an intermediate product of ethanol disposition - to acetic acid and the metabolism of endogenous or exogenous aldehydes. From observations with human subjects it is known that certain dithiocarbamates - e.g. the dimers tetraethylthiuram disulfide (disulfiram, TETD), and tetramethylthiuram disulfide (thiram, TMTD) - cause an alcohol intolerance (Freundt 1980) described as "Antabuse syndrome" showing nausea, flush, tachycardia, collapse, etc. This reaction can be traced back to a rise of the acetaldehyde concentration in the blood. The increased acetaldehyde level is due to an inactivation of ALDH. A treatment with disulfiram caused a delay of the ethanol elimination from the blood of patients (Peachey et al. 1981) in addition to ALDH inhibition. The goal of the present investigation was to examine in vitro the possible interaction of metal containing dithiocarbamates with the activity of ALDH isolated from rat livers in comparison with that of dithiocarbamates without metals. The results should allow a predictive approach to the health situation of employees occupationally exposed to the investigated dithiocarbamates (fungicides, rubber accelerators). In addition, the elucidation of an inhibitory effect on isolated liver ADH possibly caused by dithiocarbamates with or without metals could help to explain a previously found delay of ethanol elimination from rat blood (Römer et al. 1984). To this end the following substances were tested: The dimers TMTD and TETD, the monomers dimethyldithiocarbamate (DMDC) as sodium salt or as zinc salt (ziram) and diethyldithiocarbamate (DEDC) as sodium salt, as well as the related compounds tetramethylthiuram monosulfide (TMTM), manganese(II)- [N,N'-ethylenebis(dithiocarbamate)] (maneb), and zinc-[N,N'-ethylenebis(dithiocarbamate)] (zineb) (Table 1).

MATERIALS AND METHODS

Adult female SPF Sprague-Dawley rats weighing between 220 and 250 g were used. The animals were housed under standardized conditions

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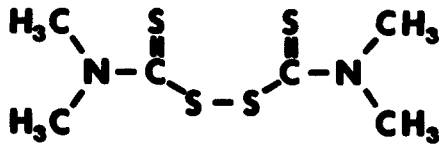
Table 1. Structural formulae of the compounds tested. TETD (used to treat alcoholism) and DEDC (complexing agent) differ from TMTD and DMDC, respectively, by additional methyl groups.

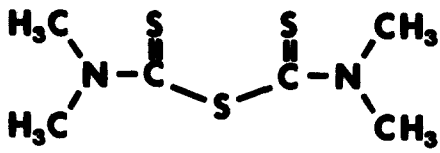
TMTD

Tetramethylthiuram disulfide
fungicide, rubber accelerator



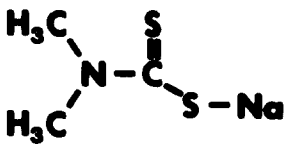
TMTM

Tetramethylthiuram monosulfide
rubber accelerator



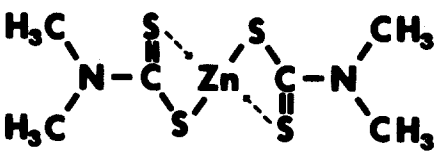
DMDC

Dimethyldithiocarbamate
complexing agent



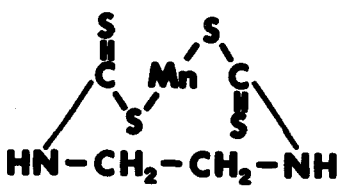
ZIRAM

Zinc-bis(dimethyldithiocarbamate)
fungicide, repellent



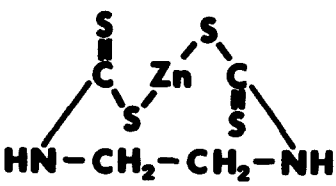
MANEB

Manganese(II)- [N,N'-ethylene-
bis(dithiocarbamate)]
fungicide



ZINEB

Zinc- [N,N'-ethylenebis(dithio-
carbamate)]
fungicide



(Schreiner and Freundt 1987). The livers were removed and a homogenate was prepared (Freundt et al. 1986, Schreiner and Freundt 1987). After centrifugation hepatic ADH was separated (Freundt et al. 1986) and ALDH was isolated from mitochondrial and microsomal membranes by solubilization (Schreiner and Freundt 1987). The obtained enzyme preparations were diluted to yield a protein concentration of 5 mg/ml. The in vitro reaction mixtures used were composed as reported previously for testing the activities of ADH (Freundt et al. 1986, Schreiner and Freundt 1984) or ALDH (Schreiner and Freundt 1984, Schreiner and Freundt 1987). In the dual-substrate reactions (ADH and ALDH) the effects of the dithiocarbamates on the binding of substrate (ethanol or acetaldehyde) or cosubstrate (NAD), respectively, were studied. In the case of the binding of substrate (ethanol or acetaldehyde) the cosubstrate (NAD) concentration amounted to 2 mmol/l (= 75 times its K_m); in the case of the binding of the cosubstrate (NAD) the concentration of ethanol amounted to 200 mmol/l (= 15 times its K_m) or that of acetaldehyde to 50 mmol/l (= 50 times its high K_m). The samples were incubated for 15 min at 37 °C. The dithiocarbamate concentrations used were 0.01, 0.1 or 1.0 mmol/l ethanol containing reaction mixture and 0.01 or 0.1 mmol/l acetaldehyde containing reaction mixture. The pH was 8.4, the incubation volume amounted to 1 ml. Following the incubation, 0.5 ml of the reaction mixture were added to 1.5 ml phosphate buffer (pH = 4.5, 0.5 mmol/l) yielding a final pH of 6.8. The reaction mixtures were kept in ice baths before and after the incubation. The reaction was terminated by lowering the pH to 6.8 in combination with the cooling. The NADH that was formed during the reactions was measured in a spectrophotometer at 346 nm (Freundt et al. 1986, Schreiner and Freundt 1987) and the concentrations of acetaldehyde or ethanol were calculated based on the change in the extinction of NADH. Protein was determined (standard: bovine albumine, fraction V; Sigma, St. Louis/USA) with Folin's reagent (Lowry et al. 1951). The following chemicals (Table 1) of analytical purity were used: TMTM (ICN Pharmaceuticals Inc., Life Science Group, Plainview, N.Y./USA); DMDC, TMTD, TETD, ziram (Fluka, Neu-Ulm/FRG); zineb, maneb (Röhm and Haas Comp., Philadelphia/USA); other chemicals were purchased from Merck, Darmstadt/FRG. From the values measured mean and SEM were calculated and the significance of differences from controls was examined using Dunnett's (1955) test (p less than 0.05).

RESULTS AND DISCUSSION

The dimers TMTD, TETD and TMTM inhibited ADH more than the metal containing dithiocarbamates zineb and maneb or the monomer salts (DMDC, DEDC, ziram) (Fig. 1) in both cases with ethanol or NAD as the main substrate. The pattern of activity displayed as a histogram with ethanol as the main substrate closely resembled that of NAD as the main substrate (Fig. 1). The results can be used in part to explain the delayed ethanol elimination from rat blood after treatment with TMTD or TMTM (Römer et al. 1984) and that from blood of humans dosed with TETD (Peachey et al. 1981). It can be concluded from the results (Fig. 1) that the risk of a delayed ethanol elimination from mammalian blood by zineb or maneb may be very low since high concentrations of 1 mmol/l or 0.1 mmol/l showed weak inhibitory actions on ADH (Fig. 1). This is confirmed by the observation that in vivo no effect on the ethanol elimination was observed in rats after treatment with zineb (Römer et al. 1984). DMDC and

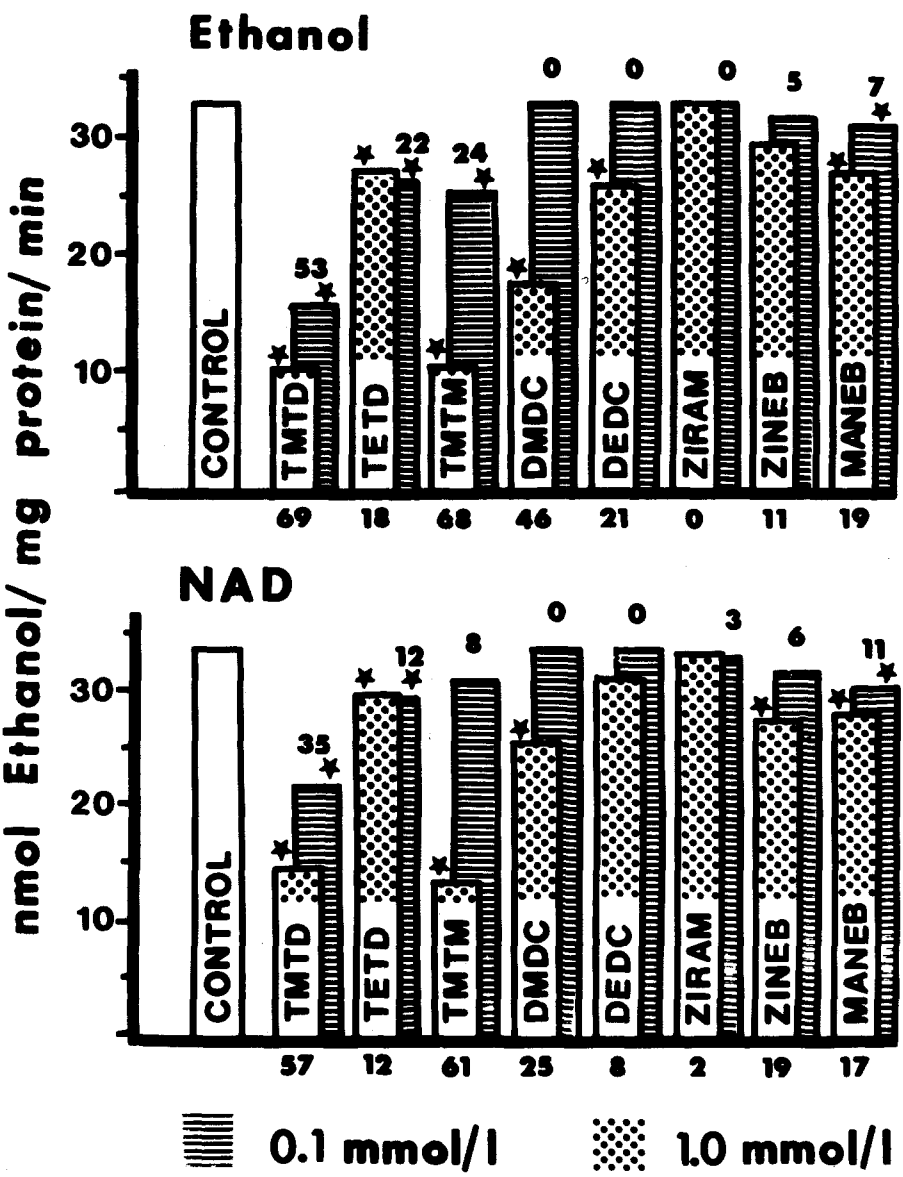


Figure 1. Activity of ADH with ethanol or NAD as the main substrate after addition of dithiocarbamates. The control columns represent the mean of 20 observations each, the other columns (dithiocarbamate concentrations in the reaction mixtures: 0.1 or 1.0 mmol/l) comprise the means of 4 observations each. The SEM was less than 4 % each. The numbers at the columns show the loss of activity as percent compared to controls. Asterisks indicate significant differences from control mean (Dunnett's test).

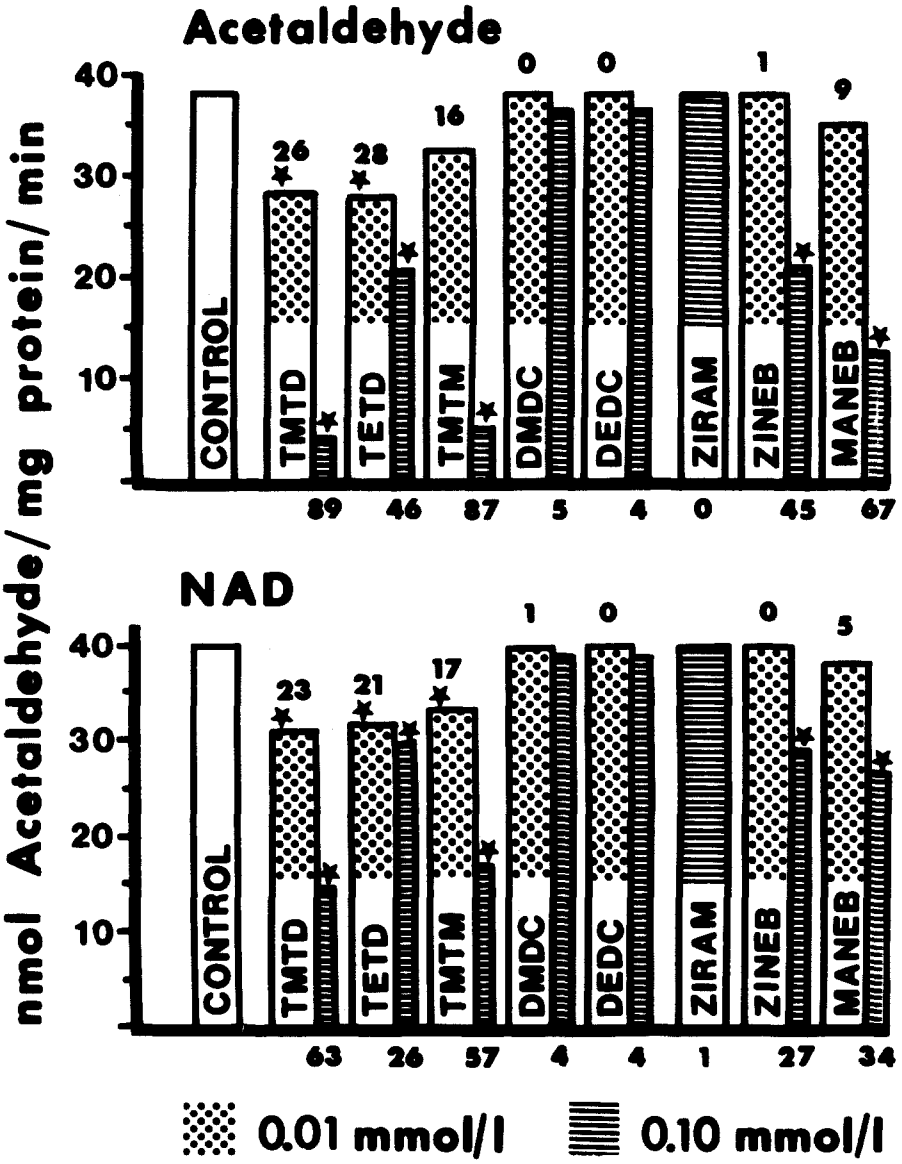


Figure 2. Activity of ALDH with acetaldehyde or NAD as the main substrate after addition of dithiocarbamates. The control columns represent the mean of 20 observations each, the other columns (dithiocarbamate concentrations in the reaction mixtures: 0.1 or 1.0 mmol/l) comprise the means of 4 observations each. The SEM was less than 4 % each. The numbers at the columns show the loss of activity as percent compared to controls. Asterisks indicate significant differences from control mean (Dunnett's test).

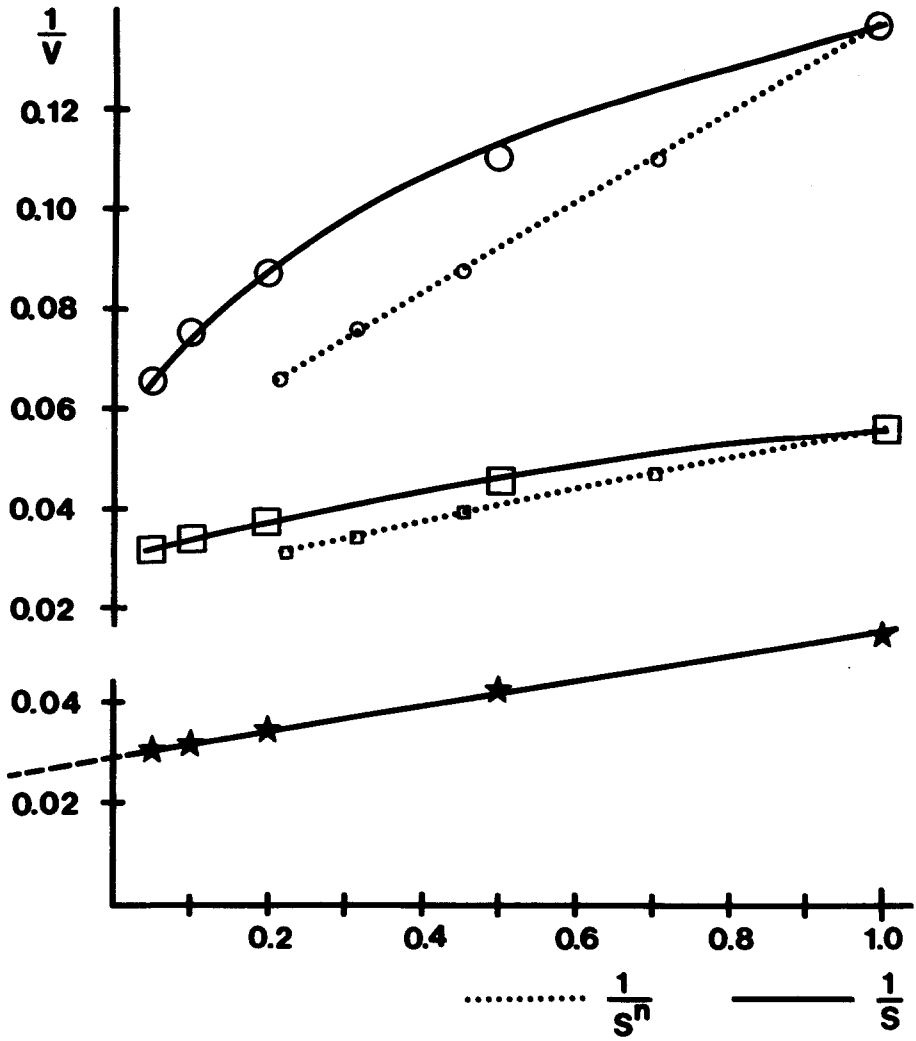


Figure 3. The curves demonstrate double reciprocal plots of the ADH reaction with ethanol as substrate; each point represents the mean of 3 observations. The lower curve (★) shows no cooperativity (control). After addition of TMTD in concentrations of 0.01 mmol/l (□) or 0.1 mmol/l (○) the reaction became negatively cooperative. The values observed fit a curve represented by the equation (Koshland 1970)

$$v = \frac{n \cdot v_{max} \cdot [s]^n}{1/K_a + [s]^n}$$

(v = reaction rate, n = number of binding sites, v_{max} = maximal reaction rate, [s] = substrate concentration, K_a = dissociation constant) with n = 2. This corresponds to an enzyme protein with two identical cooperative binding sites. This assumption is confirmed by the resulting straight lines in a plot of 1/v against $1/s^n$ after the insertion of the empirical n = 2 into the formula.

DEDC were found to be active only after a high concentration of 1 mmol/l (Fig. 1). But in vivo the ethanol elimination from blood could possibly be delayed after application of these monomers, since in the organism active dimers can be formed from the monomers (Brien and Loomis 1983). This mechanism might explain, why ziram was inactive in vitro (Fig. 1) but active after oral (gavage) application to rats 90 min before ethanol treatment (Römer et al. 1984). Ziram is a Zn-complex of the DMDC anion that may be liberated upon dissociation of the complex and then form the dimer TMTD as the active agent in the rat.

The actions on the aldehyde binding site of the studied compounds are similar to those on the NAD binding site (Fig. 2). TMTD, TETD and TMTM inhibited ALDH in both cases when acetaldehyde was the main substrate or when NAD was the main substrate (Fig. 2); these effects occurred even at a concentration of 0.01 mmol/l. Zineb and maneb initiated a less pronounced alteration of ALDH activity, while the monomers (DMDC, DEDC, ziram) were ineffective (Fig. 2). But this does not preclude an inhibitory effect of the monomers in vivo with a possible rise of ethanol-derived acetaldehyde, since dimers active on ALDH can be formed (Brien and Loomis 1983). The inhibitory action on ALDH by TMTD and TETD would explain the enhanced blood levels of acetaldehyde in alcoholized rats co-dosed with TMTD or TETD (Freundt and Netz 1977). From the inhibition of ALDH by zineb and maneb (Fig. 2) one can conclude that both substances lead to less accumulation of blood acetaldehyde than TMTD and TETD and thus induce a lower degree of ethanol incompatibility.

A comparison of ADH inhibition with that of ALDH shows that ALDH was affected by the dithiocarbamates at a concentration (0.01 mmol/l) which was about 10-fold lower than the concentration (0.1 mmol/l) affecting ADH (Fig. 1 and 2). It can be concluded that ALDH is about 10-fold more sensitive to dithiocarbamates than ADH.

The inactivation of ADH by TMTD and (to a smaller extent) by TETD is possibly due to changes of the enzyme molecule. The formation of oligomers of the enzyme is a likely mechanism. We have evidence of cooperativity from enzyme kinetic studies indicating that at lower inhibitor concentrations a dimer structure of the ADH molecule could appear (Fig. 3). Therefore, at higher dithiocarbamate concentrations more S-S-bridges may be formed which causes aggregation resulting in enzyme inactivation. DMDC or DEDC may abstract the active zinc from the ADH molecule by complex binding and may thus cause enzyme inactivation.

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