Effect of Certain Dimethyldithiocarbamate Salts on Some Intermediates of the Glycolytic Pathway in Vivo

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Blood sugar levels and tissue content of glycogen, glucose-6-phosphate, and lactic acid were determined in young adult male rats after intraperitoneal administration of ziram (I), ferbam (II), sodium dimethyldithiocarbamate (III), zinc chloride (IV), and combinations of III and IV. All of the compounds tested elicited a glycogenolytic response in vivo; however, I (10 mg. per kg.) and simultaneous injections of III (10 mg. per kg.) + IV (5 mg. per kg.) produced comparable changes in the parameters at much smaller dose levels than II (500 mg. per kg.), III (50 mg. per kg.), or IV (20 mg. per kg.). The glycogenolytic response was evidenced by a significant increase in blood sugar above control levels, by decreased liver and muscle glycogen, and by increased muscle glucose-6-P and lactic acid. The results of this in vivo study suggest that the disruption of normal carbohydrate metabolism by low parenteral doses of I is a specific effect of the compound itself rather than its zinc or dimethyldithiocarbamate moieties.

The use of dithiocarbamate derivatives as fungicides was patented by Tisdale and Williams (1934). Since that time, a vast amount of work has been performed relative to the practical applications and biological effects of these compounds. Their wide usage in agricultural programs has prompted close scrutiny of these substances with regard to the possibility of an environmental hazard to man.

One group of the dithiocarbamate derivatives that has found extensive commercial acceptance as fungicides is the dialkyl dithiocarbamates. Important representatives of this group are sodium dimethyldithiocarbamate (NaDMDC), zinc dimethyldithiocarbamate (ziram), and ferric dimethyldithiocarbamate (ferbam). In vitro studies of the inhibitory effects of these compounds and of the thiuram sulfides on various enzyme systems have been reported (Thorn and Ludwig, 1962). Excellent reviews of the chemistry and mode of action of the dithiocarbamates and related compounds have been presented by Ludwig and Thorn (1960), Owens (1963), and Thorn and Ludwig (1962). Because of the reported effects of dithiocarbamate on isolated enzyme systems which regulate carbohydrate metabolism, the authors investigated some of the toxic manifestations of a few of these compounds after parenteral administration to intact animals. Measurement of tissue levels of various intermediates of the glycolytic pathway might reveal possible inhibitory effects of the dithiocarbamate derivatives on enzyme systems involved in this phase of glucose utilization in vivo. For this study, blood glucose levels and tissue concentrations of glycogen, glucose-6-phosphate (glucose-6-P) and lactic acid of the rat were selected for measurement.

MATERIALS AND METHODS

Male Osborne-Mendel rats weighing between 150 and 200 grams were used throughout this study. They were maintained on a diet of Purina rat chow pellets and water ad libitum. The dithiocarbamate salts were injected intraperitoneally as suspensions in corn oil and were prepared so that the desired dose would be given in a total volume of 2.5 ml. per kg. Ziram was recrystallized three times from chloroform and ethanol; ferbam was recrystallized three times from methylene chloride and hexane. Reagent grade $NaDMDC \cdot 2H_2O$ was obtained commercially. Two hours after administration of the compounds, the rats were anesthetized with vinyl ether and samples of whole blood, liver, and gastrocnemious muscle were taken. The tissue samples were frozen immediately in a mixture of dry ice–acetone. The whole blood specimens were deproteinized with tungstic acid reagents and the blood sugar was determined by the Folin and Wu (1920) procedure. Weighed aliquots of the frozen tissue were digested in hot sodium hydroxide for subsequent glycogen estimation using a method described by Roe and Dailey (1966). As in a previous study by Dailey *et al.* (1966), tissue levels of glucose-6-P and lactic acid were determined enzymatically by the methods of Horecker and Wood (1957) and Olson (1962).

RESULTS AND DISCUSSION

All three of the salts of dimethyldithiocarbamic acid tested produced a glycogenolytic effect in vivo (Table I). Ziram was by far more effective, on a dosage basis, than either NaDMDC or ferbam. A dose of 10 mg. per kg. [65 microequivalents per kg. (μeq. per kg.)] of ziram resulted in an increase in blood sugar and decrease in tissue glycogen content as great as or greater than that elicited by doses of either 500 mg. per kg. (3600 μeq. per kg.) of ferbam or 50 mg. per kg. (279 μeq. per kg.) of NaDMDC. The intraperitoneal LD_{50} of ziram was reported by Hodge *et al.* (1952) to be 23 ± 2 mg. per kg. for male rats.

Dose levels of all the compounds administered were chosen to produce significant changes from control levels of about the same magnitude in blood sugar and tissue glycogen. Preliminary experiments indicated an increased response with increasing doses up to the levels used in this study. The effects of higher doses were not ascertained. A state of quiescence or depression and loss of mobility of the hind limbs was noted in most of the animals after administration of the dithiocarbamate salts.

The levels of glucose-6-P and lactic acid in liver after administration of the fungicides are shown in Table I. Striking alterations were not observed, although a large variation in glucose-6-P content of liver in individual samples was noted after ziram administration. The variation was probably influenced by the rate of glycogen mobilization in the liver, the residual level of glycogen at the time the sample was taken,

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Table I. Response of Blood Glucose and Tissue Glycogen, Glucose-6-Phosphate, and Lactic Acid to Various Dithiocarbamate Salts and Zinc Chloride[a]

Compound	Dose (I.p.)	Blood Glucose (Mg. %)	Liver Glycogen (G./100 G.)	Muscle Glycogen (G./100 G.)	Liver Glucose-6-P (μMoles/100 G.)	Muscle Glucose-6-P (μMoles/ (100 G.)	Liver Lactic Acid (Mmoles/Kg.)	Muscle Lactic Acid (Mmoles/Kg.)
Control		122 ± 11 (19)	4.14 ± 1.23 (18)	0.464 ± 0.061 (20)	47.9 ± 9.4 (19)	124 ± 23 (20)	6.04 ± 2.14 (19)	3.64 ± 1.17 (17)
Corn oil	2.5 ml./kg.	124 ± 7 (11)	3.39 ± 0.81 (11)	0.418 ± 0.064 (9)	55.0 ± 12.2 (10)	136 ± 40 (10)	6.72 ± 2.04 (14)	3.30 ± 1.44 (14)
Ziram	10 mg./kg. (65 μeq./kg.)	168 ± 26[b] (15)	0.77 ± 0.50[b] (12)	0.219 ± 0.058[b] (12)	54.3 ± 39.9 (14)	300 ± 99[b] (15)	7.43 ± 2.62 (16)	5.24 ± 2.14[c] (15)
Fcrbam	500 mg./kg. (3600 μeq./kg.)	137 ± 12[b] (14)	2.07 ± 1.41[b] (14)	0.384 ± 0.070[b] (14)	33.8 ± 16.1[b] (13)	137 ± 38 (13)	4.91 ± 1.31 (14)	3.00 ± 1.01 (14)
NaDMDC	50 mg./kg. (279 μeq./kg.)	153 ± 20[b] (21)	2.58 ± 0.98[b] (16)	0.374 ± 0.051[b] (17)	48.2 ± 13.9 (20)	134 ± 37 (20)	3.18 ± 1.07[b] (20)	3.26 ± 1.48 (20)
$ZnCl_2$[d]	20 mg./kg. (294 μeq./kg.)	140 ± 16[d] (20)	2.84 ± 1.02[b] (18)	0.392 ± 0.090[b] (20)	50.0 ± 9.8 (17)	116 ± 26 (18)	4.43 ± 1.29[e] (20)	3.81 ± 1.44 (20)
NaDMDC[d] + $ZnCl_2$	10 mg./kg. (65 μeq./kg.) + 5 mg./kg. (73 μeq./kg.)	185 ± 26[b] (20)	2.31 ± 1.09[b] (20)	0.317 ± 0.092[b] (20)	42.9 ± 18.0 (20)	151 ± 43[e] (18)	5.64 ± 2.00 (20)	4.83 ± 1.65[e] (20)

[a] Number of animals is given in parentheses. [b] $P < 0.01$. [c] $P < 0.02$. [d] Dissolved in water. [e] $P < 0.05$.

and the dynamic state of glucose-6-P metabolism in the tissue at biopsy.

In muscle tissue, levels of glucose-6-P were substantially elevated and levels of lactic acid were moderately increased over control levels after ziram administration (Table I). These increases suggest either a lack of sufficient enzyme reserve to cope with the challenge presented by rapid glycogen breakdown, enzyme inhibition by the pesticide, or both.

LD_{50} doses of three chlorinated hydrocarbon pesticides were administered to young male rats (four rats in each of three groups) to determine if significant changes in blood glucose, muscle glucose-6-P, and muscle lactic acid had taken place after the onset of toxic symptoms. Only slight elevations in blood glucose were found after *p,p'*-DDT (av. = 130 mg. %), aldrin (av. = 135 mg. %) and dieldrin (av. = 141 mg. %) as compared to five control rats (av. = 126 mg. %). Muscle glucose-6-P and lactic acid levels were essentially the same in both treated and control rats. Thus, it seems that stress could play a part in the response noted in the parameters studied after administration of high doses of any toxic compound but that the action of ziram is either more specific or is the result of the combination of factors mentioned above, since the changes were of a much greater magnitude than those seen after LD_{50} doses of the chlorinated hydrocarbons.

In view of the potency of the zinc salt of dimethyldithiocarbamic acid relative to the ferric and sodium salts, it became of interest to investigate the effect on glycogen mobilization of zinc chloride alone and in combination with NaDMDC, since ZnDMDC is formed immediately on mixing aqueous solutions of $ZnCl_2$ and NaDMDC.

Administration of 20 mg. per kg. (294 μeq. per kg.) of $ZnCl_2$ (four times the amount of zinc present in a dose of 10 mg. per kg. of ziram) resulted in increased blood sugar and decreased tissue glycogen, but smaller doses of $ZnCl_2$ (5 and 10 mg. per kg.) failed to produce significant changes. Apparently, the glycogenolytic effect of ziram is not simply a manifestation of zinc toxicity.

When aqueous solutions of $ZnCl_2$ and NaDMDC are administered simultaneously, a glycogenolytic response is seen at lower dosage levels than when either compound is given alone, and the response appeared to be additive rather than synergistic. The toxic effect of ziram in vivo may be mediated by: reaction with thiol groups of essential cell components as part of a dithiocarbamate-zinc complex, stimulation of catechol amine and/or glucocorticoid release by the adrenal, or insulin antagonism as a result of the formation of a biologically inactive complex with the hormone.

In vitro studies have suggested that dithiocarbamate toxicity might be the result of chelation of trace metals required for enzyme activity. The data reported in this study in the intact animal do not support that concept, since the far more soluble and dissociable sodium salt, NaDMDC, would be expected to be much more effective than the zinc salt in supplying the dithiocarbamate moiety required for chelation. As shown by the experimental data, at least five times as much sodium salt is required to disrupt normal glucose metabolism in vivo to the extent produced by the zinc salt.

The acute oral toxicity of ziram is relatively low in mammals (1400 mg. per kg. for rats). However, rather striking toxic manifestations were apparent after parenteral administration of small doses of the toxicant. A probable explanation for this finding is that the dimethyldithiocarbamate salts are quite unstable at the acid pH's encountered in the gastric contents and that rapid decomposition in the stomach would account for the low oral toxicity.

LITERATURE CITED

Dailey, R. E., Hines, R. A., Roe, J. H., *Arch. Biochem. Biophys.* **114**, 371 (1966).
Folin, O., Wu. H., *J. Biol. Chem.* **41**, 367 (1920).
Hodge, H. C., Maynard, E. A., Downs, W., Blanchet, H. J., Jr., Jones, C. K., *J. Am. Pharm. Assoc. Sci. Ed.* **41**, 662 (1952).
Horecker, B. L., Wood, W. A., "Methods in Enzymology," S. P. Colowick, N. O. Kaplan, Eds., Vol. **3**, p. 152, Academic Press, New York, 1957.
Ludwig, R. A., Thorn, G. D., *Advan. Pest Control Res.* **3**, 219 (1960).
Olson, G. F., *Clin. Chem.* **8**, 1 (1962).
Owens, R. G., *Ann. Rev. Phytopathol.* **1**, 77 (1963).
Roe, J. H., Dailey, R. E., *Anal. Biochem.* **15**, 245 (1966).
Thorn, G. D., Ludwig, R. A., "The Dithiocarbamates and Related Compounds," Elsevier, Amsterdam, New York, 1962.
Tisdale, W. H., Williams, I., U. S. Patent **1,972,961** (Sept. 11, 1934).

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