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Protection by D-Penicillamine against the Lethal Effects of Mercuric Chloride

The oral administration of the sulfhydryl amino acid, penicillamine, increases the urinary excretion of copper by normal individuals and by patients with hepatolenticular degeneration (*1*). Because of the afore-mentioned report and because of the interests of this laboratory in the metabolic and antimetabolic properties of penicillamine and its analogs (*2, 3*), the efficacy of this amino acid was compared with that of British antilewisite (BAL) as an antidote for heavy metal poisoning.

Male Sprague-Dawley rats (approximately 2½ months old, weight 280 to 340 g), housed in temperature- and humidity-controlled quarters (76°F, 50 percent) were used. As is demonstrated by the data of Table 1, while BAL (group II) completely protects rats against the lethal effects of a single intraperitoneal dose of 3.0 mg of mercuric chloride per kilogram, an equimolar amount of DL-penicillamine (group III) does not. When, however, an amount of D-penicillamine (group IV) equimolar to BAL, or a twice equimolar amount of DL-penicillamine (group V) is administered, a highly significant amount of protection is obtained. Since it appeared that the protective action of DL-penicillamine is due to the D-isomer (groups III, IV, and V), each of the enantiomorphs was tested. It was found that the protective action of penicillamine is primarily a property of the D-isomer (groups VII, VIII, and I). Whereas DL-cysteine (group VI) does not protect the animals, an equimolar amount of its β,β-dimethyl homolog, DL-penicillamine (group V), exerts a significant protective action.

The chronic oral use (*1, 4*) of DL-penicillamine in the treatment of various neurological disorders should be viewed with caution since weanling male Sprague-Dawley rats receiving two oral doses of 50 mg of DL-penicillamine per kilogram each day began to lose weight on the fifth day and four of ten animals were dead by the 13th day.

The biological activity of penicillamine has now been extensively studied (*2, 3, 5, 6*). Although for the rat (*5*) and *E. coli* (*3*) the L-isomer has growth inhibitory activity, the D-isomer is innocuous. While pyridoxine, ethanolamine, choline, or metabolites intermediate between the latter two compounds have been shown to reverse the growth-inhibiting activity of L-penicillamine in the rat (*5, 6*), only valine, isoleucine, leucine, or methionine will do so in *Escherichia coli* (*3*). These inhibitory properties of L-penicillamine and its relative ineffectiveness in treating heavy-metal poisoning appears to make D-penicillamine a safer and more effective agent than DL-penicillamine in the chronic treatment of hepatolenticular degeneration.

The oral effectiveness of D-penicillamine in stimulating copper excretion (*1*) and its intramuscular effectiveness in protecting rats against death due to mercuric chloride under the conditions of these experiments (*7*) presents the possibility of development of an oral prophylactic and an oral treatment for heavy-metal poisoning (*8*).

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Table 1. Mortality of rats receiving mercuric chloride and sulfhydryl compounds. Statistical analysis of the groups showed $p > 0.05$ for groups I versus III, IV versus V, I versus VIII, and II versus VII; $p < 0.05$ for group VII versus group VIII; and $p < 0.01$ for groups I versus II, I versus IV, I versus V, I versus VII, III versus IV, III versus V, II versus IV. Pen, penicillamine; Cys, cysteine; BAL, British antilewisite.

Group	Compound (mg/kg)*	Cumulative 30-day mortality: No. dead/No. started, Expt. 1	Expt. 2	Expt. 3	Expt. 4	Expt. 5	Total	Survival (%)
I	3.0 $HgCl_2$	8/10	9/10	8/10	9/10	9/10	43/50	86
II	3.0 $HgCl_2$ + 60 BAL†	0/10	0/ 5	0/10			0/25	0
III	3.0 $HgCl_2$ + 72 DL-Pen†	6/10	9/10		7/10		22/30	73
IV	3.0 $HgCl_2$ + 72 D-Pen†		6/10	0/10	4/10		10/30	33
V	3.0 $HgCl_2$ + 144 DL-Pen‡		3/10	0/10	4/10		7/30	23
VI	3.0 $HgCl_2$ + 117 DL-Cys‡			9/10	10/10		19/20	95
VII	3.0 $HgCl_2$ + 144 D-Pen‡					5/20	5/20	25
VIII	3.0 $HgCl_2$ + 144 L-Pen‡					14/20	14/20	70
IX	144 DL-Pen‡		0/10				0/10	0
X	117 DL-Cys‡		0/10				0/10	0
XI	144 D-Pen‡		0/13				0/13	0

* All sulfhydryl compounds (*9*) were injected intramuscularly 20 minutes, 1½ hours, and 3½ hours after a single intraperitoneal injection of $HgCl_2$. Recorded amounts of the sulfhydryl compounds are of the free base and are the total of the three injections.
† Equimolar amounts. ‡ Equimolar amounts.

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7. This work was supported in part by a grant from the Surgeon General, Department of the Army. A preliminary report was presented at the September 1957 meeting of the American Society for Pharmacology and Experimental Therapeutics at Baltimore, Maryland. The technical assistance of Nancy S. Pointer is gratefully acknowledged.
8. Such experiments are in progress. Different dosage schedules, as well as modifications of the chemical structure of this compound, are being studied in an attempt to obtain 100-percent protection.
9. Sources of compounds: Redistilled BAL was the generous gift of Dr. J. H. Wells, Army Chemical Center, Md. Chromatographically pure DL-penicillamine, mp 204° to 205°C, was purchased from the Aldrich Chemical Company, D-Penicillamine HCl • ½H_2O $[\alpha]_D^{30} = -59.7$ (1 percent in 1*N* NaOH) and L-penicillamine HCl • ½H_2O $[\alpha]_D^{30} = +60.2$ (1 percent in 1*N* NaOH) were purchased from the California Foundation for Biochemical Research. Mallinckrodt's A. R. mercuric chloride was used. The solutions were such that 0.1 ml of a saline solution of mercuric chloride or sulfhydryl compound was injected per 100 g of body weight.

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