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2,3,2-Tetramine—A Potent Cupruretic Agent (40653)

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The treatment of Wilson's Disease (hepatolenticular degeneration) became practical and effective with the availability of D-penicillamine, a chelator which is effective after oral administration (1). However, the incidence of hypersensitivity or toxic reactions to D-penicillamine is high (2, 3). Although some patients can be desensitized, there is need for additional therapeutic agents that can be better tolerated. The linear tetramine, triethylene tetramine (trien), or 2,2,2-tetramine,[2] has been shown to be effective in patients with increased copper stores, and therefore, a useful alternative to D-penicillamine (4–7).

The *in vitro* interaction of Cu(II) with D-penicillamine (9) involves complex redox chemistry while the interaction of Cu(II) with the 2,2,2-tetramine is a simple issue of metal-chelating agent equilibrium. The coordination of the 2,2,2-tetramine to Cu(II) results in a strained arrangement of three linked five-membered chelate rings (8). This strain can be relieved by the central six-membered chelate ring present in the Cu(II) complex of the homologous 2,3,2-tetramine with an increase in the formation constant (log K_1) from 20.2 to 23.9 (8). In addition to having a higher formation constant for the Cu(II) complex, the purification of the 2,3,2-tetramine is simpler since there are no potentially toxic isomers formed as in the synthesis of trien (6, 10, 11).

Because of these features, the 2,3,2-tetramine or (3,7-diazanonane-1,9-diamine) was investigated to determine its effectiveness *in vivo* as a chelating agent for copper in rats following intravenous and oral administration.

Materials and methods. The 2,3,2-tetramine was synthesized by the method of Van Alpen (12), and converted to its tetrahydrochloride salt. Following recrystallization from an ethanol/dilute aqueous HCl mixture, the salt melted at 281–286° with decomposition (sealed cap.). Analysis calculated for $C_7H_{24}N_4Cl_4$: C, 27.47, H, 7.90; N, 18.30; Cl, 46.33. Found: C, 27.58, H, 7.95; N, 18.30; Cl, 46.14.

Rats (Sprague–Dawley) weighing 360–380 g were placed in restraining cages after a polyethylene tube (PE-190) was surgically placed in the bladder and connected to a fraction collector. The 2,3,2-tetramine (4.0 μmole/100 g body wt) was administered as its salt by continuous infusion for 3 hr via femoral vein or by gavage. Rats were normal or copper-loaded (13). The hourly urine volumes were measured and the urinary copper measured by atomic absorption spectrophotometry (14).

Results. The intravenous infusion of 2,3,2-tetramine (4.0 μmole/100 g body wt) resulted in a prompt cupruresis which was sustained during the infusion period—see Fig. 1. Copper excretion increased 70-fold and was at least 5-fold greater than the control period for 6 hr after the infusion was discontinued. Copper excretion following the infusion exceeded the control period during the 12 hr of observation.

Figure 2 shows the urinary copper excretion follows administration of 2,3,2-tetramine by gavage (4.0 μmole/100 g body wt). There was a delay in cupruresis but by 2 hr there was a sevenfold increase in copper-loaded rats. This cupruresis was sustained for the 12 hr of the study; copper excretion was at least twice the control values for 10 hr.

Discussion. Administration of the chelating agent, 2,3,2-tetramine, results in a cupruresis when given intravenously or orally. This cu-

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[2] A convenient shorthand nomenclature has recently appeared in the chemical literature (8) whereby various linear tetramines are characterized by the number of CH_2 units in each bridge. Thus, trien ($H_2NCH_2CH_2NHCH_2CH_2NHCH_2CH_2NH_2$) is a 2,2,2-tetramine.

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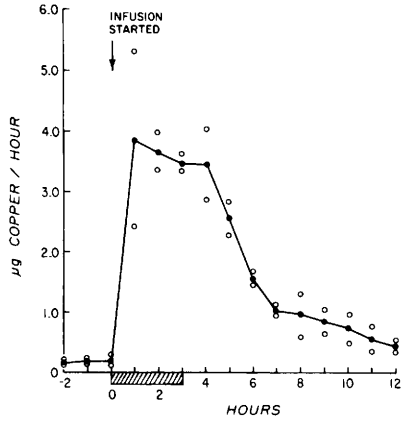

FIG. 1. Infusion of 2,3,2-tetramine, 4.0 μmole per 100 g body wt, started at 0 hr. Infusion continued for 3 hr (shaded area). Urinary copper values were obtained on two rats over a 14-hr period; individual values are designated with ○ and average by ●. The average values for the two rats are connected by the solid line.

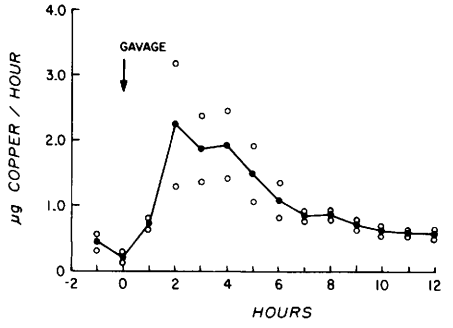

FIG. 2. The urinary excretion of copper following gavage with 2,3,2-tetramine. The dose, 4.0 μmole per 100 g body wt, was administered to two copper-loaded rats at 0 hr. Urinary copper values were obtained over a 14-hr period; individual values are designated with ○ and average by ●. The average values for the two rats are connected by the solid line.

pruresis was marked, particularly following intravenous administration, and lasted for several hours. This agent may prove to be an effective drug for those individuals requiring therapy for Wilson's disease or other diseases characterized by excessive copper stores, particularly in those individuals who show evidence of hypersensitivity or toxicity to D-penicillamine. Studies are in progress to determine effectiveness in comparison to D-penicillamine and trien.

Summary. The 2,3,2-tetramine is a potent cupruretic agent which may prove to be useful in management of disorders characterized by excessive copper stores.

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