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Silastoseal B is obtainable from Midland Silicones Ltd., Barry, Glamorgan.

Bone screws—nickel silver cheese head 16 BA × 1/8 inch obtainable from Laubscher Brothers, London, E.C.1.

Surgical Simplex C is an autopolymerizing acrylic resin obtainable from North Hill Plastics Ltd., London, N.16.

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Effect of chelating agents on copper content and tyramine response of the rat heart

Of the three enzymes involved in the conversion of tyrosine to noradrenaline, two have been shown to be metalloproteins. Tyrosine hydroxylase, which converts tyrosine to dopa in what is generally considered to be the rate-limiting step, contains iron (Udenfriend, 1966) and dopamine-β-hydroxylase, which converts dopamine to noradrenaline, contains copper (Friedman & Kaufman, 1965). Pharmacological inhibition of either of these two enzymes or of dopa decarboxylase, which converts dopa to dopamine, has been shown to lower catecholamine levels in guinea-pig heart to varying degrees (Spector, 1966). Among the substances which have been shown to inhibit dopamine-β-hydroxylase *in vitro* are various chelating agents (Goldstein, Lauber & McKereghan, 1964). *In vivo*, the acute administration of chelating agents has led to decreased levels of noradrenaline in the rat heart (Collins, 1965; Carlsson, Linqvist & others, 1966).

While studying the effect of chronic administration of three chelating agents on the copper levels of various tissues in rats, the response to tyramine of atria isolated from these rats was determined. An apparent relation between the copper levels of the heart tissue and the chronotropic response of the atria to tyramine was observed. The three chelating agents were: γ-thujaplicin (5-isopropyltropolone) (Bryant & Fernelius 1954), plicatic acid (Bock, L. H., personal communication; Gardner, Swan & others, 1966) and penicillamine (Walshe, 1960). The dosages of drugs in Table 1 are shown as equimolar quantities calculated as the sodium salt of γ-thujaplicin (GT), the potassium salt of plicatic acid (P) and free penicillamine (PA).

Table 1. *Copper content of whole heart and chronotropic response to tyramine of isolated atria after prolonged administration of three chelating agents to rats on normal and copper-supplemented drinking water*

Treatment	N	Heart copper μg/g dry weight ± s.e.	P*	Isolated atria N	Basal rate beats/min ± s.e.	P*	Response to tyramine beats/min ± s.e.	P*	Increase in rate as % of control response
				Normal diet					
Untreated control	4	24·89±0·98		12	212±5		379±10		100
GT 2 mg/day	4	21·00±0·61	=0·02	5	208±5	<0·6	304±13	<0·001	58
P 5 mg/day	4	21·79±1·24	<0·2	3	216±11	<0·8	337±15	<0·05	73
PA 1·6 mg/day	4	20·18±1·84	<0·1	7	217±6	<0·6	334±14	<0·02	70
				Excess dietary copper					
GT 2 mg/day	4	25·82±0·93	<0·6	5	197±5	=0·05	338±7	<0·005	84
P 5 mg/day	4	23·09±1·26	<0·4	3	204±8	<0·8	353±20	<0·5	89
PA 1·6 mg/day	4	22·01±0·78	<0·1	4	251±7	<0·001	387±10	<0·6	81

* Statistical significance of the difference between treated and control groups.

GT = γ-thujaplicin
P = potassium salt of plicatic acid
PA = free penicillamine

Rats (Wistar strain; male, 130 g average weight) were divided into a control group and one group for each chelating agent. A normal diet of Purina Lab Chow and tap water was supplied *ad libitum* to the control group and to half of each drug-treated group. The remaining half of each drug-treated group received the same food but had, in place of tap water, distilled water containing sufficient $CuCl_2$, $2H_2O$ to give a final copper concentration of 100 μg/ml. A preliminary estimate had indicated this would result in an approximate seven-fold increase in daily copper intake. Drugs were given daily by the intraperitoneal route on six days each week for a total of 12–13 weeks. The dose of each drug was stoichiometrically in excess of the amount of copper estimated to be absorbed.

At the end of the treatment period, hearts were removed from some rats of each group for analysis of copper. The hearts were rinsed in distilled water to remove excess blood, blotted dry and trimmed of all vessels and fat, leaving only atria and ventricles. Two hearts were pooled into one sample and after dry ashing in a muffle furnace and acid extraction of the ash, the copper content was determined colorimetrically by the method of Eden & Green (1940).

Isolated atrial preparations were obtained from the remaining rats. The atria were suspended in Krebs solution containing double glucose and gassed with a mixture of 5% CO_2 in oxygen. Activity was recorded on a Grass Model 5D Polygraph using an FT 0·03 force displacement transducer. After allowing 2 h for equilibration, a control contraction rate was determined followed by measurement of the chronotropic response to a single exposure to 10^{-6} g/ml of tyramine, an amount in the middle range of the dose-response curve. It is widely accepted that tyramine acts through liberation of endogenous catecholamines (Burn & Rand, 1958; Davey & Farmer, 1963; Muscholl, 1966). The response of isolated rat atria is reduced or abolished upon depletion of noradrenaline (Kuschinsky, Lindmar & others, 1960). Lee, Yoo & Kang (1964) found that there was a significant correlation between the catecholamine content and rate of contraction of isolated rabbit atria. In the present work therefore, a reduction in the chronotropic response to tyramine was assumed to indicate a reduction in noradrenaline content of the myocardium.

The results (Table 1) show that in rats on a normal diet all three chelating agents lowered the copper content of heart tissue, though only significantly so in γ-thujaplicin-treated rats. At the same time responses to tyramine of atria taken from rats treated similarly with the chelating agents were reduced. Table 1 shows further that addition of copper to the diet tended to offset both of these effects of the chelating agents. Extra dietary copper was most effective in offsetting the effects of γ-thujaplicin and least effective against the effects of penicillamine. This probably reflects the much greater effectiveness of penicillamine in promoting copper excretion. In our laboratory we have found that penicillamine is much more effective than plicatate which in turn is only slightly more effective than γ-thujaplicin in promoting urinary excretion of copper. The reason for the high basal rates of atria from rats which had received both copper and penicillamine is not known.

In general, the changes in copper content of the heart paralleled the changes in responses to tyramine. This relation was clearest in rats treated with γ-thujaplicin, a powerful copper chelator belonging to a class of compounds, the tropolones, shown to be potent inhibitors of dopamine hydroxylase *in vitro* (Goldstein, Lauber & McKereghan, 1964). These data are consistent with the hypothesis that chelating agents lower tissue catecholamines due to chelation of heavy metals necessary for the activity of enzymes involved in catecholamine biosynthesis.

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