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Copper: one man's meat is another man's poison

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Copper is widely distributed in nature and indeed the rich copper ores of the eastern Mediterranean made possible the brilliant civilizations of Egypt, of Crete and of Mycenae. Even away from areas of economically valuable copper deposits the metal is still present in most if not all soils and hence in the plants that grow on them. Copper is also present in the sea and it was probably its ready availability that led, early in evolution, to its use both for oxygen transport and as the final electron transfer oxidase for the reduction of molecular oxygen to water. Copper thus early became, and has since remained, an essential constituent of all living things (Adelstein & Vallee, 1961; Schubert, 1964); hence it is present in the normal diet in

quantities sufficient to meet all needs. Indeed we probably eat daily in our food between 2 and 5 mg of the metal, the concentration being highest in shell-fish, liver, cocoa, nuts, mushrooms, dried fruit and certain cereals (Scheinberg & Sternlieb, 1960*a*); whisky and chocolate are prepared in copper vats and in most homes copper pipes have replaced lead so that drinking water may also have a high concentration of the metal. The total body copper content is between 100 and 150 mg and, with no major source of loss, the requirements can easily be met from a normal diet. Although it is relatively easy to induce copper deficiency in experimental animals (Cartwright & Wintrobe, 1964; Gallagher, Judah & Rees, 1956*a*,*b*) and though copper deficiency can occur spontaneously in farm animals as Howell (1968) has already described in this symposium, yet a deficiency of the metal has never convincingly been demonstrated to occur in man as a result of purely dietary causes even when deliberate attempts have been made to induce such a deficiency in premature infants (Wilson & Lahey, 1960).

In man by far the highest concentration of copper is found in the locus caeruleus in the brain stem (Warren, Earl & Thompson, 1960); relatively high concentrations are found in the cortical grey matter and, of other organs, the liver and kidneys have the highest concentrations. However, it should be noted that the copper content of the newborn is considerably higher than of the adult. Copper is found in particularly large amounts at birth in the liver where it is apparently stored as a unique protein of 2% copper content, the neonatal hepatic mitochondrocuprein (Porter, Sweeney & Porter, 1964). By 3 months of age the liver stores of the metal have been reduced to normal levels and at the same time the newborn liver has developed an adult ability to synthesize the serum copper protein caeruloplasmin.

Owing to the high affinity of copper for proteins and amino acids it is probable that all copper in the diet is bound in organic combination, except of course the metal present in drinking water. Copper is rapidly absorbed from the stomach or upper small gut and can be detected in the plasma and red cells within 15 min of ingestion (Osborn & Walshe, unpublished). In normal individuals copper so absorbed is rapidly concentrated in the liver from which it is later returned to the plasma as caeruloplasmin copper (Bearn & Kunkel, 1954). The function of this protein and the fate of the copper attached to it are not known but the evidence suggests that 'the copper of catabolised caeruloplasmin is not directly or preferentially excreted by hepatic, pancreatic, intestinal or renal routes in the rabbit.' (Aisen, Morell, Alpert & Sternlieb, 1964). A small amount of serum copper remains attached to albumin and to amino acids, the direct reacting copper of Cartwright, and it is probably this fraction which is able to pass through a semipermeable membrane (Walshe, 1963; Osborn & Walshe, 1964), to be filtered at the glomerulus and to be deposited in the tissues. As there is no exchange of caeruloplasmin copper in vivo (Sternlieb, Morell, Tucker, Greene & Scheinberg, 1961) it may well be that the albumin copper is the normal transport form of the metal. Besides caeruloplasmin there are a number of other specific copper proteins, hepatocuprein, erythrocuprein, cerebrocuprein, tyrosinase and cytochrome c oxidase (Scheinberg & Sternlieb, 1960*b*).

Although a small amount of copper is lost daily in the urine the bile is the principal

route of excretion of the metal: up to 10% of a dose of injected radioactive copper has been recovered in the bile of dogs (Adelstein & Vallee, 1961) and also man (Osborn & Walshe, unpublished).

So much for the normal turnover of copper in the body. The function of the metal in tyrosinase is as an oxidase for the conversion of dihydroxyphenylalanine into melanin; in cytochrome c oxidase it mediates the final step in the electron transfer chain for the reduction of molecular oxygen to water and as such plays an essential part in energy supply at the cellular level; in the other copper proteins its role is unknown although caeruloplasmin has, like tyrosinase, a weak oxidase activity for polyphenols but at a reaction rate and pH which can hardly have any physiological significance (Curzon, 1961; Holmberg, 1961). The very high concentrations of copper in the locus caeruleus certainly suggest a specific function for nature is unlikely to have made this arrangement purely as an academic excercise.

Despite this essential physiological role as a prosthetic group in a number of enzymes the unbound copper ion can be extremely toxic; for instance at a concentration of 1 in 77×10^6 ionic copper is toxic to tadpoles and to spirogyra (Bayliss, 1918) and many enzyme systems are inhibited in vitro by traces of the metal (Rees, 1961), particularly enzymes dependent on an –SH radicle at the active centre (Veeger & Massey, 1960). Recently it has been shown that subarachnoid injections of a few micrograms of ionic copper in pigeons will lead to convulsions and death and that this is probably mediated by inhibition of the cerebral microsomal ATPase; in vitro, the metal will also block the oxidation of pyruvate thereby preventing its entry into the tricarboxylic acid cycle (Peters & Walshe, 1966; Peters, Shorthouse & Walshe, 1966). It is apparent that there must be mechanisms in the body which prevent this type of enzyme inhibition from taking place in the intact animal in the face of normal copper loads and, since industrial copper toxicity appears to be unknown (Davenport, 1953), some very specific mechanisms must exist for controlling the normal body burden of the metal. Ingestion of 10–15 mg of inorganic copper as a salt will lead to nausea and vomiting and, in larger doses, diarrhoea (Wylie, 1957) and the application of copper salts to a burned surface has been shown to precipitate haemolytic crises (Holtzman, Elliott & Heller, 1966), presumably by short circuiting and overwhelming the normal delicate balance between absorption and excretion. In animals, naturally occurring chronic copper toxicity can lead to haemolytic crises similar to that described in man by Holtzman and his colleagues (Goldberg, Williams, Jones, Yanagita, Cartwright & Wintrobe, 1956).

Chronic copper toxicity in man, though not an industrial or environmental hazard, does occur as a genetically determined metabolic abnormality. In this disease excess of copper is deposited in many tissues, but the highest concentrations are found in the liver, brain and kidneys whilst in the cornea the copper actually becomes visible as brown or green rings which can be seen to increase in size and density as the disease progresses. This localization of the copper deposits is so closely related to the development of symptoms that it is hard to believe, despite views to the contrary (Uzman, 1957) that there is not a cause and effect relationship. Certainly the increased amount of copper in the liver is always associated with histological changes

and abnormalities of liver function and usually, though not invariably, with macroscopic cirrhosis. Indeed the hepatic involvement may be so severe as to lead to death from subacute or chronic liver failure before the nervous system becomes involved (Chalmers, Iber & Uzman, 1957; Scheinberg & Sternlieb, 1959; Walshe, 1962). This clinical observation correlates well with the radiochemical studies of Osborn & Walshe (1967) who showed that the liver may become progressively saturated with copper as the illness progresses and that at the same time there is a reduced rate of clearance of copper from the plasma, which in turn is associated with the later involvement of the central nervous system. It was in fact the dramatic nature of the neurological disability with its wild tremor, its emotional and later intellectual changes which first led Wilson (1912) to realize that here was a new disease entity in which the brain lesion was associated with cirrhosis of the liver even when the latter organ had not given clinical evidence of disease. The renal lesion is of less significance though still an integral part of the illness for isotope studies have shown that as the liver becomes progressively saturated with copper so the kidneys take over as a secondary site of metal deposition (Pl. 1) leading to the same type of tabular reabsorptive defects that are found with other heavy metal poisonings.

Although Wilson's disease, as it has rightly come to be known, is a genetically determined inborn error of metabolism presumably mediated through a single abnormal allele, it is remarkably plemorphic in its manifestations. This must in part be due to the genetic background upon which the abnormal gene is working and in part to the environment, for it is clear that the pathological processes set in train by the build-up of excess stores of copper must be dependent upon the rate of accumulation of the metal. Presumably individuals living in an area of high copper concentration will develop symptoms faster than persons living in an area where copper is scarce. If one could imagine a copper-free world it must be supposed that the disease would not occur at all. While it may thus be possible to explain the different rate of development of symptoms it is still hard to say why some patients have a predominantly hepatic illness, some a predominantly neurological illness and some have what may be called a classical mixed syndrome. Again, in those patients in whom the brain bears the brunt of the insult, we have to explain why some suffer almost entirely from ataxia and tremor, some from parkinsonism, some from dystonia and some from choreiform or ballistic movements. Others have psychiatric disturbances, although it is usual for intellectual function to remain intact till late in the disease. Again, more mysterious still, why is the sensory nervous system never under any circumstances involved? Unfortunately we do not have the necessary meticulous estimations of the anatomical distribution of copper in the brain to know if there is any correlation between the biochemical and functional lesion in these patients. Now that a highly effective treatment is available it may well be that such information will never be forthcoming.

This mention of therapy brings me to my last point: the role of chelating agents in the management of copper deposition in this disease and its influence on the clinical outcome of the illness. Of the various chelating agents which have, at one time or another, been tried in the management of Wilson's disease I will confine my

remarks to penicillamine as it alone is clinically effective, and in the long run tolerable, for the majority of patients. I have summarized my own results with this drug up to last year (Walshe, 1967) so I will be brief here. Penicillamine is derived from penicillin by hydrolysis and splitting of the lactam ring; it is a simple amino acid, β,β-dimethyl cysteine, and should always be used as the D isomer for this is much less toxic than the L form. Given in doses of 1–2 g daily it will promote a cupriuresis of as much as 8 or 9 mg in an untreated patient, though with time this will fall to about 1 mg a day. This fall is not due to drug resistance, but, as I have shown, to depletion of the excess body stores of copper (Fig. 1). Normal copper requirements are however closely guarded so that copper deficiency has not as yet been reported even in patients with no chemically detectable copper in the plasma. This depletion of the body excess stores of copper is correlated with a return to normal of the liver's ability to concentrate the metal (Osborn & Walshe, 1967) and a striking improvement in both hepatic and neurological signs and symptoms; indeed the majority of patients can return to a normal way of life and a number have already embarked upon or are contemplating matrimony with every prospect of propagating their abnormal genes. Studies of the abnormalities of movement, using a simple photographic technique worked out in co-operation with Mr Leonard Beard in the Department of Medical Illustration, have been found valuable in recording the remarkable improvement in motor function which can be achieved in these patients (Pl. 2). Presumably the brain damage must remain, for months or years, biochemical and not structural since it can be so completely reversed. Moreover it

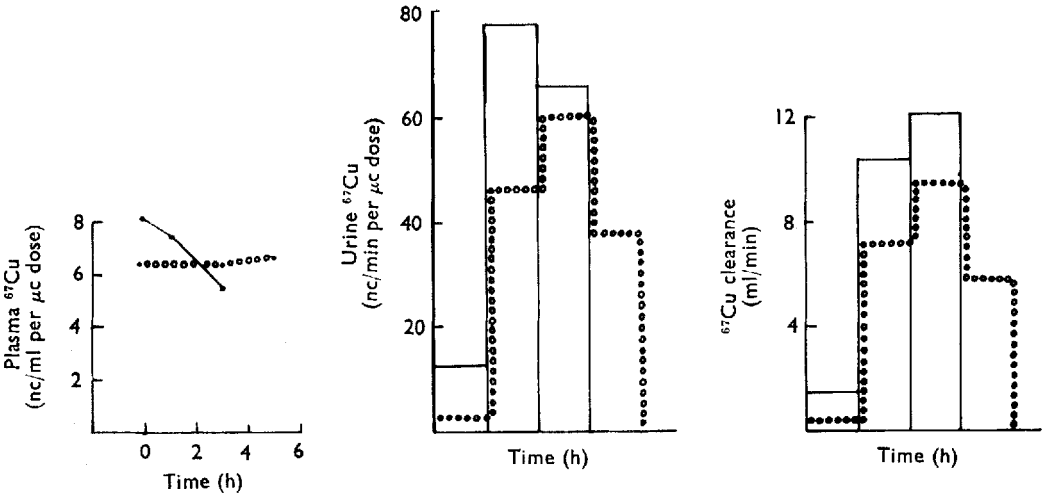

Fig. 1. Endogenous copper clearance estimated in two patients with Wilson's disease using the long half-life isotope of copper, ^{67}Cu. One patient, J.Ba., had been treated with penicillamine (β,β-dimethyl cysteine) for 5 years, the other, D.C., had only recently been diagnosed. Penicillamine was given at zero h; the response to the drug was similar for these two patients despite the differences in their treatment histories. oooooo, J.Ba. after 5 years on penicillamine; ————, D.C. untreated.

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seems reasonable to assume that as deposition of copper is associated with development of the disease and its removal is followed by improvement then copper is the toxic factor, though the primary mechanism of copper deposition remains obscure.

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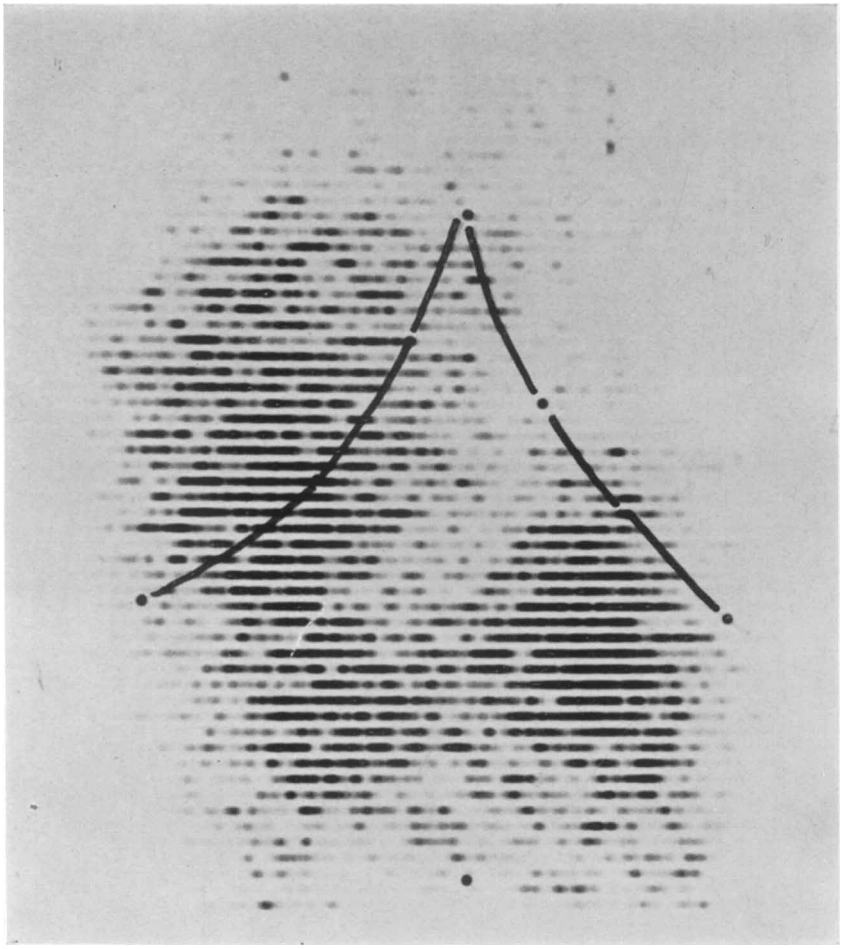
EXPLANATION OF PLATES

Pl. 1. Abdominal scintiscan of a patient with Wilson's disease (E.M.) 4 h after injection of ^{64}Cu showing rather poorly defined hepatic and renal outlines as a result of failure of the hepatic mechanism for concentrating copper.

Pl. 2. Taxograms of a patient, D.C., (*a*) before treatment and (*b*) 1 year later. On each occasion a light was attached to the index finger and the patient asked to draw circles in the air; in each case the camera exposure lasted $\frac{1}{2}$ min.



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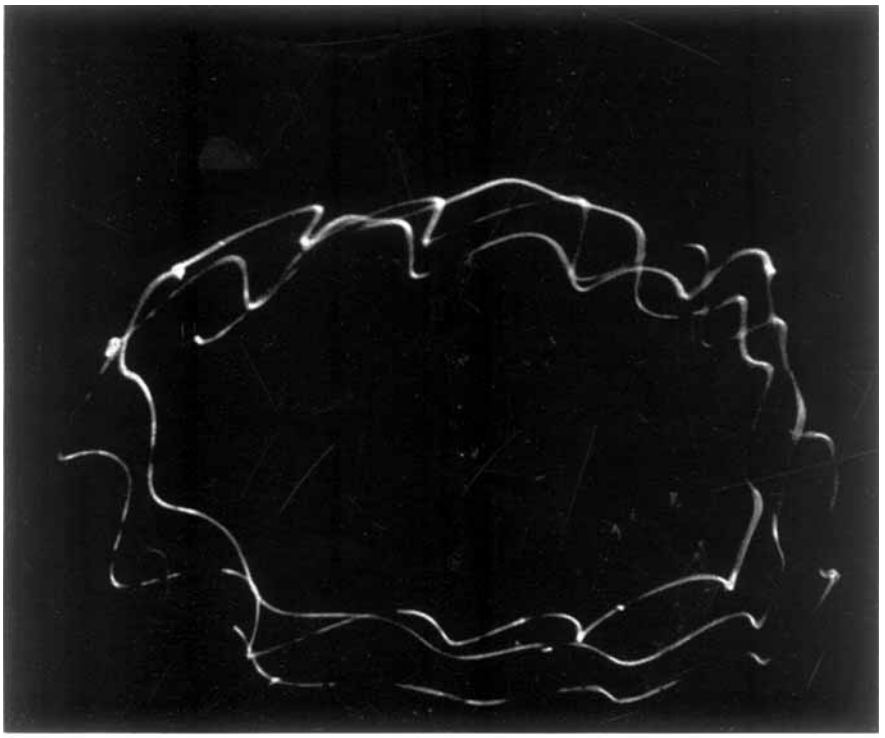
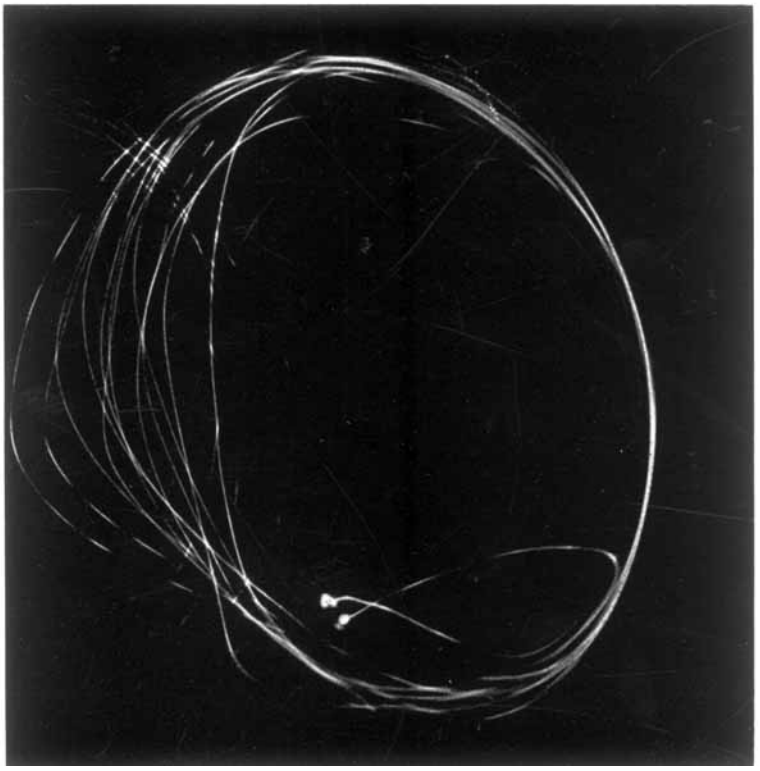


(*a*)



(*b*)

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