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SUMMARY

Copper is essential for the normal function of human tissues although the only established condition in man which results from disturbed metabolism of the metal is Wilson's disease. The disease involves an abnormal positive balance of the metal with manifestations of liver and central nervous system dysfunction. These serious implications of the disease stress the need for a greater understanding of the mechanism of and controlling factors which regulate the extent of gastrointestinal absorption and excretion of copper. The literature provides no supportive evidence for control by mucosal or humoral factors. The role of luminal factors which might be critical in this regard has received little attention.

The aim of the present investigation was to examine some facets of the gastrointestinal absorption and excretion of copper in man, with particular emphasis on the interaction of exogenous and endogenous luminal factors on the metal.

It was considered that the predilection of copper to form coordination and chelate complexes at physiological pH could be of significance in the absorption of the metal, providing that the complexes are soluble and of sufficient stability to prevent precipitation of cupric hydroxide in the alkaline environment of the small intestine. Such copper complexing agents might be encountered in the gut lumen as the products of food digestion and in the alimentary secretions. Depending on the physical

properties and chemical configuration of the complexes they may either facilitate or inhibit copper uptake.

This study confirmed earlier investigations regarding the normal equilibrium which exists between copper absorbed and excreted. A mean of 22.7% was retained from an oral dose of radiocopper and 22.2% was excreted in the faeces and urine following intravenous administration. It is probable that the principal site of copper absorption in humans is the duodenum and upper jejunum because the peak of radioactivity which was observed in plasma occurred less than one hour after an oral dose of copper^{64}.

The microdetermination of copper in the upper alimentary secretions by a colorimetric method showed that saliva and gastric juice contain much smaller amounts of copper than does bile. However when considered on the basis of their daily volumes, saliva and gastric juice represent a significant pool of luminal copper .

The remaining studies detailed in this summary have not previously been reported. A simple radio-assay was developed for estimation of the copper complexing ability of synthetic chelating agents and biological fluids. The application of this test to a number of sugars and amino acids confirmed their avidity for copper. In addition human saliva, gastric juice,

duodenal aspirate and bile each contained substances with the ability to bind copper under the alkaline conditions imposed *in vitro*. The affinity of gall bladder bile for copper greatly exceeded that of the other secretions. Even excluding the effect of bile, the binding capacity of saliva and gastric juice exceeds the amount of copper acquired from dietary sources.

Preliminary characterisation of the endogenous complexes by dialysis and gel filtration revealed that in normal saliva, gastric juice and secretin stimulated duodenal aspirate the components were of low molecular weight and most likely consisted of amino acids and short chain peptides. In patients with Wilson's disease the nature of the copper complexes formed by these secretions were the same as those observed in normal persons. Unlike the other secretions, bile contained a macromolecular binding component which was more pronounced in gall bladder bile than hepatic bile. When hepatic bile was concentrated and dialysed the pattern of copper binding closely resembled that of gall bladder bile. The absence of copper binding in the fluid obtained from a mucocoele of the gall bladder was further evidence that the complexing substances of bile originate in the liver and not in the gall bladder.

The short physical half life of copper^{64} restricted a detailed qualitative analysis of the endogenous biliary copper in man.

In vivo labelled bile was obtained at operation following an intravenous dose of ^{64}Cu, in patients with symptomatic biliary tract disease but in whom the gall bladder was functioning according to cholecystography. The excreted copper was found primarily in association with a macromolecular complex.

With further study many similarities were evident in the nature of *in vivo* and *in vitro* copper binding components of human bile. Both complexes were soluble at alkaline pH, with reversible dissociation of the copper at low pH. The molecular weights determined by gel filtration were in excess of 50,000 and less than 200,000. Resolution of both *in vivo* and *in vitro* labelled bile by electrophoresis on cellulose acetate showed the same pattern of copper binding. Finally, the staining properties of the macromolecular binding component were those of a neutral glycoprotein.

The introduction of both *in vivo* and *in vitro* ^{64}Cu labelled human bile into the duodenum of intact rats resulted in a highly significant inhibition of intestinal uptake of radiocopper. The poor absorption of copper under these circumstances may well be attributed to attachment of the metal to the macromolecular biliary glycoprotein.

The many similarities noted between the macromolecular

components of bile responsible for the binding of copper *in vivo* and *in vitro*, led to the hypothesis that these similar faculties are properties of one in the same substance. Characteristics of the substance are dissimilar to those of the acknowledged constituents of normal bile and the recognised mammalian tissue copper proteins and thus may represent a previously unrecognised factor in the maintenance of copper homeostasis in man.

The results reported in this thesis have demonstrated various factors in the gut lumen with an affinity for copper. These factors may influence the mucosal uptake of the metal. The small molecular weight soluble complexes in the products of digestion such as sugars and amino acids and those present in the gastro-intestinal secretions may facilitate the uptake and mucosal transfer of the luminal copper by virtue of their small molecular dimensions. The role postulated for the macromolecular biliary glycoprotein is a dual one, whereby it acts both in the liver as a vehicle for the excretion of copper in a form relatively unavailable for reabsorption and in an unsaturated form in the gut lumen where it binds the dietary copper and limits absorption of the metal. The net absorption of ingested copper might depend on the differential binding of the metal to the small molecular weight ligands and the macromolecules in the intestinal lumen, particularly the biliary copper binding glycoprotein.

The test of this hypothesis and its significance in the pathogenesis of Wilson's disease require further study.