EFFECTS OF HUMAN ALIMENTARY SECRETIONS ON ^{64}Cu DIFFUSION IN AN *IN VITRO* SYSTEM

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[Manuscript received December 2, 1970]

Abstract

Human alimentary secretions are known to form soluble complexes with copper. The passive diffusion rate of ^{64}Cu complexed to human saliva, gastric juice, and bile was determined and compared with that of known synthetic complexing agents. The study was performed using a two-piece glass diffusion apparatus which permitted continuous sampling from each compartment. It was shown that whereas copper diffusion was unimpaired by saliva or gastric juice, it was markedly inhibited by bile. The most likely explanation for this phenomenon is either that copper is bound by a macromolecular component of bile or that a low molecular weight complex is formed with some biliary component and undergoes polymerization. Which of these is applicable has not yet been determined. The possible role of such a mechanism in the regulation of gastrointestinal copper absorption is discussed.

I. INTRODUCTION

Although small amounts of copper occur naturally in the gastrointestinal secretions of normal human subjects (Gollan, Davis, and Deller 1971*a*) and it is recognized that bile acts as the major excretory vehicle for the metal (Bush *et al.* 1955; Osborn and Walshe 1965), we have recently demonstrated the ability of these secretions to bind further amounts of copper *in vitro* (Gollan, Davis, and Deller 1971*b*). The capacity of the alimentary fluids to form such copper complexes which are soluble under alkaline conditions is in excess of the 3–5 mg of the metal present in the normal daily diet (Gubler 1956).

As part of a study to determine whether this property of alimentary secretions might be implicated in the regulation of dietary copper absorption, the effect on radiocopper transfer in an *in vitro* dialysis system of binding by human saliva, gastric juice, and gall bladder bile has been examined. For comparison purposes the effect on dialysis behaviour of the known copper chelating agents ethylenediaminetetraacetic acid (EDTA), D-penicillamine, and sodium diethyldithiocarbamate was also investigated.

II. MATERIALS AND METHODS

(*a*) *Reagents*

The ^{64}Cu as cupric acetate was obtained from the Australian Atomic Energy Commission as approximately 1 mCi/ml with a specific activity of 2·5 mCi/mg. The ^{64}Cu stock solution was prepared by a 1 : 1600 dilution so that 1 ml was equivalent to 0·25 μg Cu.

Disodium ethylenediaminetetraacetic acid, EDTA (Analar), D-penicillamine hydrochloride (Dista), and sodium diethyldithiocarbamate (British Drug Houses) were prepared as 0·1M solutions.

(*b*) *Collection of Human Alimentary Secretions*

Saliva was collected from 10 normal subjects after the mouth had been rinsed with deionized water. Samples were centrifuged at 2000 *g* for 20 min and stored at −15°C.

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Gastric juice was obtained in the fasting state from 12 normal volunteers by nasogastric intubation. The fasting residue was aspirated and discarded, and patients were instructed not to swallow saliva or nasopharyngeal secretions during the interval of collection. Any blood- or bile-contaminated samples were discarded.

Gall bladder bile was collected by needle puncture and aspiration of the gall bladder at laparotomy in five patients. These patients exhibited normal liver function tests and the liver and biliary tract were found to be normal at operation.

(*c*) In vitro ^{64}*Cu Labelling of Alimentary Secretions*

The ^{64}Cu as cupric acetate stock solution was added with carrier at about pH 6 to the sample under study. A molar concentration of the metal sufficient to saturate all available binding sites was used. The pH was adjusted to 7·2 with sodium hydroxide in order to precipitate any unbound metal which was removed as cupric hydroxide by centrifugation. An aliquot of supernatant was subsequently applied in the study.

(*d*) In vitro *System for Radiocopper Diffusion Experiments*

The apparatus of Helbock, Forte, and Saltman (1966) was used to study the movement of radiocopper across a semipermeable membrane. The two-piece glass system provided two separate compartments for serial sampling and monitoring of pH. The volume of the compartment to which the ^{64}Cu-labelled sample was added measured 115 ml and that from which ^{64}Cu transfer was noted measured 15 ml. A semipermeable membrane of Visking dialysis tubing, flat width 1 cm with an average pore size of radius 24 Å, was the only communication between the two compartments. A constant flow of carbogen gas (95% O_2 and 5% CO_2) was maintained through a bubbling device to ensure constant circulation of fluids. The apparatus was partially immersed in a water-bath at 37°C with access allowed for serial sampling of fluid from both compartments.

The two compartments initially contained Sorensen's phosphate buffer at pH 7·2, prepared from 0·067M monopotassium phosphate and 0·067M disodium phosphate (Documenta Geigy 1962). A 5-ml aliquot of the ^{64}Cu-labelled sample to be studied was introduced to the larger section of the apparatus. Serial samples of 2 ml were made at 15-min intervals from each compartment and the radioactivity of samples measured for 100 sec in a well-type scintillation counter (Philips type PW 4003 automatic gamma counter). Immediately after counting, the samples were returned to their respective compartments in the system. All experiments were performed in duplicate.

Transfer of radiocopper across the membrane was expressed as the percentage of total activity transferred per unit time.

III. Experimental Results

At the completion of each experiment the pH of the system was stable at pH 7·2. The total radioactivity in the two compartments remained constant throughout, indicating no significant loss of radiocopper from adsorption on to the dialysis membrane or glass surface.

The effect of synthetic chelating agents on the rate of ^{64}Cu transfer across the membrane, expressed as a percentage of the total activity at 1 hr, is shown in the following tabulation:

Sample	Concn. (M)	No. of Expts.	Mean Percentage Transfer (±S.D.) of ^{64}Cu at 1 hr	Range
Control*		6	6·0±0·5	5·6–7·0
+EDTA	0·1	3	14·9±1·8	13·2–16·8
+D-Penicillamine	0·1	3	8·1±0·8	7·2–8·8
+Diethyldithiocarbamate	0·1	3	0·1±0·1	0–0·2

* Sample contained only [^{64}Cu]cupric acetate.

Time course of transfer is illustrated in Figure 1(*a*). Both EDTA and D-penicillamine resulted in a significant increase in radiocopper diffusion ($P < 0{\cdot}001$), although a more marked effect was observed with the former. The absence of transfer of Cu diethyldithiocarbamate was due to precipitation of the complex in phosphate buffer.

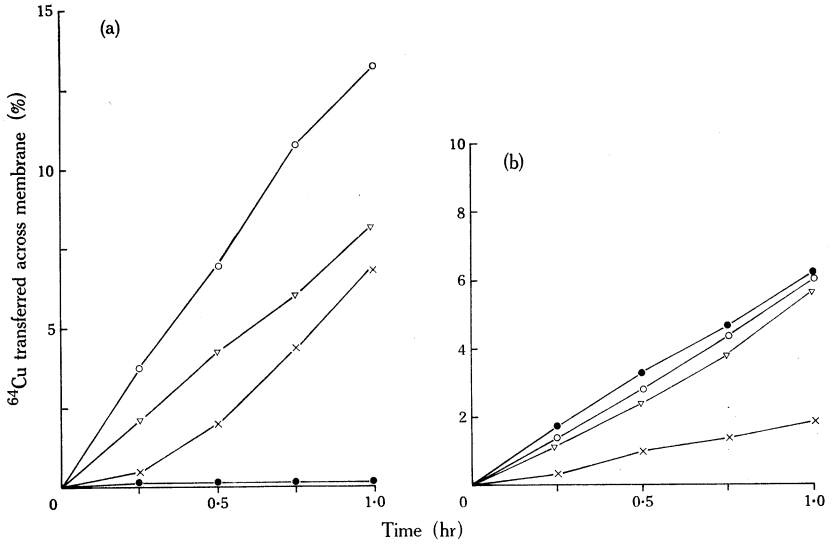

Fig. 1.—(*a*) Influence of 0·1M solutions of EDTA (○), D-penicillamine (▽), and sodium diethyldithiocarbamate (●) on radiocopper transfer across a semipermeable membrane, expressed as a percentage of total radioactivity added to the system. × [^{64}Cu]cupric acetate alone. (*b*) Effect of human saliva (●), gastric juice (▽), and gall bladder bile (×) on the transfer of radiocopper across a semipermeable membrane. ○ [^{64}Cu] cupric acetate alone.

The influence of the various gastrointestinal secretions on radiocopper diffusion is shown below:

Sample	No. of Expts.	Mean Percentage Transfer (±S.D.) of ^{64}Cu at 1 hr	Range
Control*	6	6·0±0·5	5·6–7·0
+Saliva	10	6·3±1·8	4·1–8·2
+Gastric juice	12	5·8±2·2	3·5–8·9
+Gall bladder bile	5	1·8±0·4	1·3–2·5

* Sample contained only [^{64}Cu]cupric acetate.

Time course of diffusion is illustrated in Figure 1(*b*). Values obtained for normal saliva and gastric juice are comparable to the control. However, gall bladder bile obtained from normal subjects produced a highly significant reduction in ^{64}Cu diffusion.

IV. Discussion

Free ionic copper is unlikely to exist in appreciable amounts in man because of the recognized ease with which copper complexes form at physiological pH. It thus seems likely that dietary copper may be absorbed in the form of soluble complexes which are sufficiently stable to prevent precipitation of cupric hydroxide in the

alkaline environment of the small intestine. Such complexes might take their origin in the various gastrointestinal secretions or in the products of food undergoing digestion and, depending on their chemical configuration and molecular size, may facilitate or depress copper absorption. However, other properties such as valency state, net charge on the molecule, type of bonding, and fat–water solubility may also be involved.

The results presented above indicate that, on the basis of molecular size, the copper-binding substances present in human saliva and gastric juice should not impede gastrointestinal absorption of the metal. The effect of bile, however, was to reduce markedly the passive diffusion of radiocopper through a semipermeable membrane. Although the molecular sizes of the synthetic chelating agents studied were similar, the influence on radiocopper diffusion varied widely and was unpredictable. This may be attributed to the tendency of many transition metal chelate compounds to polymerize by olation reactions, so that molecular size of the chelate complex will depend upon many factors including pH, metal concentration, denticity of the ligand, and ligand-to-metal ratio. The polymerization reactions have been examined in some detail in the case of iron(III) (Spiro, Bates, and Saltman 1967; Spiro, Pape, and Saltman 1967).

Because of the existence of the metal complex polymerization reactions, it is not possible to decide on the evidence presented above whether the large molecular weight copper–bile complex results from the interaction of copper with a macromolecular component of bile, or from the polymerization of a low-molecular-weight chelate. Whatever the mode of its formation, the incorporation of dietary copper into such a large molecule may well act to inhibit its gastrointestinal absorption and thus be involved in the overall homeostasis of the metal.

V. Acknowledgment

This investigation was supported by the National Health and Medical Research Council of Australia.

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