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Research Article

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Absorption of Copper in Malabsorption Syndromes *

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Defects in the intestinal transport of iron and calcium in malabsorptive states result in well recognized deficiencies with obvious clinical manifestations. Syndromes resulting from defects in the absorption of other essential metals—manganese, zinc, and copper, for examples—have not been recognized. Cartwright (2), however, has reported that some patients with tropical and nontropical sprue exhibit decreased concentrations of serum copper. In the present paper we report on studies of copper absorption in patients with malabsorption states.

Under normal conditions almost all of the copper of the serum circulates as an integral part of the blue α_2-globulin, ceruloplasmin, while the remainder is loosely bound to albumin (3). Therefore, normally, changes in the concentration of serum copper parallel those of ceruloplasmin. A decreased concentration of serum copper is most frequently the consequence of inadequate synthesis of ceruloplasmin, as in Wilson's disease (4); of interference with protein synthesis associated with severe malnutrition (5) or rarely severe hepatic dysfunction (6); or of excessive urinary or fecal losses from the body (7, 8). The present study presents evidence that impaired absorption of copper may also result in a decreased concentration of serum ceruloplasmin.

Methods

Patients. Forty-nine patients with a wide variety of malabsorption syndromes and varying degrees of steatorrhea (Table I) were studied. The severity of the clinical disease as judged by malnutrition and steatorrhea ranged from mild to severe. The diagnosis of the underlying disease was established by appropriate clinical, radiologic, and laboratory studies and by intestinal biopsy. All patients with nontropical and tropical sprue had the characteristic histologic features (9). In two patients the diagnosis of lymphosarcoma and schleroderma of the small bowel was confirmed at necropsy.

Quantitative determination of ceruloplasmin. The concentration of ceruloplasmin was determined by measuring the oxidase activity of serum towards paraphenylenediamine (10). Normal values in this laboratory range from 20 to 35 mg per 100 ml of serum. Since, as pointed out above, 90 to 95% of serum copper is part of ceruloplasmin, the concentration of ceruloplasmin may be approximately converted into copper concentration by multiplying it by 0.0034, since this is the weight ratio of copper to protein in ceruloplasmin.

Quantitative determination of the concentration of copper in serum and liver. Serum specimens were obtained with copper-free syringes and glassware in 14 patients. One-ml samples of serum were wet ashed with sulfuric and perchloric acids, and the concentration of copper in triplicate samples was determined spectrophotometrically with the aid of dicyclohexanoneoxalyldihydrazone by the method of Scheinberg and Morell (10). Normal values

TABLE I

Incidence of decreased serum ceruloplasmin concentration in 49 patients with malabsorption

Diagnosis	Total no. of patients	Patients with ceruloplasmin concentrations <20 mg/100 ml
Acanthocytosis	1	
Diabetic enteropathy	1	
Diverticulum of jejunum	1	
Hypogammaglobulinemia	1	
Lymphosarcoma of small bowel	1	1
Massive distal small bowel resection	4	
Nontropical sprue	24	8
Postgastrectomy malabsorption	2	
Regional enteritis	1	
Scleroderma	1	1
Tropical sprue	10	1
Tuberculous enteritis	1	
Whipple's disease	1	

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for serum in this laboratory range from 80 to 120 μg per 100 ml.

The same procedure was applied to dehydrated, weighed specimens of two livers obtained with copper-free instruments at autopsy. The concentration of copper in the livers of five control subjects ranged from 16.1 to 31 (mean, 23.6) μg per g dry liver (11).

Test procedure. In three patients with malabsorption,

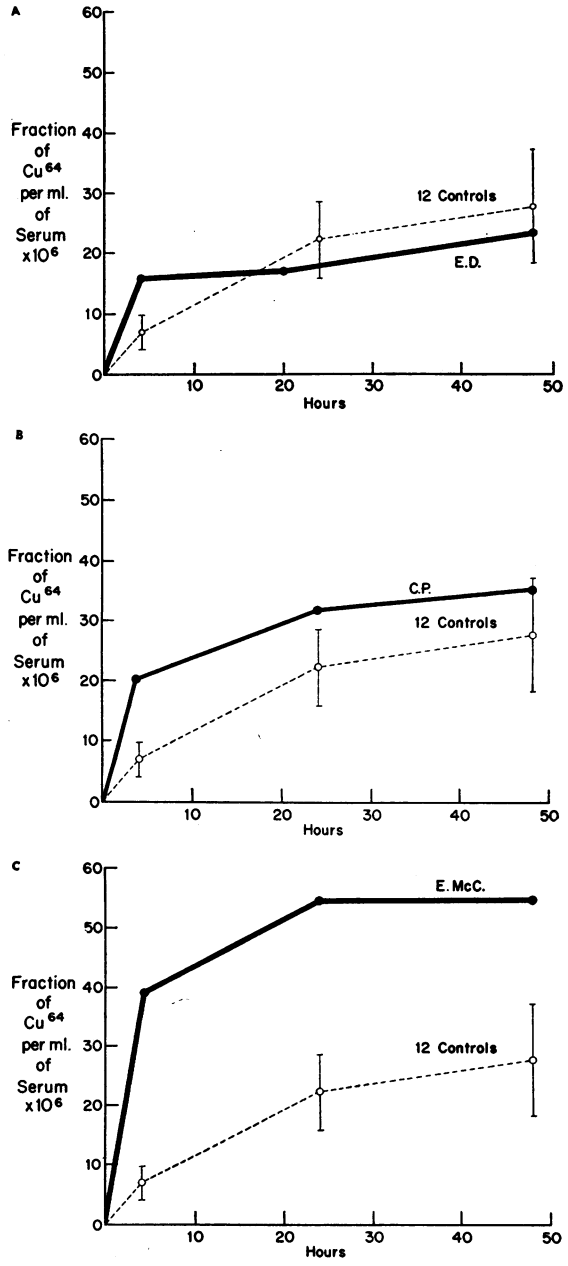

FIG. 1. CONCENTRATIONS IN SERUM OF Cu^{64}, EXPRESSED AS FRACTIONS OF THE ADMINISTERED DOSE PER MILLILITER, AFTER IV ADMINISTRATION OF 0.5 MG OF CUPRIC^{64} ACETATE TO THREE PATIENTS WITH MALABSORPTION AND HYPOCERULOPLASMINEMIA COMPARED TO THE MEAN CONCENTRATIONS ± 1 SD OF 12 CONTROL SUBJECTS. A and B: patients with nontropical sprue (E.D. and C.P.). C: patient with scleroderma involving small bowel (E.McC.).

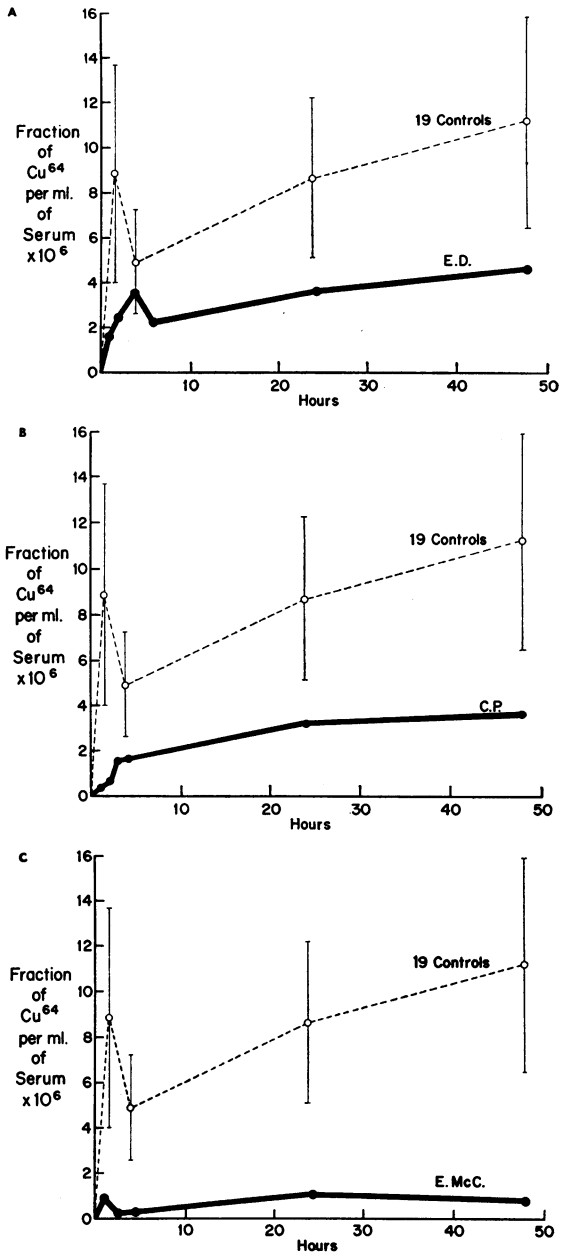

FIG. 2. CONCENTRATIONS IN SERUM OF Cu^{64}, EXPRESSED AS FRACTIONS OF THE ADMINISTERED DOSE PER MILLILITER, AFTER ORAL ADMINISTRATION OF 2.0 MG OF CUPRIC^{64} ACETATE TO THREE PATIENTS WITH MALABSORPTION AND HYPOCERULOPLASMINEMIA COMPARED TO THE MEAN CONCENTRATIONS ± 1 SD OF 19 CONTROL SUBJECTS. A and B: patients with nontropical sprue (E.D. and C.P.). C: patient with scleroderma involving small bowel (E.McC.).

TABLE II

Concentration of ceruloplasmin and radioactive Cu^{64} in the sera of four patients with malabsorption and normal ceruloplasmin concentrations

Subject	Ceruloplasmin	Fraction of administered dose of Cu^{64}/ml × 10^6						Remarks
		1 hr	2 hrs	4 hrs	5 hrs	24 hrs	48 hrs	
	mg/100 ml							
J.C.*	25.2	1.499	3.015	6.507	6.903	11.483	11.631	Delayed absorption
J.C.†	35.0	5.904	9.740	4.789		6.823	7.743	Return to normal
A.R.	35.1	2.404	3.605	4.808		9.014	9.014	Delayed absorption
T.R.	25.8	2.664	6.993	6.327	6.993	7.992	9.657	Delayed absorption
M.vB.†	30.2	6.290	8.707	6.157		7.449	8.674	Normal curve

* Before gluten-free regimen.
† After gluten-free regimen.

steatorrhea, and ceruloplasmin deficiency the absorption of copper was investigated by comparing the results of incorporation of orally administered copper[64] [1] into ceruloplasmin with the incorporation following iv administration of the same isotope according to previously published procedures (12, 13). Doses of 2 mg of the isotope as cupric[64] acetate or sulfate, with an activity of 0.5 to 0.7 mc, were administered orally. One week later, 0.5 mg of Cu^{64} as sterile cupric[64] acetate with an activity of 0.5 to 0.6 mc was diluted in 20 ml normal saline solutions and administered intravenously. Concentrations of Cu^{64} in whole serum were determined in specimens obtained at least at 1, 2, 4, 24, and 48 hours following the oral dose and at 4, 24, and 48 hours after the iv administration of Cu^{64}. The concentrations of Cu^{64} per ml of serum were plotted against time. The results were compared with similar curves previously obtained in 19 and 12 normal subjects, respectively. Five other patients with malabsorption and normal ceruloplasmin were tested with oral doses of Cu^{64} only. In these five patients iv administration of Cu^{64} was not warranted because of normal or close to normal results following the oral test.

Results

Three patients with malabsorption and low serum ceruloplasmin concentrations, two due to nontropical sprue and one to scleroderma involving the small intestine, were studied during active phases of their diseases. In these patients the results indicated that Cu^{64} was incorporated into the protein at normal rates that varied from normal in E.D. and C.P. (Figure 1, A and B) to a significantly increased rate in E. McC. (Figure 1, C) after iv administration. When the same three patients were fed Cu^{64} orally, however, they exhibited markedly diminished concentrations of radioactivity in their sera, well below 1 SD of the mean values of 19 normal subjects who had received the same oral dose of Cu^{64} (Figure 2, A–C).

The three patients with malabsorption who had normal concentrations of ceruloplasmin before therapy exhibited delayed absorption of copper as indicated by the occurrence of the initial peak of radioactivity at 4 or 5 hours after feeding (Table II). However, 24 and 48 hours later the concentration of Cu^{64} in their sera was essentially normal. In one of these this abnormality disappeared when the study was repeated 6 weeks after institution of a gluten-free regimen. The remaining two patients, one with nontropical sprue on therapy and one with a jejunocolostomy (Figure 3), displayed concentrations of Cu^{64} within the normal range after oral doses of Cu^{64}.

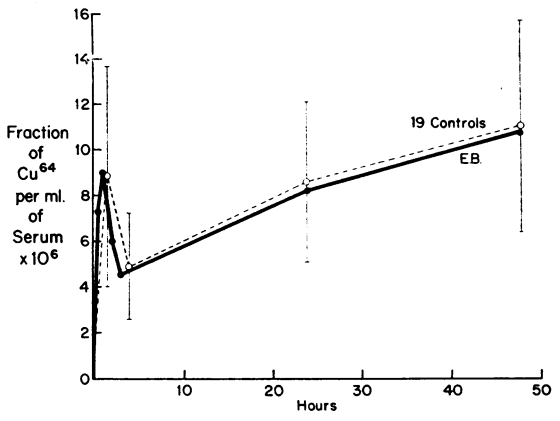

FIG. 3. CONCENTRATIONS IN SERUM OF Cu^{64}, EXPRESSED AS FRACTIONS OF THE ADMINISTERED DOSE PER MILLILITER, AFTER ORAL ADMINISTRATION OF 2.0 MG OF CUPRIC[64] ACETATE TO A PATIENT WITH NORMAL SERUM CERULOPLASMIN CONCENTRATION AND MASSIVE STEATORRHEA FOLLOWING ANASTOMOSIS OF 3½ FEET OF JEJUNUM TO THE MID-TRANSVERSE COLON.

[1] Cupric[64] acetate (half-life, 12.8 hours) was obtained from Abbott Laboratories, Oak Ridge, Tenn.

TABLE III

Significant clinical data on patients with ceruloplasmin deficiency

Patient	Serum cerulo-plasmin	Serum copper	Hemo-globin	Serum proteins	Serum albumin	Serum carotene	Serum calcium	Stool fat	$Co^{60}B_{12}$ excretion*	D-Xylose excre-tion†	Vitamin A tolerance‡
	mg/100 ml	*μg/100 ml*	*g/100 ml*	*g/100 ml*	*g/100 ml*	*μg/100 ml*	*mg/100 ml*	*g/24 hrs*	*% of dose*	*g*	*μg/100 ml*
					Nontropical sprue						
E.D.	13.0		8.0	5.2	3.0	100	8.2	Increase	1.7/0.9		
A.C.	18.9					43		10.5	0.7/1.5		
R.M.	15.1		11.6	6.3	3.6	33	6.8	6.3	26	3.4	42/248
E.J.	18.7	79	14.3	5.1	3.1	11	8.3	6.2	0/0.3	1.0	96/104
J.S.	15.1	62	10.4	5.4	3.0		8.5	Increase	1.3		
P.K.	15.4		11.0	6.0	2.8	20	6.6	27	9		49/77
W.F.	3.7	39	15.0	4.4	2.0	9	9.0	21		1.8	55/88
C.P.	12.1	50	10.2	3.7	1.6	80	7.2	Increase	0.5/1.0		
					Tropical sprue						
C.L.	13.3	54	7.0	4.6	2.3	73	6.2	6.9	0.1/0.03	0.6	73
					Scleroderma						
E.McC.	15.3	59	8.9	6.4	1.8			Increase			
					Lymphosarcoma of the small bowel						
C.DM.	14.3	75	15.4	4.2	2.3	74	7.2	16.8		3.8	48/103

* Before and after administration of intrinsic factor.
† After 25 g given by mouth.
‡ Fasting and peak values.

Eleven of the 49 patients had concentrations of ceruloplasmin below 20 mg per 100 ml of serum, the lower limit of normal in this laboratory (Table I). Significant laboratory findings in these eleven patients are given in Table III. In the patients with acanthocytosis, hypogammaglobulinemia, regional and tuberculous enteritis, respectively, and in two of four patients with small bowel resections ceruloplasmin concentrations were increased, ranging from 39.1 to 56.7 mg per 100 ml. The response of the lowered serum ceruloplasmin level to therapy of the underlying disorder is recorded for four patients (three with nontropical and one with tropical sprue) in Table IV. In a fifth patient who was first studied during a remission and again while suffering a relapse of nontropical sprue, a decrease to an abnormally low concentration was observed within 3 months.

The concentrations of hepatic copper determined in autopsy specimens of two patients with ceruloplasmin deficiency (E.McC. and C.DM.) were 14.5 and 15.5 μg per g dry liver, respectively, just below the lower limit of concentrations observed in five normal subjects (range, 16.1 to 31; mean, 23.6 μg per g dry liver).

TABLE IV

Alterations of serum ceruloplasmin concentration in patients with malabsorption and hypoceruloplasminemia

	Before therapy	Interval	After therapy
	mg/100 ml	*months*	*mg/100 ml*
C.L.	13.3	1	34
W.F.	3.7	5	29.5
E.D.	13.0	1	20.3
C.P.	12.1	1	22.9

Discussion

In this study we have attempted to determine the extent and possible significance of abnormalities of copper metabolism in patients with malabsorption syndromes, since lowered serum copper levels had been observed in some patients with nontropical (2) and tropical sprue (14). Similar lowered values were seen in two patients with intestinal lymphosarcoma and scleroderma as well. We were interested in finding out whether the decreased serum copper concentrations in these patients resulted from a defect in absorption, from an inability to synthesize ceruloplasmin, or from abnormal losses of protein via the intestine.

There was a wide range in severity of ceruloplasmin deficiency among the 11 patients whose concentrations varied from a profoundly abnormal value of 3.7 mg per 100 ml to 18.9 mg per 100 ml, which was close to the lower limit of normal subjects in our laboratory. However, the mean concentration ± SD (14.1 ± 4.2 mg per 100 ml) for the entire group was markedly below the lower limit of normal.

There was a marked discrepancy in frequency

of ceruloplasmin deficiency between nontropical and tropical sprue in our patients. This finding in eight of 24 patients with nontropical sprue in contrast to only one of ten patients who suffered from tropical sprue may reflect the diminished severity of tropical sprue as encountered in New York.

A defect in synthesis of ceruloplasmin as basis for serum copper deficiency was postulated by Butterworth, Gubler, Cartwright, and Wintrobe (14), since iv injections of 10 mg of copper failed to raise the concentrations of plasma copper in two of four patients with tropical sprue. A similar mechanism, however, cannot account for the decreased serum ceruloplasmin concentrations of our patients. From previous studies (15) we learned that *a*) Cu^{64} is incorporated into ceruloplasmin only at the time of synthesis, *b*) freshly deposited hepatic copper is preferentially utilized, and *c*) copper utilized for the synthesis of ceruloplasmin is derived from a small pool with rapid turnover. Therefore the slope of the curve of incorporation of Cu^{64} into ceruloplasmin can be used as an index of the rate of synthesis. Consequently, the rapid rates of incorporation of Cu^{64} into ceruloplasmin after iv administration of the isotope suggest a normal or increased rate of synthesis of the protein in our three patients. Although these data may not lend themselves to quantitative interpretations, they clearly demonstrate our patients' ability to synthesize ceruloplasmin when Cu^{64} is administered parenterally.

Conclusive evidence that the low serum ceruloplasmin concentration in our patients was related to impaired intestinal absorption was obtained by administering Cu^{64} orally and following its appearance in the serum. In normal subjects the concentration of the isotope rises to a peak within 1 to 2 hours and then falls promptly over the subsequent 2 to 4 hours to be followed by a slow secondary rise that reaches a plateau within 36 to 48 hours after the dose (16). The initial peak of radioactivity is due to freshly absorbed Cu^{64}, which is loosely bound to serum albumin, whereas the secondary rise results from Cu^{64} being incorporated into ceruloplasmin during synthesis of the protein. In contrast to the control subjects, the three patients E.D., C.P., and E.McC. exhibited very low concentrations of Cu^{64} in their sera at all times. Since we know that these same patients were able to incorporate Cu^{64} into ceruloplasmin at a rapid rate after iv administration, their low curves following oral Cu^{64} clearly indicate that transport across the intestinal mucosa was impaired.

Since the severity of malabsorption varies greatly among patients and even in the same patient, depending on the phase of the disease, it was not surprising that there were various degrees of alterations in Cu^{64} absorption observed in our patients. Thus, some of the patients showed evidence of impaired gut transport of Cu^{64}, although their serum ceruloplasmin concentrations were normal. This impairment was evidenced by alterations in shapes of oral absorption curves with delays in the appearance of the initial peak of radioactivity (Table II) but concentrations of Cu^{64} within a normal range at 24 and 48 hours. These changes demonstrated abnormalities of absorption of Cu^{64} of lesser severity that had not yet resulted in alterations of the concentration of serum ceruloplasmin. Reversibility of these changes was demonstrated in one patient (J.C.) in whom the study was repeated within 6 weeks after disappearance of the acute symptoms. In another patient (M.vB.), studied only during a remission, a normal curve was obtained.

We did not investigate whether loss of the copper protein via the feces might contribute to lowering of ceruloplasmin concentrations analogous to the loss of albumin noted in a few patients with sprue (17). Such a mechanism might be considered, since virtually all plasma proteins, including ceruloplasmin, have been found in gastric and intestinal juices (18) and such physiologic losses may be exaggerated in patients with various forms of intestinal pathology. Theoretically this problem could be solved for ceruloplasmin by infusing the purified protein labeled with $copper^{67}$ (half-life, 61.8 hours) and by following its rate of disappearance from the plasma (19).

Another possible mechanism which is known to interefere with absorption of calcium and magnesium (20) is the formation of insoluble soaps in the presence of abnormal amounts of fat and fatty acids in the gut. We studied this particular problem in a patient with massive steatorrhea resulting from short-circuiting of 3½ feet of jejunum to the transverse colon. In this patient there was no evidence of abnormal absorption, since the concentrations of Cu^{64} in her serum after oral ad-

ministration were normal (Figure 3). This study also indicated that copper was absorbed in the upper small bowel, a fact suspected by the promptness of the appearance of orally administered Cu^{64} in the circulation in normal subjects, but so far unproved.

Some indication that the defect in absorption of copper resulted in decreased body stores was obtained from determinations of hepatic copper concentration in two patients, since the liver is the organ with the highest concentration of copper in the body, containing on the average 23.6 μg per g of dry weight in normal subjects (11). Both patients with ceruloplasmin deficiency exhibited concentrations just below the range of normal subjects.

The clinical significance of our observations is difficult to assess. None of the features exhibited by the patients with low ceruloplasmin concentrations is distinctive, nor could we detect consistently any association with other aspects of their illness. The incidence and the severity of the ceruloplasmin deficiency correlated roughly with the severity and duration of the clinical manifestations of the underlying disease. No single clinical or laboratory criterion, however, permitted the differentiation of the patients with ceruloplasmin deficiency from other patients with sprue. Follow-up examinations in four patients indicated that specific therapy for copper deficiency was unnecessary, since successful therapy directed towards the underlying disease was followed by a prompt rise in the concentration of ceruloplasmin (Table IV). Conversely, when therapy directed toward the primary disease failed, as it did in the patient with scleroderma, the deficiency persisted to her death.

Summary

1) Significant depression of serum ceruloplasmin below the lower limit of normal values (20 mg per 100 ml) was observed in 11 of 49 patients with malabsorption due to small bowel disease.

2) Studies with Cu^{64} given by mouth and by vein to three patients suggested that the defect in copper metabolism was due to failure to absorb copper, rather than to failure to synthesize ceruloplasmin. Defects in absorption of lesser severity were encountered in three other patients given oral Cu^{64} only, who had normal levels of ceruloplasmin.

Addendum

An additional patient (J.D.S.) with nontropical sprue, severe steatorrhea, and serum ceruloplasmin concentration of 8.9 mg per 100 ml was studied with oral and iv Cu^{64}. There was virtually zero absorption after oral feeding of the isotope. After iv administration the concentrations of Cu^{64} (fractions of dose $\times 10^6$ per ml of serum) were 21.84, 22.35, and 24.15 at 4, 24, and 48 hours, respectively. The normal rate of incorporation of iv Cu^{64} into ceruloplasmin observed in this patient adds further support to our thesis.

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