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**Research Article**

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# Measurement of Gastrointestinal Protein Loss Using Ceruloplasmin Labeled with 67Copper *

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Summary. Ceruloplasmin labeled with 67copper and administered intravenously to dogs, control human subjects, and patients with excessive gastrointestinal loss was shown to fulfill the requirements for a label for quantification of gastrointestinal protein loss. The radiocopper moiety was poorly absorbed from the gastrointestinal tract, not actively secreted into the intestinal tract, and did not alter significantly the metabolism of ceruloplasmin. Approximately 70% of the body pool of ceruloplasmin in both dog and man was within the intravascular space. In control human subjects the mean ceruloplasmin concentration was 30 mg per 100 ml with total circulating and total body ceruloplasmin pools of 15.5 and 22 mg per kg, respectively. In patients with excessive gastrointestinal protein loss secondary to intestinal lymphangiectasia, the serum ceruloplasmin concentration was reduced to 16 mg per 100 ml with a comparable reduction in the total circulating and total body ceruloplasmin pools to 8.8 and 12 mg per kg.

The survival half-time of ceruloplasmin was 6.1 days in normal human subjects and 4.5 days in normal dogs. From 1.0 to 1.9% of the intravascular pool of ceruloplasmin was lost into the gastrointestinal tract of the dog per day, representing less than 11% of the over-all metabolism of this protein. In control human subjects from 1.9 to 3.9% of the intravascular pool was lost into the gastrointestinal tract each day, representing a maximum of from 11 to 22% of the over-all metabolism of this molecule. In contrast, patients with intestinal lymphangiectasia had a markedly shortened ceruloplasmin survival of 3.1 days, with from 15 to 40% of the intravascular pool of ceruloplasmin cleared into the gastrointestinal tract daily. This represented 76% of the over-all metabolism of this protein. Thus, although bulk loss of serum proteins into the gastrointestinal tract does not normally appear to be a significant factor in protein metabolism in normal dogs and men, such loss is a major factor in the pathogenesis of the hypoceruloplasminemia noted in patients with intestinal lymphangiectasia.

## Introduction

Loss of proteins into the gastrointestinal tract has been shown to be a major factor in the pathogenesis of the hypoproteinemia associated with gastrointestinal disorders (1–6). Because of rapid degradation of proteins in the gastrointestinal lumen and subsequent absorption of the constituent amino acids, nitrogen balance techniques and immunochemical analysis of the gastrointestinal se-

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cretions have given only qualitative results. Quantitation of the enteric protein loss has been attempted by using macromolecules with radioactive labels (1–8). Requirements for an ideal label for the quantitation of enteric protein loss are: *1*) The radioactive label should not alter the survival or distribution of the protein, nor should the label be eluted from the protein. If this requirement is fulfilled, the labeled protein could be used to quantitate simultaneously the rates of intestinal protein loss, endogenous catabolism, and in the steady state, protein synthesis. *2*) There should be no absorption of the label from the intestinal tract; otherwise the extent of the gastrointestinal protein loss would be underestimated. *3*) There should be no loss of label into the gastrointestinal tract in any form other than as the intact labeled protein. Excretion of unbound label would result in overestimation of the gastrointestinal protein loss. *4*) The label should be easy to detect and quantitate in the stools; it should be safe for the subject and should be excreted only minimally in the urine because of possible urinary contamination of the stool.

Iodinated serum protein (1, 2, 7), iodinated polyvinylpyrrolidone (PVP) (3), and chromium-labeled albumin (8) have been proposed for the detection of gastrointestinal protein loss. None of these materials fulfills all of the requirements for an ideal label for the quantitation of enteric protein loss. Iodine-labeled proteins cannot be used to quantitate accurately enteric protein loss because of rapid reabsorption of the radioiodide label after the catabolism of the protein in the intestinal lumen and because of secretion of radioiodide in salivary and gastric secretions. These deficiencies have not been overcome with the use of an ion exchange resin (Amberlite) given orally in conjunction with intravenous iodinated albumin, since significant gastric radioiodide secretion persists (9–11). Polyvinylpyrrolidone-131I, a large macromolecule that is poorly absorbed on oral administration, represented a considerable advance in the detection of excessive intestinal protein loss. However, it is not entirely satisfactory for this purpose since PVP is not a protein but a polymer with a wide spectrum of molecular weights and only indirectly gives information on the metabolism of normal serum proteins. In addition variable quantities of PVP-131I are absorbed from the gastrointestinal tract, ranging from 10 to 64% of the orally administered radioactivity (3, 12). Unlike the normal proteins, intravenously administered PVP disappears rapidly from the plasma and is largely excreted in the urine or taken up by the reticuloendothelial system within 48 hours (13).

51Chromium-labeled albumin presents certain significant advantages. The radioactive label is neither significantly absorbed from nor actively secreted into the gastrointestinal tract (8). The major disadvantage in the use of 51Cr-labeled albumin is that the chromium moiety is gradually eluted from the protein and therefore the 51Cr-labeled albumin has a short apparent survival. This precludes the use of 51Cr-labeled albumin in the study of endogenous catabolism or synthesis of protein.

Although each of the techniques described for the detection of gastrointestinal protein loss has certain significant limitations, it should be noted that there is agreement in qualitative terms among these methods when there is a marked increase in loss of proteins into the gastrointestinal tract. In contrast, the role of the gastrointestinal tract in the normal metabolism of the serum proteins has been the subject of considerable controversy. Studies using iodinated proteins and intestinal intubation have indicated that gastrointestinal protein loss may normally represent the major factor in the catabolism of albumin (14, 15) and gamma globulin (16), whereas albumin-51Cr studies have indicated that this mechanism is normally of little significance (11).

In an attempt to overcome the limitations of earlier methods, we evaluated 67copper-labeled ceruloplasmin as a technique for the quantitation of gastrointestinal protein loss. Ceruloplasmin, a protein of 160,000 molecular weight (17) containing eight copper atoms per molecule, is normally found in serum at concentrations of 20 to 35 mg per 100 ml. Sternlieb and co-workers (18) demonstrated previously that although the copper atoms of ceruloplasmin can be exchanged *in vitro,* there is no exchange of the copper moiety *in vivo.* Furthermore, exchange labeling of ceruloplasmin *in vitro* with 67Cu did not alter the biological survival of the protein.

In the present study, 67copper-labeled ceruloplasmin administered intravenously was shown to fulfill the major requirements for a label for quan-

titation of gastrointestinal protein loss. The radio-copper moiety was relatively poorly absorbed from the gastrointestinal tract, not actively secreted into the intestinal tract, and did not significantly alter the metabolism of ceruloplasmin. Radiocopper-labeled ceruloplasmin was used to estimate the size of the circulating and total body ceruloplasmin pools, the rates of synthesis and catabolism of ceruloplasmin, and the role of gastrointestinal loss in ceruloplasmin metabolism of dogs, control human subjects, and patients with intestinal lymphangiectasia. Loss of ceruloplasmin into the gastrointestinal tract was not a significant factor in the metabolism of ceruloplasmin of normal dogs and men. Gastrointestinal protein loss was, however, the major factor in the pathogenesis of the hypoceruloplasminemia noted in patients with intestinal lymphangiectasia.

## Methods

### Preparation and labeling of ceruloplasmin

The ceruloplasmin used in these experiments was prepared from Cohn Fraction IV-1 of pooled human plasma, with DEAE-cellulose column chromatography and precipitation by an ethanol-chloroform mixture (19). The ceruloplasmin thus obtained for labeling was a clear blue 2% solution of at least 90% purity that gave a single precipitation line on Ouchterlony immunodiffusion studies when reacted with antibody to whole human serum and with a specific antihuman ceruloplasmin antibody.

A previously published method for labeling of ceruloplasmin with radiocopper was utilized (18). Ceruloplasmin was incubated with [67]copper nitrate,[1] ascorbic acid, and a 0.2 M sodium acetate pH 5.5 buffer in vacuo at 37° for 2 hours. The radiocopper that had not been incorporated into the ceruloplasmin molecule was removed by passing the preparation through a column containing Amberlite IRA 400 resin [2] between two layers of chelex 100 resin[3] (19). After storage at room temperature overnight, the effluent solution was first passed through a column containing chelex 100 resin and was then filtered through a sterile Millipore filter. The ceruloplasmin used in the studies in man was heated to 60° F for 10 hours to exclude any viable hepatitis virus. Over 98% of the radiocopper of the final product was precipitable with 10% phosphotungstic acid.

Other parts of the same lot of ceruloplasmin that had not been labeled with the radioactive copper were labeled with [125]iodine by the iodine monochloride method of Mc-Farlane (20). The final material contained from 0.3 to 1 mole of iodine per mole of ceruloplasmin. Over 99%

[1] Cyclotron-produced [67]Cu was obtained from Brook-haven National Laboratory, Upton, Long Island, N. Y.

[2] Mallinckrodt Chemical Works, St. Louis, Mo.

[3] Bio-Rad Laboratories, Richmond, Calif.

of the radioiodine of the final product was precipitable with 10% phosphotungstic acid.

### Quantitative determination of ceruloplasmin

The serum ceruloplasmin concentrations were measured by determining the oxidase activity of serum toward paraphenylenediamine (21) in 185 normal control subjects and in 14 patients ranging in age from 9 to 60 years, with excessive gastrointestinal protein loss demonstrated by using [51]chromium-labeled albumin (8). Included were 9 patients with intestinal lymphangiectasia (22), 2 with allergic gastroenteropathy (23), 1 with giant rugal hypertrophy, 1 with regional enteritis, and 1 with protein-losing gastroenteropathy secondary to constrictive pericarditis. None of the subjects had proteinuria, abnormalities of liver function, or significant malabsorption, any of which may affect serum ceruloplasmin concentration.

### Determination of intestinal copper absorption

In order to determine the absorption from the intestinal tract of the [67]Cu label of the ceruloplasmin, we administered 5 μc (10 mg) of ceruloplasmin-[67]Cu orally to six 250-g Sprague-Dawley rats, four mongrel dogs, and eight patients with diseases that did not affect the gastrointestinal tract. The subsequent stools were then collected and counted with appropriate standards to determine the percentage of the orally administered dose of radioactivity that appeared in the stools and was therefore not absorbed. The eight patients received 10 mg of copper sulfate orally three times a day for 2 days before oral administration of ceruloplasmin-[67]Cu and throughout the study period to inhibit absorption of any free [67]copper.

### Intravenous administration

*Studies in dogs.* Approximately 100 mg of ceruloplasmin labeled with 20 μc of [67]Cu was injected intravenously into each of five dogs. Four were normal mongrel dogs ranging in weight from 10 to 30 kg. The fifth dog was given 1 mg per kg nitrogen mustard intraperitoneally before infusion of labeled ceruloplasmin to produce intestinal protein loss similar to that observed in patients with exudative enteropathy. Each dog had been inoculated against distemper, rabies, and hepatitis, dewormed with butyl chloride, and observed for a 2-month period for evidence of infectious disease. They were housed in individual metabolic dog cages, from which the urine and stools could be collected separately.

*Studies in man.* Approximately 100 mg of ceruloplasmin labeled with 20 μc of [67]copper was administered intravenously to four control subjects and to four patients with excessive gastrointestinal protein loss secondary to intestinal lymphangiectasia. This last group had marked hypoalbuminemia, hypogammaglobulinemia, and lymphocytopenia. The half-times of survival of albumin, IgG, and IgM were markedly shortened in all of them. We demonstrated excessive gastrointestinal protein loss in each of these patients with PVP-^{131}I and albumin-[51]Cr. Three of the subjects used as controls had myotonia dystrophica, and one had aplastic anemia. All of the

control subjects had normal liver function, total serum protein, and normal albumin concentrations and normal rates of albumin synthesis and catabolism as estimated by albumin-¹³¹I turnover studies.

All subjects received 10 mg of copper sulfate orally three times a day throughout the study period for the purpose of blocking reabsorption of radiocopper by dilution in a relatively large intestinal copper pool.

### Quantitative determination of radioactive ⁶⁷copper

Three to 5 ml of blood was removed at 10 minutes, 4 hours, and at least daily thereafter for the 10-day period of the study. Urine and stool samples were collected in 24-hour lots throughout the study. Two-ml serum samples were counted with appropriate standards in a well-type scintillation counter. The 24-hour urine and stool pools were counted with appropriate standards in a bulk-type scintillation counter, with three 2-inch sodium iodide crystals at 120° angles.

### Calculations

Plasma radioactivity curves were used to determine plasma volume, fraction of body ceruloplasmin within the intravascular space, rate of transfer of ceruloplasmin between the intra- and extravascular spaces, half-time of ceruloplasmin disappearance, fraction of the intravascular pool of ceruloplasmin catabolized per day, and in the studies in man, rate of ceruloplasmin synthesis. Plasma volume was determined from the quotient of the total radioactivity administered and the activity present per milliliter of plasma at 10 minutes after injection. The protein distribution ratios and the fraction of the intravascular pool catabolized per day were determined according to the method of Matthews (24) as summarized briefly below.

As indicated in Figure 1, plasma radioactivity was plotted as a function of time on semilogarithmic graph paper. The data were analyzed into the sum of two exponentials by the method of "curve peeling." The half-time ($t_\frac{1}{2}$, days) of survival of the protein was determined from the slow exponential curve.

The fraction of the intravascular pool catabolized per day ($K_{2,1}$) is given by the following equation: $K_{2,1} = 1/$ $[(C_1/b_1) + (C_2/b_2)]$, where $-b_1$ is equal to the slope of the slower exponential straight line $= 0.693/t_\frac{1}{2}$, $C_1 =$ the intercept of this slower exponential straight line with the ordinate, $-b_2 =$ the slope of the more rapid exponential straight line, and $C_2 =$ the intercept of this line with the ordinate.

The fraction of the intravascular protein transferred to the extravascular pool each day ($K_{3,1}$) is given by: $K_{3,1} = [(C_1 C_2) \times (b_2 - b_1)^2]/[(C_1 b_2) + (C_2 b_1)]$. The fraction of the extravascular protein transferred to the intravascular compartment per day ($K_{1,3}$) is given by: $K_{1,3} = C_1 b_2 + C_2 b_1$. The distribution of the protein between intra- and extravascular compartments may be calculated from $K_{3,1}$ and $K_{1,3}$ with the relation: extravascular protein mass/intravascular protein mass $= K_{3,1}/K_{1,3}$.

The rate of ceruloplasmin turnover, which in the steady state equals the rate of synthesis, is given by: ceruloplasmin synthesis $= K_{2,1} \times$ plasma volume $\times$ serum ceruloplasmin concentration per milliliter.

The rate of loss of ceruloplasmin into the gastrointestinal tract was determined by a clearance method. The fraction of the plasma pool of ceruloplasmin lost into the gastrointestinal tract per day ($K_s$) = the counts in the stool in the 24-hour period/the counts per milliliter of serum in the period × the plasma volume. We related the counts in the stool to those in the plasma 24 hours before the stool period, thus assuming a 24-hour delay in excretion of material passed into the gastrointestinal tract. The mean of the 24-hour periods from days 2 to 10 was used to determine the $K_s$ value. The fraction of the over-all metabolism of ceruloplasmin that is accounted for by gastrointestinal loss is equal to $K_s/K_{2,1}$.

The values for $K_s$ obtained by this method represent a theoretical maximal figure for the fraction of plasma ceruloplasmin ⁶⁷copper cleared into the gastrointestinal tract daily, since unbound ⁶⁷copper freed from catabolized ceruloplasmin could also enter the gastrointestinal tract in the bile (25) and thus contribute to the radioactivity ultimately measured in the stool. In order to correct for any such loss of unbound ⁶⁷copper into the gastrointestinal tract and thus determine the clearance of ceruloplasmin into the bowel more accurately, we also evaluated the data
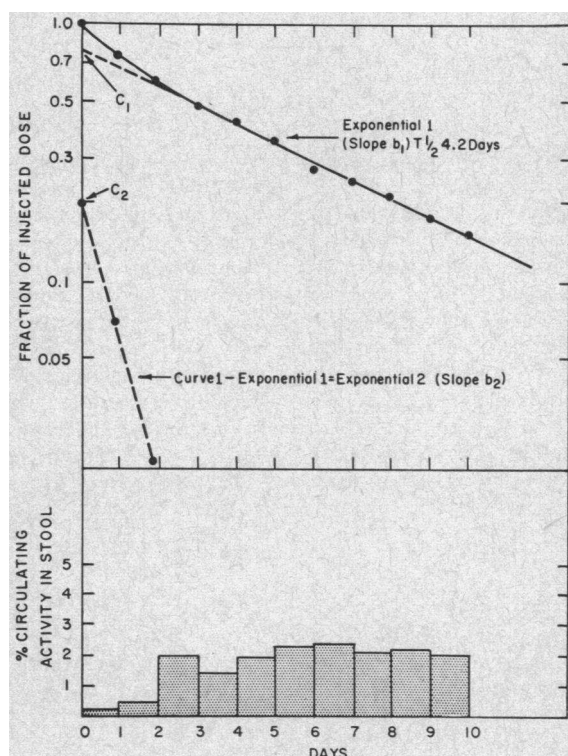


FIG. 1. ANALYSIS OF PLASMA RADIOACTIVITY CURVE AFTER INTRAVENOUS ADMINISTRATION OF CERULOPLASMIN-⁶⁷Cu TO THE NORMAL DOG. The fecal clearance of ⁶⁷Cu derived from the activity excreted in the feces in 24 hours and the corresponding mean plasma activity are also shown.
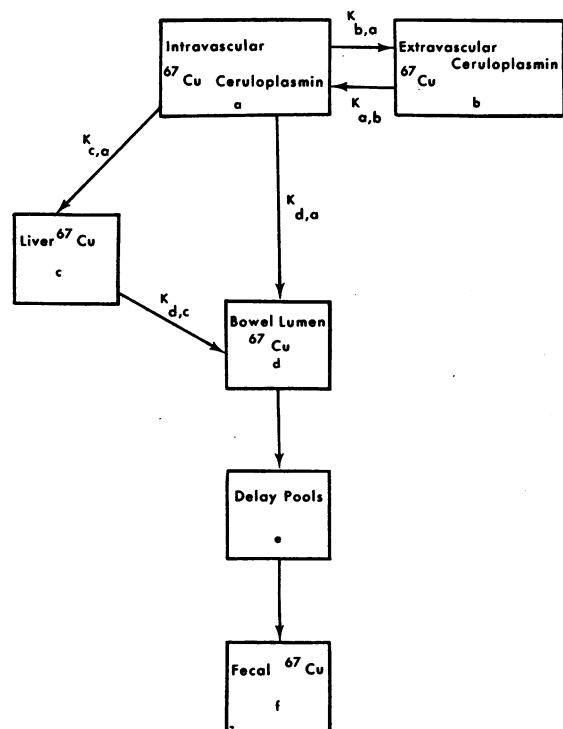
FIG. 2. KINETIC MODEL OF CERULOPLASMIN-⁶⁷CU METABO-
LISM. See text for explanation.

with a method of compartmental analysis elaborated by
Berman, Shahn, and Weiss (26). The model used is
shown in Figure 2. Compartment a represents the plasma
⁶⁷copper radioactivity; compartment b, the extravascular
⁶⁷copper-labeled ceruloplasmin; compartment c, the liver
unbound ⁶⁷copper pool; compartment d, the ⁶⁷copper in
the bowel lumen; compartment f, the fecal radioactivity
pool; and compartment e, a series of delay pools repre-
senting the time required for radioactivity entering the
intestinal lumen to make its appearance in the stool.

The transfer rate ($K_{d,a}$) represents the rate of loss of
⁶⁷copper-labeled ceruloplasmin from the plasma into the
bowel lumen. $K_{c,a}$ represents the rate of transfer of
⁶⁷copper from the plasma to the liver on catabolism of

ceruloplasmin, and $K_{d,c}$ represents the rate of transfer of
unbound ⁶⁷copper from the liver into the gastrointestinal
tract through the bile.

Experimental data were available for compartments a
and f. Initial estimates for the time between loss of ra-
dioactivity into the intestinal lumen and its appearance
in the stool were obtained from oral ceruloplasmin-⁶⁷Cu
studies. Radioactive ⁶⁷copper-labeled ceruloplasmin was
injected into compartment a. Subsequent distribution was
assumed to be governed by first-order exponential proc-
esses; the amounts of residual labeled material in the
various compartments could thus be described by sets of
first-order linear differential equations.

Accordingly, the data in appropriate form were fit to
sets of equations by a digital computer so that the values
for intercompartmental transfer rates ($K_{i,j}$) that best fit
the data could be calculated. The compartmental analysis
corrects for loss of unbound ⁶⁷copper into the bowel and
thus provides a more accurate estimate of the true rate of
loss of ceruloplasmin-⁶⁷Cu into the bowel ($K_{d,a}$) than
does the clearance method ($K_s$).

*Effect of method of labeling on survival of protein
molecules*

The effect of introduction of radioactive copper into the
ceruloplasmin molecule was studied by determining the
metabolism of the same lot of ceruloplasmin labeled with
¹²⁵I. Subjects for these studies included the four patients
and four control subjects used in the intravenous ceru-
loplasmin-⁶⁷Cu studies and six dogs other than those that
received the ⁶⁷copper-labeled ceruloplasmin. The patients
received 10 drops of saturated solution of potassium io-
dide each 8 hours during the study period, and the dogs
received water containing 0.25% potassium iodide to in-
hibit thyroidal uptake of the radioiodine. Fifty micro-
curies of ¹²⁵I-labeled ceruloplasmin was administered in-
travenously, and 3- to 5-ml blood samples were removed at
10 minutes, 4 hours, and daily throughout the 10-day study.
The serum samples were counted and the data analyzed
according to the method of Matthews (24) as presented
above.

## Results

*Gastrointestinal absorption of ceruloplasmin-
⁶⁷Cu.* After oral administration of ⁶⁷Cu-labeled

TABLE I

*Ceruloplasmin-⁶⁷Cu turnover in the dog**

| Dog | % oral ⁶⁷Cu not absorbed | Plasma volume | % body ceruloplasmin in intravascular pool | Ceruloplasmin survival t½ | $K_{2,1}$ | $K_s$ | $\dfrac{K_s \times 100}{K_{2,1}}$ |
|---|---|---|---|---|---|---|---|
| | | *ml/kg* | | *days* | | | |
| 1 | 97.1 | 41 | 91 | 4.3 | 0.18 | 0.019 | 10.6 |
| 2 | 101.0 | 45 | 94 | 4.8 | 0.15 | 0.010 | 6.7 |
| 3 | 82.0 | 51 | 73 | 4.6 | 0.21 | 0.018 | 8.6 |
| 4 | 88.7 | 39 | 79 | 4.2 | 0.21 | 0.017 | 8.1 |
| Mean | 92.2 | 44 | 84 | 4.5 | 0.19 | 0.016 | 8.5 |

* Abbreviations: $K_{2,1}$ = fraction of plasma pool catabolized per day; $K_s$ = fraction of plasma pool cleared into
gastrointestinal tract per day.

ceruloplasmin, from 95 to 100% (mean 98) of the $^{67}$Cu appeared in the stools of the six rats. In the four dogs from 82 to 100% (mean 92) of the administered dose was excreted in the subsequent stool collections. The eight patients excreted from 70 to 99% (mean 88) of the orally administered copper-labeled ceruloplasmin in the subsequent 5-day stool collections. Thus, the copper moiety of $^{67}$copper-labeled ceruloplasmin is relatively poorly absorbed from the gastrointestinal tract under the conditions of this experiment.

*Metabolism of intravenously administered ceruloplasmin-$^{67}$Cu in the dog.* The data on turnover of ceruloplasmin-$^{67}$Cu and ceruloplasmin-$^{131}$I in the dog are shown in Tables I and II and Figure 1. On the basis of these studies from 61 to 94% of the body pool of ceruloplasmin was present in the vascular compartment, with a mean of 84% for those studies using ceruloplasmin-$^{67}$Cu and 75% for ceruloplasmin-$^{131}$I. The $^{67}$Cu-labeled ceruloplasmin had a mean half-time of survival of 4.5 days with 19% of the intravascular pool catabolized per day. The iodinated ceruloplasmin had a mean $t_\frac{1}{2}$ of survival of 3.8 days, with 25% of the intravascular protein pool catabolized per day.

The ceruloplasmin-$^{67}$Cu clearance studies showed that from 1 to 1.9% of the intravascular pool of radioactive ceruloplasmin was lost into the gastrointestinal tract per day. Thus, gastrointestinal loss could account for only 6.7 to 10.6% of the total daily catabolism of $^{67}$Cu-labeled ceruloplasmin. In contrast, the dog that received intraperitoneal ni-

TABLE II

*Ceruloplasmin-$^{131}$I turnover in the dog*

| Dog | % of body ceruloplasmin in intravascular pool | Ceruloplasmin survival $t_\frac{1}{2}$ | $K_{2,1}$ |
|-----|-----|-----|-----|
| | | *days* | |
| 5 | 74 | 3.6 | 0.26 |
| 6 | 74 | 4.6 | 0.18 |
| 7 | 88 | 3.0 | 0.27 |
| 8 | 83 | 3.0 | 0.30 |
| 9 | 71 | 4.9 | 0.16 |
| 10 | 61 | 3.7 | 0.34 |
| Mean | 75 | 3.8 | 0.25 |

trogen mustard had a survival $t_\frac{1}{2}$ that was markedly reduced to 1.5 days, with over 30% of the plasma pool of ceruloplasmin lost into the gastrointestinal tract per day.

Only minimal quantities of radioactivity appeared in the urine throughout the period of study in the dog. From 0.01 to 0.3% of the circulating radioactivity appeared in the urine per day, with a mean of 0.13% of the circulating radioactivity pool appearing in the urine for a 24-hour period.

*Metabolism of ceruloplasmin-$^{67}$Cu in control human subjects.* The data on the metabolism of intravenously administered $^{67}$copper-labeled ceruloplasmin in the four control subjects and the patients with intestinal lymphangiectasia are summarized in Tables III and IV and Figure 3. Approximately 70% of the body pool of ceruloplasmin was within the intravascular space in the control subjects. These subjects had a mean total circu-

TABLE III

*Ceruloplasmin distribution and pool size**

| Patient | Ceruloplasmin concentration | Plasma volume | $K_{3,1}$ | $K_{1,3}$ | % of body pool intravascular | Total circulating ceruloplasmin | Total body ceruloplasmin |
|-----|-----|-----|-----|-----|-----|-----|-----|
| | *mg/100 ml* | *ml/kg* | | | | *mg/kg* | |
| *Control* | | | | | | | |
| W.J. | 45.3 | 44 | 0.37 | 0.86 | 70 | 19.9 | 28.4 |
| J.C. | 34.0 | 33 | 0.41 | 0.67 | 67 | 11.2 | 16.8 |
| A.S. | 44.4 | 47 | 0.36 | 0.87 | 71 | 20.9 | 29.8 |
| B.J. | 33.7 | 30 | 0.36 | 0.79 | 69 | 10.1 | 14.7 |
| Mean | 39.4 | 38 | 0.38 | 0.80 | 69 | 15.5 | 22.4 |
| *Intestinal lymphangiectasia* | | | | | | | |
| L.H. | 26.5 | 36 | 0.68 | 1.62 | 70 | 9.54 | 13.6 |
| J.T. | 25.7 | 42.4 | 0.46 | 1.00 | 69 | 10.9 | 15.7 |
| N.B. | 15.9 | 43.7 | 0.14 | 0.97 | 87 | 6.95 | 8.0 |
| I.S. | 21.9 | 35.9 | 0.28 | 0.87 | 76 | 7.86 | 10.4 |
| Mean | 22.5 | 40 | 0.39 | 1.11 | 76 | 8.8 | 11.9 |

* Abbreviations: $K_{3,1}$ = fraction of iv (intravascular) pool transferred to ev (extravascular) pool per day; $K_{1,3}$ = fraction of ev pool transferred to iv pool per day.

TABLE IV

*Rates of ceruloplasmin catabolism and gastrointestinal clearance**

| Patient | $^{67}$Cu-labeled ceruloplasmin survival t½ | $K_{2,1}$ | Fraction of iv pool catabolized per day with ceruloplasmin-$^{125}$I | Ceruloplasmin turnover (synthesis) | $K_s$ | $K_{d,s}$ | $\frac{K_s}{K_{2,1}} \times 100$ |
|---|---|---|---|---|---|---|---|
| | *days* | | | *mg/kg/day* | | | |
| *Control* | | | | | | | |
| W.J. | 6.5 | 0.16 | 0.21 | 3.18 | 0.019 | 0.0006 | 11.9 |
| J.C. | 6.6 | 0.18 | 0.21 | 2.02 | 0.030 | 0.013 | 16.7 |
| A.S. | 5.8 | 0.18 | 0.22 | 3.76 | 0.039 | 0.040 | 21.7 |
| B.J. | 5.5 | 0.19 | 0.22 | 1.92 | 0.028 | 0.011 | 14.7 |
| Mean | 6.1 | 0.18 | 0.21 | 2.72 | 0.029 | 0.016 | 16.3 |
| *Intestinal lymphangiectasia* | | | | | | | |
| L.H. | 3.3 | 0.31 | 0.45 | 2.96 | 0.29 | 0.25 | 93.5 |
| J.T. | 3.2 | 0.33 | 0.31 | 3.60 | 0.18 | 0.16 | 54.5 |
| N.B. | 2.3 | 0.40 | 0.47 | 2.78 | 0.40 | 0.32 | 100.0 |
| I.S. | 3.6 | 0.26 | 0.38 | 2.04 | 0.15 | 0.14 | 57.8 |
| Mean | 3.1 | 0.33 | 0.40 | 2.84 | 0.26 | 0.22 | 76.5 |

* Abbreviations: $K_{2,1}$ = fraction of iv pool catabolized per day using $^{67}$Cu; $K_s$ = fecal clearance of $^{67}$Cu as fraction of iv pool; $K_{d,s}$ = fraction of circulating ceruloplasmin-$^{67}$Cu lost into bowel per day by multicompartmental analysis; $(K_s/K_{2,1}) \times 100$ = per cent of catabolism due to intestinal loss.

lating ceruloplasmin of 15.5 mg per kg and a total body ceruloplasmin pool of 22.4 mg per kg. The mean ceruloplasmin half-time was 6.1 days, with 17.8% of the intravascular pool of ceruloplasmin catabolized per day. With ceruloplasmin-$^{125}$I, the estimates of fractional catabolic rate were slightly



FIG. 3. TURNOVER OF INTRAVENOUSLY ADMINISTERED CERULOPLASMIN-$^{67}$CU IN MAN. The radioactivity remaining in the plasma and the fecal clearance of $^{67}$Cu for a control subject (W.J.) and a patient with intestinal lymphangiectasia (L.H.) are shown. The patient has a shorter ceruloplasmin-$^{67}$Cu survival and a markedly increased fecal clearance of $^{67}$Cu from the plasma.

higher than with $^{67}$copper-labeled ceruloplasmin. The control subjects synthesized a mean of 2.7 mg of ceruloplasmin per kg per day. The fraction of the plasma pool of ceruloplasmin lost into the gastrointestinal tract per day ($K_s$) estimated from the clearance method ranged from 1.9 to 3.9% of the intravascular pool per day. Thus, as a maximum, only 11 to 22% of the total catabolism of ceruloplasmin could be accounted for by gastrointestinal protein loss. The mean fecal clearance of $^{67}$copper-labeled ceruloplasmin estimated from the multicompartmental analysis was 1.6% of the intravascular pool per day; this is less than 10% of the mean catabolic rate of 18% and confirms that gastrointestinal protein loss normally represents only a minor factor in ceruloplasmin metabolism.

*Ceruloplasmin metabolism in patients with intestinal lymphangiectasia and gastrointestinal protein loss.* The mean ceruloplasmin concentration of 14 patients with excessive gastrointestinal protein loss was 16.4 ± 5 mg per 100 ml of serum, compared to 30.7 ± 3.5 in 185 normal control subjects (p < 0.01). The plasma volume, rates of transfer of ceruloplasmin between the intra- and extravascular pools ($K_{1,3}$, $K_{3,1}$), and the fraction of the body pool of ceruloplasmin in the intravascular compartment were comparable in patients with intestinal lymphangiectasia and control subjects. In patients with intestinal lymphangiectasia, the total circulating ceruloplasmin and total body ceru-
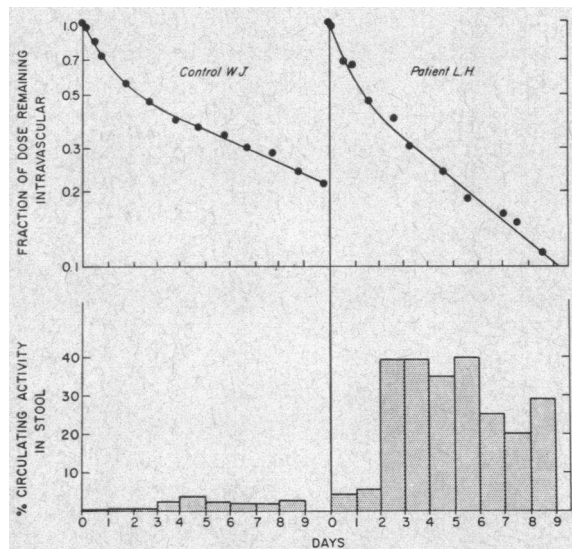
loplasmin pools were significantly reduced to 8.8 and 11.9 mg per kg, respectively (Table III). The mean half-time of survival of ⁶⁷copper-labeled ceruloplasmin was reduced to 3.1 days, one-half that seen in the control subjects, and the fraction of the intravascular pool of ceruloplasmin-⁶⁷Cu catabolized per day was increased to 32.4% (Table IV, Figure 3). The rate of ceruloplasmin synthesis was 2.8 mg per kg per day, quite comparable to the 2.7 seen in the control subjects. The patients with intestinal lymphangiectasia had a markedly increased rate of loss of ceruloplasmin into the gastrointestinal tract ($K_8$), with 25.5% of the intravascular pool cleared per day compared to 2.9 in control subjects. Thus, in the patients with intestinal lymphangiectasia, approximately 75% of the catabolism of ceruloplasmin was due to intestinal loss of ceruloplasmin, and in contrast to normal subjects this loss represents a major factor in the metabolism of this protein.

## Discussion

In the present study ⁶⁷copper-labeled ceruloplasmin was used to study the turnover of ceruloplasmin in dogs, control human subjects, and patients with excessive gastrointestinal protein loss and was evaluated as a technique for the quantitation of loss of protein into the gastrointestinal tract. The studies reported in this paper could not have been performed with the commonly available ⁶⁴copper because of the short physical half-life (12.8 hours) of this isotope. Availability of the longer lived ⁶⁷copper (half-life, 61.8 hours) enabled us to obtain meaningful measurements of the radioactivity of the serum and stools for sufficiently long periods of observation.

Previous studies in human subjects showed that ceruloplasmin labeled *in vitro* with ⁶⁷copper was a satisfactory material for the study of the metabolism and distribution of this protein because no exchange of the incorporated copper occurred during the life-span of the molecule *in vivo,* and thus the radiocopper served as a lifelong label of the protein molecule (18). This conclusion was based on results of studies in two aceruloplasminemic patients with Wilson's disease in whom the rates of disappearance of ⁶⁷copper and of the oxidase activity of the serum were similar after infusion of 1.3 g of ceruloplasmin-⁶⁷Cu. The half-lives observed in these two patients and in a third normal

subject who was infused with ceruloplasmin labeled with ⁶⁷copper *in vivo* ranged from 4.16 to 5.4 days (18). These values were quite comparable to the results using ¹³¹I-labeled ceruloplasmin in patients with chronic diseases unrelated to Wilson's disease (27).

In the present study, the behavior of the ⁶⁷copper-labeled ceruloplasmin and that of the iodine-labeled ceruloplasmin were comparable, although the half-lives obtained with the copper-labeled ceruloplasmin were slightly longer than those obtained with the iodinated ceruloplasmin. This discrepancy might represent a short survival of the iodinated ceruloplasmin due to the iodination procedure. Another possible explanation for this difference is reincorporation of ⁶⁷copper released from catabolized ceruloplasmin into newly synthesized ceruloplasmin molecules. Such reincorporation of the radiocopper was minimized in the present studies in man by diluting the liver copper pool with unlabeled copper salts during the experimental period.

In the present studies it was shown that the copper moiety of ceruloplasmin is not very actively absorbed from or secreted into the gastrointestinal tract, in contrast to radioiodine. Although bile is a major route of excretion of nonceruloplasmin copper, it is known from other studies (25), as well as the present studies in normal subjects, that only a small fraction of the daily turnover of ceruloplasmin-copper is excreted directly into the bile. Thus, ⁶⁷copper-labeled ceruloplasmin is the only labeled macromolecule proposed so far that fulfills the three major requirements for adequate quantitation of transfer of serum protein into the gastrointestinal tract. Although it appears to have significant advantages over the other techniques for quantitating gastrointestinal protein loss, the short physical half-life of ⁶⁷copper (61.8 hours) and the difficulty in its production preclude its widespread use in clinical studies where all that is required is the semiquantitative detection of excessive gastrointestinal protein loss. Copper-labeled ceruloplasmin appears to have considerable promise, however, in the study of the role of the gastrointestinal tract in the metabolism of serum proteins where other techniques give conflicting results.

*Metabolism of ceruloplasmin-⁶⁷Cu in normal subjects.* Ceruloplasmin was shown to be confined

largely to the intravascular compartment with approximately 70 to 80% of the body pool of this protein within the intravascular space. Albumin with a molecular weight of 68,000 and gamma globulin (IgG) with a molecular weight of 160,000 have a much lower percentage (45%) of their body pool within the intravascular compartment than does ceruloplasmin with a molecular weight of 160,000. The space of distribution of ceruloplasmin is comparable to that seen previously in studies with fibrinogen-[131]I (28) and gamma macroglobulin (29). The total body ceruloplasmin pool of 22 mg per kg seen in normal subjects in the present study is quite small compared to more than 4,000 for albumin (30), 1,200 for IgG (31), and 190 for fibrinogen (28). The rate of ceruloplasmin synthesis was also markedly less than that observed for these other proteins with a synthetic rate of less than 2% that of albumin and less than 10% that of IgG and fibrinogen. The 6.1-day half-time survival of ceruloplasmin seen in the control subjects in the present study is comparable to that observed previously in patients with Wilson's disease (18) and patients with chronic diseases unrelated to Wilson's disease (27). The survival half-time of ceruloplasmin in the dog of 4.5 days is slightly less than that seen in man (25) but considerably longer than 56 hours for rabbit ceruloplasmin in rabbits (25) and 18 to 22 hours for human or mouse ceruloplasmin in the mouse (32).

*Role of the normal gastrointestinal tract in ceruloplasmin metabolism.* Although there is general agreement that serum proteins can be demonstrated in normal gastrointestinal secretions (33–35), the techniques now available have given conflicting estimates of the quantitative significance of this gastrointestinal loss in the over-all metabolism of serum proteins. A number of workers (14–16) have approached this problem by administering ^{131}I-labeled albumin or gamma globulin intravenously and determining the quantity of protein-bound radioiodine appearing in the lumina of isolated segments of the gastrointestinal tract. These authors have concluded that loss of albumin and gamma globulin into the gastrointestinal tract may account for 40 to 100% of the over-all catabolism of these proteins (14–16). However, other studies suggest that the gastrointestinal tract plays a less significant role in the catabolism of serum

proteins. Removal of the subdiaphragmatic gastrointestinal tract has resulted in only a 0 to 10% prolongation of the survival of iodinated albumin (36–38). [51]Chromium-labeled albumin has been used to quantitate gastrointestinal protein loss in normal human subjects by relating the daily fecal excretion of chromium to the serum radioactivity curve in a manner comparable to that used for copper-labeled ceruloplasmin in the present study (11). The results of these studies also suggest that less than 10% of the catabolism of the albumin could be accounted for by enteric protein loss.

The present study using [67]copper-labeled ceruloplasmin in dogs and control human subjects is in accord with the data using [51]chromium-labeled albumin in man which suggest that less than 20% of the daily turnover of ceruloplasmin is accounted for by gastrointestinal loss of the protein. The conclusions of the present study may not necessarily be extended to serum proteins other than ceruloplasmin, since gastrointestinal excretion of these proteins may not parallel that of ceruloplasmin. However, it can be stated that bulk loss of serum or of materials with comparable protein compositions into the gastrointestinal tract is of only minor significance in the protein metabolism of the normal dog and man.

*Metabolism of ceruloplasmin in patients with intestinal lymphangiectasia.* The serum ceruloplasmin concentration in patients with intestinal lymphangiectasia was reduced below the lower limits of normal. Such reduction may be the result of either diminished synthesis or of increased loss of the protein. Diminished synthesis on a genetic basis accounts for the deficiency that characterizes patients with Wilson's disease (hepatolenticular degeneration) (39). In neonates a diminished rate of synthesis is physiological up to 6 months of age (40). Acquired hypoceruloplasminemia, probably secondary to diminished synthesis, was observed in rare instances of marked hepatic insufficiency (41, 42), profound malnutrition (43), and severe intestinal malabsorption (44). In contrast, abnormal losses of ceruloplasmin occur in patients with nephrotic syndrome (40).

In this study patients with intestinal lymphangiectasia were shown to have hypoceruloplasminemia as a result of excessive loss of the copper protein into the bowel. They excreted ceruloplasmin at approximately ten times the rates measured in

control subjects. The shortened survival of ceruloplasmin as well as that of albumin, fibrinogen, and immunoglobulins G, M, and A in the same patients suggested a generalized enteric loss of all serum proteins. Thus, although gastrointestinal excretion plays only a minor role in ceruloplasmin catabolism in normal subjects, bulk fecal loss of ceruloplasmin, as well as of other serum proteins, is the major factor producing the deficiency of ceruloplasmin in patients with intestinal lymphangiectasia.

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