GASTROINTESTINAL ABSORPTION OF COPPER: STUDIES WITH ^{64}Cu, ^{95}Zr, A WHOLE-BODY COUNTER AND THE SCINTILLATION CAMERA

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Copper has been recognized for many years as an essential nutrient (*1*). If it is assumed that the normal adult is in copper balance (i.e., that his total quantity of body copper does not increase or decrease significantly with time), then it is reasonable to infer that the amount of copper absorbed by him must equal the amount excreted (*2*). Urinary excretion of this metal is usually negligible—10–60 μg/day (*2*). Maintenance of an average total-body copper content of 100 mg (*3–5*) must therefore represent a balance between absorption and excretion in the gastrointestinal tract.

Metabolic balance studies have been greatly simplified by using a whole-body counter capable of detecting small amounts of gamma-emitting nuclides *in vivo*. Nevertheless, variations in data resulting from urinary excretion of a small fraction of absorbed copper and from elimination in the stool of a larger fraction of orally absorbed copper together with the limitation placed upon observations of whole body and excreta by the short physical half-life of ^{64}Cu (12.8 hr) have made necessary the development of a fairly complex model for the present inquiry. Comparisons are reported of copper absorption and gastrointestinal excretion in seven normal subjects, in two patients with Wilson's disease (*6*) and in one patient with an undiagnosed disorder of copper metabolism.

SUBJECTS AND METHODS

Subjects. Ten subjects were studied. The seven controls were asymptomatic volunteers in whom no abnormality believed to affect copper metabolism was found. They were patients who had been admitted to the hospital for treatment of conditions unrelated to the subject of this inquiry and without cirrhosis or abnormalities of liver function. Of the three patients with disease pertinent to this inquiry, two had Wilson's disease and were receiving penicillamine. The third, a 16-year-old male, had an undiagnosed abnormality of copper metabolism that was not Wilson's disease.

Throughout the investigation, all of the 10 subjects were maintained on a constant metabolic diet in the Clinical Study Center of the San Francisco General Hospital. One complete absorption study was performed in each of five control subjects; three studies were performed in one control and four studies in one. One complete study was performed in each of the three patients.

Method. To each fasting subject ^{95}Zr, as carrier-free zirconium oxalate, was given orally in doses varying from 0.5 to 10.0 μCi. This was followed immediately by the oral administration of 100–500 μCi of ^{64}Cu as copper acetate with specific activity of 0.3–1.3 μCi/μg copper.

Counting was performed in a low-background whole-body counter with 2-ft-thick concrete walls lined interiorly with ⅜-in. lead. An 11.9 × 4-in. NaI(Tl) crystal was placed at the center of the arc formed by a counting couch with a 1-meter radius of curvature (1-meter arc geometry). The output of the crystal was analyzed by a 400-channel analyzer (RIDL Model 34-12 B). The ^{64}Cu and ^{95}Zr were counted in 400–600-keV and 650–850-keV windows, respectively. Appropriate corrections were made for Compton events of zirconium appearing in the copper window.

In each subject, counting was performed daily for 4 days beginning with the administration of the oral dose; this is the maximum possible counting time in view of the 12.8-hr half-life of ^{64}Cu. The bolus of orally administered radioactive copper was followed through the gastrointestinal tract by scintiphotographs obtained sequentially with an Anger scintillation camera (Nuclear-Chicago). All urine and stools were collected and counted daily in the whole-body counter under appropriate geometric control, by calculations based on comparison with appropriate standards similarly counted.

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One week after administration of the oral dose, 0.5–10 μCi of ^{95}Zr oxalate was given again to each fasting subject, this time followed immediately by the intravenous administration of 500 μCi of ^{64}Cu which had been incubated with 10–20 ml of the subject's plasma for 30 min at 37°C to insure protein binding of the copper. Thereafter, each of the 10 subjects was again counted in the whole-body counter, daily for 4 days. All urine and stools were again collected and counted daily and related to appropriate standards.

The net dose (oral or intravenous) was determined by applying to the administered dose the ratio obtained by relating the whole-body counting rate of an intravenous dose of ^{64}Cu to the counting rate of the syringe counted on the 1-meter arc, corrected for counts remaining in the syringe after injection. In preparatory studies we had determined this ratio at frequent intervals up to the first 4 hr and had found it to be most constant immediately after injection (0.73 ± 0.035 in 12 normal subjects). Since at this time hepatic extraction has not yet begun, all of the radiocopper must be within the vascular space so that this factor must represent self-absorption by the subject. Plasma radioactivity was measured in a standard well gamma-ray counter using a 400–600-keV window.

The model and the calculation method for net copper absorption are shown schematically in Fig. 1. Copper absorption, A, is equal to the ratio of the net retention of radiocopper in the whole body after oral administration of copper, R_o, to the net retention of radiocopper in the whole body after intravenous administration of copper, $1\text{-}E_{iv}$, where E_{iv} is the fractional excretion of radiocopper into the gastrointestinal tract after intravenous administration:

$$A = \frac{R_o}{1\text{-}E_{iv}}.$$

Excretory data for both R_o and E_{iv} are incomplete because of the short half-life of ^{64}Cu. Both were corrected for this incompleteness on the basis of the data obtained from the zirconium which had been administered orally as a nonabsorbed stool marker (7) as follows:

$$R = 1 - \frac{D_o\text{-}Ru_o}{S_oD_o} \text{ and } E = \frac{D_{iv}\text{-}Ru_{iv}}{S_{iv}D_{iv}} \text{ (See Appendix).}$$

D_o and D_{iv} are the net oral and intravenous dose, respectively; Ru_o and Ru_{iv} represent the copper retained in the whole body, uncorrected for stool recovery; S_o and S_{iv} are the fractions of zirconium recovered in stool during the oral and intravenous phase of the study, respectively. Both Ru_o and Ru_{iv} are corrected for counts recovered in the urine during the course of the study although this fraction was trivial except in subjects receiving penicillamine. This calculation presumes that ^{95}Zr is excreted at the same rate as unabsorbed gastrointestinally excreted ^{64}Cu. This presumption has been validated experimentally when more than 25% of zirconium oxalate was recovered in stool.

This technique is valid only if three additional assumptions are correct: (1) That copper absorption is equal to net copper retention plus the fraction excreted after absorption. This is self-evident from the definition of absorption. (2) That the excreted fraction of the absorbed oral fraction is equal to the fraction excreted after the intravenously administered dose. Studies related to the validity of this assumption have been in progress in our laboratory for some time, and present data suggest that it is correct. Externally placed probes and whole-body scanning techniques have shown that approximately the same fraction of orally absorbed and intravenously injected copper localize in the liver. Further support is lent to this assumption by the similarity of plasma radioactivity curves after oral and intravenous copper administration, and we are now collecting data on the labeled copper content of bile which we hope will be definitive in this regard (such studies have been made feasible only within the past year by the availability of ^{67}Cu). Our results suggest then that the hepatoenteric metabolism of copper differs qualitatively from that of other metals such as iron. (3) That the fractional excretion after intravenous injection is constant from week to week. This as-

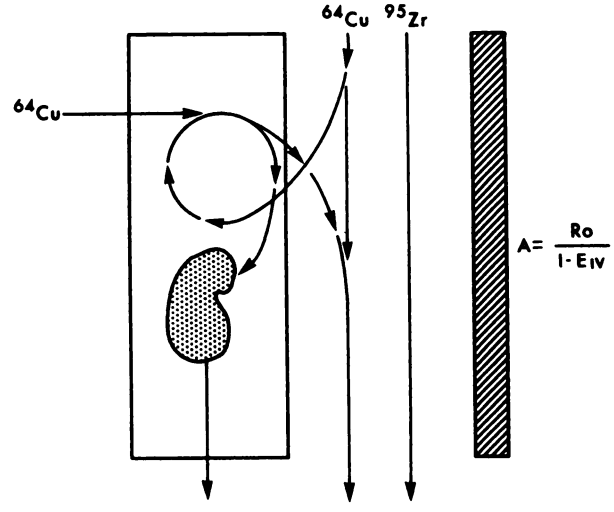

FIG. 1. Schematic model for calculation of net copper absorption (A). R_o is net retention of radiocopper after oral administration and E_{iv} is fractional excretion of radiocopper into gastrointestinal tract after intravenous administration.

TABLE 1. SUMMARY OF COPPER ABSORPTION AND GASTROINTESTINAL EXCRETION

Subject No.	Age (yr)	Sex	Fraction of oral dose absorbed (%)	Fraction excreted after intravenous administration (%)
Controls:				
1	19	M	75	23
2	29	M	35	0
3	43	M	75	19
			37	
			86	
4	21	F	43	20
5	28	M	71	< 1
6	60	M	63	14
7	66	M	15	1
			87	14
			97	
			33	
		Mean	59.8	11.5
Patients				
8*	19	M	87	14
9*	41	F	100	19
10†	16	M	74	24

* Wilson's disease; taking penicillamine.
† Undiagnosed abnormality of copper metabolism; not taking penicillamine.

TABLE 2. CARRIER COPPER ADMINISTERED, RELATED TO FRACTION OF RADIOACTIVE COPPER ABSORBED, IN SEVEN NORMAL SUBJECTS GROUPED BY CARRIER DOSE

Dose of carrier Cu administered (mg)	Subjects (No)	Fraction of ^{64}Cu absorbed Mean (%)	Range (%)
<0.1	3	66	37–87
0.1–0.9	7	58	15–86
0.9–1.6	3	49	35–63
5.1	1	33	—
10.1	1	97	—

sumption is relevant because the studies are performed sequentially. This variable was controlled as well as possible by maintaining the patients in the clinical study center on metabolic diets.

RESULTS

Net absorption of orally administered radiocopper and net excretion of the intravenously administered nuclide are shown in Table 1. In the normal subjects, net absorption of the orally administered dose varied from 15 to 97% with a mean of approximately 60%. The fraction excreted after intravenous administration varied through a much narrower range—0–23% with a mean of 11.5%. This wide variation in the fraction absorbed by control subjects suggests that the normal range is very broad and that absorption of copper, unlike that of other elements such as iron, is not limited. In the two subjects with Wilson's disease who were receiving penicillamine, almost all orally administered radiocopper was absorbed while gastrointestinal excretion of the intravenously administered dose was within the normal range.

The amount of carrier copper given with the oral dose, related to the fractional absorption of radiocopper during the oral phase, is shown for normal subjects in Table 2. The quantities of carrier given varied over a 1,000-fold range to span the range of copper generally presented to the gastrointestinal tract at one time (estimated to be 1.5–5.0 mg of copper per meal). Five groups are defined according to the quantity of carrier administered. While the average absorption values suggest that the fraction of radiocopper absorbed decreased as the quantity of carrier increased, the ranges of absorption are extremely wide within each group and they overlap markedly. In addition, the subject with the highest percentage of absorption was the one who had been given the largest amount of carrier; when this subject was studied by varying the amounts of carrier presented with the oral dose, fractional absorptions showed no direct relationship to those amounts.

When all data were plotted with fractional absorption on the x-axis and amount of carrier copper

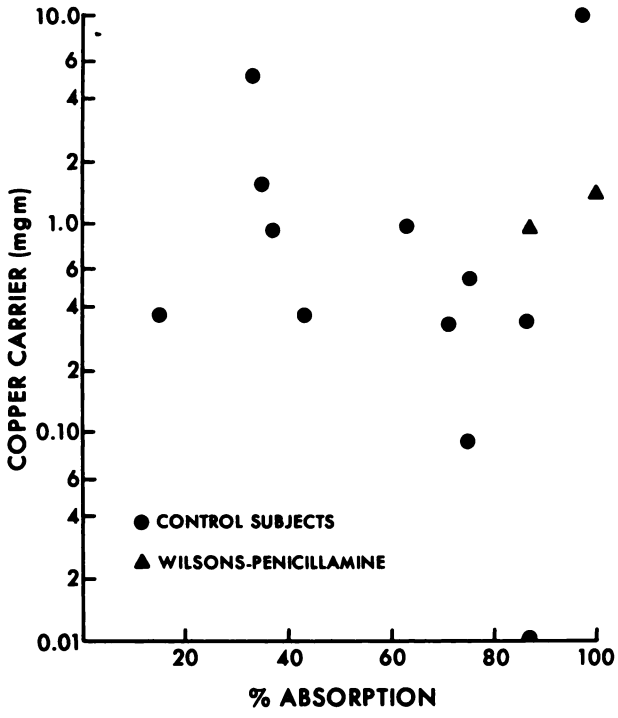

FIG. 2. Relationship between copper absorption and carrier copper, latter on a logarithmic scale.

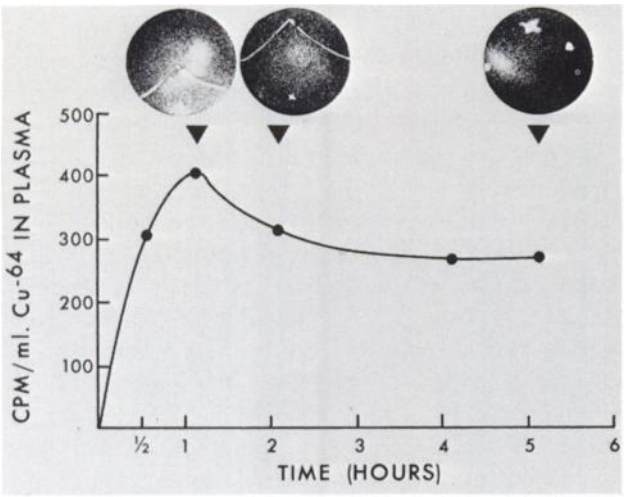

FIG. 3. Plasma copper radioactivity related to time after oral administration. Scintiphotographs of abdomen at 1, 2 and 5 hr are superimposed on curve and show activity in stomach and duodenum when adsorption is maximal.

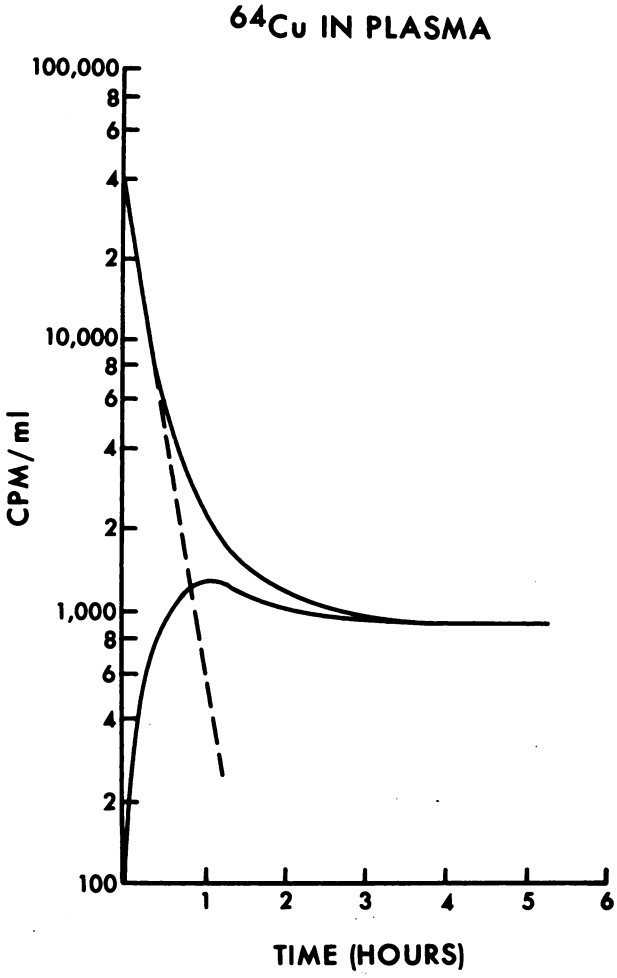

FIG. 4. Relationship between plasma radioactivity curves following intravenous and oral administration of radiocopper with oral curve normalized as to time relative to the intravenous data.

along a logarithmic scale on the y-axis (Fig. 2), no significant relationship between these two variables emerged.

Plasma copper radioactivity is plotted against time after oral administration of radioactive copper in Fig. 3. Absorption was maximal in the first hour but continued for several hours. Scintiphotographs of the abdomen show that at 1 hr, the time of maximal absorption, the radiocopper was in the stomach and duodenum; at 2 hr, in the small intestine. At 5 hr, when the bolus of radioactivity was largely in the ileocecal region and first portion of the large bowel, absorption was probably completed.

Figure 4 relates the plasma radioactivity curves after intravenous administration to those after oral administration of radiocopper, the oral curve being normalized with respect to the intravenous data as to time. During plasma clearance of the intravenously administered isotope, feedback to plasma began at about 25 min, too early to permit determination of the total length of the period of absorption of the oral dose; however, the plateau reached by the oral curve about an hour before there is a plateau of the intravenous curve indicates that some of the orally administered copper was still being absorbed, up to 3½ hr after it had been received.

Data before and after administration of penicillamine were available in three control subjects. The effect of penicillamine on their gastrointestinal excretion of intravenously administered copper and on their net absorption of orally administered copper is shown in Table 3. In Subjects 3 and 6 gastrointestinal excretion diminished after the administration of penicillamine. In Subject 5 gastrointestinal excretion was insignificant initially and did not change in response to penicillamine. The percent of copper absorbed after penicillamine is available only for Subjects 5 and 6; in both, there was a modest decrease from pre-penicillamine values. The primary effect of penicillamine was a marked increase in the urinary excretion of copper.

TABLE 3. EFFECT OF PENICILLAMINE ON ABSORPTION AND GASTROINTESTINAL EXCRETION OF ^{64}Cu IN 3 NORMAL SUBJECTS

Subject (No)	Fraction of ^{64}Cu absorbed		Fraction of ^{64}Cu excreted in stool	
	With penicillamine (%)	Without penicillamine (%)	With penicillamine (%)	Without penicillamine (%)
3	75 86 37		19	10
5	71	53	<1	1
6	63	49	14	4

DISCUSSION

The availability of only a single radioactive isotope of copper (^{64}Cu) has precluded studies of the metabolism of this metal by methods such as the double-isotope technique used by Fawwaz and coworkers (*8*) in investigating the disposition of iron. With the very recent availability of the nuclide ^{67}Cu, we are beginning to explore the application of multiple-isotope procedures to the physiologic handling of copper. The studies with ^{64}Cu (half-life 12.8 hr) reported here were designed to offset the limitations of the single isotope in providing new information on gastrointestinal absorption and excretion in man.

Our findings are somewhat at variance with some of the admittedly limited data from studies in man reported by others. Bearn and Kunkel (*9*) reported up to 33% stool recovery of intravenously administered ^{64}Cu, figures higher than any found by us. Bush and coworkers (*10*) found an average of 12.4% of intravenously administered ^{64}Cu in the stool of normal subjects, the highest value being 16.1%—close to our normal values. Bearn and Kunkel (*9*) reported recovery of 26% of orally administered ^{64}Cu in the stool over 4 days; while Bush and his group (*10*) recovered up to 95% of an oral dose in the stool over a similar period.

The wide variations in net absorption in our normal subjects was most surprising. While at variance with the findings of the investigators just cited, they are not inconsistent with the broad discrepancy between the results reported separately by them. These observations suggest that the control mechanism regulating copper absorption is much more labile than that regulating the absorption of, for example, iron. Such lability might be expected, as requisite to homeostasis of the small total-body pool of copper (in the 100-mg range), in contrast to the total-body pool of iron (probably close to 4,000 mg). Since the fractional excretion of copper varies within relatively narrow limits, the findings suggest that variation in absorption is a primary control mechanism.

The other possible interpretation of these data is that the subjects were not in a steady state even though they were hospitalized in a metabolic ward. Variations in copper absorption noted in repeated studies of the same subjects suggest that this might be the case. Perhaps even a metabolically controlled diet contains elements or compounds that can limit or enhance copper absorption by such mechanisms as copper binding or even by competition for absorption sites.

The high absorption levels in our normal subjects do not imply positive copper balance, for once radiocopper is absorbed, its fractional excretion depends primarily upon pool size and turnover rate. Evaluation of these data requires the longer observation periods now possible with ^{67}Cu. One would expect that over a sufficient interval, equality of copper absorption and excretion should be demonstrable.

The observations reported here do not lend themselves to interpretation relative to mechanisms involved in the positive copper balance known to exist in Wilson's disease (*11–13*), largely because subjects were limited in number and were studied only while under therapeutic control by penicillamine. Patient 10, without Wilson's disease, is unusual. When measured on several occasions by oxidative and spectrophotometric methods, ceruloplasmin was found low; but when measured immunochemically, it was found quantitatively normal. Final diagnosis has not been made; but his copper absorption and excretion data do not differ significantly from those of the control subjects reported.

The three control subjects studied before and after penicillamine excreted less ^{64}Cu into the stool while taking this drug than when not taking it. This is in contrast to our observation in a previous metabolic balance study (*14*) that penicillamine appeared to increase gastrointestinal excretion of copper in patients with Wilson's disease. The normal range of gastrointestinal excretion of intravenously administered radiocopper during penicillamine administration in both of the Wilsonian homozygotes in the present study may be interpreted as support for this finding for it is virtually certain that without penicillamine their gastrointestinal excretion of copper would have been significantly less. Further studies are needed on the effect of penicillamine on the gastrointestinal excretion of copper in Wilson's disease and on the apparent decrease in net copper absorption in response to penicillamine in two normal subjects found here.

CONCLUSION

A technique for measuring copper absorption has been described in which ^{64}Cu is administered twice. The first dose is given orally. One week later, the second dose is administered intravenously. Simultaneously with each dose, ^{95}Zr oxalate is given orally as a nonabsorbable stool marker. Data so obtained indicate a wide range of absorption values in normal subjects and absence of a well-defined carrier effect. After intravenous administration of copper, an average of 11.5% is excreted into the gastrointestinal tract (range 0–23%).

In the normal subject, copper absorption is maximal during the first hour while the metal is in the stomach and duodenum; absorption continues for at least 3.5 hr.

Urinary excretion of copper is negligible except during the administration of penicillamine.

In a limited number of control subjects, penicillamine tended to decrease both net absorption (or retention) of orally administered copper and fractional gastrointestinal excretion.

Two patients with Wilson's disease, while taking penicillamine, showed nearly maximal absorption but "normal" gastrointestinal excretion of copper.

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REFERENCES

1. ELVEHJEM, C. A.: The biological significance of copper and its relation to iron metabolism. *Physiol. Rev.* **15**: 471, 1935.

2. CARTWRIGHT, G. E. AND WINTROBE, M. M.: Copper metabolism in normal subjects. *Am. J. Clin. Nutr.* **14**:224, 1964.

3. STRAIN, W. H.: *AAAS Symposium on Geochemical Evolution.* Table I. Denver, 1961.

4. CHOU, TUNG-PI AND ADOLPH, W. H.: Copper metabolism in man. *Biochem. J.* **29**:476, 1935.

5. GUBLER, C. J.: Copper metabolism in man: report to Council on Foods and Nutrition. *J. Am. Med. Assoc.* **161**: 530, 1956.

6. O'REILLY, S.: Problems in Wilson's disease. *Neurology* **17**:137, 1967.

7. MACDOUGALL, L. D.: Estimation of fat absorption from random stool specimens: measurement by zirconium-95 and iodine-131. *Am. J. Diseases Children* **108**:139, 1964.

8. FAWWAZ, R. A., WINCHELL, H. S., POLLYCOVE, M., SARGENT, T., ANGER, H. AND LAWRENCE, J. H.: Intestinal iron absorption studies using iron-52 and Anger positron camera. *J. Nucl. Med.* **7**:569, 1966.

9. BEARN, A. G. AND KUNKEL, H. G.: Metabolic studies in Wilson's disease using Cu-64. *J. Lab. Clin. Med.* **45**:832, 1955.

10. BUSH, J. A., MAHONEY, J. P., MARKOWITZ, H., GUBLER, C. J., CARTWRIGHT, G. E. AND WINTROBE, M. M.: Studies on copper metabolism. XVI. Radioactive copper studies in normal subjects and in patients with hepatolenticular degeneration. *J. Clin. Invest.* **34**:1,766, 1955.

11. BICKEL, H., NEALE, F. C. AND HALL, G.: A clinical and biochemical study of hepato-lenticular degeneration (Wilson's disease). *Quart. J. Med.* **26**:527, 1957.

12. SUNDERMAN, F. W., JR., WHITE, J. C. AND SUNDERMAN, F. W.: Metabolic balance studies in hepatolenticular degeneration located with diethyldithiocarbamate. *Am. J. Med.* **34**:875, 1963.

13. GOLDSTEIN, N. P., RANDALL, R. V., GROSS, J. B. AND MCGUCKIN, W. F.: Copper balance studies in Wilson's disease: observations on the effect of penicillamine, carbacrylamine resins, and potassium sulfide. *Arch. Neurol.* **12**:456, 1965.

14. O'REILLY, S. AND BANK, W.: Treatment of Wilson's disease—a new oral chelating agent. *Nature* **212**:1,597, 1966.

APPENDIX

D_o, D_{iv} — Net oral and intravenous dose, respectively, in cpm, obtained by multiplying net counts injected by 0.73 as described.

Ru_o, Ru_{iv} — Radiocopper retained in the whole body in cpm uncorrected for completeness of stool recovery after oral and intravenous dose, respectively. Data obtained either from whole-body counter or from stools.

S_o, S_{iv} — Fractions of zirconium recovered in stool during the oral and intravenous phase of the study, respectively.

R_o — Fractional net retention of ^{64}Cu in the whole body after orally administered copper corrected for completeness of stool recovery.

$$R_o = 1 - \frac{D_o\text{-}Ru_o}{S_o/D_o} = 1 - \frac{D_o\text{-}Ru_o}{S_oD_o}$$

E_{iv} — Fractional excretion of ^{64}Cu in stool after i.v. administration of copper corrected for completeness of stool recovery.

$$E_{iv} = \frac{D_{iv}\text{-}Ru_{iv}}{S_{iv}/D_{iv}} = \frac{D_{iv}\text{-}Ru_{iv}}{S_{iv}D_{iv}}$$

A — Net fractional absorption of ^{64}Cu.

$$A = \frac{R_o}{1\text{-}E_{iv}}$$