ABSORPTION OF COPPER IN HOMOZYGOTES AND HETEROZYGOTES FOR WILSON'S DISEASE AND CONTROLS: ISOTOPE TRACER STUDIES WITH $^{67}$Cu AND $^{64}$Cu

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SUMMARY

1. Absorption of copper was determined by the simultaneous administration of
$^{64}$Cu orally and $^{67}$Cu intravenously to six patients with Wilson's disease (WD),
eighteen of their parents and siblings, four normal subjects and three subjects with
cirrhosis of the liver. Absorption was calculated by three methods: (1) the mean
ratio of $^{64}$Cu to $^{67}$Cu body retention at 3 and 4 days as determined by whole-body
counting; (2) the mean ratio of $^{64}$Cu to $^{67}$Cu at 3 and 4 days as determined by faecal
excretion; and (3) the mean ratio of $^{64}$Cu to $^{67}$Cu plasma radioactivity 6–24 h after
administration.

2. The total-body counting and faecal methods for determining copper absorption
agreed with each other, demonstrating that the normal absorption of copper is
40–70% (mean 56%) of the dose and that absorption is not influenced by cirrhosis
of the liver, age or sex; but it appears to be inversely related to the amount of carrier
copper. The absorption of copper in both homozygotes and heterozygotes for WD
did not differ significantly from that of the control subjects. Therefore, the increased
body burden of copper in WD does not appear to be due to over absorption, but rather
to decreased biliary excretion of copper.

Key words: copper metabolism, Wilson's disease, copper absorption.

Wilson's disease (WD) is a rare malady inherited in an autosomal-recessive manner. The
onset of clinical symptoms is generally in the second or third decade and findings include
neurological abnormalities, cirrhosis of the liver, and renal dysfunction (Bearn, 1966; Schein-
berg & Sternlieb, 1965; Walshe, 1970). Excessive copper deposition in the tissues occurs in
both homozygous and heterozygous individuals with the WD gene (Sternlieb & Scheinberg,
1968) and is associated with the under excretion of copper in the bile (O'Reilly, Weber,
Oswald & Shipley, 1971; Strickland, Beckner, Leu & O'Reilly, 1972). In the present study we report the intestinal absorption of copper in both homozygotes and heterozygotes for WD as compared with normal subjects and subjects with hepatic cirrhosis (HC). The heterozygote with decreased biliary excretion of copper is of particular interest since he should decrease his intestinal absorption of copper to remain in copper balance.

Previous studies of copper absorption, with the exception of a report by Weber, O'Reilly, Pollycove & Shipley (1969), have failed to take into consideration the absorbed and then excreted $^{64}$Cu (Fig. 1) in their subjects (Bush, Mahoney, Markowitz, Gubler, Cartwright & Wintrobe, 1955; Jenson & Kamin, 1957; Matthews, 1954). Hence, these reports concern body retention, not absorption of copper. To ascertain the absorption of radioactive copper we assayed the radioactivity in the subject, his stools and his plasma at intervals after the simultaneous oral administration of $^{64}$Cu and intravenous administration of $^{67}$Cu (Fig. 1).

**METHODS**

**Subjects**

Six patients with WD, seven of their parents, eleven of their siblings, four normal subjects and three subjects with HC have been described (Strickland et al., 1972). The patients with WD had hypocaeruloplasminaemia, hypocupuraemia and hypercupriuria. Results of standard
liver-function tests varied from normal to moderately deranged. Family members and normal subjects had normal liver function, and biochemical tests of copper transport and storage were not compatible with the diagnosis of WD. The HC subjects, confirmed by liver biopsy, had mild abnormalities in liver function tests.

**Method of study**

*Determination of a geometry (g) factor for $^{64}$Cu.* The informed consent of the subjects was obtained (Strickland *et al.*, 1972). The average calculated radiation exposure to $^{64}$Cu in these studies was 0.177 rad to the liver and 0.033 rad to the total body. The calculated average total exposure from both $^{67}$Cu and $^{64}$Cu to the liver was 0.545 rad and to the total body was 0.065 rad. These exposures might be slightly higher in homozygotes and heterozygotes for WD with a high hepatic uptake and decreased excretion of copper (Strickland *et al.*, 1972) and would be less in individuals with normal excretion of copper. One to 6 weeks before the dual-isotope study eighteen of thirty-one subjects and eight others received $^{64}$Cu as copper acetate (National Tsing Hua University reactor, Hsinchu, Taiwan, Republic of China) intravenously at a dose of 0.3–6.0 μCi/kg body weight to determine a $g$ factor [the relationship between counts per minute (c.p.m.) from the subject to c.p.m. from a standard containing the same activity]. Subjects and standards were counted at 4 h and at 1 day in a whole-body counter (WBC) with the same geometry as in the later simultaneous $^{64}$Cu and $^{67}$Cu studies. Thirteen subjects were also counted on day 2. Counts in the energy range 440–3000 keV were recorded. Urine- and stool-collection periods coincided with the time of the subject's counts on days 1 and 2. The method for determining $^{64}$Cu radioactivity in the urine and stool was the same as that used for determining stool $^{67}$Cu radioactivity (Strickland *et al.*, 1972) except that counts were recorded over an energy range of 440–3000 keV. The $g$ factor at the respective counting times was calculated as follows: 

$$g = \frac{(a-b)}{cd(1-e)}$$

where $a =$ c.p.m. of subject; $b =$ c.p.m. of subject before study; $c =$ c.p.m. of standard; $d =$ ratio of weight of dose to subject to weight of dose to standard; $e =$ cumulative dose of $^{64}$Cu excreted before subject's WBC count.

The average $g$ factor was 0.766 ± 0.091 (SD) at 4 h, 0.748 ± 0.078 at 1 day, and 0.800 ± 0.063 at 2 days. The mean $g$ factor by subject diagnosis for all observations was 0.788 ± 0.089 for normal and HC controls, 0.753 ± 0.071 for siblings and parents of WD patients, and 0.768 ± 0.041 in patients with WD. Since there was not a significant difference in the $g$ factor as a function of time or diagnosis, the average $g$ factor for all observations of 0.764 was used in calculating absorption by the WBC method.

$^{67}$Cu and $^{64}$Cu simultaneous study. Carrier-free $^{67}$Cu with a physical half-life ($t_1/2$) of 61.9 h, from Oak Ridge National Laboratory, Oak Ridge, Tennessee, was processed for administration to humans and assayed (Strickland *et al.*, 1972). An intravenous injection of 1–2 μCi of $^{67}$Cu/kg body weight and an oral administration of 1–6 μCi of $^{64}$Cu/kg body weight were given simultaneously to all subjects in a fasting state. The quantity of oral copper ranged from 0.41 to 4.54 mg.

Heparinized venous blood samples were collected at 10, 20, 30, 45 and 60 min, 2, 3, 4, 6, 8 and 24 h, and daily for 10 days. The blood was centrifuged and 3 ml plasma samples pipetted. Samples (3 ml) of a known dilution of the $^{67}$Cu and $^{64}$Cu doses were used for standards. Volumetric pipettes and flasks were used throughout the procedure. The plasma samples and standards were counted to achieve a statistical error of less than 3% relative error with 95%
confidence in a 7.6 cm NaI(t1) well crystal attached to a three-channel gamma-ray spectrometer (model 3375 liquid-scintillation counter with a model 5023 Autogamma Attachment, Packard Instrument Co., Downers Grove, Ill.). Channel 1 ($^{67}$Cu) was set to record counts over an energy range of 25–210 keV, and channel 2 ($^{64}$Cu) recorded all counts above an energy of 440 keV. Channel 1 counts were corrected for the counts contributed by $^{64}$Cu. The radioactivity of each

![Graph](image)

Fig. 2. Example of whole-body counter (WBC) method for calculating copper absorption in a normal control and patient with WD. The biological disappearance of $^{67}$Cu (○) was plotted for both subjects extrapolating back to 100% at zero time. Daily WBC of $^{64}$Cu (●) on both patients for 4 days were plotted as % of dose ingested. A line was then extrapolated from day 4 to zero time using the same slope as the biological $t_1$ of $^{67}$Cu (copper excretion). In both cases this line approximates to the previous point, suggesting that the bolus of unabsorbed $^{64}$Cu has passed from the gut by day 3 (C.Y.O.) or day 2 (S.P.C.). The calculated absorption for C.Y.O. is 55% and for S.P.C. is 64%.

plasma sample was expressed as % dose/litre of plasma for each radioactive isotope. Techniques of stool collection and total-body counting have been described (Strickland et al., 1972). The stools and standards were prepared by the same technique and were counted in a WBC to determine their $^{67}$Cu and $^{64}$Cu radioactivities. The stool excretion results were expressed as % of the respective administered doses.

$^{64}$Cu retention ($r$) by whole-body counting was calculated as follows: $r = (a-b)/cdg$, where $a =$ c.p.m. of subject; $b =$ c.p.m. of subject before study; $c =$ c.p.m. of standard; $d =$ ratio of weight of dose to subject to weight of dose to standard; $g =$ geometry factor for $^{64}$Cu. Retention of $^{64}$Cu was derived from the biological turnover curve calculated with $^{67}$Cu.
Absorption of copper in Wilson's disease

Absorption of $^{64}$Cu measured by whole-body counting was calculated as the mean ratio of $^{64}$Cu body retention to $^{67}$Cu body retention at 3 and 4 days. Fig. 2 graphically illustrates this calculation for the fourth day in two subjects.

Absorption of $^{64}$Cu measured by the faecal method was taken as the mean of the 3- and 4-day absorption values calculated by the formula $a = (1 - b)/(1 - c)$, where $a =$ fraction of dose of $^{64}$Cu absorbed; $b =$ accumulative fraction of dose of $^{64}$Cu in stool; $c =$ accumulative fraction of dose of $^{67}$Cu in stool.

The mean ratio of the % dose of $^{64}$Cu to % of the dose of $^{67}$Cu/litre of plasma at 6–24 h administration was considered to represent $^{64}$Cu absorption measured by the plasma method.

RESULTS

Absorption measured by whole-body counting (WBC method) (Fig. 3)

The mean absorption for the four groups was: normal and HC subjects 57% ± 14% (SD); WD patients 62% ± 17%; parents of WD patients 52% ± 8%; and siblings of WD patients 52% ± 15%. There were no statistically significant differences between absorption in the control group and any of the other groups.

Absorption measured by the faecal method

The results obtained by calculating absorption measured by the faecal method are given in Fig. 4. The mean absorption was: normal and HC subjects 54% ± 23%; WD patients 38% ± 22%; parents 43% ± 18%; and siblings 53% ± 18%. Again, no statistically significant difference was found between absorption for the control group and any of the other groups.
FIG. 4. Mean ± 1 SD and individual values for absorption of copper in the four groups as determined by the faecal method. The symbols are as in Fig. 3.

FIG. 5. Example of plasma method of measuring absorption in an HC control and patient with WD. At zero time $^{67}\text{Cu}$ (○) was injected intravenously and $^{64}\text{Cu}$ (●) was taken orally. Equilibrium was quickly reached and plasma radioactivity of the two isotopes were parallel after 4 h. The average ratio of the $^{64}\text{Cu}/^{67}\text{Cu}$ plasma radioactivity from 6 to 24 h was considered a measure of absorption of $^{64}\text{Cu}$. 
Absorption of copper in Wilson's disease

Absorption measured by the plasma method

This technique demonstrated apparent equilibrium between plasma $^{64}$Cu and $^{67}$Cu concentrations in thirty of thirty-one subjects (Fig. 5). The mean absorption calculated from plasma curves was $37\% \pm 21\%$ for the normal and HC subjects; $53\% \pm 34\%$ for WD patients; $70\% \pm 28\%$ for parents; and $64\% \pm 24\%$ for siblings. Absorption of $^{64}$Cu was increased in siblings and parents of WD patients when compared with normal and HC subjects ($P<0.05$). There was no statistical difference between absorption in the WD patients and the control group.

Correlation of the three methods

Copper absorption as calculated by whole-body counting and by faecal excretion was in fair agreement. In most cases the two methods agreed within $10\%$, with a correlation coefficient of $r = 0.44$. Agreement was diminished in six subjects because of a lower absorption as measured by the faecal method. Copper absorption by the WBC method did not correlate with absorption calculated by the plasma method ($r = 0.01$), nor did the plasma method correlate with the faecal method ($r = 0.17$).

Correlation of copper absorption with quantity of oral copper

The relationship between copper absorption and mg of oral copper showed the following correlation: plasma method, $r = -0.42$, WBC method $r = -0.41$, and faecal method $r = -0.02$.

DISCUSSION

Over absorption of copper has been thought to cause the excessive tissue copper concentration in Wilson's disease (WD) (Bush et al., 1955; Jenson & Kamin, 1957; Matthews, 1954). Since those investigators did not separate absorption from retention of copper, and radioactive copper is rapidly absorbed from and excreted into the gastrointestinal tract, the over absorption they noted could be due to decreased biliary excretion in subjects with normal absorption (Fig. 1). Bearn & Kunkel (1955) proposed this explanation for their findings in studies with $^{64}$Cu. Nevertheless, Bearn (1966) stated that the 'use of radioactive copper has enabled the increased absorption of copper to be demonstrated clearly.' Sternlieb (1967) noted that the cause of chronic copper toxicity seen in patients with WD was unresolved and proposed the value of $40\%$ for average absorption of copper. If faecal excretion of absorbed copper, $20\%$ in 5 days for normal subjects (Strickland et al., 1972), is taken into account, then Sternlieb's estimate approximates to the $56\%$ of the present report and the $60\%$ value given by Weber et al. (1969).

This disagreement between the plasma method and WBC and faecal methods may be due to the fact that the plasma method measures only the copper absorbed during the first 24 h, whereas the latter two methods determine absorption over the entire 4-day study period. Absorption of $^{64}$Cu although decreased, occurs in rats, at a steady rate from 2 to 8 h (Marceau, Aspin & Sass-Kortsak, 1970). If these differences in absorption by the three methods are valid, it would suggest that normal and HC subjects absorb a considerable fraction of copper later and lower in the gastrointestinal tract, whereas homozygotes and heterozygotes for WD
have complete absorption of copper very early, presumably while the $^{64}$Cu is still in the stomach and upper small intestine.

Correlation of absorption with oral copper load suggested that the percentage of copper absorbed decreases as the amount of carrier-bound copper increases. This seems more physiological than the absence of correlation noted by others (Weber et al., 1969) and agrees with findings of the absorption of copper in rats (Marceau et al., 1970) and the absorption of iron in humans (Bothwell, Pirzio-Biroli & Finch, 1958). The wide variation in specific radioactivity of the orally administered $^{64}$Cu (11-fold range in quantity of stable copper) resulted from the difficulties in coordinating studies with the simultaneous administration of two isotopes with short half-lives. We do not believe that this invalidates our results since subjects in all four groups received the extremes of oral copper load, and even the maximum dose (4.5 mg) was within physiological limits. Absorption of copper did not vary with age or sex and was not influenced by cirrhosis of the liver.

The amount of storage copper did not appear to influence copper absorption since absorption of copper in a patient with severe untreated WD was similar to that observed in two presymptomatic patients without K-F rings and another patient who had received penicillamine therapy for 10 years. The first patient should have maximum copper stores, the presymptomatic patients should still be depositing copper in their tissues, while the latter patient should have had much of his tissue copper stores diminished from prolonged penicillamine therapy.

Reabsorption of biliary-excreted copper may occur, but we are unaware of any published results on this subject in humans. All three methods used to determine copper absorption take into account the reabsorption of excreted copper. The ratio of $^{64}$Cu to $^{67}$Cu activities was used in the calculations and both isotopes should be excreted into the bile and reabsorbed in similar proportions.

Correlation between the three methods was disappointing, but the following important points should be stressed. (1) The average absorption of copper in control subjects using the WBC and faecal methods was very similar, 57% and 54% respectively. (2) By these two methods there was no statistically significant difference in copper absorption among the four groups of subjects. Therefore, copper absorption does not appear to be increased in WD. (3) Since absorption of copper in heterozygotes did not differ from that in normal subjects, and since these individuals have a decreased biliary excretion of copper (Strickland et al., 1972), it follows that they should have a body retention of the metal.

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