STUDIES ON COPPER METABOLISM. XVI. RADIOACTIVE COPPER STUDIES IN NORMAL SUBJECTS AND IN PATIENTS WITH HEPATOLENTICULAR DEGENERATION [1]

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(Submitted for publication June 21, 1955; accepted August 24, 1955)

A number of abnormalities in the metabolism of copper in hepatolenticular degeneration (Wilson's disease) have now been described. These may be enumerated briefly as follows: the total plasma copper level is usually decreased (1–3); there is a decrease in the concentration of ceruloplasmin (3, 4), the blue copper containing alpha globulin of plasma (5); the direct-reacting fraction of plasma copper (6), that which is bound probably to albumin (7) is increased (2, 7); the urinary excretion of copper is greatly increased (1, 2); the concentration of copper in the tissues is elevated (2, 8); and the patients retain a greater amount of dietary copper than do normal subjects (2, 9, 10).

The pathogenesis of this disease is poorly understood. It has been suggested by several observers (1, 2) that the excessive accumulation of copper in the brain and liver causes the characteristic lesions in these organs. Whether the excessive deposition of copper is due to increased absorption or to a decreased rate of excretion has not been clarified by non-isotopic copper balance studies (2). The observation of Matthews (10) that a larger proportion of an orally administered dose of radioactive copper was recovered in the feces of control subjects than in those of patients with Wilson's disease does not answer this question, because it is not possible to determine by such a study whether more radiocopper was absorbed or whether less had been re-excreted.

The purpose of this paper is to present studies on the excretion of radiocopper in the urine and stools of normal subjects, patients with Wilson's disease, and patients with alcoholic cirrhosis of the liver following the oral and intravenous administration of radiocopper. In addition, the uptake of the isotope into plasma, erythrocytes, and liver will be reported. The amount of radioactivity in the direct-reacting fraction (albumin-bound) of plasma copper and in the indirect-reacting fraction (globulin-bound or ceruloplasmin) was also studied. For this purpose plasma was fractionated in some instances with rabbit anti-human ceruloplasmin serum, and in other cases with ammonium sulfate, because of limitations in the amount of available anti-serum.

Several preliminary reports of this work have been published (11, 12). While the present paper was in preparation, Bearn and Kunkel (13) have reported that the fecal excretion of intravenously administered radiocopper was significantly less in two patients with Wilson's disease than in two control subjects.

METHODS

Eleven normal subjects, four patients with hepatolenticular degeneration, and two patients with alcoholic cirrhosis of the liver were used in these studies. All individuals were hospitalized in the metabolic ward. The normal subjects were healthy males, 25 to 30 years of age. A complete physical examination, urine analysis and determination of volume of packed red cells, leukocyte count, sedimentation rate, and total plasma copper were carried out on each. The four patients (B. S., D. C., J. Si., and Dar. H.) with Wilson's disease have been described previously (2, 3). Blood copper values in these four patients are presented in Table I.

Radioactive copper (Cu^{64}) was supplied as a solution of cupric acetate by Abbott Laboratories on allocation from the United States Atomic Energy Commission. The standard solutions, blood, plasma, urine, and stool

[1] This investigation was supported in part by a contract (AT (11-1)-82), Project No. 6, between the University of Utah and the U. S. Atomic Energy Commission and in part by a research grant (C-2231) from the National Cancer Institute, National Institutes of Health, Public Health Service.

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specimens were counted in a well-type, thallium activated sodium iodide crystal, scintillation counter with an efficiency of 5 per cent for Cu^{64}. Two standard solutions were prepared: one a 1:1,000 and the other a 1:100,000 dilution of the stock solution. The syringe from which the material was delivered to the patient was used for the preparation of the concentrated standard. Since the half life of Cu^{64} is 12.8 hours, a record was kept of the exact time at which each count was performed in order to correct the observed activity for radioactive decay. The dilute standard was used to correct the counts for the first 48 hours, and thereafter the concentrated standard was used. The standards were counted twice a day. The counts of the standards were plotted against time on semi-logarithmic paper as "counts per minute injected." The observed counts per minute obtained on samples from the subjects were corrected for decay.

In order to make the data more comparable, each subject or patient was given a dose of one mg. of copper. Since the specific activity of the material at the time of administration varied from 0.8 to 1.2 mc. per mg. of copper, the final data were adjusted to CPM/one mc. of copper administered.

The stools collected during the first 72 hours were pooled, weighed, and thoroughly mixed in a Waring blendor. Triplicate aliquots of each stool were counted. After 72 hours, single stool specimens were weighed, mixed, and counted in triplicate. Stool collections were continued until less than one per cent of the injected dose of Cu^{64} appeared in a single stool (72 to 120 hours).

Twenty-four-hour urine specimens were collected for three consecutive days after the administration of the isotope. During the first 24 hours one-ml. aliquots were taken for counting. After the first 24 hours the urine was concentrated 10:1 or 20:1 and digested by boiling with concentrated sulfuric, nitric, and perchloric acids (14). A one-ml. aliquot of the digest was counted.

The activity in one ml. of whole plasma was determined 0.5, 1, 2, 3, 4, 5, 6, 8, 12, 24, 36, and 48 hours after the administration of copper by the oral route and 10 minutes, 0.5, 1, 2, 4, 6, 12, 24, 36, and 48 hours after the intravenous administration of radiocopper. In most instances an additional aliquot of plasma was fractionated with either rabbit anti-human ceruloplasmin serum (3) or ammonium sulfate or both.

Plasma fractionation with rabbit anti-human ceruloplasmin serum was carried out as follows. One ml. of plasma was added to a sample of rabbit anti-ceruloplasmin serum calculated to contain excess antibody. After incubation at 37° C. for one hour, the mixture was centrifuged in the cold,[3] and the precipitate was washed successively with 3.0 ml. and 2.5 ml. of cold 0.85 per cent saline. It was then dissolved in 1.0 ml. of 0.1 N sodium hydroxide solution and counted. The washings were added to the supernate and the activity in the supernate ("non-ceruloplasmin" copper) was measured. The supernates from the precipitin reactions were tested for excess antibody in order to be certain that all ceruloplasmin had been precipitated.

Plasma was fractionated with ammonium sulfate by a modification of the method of Earl, Moulton, and Selverstone (15). One ml. of a saturated solution of ammonium sulfate was added to an equal volume of plasma. After standing for 15 minutes at room temperature, the mixture was centrifuged at 8,000 rpm for 15 minutes in a Custom Scientific Instrument Angle Head Centrifuge. The supernatant solution was decanted and the precipitate washed with 2 ml. of 50 per cent saturated ammonium sulfate solution. After centrifugation the precipitate was dissolved in 0.1 ml. of concentrated ammonium hydroxide, made up to one ml. with water, and the activity determined. The washing was added to the origi-

TABLE I

Plasma copper and ceruloplasmin values in four patients with hepatolenticular degeneration

Patient	Total plasma copper μg./100 ml.	Direct-reacting copper μg./100 ml.	Indirect-reacting copper μg./100 ml.	Ceruloplasmin mg./100 ml.
B. S.	57	50	7	2
J. Si.	50	37	13	4
Dar. H.	53	34	19	8
D. C.	86	26	60	19
Normal	108±9	5±6	103±11	34±4

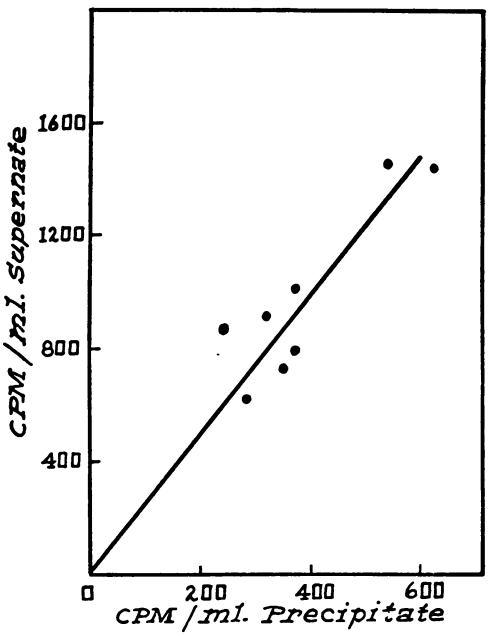

FIG. 1. CORRELATION BETWEEN RADIOACTIVITY OF SUPERNATE AND PRECIPITATE AFTER AMMONIUM SULFATE (50 PER CENT SATURATION) FRACTIONATION OF PLASMA CONTAINING NO DEMONSTRABLE ACTIVITY IN CERULOPLASMIN

The values on the ordinate and abscissa refer to CPM/mc. of radiocopper injected, in the supernate or precipitate obtained from one ml. of plasma.

[3] International PR-1 refrigerated centrifuge.

nal supernate. The total volume was adjusted to four ml. with water. One ml. of the pooled, diluted supernate was counted.

Fractionation of plasma with ammonium sulfate by the above method did not give complete separation of ceruloplasmin from non-ceruloplasmin copper. The results of ammonium sulfate fractionation of plasma samples from patients with Wilson's disease which contained no activity in the precipitate obtained with anti-serum are shown in Figure 1. It can be seen that "non-ceruloplasmin" copper was carried down with the ammonium sulfate precipitate. This is further illustrated in Figure 4, where, in addition, it can be seen that not all of the ceruloplasmin was precipitated with ammonium sulfate in normal subject S. F.

Total plasma copper (16), direct-reacting plasma copper (6), and ceruloplasmin (3) were measured by methods described previously.

RESULTS

Excretion of radiocopper in stools and urine following oral administration of radiocopper

One mg. of radiocopper was administered orally to each of four normal subjects and four patients with Wilson's disease (Table II). The normal subjects excreted 0.1 per cent of the administered activity in the urine; whereas, the patients with hepatolenticular degeneration excreted 14 to 44 times this amount. With the exception of one patient (J. Si.), a significantly smaller amount of radiocopper was recovered in the stools of the patients with Wilson's disease than in the control subjects. Despite the greater amount of activity in the urine of the patients, the total amount of radiocopper recovered was greater in the normal subjects than in the patients, again with the exception of patient J. Si.

TABLE II

Radioactivity in urine and stools following the oral administration of radiocopper

Group	Patient	Per cent of administered activity		
		Urine	Stool	Total
Normal subjects	J. C.	0.1	67.0	67.1
	D. L.	0.1	66.2	66.3
	S. F.	0.1	94.6	94.7
	R. H.	0.1	61.8	61.9
	Mean	0.1	72.4	72.5
Hepatolenticular degeneration	B. S.	4.4	48.8	53.2
	D. C.	1.8	35.5	37.3
	J. Si.	2.2	82.0	84.2
	Dar. H.	1.4	41.9	43.3
	Mean	2.5	52.0	54.5

TABLE III

The influence of potassium sulfide given orally on the radioactivity in the stool, urine, and plasma, following the oral administration of radiocopper

	Patient	No sulfide	With sulfide
Stool Per cent of administered activity	B. S.	48.8	73.8
	D. C.	35.5	110.4
Urine Per cent of administered activity	B. S.	4.4	1.2
	D. C.	1.8	2.1
Plasma maximum *CPM/mc./ml.*	B. S.	2,270	1,730
	D. C.	1,310	472

In order to study the influence of potassium sulfide on the absorption of copper (2), two patients with hepatolenticular degeneration were given one mg. of radioactive copper orally. The activity in the stools, urine, and plasma was measured. Two weeks later, the same two patients were given the same amount of labelled oral copper simultaneously with 20 mg. of potassium sulfide [4] in a gelatin capsule. On both occasions the patients were in a fasting state.

In both patients the per cent of administered activity recovered in the stools increased with administration of potassium sulfide (Table III). In one patient (D. C.), obviously as a result of technical error, the recovery in the stools exceeded 100 per cent. The activity in the urine was less in one patient (B. S.) when sulfide was administered; whereas in the second patient there was no decrease in the activity in the urine. In both patients the maximal activity in the plasma, expressed as CPM/mc. of radiocopper injected per ml. of plasma, was less when sulfide was given than without it.

Excretion of radiocopper in stools and urine following intravenous administration of radiocopper

One mg. of radiocopper was administered intravenously to each of four normal subjects, three patients with Wilson's disease, and two patients with alcoholic cirrhosis of the liver (Table IV). The patients with Wilson's disease excreted

[4] Potash sulfurated technical (Mallinckrodt).

more of the radioactivity in the urine and much less in the stools than the normal subjects. The quantity of radioactive material excreted in the stools of the patients with cirrhosis of the liver was not significantly different from the quantity excreted by the normal subjects. Slightly more activity was recovered in the urine of the patients with cirrhosis than in the urine of control subjects. The total excretion of radiocopper by the normal subjects and the patients with alcoholic cirrhosis of the liver was somewhat greater than by the patients with hepatolenticular degeneration, with the exception of patient J. Si. who excreted a total of 11.2 per cent of the administered activity due to the large percentage (7.8) of the administered dose which was recovered in the urine.

There was no correlation between the degree of impairment of liver function, determined by routine liver function tests, and the amount of radiocopper which was recovered in the stools (Tables IV and V). The amount of radioactive material which was recovered in the stools of the three patients with Wilson's disease was reduced about equally, even though there was marked impairment of liver function in patient D. C.; whereas, in B. S. and J. Si. there was no demonstrable abnormality in liver function, either clinically or by liver function tests. Furthermore, the two patients with alcoholic cirrhosis of the liver excreted more than 3 times as much of the administered radiocopper as the patients with Wilson's disease, in spite of the fact that there was marked impairment of liver function in one (J. St.) and moderate impairment in the other (C. L.).

TABLE IV

Radioactivity in urine and stools following the intravenous administration of radiocopper

Group	Patient	Per cent of administered activity		
		Urine	Stool	Total
Normal subjects	O. H.	0.2	9.3	9.5
	D. Ca.	0.3	16.1	16.4
	A. P.	0.2	14.2	14.4
	R. W.	0.3	9.9	10.2
	Mean	0.2	12.4	12.6
Hepatolenticular degeneration	D. C.	3.2	2.3	5.5
	B. S.	5.2	1.8	7.0
	J. Si.	7.8	3.4	11.2
	Mean	5.4	2.5	7.9
Alcoholic hepatic cirrhosis	J. St.	1.0	7.0	8.0
	C. L.	0.7	9.9	10.6
	Mean	0.8	8.5	9.3

Plasma activity following oral administration of radiocopper

The activity in whole plasma was studied in four normal subjects and in all four of the patients with Wilson's disease given radiocopper by mouth (Figure 2). The plasma of one normal subject (S. F., Figures 3 and 4) and of one patient with Wilson's disease (J. Si., Figure 4) was fractionated by means of both anti-ceruloplasmin serum and ammonium sulfate. The plasma of one patient (B. S., Figure 3) was fractionated only with anti-serum; whereas, the plasma samples of one normal subject (D. L.) and one patient with hepatolenticular degeneration (D. C.) were fractionated only with ammonium sulfate (Figure 4).

Following the oral administration of radioactive copper, there was a prompt increase in activity in the plasma of both the normal subjects and the patients with Wilson's disease. The peak value in all subjects occurred between one and three hours after ingestion of the isotope, and this tended to be higher in the patients with Wilson's disease than in the normal subjects. However, in two of the normal subjects (S. F. and R. H.), the values were equal to the two lowest in the patient group (D. C. and J. Si.). There was poor correlation (correlation coefficient + 0.48, $P = 0.25$) between the peak value for plasma activity and the amount of radioactivity absorbed and retained (100 − stool and urine activity) (Tables II and III).

In the normal subjects, the activity in the plasma decreased rapidly during the 4 to 6 hours following maximal activity, and thereafter increased slowly. On the other hand, in the patients with Wilson's disease, the activity declined more slowly than in the control subjects and no secondary increase was observed.

The activity of the plasma during the initial peak in both the normal subjects and the patients was almost entirely in the supernatant solution remaining after precipitation with anti-ceruloplasmin serum (Figure 3). In the normal subjects, incorporation of radiocopper into the precipitate (ceruloplasmin) could be detected within two hours after the administration of radiocopper and the radioactivity of this fraction increased with

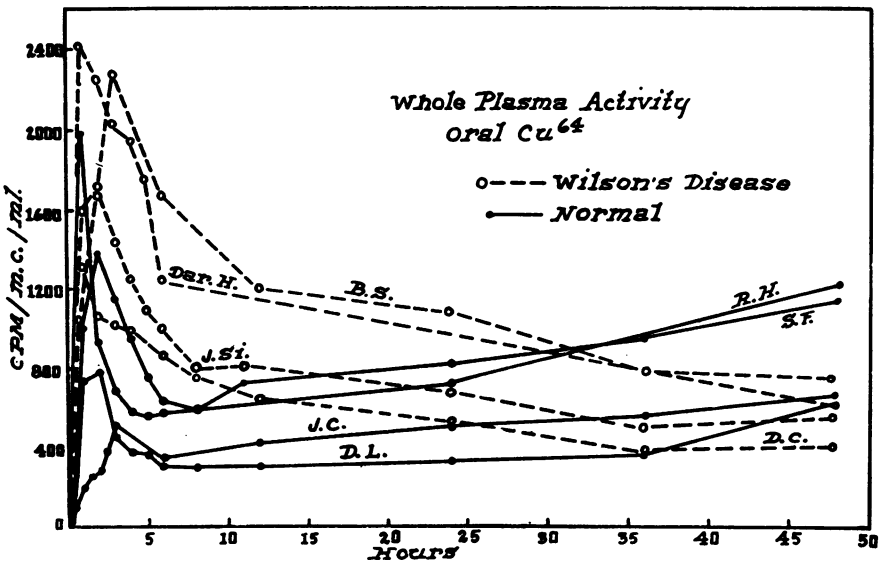

FIG. 2. WHOLE PLASMA ACTIVITY FOLLOWING ORAL ADMINISTRATION OF RADIOCOPPER
The values on the ordinate refer to CPM/mc. of ingested radiocopper in one ml. of whole plasma.

time. As a result, the secondary rise in whole plasma activity (Figure 2) was due to the appearance of radioactivity in the anti-serum precipitate (ceruloplasmin) (Figures 3 and 4). In contrast, no measurable quantities of radiocopper were incorporated into the precipitate (ceruloplasmin) in the two patients with Wilson's disease (B. S. and J. Si.), and no secondary rise in whole plasma activity occurred.

When plasma was fractionated with ammonium sulfate, activity was present in the precipitate from the plasma of the patients with Wilson's disease. By comparison of the activity in the ammonium sulfate precipitate with the activity in

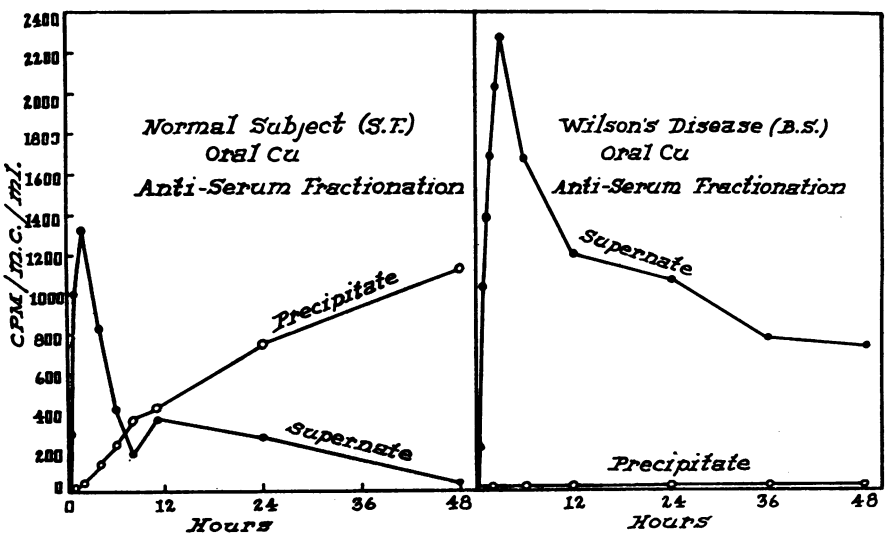

FIG. 3. RADIOACTIVITY IN FRACTIONS OF PLASMA PRODUCED BY ADDITION OF RABBIT ANTI-HUMAN CERULOPLASMIN SERUM
The values on the ordinate refer to CPM/mc. of radiocopper ingested appearing in fractions derived from one ml. of plasma.

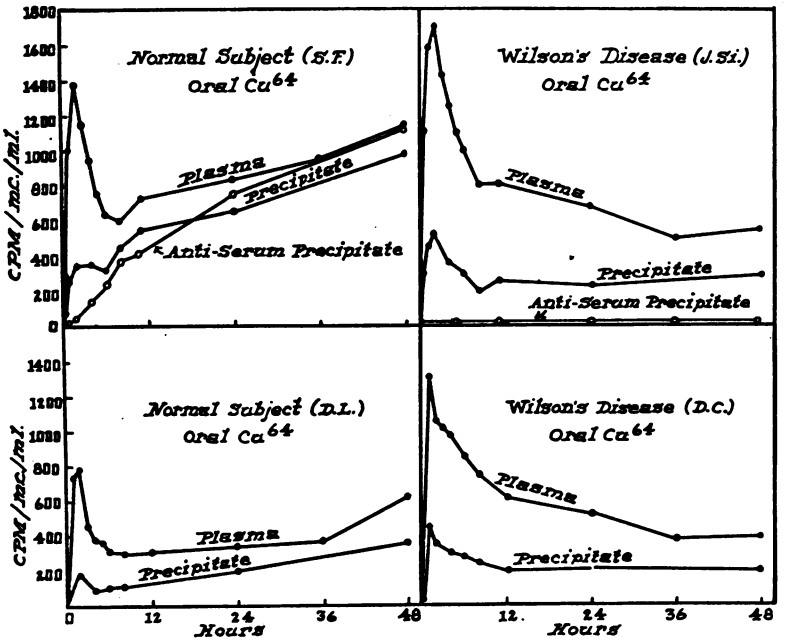

FIG. 4. RADIOACTIVITY IN WHOLE PLASMA AND IN FRACTIONS PRODUCED BY ADDING AMMONIUM SULFATE (50 PER CENT SATURATION)

For purposes of comparison, the activity in the precipitate after the addition of rabbit anti-human ceruloplasmin serum in one normal subject (S. F.) and in one patient with Wilson's disease (J. Si.) is also shown. The difference between the curves of activity in whole plasma and in the ammonium sulfate precipitate represents the activity in the supernate after 50 per cent saturation with ammonium sulfate. The values on the ordinate refer to CPM/mc. of ingested radiocopper in one ml. of whole plasma or in the precipitate obtained from one ml. of plasma.

the precipitate after the addition of anti-serum it can be seen that the activity in the former was due to non-ceruloplasmin copper which was carried down with the precipitate (Figure 4).

Plasma activity following intravenous administration of radiocopper

The activity of whole plasma was measured in three normal subjects, four patients with hepatolenticular degeneration, and two patients with alcoholic cirrhosis of the liver. The plasma of two patients in each group was fractionated with ammonium sulfate. The results are presented in Figures 5, 6, and 7.

The activity of whole plasma in the normal subjects (Figures 5 and 6) decreased rapidly during the first three hours after injection. Thereafter, the activity increased progressively with time. During the first three hours the activity was almost entirely in the supernatant solution after fractionation with ammonium sulfate. Thereafter, the activity in the precipitate after ammonium sulfate fractionation increased progressively and most of the activity of the whole plasma was accounted for in this fraction.

In the patients with Wilson's disease (Figures 5 and 6), the activity of the whole plasma decreased more slowly than in the normal subjects and a secondary rise in activity was not observed. The activity in the precipitate from ammonium sulfate fractionation was high during the first hours following the injection (due to non-ceruloplasmin copper carried down with the precipitate [Figures 1 and 4]) and decreased rapidly at first and then more slowly. A progressive increase in the activity in the precipitate, such as was observed in the normal subjects, did not occur.

In one of the patients (J. St., Figure 7) with alcoholic cirrhosis of the liver and a normal plasma

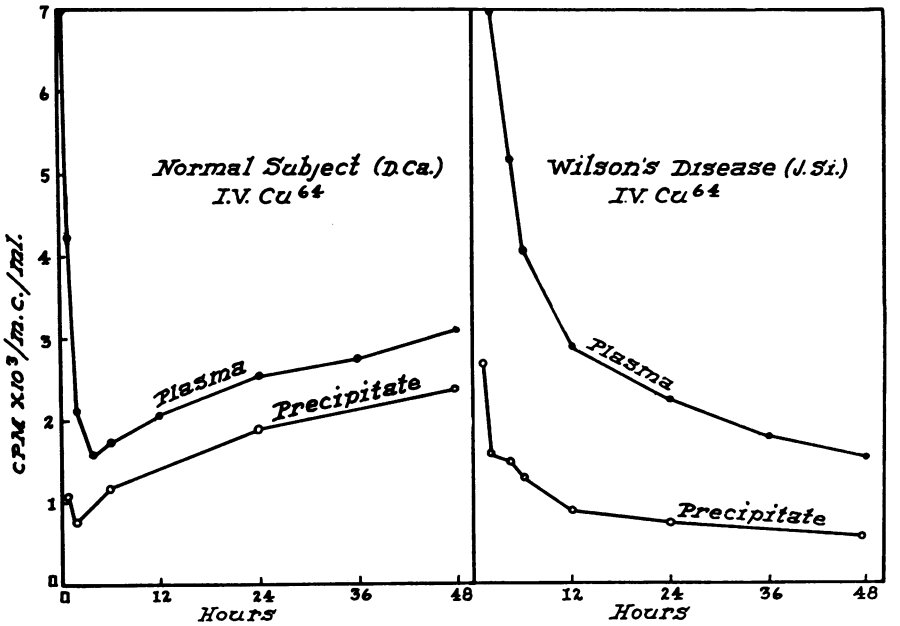

FIG. 5. RADIOCOPPER IN WHOLE PLASMA AND IN PRECIPITATE FOLLOWING FRACTIONATION WITH AMMONIUM SULFATE (50 PER CENT SATURATION)

The difference between the curves of activity in whole plasma and in the ammonium sulfate precipitate represents the activity in the supernate. The values on the ordinate refer to CPM/mc. of injected radiocopper in one ml. of whole plasma or in the precipitate obtained from one ml. of plasma.

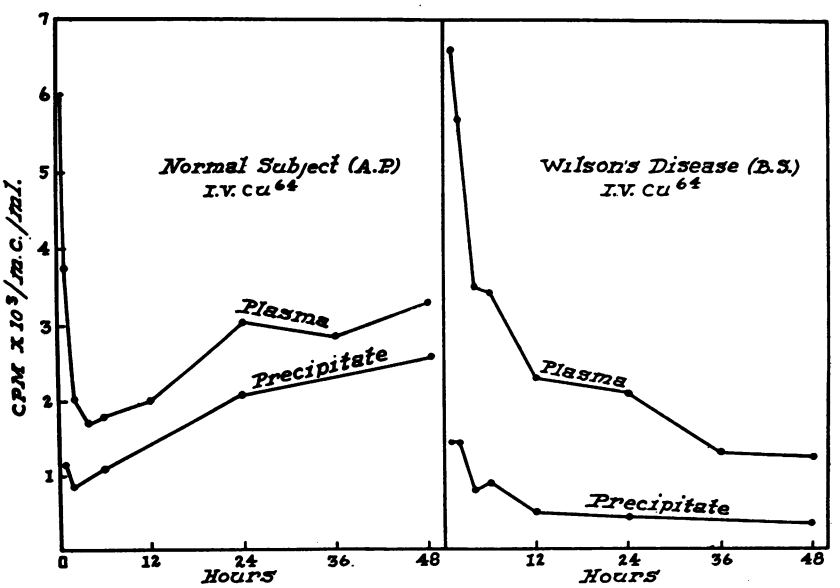

FIG. 6. RADIOCOPPER IN WHOLE PLASMA AND IN PRECIPITATE FOLLOWING FRACTIONATION WITH AMMONIUM SULFATE (50 PER CENT SATURATION)

The difference between the curves of activity in whole plasma and in the ammonium sulfate precipitate represents the activity in the supernate. The values on the ordinate refer to CPM/mc. of injected radiocopper in one ml. of whole plasma or in the precipitate obtained from one ml. of plasma.

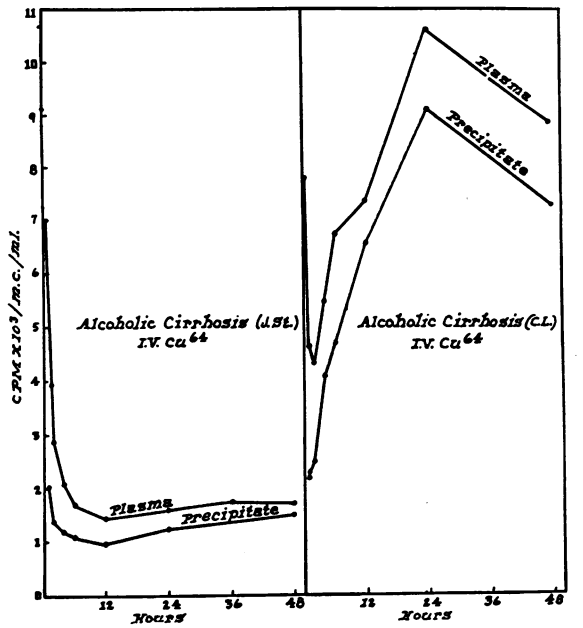

FIG. 7. RADIOCOPPER IN WHOLE PLASMA AND IN PRECIPITATE FOLLOWING FRACTIONATION WITH AMMONIUM SULFATE (50 PER CENT SATURATION)

The difference between the curves of activity in whole plasma and in ammonium sulfate precipitate represents the activity in the supernate. The values on the ordinate refer to CPM/mc. of injected radiocopper in one ml. of whole plasma or in the precipitate obtained from one ml. of plasma.

copper level (110 μg. per cent), the pattern did not differ from that observed in the normal subjects. The second patient (C. L., Figure 7) had hypercupremia (170 μg. per cent) and there was a striking increase in the activity in the whole plasma beginning two hours after the injection. This secondary increase was due entirely to increased activity in the precipitate.

The disappearance of radiocopper from plasma

The rate of disappearance of radioactivity from whole plasma after the oral (Figure 2) or intravenous (Figures 5 and 6) administration of one mg. of radiocopper was slower in the patients with hepatolenticular degeneration than in the normal subjects. In order to determine if the radiocopper disappeared according to a first order equation, the data were plotted on semi-logarithmic paper. A straight line was not obtained in either group. In these studies a relatively large dose of copper (1 mg.) was employed. In order to study the disappearance rate after a "tracer dose," 50 μg. of copper were injected intravenously into three normal subjects and four patients with Wilson's disease.

As shown in Figure 8, even under these circumstances, the copper did not disappear in a simple exponential fashion. The disappearance rate of the radioactivity from the plasma was slower in all four patients with Wilson's disease than in three normal subjects.

Since the curve for the disappearance rate of copper does not follow the equation of a first order reaction, it is not possible to calculate the turnover rate of the "direct-reacting" plasma copper. It seems likely, however, that since the amount of "direct-reacting" or albumin-bound copper in patients with Wilson's disease was at least three times that in normal subjects (3), the amount of

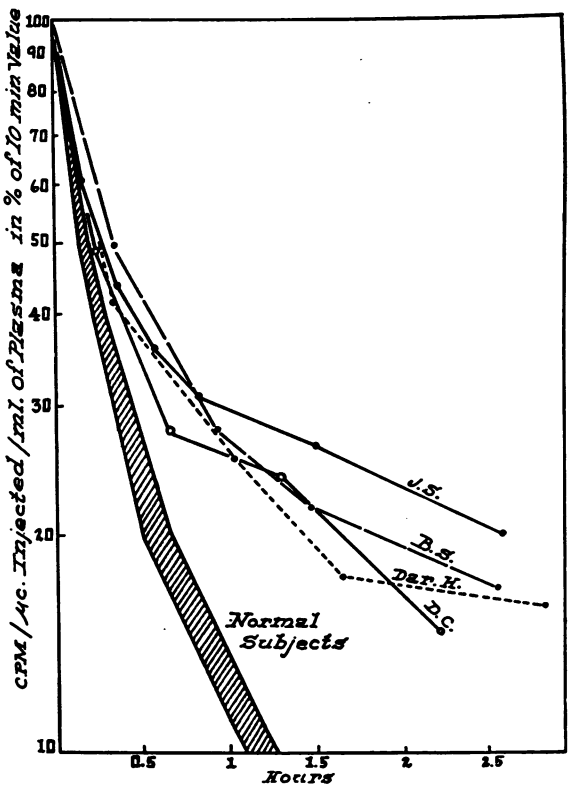

FIG. 8. DISAPPEARANCE RATE OF INTRAVENOUSLY ADMINISTERED RADIOCOPPER (50 μg.) FROM PLASMA OF NORMAL SUBJECTS AND PATIENTS WITH WILSON'S DISEASE

The value obtained 10 minutes after the intravenous injection was taken as 100 per cent. Subsequent values are plotted as per cent of the activity in the 10-minute specimen.

"direct-reacting" plasma copper turned over per unit of time may have been equal to or greater than the amount in the normal subjects, even though the disappearance rate of radiocopper was slower in the former than in the latter.

The uptake of radiocopper by liver

The uptake of radiocopper by the liver was estimated by the use of a mobile body-surface scintillation counter placed over the liver. This determination was performed following the intravenous administration of 50 μg. of radioactive copper to two normal subjects, four patients with Wilson's disease, and two patients with alcoholic cirrhosis of the liver. Prior to the injection of the isotope, the point of maximal liver dullness was determined and marked with ink. Care was taken to place the counter in the same position at the time of each counting. The determined counts were corrected for radioactive decay and for activity due to blood circulating through the liver. The latter was calculated by the method of Huff, Elmlinger, Garcia, Oda, Cockrell, and Lawrence (17) from the determined activity in the plasma at the time of the body-surface count. The data are presented in Figure 9 and are expressed as CPM/μc injected.

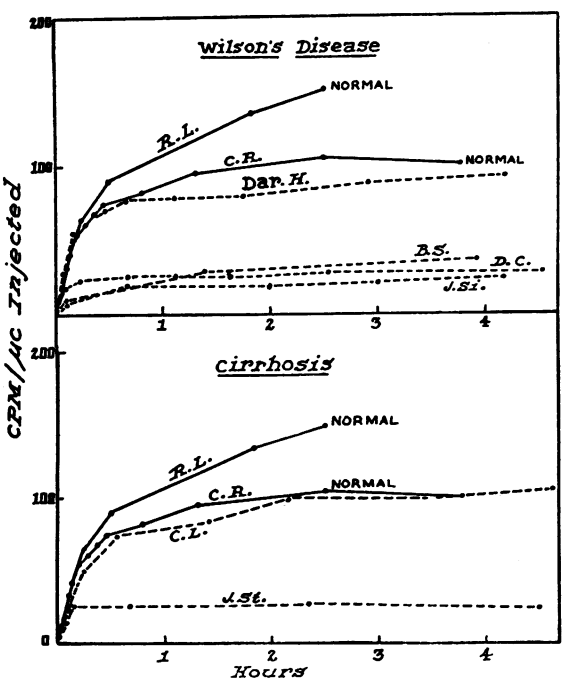

FIG. 9. UPTAKE OF RADIOCOPPER BY LIVER OF NORMAL SUBJECTS (R. L. AND C. R.), PATIENTS WITH WILSON'S DISEASE (DAR. H., B. S., D. C., AND J. SI.), AND PATIENTS WITH CIRRHOSIS OF LIVER (C. L. AND J. ST.) AS MEASURED WITH MOBILE BODY-SURFACE SCINTILLATION COUNTER

The uptake of radiocopper by the liver was depressed in three of the four patients with Wilson's disease as compared with the uptake curves in the normal subjects. In the fourth patient (Dar. H.) the uptake curve was not significantly different from the normal. In one of the patients (C. L.) with alcoholic cirrhosis of the liver, the uptake curve was normal; in the other (J. St.) it was depressed.

In order to determine whether or not the loss of large amounts of radioactivity in the urine of the patients with Wilson's disease accounted for the low uptake in the liver, the activity in the urine during the five-hour period was measured. In none of the patients was more than two per cent of the administered activity recovered in the urine during the period of study.

In an effort to localize the major portion of the injected copper in the patients with hepatolenticular degeneration, the counter was placed over the skull, sacrum, lungs, heart, spleen, and kidney. No localized accumulation of radioactivity could be detected in any of these sites.

The uptake of radiocopper by erythrocytes

The uptake of radiocopper by erythrocytes was studied in four normal subjects and in four patients with Wilson's disease. Representative results in two normal subjects and in two patients are presented in Figure 10.

The activity in one ml. of whole blood and in one ml. of plasma was measured. The differences between these values were considered to represent the activity in one ml. of packed erythrocytes after appropriate correction for relative plasma and red cell volume as determined by the volume of packed red cells. The red cell values have not been corrected for "trapped plasma" and are expressed in CPM per ml. of plasma or red cell water, on the assumption that the plasma contained 90 per cent water and the erythrocytes 64 per cent water.

Following the oral administration of radiocopper there was a rapid uptake of activity by the red cells in both the normal subjects and the patients with Wilson's disease. Thereafter there was a

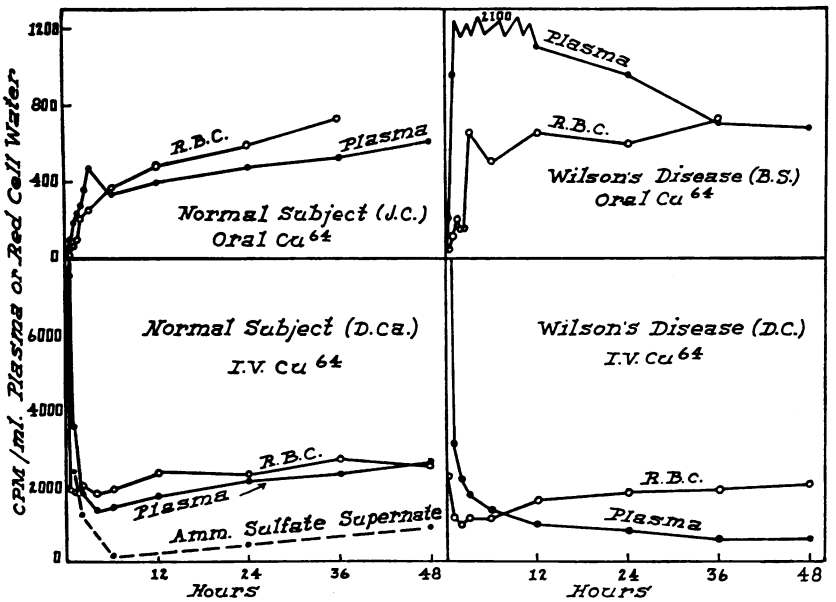

FIG. 10. THE UPTAKE OF RADIOCOPPER BY ERYTHROCYTES

slow progressive increase in the activity in the cells. Similarly, following the intravenous administration of the isotope, considerable activity was found in the erythrocytes in 10 minutes. During the next two hours, as the plasma activity declined rapidly, there was a decline in the activity in the cells. Thereafter, the activity in the red cells increased slowly despite the fact that the activity in the supernatant solution after ammonium sulfate fractionation remained low in the normal subjects.

DISCUSSION

The proportion of the intravenously administered radiocopper which was recovered in the stools of the patients with hepatolenticular degeneration was distinctly less than the amount recovered in the stools of the normal subjects. A similar observation has been made by Bearn and Kunkel (13). Two explanations for these results seem reasonable to us. Since it has been demonstrated in dogs (18) that the main excretory route for copper is through the biliary system,[5] it follows that the ability of the liver to excrete copper into the bile may be impaired in Wilson's disease. If so, this defect appears to be related to the nature of the hepatic disorder rather than to the degree of impairment of liver function. Thus, the functional status of the liver of the patients with Wilson's disease ranged from normal to markedly impaired; nevertheless, the excretion of radiocopper was depressed to approximately the same degree in each patient. Furthermore, the two patients with alcoholic cirrhosis of the liver who were studied excreted a normal quantity of radiocopper.

Another possibility is that the excretion of copper by the liver is not actually impaired in hepatolenticular degeneration. There are several experimental observations to support this suggestion. In this disease the quantity of copper throughout the body is greatly increased as compared with normal individuals (2, 8). Consequently, it could be expected that a given amount of isotopic copper would be distributed widely and a smaller proportion would be deposited in the liver. That this was the case was suggested by the body surface counts which showed that three of our patients with Wilson's disease did not concentrate the isotope in the liver to the same degree as did normal subjects.

In addition to the likelihood that a smaller proportion of radiocopper is carried to the liver in the cases of Wilson's disease than in the normal

[5] We have recently given radiocopper intravenously to a patient with complete obstruction of the bile duct and a cutaneous bile fistula. In 72 hours, 7.8 per cent of the radioactivity was recovered in the bile; 0.8 was recovered in the urine; and 2.1 per cent in the stools.

subjects, the isotopic copper in the liver becomes part of a larger hepatic "pool" of copper than occurs normally. Consequently, the specific activity of the copper excreted in the bile could be reduced even though the total quantity of copper excreted by this route is not decreased. That this is a plausible explanation is suggested by our earlier observation (2) in a single patient that the concentration of copper in the bile removed directly from the gall-bladder at autopsy was normal and the finding by Denny-Brown and Porter (19) of a normal concentration of copper in the bile of three patients.

Following the oral administration of radiocopper a greater proportion of the activity was recovered in the stools of the normal subjects than in the stools of the patients with Wilson's disease. This observation could be explained by reduced excretion of isotope through the bile or by increased absorption in the patients with Wilson's disease. Although the facts are unknown, if it were assumed that radiocopper which is absorbed from the gastro-intestinal tract is handled in the same manner as intravenously injected copper, this question lends itself to mathematical solution, thus:

$$x = \frac{100\,A}{100 - E}$$

x is the per cent of ingested radiocopper absorbed,

A is equal to 100 minus the per cent of radiocopper recovered in the stool,

E is the sum of the per cent of copper excreted through the kidneys, liver, and intestinal wall following the intravenous administration of radiocopper.

According to this calculation the normal subjects absorbed an average of 32 per cent (6 to 43) of the ingested radiocopper and the patients absorbed an average of 49 per cent. Three patients with Wilson's disease absorbed 55, 68, and 52 per cent. A fourth patient (J. Si.) absorbed only 20 per cent. Thus, it appears that three of the four patients with Wilson's disease absorbed more radiocopper from the gastrointestinal tract than did the normal subjects.

The observation that the intestinal absorption of copper appeared to be increased in patients with Wilson's disease is in agreement with the conclusions of previous studies in which the nonradioactive copper balance (2, 9) and the radiocopper balance techniques (10, 13) were used. In the present study, however, the proportion of the ingested radiocopper recovered in the stools of both the normal subjects and the patients was considerably higher than the values obtained by Matthews (10) and by Bearn and Kunkel (13). The reason for this difference is not apparent.

The oral administration of potassium sulfide together with the radiocopper was effective in reducing the proportion of the ingested cupric acetate that was retained. If one accepts the theory that the pathological lesions of Wilson's disease are due to the deposition of copper, this observation provides a rationale for long-term therapy with oral potassium sulfide.

In a previous publication (2), it was suggested that the absorption of copper may be increased in this disease because the ceruloplasmin level is decreased. This theory is not supported by our present data since D. C., with ceruloplasmin level of 19 mg. per 100 ml., absorbed about 68 per cent of the radiocopper; B. S., with a level of 2 mg. per 100 ml., absorbed 55 per cent; and J. Si., with a level of 4 mg. per 100 ml., absorbed 20 per cent.

The uptake of radiocopper into ceruloplasmin was greatly impaired in the patients with Wilson's disease. A similar observation has been made by Bearn and Kunkel (13), Earl, Moulton, and Selverstone (15), and Jensen (20). This could be due either to an impaired rate of ceruloplasmin synthesis or to dilution of the isotope in a large pool of copper before it was incorporated into ceruloplasmin. Since the uptake of radiocopper into ceruloplasmin was no greater in patient D. C. (Figure 4) with a ceruloplasmin concentration of 19 mg. per 100 ml. than in patient J. Si. (Figure 4) with 4 mg. per 100 ml., it seems likely that deficient ceruloplasmin in the plasma is not the only explanation for the low uptake.

The finding that the albumin-bound (direct-reacting) copper is markedly increased in Wilson's disease confirms our earlier observations (2) and the observations of others (7, 13, 15). The increase in this fraction is probably a reflection of the extremely high tissue levels of copper, and the increased absorption of copper from the gastro-intestinal tract. The high concentration of this loosely bound copper, as suggested by Bearn

TABLE V

Liver function studies

Patient	Plasma protein			B.S.P.*	Thymol	Ceph.	Total	Alkaline	Pro-
	Total	Albumin	Globulin	%	turbidity	floc.	plasma	phos-	thrombin
	gm./100 ml.	*gm./100 ml.*	*gm./100 ml.*	*retention*	*units*		bilirubin *mg./100 ml.*	phatase *units*†	time *% of normal*
D. C.	6.6	3.8	2.8	35	13	4+	3.2	10	59
B. S.	6.3	4.7	1.6	5	3	0	0.8	5	79
J. Si.	6.0	3.8	2.2	5	1	0	0.9	1	100
Dar. H.	6.8	4.7	2.1	10	6	3+	0.7	12	100
J. St.	7.7	3.0	4.7	40	13	4+	3.6	8	40
C. L.	8.6	5.1	3.5	17	5	0	1.4	6	100

* Forty-five minutes after 5 mg. per kg. of body weight.
† King and Armstrong.

and Kunkel (13), explains, in part at least, the increased excretion of copper via the kidneys since it seems likely that the urinary copper is derived from this fraction.

The uptake curves of radiocopper into the erythrocytes of patients with Wilson's disease and normal subjects were similar after the first 12 hours. The incorporation of copper into normal erythrocytes is now being investigated in detail and the results will be reported later.

SUMMARY

1. One mg. of copper containing 1 mc. of radioactivity was administered orally to four normal subjects and four patients with Wilson's disease. The same amount of radiocopper was injected intravenously into four normal subjects, three patients with Wilson's disease, and two patients with alcoholic cirrhosis of the liver. The proportion of the administered radiocopper excreted in the urine and feces was determined. In addition, the uptake of the isotope into the albumin and globulin (ceruloplasmin) fractions of plasma was studied. After the administration of a dose of 50 μg. of radiocopper, the curve of disappearance of Cu^{64} from the plasma was determined in three normal subjects, four patients with Wilson's disease, and two patients with alcoholic cirrhosis of the liver. The uptake of radiocopper into the liver was determined with a body-surface scintillation counter in two normal subjects, four patients with hepatolenticular degeneration, and two patients with alcoholic cirrhosis of the liver.

2. Following the oral administration of radiocopper to normal subjects, an average of 0.1 per cent of the administered dose was recovered in the urine and 72.4 per cent in the stools; in the patients with Wilson's disease, an average of 2.5 per cent was recovered in the urine and 52 per cent in the stools. The administration of potassium sulfide together with radiocopper to two of the patients with hepatolenticular degeneration resulted in an increase in the amount of activity recovered in the stools.

3. Following the intravenous administration of radiocopper to normal subjects, an average of 0.2 per cent of the activity was recovered in the urine and 12.4 per cent in the stools; in the patients with Wilson's disease, an average of 5.4 per cent was recovered in the urine and 2.5 per cent in the stools. In the patients with alcoholic cirrhosis of the liver, an average of 0.8 per cent was recovered in the urine and 8.5 per cent in the stools.

4. The uptake of radiocopper into ceruloplasmin was impaired in the patients with hepatolenticular degeneration as compared with that in normal subjects. The uptake was not impaired in the patients with alcoholic cirrhosis of the liver.

5. The uptake of radiocopper by the liver, as measured by a body-surface scintillation counter, was depressed in three of the four patients with hepatolenticular degeneration and in one of two patients with alcoholic cirrhosis of the liver, as compared with the uptake in two normal subjects.

6. The uptake of radiocopper by the erythrocytes of patients with hepatolenticular degeneration was not different from that of the red corpuscles of normal individuals.

7. These findings are interpreted as indicating that the excessive accumulation of copper in the tissues of patients is due primarily to increased absorption of copper from the gastro-intestinal tract. Although a smaller proportion of the isotope administered intravenously was recovered in

the stools of the patients with Wilson's disease than was recovered in the stools of normal subjects, evidence has been presented in support of the concept that the excretion of copper in the bile may not be impaired.

8. The absorption of cupric acetate can be effectively inhibited by the administration of potassium sulfide.

ACKNOWLEDGMENTS

We are indebted to Miss Helen Ashenbrucker and to Miss Marilyn Jacobson for technical assistance. Dr. Frank Tyler kindly made available the use of the metabolic ward.

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