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Blood stool
copper urine
Menkes Kinky-hair disease

Distribution of Ingested and Injected Radiocopper in Two Patients with Menkes' Kinky Hair Disease

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Summary

The distribution of ^{64}Cu is reported in whole blood, plasma fractions, urine, and stool in an adult volunteer and two patients with Menkes' kinky hair disease (trichopoliodystrophy) after intragastric and iv administration. In the adult, after oral ^{64}Cu, circulating ^{64}Cu showed an initial peak at 40–60 min which was 4% of the total dose (or 5% of the amount calculated to be absorbed), a small secondary peak at 3.5% by 4 hr, then a gradual rise to 32 hr. Approximately 55% of the whole blood radioactivity was in the plasma from 8–48 hr. In the two patients, after ingestion or injection, there was no early blood peak and about 10% of both the absorbed or injected copper persisted in the circulation from the 4th hr to beyond 24 hr in a pattern similar to that in the adult, but less than 40% of the ^{64}Cu was in the plasma fraction after 4 hr. In all subjects, the albumin/globulin ratio of radioactivity fell progressively, suggesting prompt ceruloplasmin synthesis by the liver. No radioactivity was detected in cerebrospinal fluid 10 hr after ^{64}Cu injection in patient 2. Urinary excretion of ^{64}Cu in the adult appeared to be linear after 6 hr (approximately 0.08% of the total dose per 24 hr). In patient 1, it was half as great (0.04%/24 hr), but if calculated on the assumption that only 6% of ingested copper was absorbed in patient 1, his urinary excretion rate, 0.67%/24 hr would be eight times that of the control, strongly suggestive of renal inability to conserve copper. In the adult, stool radioactivity totaled 24% of the ingested dose by 48 hr. In patient 1, stool radioactivity was already 94% by 48 hr and in patient 2, after iv ^{64}Cu, stool radioactivity was only 2.8% by 96 hr. This low stool radioactivity after injected copper indicated minimal biliary loss of radiocopper, and ruled out a rapid enterohepatic recycling of copper in this disease. Because 94% of a physiologic dose of ^{64}Cu was in the 48-hr stool in patient 1, <8% of his ingested copper could have been absorbed, compared with over 80% in the adult control. This confirms that there is an absorption defect for copper in Menkes' disease, resulting in approximately 10% of normal copper uptake.

Speculation

The data are interpreted as confirming that there is a mucosal block to copper absorption in Menkes' disease, but that this block is incomplete. Despite depleted circulating copper, erythrocyte uptake appeared normal or high in these patients, and hepatic incorporation into ceruloplasmin seemed to be unimpaired. There may be renal transport abnormalities in Menkes' disease as well, because of the unexpectedly high urinary ^{64}Cu excretion in the presence of hypocupremia. Perhaps an abnormality of blood-brain copper distribution is responsible for the severe neurologic features of this disease.

Menkes' kinky-hair disease (trichopoliodystrophy) is an X-linked recessively inherited condition associated with infantile seizures, profound psychomotor retardation, cerebrocerebellar degeneration, hypopigmentation, tortuous arteries, fragile bones and abnormal hair (4, 21). Danks *et al.* (9–11) first noted that these patients had abnormally low levels of serum copper and ceruloplasmin, unresponsive to oral copper, although parenteral copper would correct the hypocupremia. On the basis of increased copper in jejunal biopsies from from their patients, compared to infants with nonspecific diarrhea, they postulated a defect in the mucosal transport of copper. Clinical trials of parenteral copper, even from early infancy, have failed to correct or reverse the neurological sequelae (5, 12, 15, 19). Thus, this condition is not due to a simple mucosal block with consequent copper deficiency. One paradoxical observation has been the absence of anemia and neutropenia in all but one of these patients (1) despite their severe hypocupremia. This is in contrast to infants and adults with nutritional hypocupremia who have severe hematologic manifestations with marked lethargy and hypotonia (2, 3, 18), but not the grave neurologic abnormalities or the *pili torti* of Menkes' disease (16, 22, 25).

Dekeban *et al.* (12) have studied the fate of ^{67}Cu in three patients with Menkes' disease. Two further cases are reported in which the distribution of ^{64}Cu was followed in whole blood, plasma fractions, urine, and stool, after intragastric and iv administration.

PATIENTS

Patient 1, a black male, was 18-months-old at the time of study (Fig. 1); his clinical presentation has been reported previously (27). He was born 3 wk early after premature rupture of membranes, his birth weight was 3.3 kg, his Apgar was 10. The neonatal period was complicated by transient mild hyperbilirubinemia. His mother reported that he had normal development until age 4 months, when he was holding up his head, looking about, but not yet turning over. At that age, he was first hospitalized for seizures and had several episodes of hypothermia with temperatures of 35°C. A pneumoencephalogram showed dilated ventricles, a brain biopsy was nondiagnostic, and long bone X-rays showed metaphyseal flaring. His hair showed microscopic *pili torti.* His eyes were blue, although his parents and both sisters had brown eyes. The diagnosis of Menkes' was considered and a serum copper was found to be 19 μg/dl. He continued to have numerous minor motor seizures, not responsive to anticonvulsant medication. His development had regressed by the time of study to having only a sucking reflex. There was no family history of similar disease. His serum copper has ranged from 10–19 μg/dl (normal 75–135), his serum ceruloplasmin from 2–16 IU (normal 35–65), and his urine copper excretion was as low as 6 μg/24 hr (normal <50 for adults).

Patient 2, a while male, was 5-years-old at the time of study (Fig. 2). He was the first child of healthy parents who had no family history of this condition. In infancy, he fed poorly and had episodes of hypothermia and frequent infections. At age 6 months, he presented with seizures, hypotonia, and the characteristic hair findings diagnostic of Menkes' syndrome (Fig. 3). Further studies revealed hypocupremia with serum copper ranging from 20–58 μg/dl, serum ceruloplasmin was 6–17 IU, and urine copper was as low as 2 μg/24 hr. His neurologic status has declined progressively

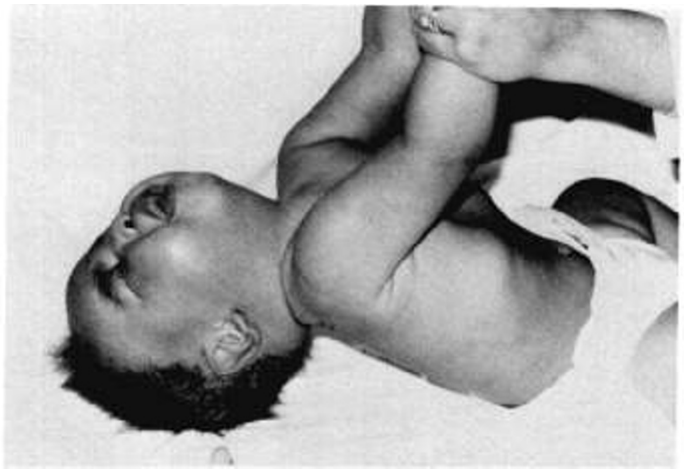

Fig. 1. Patient 1: age, 18 months. Note poor head control and abnormal hair.

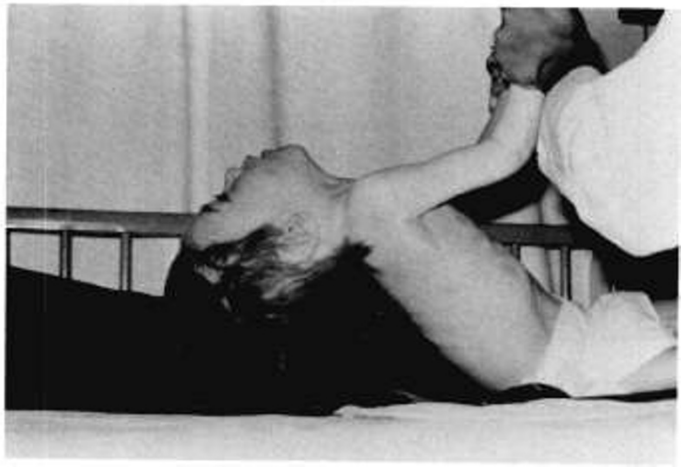

Fig. 2. Patient 2: age, 5 yr. Note poor head control.

with loss of all developmental milestones, uncontrollable seizures, and need for nasogastric feeding.

The adult subject was a 42-year-old male in good health.

MATERIALS AND METHODS

Radioactive copper ^{64}Cu (28) (T ½ = 12.8 hr) was supplied as cupric nitrate in 1 N nitric acid, 25–30 mCi/mg at time of delivery. For oral and intragastric administration, the calculated dose of ^{64}Cu was diluted in 10 ml of 0.9% saline, and given after an overnight fast (without breakfast). For iv administration, the solution was brought to neutral pH by titration with 1.2 M sodium bicarbonate, diluted in 0.9% saline, and then passed through a 0.22 μ millipore filter; then, after testing for pyrogenicity in a rabbit, 4 ml were infused iv over 10 min into patient 2 with no adverse reactions. The dose of elemental copper was estimated to be less than 30% of each subject's daily dietary copper (Table 1). Heparinized venous whole blood samples were counted for radioactivity on a gamma spectrometer (29) adjusted for ^{64}Cu. Plasma was counted separately. Portions of plasma were centrifuged through ultrafilters (30) at 3000 × *g* to separate ultrafiltrable from protein-bound copper; other portions were mixed with an equal volume of saturated ammonium sulfate for 1 hr at 20°C and centrifuged at 10,000 × *g* for 20 min. The supernatant (defined as the albumin fraction), and the precipitate (defined as the globulin fraction which contains ceruloplasmin, an α-globulin), were counted. Each urine voided and each 24-hr collection of stool (incinerated to a fine ash [31]) was also counted. Standard ^{64}Cu samples were counted with each batch to calibrate and monitor the decay rate of radioactivity.

Plasma volume was determined in the two subjects with I^{125} albumin. Total circulating radiocopper was calculated by multiplying concentration times estimated blood volume, (plasma volume) × (1-hematocrit)/1. Hepatic uptake of radioactivity was assessed by gamma-scanning.

Results are expressed as the percent of the initial dose or of the absorbed dose of ^{64}Cu calculated to be in the total circulating blood volume at each time point. The cellular fraction was the difference between whole blood and (plasma radioactivity) × (1-hematocrit).

Informed consent, specifying that these studies would have no therapeutic benefit for the patients, was obtained from the parents or legal guardians of the children, and from the male adult subject. The studies were approved by the Yale Radiation Safety and Human Investigation Committees.

RESULTS

PRELIMINARY FINDINGS

In a preliminary experiment, 26 μg of ^{64}Cu (initial radioactivity, 440 μCi) was administered *via* nasogastric tube to each of the patients. Circulating radioactivity was readily measurable; and was about 0.1% of the administered dose by 20 min rising to a plateau of 0.2% (patient 1) and 0.3% (patient 2) from 8–24 hr. A jejunal biopsy (20 mg) at 4 hr in patient 2 had no detectable radioactivity. (The specimen would have to contain at least 10 times the radioactivity of an equal volume of blood for any to be detected.) Electron-microscopy of a portion of this specimen revealed no structural abnormalities of the jejunal mucosa. Gamma-scanning at 6 hr revealed diffuse radioactivity in the abdomen, but no concentration over the liver. Urine radioactivity was also detectable, about 0.01% of the absorbed dose being excreted by 4 hr, and 0.02% by 9 hr.

INTRAGASTRIC ^{64}Cu IN PATIENT 1

After a steady rise in total circulating radioactivity to a peak at 4 hr of 0.55% of the administered dose, there was a gradual

Table 1. *Doses of ^{64}Cu given*

Subject	Copper (μg)	^{64}Cu (μCi)	Route
Case 1	26	740	Gastric
Case 2	8.6	275	iv
Control	31	880	Oral

[1] Radioactivity at time of administration.

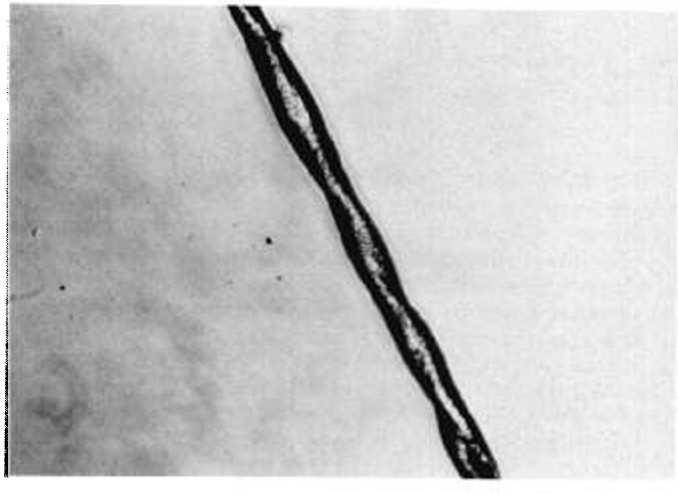

Fig. 3. Hair from patient 2 shows the classic *pili torti.*

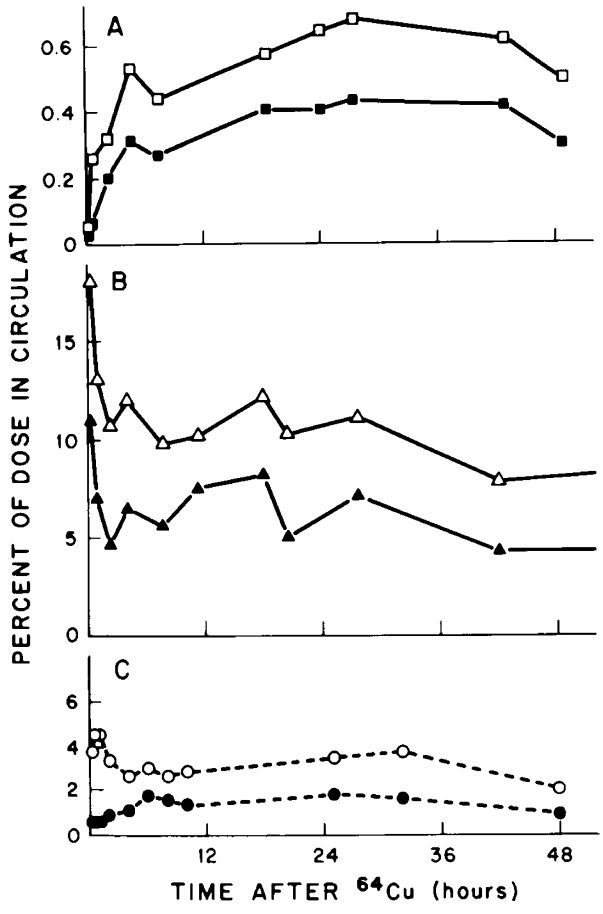

Fig. 4. Distribution of circulating radioactivity in whole blood and plasma. The *symbols* represent percent of administered radioactive dose calculated to be in the circulation at each time point in whole blood (*open symbols*) and cellular fraction (*closed symbols*). *A:* intragastric ^{64}Cu in case 1 (*squares*); *B:* iv ^{64}Cu in case 2 (*triangles*); *C:* oral ^{64}Cu in adult (*circles*). The vertical scales are adjusted to be proportionate to the absorbed fraction of the administered dose (6% for case 1, 100% for case 2, 80% for the adult).

secondary rise to 0.6% by 28 hr. The distribution of radioactivity in the whole blood (Fig. 4a, 5), showed less than 40% in the plasma fraction after 4 hr. The plasma ultrafiltrate (presumably amino acid-bound copper) had detectable radioactivity throughout the 24 hr; the albumin/globulin radioactivity ratio was about 0.40 for the first 4 hr, this then decreased to <0.08 by 42 hr.

Urine excretion of ^{64}Cu (Fig. 6) was approximately linear at the rate of 0.04%/24 hr of the administered dose. Stool radioactivity (Fig. 7) was already 83% of the administered dose by 10 hr, 94% by 48 hr, and greater than 99% by 72 hr.

IV ^{64}Cu IN PATIENT 2

Circulating radioactivity (Fig. 4b) fell rapidly to 10% of the administered dose by 8 hr, rose to 12% by 16 hr, then declined to 8% by 48 hr. The proportion of whole blood radioactivity in plasma, after the first 2 hr, was similar to that after ingested ^{64}Cu in patient 1. Less than 1% of plasma radioactivity, however, was found in the ultrafiltrate at 2 hr (Fig. 5) and none by 18 hr; the albumin/globulin ratio was 0.78 at 2 hr and 0.14 at 18 hr. Two ml of cerebrospinal fluid, obtained at 10 hr, had no detectable radioactivity (if the specimen had >1% of the radioactivity in 2 ml of blood or plasma, it would have been measurable). Intense hepatic radioactivity was shown by gamma-scanning at 8 hr, but not at 96 hr (after eight half-lives, when only intense concentrations would have remained detectable).

Urine excretion of ^{64}Cu (Fig. 6) was not linear. The excretion rate was about 0.6%/24 hr for the first 4 hr, but after 24 hr, the rate had declined to <0.04%/24 hr. Stool radioactivity (Fig. 7) was very low, <1.5% of the iv dose had appeared in the stool by 48 hr and 2.8% by 96 hr.

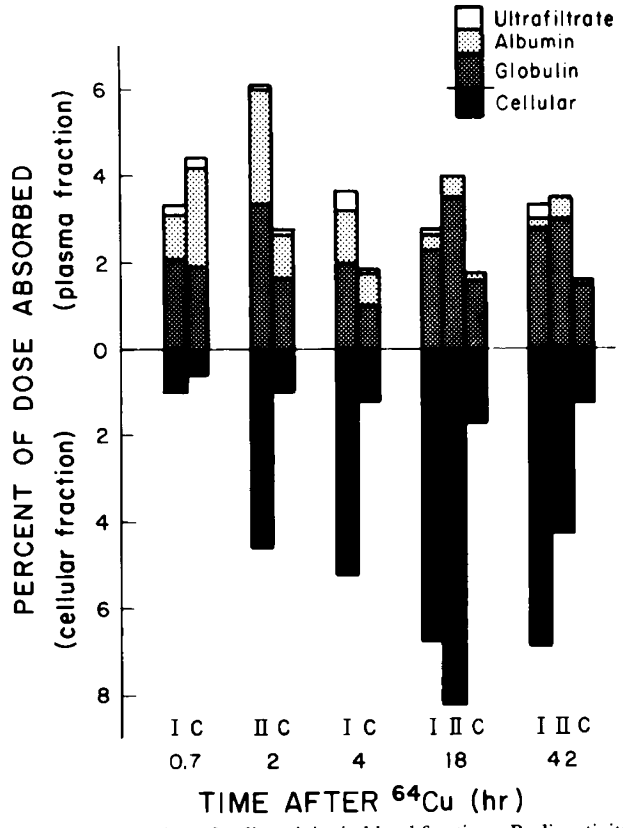

Fig. 5. Distribution of radioactivity in blood fractions. Radioactivity in the cellular fractions are plotted below the ordinate, and the ultrafiltrate, albumin and globulin fractions above the ordinate. I = intragastric ^{64}Cu in case 1, II = iv ^{64}Cu in case 2, C = oral ^{64}Cu in adult. Note that no data are presented for case 1 at 2 hr or for case 2 at 40 min and 4 hr. The vertical scale has been adjusted to represent percent of the estimated absorbed radioactivity.

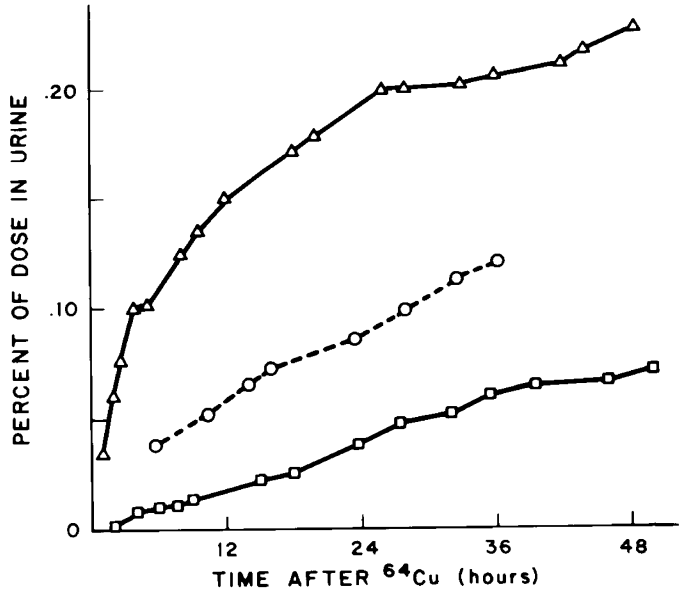

Fig. 6. Radioactivity recovered in urine. Cumulative recovery of radioactivity in urine after intragastric ^{64}Cu in case 1 (*squares*), iv ^{64}Cu in case 2 (*triangles*), and oral ^{64}Cu in the adult (*circles*). The vertical scale represents percent of administered dose.

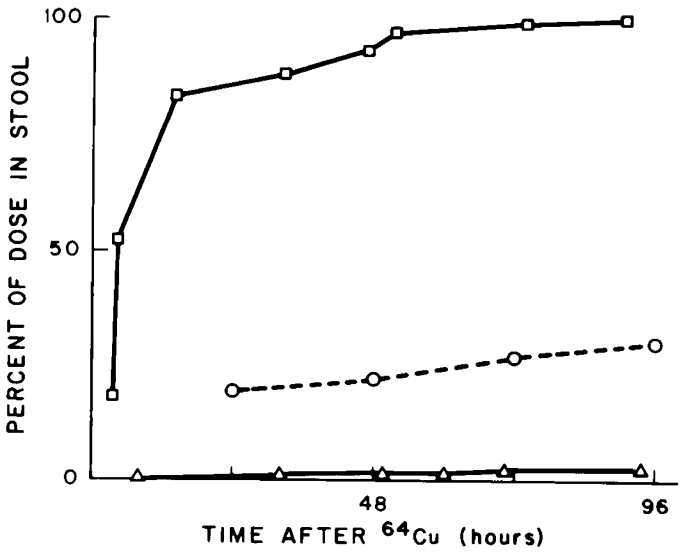

Fig. 7. Radioactivity recovered in stool. Cumulative recovery of radioactivity in the stool; the symbols are the same as for Figure 6.

ORAL ^{64}Cu IN THE ADULT

Circulating ^{64}Cu (Fig. 4c) showed an initial peak at 40–60 min, which was 4% of the total dose (or 5% of the absorbed ^{64}Cu), a smaller peak of 3.5% by 4 hr, then a very gradual rise until 32 hr. The plasma radioactivity (Figs. 4c, 5) declined to 50% of whole blood at 8 hr, after which it remained steady at about 55%. The ultrafiltrate contained 4% of plasma radioactivity at 2 hr declining to 2% at 18 hr (Fig. 5); the albumin/globulin ratio fell to 0.60 by 2 hr and 0.14 by 18 hr. Hepatic uptake of ^{64}Cu was intense by gamma-scanning both at 4 hr and at 48 hr.

Urine excretion of ^{64}Cu (Fig. 6) appeared to be linear after 6 hr, at the rate of about 0.08%/24 hr of the ingested dose. Stool radioactivity was 20% of the ingested dose by 24 hr, 24% by 48 hr and 30% by 96 hr (Fig. 7).

DISCUSSION

Normally (13), as illustrated by the findings in the adult control, a major portion of the 3–6 mg/day of dietary copper is absorbed from the intestine. Of circulating copper, a small fraction is ionically bound to plasma amino acids (23), a large fraction is absorbed to albumin, most plasma copper is covalently bound to ceruloplasmin, and another large fraction is bound to metallothioneins in the red cell (6, 7, 13, 23, 24, 26). Radiocopper tracer studies have shown intensive hepatic uptake of copper in the first 4 hr, followed by retention in liver cells and incorporation into ceruloplasmin for recirculation in the globulin fraction of plasma, or excretion into the bile. In normal subjects, 8–10% of injected or 20–80% of ingested radiocopper ends up in the stool in the first few days (7, 13, 26). Radiocopper uptake by other tissues is minimal compared with hepatic uptake. Even with intestinal reabsorption of biliary copper, the major route of copper excretion eventually is *via* the bile into the stool, so stool copper content nearly equals food copper, <50μg/day being lost in the urine, and smaller amounts through the skin. Urine recovery of injected radiocopper is normally 0.1–0.3%; of ingested radiocopper, it is 0.06–0.18% (7, 20, 26).

In Menkes' disease, the abnormally low stool radioactivity found in case 2 after injected ^{64}Cu indicates reduced biliary loss of copper, and rules out any rapid enterohepatic recycling of copper. Because 94% of a physiologic dose of ingested ^{64}Cu was in the 48-hr stool in patient 1 (and some still remained in his abdomen, probably in his colon) <6% of his ingested copper was absorbed compared with over 80% in our adult control (who would already have excreted some absorbed ^{64}Cu into his bile [7, 13]). This confirms that there is an absorptive defect for copper in Menkes' disease, resulting in approximately 10% of normal copper uptake.

Comparison of circulating and urinary ^{64}Cu after injected and ingested copper showed interesting similarities in these patients. Assuming that only 6% of the ingested dose in patient 1 was absorbed, after the 4th hr, a higher proportion of absorbed ^{64}Cu was in his circulation, particularly in his cellular fraction, than in the adult (Fig. 5). There was a similar disproportionate excess of radioactivity in the whole blood and cellular fraction of patient 2 after inejcted ^{64}Cu. The persistence of radioactivity in the ultrafiltrate of patient 1 after ingested ^{64}Cu (Fig. 5) can be attributed to prolonged uptake from his intestine. Although the radioactivity in the crude ammonium sulfate precipitate would be contaminated by trapped albumin, shifts in radioactivity from the supernatant to the precipitate must be indicative of shifts in the distribution of copper between albumin ceruloplasmin (excluding the remote possibility of an abnormal copper binding globulin). Both after ingested and injected ^{64}Cu, a definite shift of radioactivity from the albumin fraction into the globulin fraction was suggestive of prompt ceruloplasmin synthesis by the liver. This concurs with other reports of normal hepatic synthesis of ceruloplasmin in Menkes' disease (5, 15, 19).

The differences between early urinary excretion of ingested copper and injected copper in the two patients is accounted for by the slow uptake of ingested ^{64}Cu versus the sharp uptake of injected ^{64}Cu. As a proportion of the administered dose, the rate of urinary excretion in Menkes' was about half of the adult's, but when calculated on the assumption that only 6% of ingested copper was absorbed in patient 1, his urinary excretion rate, 0.67%/24 hr, would be eight times that of the adult, strongly suggestive of renal inability to conserve copper in Menkes' disease.

These data are consistent with those of Dekaban *et al.* (12) who used ^{67}Cu (T ½ = 61.7 hr) and also found decreased absorption, increased urine excretion, and preferential uptake by red cells.

Finally, in some preliminary experiments, unpublished evidence is available that *in vitro* uptake of ^{64}Cu by the patient's red cells, suspended either in their own or in control plasma, was equal to or greater than that of normal subjects. Cultured cells from our patients (Milunsky *et al.*, unpublished data) have shown the same increased copper content reported by Goka *et al.* (14). The abnormal distribution and turnover of radiocopper we have demonstrated *in vivo* correlate well with *in vitro* evidence that the molecular defect in Menkes' disease must be due to abnormal cellular transport or retention of copper (8, 17).

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