Copper infusion therapy in trichopoliodystrophy

The effect of repeated intravenous infusions of cupric salts was studied in two patients (cousins) with trichopoliodystrophy identified at the ages of 3 days and 2 1/2 mo, respectively. The intravenous doses of copper were gradually increased and normal or near-normal serum values were achieved after 150 µg Cu²⁺/kg were administered daily for 5 days. At that time the hepatic concentration of copper in the two patients increased from 14 to 38 µg/gm of dry weight from 7 to 45 µg/gm of dry weight, respectively, and the muscle homogenate exhibited the capacity to oxidize pyruvate-3-¹⁴C. Continued infusions of cupric salts in doses of 190-220 µg/kg/day once or twice weekly were necessary to maintain elevated hepatic and serum concentrations of copper. At 6 mo of age the younger infant had reached a functioning level of 4 mo. The older infant demonstrated progressive loss of neurologic functions and died at 15 mo of age. These observations suggest that the neurologic and biochemical dysfunctions of trichopoliodystrophy may be altered by infusion of cupric salts early in the course of the disease.


TRICHOPOLIODYSTROPHY (Menkes syndrome, kinky hair disease) is a disease characterized by a sex-linked pattern of inheritance of kinky, friable hair, and by progressive neurologic dysfunction. The earliest age of diagnosis in published reports is 3 mo.¹ Death usually occurs by 4 yr of age. Abnormally low levels of copper are found in both plasma and tissues and it has been suggested that the disease results from a defect in the absorption of copper from the gastrointestinal tract. Therapy with Cu(II)-EDTA given by subcutaneous injection has been used by other investigators without prolonged reversal of neurologic symptoms.² We have found no reports of long-term intravenous administration of copper.

Identification of a 3-day-old male infant and a 2 1/2-mo-old male cousin with trichopoliodystrophy permitted studies on the effect of intravenous infusions of copper on the course of this disease. Intravenous administration of copper sulfate or copper acetate increased serum and hepatic levels of copper in each patient. Early therapy has been associated with some achievement of developmental abilities in the younger patient; similar improvement was not noted in the older patient, who died at 15 mo of age.

METHOD

Copper concentrations were estimated by atomic absorption spectrophotometry at 324.7 nm by means of an Aztec Instrument Model AAA-3 atomic absorption spectrophotometer equipped with a Honeywell Model 15 Electronik recorder. For determination of serum concentration of copper, serum samples were diluted two- to fivelfold with twice-distilled water in the presence and absence of an aliquot (0.25 µg of Cu/ml) of standard Cu²⁺ solutions. These solutions were aspirated directly into the spectrophotometer. The concentration of copper in the serum sample was calculated from the increment in absorbance which results from addition of the aliquot of the Cu²⁺ standard to this sample.

Liver and muscle biopsy samples (2-10 mg dry weight) were solubilized by incubation with 0.1 ml concentrated H₂SO₄ + 0.2 ml of concentrated HNO₃ (Veripur

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reagent grade, PCR Inc.) at elevated temperatures in 10 ml Kjeldahl flasks. After heating until white fumes were observed (15-30 min) the flasks were cooled and distilled water was added to achieve a final concentration equivalent to 1-4 mg (liver), or 4-12 mg (muscle) of sample/ml of solution. Copper analysis was performed on these solutions as described above and the concentration of the metal calculated by reference to the increment in absorbance resulting from addition of an aliquot (0.1-0.25 μg of Cu/ml) of a standard Cu²⁺ solution. The range observed for the copper content of normal adult human liver (20-50 μg/gm of dry weight) using this procedure is in good agreement with that reported by Tipton and Cook.³

The copper acetate solution was prepared from cupric acetate [(CH₃COO)₂CuH₂O, product No. 1766, J. T. Baker Co., Phillipsburg, N. J.] The final concentration was 20 μg of Cu/ml administered with 5% glucose in 0.29 saline to make a volume of 150-200 ml. The infusions were given over periods of time ranging from 30-50 min.

CASE REPORTS

Case 1. J. D. was admitted to St. Christopher's Hospital for Children at 3 days of age for evaluation of kinky hair and jaundice. He was born weighing 2.9 kg and was the second child of his mother, who reported no complications of gestation. The second stage of labor was rapid but the Apgar score was 10 at 1 min of age. Examination at 8 hr of age at the referring hospital showed knots of friable kinky hair covering the scalp, a cephalohematoma, a small mandible, talipes equinovarus, thrombocytopenia (platelets 80,000/mm³), and icterus. A skull radiograph revealed multiple wormian bones.

Examination on admission showed mild lethargy and hypotonia but an intact Moro response, postural reflexes and deep tendon reflexes. Initially the serum bilirubin concentration was 17 mg/dl and it rapidly increased to 23 mg/dl. An exchange transfusion was performed without complications.

The family history indicated that a female sibling had died at 7 mo of age after a short illness complicated by vomiting and aspiration. A male cousin, W. D., was receiving therapy with intravenous copper for trichopoliodystr0phy in the Clinical Research Center of St. Christopher's Hospital for Children at the time of admission of J. D.

Studies to define the hair abnormality and abnormalities in copper metabolism were begun in the second week of life. A scalp biopsy showed miniature hair follicles, pili torti, and monilethrix. An abnormal fluorescent pattern indicated defective hair protein. As shown in Fig. 1, the serum concentration of copper at 21 days of age was 19 μg/dl (normal range (21 days), 75-90 μg of Cu/dl)⁴ and the hepatic concentration of copper was 14 μg/gm of dry weight. Examination of tissue from the liver biopsy showed extramedullary hematopoiesis and hemosiderin-laden Kupffer cells compatible with the healing phase of a hemolytic episode. Histologic sections obtained from a muscle biopsy performed at 3 wk of age showed abnormal subsarcolemal vacuolization and large, densely staining mitochondria. Normal serum levels for electrolytes, calcium, phosphorus, alkaline phosphatase, magnesium, serum glutamic oxalacetic transaminase, and serum glutamic pyruvic transaminase were repeatedly found.

Therapy with intravenous infusions of copper sulfate was started at 28 days of age with the administration of 10 μg/kg/day to two three times weekly (Fig. 1). The dose was gradually increased over a 9 wk period to 150 μg/kg/day. The serum levels of copper were measured daily, and serum glutamic oxalacetic transaminase and serum glutamic pyruvic transaminase levels were monitored twice weekly. No significant elevations of serum transaminase levels were observed during therapy. Hepatic levels of copper increased from 14 μg/gm of dry weight at 3 wk of age to 38 μg/gm of dry weight at 3 mo of age (Fig. 1). From 4 mo of age onward we were able to maintain the serum concentration of copper within normal range for age by intravenous therapy (Fig. 1). Measurement of copper levels in urine obtained during copper infusions indicated excretion of only 10% of the administered dose. Substitution of acetate for copper sulfate at the same dosage (150 μg/kg/day) had no effect on the pattern of response observed in the serum copper concentrations. No changes in pulse or respiration or neurologic signs were observed during copper infusions.

Growth and development were recorded during therapy. Measurement for height and weight followed the tenth percent-

Fig. 1. Increase in fasting serum (mean value of 5-7 daily levels) and hepatic copper levels in Patient J. D. with administration of Cu²⁺. Bar (upper section), μg Cu²⁺/kg/wk; NSR, normal serum level.⁴
Laboratory data were similar to those of other infants with trichopoliodystrophy. Four weeks after starting infusions of copper at 4 mo of age (Fig. 2) the serum ceruloplasmin was 38 U (normal adult values, 280-570 U), the hepatic copper concentration in a percutaneous hepatic biopsy specimen was 7 μg/gm of dry weight; the serum copper concentration was 12 μg/dl (normal range, 90-105 μg/dl). The changes in biopsied scalp tissue were similar to those of J. D. Sections of a muscle biopsy obtained at 4 mo of age demonstrated excessive subsarcolemmal vacuolization and pleomorphic, densely staining mitochondria. Mitochondria isolated from the muscle biopsy did not consume oxygen at a significant rate when incubated with glutamate, malate, and succinate, indicating defective energy metabolism. Roentgenograms showed wormian bones in the skull and multiple metaphyseal spurs with slight cupping in the humeri. Although an initial electroencephalogram was interpreted as normal, serial records demonstrated slow background frequencies with generalized paroxysmal discharges. Electroretinography and visual responses were normal. Values within the normal range were obtained for serum electrolytes, magnesium, zinc, calcium, phosphorus, alkaline phosphatase, transaminase, bilirubin, blood glucose, and blood urea nitrogen. Urinalysis and urine amino acid chromatography were not abnormal.

Therapy with intravenous infusions of copper sulfate was started at 4 mo of age in a dose of 10 μg/kg/day two to three times weekly and increased 150 μg/kg/day over a 14 wk period (Fig. 2). Eighteen weeks after the start of this regimen, the serum concentration of copper approached the normal range for this age (125-140 μg/dl). The hepatic concentration of copper at that time was 45 μg/gm of dry weight (Fig. 2). Serial transaminase levels were measured to monitor hepatic toxicity. When the serum copper concentration reached 154 μg/dl, an increase of the serum glutamic oxalacetic transaminase values to 90 U (normal, 3-27 U) was observed. However, the transaminase values were not further increased by continued copper infusions and returned to the normal range within 48 hr. A repeat muscle biopsy obtained when the serum values of copper approached the normal range showed no morphologic changes; a homogenate of the muscles showed intact energy metabolism as evidenced by a normal rate of 14CO2 production when incubated with pyruvate-14C. No alterations in vital signs or neurologic symptoms were observed during copper infusions.

The gradual increase in serum and hepatic concentrations of copper did not appear to alter progressive neurologic dysfunction. Hypotonia and a decrease in head control were noted 6 wk after initiation of therapy. Nasogastric tube feedings were required 2 wk later. At 9 mo of age, the patient had no head control and displayed only irritability when manipulated. Serial electroencephalograms showed progressive slowing and disorganization of background frequencies. No change in background frequencies or increase in paroxysmal features were noted in tracings obtained during administration of copper. No change in blood gas values or evidence of hemolysis was noted during or after copper infusions. At 12 mo of age (9 mo after the initiation of therapy) the child developed recurrent dia-
rhea. The serum copper values decreased to the 60-70 µg/dl range (normal, 120-140 µg/dl). Proteinuria, hypoproteinemia (total proteins 3.7 gm), and hypostenuria without evidence of glomerular failure were noted. Total intravenous ralimentation (FreAmine) was associated with an elevation of serum protein levels to 5.7 gm and serum copper values to the 100-120 µg/dl range without further infusions of cupric salts. The patient gradually became less responsive and died at 15 mo of age following an apneic episode. Normal values for serum electrolytes, blood urea nitrogen, and blood glucose were obtained prior to death. Tissue obtained at the time of postmortem examination showed the following copper values (µg/gm of dry wt): liver, 20.0; renal parenchyma, 240.0; frontal cortex, 1.0; corpus callosum, 1.0; cerebellar folia, 4.0.

DISCUSSION

The progressive neurologic dysfunction and the hair abnormality in trichopoliodystrophy were described in 1962, but the abnormality in copper metabolism characteristic of this disease has been documented only recently. Menkes and associates described five male infants who demonstrated a sex-linked recessive disorder characterized by failure to gain weight, abnormal hair (pili torti, monilethrix, trichorrhexis nodosa), seizures, spasticity, and death at ages ranging from 7 to 42 mo. Other investigators have emphasized typical facies and abnormalities in morphology and function of the optic fundus, including diminished retinal ganglion cells and microcyst of iridal pigment. Abnormalities in electroretinographic and visual evoked responses have been described. Roentgenographic studies have demonstrated occipital wormian bone formation, flaring of anterior rib ends, metaphyseal and diaphyseal periostial reaction, and abnormal cerebral vasculature (angiography). Danks and associates demonstrated defective absorption of copper from the gastrointestinal tract and low levels of serum, hepatic, and brain copper. A variety of both gross and specific biochemical abnormalities have been observed in studies on trichopoliodystrophy and suggest a defect in mitochondrial and microsomal oxidative metabolism which could be caused by low tissue copper levels. It is well established that copper is an essential component of many tissue oxidases, including cytochrome oxidase (a + a3) and ascorbic acid oxidase. The tissue copper deficiency provides an explanation for the observed neurologic dysfunction and for the osseous changes, which resemble those described in scurvy. Danks and associates have suggested that the copper deficiency may cause abnormalities of cross-linking in certain structural proteins and that these changes account for the characteristic vascular and hair alterations.

The studies reported by Danks and associates demonstrated that patients with trichopoliodystrophy failed to absorb Cu from the gastrointestinal tract but cleared this isotope normally from the serum after intravenous administration. This observation suggested that effective therapy for the disorder might be achieved by intravenous administration of cupric salts.

Our regimen was designed to raise the serum concentration of copper and to replenish tissue stores of this metal ion without causing a toxic reaction. We observed that a single intravenous administration of Cu2+ in dosages of 20-200 µg/kg/day resulted in a peak level in serum within 3 to 4 hr; a return to baseline levels occurred within 24 hours. The amount and frequency of intravenous Cu2+ dosage was increased progressively in order to achieve the normal serum concentration of copper of this metal ion; no significant sustained increase was reached in either patient until the dosage reached 550 to 850 µg/kg, respectively, divided into five daily doses over a 1 wk period. An elevation of approximately 20 µg/dl from baseline values was then noted 24 hr after the last dose. We also observed a concomitant elevation in serum ceruloplasmin, a finding reported previously by other investigators. The serum copper level then increased for 3 days without further infusions; after normal or near-normal concentrations were achieved, infusions of Cu2+ acetate, 190-220 µg/kg/day, were necessary once or twice weekly to maintain these concentrations. In one patient (W.D.) administration of Cu2+ was discontinued in order to determine the rate at which the serum concentration of copper declined without infusions. The serum concentration decreased from 146 to 64 µg/dl over a 13 day period. A decrease in hepatic copper concentration from 45 to 18 µg/kg of dry weight was observed during this period (Fig. 2). After normal serum copper values were again achieved by infusions, intravenous therapy was stopped and Cu2+ (3 mg/day) was administered to W. D. in combination with a protein hydrolysate preparation (Nutra-migen). A similar fall in serum copper concentration was observed, indicating that the provision of Cu2+ in the form of complexes with amino acids failed to bypass the postulated defect in gastrointestinal absorption. In both instances, the daily copper levels measured when infusions were discontinued show a decay curve suggesting that copper stores in various tissues are mobilized at different rates or different serum thresholds to support the serum copper concentration.

The clinical findings and course of our youngest patient have important implications in the pathogenesis of trichopoliodystrophy. We have found no reports of any other patient in whom the clinical findings of this disease could be observed in the first day of life. The ab-
normal hair and the low hepatic copper concentration measured to 3 wk of age (Fig. 1) suggest that inadequate copper was stored in fetal tissue during the latter part of gestation. Significant neurologic impairment in early infancy could be anticipated because copper is an important element in the developing central nervous system and it is therefore of interest that replacement of tissue copper as indicated by the rise in hepatic copper concentration (Fig. 1) has been associated with some gain in developmental skills in the younger patient. Although his level of functioning is 2 mo below his chronologic age, such factors as prolonged hospitalization, excessive manipulation, and delay in achieving normal serum and tissue concentrations of copper should be considered in judging the value of therapy. We found no description of spontaneous improvement in published cases of trichopoliodystrophy. Our data suggest that early diagnosis by determining tissue and serum concentrations of copper, and early institution of therapy to produce rapid elevation of these concentrations to values within the normal range may influence favorably the progressive neurologic dysfunction in trichopoliodystrophy.

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Wayne C. Johnson, M.D., Dermal Pathology Laboratory, The Skin and Cancer Hospital of Philadelphia, Temple University School of Medicine interpreted the scalp biopsies. Peter Koblener, M.D., was the Dermatological Consultant.

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REFERENCES
2. French, JH: Personal communication.