STUDIES ON COPPER METABOLISM. XIII. HEPATOLENTICULAR DEGENERATION [1]

By G. E. CARTWRIGHT, R. E. HODGES, C. J. GUBLER, J. P. MAHONEY, K. DAUM, M. M. WINTROBE, AND W. B. BEAN

(From the Departments of Medicine, University of Utah College of Medicine, Salt Lake City, and the State University of Iowa, Iowa City, Ia.)

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It has now been demonstrated repeatedly that in patients with hepatolenticular degeneration there is a disturbance in the metabolism of copper and amino acids. The amount of copper in the tissues, particularly in the liver and involved areas of the brain, is increased about ten-fold above the normal (1–4). The pigmented Kayser-Fleischer rings have been shown to contain copper (5, 6). Increased excretion of copper in the urine has also been demonstrated (7–9). The plasma copper level was originally considered to be normal or increased (3, 9, 10), but recent studies have shown that the plasma copper level is decreased (11–13). Normally, about 96 per cent of the copper in plasma is bound to a specific alpha-2 globulin, ceruloplasmin (14, 15). The remainder of the copper in plasma is more loosely bound to protein and is capable of reacting directly with sodium diethyldithiocarbamate (15). This direct-reacting fraction actively transports copper (15). It has now been demonstrated that in patients with Wilson's disease there is a reduction in the ceruloplasmin fraction (16, 17). Measurements of the direct-reacting fraction have not been made, but it has been noted that the ceruloplasmin content of the serum is consistently too low to account for the copper present (13, 16).

The aminoaciduria which accompanies this disease (18) involves most of the amino acids found in normal urine, but, in addition, proline and citrulline are excreted (19, 20). The urinary amino acid pattern varies widely, depending upon the stage of the disease and the protein composition of the diet. The aminoaciduria is independent of the severity of the hepatic disorder. It occurs even in patients with no manifest liver disease (21). No significant elevation of the blood alpha amino nitrogen level has been observed (10, 20, 22). It has been suggested that the aminoaciduria is the result of a renal lesion (20).

In addition to the aminoaciduria, an increased excretion in the urine of oligopeptides with dicarboxylic acids as terminal residues has been observed (21). Evidence has been presented that the copper is excreted in the urine, at least in part, in the form of an oligopeptide-copper chelate (23).

Various workers have demonstrated that following the administration of B.A.L. (dimercaptopropanol) (6, 7, 9, 10), glycine (10), alanine (10), "Versene" (calcium disodium salt of ethylenediamine tetra-acetic acid) (13), a high protein diet (13), and cortisone (13), the excretion of copper in the urine is enhanced. Clinical improvement has been reported in some patients following repeated courses of B.A.L. (6).

At the present time there is no agreement as to which of the metabolic defects is inherited and primary. Uzman (23) has proposed that the aminoaciduria is the primary defect since he observed this abnormality to be the single most constant metabolic feature of the disease, present even in five asymptomatic siblings of affected patients (21). However, Bearn and Kunkel (13) point out that the family studied by Uzman and Hood (21) is genetically atypical because of the large number of biochemically affected individuals described. A study of 35 siblings of affected patients failed to reveal aminoaciduria in any individual. Furthermore, increased amino acid excretion was absent in one patient. Bearn and Kunkel (13) conclude that aminoaciduria is not the primary defect or earliest manifestation of this disease and suggest that the fundamental defect is an increased absorption of copper. Scheinberg and Gitlin (16) have proposed that the inherited defect is a deficiency of ceruloplasmin. As yet

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abnormalities in copper metabolism have not been sought adequately in asymptomatic siblings.

Two different concepts concerning the metabolic factors operative in the pathogenesis of hepatolenticular degeneration have been proposed. Uzman (23) and Brick (24) suggested that the development of cirrhosis of the liver is a result of the chronic loss of amino acids. They call attention to the similarity of the de Toni-Fanconi syndrome with the same combination of aminoaciduria and cirrhosis. These investigators attempt to explain the accumulation of copper in the tissues as the result of a chelation between the copper and peptide residues, the latter accumulating in the tissues as a consequence of a fault in the metabolism of dicarboxylic amino acid peptides. They suggest, furthermore, that the oligopeptide-copper chelates pass from the tissues to the plasma and into the glomerular filtrate. The increased urinary excretion of copper would then be the result of the competition for tubular reabsorption between the oligopeptide complex and the amino acids present in high concentration in the tubular fluid.

On the other hand, Bearn and Kunkel (13) suggest that, as a result of increased absorption, copper accumulates in the tissues and, as a consequence of excessive deposition of copper in the liver and kidney, cirrhosis and damage to the tubular epithelium occur. This would be somewhat analogous to the development of cirrhosis of the liver secondary to the deposition of excessive amounts of iron in the liver, such as occurs in patients with hemochromatosis. The increased excretion of amino acids, peptides, and occasionally glucose would then be the result of the damage to the tubular epithelium, and the increased excretion of copper in the urine is interpreted as the consequence of the high tissue level of copper and the aminoaciduria and peptiduria.

The purpose of this communication is to report studies undertaken to explore further the abnormalities in copper metabolism in patients with hepatolenticular degeneration. On the basis of these studies a modified concept of the pathogenesis of the disease will be presented.

METHODS

The methods for the determination of total plasma copper (25), the direct-reacting fraction of plasma copper (15), erythrocyte copper (25), tissue copper (26), urine copper (27), plasma iron (28), the total iron-binding capacity of the plasma (29), urine protein (27), and electrophoretic analysis of the plasma proteins (30) have been described in previous publications. Total plasma protein, albumin, and globulin were determined by the biuret method (31) with the modification of Weichselbaum (32). Urinary alpha amino nitrogen was determined by the gasometric ninhydrin method of Van Slyke, MacFadyen, and Hamilton (33).

During the period of the copper balance studies the patients were hospitalized on a metabolic ward. The exact amount of each foodstuff eaten by each patient was weighed daily. An equal amount of each foodstuff was placed in a suitable container, sulfuric acid was added, and the material was digested slowly over a hotplate. The digests were then combined for each day and homogenized in a Waring blendor. The total volume was recorded and the amount of copper in an aliquot was determined. Total 24-hour urine collections were made daily. All stools were collected for the period, combined and processed as noted above for the food. The water intake was recorded daily and the amount of copper in the water was determined but was found to be insignificant (0.2 μg per 100 ml.). Particular care was exercised to avoid contamination of the specimens by copper. All glassware used was carefully cleaned with acid, rinsed with copper-free water, and air-dried. The amount of copper in the stool and food digests was determined by the method used for the determination of copper in urine.

RESULTS

Clinical data

Seven patients with hepatolenticular degeneration have been studied in detail. Total plasma copper and red cell copper were determined in one additional patient.[2] All seven patients had well developed Kayser-Fleischer corneal rings. With one exception (Dar. H.) to be discussed later, all of the patients manifested signs of neurologic disease such as tremor, dysarthria, and mental disturbances. Rigidity of the skeletal muscles was moderate to advanced in four of the patients. All patients except one (Del. H.) are still living. Additional clinical details are given in Table I. Liver function studies, plasma iron, and the total iron-binding capacity of the plasma are presented in Table II. No patient had glycosuria.

Family H. is of particular interest. The parents are fourth cousins and have had five children. The oldest child (Del. H.) is described in detail

[2] We are indebted to Dr. Ian Brown, Department of Neurology, University of Minnesota, for sending us a specimen of blood from this patient.

TABLE I

Clinical data

Patient	Age *yrs.*	Age at onset *yrs.*	Sex	Parental consanguinity	Siblings No.	Siblings No. involved	Type of onset	Hepatomegaly	Splenomegaly
D. C.	28	25	F	None	8	0	Tremor of arms	+	+
Del. H.	15	9	M	4th Cousin	4	2	Dysarthria	0	+
Dar. H.	10	8	F	4th Cousin	4	2	Hemolytic anemia	+	+
R. K.	21	9	M	None	1	0	Purpura	+	+*
W. K.	30	26	M	None	2	0	Tremor of arms	+	+
M. L.	20	18	M	None	5	0	Dysarthria	0	0
J. S.	29	26	F	None	2	0	Dysarthria	0	0

* Splenectomy for purpura at age 9.

in this paper. The next oldest child (La. H.), at the age of six, suddenly developed jaundice which was accompanied by vomiting, fever, anemia, hepatomegaly, and ascites. The degree of jaundice deepened progressively and the child died six weeks later. She had apparently been quite well prior to the onset of this illness and neither before or during the illness were any neurological abnormalities noted. The patient was not under our observation at any time and died in another hospital. Autopsy revealed cirrhosis of the liver. Examination of the brain was not performed and the eyes were not examined for the presence of Kayser-Fleischer rings.

The third child (Dar. H.) was examined after the diagnosis of Wilson's disease was made in the oldest sibling (Del. H.). Physical examination revealed a healthy looking, well-nourished, alert girl nine years of age. Spider angiomata were observed over the shoulders. The liver and the spleen were both palpable 2 cm. below the costal margin. The remainder of the physical examination was not unusual. No neurologic abnormalities were present. Past history revealed that at age eight the patient had an illness characterized by nausea, vomiting, fever, and jaundice. Laboratory examinations performed in another hospital revealed a hemoglobin of 4.4 gm. per 100 ml.; reticulocytes, 24 per cent; leukocyte count, 22,800 per mm.[3]; total serum bilirubin, 7 mg. per 100 ml. Examination of the blood smear showed anisocytosis, a marked degree of polychromatophilia and a few nucleated red blood cells. The patient was given several transfusions of whole blood and was thought to have recovered rapidly and completely. Since this illness she has remained asymptomatic and active.

Examination of the fourth child (Le. H.), age 5, and the fifth child (C. H.), age 1, has failed to reveal any significant abnormalities on physical examination. The plasma copper levels were 155 and 169 μg per 100 ml., respectively. The urinary levels of copper and alpha amino nitrogen were within the normal range and liver function studies were not remarkable.

Examination of both parents and the four grandparents has also failed to reveal any signifi-

TABLE II

Liver function studies, plasma iron, and total iron-binding capacity of the plasma

Patient	Total plasma proteins *gm./100 ml.*	Plasma albumin *gm./100 ml.*	Plasma globulin *gm./100 ml.*	B.S.P.* % retention	Thymol turbidity units	Ceph. floc.	Total plasma bilirubin *mg./100 ml.*	Plasma iron† *μg/100 ml.*	T.I.B.C.‡ *μg/100 ml.*
D. C.	6.0	3.2	2.8	32	6	2†	2.2	97	187
Del. H.	4.8	1.0	3.8	28	12	4†	2.8	23	101
Dar. H.	5.7	2.9	2.8	26	7	0	1.6	82	217
R. K.	6.7	3.7	3.0	8	5	0	0.6	24	184
W. K.	7.4	3.8	3.6	5	7	4†	0.5	70	180
M. L.	6.2	4.1	2.1	11	4	1†	0.8	71	221
J. S.	6.5	3.3	3.2	2	5	0	0.6	122	172

* Forty-five minutes after 5 mg. per kg. of body weight.
† Normal value, 110 ± 31 μg per 100 ml.
‡ T.I.B.C., total iron-binding capacity of the plasma. Normal value, 359 ± 30.8 μg per 100 ml.

cant abnormalities either by physical examination or by laboratory studies.

Plasma copper

The total plasma copper levels were significantly reduced in all eight patients (Table III). The lowest plasma copper level which we have observed in a study of a total of 228 normal subjects is 68 μg per 100 ml. The values in all eight patients were below this lowest normal value. The mean plasma copper value for the patients with Wilson's disease was 50 μg per 100 ml. as compared with a mean normal value of 116 μg per 100 ml.

The absolute amount of direct-reacting copper in the plasma of the patients was significantly increased (Table III) above the normal. The mean value for the group was 26 μg per 100 ml. as compared with a mean value of 8 μg per 100 ml. in the normal control group. In only one of the patients was the value within the normal range. In the normal subjects, 7 per cent (range, 0 to 23) of the total plasma copper was direct-reacting. In the patients with hepatolenticular degeneration, 52 per cent (range, 42 to 68) of the total plasma copper was in the direct-reacting fraction.

Subtraction of the direct-reacting fraction from the total plasma copper (Table III) results in a mean value of 24 μg per 100 ml. for the indirect-reacting fraction in the patients, as compared with a mean value of 108 μg per 100 ml. for the indirect-reacting fraction in normal subjects.

Spinal fluid copper

The concentration of copper in the spinal fluid was determined in six of the patients. The results are presented in Table III and are compared with the values obtained in 11 patients with various forms of neurologic disease or suspected neurologic disease other than hepatolenticular degeneration. The control group cannot be considered as normal, since in many of the patients studied the plasma copper level was elevated. In spite of this, in all six of the patients with Wilson's disease, the concentration of copper in the spinal fluid was increased above the control range and the mean value was three times the mean value observed in the control patients. Determination of the amount of spinal fluid copper reacting directly with sodium diethyl-dithiocarbamate revealed that in all six fluids obtained from the patients with Wilson's disease, all of the copper reacted directly. In the control group only about one-half of the spinal fluid copper reacted directly.

Erythrocyte copper

The amount of copper in the erythrocytes was determined in eight of the patients (Table III). The mean value (129 μg per 100 ml.) was not significantly different from the normal mean (115 μg per 100 ml.). In one patient (R. K.), the red cell copper was increased above the normal range.

TABLE III

Plasma, spinal fluid, erythrocyte and urine copper and urine alpha amino nitrogen and protein

Patient			(A) Total plasma copper μg/100 ml.	(B) Direct-reacting plasma copper μg/100 ml.	A−B μg/100 ml.	Spinal fluid copper μg/100 ml.	R.B.C. copper μg/100 ml.	Urine copper μg/day	Urine α-amino nitrogen mg./day	Urine protein gm./day
	Normal	No.	228	19	19	11	31	10	15*	10
		Mean	116	8	108	6	115	9	164	0.05
		±Stand. dev.	14	6.8		2.3	22	8.1		0.02
		Range	68–161	0–20	66–150	3–9	84–159	0–26	118–204	0.02–0.08
D. C.			65	27	38	10	97	415	397	0.22
Del. H.			33	12	21	16	130	115	295	0.30
Dar. H.			40	27	13		111	122	90	0.16
R. K.			44	23	21	18	212	119	484	0.33
W. K.			60	31	29	16	103	493	519	0.17
M. L.			47	22	25	24	138	611	271	0.34
J. S.			60	41	19	24	112	236	303	0.12
J. T.			52				130			
Mean			50	26	24	18	129	302	337	0.23

* Values given by Cooper, Eckhardt, Faloon, and Davidson (22).

Urine copper

Representative values for the daily excretion of copper in the urine are given in Table III. In all of the patients, the urinary excretion of copper was increased well beyond the normal range of 0 to 25 μg per 24 hours. The amount of copper excreted per day by a given patient varied somewhat from one period to another but rarely was it less than 100 μg per day. The highest single value observed in any patient without therapy was approximately 850 μg per 24 hours. In general, the excretion of copper in the urine ranged from 4 to 30 times the maximal normal value.

It should be noted that during the periods of study no attempt was made to maintain a constant intake of protein. Bearn and Kunkel (13) have shown that with an increase in dietary protein, there is an increase in the excretion of amino acids and copper in the urine.

It has been demonstrated in this laboratory (27) that in patients with the nephrotic syndrome, the amount of copper excreted in the urine is directly proportional to the amount of protein in the urine. In such individuals approximately 31 μg of copper were excreted per gram of protein. As shown in Table III, in patients with hepatolenticular degeneration, only traces of protein were excreted in spite of pronounced hypercupriuria.

Urine alpha amino nitrogen

The urinary excretion of alpha amino nitrogen was increased in six of the seven patients studied (Table III). In the seventh patient (Dar. H.) the values of 73 and 107 mg. per 24 hours (1.12 and 1.64 mg. per lb. of body weight per 24 hours) were within the normal range of 0.71 to 1.98 mg. per lb. of body weight per day given for children (34).

The values recorded in Table III represent the means of several determinations. Measurements of the urinary copper were not made on the same specimens as those in which amino acid was measured. When alpha amino nitrogen and copper determinations were performed on the same sample of urine, there was good correlation (correlation coefficient, + 0.78) between the amounts of alpha amino nitrogen and copper. This is similar to the finding of Bearn and Kunkel (13). It should be noted, however, that in the patient (Dar. H.) who excreted normal amounts of alpha amino nitrogen there was a significant degree of hypercupriuria.

Electrophoretic analysis of plasma proteins

Electrophoretic analyses were performed on the sera of two patients and on the plasma of five patients (Table IV). In several of the patients there was a reduction in the albumin fraction and an increase in the gamma globulin fraction. These changes were consistent with the degree of liver damage in these patients. The amount of alpha globulin was not reduced. This indicates that in such patients there is no over-all inability to maintain the alpha globulin level even though the alpha-2 globulin, ceruloplasmin, is greatly decreased.

Tissue copper

Analyses of the tissues for copper were made on the one patient (Del. H.) who died during the course of the study. The results are presented in Table V and are compared with those obtained on a 30-year old male subject, weighing 250 pounds, who was killed in an automobile accident. The

TABLE IV

Electrophoretic analyses of the plasma proteins

Patient	Total protein gm./100 ml.	Albumin gm./100 ml.	α_1 gm./100 ml.	α_2 gm./100 ml.	α_2 gm./100 ml.	β_1 gm./100 ml.	$\beta_2+\beta_3$ gm./100 ml.	γ gm./100 ml.	ϕ gm./100 ml.
D. C.*	5.80	2.62	0.35		0.47	0.40	0.34	1.62	
Del. H.*	6.22	1.87	0.62	0.43	0.37	0.50	0.43	1.99	
Dar. H.	5.90	3.02	0.41	0.41	0.06	0.53	0.18	1.00	0.29
R. K.	7.27	3.71	0.29		0.46		1.20	0.87	0.74
W. K.	8.12	4.40	0.41		0.85		1.00	1.00	0.46
M. L.	7.35	4.38	0.52		0.96		0.71	0.65	0.13
J. S.	6.22	3.18	0.55		0.73		0.69	0.67	0.40

* Serum.

TABLE V

Tissue copper analyses on a normal control subject and on a patient (Del. H.) with hepatolenticular degeneration

Tissue	Normal subject			Del. H.		
	Dry wt. μg/gm.	Wet wt. μg/gm.	Total organ μg	Dry wt. μg/gm.	Wet wt. μg/gm.	Total organ μg
Liver	12.2	3.9	8,112	532.0	98.4	49,200
Spleen	3.3	0.7	126	7.7	1.5	300
Kidney (R)	10.0	1.9	256	47.2	8.7	2,310
Adrenal gland (R)	5.0	1.5	8	9.6	2.6	30
Heart	12.2	3.8	1,178		3.0	510
Lung (R)	6.7	1.2	330	15.2	1.6	320
Muscle	4.6	1.0		10.0	1.9	
Pancreas				4.2	1.7	
Bile (*per 100 ml.*)		24.0			19.0	
Spinal cord	12.0	3.0		109.0	27.3	
Cortex (white matter)	13.9	4.3		144.0	41.7	
Cortex (gray matter)	15.9	3.8		327.0	65.3	
Cerebellum (white matter)	23.3	5.3		247.0	74.2	
Cerebellum (gray matter)	33.0	7.0		337.0	67.4	
Basal ganglion (R)	24.6	6.0		225.0	56.4	
Brain stem	8.0	2.0		237.0	58.6	

results are not entirely comparable since Del. H. was 15 years of age and weighed 80 pounds.

In the patient with Wilson's disease, as compared with the normal subject, the concentration of copper was increased in all of the tissues except the heart muscle. The increase was most marked in the liver and brain stem. However, the concentration of copper (wet weight) was increased 9 to 17 times that of the control value in all areas of the nervous system which were studied, including the spinal cord. The concentration of copper in the lung and in muscle was increased only slightly.

Copper balance studies

Copper balance studies were carried out in four patients with Wilson's disease and in three normal control subjects. Because it has been demonstrated that in patients with infection or with active rheumatoid arthritis there is an increase in the plasma copper level (35) and that under these circumstances there is an increased retention of copper in the body (36), balance studies were also carried out for comparison in two patients with active rheumatoid arthritis.

The results are presented in Table VI. Because the average daily copper intake was not the same in all individuals, the differences between intake and output are expressed in proportion to each mg. of copper ingested as well as in absolute amounts. It must be emphasized that, because of difficulties in analysis and collections, copper balance studies are extremely difficult to carry out accurately and the values given in the table represent only approximations.

In the normal control subjects, intake and output of copper were essentially the same (+ 0.01 mg. of copper per day per mg. of copper intake). The patients with hepatolenticular degeneration were in positive copper balance to the extent of about 0.56 mg. of copper per day per mg. of copper ingested. The patients with active rheumatoid arthritis were also in positive copper balance (0.27 mg. of copper per day per mg. of copper ingested), although less so than the patients with Wilson's disease.

The influence of potassium sulfide and of casein hydrolysate on copper balance

In order to determine whether a state of negative copper balance could be produced in patients with Wilson's disease, one of the patients (J. S.), after a control period of 10 days was given 20 mg. of potassium sulfide [8] by mouth three times daily with meals for a total of ten days. The rationale of giving potassium sulfide orally was that insoluble copper sulfide would be formed in the gastro-intestinal tract. It has been demonstrated in animals that copper sulfide is not absorbed (37). The period of potassium sulfide administration was then followed by a second control period of 11 days, and then one liter of casein hydrolysate was given slowly by the intravenous

[8] Potash sulfurated technical (Maelinckrodt).

TABLE VI

Copper balance studies

Group	Normal controls			Rheumatoid arthritis		Hepatolenticular degeneration			
Subject	A. G.	T. C.	B. H.	H. S.	V. H.	J. S.	W. K.	R. K.	M. L.
Period, *days*	10	10	10	7	7	10	11	11	12
Intake, *mg./day*	2.62	2.62	2.62	1.87	2.57	1.38	3.82	3.79	2.44
Output:									
Urine, *mg./day*	0.04	0.01	0.04	0	0	0.26	0.40	0.11	0.15
Stool, *mg./day*	2.99	2.55	2.21	1.41	1.79	0.53	1.30	0.96	1.02
Total, *mg./day*	3.03	2.56	2.25	1.41	1.79	0.79	1.70	1.07	1.17
Intake minus output *mg./day*	−0.41	+0.06	+0.37	+0.46	+0.78	+0.59	+2.12	+2.72	+1.27
Intake minus output *mg./day/1 mg. Intake*	−0.15	+0.02	+0.14	+0.25	+0.30	+0.43	+0.56	+0.72	+0.52
Mean for each group *mg./day/1 mg. Intake*		+0.01		+0.27			+0.56		

route daily for eight days in order to increase the excretion of copper through the kidneys.

The results are presented in Figure 1. The administration of potassium sulfide was associated with an increased excretion of copper in the stools but not in the urine, with the result that the overall copper balance changed from + 0.43 to − 0.14 mg. per day per mg. of copper intake. During the second control period, positive copper balance was re-established. The administration of casein hydrolysate was associated with an increased excretion of copper in both the urine and feces with the result that the copper balance was again changed from positive (0.21 mg. per day per mg. of copper intake) to negative (− 0.75 mg. per day per mg. of copper intake).

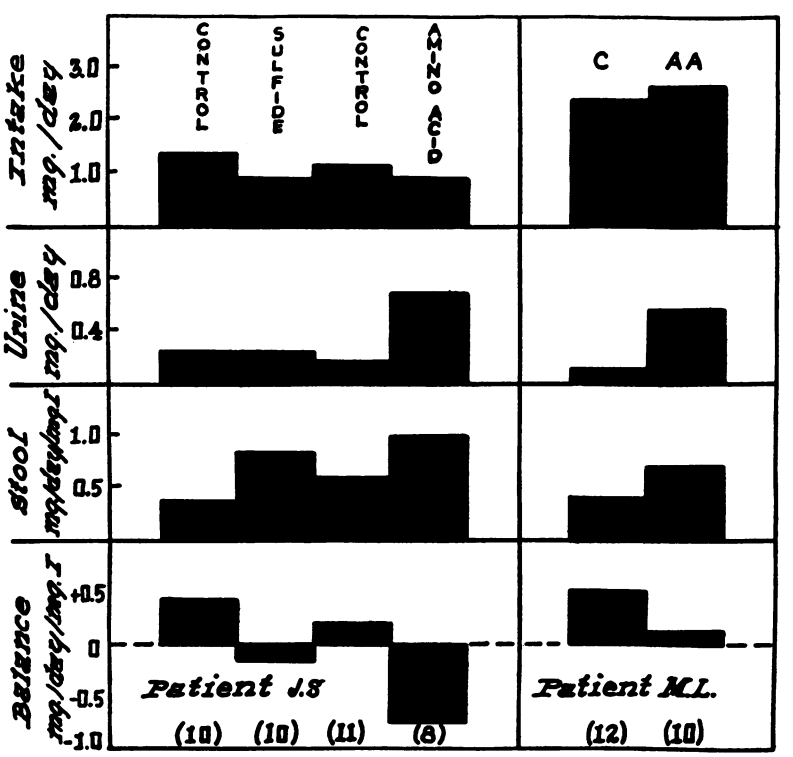

FIG. 1. THE INFLUENCE OF POTASSIUM SULFIDE (20 MG., THREE TIMES DAILY), AND CASEIN HYDROLYSATE (AMINO ACIDS, 50 GRAMS DAILY) ON COPPER BALANCE

TABLE VII

The influence of various agents on the excretion of copper in the urine

Agent	Patient	Period		Increase	%
		Control μg/day	Therapy μg/day	μg/day	Increase
B.A.L.	D. C.	423	974	551	130
	D. C.	287	1,237	950	330
	D. C.	241	1,085	844	350
	Del. H.	187	562	375	200
	Del. H.	220	563	343	156
Casein hydrolysate	D. C.	312	705	393	126
	Del. H.	101	538	437	432
	M. L.	152	565	413	272
	J. S.	180	693	513	286
	R. K.	106	381	275	260
	W. K.	442	907	465	105
B.A.L. + Casein hydrolysate	D. C.	256	1,111	855	367
	Del. H.	114	896	782	636
I.V. "Versenate"	D. C.	395	817	422	107
	D. C.	218	656	438	200
	Del. H.	161	217	56	35
	W. K.	362	785	423	117
Oral "Versenate"	D. C.	306	548	242	79
	R. K.	110	87	0	0
Sulfide	J. S.	267	273	6	4

A second patient (M. L.) was given one liter of casein hydrolysate intravenously daily for a 10-day period following a 12- day control period (Figure 1). Simultaneously with the administration of the amino acid mixture there was an increase in stool and urine copper and the over-all balance decreased from + 0.52 mg. per day per mg. of intake to + 0.13.

The influence of various chelating agents and potassium sulfide on the urinary excretion of copper

To study the influence of various agents on the urinary excretion of copper, B.A.L. (dimercaptopropanol), casein hydrolysate (amino acids), "Calcium Versenate" (calcium disodium salt of ethylene-diamine tetra-acetic acid), and potassium sulfide were administered to patients. B.A.L. was given intramuscularly in oil twice a day in an amount of 2.5 mg. per kg. of body weight. One liter of casein hydrolysate (5 per cent solution) was given intravenously over a two to four-hour period each day. Two hundred and fifty mg. of "Calcium Versenate" were given orally every hour for a total of 13 doses daily. "Calcium Versenate" was administered intravenously each day by adding two grams to 200 ml. of 5 per cent glucose. Potassium sulfide was given orally in an amount of 20 mg. three times a day with meals.

The results are summarized in Table VII. The administration of B.A.L., casein hydrolysate and "Calcium Versenate" was associated with a marked increase in the excretion of copper in the urine. These agents, in the amounts given, increased the urinary excretion of copper about 300 to 900 μg per day. This represented an increase of about 100 to 400 per cent. The mean increase with B.A.L. was 233 per cent; with casein hydrolysate, 247 per cent; with "Calcium Versenate," 115 per cent. When B.A.L. was given simultaneously with casein hydrolysate, the greatest increase in urinary copper occurred. The mean increase was 501 per cent.

The oral administration of "Calcium Versenate" was not accompanied by a significant increase in urinary copper. Although in one patient (D. C.) an increase occurred, this was probably due to the fact that "Calcium Versenate" was given intravenously in the period immediately prior to the oral administration of the compound (Figure 2).

The oral administration of potassium sulfide, as would be expected, was not associated with a rise in urinary copper (Table VII and Figure 1).

In Figures 2, 3, and 4, the influence of various

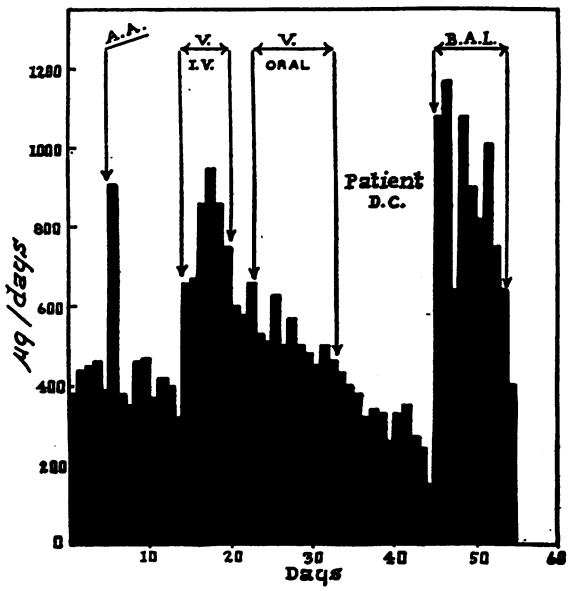

FIG. 2. THE INFLUENCE OF CASEIN HYDROLYSATE (AA), "CALCIUM VERSENATE" (V), AND B.A.L. (DIMERCAPTOPROPANOL) ON THE EXCRETION OF COPPER IN THE URINE

For experimental details consult the text.

agents on the excretion of copper in the urine is depicted graphically for purposes of comparison. It should be noted that there was no tendency for the amount of copper eliminated in the urine to decrease with each additional course of therapy. Likewise, after repeated courses, the baseline level of copper excreted was changed little or not at all. One patient (Del. H., Figure 4) was given B.A.L. for 15 consecutive days. In spite of the long course of B. A. L., and in spite of the fact that this followed soon after four other courses of therapy, there was little tendency for the amount of copper excreted to decrease.

Approximately 58 per cent of the copper excreted in the urine was dialyzable under the conditions stated in Table VIII. Following the intravenous administration of casein hydrolysate, the proportion of copper which was dialyzable remained the same, in spite of the fact that the total amount of copper excreted increased. Thus, following the administration of casein hydrolysate, there was also an increase in the non-dialyzable fraction of urinary copper. As would be expected, the per cent of dialyzable copper increased significantly following the administration of B.A.L. or "Versenate."

Five of the patients (Table IX) were treated with 20 mg. of potassium sulfide by mouth with meals three times daily for periods of four to 12 months. After such therapy, there was no significant change in the plasma copper level and the urinary excretion of copper was not significantly less. Four of the patients received no other form of therapy during this period of time. Clinical evaluation of the results was difficult. Although striking clinical improvement did not occur in any of the patients, it was our impression that the disease did not progress in any of them.

TABLE VIII

Per cent of urine copper dialyzable *

Agent administered	Number of determinations	% Dialyzable
None	10	58 (44–74)
Casein hydrolysate	6	53 (41–67)
B.A.L.	3	77 (72–81)
"Calcium Versenate," I.V.	1	84

* Cellophane casing (Visking Corp.). Twenty ml. of urine were dialyzed against one liter of distilled water for 24 hours at 5° C. The water was changed four times during this period.

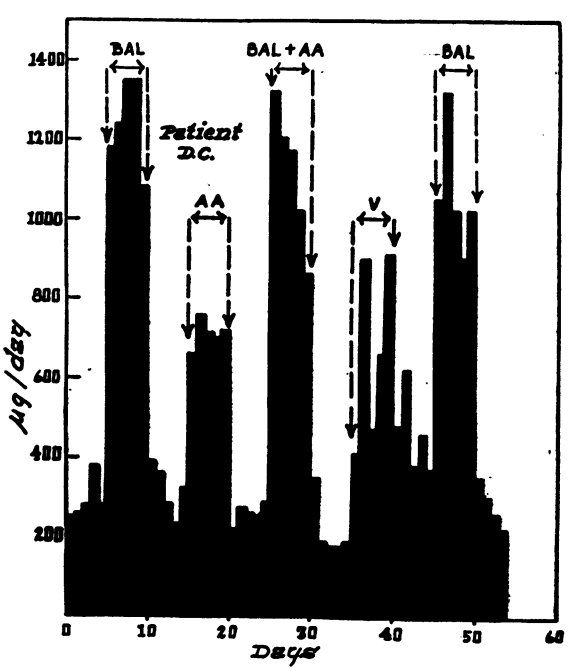

FIG. 3. THE INFLUENCE OF B.A.L. (DIMERCAPTOPROPANOL), CASEIN HYDROLYSATE (AA), AND "CALCIUM VERSENATE" (V, GIVEN INTRAVENOUSLY) ON THE EXCRETION OF COPPER IN THE URINE

For experimental details, consult the text.

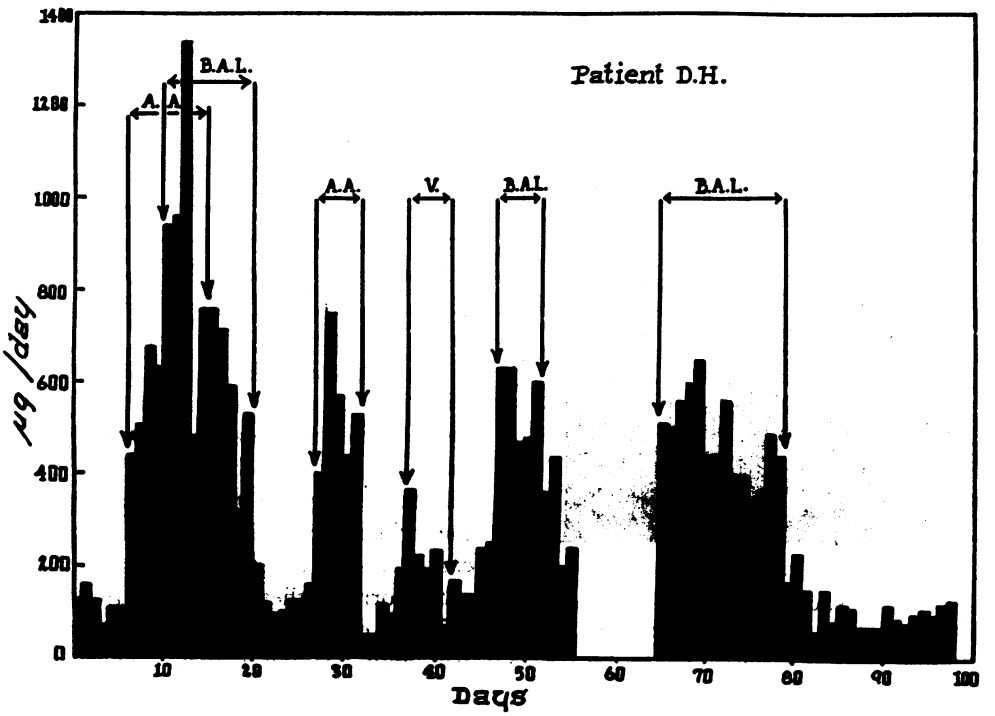

FIG. 4. THE INFLUENCE OF CASEIN HYDROLYSATE (AA), B.A.L. (DIMERCAPTOPROPANOL), AND "CALCIUM VERSENATE" (V, GIVEN INTRAVENOUSLY) ON THE EXCRETION OF COPPER IN THE URINE

For experimental details, consult the text.

TABLE IX

The influence of the prolonged administration of potassium sulfide on the plasma and urine copper levels

Patient	Duration of therapy *months*	Plasma copper *μg/100 ml.*	Urine copper *μg/day*
D. C.	0	71	415
	12	71	408
R. K.	0	43	185
	7	39	100
J. S.	0	57	181
	11	57	260
W. K.	0	43	297
	8	59	440
M. L.	0	36	837
	4	41	762

DISCUSSION

The total plasma copper level was significantly reduced in all of the patients in this study. This has also been observed by Bearn and Kunkel (12, 13, 38) whose reports appeared while these investigations were in progress. In addition, it has been demonstrated in this study that there is an absolute increase in the amount of direct-reacting copper, with the result that there is an even more striking reduction in the indirect-reacting fraction (ceruloplasmin) than is indicated by the reduced total plasma copper level. This adds support to the more direct observations of Scheinberg and Gitlin (16) that there is a marked deficiency of ceruloplasmin in patients with Wilson's disease.[4] The increase in the direct-reacting fraction is probably a manifestation of the extremely high tissue levels of copper and probably reflects the increased turnover of copper from the gastro-intestinal tract to the tissues and from the tissues to the excretory organs, the kidney, liver, and intestine. Previous studies have shown that the direct-reacting fraction is concerned in the transportation of copper (15). The copper in the direct-reacting fraction is not dialyzable *in vitro* and the evidence available suggests that this

[4] Recent studies in our laboratory (H. Markowitz) indicate that there is a high degree of correlation between the indirect-reacting fraction of plasma copper and ceruloplasmin determined immunologically.

copper may be bound to serum albumin (17). The observation that the concentration of copper in the spinal fluid is about one-half the value of the direct-reacting fraction in plasma suggests that the direct-reacting copper is not completely dialyzable *in vivo*. However, that a portion of it is dialyzable is suggested by the finding that both the direct-reacting fraction in the plasma of the patients and the concentration of copper in their spinal fluids was three times greater than the mean values found in the control subjects.

It is of considerable interest that there was a marked reduction in the indirect-reacting fraction of plasma copper in the patient (Dar. H.) with relatively early Wilson's disease, in the absence of an increase in the excretion of amino acids in the urine. Stein, Bearn, and Moore (20) studied one patient with well-advanced Wilson's disease with hypocupremia in whom there was no significant aminoaciduria. These observations suggest that the deficiency of ceruloplasmin may precede development of aminoaciduria, from which it may be suspected that the disturbance in copper metabolism may be the primary defect in this disease rather than the disturbance in amino acid metabolism. Earlier observations (3, 9, 10) of a normal or elevated plasma copper level with which our observations and those of Bearn and Kunkel are not in agreement might be explained by assuming that the copper analyses were in error, or, more likely, the direct-reacting fraction was increased to such a degree as to completely obscure the decrease in ceruloplasmin.

The observation that the tissue copper level is increased and that patients with Wilson's disease receiving a normal diet are in positive copper balance, must mean that in hepatolenticular degeneration, either the absorption of copper from the gastro-intestinal tract is increased, or the excretion of copper from the body is decreased. Theoretically, a decreased excretion of copper could result from an increased avidity of the tissues for copper or from a failure of one or more of the main excretory routes for copper to function properly. From the available evidence it cannot be stated dogmatically which of these mechanisms might be at fault in Wilson's disease. In normal subjects the main excretory route for copper is through the bile (39). In addition, copper may be excreted directly through the intestinal wall. The observation in one of our patients that the concentration of copper in the bile removed directly from the gall-bladder at autopsy was normal, together with the finding of Denny-Brown and Porter (6) of a normal concentration of copper in the bile in three of their patients, suggests that the biliary copper excretion is normal. Measurement of the amount of copper excreted directly through the intestinal wall has not been possible. That the affinity of the tissues for copper is increased, seems rather unlikely since the excretion of copper in the urine is increased and since the tissue copper can be readily mobilized by B.A.L. or casein hydrolysate. The administration of these agents to normal subjects is not associated with an increase in the excretion of copper in the urine (10, 13).

From the above observations and deductive reasoning it would seem logical to conclude that in patients with Wilson's disease, the absorption of copper from the gastro-intestinal tract is increased. This conclusion is in agreement with that of Zimdahl, Hyman, and Cook (40). However, it must be admitted that the studies to date have not completely eliminated the possibility that the excretion of copper into the intestinal tract is decreased. In fact, Bearn and Kunkel (17) have observed that the fecal excretion of intravenously administered copper^{64} is diminished in patients with Wilson's disease as compared with normal subjects.

Our determinations of the concentration of copper in the tissues of the one patient who died confirm the earlier observations (1, 3, 4) that in Wilson's disease there is an accumulation of copper in the tissues. The concentration of copper was increased about 10-fold in all of the tissues, except heart muscle, skeletal muscle, lung, and erythrocytes. This was true in spite of the fact that the patient had been given several courses of B.A.L., casein hydrolysate, and "Calcium Versenate" (Figure 4) four months prior to his death. It should be noted that these results are at variance with those of Palmer, Drew, and Chenoweth (2) who found essentially normal tissue copper levels in a patient with hepatolenticular degeneration who had been given four courses of B.A.L. therapy.

It is tempting to conclude, as have others (13), that the pathological lesions in the liver and brain,

and the functional renal defect, are the consequence of the accumulation of excessive amounts of copper in these organs. That copper may have such an effect is suggested by the cirrhosis of the liver which has been produced experimentally in rabbits by the administration of large amounts of copper (41) and the cirrhosis which has been described in sheep under natural conditions which are conducive to the retention of copper in the tissues (42, 43). Furthermore, in this laboratory it was possible to damage the renal tubular epithelium of rats by the administration of excessive amounts of copper (44). In further support of this concept is the observation that, after the mobilization of copper from the tissues, clinical improvement occurred in some patients (6). In one of our patients (D. C.) the degree of improvement has been extremely impressive. Prior to the first course of B.A.L. and potassium sulfide therapy, the patient was frankly psychotic and tremors and rigidity were marked. Approximately a month after therapy was initiated, the patient showed no mental abnormalities at all, the rigidity had entirely disappeared and the tremors were much less severe. With each subsequent course of therapy, the neurologic state has improved. That all patients do not improve, or that the improvement is only partial and temporary is not at all surprising, if consideration is given to the quantitative aspects of the problem. According to our findings, patients with this disease may be in positive copper balance to the extent of 0.5 to 2.5 mg. of copper per day. The chelating agents, in the amounts which have been used, mobilize about 0.5 to 1.0 mg. per day. In one patient the influence of the agents was not great enough to establish a negative balance. Furthermore, these agents are not given continuously and positive balance is reestablished as soon as therapy is discontinued. When these facts are taken into consideration, it is more surprising that a few of the patients improve temporarily, rather than that all of the patients are not benefitted. To produce marked improvement it may be expected that therapy with the available agents will have to be more energetic and more continuous than heretofore or that more effective agents will have to be found.

With our present knowledge and the agents available, the most effective combination of drugs and schedule of therapy might be outlined as follows. The patients should be given as high a protein diet as possible. In this way, the aminoaciduria will be increased, and greater quantities of copper will be excreted in the urine. In addition, the patients should be given 20 mg. of potassium sulfide with each meal in order to decrease the absorption of copper. Five day courses of B.A.L. (2.5 mg. per kg. twice daily) or casein hydrolysate (one liter of a 5 per cent solution per day) should be given every 10 days until maximum improvement occurs. Ideally, the above regimen should be maintained continuously.

From the data presented in this paper a working hypothesis to explain the pathogenesis of Wilson's disease may be outlined. It is anticipated that as further data accumulate, this hypothesis will require modification. Furthermore, it must be conceded that this hypothesis does not explain adequately all of the observations which have been made to date.

According to this hypothesis, the primary defect in hepatolenticular degeneration is a congenital inability to synthesize ceruloplasmin. As a result of the deficiency of ceruloplasmin, the absorption of copper from the gastro-intestinal tract is increased and the direct-reacting fraction of plasma copper is increased. The deposition of excessive amounts of copper in the brain and liver causes the characteristic pathologic lesions in these organs. The accumulation of copper in the kidneys produces a functional impairment in the reabsorption of certain amino acids and peptides. Aminoaciduria and peptiduria result. As a consequence of the accumulation of copper in the tissues, copper is chelated by amino acids and peptides and is excreted in increased amounts in the urine.

The above concept is in harmony with that of Scheinberg and Gitlin (16) and with that of Bearn and Kunkel (12, 13, 17).

This hypothesis assigns to ceruloplasmin an important role in regulating the amount of copper absorbed. It does not explain how this function is achieved. Admittedly, it is difficult to visualize how ceruloplasmin regulates absorption since the copper in ceruloplasmin is apparently not in equilibrium with the direct-reacting copper. Perhaps as a result of the deficiency of ceruloplasmin, cop-

per is absorbed and retained in excessive quantities in an effort to make more ceruloplasmin, or a precursor of ceruloplasmin might accumulate.

From these speculations it is apparent that information is very much needed concerning the form in which copper is deposited in the tissues in Wilson's disease. The possibility must not be overlooked that ceruloplasmin may not be the only copper protein which is deficient. Studies concerning other copper proteins such as hepatocuprein, tyrosinase, and butyryl Co A dehydrogenase should be undertaken. Our observation that the erythrocyte copper level is normal, and the fact that anemia is not consistently observed in patients with this disorder suggests, although it does not prove, that the copper protein of the red cells is intact, and that, therefore, the impairment of copper protein synthesis does not involve all of the copper proteins.

It is of interest to speculate on one additional observation. A severe hemolytic anemia of sudden onset preceded by one year the diagnosis of Wilson's disease in one patient (Dar. H.). In three of Wilson's patients (45) an attack of jaundice antedated the onset of the clinical syndrome. Others have reported a similar event. A disease, known as "Enzootic Jaundice," has been described in sheep in Australia (42, 43) and has been produced experimentally (46). In this condition, for reasons which are not understood, the tissues retain excessive quantities of copper. The sheep suffer no apparent ill health until suddenly there is a "catastrophic liberation" of copper into the blood stream. The plasma level of copper increases to values of more than ten times normal. Severe hemolytic anemia, jaundice, and hemoglobinuria follow. Death frequently supervenes as a result of severe renal failure. If the animals survive and suffer two or three such crises, cirrhosis of the liver then results. By comparison it is possible that such crises may occur in patients with Wilson's disease. The severe hemolytic anemia in our patient may have been related to such an event. This suggestion is made in the hope that it will encourage investigators who should have the opportunity to study in detail courses of jaundice occurring in patients with this disease.

SUMMARY

A. Various aspects of copper metabolism have been studied in seven patients with hepatolenticular degeneration. The following abnormalities have been demonstrated:

1) A decrease in the plasma total copper level;
2) An increase in the direct-reacting fraction of plasma copper;
3) a decrease in the indirect-reacting fraction (ceruloplasmin) of plasma copper;
4) an increase in the concentration of copper in the spinal fluid, all of which was direct-reacting;
5) a large increase in the concentration of copper in all of the tissues studied, except heart muscle, skeletal muscle, lung, and erythrocytes;
6) a state of positive copper balance; and
7) an increased excretion of copper in the urine.

B. A patient is described with early Wilson's disease, in whom the indirect-reacting fraction of plasma copper was markedly depressed in the absence of an increased excretion of amino acids in the urine.

C. As a tentative hypothesis to explain the observations, it is proposed that in such patients there is a congenital deficiency in the formation of ceruloplasmin. As a consequence, the absorption of copper is increased and copper accumulates in the tissues. As a result of the accumulation of copper in the brain, liver, and cornea, the characteristic pathological lesions of the disease appear. The accumulation of copper in the kidney causes a functional impairment in the reabsorption of certain amino acids and peptides, and aminoaciduria and peptiduria result. With the accumulation of copper in the tissues, copper is chelated by amino acids and peptides and is excreted in increased amounts in the urine. The increase in the direct-reacting fraction of copper in the plasma is a manifestation of the extremely high tissue levels of copper and reflects the increased turnover of copper between the gastro-intestinal tract, the tissues, and the excretory routes.

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