HEPATOCEREBRAL DISEASE AND COPPER METABOLISM

MIKIO ASAKURA

1st Department of Internal Medicine, Nagoya University School of Medicine
(Director: Prof. Susumu Hibino)

In 1912 Kinner Wilson[1] studied 6 cases of a rare nervous disease accompanied by cirrhosis of the liver. He studied in addition six similar cases which Gower, Ormed, Freichs and others had reported. Wilson named this disease "progressive lenticular degeneration" and published detailed clinical and histopathological investigations of this disease which was characterized by marked pathological changes in the liver and brain stem. Hall later in 1921 called this same disease "hepatolenticular degeneration".

Recently the existence of an abnormal copper metabolism in this disease was reported by Glazebrook,[2] Mandelbrote,[3] Cumings,[4] Porter,[5] Sullivan,[6] Hyman[7] etc., and aminoaciduria by Uzman, Denny-Brown,[8] Cooper,[9] Bean,[10] Davidson[11] etc.

Another disease which differs from hepatolenticular degeneration has been called hepatocerebral disease by N. Konovalov[12] and the name is used quite often today for patients with diseased liver accompanying extrapyramidal manifestation.

Furthermore the name of "portal systemic encephalopathy" was given to a associated syndrome of neuropsychiatric changes and liver disease by Scherlock[13] *et al.* recently.

Thus the entity of hepatocerebral disease seems to have become clearer step by step by clinical, neurological and histopathological studies. However reports of systematic studies on copper metabolism to clarify the entity of hepatocerebral disease are very rare.

This paper deals with the following aspects.

1) Method of copper determination (Brawn-Schaeffer-Asakura's method).

2) Correlation between cerebellar dysfunction and blood copper level.

3) Studies on copper metabolism and hepatic function of various liver diseases at different clinical stages.

4) Studies on the mechanism of abnormal copper metabolism in Patients with nephrotic syndrome.

5) Studies on copper metabolism in patients with copper intoxication.

6) Correlation between blood copper level and various plasma protein fractions.

7) Studies on the influence of various substances on blood and urinary copper.

Received for publication September 15, 1958.

1. Copper Determination (Brawn-Schaeffer-Asakura's Method)

The appearence of a colorimetric method instead of a weighing method, namely a micro-quantitative analysis of copper (in gamma unit) rendered the method of analysis in addition to a more accurate one. At this point, Elvejem[14]-Sindow's modification method seems to be classical. On the contrary the dithion method is the most accurate procedure at present. This method has been studied by Callan, Handerson, Tompsel, Schmidt etc. and in 1940 Brawn and Schaeffer[15] made possible the determination of such small quantity 0.2 to 2.0 gamma of copper, but the only trouble with this method is the timeconsuming and cumbersome, technique of wet ashing for the destruction of organic materials in order to librate the copper from metal protein.

In 1952 the Author[16] modified this method completely especially the wet ashing method. Recently this Brawn-Schaeffer-Asakura's method has been proved to be accurate, simple and rapid by other investigators. This procedure can also be used for checking the accuracy of other methods of copper determination.

Proposed method for blood and urinary copper determination.

In Brawn-Schaeffer-Asakura's method the experiment is carried out in one apparatus all the way from wet ashing to colorimetry, without transferring the contents to another flask after the wet ashing. Fig. 1 shows the apparatus for copper determination.

With this method, there is no fear of losing the copper by spattering during wet ashing or by transferring the contents to another flask from 100 ml Kjeldal flask.

The time needed is only 10 to 15 min. for the wet ashing of one sample of blood or urine.

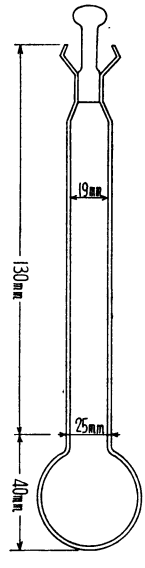

FIG. 1

Reagents

1) Redistilled copper free water.
2) Concentrated sulfuric acid.
3) Concentrated perchloric acid.
4) Concentrated Isoamylalcohol.
5) A mixture of 50% K_2CO_3 100 cc and 4 gm of $Na_4P207 \cdot 10H20$.
6) 0.5% solution of sodium diethyl dithiocarbamate ("Wako").
7) Standard copper solution (400 gamma % solution).
   Resolve 15.7 mg of $CuSO_4 \cdot 5H_2O$ in 1 000 ml. All glassware must be cleaned, acid washed and finally rinsed 3 times with redistilled water.

Determination of whole blood copper

1.0 ml of whole blood is placed in the apparatus shown in Fig. 1. Add 0.8 ml of concentrated sulfuric acid and 0.6 ml of concentrated perchloric acid. Wet ashing should next be watched carefully and continued until the mixture

become approximately 1.0 ml of a water clear solution determined by holding the 2 apparatus with a wooden holder.

After cooling, add approximately 5 to 8 ml of redistilled water, and 5 ml of pyrophosphoric-carboxylate solution. Shake well and add redistilled water up to the mark, Number 25. Add 5 ml of amylalcohol and 0.3 ml of carbamate solution. Then the stopper is set. Shake again, and place it upright for approximately 15 to 20 min. without the stopper. The supernatent of amylalchohol (yellow) is used for reading, in a Beckman spectrophotometer at a wave length of 434 mμ. A blank is prepared in a similar manner.

Determination of urinary copper

10 or 20 ml of urine is transferred into apparatus of Fig. 1. The procedure is carried out in a manner similar to that for blood copper determination.

Standard curve

Different dilutions of copper solution as shown in Table 1 are prepared from the 400 gamma % standard copper solution. Wet ashing and colorimetry by a Beckman spectrophotometer for each one ml of the solution are carried out. From the reading of the Beckman spectrophotometer, a standard curve can be obtained. The concentration of copper is calculated from the following formula.

TABLE 1

Redistilled water (ml)	400 γ% Cu-standard solution (ml)	Concentration (diluted) (%)	Actual copper	Optical density	$E-E_0$
0	0			0.075	
7	1	50	0.5	0.091	0.016
3	1	100	1.0	0.115	0.04
1	1	200	2.0	0.164	0.086
1	3	300	3.0	0.208	0.133
		400	4.0	0.247	0.172

Whole blood copper

Copper in gamma per 100 ml blood $2\,330\ (E-E_0)$

Urinary copper

Copper in gamma per 100 ml urine

$116\ (E-E_0)$ When 20 ccm of urine used

$233\ (E-E_0)$ When 10 ccm of urine used,

Where E is optical density of the sample and E_0 the optical density of the blank.

Determination of the wave length of 434 mμ

As shown in Table 2, 100 gamma % and 300 gamma % of copper solutions are studied. The maximum optical density is obtained from the Various optical densities at different wave lengths of both copper solutions,

TABLE 2. Optical Densities of Wave Lengths 410 mμ to 490 mμ

100 γ% Cu solution		300 γ% Cu solution	
Wave length (mμ)	O.D.	Wave length (mμ)	O.D.
410	0.071	410	0.129
420	0.096	420	0.182
430	0.113	430	0.213
432	0.116	432	0.218
434	0.116	434	0.219
436	0.116	436	0.218
440	0.115	440	0.218
450	0.105	450	0.1975
460	0.084	460	0.161
470	0.062	470	0.125
480	0.050	480	0.098
490	0.038	490	0.007

Recovery (%) of Copper added to Whole Blood and Urine

Sample	Original	Added	Total		Recovery (%)
			Calculated	Showed	
Whole blood	117	560	167	163	97.6
〃	117	100	217	209	96.3
〃	128	150	278	274	98.5
Urine	82	50	132	135	102.2
〃	58	100	158	163	103.1
〃	62	150	212	217	102.3

Normal values of blood and urinary copper

The mean value of blood copper level of 43 normal individuals (adult) was 127±12 gamma per 100 ml. As shown in Table 3, in 13 subjects in whom the plasma protein fractions were estimated electrophoretically, the mean was 115 gamma per 100 ml. In 3 of 13, the value was 70 (M), 82 (F) and 82 (F) gamma per 100 ml. The mean value of urinary copper in 10 normal subjects was 56 gamma per day with a range of 16 to 96 gamma per day.

TABLE 3. Normal Subjects

Subject	Blood Cu (γ%)	T.P. (g/dl)	Alb. (%)	Glob. (%)	(%)	(%)	(%)	(%)	A/G
1	117	7.8	58.8	35.3	5.9	11.8	5.9	17.6	1.66
2	140	7.4	60.0	33.2	6.3	12.6	6.8	14.3	1.80
3	117	7.3	59.0	35.1	6.0	11.9	5.9	17.2	1.68
4	128	8.1	61.6	33.3	4.3	11.3	5.0	17.7	1.84
5	117	8.4	56.3	39.0	5.5	11.7	4.7	21.8	1.44
6	70	8.2	60.4	33.3	7.3	8.3	6.3	17.7	1.81
7	140	8.2	59.2	34.6	6.2	9.9	6.2	18.5	1.71
8	93	7.4	58.7	34.7	8.0	9.4	6.6	17.3	1.69
9	82	7.9	59.7	35.6	6.6	12.2	4.7	16.8	1.67
10	140	8.8	54.8	38.4	6.9	15.1	6.8	16.4	1.47
11	93	7.4	58.9	34.3	6.9	11.0	6.8	16.4	1.71
12	82	7.2	57.4	36.1	6.6	13.1	6.5	16.4	1.59
13	186								
Mean	115	7.8	58.7	35.2	6.4	11.5	6.0	17.3	1.67

2. Correlation between the Degree of Cerebellar Dysfunction and Blood Copper Level

Clinical analysis of dyskinetic phenomenon

The evaluation of the motor phenomenon is one of the most important clues in the diagnosis of hepatolenticular degeneration. The characteristics of the clinical picture and of the pathologic changes in the brain stem should be stressed. In 1950 E. Herz[17] emphasized that careful observation of the various forms of tremor in organic lesions is very important. There have been a number of observations on nervous abnormalities associated with various liver diseases in human so called hepatocerebral disease. These nervous changes are often very similar to those of hepatolenticular degeneration and some of parkinsonism. In this chapter a careful systematic analysis of the dyskinetic phenomenon in 2 patients with hepatolenticular degeneration, 45 patients with various nervous diseases, 3 cases of copper intoxication and 21 patients with various liver diseases were made, together with blood copper determinations.

METHODS

The pathologic process in hepatolenticular degeneration is not confined to the lenticular nuclei. Konovalof,[10] Miskolczy and others have called attention to lesions in the cerebellum. Discoordination like that caused by lesions of the cerebellum or its pathway may be present without any spontaneous abnormal involuntary movement, such as that produced by striatal lesions. So clinical analysis of the dyskinetic pattern presented here was based mainly on the cerebellar function tests. A total of 69 patients were studied for the following cerebellar function tests, which is used routinely daily at our department of neurology.

Most patients were in-patients and some out-patients.

1) Manifestation of asthenia, fatiguability and slowness of movement
2) Manifestation of hypotonia or muscle flaccidity
   Palpation of extremities (muscle)
   Babinski tonus test (elbow)
   Shoulder shaking test
   Arm tapping test
   Barognosis
   Tendon reflex (knee jerk)
   Pendulation of the legs
3) Manifestation of abnormal posture and gait
   Deviation of head or body toward affected side (hemisphere)
   Fall backward or forward (vermis) toward side in standing (H)
   Spontaneous deviation in stretched extremities (H)
   Barany's test (H)
   Romberg's sign and one foot with eye opened and closed gait;
      Wartenberg's sign
      Fournier's test
      Mittelmeyer's test

Compass gait test

4) Manifestation of speech disturbances
Slow, slurred, scanning, staccato, ataxic, drawing, explosive, jerky, monotony etc.

5) Manifestation of nystagmus
Deviation nystagmus
Vestibular nystagmus
Asthenic nystagmus

6) Abnormal handwriting
Macrographia or difficulty with writing

7) Manifestation of asynergy;
Finger to finger test, F to N test
Heel to knee test
Nose-finger-nose test
Elevation of the leg in a supine position
Sitting up from the recumbent position
Leaning backward, forward and lateraly

8) Manifestation of dysmetria
Knee bending test (imitation test)
Dividing test
Line drawing test

9) Manifestation of dysdiadokokinesis
Rapid tapping of the fingers
Alternate opening and closing of the fist
Alternate successive pronation and supination of the hands
Rebound pheomenon of Gordon Holmes

10) Manifestation of involuntary movement
Static tremor, kinetic tremor, static intentional tremor
Athetoid movement, choreatic movement.

To facilitate appraisement of the degree of cerebellar dysfunction, each of the above ten items were scored as follows;

Plus 1·····one test is positive.
〃 2·····two test are positive or stronger reaction in one test.
〃 3·····more than 3 tests positive or strongest in one test.
+·····slight reaction in one test.
−·····no test positive.

Quantitative appraisal is shown in Tables 4, 5, 6 in which the total score of each patient is presented.

Asthenia, slowness and fatigability were examined by questioning the patient and by inspection during the performance of other tests on cerebellar function. Knee jerk and Babinski tonus tests were most reliable for the study of hypotonia. For gait examination, few patients were positive only in Fournier's test and Mittelmeyer's test. Turning around seems to be difficult in some patients and found to be valuable for gait examination. Handwriting was studied all round by print and script, of capacity to write his name, address and date etc. spontaneously, from dictation, and by copying. A letter written by the patient recently was a great help.

TABLE 4. Degrees of the Cerebellar Dysfunction

Name	Sex	Age	Diagnosis	Blood copper (γ%)	1	2	3		4	5	6	7			8		9		10					Total score
							A	B				A	B	C	A	B	A	B	A	B	C	D	E	
1 W.A.	♂	56	Wilson's d.	183	2	3	P 1	G 2	3	0	2	U 1	L 1	T 2	U 1	L 1	F 2	H 1	S 1	I 1	SI 1	A 0	C 1	26
2 M.H.	♂	10	〃	163	0	1	0	1	3	0	2	0	0	1	0	1	2	1	0	0	1	1	0	14
3 O.Y.	♂	70	Parkinsonism	326	1	0	0	1	1	0	2	1	0	0	0	0	0	2	0	0	1	1	0	10
4 M.Z,	♂	71	〃	128	3	1	1	1	1	0	1	1	1	2	1	1	1	2	1	0	0	0	0	18
5 A.K.	♂	55	〃	408	2	1	1	1	2	0	1	1	1	2	0	1	1	1	1	0	0	0	0	16
6 I.N.	♀	56	〃	221	1	1	0	0	0	0	1	1	0	1	0	1	1	1	1	0	1	0	0	10
7 I.T.	♀	58	Cerebellar ataxia	47	0	0	1	1	0	0	1	1	0	1	0	1	2	1	1	1	1	0	0	12
8 Y.G.	♂	70	〃	46	0	1	0	1	I/2	0	I/2	0	0	0	0	1	1	0	0	2	1	0	0	8
9 M.H.	♂	48	〃	233	1	0	0	1	1	1	0	0	1	0	0	0	I/2	1	0	0	0	0	0	6.5
10 H.U.	♂	68	〃	82	1	1	0	0	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	4
11 Y.H.	♂	38	〃	326	2	1	0	2	1	1	0	1	1	1	0	1	0	1	0	1	1	0	0	14
12 M.A.	♀	21	M.S.	221	0	0	0	1	1	0	0	1	1	1	0	1	0	0	0	1	1	0	0	8
13 N.M.	♂	43	〃	105	2	1	0	1	2	1	1	1	1	1	0	1	1	1	0	1	1	0	0	16
14 N.U.	♀	33	〃	82	0	I/2	0	1	I/2	1	0	0	0	1	1	0	0	I/2	0	0	0	0	0	5.5
15 W.Z.	♂	38	〃	130	1	0	0	1	I/2	1	0	1	0	0	0	0	1	0	0	0	1	0	0	6.5
16 I.T.	♀	19	〃	105	1	0	0	1	0	1	0	1	0	0	I/2	0	0	1	0	0	0	0	0	5.5
17 F.U.	♂	23	〃	58	1	0	I/2	1	2	0	2	1	2	1	1	0	2	2	1	1	0	0	0	17.5
18 T.T.	♂	35	Cerebellar tumor	226	1	0	0	1	I/2	0	I/2	I/2	I/2	1	I/2	1	1	I/2	0	0	1	0	0	9

TABLE 4. (*Continued*)

Name	Sex	Age	Diagnosis	Blood copper (γ%)	1	2	3		4	5	6	7			8		9		10					Total score
							A	B				A	B	C	A	B	A	B	A	B	C	D	E	
19 F.D.	♀	49	Cerebellar tumor	214	1	0	P 0	G 0	0	0	I/2	U I/2	L 0	T 1	U 0	L 0	F 1	H I/2	S +	I +	SI +	A 0	C 0	7.5
20 K.Z.	♀	62	Cerebral thrombosis	256	2	1	0	3	2	0	2	1	1	1	1	1	2	1	0	0	0	0	0	18
21 I.E.	♂	53	〃	559	2	I/2	I/2	3	2	0	1	1	1	1	0	1	2	1	0	0	0	0	0	16
22 F.K.	♂	64	〃	133	1	I/2	0	1	I/2	0	0	0	I/2	1	I/2	0	I/2	0	0	0	1	0	0	6.5
23 Y.K.	♀	45	〃	198	1	I/2	0	0	0	0	0	0	0	I/2	0	I/2	I/2	0	0	0	0	0	0	3
24 K.K.	♀		Syringomyelia	256	1	1	0	I/2	0	1	0	I/2	0	0	I/2	0	I/2	0	0	0	0	0	0	5

P: posture, G: gait. U: upper extremities, L: lower extremities, T: trunk. F: finger, H: hand. S: static tremor, I: intention tremor, SI: static intentional tremor, A: athetoid movement, C: choreatic.

TABLE 5. Degrees of the Cerebellar Dysfunction

Name	Sex	Age	Diagnosis	Blood copper (γ%)	1	2	3		4	5	6	7			8		9		10					Total score
							P	G				U	L	T	U	L	F	H	S	I	SI	A	Ch	
25 K.M.	♀	51	Right sided hemichorea	140 233	2 3	0 0	1 2	3 3	1 2	0 0	1 2	1 1	1 1	1 1	1 1	1 1	1 2	1 1	0 0	0 0	0 0	0 0	1 2	16 21
26 T.T.	♂	52	Cranial hypertension	70	0	1	0	I/2	0	0	0	0	0	1	0	0	I/2	0	I/2	1	1	0	0	5.5
27 Y.Y.	♂		〃	133	I/2	0	0	I/2	0	0	I/2	I/2	0	0	0	0	I/2	0	0	0	0	0	0	2.5
28 O.K.	♂	22	Spinal ataxia	175	0	1	0	1	1	1	0	0	0	1	0	1	0	0	0	0	I/2	0	0	6.5
29 M.S.	♂	36	Spinal syphilis	117	0	I/2	0	1	0	0	I/2	I/2	0	0	0	0	0	0	0	0	0	0	0	2.5
30 F.T.	♂	48	〃	123	1	I/2	0	1	I/2	0	I/2	I/2	0	I/2	0	0	1	0	0	0	I/2	0	0	6.0
31 K.Z.	♂	58	Progressive paralysis	303	0	0	0	1	0	0	0	0	0	1	0	I/2	1	1	0	1	0	0	0	5.5

32 K.M.	♂	53	〃	233	2	1	0	1	1	0	1	1	1	1	0	1	2	1	0	0	0	0	13
33 E.T.	♂	43	Spinal tumor	160	1	0	0	1	0	0	I/2	0	0	1	0	0	1	1	0	0	0	0	5.5
34 F.H.	♀	21	Postencephalitic	82	0	0	0	I/2	0	0	I/2	0	0	0	0	0	0	0	0	0	0	0	1
35 H.S.	♀	28	Myasthenia gravis	222	1	1	0	1	0	0	I/2	0	I/2	I/2	0	0	I/2	I/2	0	0	0	0	5.5
36 I.S.	♀	22	〃	193	1	1	0	1	0	0	0	0	0	I/2	0	0	0	0	0	0	0	0	3.5
37 S.T.	♀	25	Polio	186	I/2	0	0	1	0	0	0	0	I/2	0	0	I/2	0	0	0	0	0	0	2.5
38 M.U.	♂	23	〃	117	0	0	0	1	0	0	0	I/2	0	0	0	0	0	0	0	0	0	0	1.5
39 T.M.	♂	24	Schizophrenia	93	I/2	0	0	0	0	0	0	0	0	0	0	I/2	0	0	0	0	0	0	1.0
40 O.R.	♂	24	〃	152	1	I/2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1.5
41 S.H.	♀	23	〃	151	I/2	0	0	0	0	0	I/2	0	0	0	0	0	0	0	0	0	0	0	1.0
42 S.T.	♂	38	〃	101	1	I/2	0	0	0	0	I/2	0	0	0	0	0	I/2	0	0	0	0	0	2.5
43 F.Z.	♂	48	〃	86	1	0	0	0	0	0	0	I/2	0	0	0	I/2	I/2	0	0	0	0	0	2.5
44 F.H.	♂	32	〃	128	0	0	0	0	0	0	I/2	I/2	0	0	0	0	0	0	0	0	I/2	0	1.5
45 O.R.	♂	25	〃	93	0	0	I/2	0	0	0	I/2	0	0	0	0	0	I/2	0	0	0	0	0	1.5
46 H.T.	♂	40	Copper intoxication	209	1	0	0	0	0	0	0	I/2	0	0	0	I/2	0	0	I/2	1	1	0	0	4.5
47 Y.F.	♂	51	〃	151	I/2	0	0	0	0	0	0	0	I/2	0	0	0	I/2	0	0	0	I/2	0	0	2.0
48 I.K.	♂	50	〃	144	I/2	0	0	0	0	0	0	I/2	0	0	0	0	0	0	0	0	0	0	0	1.0

TABLE 6. Degrees of the Cerebellar Dysfunction

Name	Sex	Age	Diagnosis	Blood Cu (γ%)	1	2	3		4	5	6	7			8		9		10					Total score
							P	G				U	L	T	U	L	F	H	S	I	SI	A	C	
49 K.M.	♂	64	Hepatocerebral d.	178	1	2	I/2	1	I/2	0	I/2	2	I/2	1	0	I/2	1	I/2	1	2	I/2	0	0	14.5
				93	1	2	I/2	I/2	0	0	0	1	I/2	1	0	I/2	1	I/2	1	2	I/2	0	0	12.0
50 M.T.	♂	20	Banti's disease	443	1	0	0	0	0	0	0	I/2	0	0	0	0	0	0	0	0	0	0	0	1.5

TABLE 6. (*Continued*)

Name	Sex	Age	Diagnosis	Blood Cu (γ%)	1	2	3		4	5	6	7			8		9		10					Total score
							P	G				U	L	T	U	L	F	H	S	I	SI	A	C	
51 K.T.	♂	38	Banti's disease	458	1	0	0	0	0	0	0	I/2	0	I/2	0	0	0	0	0	0	0	0	0	2.0
52 M.G.	♂		〃	512	0	0	0	0	0	0	0	0	0	0	0	I/2	0	0	0	0	0	0	0	0.5
53 K.K.	♂	12	〃	290	0	0	0	0	0	0	0	0	0	0	I/2	0	0	0	0	0	0	0	0	0.5
54 A.G.	♂	23	〃	167	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
55 O.A.	♀	39	〃	167	I/2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.5
56 T.G.	♂	46	〃	170	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1.0
57 A.T.	♂	38	〃 (operated)	70	I/2	0	0	0	0	0	0	I/2	0	0	1	0	1	0	1	0	I/2	0	0	4.5
58 O.F.	♀	50	〃 〃	105	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
59 S.H.	♀	22	〃 〃	138	I/2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.5
60 S.K.	♀	43	〃 〃	147	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
61 I.M.	♀	60	Liver cirrhosis	128	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1.0
62 A.T.	♂	70	〃	118	I/2	0	0	0	0	0	0	I/2	0	0	0	I/2	0	0	0	0	0	0	0	1.5
63 H.Y.	♂	53	〃 +(Cystitis)	128	I/2	0	0	0	0	0	0	I/2	0	I/2	0	0	I/2	0	0	0	0	0	0	2.0
64 Y.F.	♂	49	〃	152	1	0	0	0	I/2	0	0	0	0	0	0	1	1	0	I/2	0	I/2	0	0	4.5
65 T.M.	♂	57	〃 (biliary)	101	I/2	0	0	1	0	0	I/2	0	0	0	0	0	0	0	0	0	0	0	0	2.0
66 Y.Z.	♂	70	〃	117	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1.0
67 K.S.	♂	50	〃	101	I/2	0	0	0	0	0	0	I/2	0	0	0	0	I/2	0	0	0	0	0	0	1.5
68 H.F.	♀	34	〃 (precirrhotic)	93	1	I/2	0	0	0	0	I/2	I/2	I/2	0	I/2	0	I/2	0	0	0	I/2	0	0	4.5
69 M.I.	♂	47	〃	169	1	1	0	I/2	I/2	0	I/2	I/2	I/2	I/2	I/2	I/2	0	0	0	0	I/2	0	0	6.5

Asynergy Manifestation

	Liver disease	Nervous disease	Schizoph-renia	Cu-intoxi-cation	Total score
Upper extr.	7	20.5	1	1	29.5
Lower extr.	2	16	0	0.5	18.5
Trunk	3.5	28	0	0	31.5

In nervous disease the trunk is more affected than the upper extremity, and lower extremity also considerably affected.

Manifestation of Dysmetria

	Liver disease	Nervous disease	Schizoph-renia	Cu-intoxi-cation	Total score
Upper extr.	2.5	9	0	0	11.5
Lower extr.	2.5	18.5	1.0	0.5	22.5

Dysmetria was found equally both in upper and lower extremities in liver disease, but in nervous disease the lower extremities were more affected than the upper extremities.

Manifestation of Dysdiadokokinesis

	Liver disease	Nervous disease	Schizoph-renia	Cu-intoxi-cation	Total score
Finger	5.5	31.5	1.5	0.5	39
Hand	1.0	23.0	0	0	24.0

In 71 patients finger dysdiadokokinesis was found in a score of 39 compared with hand dysdiadokokinesis of 24.0. These were 31.5 and 23.0 in patients with nervous disease. There were no patients positive in Rebound phenomenon.

Manifestation of Involuntary Movements

Staic tremor	Kinetic tremor	Static intentional tremor	Nervous disease	Schizoph-renia	Liver disease	Cu-intoxi-cation
Negative	N	N	19	6	16	1
N	N	P	7	1	2	0
N	P	N	1	0	0	1
N	P	P	4	0	0	0
P Positive	N	N	2	0	0	0
P	P	N	1	0	0	0
P	P	P	4	0	2	0
P	N	P	1	0	2	0

Tests for kinetic tremor and static intentional tremor (holding upper extremities in horizontal position for few minutes) should be carried out in each

patient, because as presented above each of the tests were positive in 12 of 39 patients with nervous disease inspite of negative static tremor. 2 of 4 patients with parkinsonism did not show either kinetic or static intentional tremor, though they had positive static tremor.

Schizophrenic patients. In 3 of 7 patients (case 39, 40, 41) the schizophrenic process was acute, and in the other 4 cases it was chronic. The former group has been hospitalized for less than one half year, and the latter longer than 2 years. 1 patient (case 42) was of the dementia simplex type, and the other 6 was of the depressive dementia type.

3 patients with copper intoxication had been working together in making copper molds at the Mitsubishi Heavy Industry Co. Ltd. for the past 2 years and developed blue sweating so that they had to change their undershirts often and one of 3 patients manifested apparent static tremor of the hand which responded well to oral versenate administration.

Athetoid movements were seen in 1 patient with Wilson's disease and 1 patients with parkinsonism (case 3). There was static tremor in both patients. Choreatic movement was seen in only 1 patient with hemichorea.

RESULTS

4 groups could be considered from the degree of cerebellar dysfunction studied here.

Group 1·····with scores over 10
〃 2····· 〃 5.5 to 9

	Score					Blood copper (gamma%)				
	1	2	3	4		1	2	3	4	
Group 1:										
Wilson's disease	26	14				183	163			
Hemichorea	21	16				233	140			
Parkinsonism	18	16	10	10		128	408	221	326	
Cerebral thrombosis	18	16	(6.5)	(3)		256	559	133	198	
M.S.	17.5	16	(8)	(6.5)		58	105	221	130	
Hepatocerebral dis.	14.5	12				178	93			
Cerebellar ataxia	14	12	(8)	(6.5)		326	47	46	233	
Prog. paralysis	13	5.5				233	303			
Group 2:										
Cerebellar tumor	9	7.5				226	214			
〃 ataxia	8	6.5	(4)			46	233	82		
M.S.	8	6.5	5.5	5.5		221	130	105	82	
Cerebral thromb.	6.5	(3)				133	198			
Liver cirrhosis	6.5	(4.5)	(4.5)	(2)	(1.5)	163	152	93	101	101
Spinal ataxia	6.5					175				
〃 syphilis	6	(2.5)				123	117			
Cranial hypertention	5.5	(2.5)				70	133			
Spinal tumor	5.5					160				
Myasthenia gravis	5.5	(3.5)				222	193			
Group 3:										
Syringomyelia	5					256				
Cu-intoxication	4.5	(2)	(1)			209	151	144		
Banti's disease	4.5	(2)	(1.5)	(1)	(1)	70	453	443	170	167

Group 3·····with scores over 4 to 5
〃 4····· 〃 0.5 to 3.5

Maximum score was given to 1 patient with Wilson's disease (26), followed by hemichorea (21), cerebral thrombosis (18), parkinsonism (18), M.S. (17.5) and hepatocerebral disease (14.5).

As shown above, there was in general no correlation between blood copper level and the degree of cerebellar dysfunction, but some correlation was present partially in the following diseases:

Wilson's disease	Spinal syphilis
Hemichorea	M.G.
Cerebellar ataxia	Unoperated Banti dis.
Hepatocerebral dis.	Cu-intoxication
M.S.	Parkinsonism
Liver cirrhosis	Cerebral thrombosis

In 2 cases of Wilson's disease (Table 4) urinary copper was 72 gamma per day (case 1) and 612 gamma per day (case 2) respectively.

3. Copper Metabolism and Hepatic Function in Various Liver Diseases at Different Clinical Processes

In 1912 K. Wilson emphasized the very slight signs and symptoms of hepatic dysfunction during life in patients with progressive lenticular degeneration though advanced cirrhosis of the liver was a constant finding at necropsy. Since then there have been many reports on clinical evidence of liver dysfunction in Wilson's disease.

In 1953 A. Bauman[18] reported that the clinical signs of liver disease were apparent in 7 of 11 patients with hepatolenticular degeneration, in 5 of whom there developed symptoms of liver disease prior to any neurological disorder and in the remaining 2, symptoms and signs of hepatic failure during the course of the illness. He concluded that liver dysfunction is not unusual in Wilson's disease and frank hepatic decompensation common in hepatolenticular degeneration.

In 1945 Glazebrook[2] reported a patient with Wilson's disease whose liver and brain at autopsy revealed a high copper content though the level of serum copper had been normal. In 1948, Mandelbrote[3] *et al.* reported on a patient with Wilson's disease who excreted an increased amount of copper in the urine. In 1953 F. L. Sullivan[6] reported hypocupremia in 2 cases of Wilson's disease. On the other hand portal-systemic encephalopathy has been emphasized by Kirk and Scherlock.[13] They suggested that patients with liver disease going into hepatic coma, are suffering from cerebral intoxication due to intestinal contents which had not been metabolized by the diseased liver. They related the high blood ammonium level in the systemic veins in patients with cirrhosis. In 1954 I. Hyman[7] reported increased absorption of copper from the gut in 3-patient's with Wilson's disease. Tables 7, 8 and 9 present studies on various liver dis-

eases in relation to plasma protein, blood copper level, hepatic function and renal function in part (urinalysis). The patients presented here consist of 11 cases of Bantis's disease, 10 of liver cirrhosis, 7 of subacute hepatitis, 6 of cholelithiasis and 5 of malignant tumor of the liver totalling 9 cases of various liver diseases.

Banti's disease. In Table 7 are presented 4 cases operated for Banti's disease previously. Case 2 was operated on for splenectomy, and porto-caval shunt 2 years prior to the present addmission; Case 8, splenectomy and ligation of esophageal veins 1 month previously; Case 6, splenectomy and splenorenal shunt 2 years previously; Case 9, splenectomy few month previously. Case 2 developed hepatic coma postoperatively on several occasions and urinalysis showed 1+ urobilinogen, 1+ sugar and 1+ protein. Case 1 showed growth anomaly besides Banti's disease, and his weight and height were below normal value for his age. All 7 of the unoperated cases showed apparent hypercupremia, ranging from 167 to 513 gamma per ml. In 4 cases of the operated patients there was no hypercupremia, with values ranging from 70 to 147 gamma per 100 ml.

Urinary copper. In the operated cases the urinary copper was within normal limits ranging from 20 to 64 gamma per 24 hours. In 5 of 7 unoperated cases hypercupriuria with a range of 90 to 630 gamma per day was present.

There was hypoproteinemia in 7 of 11 cases with a range of 4.8 to 6.8 g/dl. There was no correlation between blood copper level, urinary copper and plasma

TABLE 7. Patients

Name	Operation	Sex	Age	Blood Cu (γ%)	Urine Cu (γ/24 hr.)	T.P. (g/dl)	R.B.C.	Hb.
1 T.M.	−	♂	20	343	350	6.8	310	55
2 T.A.	+	♂	38	70	53	6.0	402	85
3 T.K.	−	♂	38	454	46	7.2	345	42
4 G.M.	−	♂	46	513	104	6.7	280	66
5 K.K.	−	♂	12	290	630	5.2	16	58
6 F.O.	+	♀	50	105	32	4.8	216	60
7 G.A.	−	♂	23	167	90	7.0	260	45
8 H.S.	+	♀	22	138	64	6.7	447	90
9 K.S.	+	♀	43	147	20	7.0	318	54
10 A.O.	−	♀	39	167	148	7.8	215	35
11 G·T.	−	♂	46	170	53	6.8	321	69

M.Gr.: Meulengracht.

protein level. Hepatic dysfunction was present in all cases and there was no correlation with blood copper level.

Hypocupremia associated with hypercupriuria was not found in all cases of Banti's which is said to be present in Wilson's disease.

Liver cirrhosis. 10 patients suffering from various types of liver cirrhosis were studied. Case 3 and case 5 shown in Table 8 were confirmed to be biliary cirrhosis by needle biopsy. Case 3 was suffuring from cystitis besides biliary cirrhosis. Case 2 was diagnosed as Lannec's cirrhosis by needle biopsy. Case 8 was found to be precirrhotic in the liver by the needle biopsy and she has had several attacks of hepatic coma with a high blood ammonium level (198.3 microgram per 100 ml). As presented in Table 8 the blood copper level was within normal limits and no definite hypocupremia was found. In this series there were no evident variations in blood copper level due to the type of cirrhosis concerned, though in the other 6 cases needle biopsy was not performed. There was no correlation between blood copper and hepatic dysfunction in degree. Cases 7 and 8 with positive protein in urine, showed lower normal value in blood copper.

Subacute hepatitis. 7 patients with subacute hepatitis (except case 8) were studied and the results of the blood copper analysis are shown in Table 9 (case 7 to case 13). In 5 of 7 patients there was found hypercupremia. Case 9 had myocardial damage confirmed by electrocardiogram, besides hepatitis. Case 12 has been suffering from diabetes mel. besides hepatitis. Case 7 with hyper-

with Banti's Disease

W.B.C.	Hepatic function					Urine		
	Takata	B.S.P.	C.C.F.	M.Gr.	Others	U	P	S
6.25	##	32	+			+	+	−
6.2	##	60	##	5		+	±	+
5.6	##	30	+		Cobalt R_8 T.T. 3.6	##	−	−
5.2	##		+			+	−	−
4.9	##	16	±		T.T. (5)	−	−	−
9.2	##		−		〃	+	−	−
5.2	+		±			N	−	−
7.8	+	5	+			±	−	−
2.2	##	20	+	13	Gros (##)	##	−	−
4.0	+	5	##	2	T.T. 8 cobalt R_6	−	−	−
2.0	##	43	±		Cobalt R_{10}	±	−	−

U: Urobilinogen, P: Protein, S: Sugar.

TABLE 8. Patients with Liver Cirrhosis

	Name	Sex	Age	Diagnosis	Blood Cu (γ%)	T.P. (g/dl)	R.B.C.	Hb.
1	M.I.	♀	60	Cirrhosis	128	5.7	311	68
2	T.A.	♂	70	〃	112	6.0	387	72
3	Y.H.	♂	53	〃 +Cystitis	128	6.3	460	100
4	T.Y.	♂	49	〃	152	7.0	320	58
5	M.T.	♂	57	〃	101	7.5	329	76
6	Z.Y.	♂	70	〃	117	6.0	362	66
7	S.K.	♂	50	〃	101	7.3	353	75
8	F.H.	♀	34	〃	93	7.8	297	59
9	M.I.	♂	47	〃	163	8.2	420	78
10	K.M.	♂	64	〃 +H.C.D.	93	6.2	409	80
11	G.H.	♂	64	Cancer	275	7.0	284	56
12	K.A.	♂	39	Reticulosarcoma	175	9.0	378	82
13	K.H.	♂	46	Cancer	268	7.0		68
14	A.A.	♀	37	〃	163	4.7		55
15	H.H.	♀	60	G.B. Cancer cholecystitis	138	4.9		70

H.C.D.: Hepatocerebral disease. T.T.: Thymol turbidity,

TABLE 9. Patients with Cholelithiasis

	Name	Sex	Age	Postoperative diagnosis	Blood Cu (γ%)	T.P. (g/dl)	R.B.C.	Hb.
1	K.S.	♂	57	Cholelithiasis	201	7.24	420	78
2	K.H.	♀	59	〃	267	6.74	335	85
3	Z.O.	♂	54	〃	290	5.24	350	90
4	K.H.	♂	67	〃	210	5.12	229	60
5	S.H.	♂	53	〃 +Ca(pancreas)	156	6.86	355	88
6	M.K.	♀	60	〃	152	5.87	312	60
7	T.K.	♀	54	Subacute hepatitis	233	6.9	480	80
8	T.M.	♂	48	Acute hepatitis	101	6.1	492	95

or Malignant Tumor of the Liver

W.B.C.	Hepatic function				Urine			Liver palm
	Takata	B.S.P.	C.C.F.	Others	U	P	S	
6.2	##	40	±	M.Gr. (9) Cobalt (R_{8-9})	+	±	−	+
5.4	+	10	−	Cobalt (R_7)	±	−	−	+
5.4	±	5	−	T.T. (5.8)	+	−	−	±
5.5	+	30	##	G.O.T. (109)	##	−	−	±
3.9	±	15	+	S.G.O.T. (380) T.T. (16)	N	−	−	±
11.2	##		+	M.Gr. (40) Al.Ph. (21.4)	+	−	−	+
6.8	###	40	##	M.Gr. (4)	N	+	−	±
7.8	##	4.6	+	NH_3 198 γ/dl M.Gr. (15)	±	+	−	±
6.4	##		+		±	−	−	−
6.2	##	40	+	E.S.R. 37.8 NH_3 325 γ%	+	−	−	##
7.8	−	5	−		+	−	−	−
7.5	−	10	−	M.Gr. (12)	N	−	−	−
9.2	−		−	M.Gr. (165)	±	+	−	±
4.8	−	5	+	T.T. (1.9)	+	−	−	−
5.2	−	15	−	T.T. (2.5)	±	−	−	±

Al.Ph: Alkaline phosphatase. U: Urobilinogen, P: Protein, S: Sugar.

or Subacute Hepatitis

W.B.C.	Hepatic function				Urine			Liver palm
	Takata	B.S.P.	C.C.F.	Others	U	P	S	
8.0	##		−	T.T. 7.2	±	−	−	−
4.0	##	40	−	T.T. 5.5	±	−	−	−
6.8	−		+		N	−	−	−
11.8	−	23	−	T.T. 7.5	+	−	+	−
4.2	##		+	T.T. 5.0	N	−	−	±
7.8	−	7.5	−	T.T. 3.6	##	+	−	−
8.0	##		−	T.T. 4.4	+	−	−	−
4.2	##	35	+	T.T. 5.8	+	−	−	−

TABLE 9.

	Name	Sex	Age	Postoperative diagnosis	Blood Cu (γ%)	T.P. (g/dl)	R.B.C.	Hb.
9	H.H.	♂	50	Subacute hepatitis +Myocard damage	221	7.6	266	50
10	T.H.	♂	68	〃	140	9.0	300	69
11	K.A.	♂	61	〃	186	6.2	310	68
12	K.A.	♀	70	Subacute hepatitis +Diabetes m.	163	8.6	476	95
13	T.K.	♀	30	〃	198	7.1	163	23

T.T.: Thymol turbidity.

cupremia had been operated for gall stone 1 year previously. Case 8 was suffering from acute virus hepatitis with normal blood copper level. There was no correlation between blood copper and the degree of hepatic dysfunction, and proteinuria.

Cholelithiasis. 6 patients with cholelithiasis were studied and the blood copper analysis is shown in Table 9. All 6 showed hypercupremia with a range from 152 to 290 gamma per 100 ml. The diagnosis of cholelithiasis was made postoperatively. Case 1 (bilirubinate stone), Case 2 (cholesterol-bilirubinate-calcium), Case 3 (bilirubinate + G.B. – cancer), Case 4 (bilirubinate), Case 5 (cholesterol stone), Case 6 (cholesterol). Patients with cholesterol stone showed lower blood copper level when compared with the other cases. There was no correlation between blood copper, the degree of anemia, plasma protein and the degree of hepatic dysfunction. Case 6 with proteinuria showed the lowest blood copper level in all 6 cases of patients with cholelithiasis.

Malignant tumor of the liver. 5 patients with hepatic malignant tumor were studied and the blood copper analysis is presented in Table 8 (case 11 to case 15). Case 12 was suffering from reticulosarcoma with metastasis in the liver. 4 of 5 patients showed hypercupremia with a range from 163 to 275 gamma per 100 ml. Case 15 with gall bladder cancer and cholecystitis showed upper normal value in blood copper. Case 13 with liver cancer died a few weeks after a blood copper study, which was 268 gamma per 100 ml and his urinalysis revealed 1+ protein, 1+ bile, few leucocytes and few erythrocytes. It is of interest to note that the hepatic functions in all cases was preserved considerably.

SUMMARY

Hypercupremia was found in patients with Banti's disease, hepatitis, cholelithiasis and malignant tumor of the liver. In 4 of 7 unoperated cases of Banti's disease, hypercupremia was associated with hypercupriuria. All 8 patients with liver cirrhosis showed normal cupremia. There was no hypocupremia in 39 patients with various liver diseases except for 2 cases. Case 2 in Table 7, of

(*Continued*)

W.B.C.	Hepatic function				Urine			Liver palm
	Takata	B.S.P.	C.C.F.	Others	U	P	S	
9.4	−	5	−	E.S.R. 71 T.T. 11.6	N	+	−	−
6.2	++	45	±	Gross (++) Cholesterol 378 mg/dl	+	++	++	±
20.0	++	60	−		++	+	−	−
5.1	−		++	Alk. Phosph 13	+	±	±	−
6.8	++			N	N	++	−	

U: Urobilinogen, P: Protein, S: Sugar.

Banti's disease who had developed several attacks of hepatic coma postoperatively, and case 8 in Table 8 of liver cirrhosis who had also developed hepatic coma, showed considerably lower blood copper levels, 70 and 93 gamma% respectively. Liver palm (mottled flushing of palms) due to hyperestrinism was seen in 9 of 10 patients with liver cirrhosis.

Normal or subnormal blood copper levels were seen in all operated cases of Banti's disease. The state of operated Banti's disease is similar to that of Wilson's disease in which the shunt might sometimes be through the diseased liver itself. As mentioned in the previous study[16] temporary rise of blood copper level was seen after hypodermic injection of 150 mg of acetylcholine and it was confirmed by Senda[19] in his irrigation test of the liver that copper and iron are driven out from the liver. Thus the liver must play an important role in copper metabolism.

4. Studies on Copper Metabolism in the Nephrotic Syndrome

It has been said that there is an extremely high aminoaciduria in hepatolenticular degeneration accompanied by hypocupremia. In 1950 C. S. Davidson[21] concluded that the kidney is one that causes the high amount of aminoaciduria in Wilson's disease. However, the mechanism of aminoaciduria by the diseased kidney has not been explained. By postulating a defect of renal function, the diseased kidney may cause a great loss of other substances besides aminoacids. We often experienced renal glycosuria and albuminuria in a patient with Banti's disease who developed several attacks of hepatic coma and a patient with Wilson's disease who also developed several mental attacks. It has been recently demonstrated that serum albumin is necessary as a binding and transporting agent not only for minerals and metals but also for fatty acids.

C. G. Holmberg[21] and Scheimberg[22] reported that most of the copper normally present in serum are bound to cereuloplasmin which is a blue alpha globulin with a molecular weight of 151 000, containing 0.34% copper.

No evidence has been found as to whether the ceruloplasmin is excreted into the urine of patients with advanced proteinemia. Supposing depletion of

the ceruloplasmin continued, hypocupremia could have occurred. In the nephrotic syndrome, protein leaks through the diseased glomerulus. In this chapter the relationships between blood and urinary copper, plasma protein, proteinuria, and clearance test of renal function were studied.

Selection of patients

Patients who showed marked edema, 4+ proteinuria, hypoproteinemia and hyperlipemia were selected for studies on copper metabolism in nephrotic syndrome.

RESULTS

The blood and urinary copper levels, electrophoretically determined plasma protein fractions, the value of urinary protein excretion, the degree of hepatic dysfunction and the value of the clearance test in 10 patients with nephrotic syndrome are presented in Table 10. The mean value of blood copper was 164 gamma%, which is much higher than the normal mean of 115 gamma% in 10 normal subjects, with a range from 110 to 210 gamma%. This was an unexpected evidence in this series. Hypercupremia was seen in 4 of 10 patients with nephrotic syndrome (case 3, 6, 7, 8).

Total plasma protein (mean value) was 6.2 g per 100 ml with a range from 4 to 7.6 g per 100 ml and hypoproteinemia was apparent. The mean value of plasma albumin was 48.5% with a range of 28 to 66.2% which is hypoalbuminemic when compared with the normal mean 58.7%. The mean value of plasma globulin was 46.0%, with a range of 24.8 to 60.6%, which is hyperglobulinemic compared with the normal mean 35.2%. The 2 cases showed hypoglobulinemic

TABLE 10. Patients with

Subjects	Blood Cu (γ%)	Urine Cu (γ/24 hr.)	T.P. (g/dl)	Alb. (%)	Glob. (%)	α (%)	β (%)	γ (%)	A/G
1 T.K.	139	40	7.0	55.2	40.4	10.9	14.2	15.3	1.11
2 H.U.	110	96	6.8	66.2	24.8	9.6	4.3	10.9	2.22
3 T.I.	203	62	5.1	51.1	48.9	15.7	19.1	14.1	1.05
4 Z.W.	133	46	7.6	50.8	40.9	10.4	11.4	18.7	1.21
5 H.N.	122	293	5.2	30.8	60.6	32.8	16.9	10.9	0.50
6 S.M.	210	92	7.5	65.0	35.0	7.0	13.0	15.0	1.85
7 Z.Y.	205	38	6.0	34.3	60.5	19.1	33.3	13.1	0.56
8 S.M.	205	42	4.0	55.1	44.9	7.1	18.4	25.6	1.20
9 K.H.	152	60	6.6	48.7	44.8	13.0	12.8	19.4	1.08
10 H.O.	163	36	5.8	28.0	58.7	22.5	25.3	10.9	0.48
Mean	164		6.2	48.5	46.0	14.4	16.9	15.4	1.13

(case 2, 6). It is of great interest that the plasma of patients with nephrotic syndrome contains increased amounts of alpha-globulin and the mean value of alpha-globulin here was 14.9% which is more than twice the normal mean of 6.4%. As presented later there was no correlation between blood copper level and plasma alpha-globulin value. Urinary copper in 10 patients ranged from 36 to 293 gamma per day. There was no apparent correlation between blood copper and urinary copper. Urine protein ranged from 1.5 to 16 g per 1 000 ml. It is very interesting to note that case 5 with the highest urinary copper (293 gamma per day) was accompanied by the highest urinary protein of 16 g per 1 000 ml. In 1 patient with uremia, U. K. ♂ 50, no hypocupremia was observed (112 γ%). As shown in Table 10, a mild degree of anemia was present in 3 patients and severe anemia in 2 patients.

DISCUSSION

The hypothesis that hypocupremia in hepatolenticular degeneration is due to the loss of cereuloplasmin into the urine is conceivable. However the data in patients with hyperproteinuria presented here failed to show hypocupremia. It seems that there is a correlation between the degree of proteinuria and the urinary copper, seen in case 5, indicating that copper is bound to protein. Hypercupriuria in case 5 could be explained by not only the great loss of albumin into the urine but also by some grade of hepatic dysfunction, increased renal blood flow, glomerular filtration rate and urinary flow. There was no hypercupriuria in the other 9 cases with normal or decreased glomerular filtration rate. In case 9 with normal cupriuria there was seen decreased glomerular filtration rate and increased urine flow. In case 6 with normal cupri-

Nephrotic Syndrome (Group III)

Urine protein	R.B.F. C.	G.F.R. C. per	Urine flow (min)	Ht. (%)	E.S.R.	R.B.C.	W.B.C.	Cholesterol (mg%)	Hepatic dysfunction
1.5	652	69.3	1.6	40	30	392	6.8	334	−
3.0	364	30.5	1.2	40	29	341	5.6	265	−
6.0	18	3.5	1.0	16	124	129	4.0	378	+
5.0	504	89.3	1.6	45	5	415	8.2		−
16.0	1 137	123.3	6.8	43	106	470	5.4	680	±
3.0	1 035	104.3	1.4	38	6	415	6.8	624	++
4.0	608	65.1	1.6	53	51	355	5.8	855	++
6.0					4	226	3.5	398	−
2.5	624	73.9	7.8	50	11	510	6.2	215	−
5.0	672	80.8	1.2	49	78	456	8.8	754	−

uria there was seen increased renal blood flow and decreased urine flow. So it can be considered that the renal function might play an important role in hypercupriuria of patients with nephrotic syndrome.

5. Copper Metabolism in Patients with Copper Intoxication

From the stand point of industrial hygiene, 3 patients with copper intoxication were studied. They had been working together in making mold with 84% of copper, 12% alminum and 4% iron for the past 2 years and developped blue sweating and signs of discoordination. One of the 3 patients was treated with oral versenate administration with improvement. The blood and urinary copper, plasma protein fractions, the degree of hepatic dysfunction and discoordination, and some hematological studies are presented in Table 11. Case 1 showed hypercupremia with urinary copper of 87 gamma%. and eosinophilia. He excreted marked amounts of copper into the urine (604% of primary urinary copper) after oral versenate treatment. He has been away from his work since then, with marked improvement and no more blue sweating but a very mild tremor. Cases 2, and 3 showed higher normal value in blood copper and a mild degree of discoordination as was presented previously. Here again there was no correlation between blood and urinary copper, plasma protein fractions and the degree of hepatic dysfunction. However there was correlation between blood copper and the degree of cerebellar dysfunction.

6. Correlation between Blood Copper and the Various Plasma Protein Fractions

During the course of study on blood copper in various patients, the elevation in the erythrocyte sedimentation rate accompanying high blood copper level was often experienced in some patients.

As stated above, most of the plasma copper had been said to bind to metal protein. In previous studies hypercupremia was present in the following conditions

1) Chronic infection: (some pulmonary tuberculosis and chronic hepatitis)
2) Acute leukemia:
3) Pregnancy:
4) Parkinson's disease:
5) Hyperthyroidism:

TABLE 11. Patients with

Name	Age	Blood Cu (γ%)	Urine Cu (γ/24 hr)	T.P. serum (g/dl)	Alb. (%)	Glob. (%)	α_1/α_2 (%)	β (%)	γ (%)	A/G
H.T.	40	209	87	7.5	51.7	48.3	2.8/6.0	10.0	29.5	1.1
Y.Y.	51	151	56	8.5	51.4	45.6	4.4/2.5	9.7	29.0	1.2
I.K.	50	144	63	8.0	43.0	57.0	2.0/7.0	16.0	30.0	0.8

6) Myasthenia gravis:
7) Aplastic anemia:
8) Banti's disease:

As mentioned in previous chapters, these studies were made on 3 groups of patients with liver disease, nephrotic syndrome and cerebellar dysfunction to clarify the entity of hepatocerebral disease. The blood copper level in each group is summarized as follows:

Group 1·····28 patients with cerebellar dysfunction and blood copper level ranging from 46 to 359 gamma%.
〃 2·····18 patients with various liver diseases and blood copper level ranging from 70 to 343 gamma%.
〃 3·····10 patients with nephrotic syndrome and blood copper ranging from 110 to 210 gamma%.

As shown in Tables 10, 12, and 13 the mean values of blood copper in all 3 groups of patients, were higher than the normal mean (115 gamma%). The difference from the normal mean was highest in group 1 (+60), followed by group 3 (+49) and group 2 (+39). Based upon these observations and results, the correlation between blood copper level and plasma protein fraction was analyzed, and presented in Figs. 2, 3, 4 and 5.

		Correlation coefficient (r)
Group 1·····	albumin	−0.4009
〃 2·····	albumin	+0.582
	total globulin	−0.602
〃 3·····	beta-globulin	+0.576

There seems to be some correlation existing between albumin, total globulin and blood copper in group 2, and also some correlation between beta-globulin and blood copper in group 3. Correlation coefficient (r) was calculated by the method of least squares. It was difficult to state that a correlation exists between albumin and blood copper in the series of group 1. There was no correlation whatsoever between alpha-, gamma-globulin and blood copper in all 3 groups of patients.

The mean value of plasma protein fractions

Albumin. In all 3 groups the mean value of albumin was lower than the normal mean. However the rates of difference between the means of patients and the normal of blood copper level and that of albumin in each group was different.

Copper Intoxication

Urobilinogen urine	R.B.C.	W.B.C.	Sp.Gr. whole blood	Reticulocytos (%)	N/L	Eosinocytes	Cerebellar dysfunction
N	374	5.3	1 054	1	0.78	250	Tremor dysfunction (+) 4.5
N	512	7.45	1 056	3	0.61	188	Discoordination (+) 2
±	395	6.1	1 056	4	1.20	100	Discoordination (+) 1

TABLE 12. Patients with Various Liver Diseases (Group II)

Name	Sex Age	Diagnosis	Blood Cu (γ%)	T.P. (g/dl)	Alb. (%)	Glob. (%)	α (%)	β (%)	γ (%)	A/G	Urine U	Urine P	Urine S	Hepatic dysfunction
1 M.I.	♀ 60	Liver cirrhosis	128	5.72	31.4	52.8	7.5	12.6	32.7	0.59	+	±	−	##
2 T.Y.	♂ 44	〃	152	6.8	43.5	47.8	4.3	9.3	34.7	0.91		−	−	##
3 M.T.	♂ 51	〃	101	7.5	26.9	73.1	15.4	25.0	32.7	0.37	+	−	−	##
4 Z.Y.	♂ 70	〃	117	6.8	42.6	52.2	11.3	18.3	22.6	0.81	+	−	−	##
5 S.K.	♂ 50	〃	101	7.3	28.6	58.0	7.1	8.9	42.0	0.49	N		−	##
6 F.H.	♀ 34	〃	93	7.8	41.0	50.6	8.8	16.8	25.0	0.81	±	+	−	##
7 I.M.	♂ 47	〃	163	8.2	53.1	41.5	4.6	5.4	31.5	1.27	+	−	−	+
8 K.M.	♂ 64	(H.C.D.) Atack	130	6.8	38.7	61.3	8.7	23.2	29.4	0.63	+	−	−	##
9 K.M.	♂ 64	(H.C.D.)	93	6.2	38.5	51	7.8	19.5	23.7	0.75	+	−	−	##
10 T.H.	♂ 68	Hepatitis	140	9.0	36.5	50.9	9.4	8.3	33.2	0.72	−			##

TABLE 13. Patients with Cerebellar

Name	Sex	Age	Diagnosis	Blood Cu (γ%)	T.P. (g/dl)	Alb. (%)	Glob. (%)	α (%)
1 W.A.	♂	56	Wilsons dis.	156	8.61	48.5	44.5	9.3
2 〃	♂	56	〃	168	7.83	47.2	46.6	7.4
3 〃	♂	56	〃	183	7.24	47.7	44.2	6.8
4 M.H.	♂	10	〃	163	6.6	58.6	33.1	5.0
5 O.Y.	♂	70	Parkinsonism	326	9.0	51.5	40.2	8.3
6 M.Z.	♂	71	〃	128	7.8	52.5	38.5	5.0
7 A.K.	♂	55	〃	208	7.2	61.5	33.6	4.9
8 M.A.	♀	21	M.S.	222	7.8	47.6	45.7	6.7
9 N.M.	♂	43	〃	105	7.0	61.6	34.5	4.6
10 N.U.	♀	33	〃	82	6.6	56.8	38.8	7.1
11 I.T.	♀	19	〃	105	7.8	47.2	47.7	3.7
12 H.U.	♂	23	〃	58	8.0	63.2	31.9	8.3
13 Y.K.	♀	45	Cerebral thrombosis	198	8.0	53.5	40.4	6.2
14 K.Z.	♀	62	〃	256	9.0	47.3	49.6	6.0
15 I.E.	♂	53	〃	359	7.3	48.8	37.6	10.3
16 I.T.	♀	58	Cerebellar ataxia	147	8.6	46.4	47.3	9.5

TABLE 12. (*Continued*)

Name	Sex Age	Diagnosis	Blood Cu (γ%)	T.P. (g/dl)	Alb. (%)	Glod. (%)	α (%)	β (%)	γ (%)	A/G	Urine U	Urine P	Urine S	Hepatic dysfunction
11 K.A.	♀ 70	Hepatitis	163	8.6	39.4	52.5	8.8	18.7	25.0	0.75	+	±	±	++
12 T.K.	♂ 30	〃	198	7.1	57.0	33.1	6.1	9.9	17.1	1.72	−		−	++
13 T.H.	♂ 20	Banti's disease	343	6.8	52.4	39.9	4.9	10.5	24.5	1.31	+	+	−	+++
14 T.A.	♂ 38	〃	175	7.0	34.9	57.1	3.4	8.0	45.7	0.61	+	±	±	+++
15 A.O.	♀ 39	〃	167	7.8	50	41.3	8.6	12.0	20.6	12	−	−	−	++
16 K.A.	♂ 39	Liver reticulosarcoma	175	9.0	47.1	46.3	7.6	10.0	28.7	1.01	N	−	−	±
17 K.H.	♂ 46	Liver cancer	268	7.0	43.1	39.8	12.6	16.8	10.4	1.08	N	+	−	±
18 T.A.	♂ 38	Banti's disease	70	6.0	37.2	54.5	4.1	8.3	42.1	0.68	+	±	±	+++
Mean			154		41.2	50.3	7.8							

H.C.D.: Hepatocerebral disease. U: Urobilinogen, P: Protein, S: Sugar.

Dysfunction (Group I)

β (%)	γ (%)	A/G	Urine U	Urine P	Urine S	R.B.C.	Hb.	W.B.C.	Hepatic dysfunction	F.E.G.	Cerebellar dysfunction
16.9	18.3	1.09	++	−	−	480	90	7.8	++	+	+++
17.1	22.1	1.00	+	−	−						
15.3	22.1	1.08	++	±	−						
12.7	15.4	1.77	+	−	−	423	64	3.2	+++	+	+++
11.4	20.5	1.28	+	−	−	440	72	4.5	+		++
11.9	21.6	1.36	IV	+	−						+++
9.8	18.9	1.83	+	−	−	416	82	5.4	±	N	+++
14.3	24.7	1.04	+	+	−	395	78	9.2	±	+	++
10.4	19.5	1.78	±	−	−	460	86	4.8	±	+	+++
14.3	27.4	1.46	+	−	−	385	75	6.2	−	+	++
9.3	34.3	0.98	−	−	−	405	82	6.8	−	N	+
9.7	13.9	1.98	−	−	−	418	80	8.2	−	+	+++
7.9	26.3	1.32	−	−	−						+
14.0	24.0	1.07	IV	−	−	381	87	5.6	−		+++
9.5	17.8	1.29	+	+	−	448	104	8.4	±		+++
18.5	19.3	0.98	IV	−	−	438	74	6.8	−		++

TABLE 13.

Name	Sex	Age	Diagnosis	Blood Cu (γ%)	T.P. (g/dl)	Alb. (%)	Glob. (%)	α (%)
17 Y.G.	♂	70	Cerebellar ataxia	46	7.0	60.8	32.0	7.2
18 M.H.	♂	48	〃	233	8.0	58.1	33.8	6.8
19 H.U.	♂	68	〃	82	7.4	58.7	35.7	3.3
20 Y.H.	♂	38	〃	326	7.2	61.8	33.6	6.6
21 K.M.	♂	51	Hemichorea	233	7.0	53.8	40.0	4.6
22 K.M.	♂	51	〃	140	7.0	60.2	34.5	4.4
23 K.Z.	♂	58	Progressive paralysis	303	8.9	45.7	43.9	9.4
24 K.M.	♂	53	〃	203	7.4	60.3	34.7	5.0
25 O.K.	♂	22	Spinal ataxia	175	5.4	60.1	33.9	6.8
26 E.T.	♂	43	Spinal tumor	148	8.0	59.3	33.9	5.9
27 T.T.	♂	52	Cranial hypertension	70	7.4	59.3	34.4	6.2
28 H.M.	♀	21	Post-encephalitic state	82	8.4	63	32	5.0
Mean				175		55.0	38.4	

E.E.G. {N: Normal / +: Abnormal finding present

The rate calculated in each group is as follows;

Group 1..... $\frac{\text{Difference between the means of patients and normal (Copper level)}}{\text{Difference between the means of patients and normal (Albumin level)}}$

$$= \frac{\text{Increased}}{\text{Decreased}} = \frac{60}{-3.7} = -16.2$$

〃 2..... 〃 $= \frac{\text{Increased}}{\text{Decreased}} = \frac{39}{-17.5} = -2.2$

〃 3..... 〃 $= \frac{\text{Increased}}{\text{Decreased}} = \frac{49}{-10.2} = -4.8$

Only in group 2 relative hypocupremia may occur in hypoalbuminemic states. Similar calculation was made for each protein fraction.

Globulin. In all 3 groups the mean value of total globulin increased when compared with the normal mean.

Group 1..... $\frac{60}{4.4} = +13.95$

〃 2..... $\frac{39}{4.2} = +2.74$

(*Continued*)

β (%)	γ (%)	A/G	Urine U	Urine P	Urine S	R.B.C.	Hb.	W.B.C.	Hepatic dysfunction	F.E.G.	Cerebellar dysfunction
12.4	12.4	1.90	IV	−	−	452	80	5.9	−		++
8.1	18.9	1.71	+	+	−		100			+	++
10.0	22.7	1.64	IV	−	−	423	85	5.4	−		+
7.2	19.8	1.83	IV	−	−	435	100	4.8	−	N	+++
9.2	26.2	1.34	IV	−	−						++++
10.6	19.5	1.74	IV	−	−	418	76	6.0	−	+	+++
18.9	15.6	1.04	IV	−	−	464	93	9.6	±	+	++
10.7	19.0	1.73	IV	−	−	446	79	7.4	−		+++
12.0	15.1	1.77	IV	−	−	513	104	8.3	−		++
9.3	18.7	1.74	IV	−	−				−		++
12.5	15.7	1.72	IV	−	−	454	91	7.3	±	N	++
9.0	18.0	1.93	IV	−	−	353	73	6.2	−	+	+
	19.9										

Urine {U: Urobilinogen S: Sugar
P: Protein

Group 3 $\cdots\cdots\cdots \frac{49}{10.8} = +4.53$

Alpha- and beta-globulin. In all 3 groups the mean values of alpha- and beta-globulin increased when compared with the normal mean.

	Alpha-globulin	Beta-globulin
Group 1 $\cdots\cdots\cdots$	$\frac{60}{0.1} = +60$	$\frac{60}{1.0} = +60$
〃 2 $\cdots\cdots\cdots$	$\frac{39}{1.4} = +27.8$	$\frac{39}{0.9} = +43$
〃 3 $\cdots\cdots\cdots$	$\frac{49}{8.4} = +5.8$	$\frac{49}{5.4} = +9.1$

Gamma-globulin. The mean value of gamma-globulin was decreased only in the group 3.

Group 1 $\cdots\cdots\cdots \frac{60}{2.9} = +23.1$

〃 2 $\cdots\cdots\cdots \frac{39}{12} = +3.25$

〃 3 $\cdots\cdots\cdots \frac{49}{-1.9} = -26.3,$

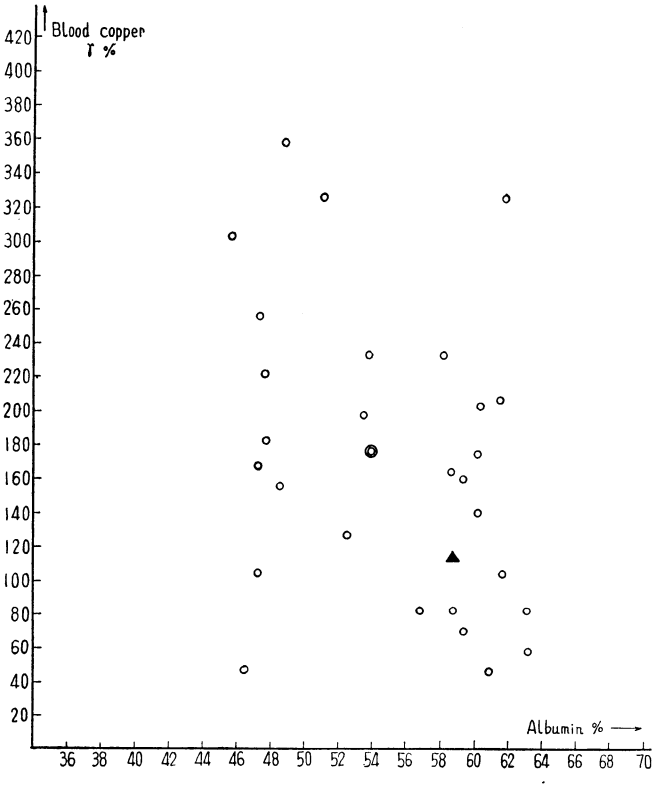

FIG. 2. Correlation between blood copper and plasma albumin in group I.

$r = -0.4$
◎: the mean in group I
▲: normal mean

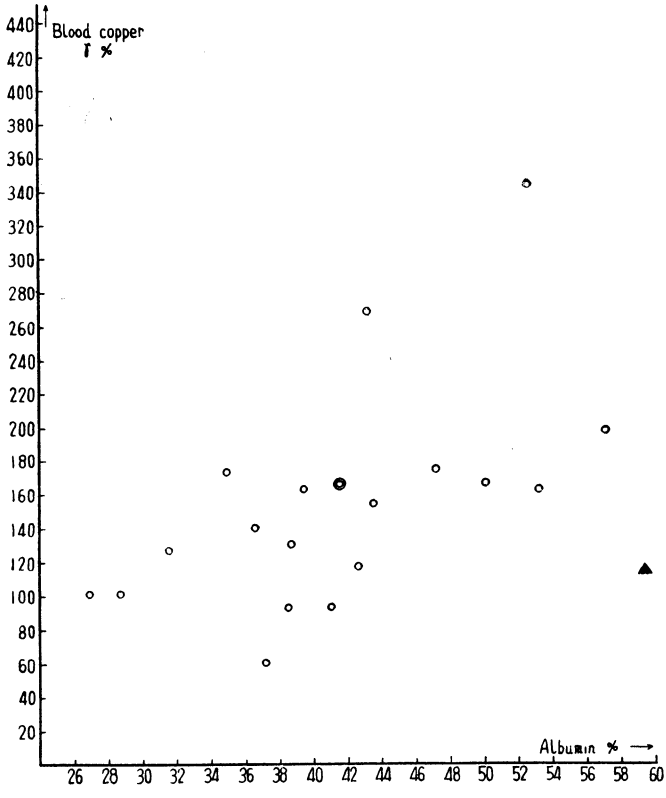

FIG. 3. Correlation between blood copper and plasma albumin in group II.

$r = +0.58$
◎: the mean in group II
▲: normal mean

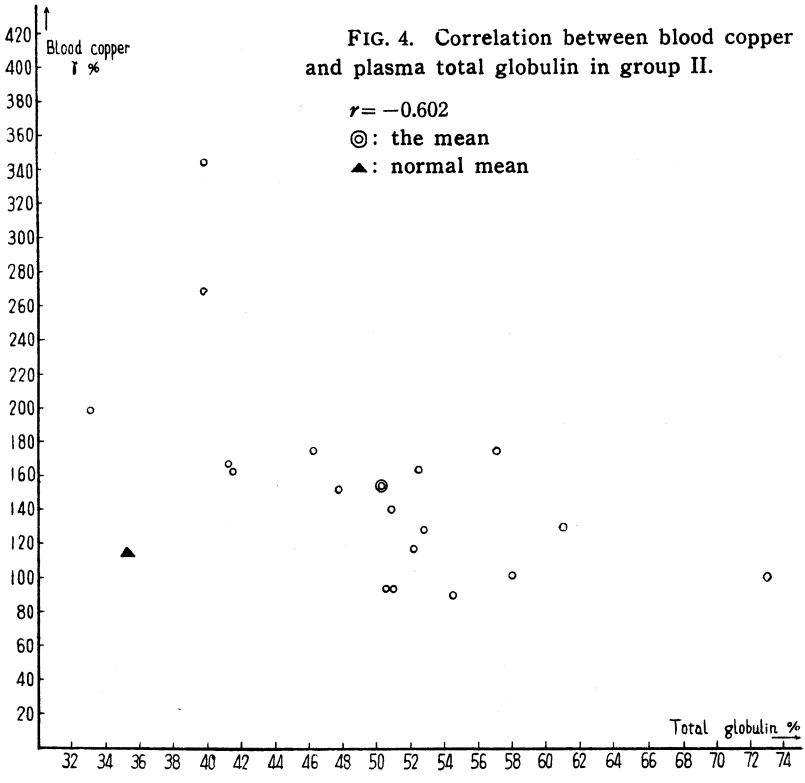

FIG. 4. Correlation between blood copper and plasma total globulin in group II.

$r = -0.602$

◎: the mean

▲: normal mean

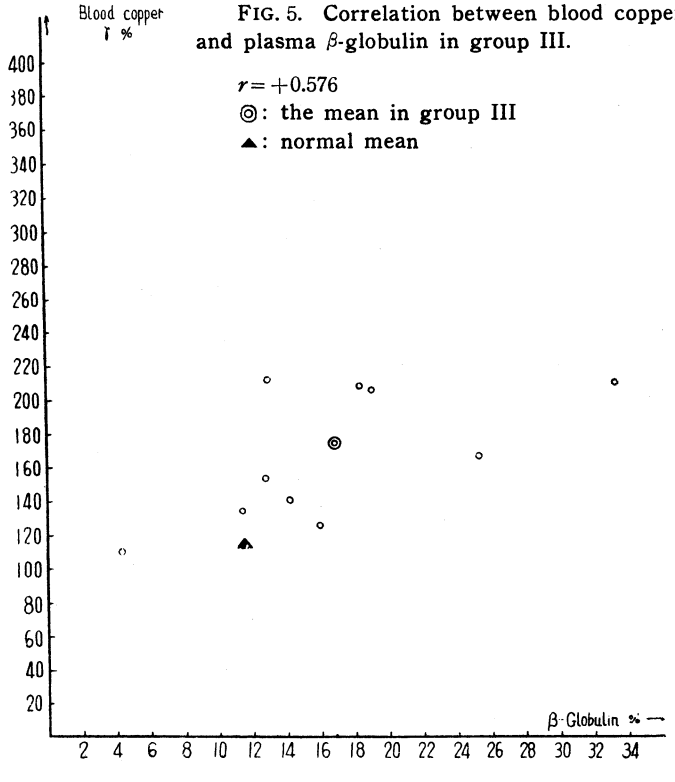

FIG. 5. Correlation between blood copper and plasma β-globulin in group III.

$r = +0.576$

◎: the mean in group III

▲: normal mean

DISCUSSION

From the studies presented above it is conceivable that the blood copper is closely related to an alteration in plasma proteins, especially in albumin, total globulin and beta-globulin. But the pattern of the correlation in various diseases is different. Blood copper in patients with liver disease is moderately and negatively related to total globulin ($r=-0.602$) and positively to albumin ($r=+0.582$). Blood copper in patients with nephrotic syndrome is moderately and positively related to beta-globulin ($r=+0.576$). Blood copper in patients with cerebellar dysfunction may have a correlation with albumin. It is interesting to note that blood copper is negatively related to albumin in patients with cerebellar dysfunction when compared with patients with liver disease in whom it is positively related to albumin. The entity of such a pattern is obscure, though Holmberg,[23] Ravin[24] and Haffon[25] have reported on evidence that serum copper protein (ceruloplasmin) functions as an oxidase (laccase). In patients with cerebellar dysfunction the difference between the means of albumin, globulin, alpha-, beta-, and gamma-globulin level in patients and normal are markedly smaller when compared with the blood copper difference. Such a pattern was not seen in the group of patients with liver disease and nephrotic syndrome.

7. Studies on the Influence of Various Substances on Blood Copper and Urinary Copper

Hypodermic injection of 0.7 ml of adrenalin or 20 units of insulin had no effect on the blood copper level, but hypodermic injection of 150 mg of acetylcholin caused temporary rise of blood copper. Substances with S-atom (methionine hypo. or 20% detoxyl I.V. injection) caused considerable rise in blood copper level.

Versenate P.O. or B.A.L. I.M. administration seem to be best for investigating the alterations in blood and urinary copper levels. Table 14 shows the alteration rate in blood and urinary copper before and after 800 mg B.A.L. I.M. or

TABLE 14. Alteration Rate in Blood and Urinary Copper before and after B.A.L. or Versenate Administration

Name	Sex	Age	Diagnosis	Alteration blood Cu (%)	Alteration urinary (Cu/24 hr.) (%)	Blood copper (γ%)	Urinary copper (γ/24 hr.)	Clinical improvement
1 W.A.	♂	56	Wilson's disease	B.A.L. 800 mg +160.6	+740.8	183	72	+
2 W.F.	♂	29	Pulmonary tuberculosis	〃 +23.3	+254.7	193.4	105.6	−
3 H.K.	♂	32	Bulbar malacia	〃 +193.3	+2796.1	104.9	25.2	−
4 K.S.	♂	26	Muscular dystrophy	〃 −5.9	+663.2	174.8	49.2	−
5 K.M.	♂	64	Hepatocerebral disease	〃 +25.7	+188	130	70	+
6 H.M.	♂	40	Cu-intoxication	Versenate 3 g +28.4	+604	245	158	+

3 gm versenate P.O. administration. Increased alteration rates of urinary copper are much higher than that of blood copper and the increased rate is not specific for various diseases. There is no way to diagnose the disease by B.A.L. or Versenate administration, which showed therapeutic effect only in the neurological field in patients with Wilson's disease, hepatocerebellar disease and copper intoxication.

SUMMARY AND CONCLUSION

1) Brawn-Schaeffer-Asakura's method was presented for urine and blood copper determination.

2) Quantitative analysis of cerebellar dysfunction and blood copper was studied in 4 groups of 69 various patients (with nervous disease, liver disease, schizophrenia and copper intoxication). In general, there were no correlations between the blood copper level and the degree of cerebellar dysfunction but partially some correlation (plus factor or minus factor) was seen in some cases.

The quantitative analysis of cerebellar dysfunction was also discussed. 2 cases of Wilson's disease showed no hypocupremia associated with hypercupriuria.

3) No hypocupremia was observed in 39 patients with various liver diseases except for 2 cases with Banti's disease and liver cirrhosis who developed several attacks of hepatic coma. Hypercupremia was found in patients with unoperated Banti's disease. subacute hepatitis, cholelithiasis and malignant tumor of the liver. An important role of the liver in copper metabolism was discussed.

4) No hypocupremia was found in 10 patients with nephrotic syndrome. There was correlation between massive proteinuria and urinary copper in one case which showed increased renal blood flow, glomerular filtration rate and urinary flow. And the significance of such abnormal renal function in hypercupriuria was emphasized.

5) There was correlation between the blood copper level and the degree of cerebellar dysfunction in 3 patients with copper intoxication. One of them responded well to oral versenate administration.

6) Blood copper and various plasma protein fractions in 3 groups of 56 patients with cerebellar dysfunction, liver disease and nephrotic syndrome were studied. Some correlation was suggested as existing between albumin or total globulin and blood copper in patients with liver disease, also between beta-globulin and blood copper level in patients with nephrotic syndrome. In patients with cerebellar dysfunction, it is not possible to state that there is any negative correlation between albumin and blood copper level, but the differences between the mean values of the plasma protein fractions in patients and normal were markedly smaller when compared with the blood copper difference.

The different patterns of corelation between blood copper and plasma protein fractions in each group were discussed.

7) There was some effect on the alteration in blood copper level by the administration of acetylcholine, methionine, and detoxyl. Administration of BAL or versenate has some value in the treatment of patients with Wilson's disease, copper intoxication and hepatocerebral disease, but it is not valuable

diagnostically.

8) In this together with a study of blood copper made on about 120 patients with various diseases, the definite hypocupremia associated with hypercupiuria was not present.

ACKNOWLEDGEMENTS

I am very much indebted for constant aid and advice to prof. Dr. S. Hibino and Dr. I. Sobue at whose department this work and the preparation of this paper were carried out.

REFERENCES

1. WILSON, A. K. *Brain* **34**: 295, 1912.
2. GLAZEBROOK, A. J. *Edin. Med. J.* **52**: 83, 1945.
3. MANDELBROTE, B. M. *Brain* **71**: 212, 1948.
4. CUMINGS, J. N. *Brain* **71**: 410, 1948.
5. PORTER, H. J. *Lab. Clin. Med.* **34**: 1623, 1949.
6. SULLIVAN, F. L. *Arch. Neurol. Psych.* **69**: 216, 1953.
7. HYMAN, I. J. *Lab. Clin. Med.* **43**: 774, 1954.
8. UZMAN, L. AND D. DENNY-BROWN. *Am. J. Med. Sci.* **215**: 599, 1948.
9. COOPER, A. M. *J. Clin. Invest.* **29**: 265, 1950.
10. BEAN, A. G. *Am. J. Med.* **19**: 442, 1953.
11. DAVIDSON, C. S. *J. Clin. Invest.* **29**: 265, 1950.
12. KONOVALOV, N. V. *Yoroppa-Igaku* **3**: 1, 1948 (Japanese).
13. SCHERLOCK, S. *Am. J. Med.* **24**: 805, 1958.
14. ELVEJEM, C. A. *Physiol. Rev.* **15**: 471, 1935.
15. BRAWN, L. UND L. SCHAEFFER. *Biochem. Z.* **304**: 397, 1940.
16. SOBUE, I. AND M. ASAKURA. *Brain and Nerve* **6**: 273, 1953 (Japanese).
17. HEREZ, E. AND A. L. DREW. *Arch. Neurol. Psych.* **63**: 843, 1950.
18. BAUMAN, A. *Am. J. Med.* **19**: 450, 1953.
19. SENDA, N. *Ketuekigaku-Togikai-Hokoku* **2**: 1, 1949 (Japanese).
20. DAVIDSON, C. S. *J. Clin. Invest.* **29**: 265, 1950.
21. HOLMBERG, C. G. *Acta Chem. Scandinv.* **1**: 944, 1947.
22. SCHEINBERG, H. *Science* **116**: 484, 1952.
23. HOLMBERG, C. G. *Scand. J. Clin. and Lab.* **3**: 103, 1951.
24. RAVIN, H. A. *Lancet* **1**: 726, 1956.
25. HAFFON, G. *Arch. Neurol. Psych.* **78**: 227, 1957.