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HEPATOLENTICULAR DEGENERATION

CLINICAL AND BIOCHEMICAL STUDY OF THREE CASES

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The history of hepatolenticular degeneration started with the cases of "pseudosclerosis" reported by Westphal [53] in 1883 and by Strümpell [44, 45] in 1898 and 1899. In 1902, Kayser [30] made the description of a yellow-greenish or brownish ring in the corneal edge, supposedly inborn, in a patient with the diagnosis of "multiple sclerosis". In the following year, Fleischer [16] observed a case of "pseudosclerosis" and other of "multiple sclerosis" with a similar ring, advancing the view that it was an acquired manifestation. In 1908, Salus [38] reported a case of "multiple sclerosis" with a corneal ring and, in 1909, Fleischer [17] published again his two cases, jointly with Kayser's case, considering the corneal ring a manifestation of a new disease process.

In 1911, Voelsch [51] reported the pathological examination of a case of "pseudosclerosis", calling attention to the atrophic nodular cirrhosis of the liver. In 1912, Fleischer [18] stressed the lack of hepatic symptomatology in spite of the severe nodular cirrhosis. In the same year Alzheimer found, in a case clinically studied by von Hoesslin [27], extensive lesions in corpora striata, subthalamus, dentate nucleus and pons, characterized by glial hyperplasia; he described then the giant cells of the neuroglia, currently known as Alzheimer's cells.

Still in 1912, in his masterwork, Kinnier Wilson [54] described the clinical manifestations and the pathological findings, emphasizing the frequently asymptomatic liver cirrhosis, and stressing the familial incidence of the disease; he named it progressive lenticular degeneration. Wilson refuted

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the worth of Alzheimer's histological findings for the characterization of the "pseudosclerosis", considering them as inespecific features. In 1920, Spielmeyer [42] draw the conclusion that, under the pathological viewpoint, Westphal and Strümpell's "pseudosclerosis" and Wilson's disease were two forms of the same disease, which Hall [25], in 1921, named hepatolenticular degeneration.

Some conceptions from this period of the history of the disease are still valid: (a) presence of the Kayser-Fleischer corneal ring, which is granted as a truly pathognomonic sign of the disease; (b) familial incidence; (c) nodular cirrhosis of liver, asymptomatic in most cases; (d) lesions of the lenticular nucleus, mainly the putamen, with destruction of the parenchyma, followed by hyperplasia of the neuroglia and then cavitation; multiple nucleated giant astrocytes of Alzheimer may also be found.

From 1921 to 1947 many clinical reports of the disease were published. But it was not overlooked by the investigators in biochemistry: in 1930, Haurowitz [26], in 1931 Lüthy [32], and in 1945 Glazebrook [21] emphasized the increase of copper content in the liver. Gerlach and Rohrschneider [20], in 1934, showed that the corneal ring is made up of copper granules, a fact corroborated in 1936 by Policard et al. [34] and later by other investigators.

The year of 1948 had great significance in the study of Wilson's disease, since its metabolic nature was evidenced by way of four great investigations: Uzman and Denny-Brown [49] showed the occurrence of increased aminoaciduria, Cumings [10] irrefutably proved the hepato-cerebral overload of copper, Mandelbrote et al. [33] found a permanent increase of the copper excretion in urine, and Holmberg and Laurell [29] demonstrated the existence, in blood serum, of a cuproprotein bound to the globulin fraction, named ceruloplasmin.

From this year on the studies were directed to the investigation of copper metabolism. In 1952, Scheinberg and Gitlin [39] pointed out the low blood ceruloplasmin content, in 1953 Zimdahl et al. [55] evidenced the increased retention of the ingested copper, and, in 1954, Bearn and Kunkel [4] demonstrated the low blood copper content. Still in 1954 Cartwright et al. [8] evidenced an increase of the non ceruloplasmin copper, which reacts directly with diethyldithiocarbamate and is probably bound to the albumin.

In 1953 Bearn [2] showed that the hepatolenticular degeneration is inherited in an autosomic recessive way.

In this other historical period the following concepts were established: (a) increased copper content in the tissues, especially in liver and brain; (b) abnormally increased absorption (with retention) of the ingested copper; (c) increased aminoaciduria; (d) increased excretion of copper in urine; (e) lowering of the serum or plasma total copper; (f) increase of the direct reacting copper in blood serum; (g) low blood ceruloplasmin content; (h) autosomic recessive heredity.

From 1954 on the researches were aimed at the explanation of the pathogenesis of hepatolenticular degeneration, a goal still not attained.

Porter and Folch [35] have identified and extracted from the brain a cuproprotein called cerebrocuprein I. Everything seems to stress the im-

portance of this protein, which is the only human protein with a loose binding of copper (Holmberg [28]).

The studies of Taylor et al. [46] on the presence and dynamics of the liver circulation showed that, in hepatolenticular degeneration, an obstruction of the lesser intra-hepatic branches of the vena porta occurs. So, there is a pre-sinusoidal pattern of obstruction, which justifies the absence of changes in the liver function tests of the majority of cases.

The investigations of Stein et al. [43] and Bickel et al. [6] brought the evidence that the increase of the aminoaciduria is due to the massive increase of glycine, histidine, threonine, cystine, serine, alanin, glutamine, tyrosine, lysine, glutamic acid, leucine and phenylalanin, and to a slight increase of valin. Excretion of taurin is lesser than in normals. The authors draw the conclusion that the aminoaciduria is secondary and not due to an error of the metabolism of proteins, as Uzman et al. [49, 50] had suggested.

The treatment of Wilson's disease aims at the maintenance of a negative copper balance, and for this purpose the following measures are indicated: (a) high protein and low copper diet (about 1 mg of copper a day); (b) potassium sulfide by oral route (one capsule of 40 mg at each meal) in order to render unsoluble the ingested copper, making it to be excreted in the feces; cation exchanging resins can also be used; (c) penicillamine ($\beta\beta$-dimethylcysteine) in daily doses from 500 to 4,800 mg, by mouth [31].

We have studied 3 cases of Wilson's disease from the clinical, biochemical, and genetic viewpoints. We consider the results obtained — some of them not yet recorded in the literature — worthy of publication.

CASE SUMMARIES

CASE 1 — A. O., a 16 year-old girl, Brazilian, white, register no. HC-641290, admitted to the Department of Neurology in 11-11-1961. Full-term pregnancy, normal birth. She frequented primary school in a satisfactory way. At the age of 10 years, she had jaundice, which waned spontaneously; she suffered from occasional epistaxes. The physical, intellectual and gregarious development was normal until the age of 12, when she began to present shaking of the four limbs, followed by progressive disturbance of equilibrium and speech, as well as mental impairment. Only 3 menses untill the age of 16. She does not know a similar disease in her family; she has an elder brother and a younger sister, both healthy.

Good general conditions. Hyperhidrosis. Liver and spleen not palpable. Disorders of behavior and humor, with exhibitionism and crises of aggressivity. Wilsonian facies, with mid-open mouth, slight protrusion of tongue, and "spastic" smile. Normal praxia and language. The patient could not stand. Active movements were present but they were restrained by a marked elastic stiffness in flexion, especially in the lower limbs, with painful crises of aggravation. Severe tremor with a wing-flapping pattern ("Flügelschlagen") interfered in the voluntary movements, particularly of the distal parts of the limbs. There were bradykinesia, asynergy between trunk and limbs, bilateral dysdiadochokinesia and slurred cerebellar speech. Normal deep reflexes in the upper limbs, hyperactive in the lower limbs; transient ankle clonus; the nasopalpebral, orbicularis oris, abdominal, plantar, and tonic postural reflexes were normal. Sensation and cranial nerves not impaired. Bilateral Kayser-Fleischer corneal ring, confirmed at slit-lamp examination. The oto-

logic examination disclosed a normal hearing, uncharacterized provoked nystagmus, and absence of vertigo at the vestibular stimulation. The results of laboratory examination are summarized in tables 1 to 4, and the study of copper metabolism in table 7.

Treatment with Versenate (calcium disodium ethylenediaminotetraacetate) and Carbo-resin (cation exchanging resin) was started, but an infectious picture appeared soon after, causing a sudden worsening of the general conditions. In spite of treatment, the patient died in 12-19-1961.

Post-mortem examination (SS 59583/61, Dr. O. A. Behmer) — Bilateral confluent bronchopneumonia. Liver weighing 920 g, with reduced volume and increased consistency; external surface with brownish nodules 3 to 4 mm wide, involved by fibrous capsule-like tissue (post-necrotic liver cirrhosis). Acute splenitis. Kidneys weighing 90 g each, with normal shape, size and consistency; the cut surface showed focal areas of steatosis, with slight congestion of cortex and medulla. The lenticular (mostly putamen) and caudate nuclei evidenced retraction, brownish pigmentation and a spongy aspect; the histochemical examination showed the presence of intracellular copper granules. The study of copper content of the tissues is summarized in table 5.

CASE 2 — J. S., a 22-year-old industrial worker, white, male, Brazilian from Yugoslav ascent, reg. HC-638026, admitted to the emergency ward of the "Hospital das Clinicas de São Paulo" in 11-24-1961, in state of unconsciousness. His relatives informed that the patient was in good health untill the middle of 1959, when he suffered a fall owing to a transient lack of consciousness. Soon afterwards he began to display speech disorders, lack of interest for his customary activities, and crises of excitation. He was admitted to an inland hospital, where he stayed for 45 days in drowsiness. When his conditions did further deteriorate, he was removed to the emergency ward of our hospital, and admitted to the Neurologic Department 5 days later. The familial history could not be ascertained.

Good physical development; slight jaundice; liver and spleen not palpable. The patient was unconscious, reacting only to painful stimuli. He exhibited spontaneous and pain-induced movements. Waxy stiffness with the cog-wheel phenomenon in the four extremities. The deep reflexes of the limbs were absent, while the orbicularis oris and naso-palpebral reflexes were hyperactive; the Babinski sign was present firstly on the left and later on the right too; the abdominal reflexes were absent; the cough, swallowing, and corneo-palpebral reflexes were present. The patient had a tremor with a parkinsonian rhythm, but with greater amplitude, in the left limbs, especially in the upper left extremity. Bilateral Kayser-Fleischer corneal rings. The results of laboratory examinations are summarized in tables 1 to 4, and the study of copper metabolism in table 7. Soon after admission hyperthermia appeared and the general conditions went worse. In 12-1-1961 hematuria and tracheobronchial oversecretion were noted, the patient dying in 12-3-1961.

Post-mortem examination (SS-59422/61, Dr. J. T. Wainman) — Bronchopneumonia and pulmonary emphysema. Liver weighing 1,150 g, with a globoid shape and a reduced volume; at the external surface nodules 10 to 20 mm wide were noted; in the cut surface greenish and yellowish nodules were seen (post-necrotic liver cirrhosis). Splenomegaly and hyperplasia of the splenic red pulp. Horseshoe kidneys, otherwise normal. Brain with normal shape and consistency, reduced sulci and congested blood vessels. The lenticular nuclei, mostly the putamen, showed reduced consistency and a brownish tinge; slight and proportioned dilatation of the lateral ventricles. The histochemical examination showed the presence of intracellular copper granules. The study of copper content of the tissues is summarized in table 5.

CASE 3 — H. P., a 28-year-old boy, white, Brazilian from Italian ascent, reg. HC-677597, admitted in 1-14-1963. The patient had a normal birth after full-term pregnancy. The psychomotor development was normal. At the age of 18,

he started to abuse of alcoholic drinks, only stopping it when the first symptoms of the disease appeared. He was in good health untill 1962, when he noted that the right fingers were loosing their agility and strength, and started to present rapid and irregular involuntary movements; the disorder did aggravate especially when he attempted to observe the phenomenon, when he tried to stop it or if someone called his attention for it. Soon afterwards he noted difficulty to walk, caused by stiffness and a slight and continuous tremor of the legs. The shaking of the right upper limb got so severe that he could not grasp the objects any more. At the same time the speech became slurred. Some days prior to the admission a tremor appeared in the left hand.

The patient's mother died at the age of 56 years, from arterial hypertension; his father is obese and hypertensive. The patient has 9 brothers and 2 young children. In one of his sisters the slit-lamp examination disclosed a Kayser-Fleischer corneal ring; she suffered from sporadic and transient episodes of slight jaundice but the neurological examination was normal (for more details, see the genetic study and figure 1).

The patient had a good physical constitution. Hyperchromic spots were seen in the right interscapular and lumbar regions. Liver and spleen not palpable. No apparent psychic disorder. Normal language and praxia. Wilsonian facies. When lying, the patient preferred the right lateral decubitus, the left hand holding the right against the bed; trunk and limbs showing a contracture-like aspect. When seated, he held with both hands te bed's borders and bent the trunk forward; the neck shaked continuously; the legs were crossed and flexed. When he stood up, the arms were kept behind the body, the base was broadened, and he assumed a rigid attitude with the head shaking to-and-fro. The gait was rigid. Voluntary movements were satisfactory. The finger-to-nose test was hindered by a tremor which greatly increased at the end of the movement; though the heel-to-knee-to-toe test was also hindered by the tremor and stiffness, errors in direction were not noted even after closing the eyes. The tests for synergy were embarrassed by the rigidity. The swallowing was normal. The speech was slurred. In the limbs, neck and in the protruded tongue there was a continuous alternating tremor with variable intensity. The right arm and especially the right hand shook violently in a wing-flapping way. The deep reflexes were hypoactive; the naso-palpebral, orbicularis oris, abdominal and cremasteric reflexes were normal; the plantar response was variable, more frequently in flexion. Moderate waxy stiffness of the neck, trunk and extremities; the cog-wheel phenomenon was not present. The sensation and the cranial nerves were normal. The eyes sometimes showed transient and short lateral jerks. Kayser-Fleischer rings in both eyes, confirmed at the slit-lamp examination.

The electroencephalogram was normal. The skull X-rays disclosed a slight degree of basilar impression; the hands and feet showed diffuse decalcification, but the joint spaces were preserved and no sign of fracture was seen; hallux valgus. The excretion urography and the electrocardiogram were normal. The needle biopsy of the kidney was normal. The liver biopsy revealed postnecrotic cirrhosis. The results of the laboratory examination are summarized in tables 1 to 4, the study of copper content of tissues in table 5, the metabolic balance of copper in table 6 and figure 3, and the study of copper metabolism in table 7 and figure 4.

Treatment with low copper diet (1.25 mg each day) was associated with BAL (200 mg daily, by intramuscular route), and later with D-penicillamin * (daily doses ranging from 1 to 2 g). Carbo-resin or potassium sulfide was also used. Two months after the use of D-penicillamin and especially after estrogens (diethylstilbestrol 15 mg/day) were associated, a moderate improvement in tremor and rigidity was noted; the biochemical results were definitely good.

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* Courtesy of Merck Sharp & Dohme International, New York, U.S.A.

DISCUSSION

The *neurologic manifestations* in our cases molded to the classic picture of Wilson's disease. All the patients showed a combination of static and kinetic tremor, which had a wing-flapping pattern ("Flügelschlagen") in two. Waxy stiffness of limbs was noted in cases 2 and 3, whereas case 1 showed a marked spasticity in flexion, especially in the lower limbs. In case 1 the tendon reflexes were hyperactive; in case 2 the Babinski sign was present and the abdominal reflexes were absent. Case 1 showed also cerebellar signs. Mental disorders were evident in cases 1 and 2.

In cases 2 and 3 the short course of the disease is striking. The patient J. S. (case 2) was admitted in coma at the emergency ward of the hospital.

The Kayser-Fleischer corneal ring was always complete and bilateral, being visible at naked eye; its presence was confirmed at the slit-lamp examination, which disclosed no "sunflower cataract" in any case.

From the clinical point of view it must be emphasized that the *functional impairment of liver* was mild, despite the severeness of the damage seen at the histologic examination, thus confirming a rule of the literature. As table 2 shows, there is no agreement between the severeness of the neurologic picture and the functional state of liver, at least when it is esteemed through the tests currently used. Such disagreement occurs in spite of the fact that the histologic involvement of liver is a rule in Wilson's disease [48].

This is more striking in case 1, where the usual protein function tests and particularly the bromsulphalein test were normal. However, in case 2 the liver damage, at least according to the results of the flocculation tests, was parallel to the nervous damage; taking account of his excellent physical constitution and the good nutritional state, the low plasma albumin, leading to a positive flocculation test, must be ascribed to the liver damage [41].

According to the absolute figures of the electrophoresis (table 2), the plasma proteins fit the normality range in case 3, and are obviously associated with the normality of the protein function tests, in spite of a slight increase of the transaminases. However, the wilsonian cirrhosis being post-necrotic [1], such disagreement may occur in much the same way as it is seen in the evolution of functional tests in the cirrhosis in general [40].

Finally, the analysis of the whole table 2 leads one to consider not much severe, from the biochemical viewpoint, the liver damage, at least at the time those tests were performed. Besides, the normal bilirubin, alkaline phosphatase, cholesterol and bromsulphalein levels allow the conclusion that the hepatocellular involvement is more marked than the cholangitic damage, a finding conforming more to the post-necrotic than to the biliary cirrhosis.

Laboratory data in blood	*Cases*			*Normal Values*
	1	2	3	
Na (mEq/1)	138	151	139	135 — 143
K (mEq/1)	3.8	5.0	4.0	3.5 — 5.2
Cl (mEq/1)	—	123	97 (5)	100 — 110
Ca (mg/100 ml)	9.8	9.6	10.5 (5)	9.0 — 11.0
P (mg/100 ml)	—	7.4	2.9 (10)	3.0 — 5.0
Mg (mEq/1)	3.00	2.50	2.45	1.7 — 2.3
Co_2 (vol. %)	—	32.4	53.8	45 — 56
Cholesterol (mg/100 ml)	—	—	222	150 — 250
Glucose (mg/100 ml)	120	—	79	60 — 110
Uric acid (mg/100 ml)	1.7	—	2.1	1.5 — 4.5
Mucoproteins (mg/100 ml)	—	—	48	30 — 75
Sialic acid (optical density)	—	—	0.319	0.198 — 0.272
Proteins (g/100 ml)	7.4	6.8	8.3	7.3 ± 0.7
albumin (%)	—	—	60.0	46.0 — 58.5
α_1 globulin (%)	—	—	5.0	2.5 — 5.2
α_2 globulin (%)	—	—	8.8	6.1 — 13.9
β-globulin (%)	—	—	11.0	10.7 — 18.2
γ-globulin (%)	—	—	15.2	15.2 — 22.5
A/G ratio	1.9	0.84	2.3	1.1 — 1.5

Table 1 — Laboratory data in blood. The figures into parentheses indicate the number of determinations, the result being the arithmetic mean.

Tests	*Cases*			*Normal Values*
	1	2	3	
Bromsulphalein clearance (5 mg/kg, % after 45 minutes)	1	6	0	< 5
Weltmann's test (tube reading)	< 6½	< 7½	—	< 6½
Thymol turbidity (MacLagan u.)	2.5	7.1	3.7	< 2.5
Thymol flocculation	+	++	+	0 to +
Hanger's test	++	++++	++	0 to +
Serum albumin (g/100 ml)	4.9	3.1	5.8	3.4 — 5.2
Serum globulins (g/100 ml)	2.5	3.7	2.5	2.0 — 3.5
Serum A/G ratio	1.9	0.84	2.3	1.1 — 1.5
Total serum bilirubin (mg/100 ml)	1.0	—	0.4	0.2 — 1.0
Serum alkaline phosphatase (King-Armstrong units)	8.8	—	9.6	4.5 — 13.5
Glutamic-oxalacetic transaminase (Frankel units/ml)	—	—	146	8 — 40
Glutamic-pyruvic transaminase (Frankel units/ml)	—	—	120	5 — 35
Urine urobilinogen (mg/day)	0	—	—	0 — 3.5
Qualitative urobilinogen (diluting)	0	< dil. 1/100	—	< dil. 1/20

Table 2 — Study of liver function.

Regarding the *renal function,* the hyperphosphaturia, described by Bearn et al. [5] in 1957, was inconstant in case 3, and the metabolic balance trended to be negative. The finding of reducing substances in the urine of cases 1 and 2 was occasional; however, other tests for confirmation of tubular glycosuria, also described by Bearn et al. [5], were not performed.

In hepatolenticular degeneration, the kidney damage is secondary to the disordered copper metabolism [6] in such a way that the increased aminoaciduria of cases 1 and 3, associated with normal or low blood aminoacid content, and thus allowing an increase of its clearance to be foreseen, is a clear-cut example of true renal aminoaciduria. Moreover, the slow and progressive impairment of the renal function [7], though not severe, is evident in case 3, as the slight decrease of tubular excretion in the bromsulphalein test and urea clearance shows (table 3).

Tests	*Cases*			*Normal Values*
	1	2	3	
Bromsulphalein (excretion %) — First	—	—	22	40 — 50
Bromsulphalein (excretion %) — Second	—	—	7	20 — 25
Concentration test — Volhard e Fahr (D)	—	—	1,002	1,025 — 1,030
Dilution test — Volhard e Fahr (D)	—	—	1,004	1,003
Urea clearance — Van Slyke (%)	—	—	55.5	60
Isolated density of urine	1.024	1.027	—	1,017 — 1,020
Albuminuria (g/l)	1.5	3.0	—	0
Reducing substances in urine (g/l)	+	2.3	0	0
Red blood cells in urine (No./field)	60	100	—	< 5
Hematic cylinders in urine (No./field)	—	3	—	0
Granulous cylinders in urine	+ + +	—	—	0
Aminoaciduria (mg/24 hours)	334.4	—	409.0	100 — 300
Phosphaturia (mg/24 hours)	—	—	550 (20)	500 — 1.500*
Calciuria (mg/24 hours)	—	—	281 (20)	100 — 300*
Urine urea (g/l)	—	—	3	10 — 35 g/24h*
Urine magnesium (mEq/24 hours)	5	—	2.0 (20)	4 — 9
Urine creatinine (mg/24 hours)	—	—	1,220 (20)	1,000 — 1,300
Urine sodium (mEq/24 hours)	—	—	125 (20)	100 — 300
Urine potassium (mEq/24 hours)	—	—	62 (20)	25 — 70
Urine chlorides (mEq/24 hours)	—	—	135 (20)	150 — 270
Blood urea (mg/100 ml)	—	148	31	20 — 40

*Table 3 — Study of kidney function. The figures into parentheses indicate the number of determinations, the result being the mean. * Variable with the diet.*

The finding of changes in the urine sediment of two cases, including proteinuria, does not permit other conclusions, because the patients were submitted to drainage of the urinary bladder for several days, and a secondary infection of kidney could not be discarded.

The patient J. S. had a blood urea content of 140 mg/100 ml when he was dehydrated, just before the death.

The blood sialic acid concentration was increased in case 3 (table 1).

Confirming the references of the literature, we found low blood uric acid content and high aminoacid concentration in urine (cases 1 and 3). We found no reference to the low blood phosphorus and the high blood magnesium contents noted in our cases.

The routine examination of the cerebrospinal fluid gave normal results. The electrophoresis of the proteins (table 4) showed absence of prealbumin in case 1 and 3, low α_1 and α_2 globulins in two cases, reversal of the α_1/α_2 ration in case 3, and high β globulin in case 2.

Cerebrospinal fluid	*Cases*			*Normal Values*
	1	2	3	
Magnesium (mEq/1)	3.50	—	1.83	—
Total proteins (mg/100 ml)	22.0	16.0	32.0	< 30.0
Pre-albumin (%)	0.0	6.6	0.0	0.1 — 7.0
Albumin (%)	59.7	44.0	64.0	43.4 — 60.5
α_1 globulin (%)	2.3	2.7	3.7	3.4 — 7.9
α_2 globulin (%)	8.1	3.2	3.1	5.5 — 12.8
β globulin (%)	20.5	29.7	17.0	14.9 — 27.5
γ globulin (%)	9.4	13.8	12.0	7.8 — 14.2
α_1/α_2 ratio	0.28	0.84	1.19	< 1.0

Table 4 — Magnesium content and proteinogram of the cerebrospinal (cisternal) fluid.

The *genetic features,* which were evident through the biochemical investigation of the relatives of case 1, could be more fully analyzed in case 3 (table 7, figs. 1 and 2). A sister of this patient (Ad. P.) evidenced abnormally low levels of blood copper and ceruloplasmin; although she was asymptomatic from the neurologic and hepatic viewpoints, a Kayser-Fleischer corneal ring was disclosed at the slit-lamp examination. A son of the patient (W. P.), 18 month old, had the same biochemical disorders, though no neurologic or ocular sign was present. The other son, 3 years old and also asymptomatic, had a low blood ceruloplasmin level. In other sister (Er. P.) the same biochemical disorder was present. Regarding the urine copper contents, high levels were found in the father, a brother (Al. P.) and in a sister (Ad. P.) of the patient. Eight relatives of the patient were submitted to the slit-lamp examination but the Kayser-Fleischer ring was found only in the mentioned sister. The cytogenetic study of bone marrow of the patient revealed a normal karyotype (fig. 2).

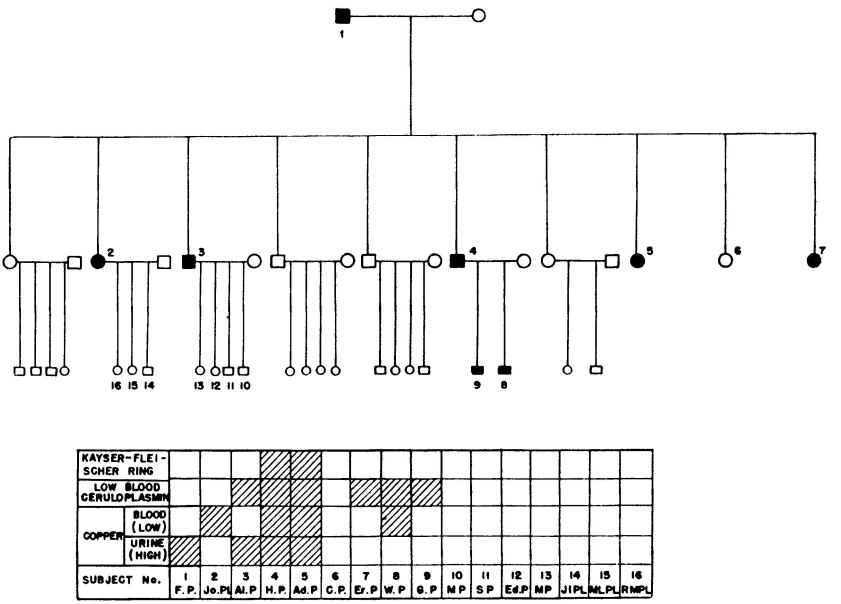

Fig. 1 — Pedigree of patient H.P. (No. 4), showing in black the biochemically affected relatives. At bottom, a schematic chart of the disorders of copper metabolism in this family.

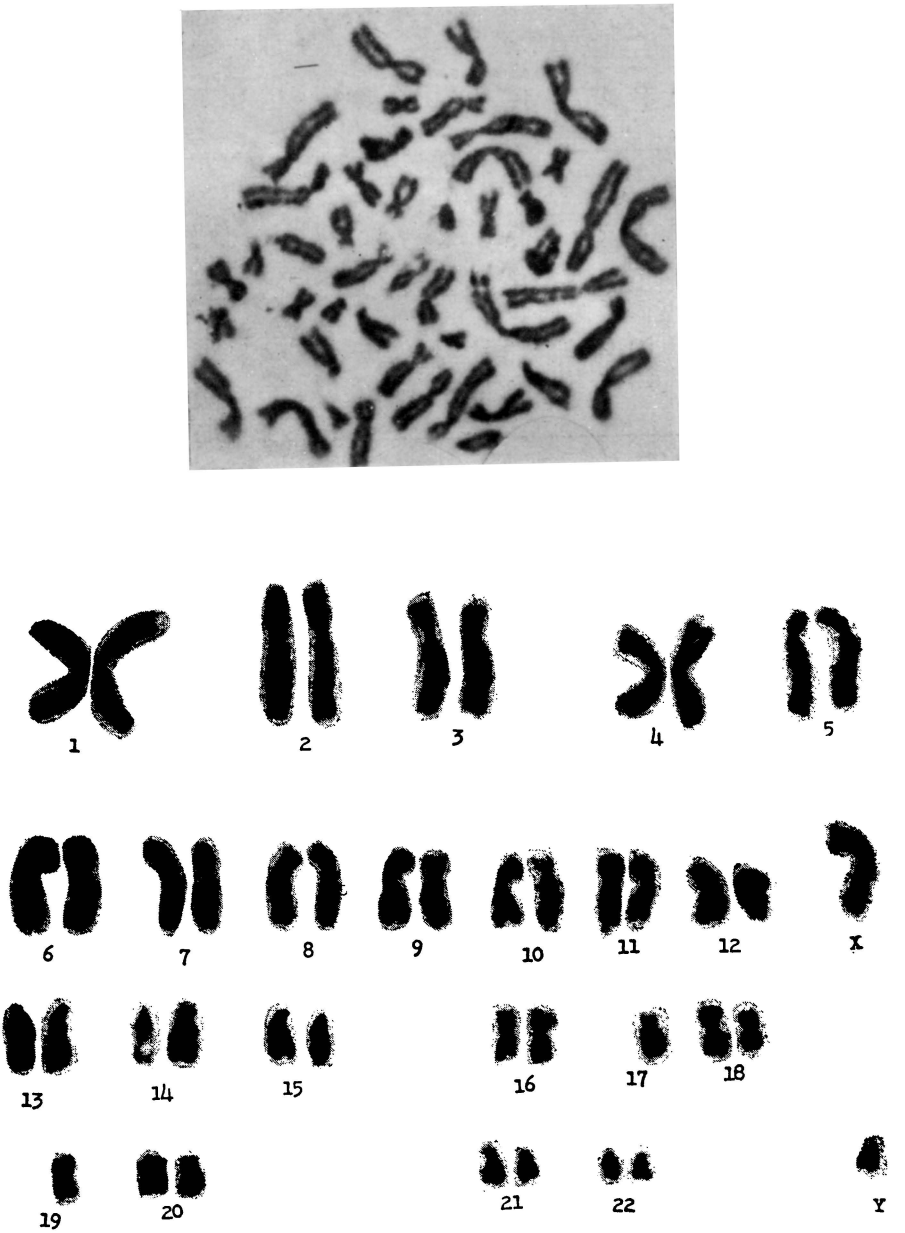

Fig. 2 — Cytogenetic study of patient H. P. (case 3).

Tissue	Case 1		Case 2		Case 3	Normal Values	
	Fresh material	Dry material	Fresh material	Dry material	Dry material	Fresh material	Dry material
Temporal lobe (white matter)	4.95	23.14	4.93	25.21	—	0.54 (47)	1.40 (52)
Temporal lobe (gray matter)	—	—	5.78	29.87	—	0.80 (47)	2.66 (52)
Frontal lobe (white matter)	1.48	5.73	2.02	7.32	—	0.43 (47)	1.28 (52)
Lenticulo-caudate nuclei	1.50	9.00	3.10	16.55	—	0.64 (24)	2.94 (52)
Thalamus	2.12	15.80	4.65	21.69	—	0.56 (24)	2.49 (24)
Substantia nigra	5.93	35.59	4.28	16.42	—	—	5.99 (52)
Red nucleus	4.19	30.18	3.93	18.45	—	—	2.27 (52)
Cerebellum (cortex)	4.42	26.52	3.66	19.92	—	0.70 (8)	3.30 (8)
Pancreas	2.43	10.61	1.11	4.16	—	0.60 (19)	0.85 (12)
Submaxillary gland	0.63	3.10	0.64	2.37	—	—	1.43 (12)
Adrenal gland	1.28	3.91	—	—	—	0.15 (8)	0.50 (8)
Kidney	1.86	9.61	3.11	15.92	22.32 *	0.19 (8)	1.00 (8)
Liver	4.95	21.29	4.84	20.27	35.00 *	1.66 (24)	5.92 (24)
Hair	—	—	—	—	0.33	—	1.50 (37)
Nail	—	—	—	—	5.52	—	0.9-8.1 (22)
Cerumen	—	—	—	—	7.17	—	2.43 (14)

*Table 5 — Copper contents (mg/100 g) in the tissues. * Biopsy. Normal mean values: into parentheses, the references.*

Copper contents in the tissues — The samples were removed either at the post mortem examination (cases 1 and 2) or by needle biopsy (case 3).

In all the examined tissues the copper levels were increased (table 5). Other authors have already evidenced this copper overload in pancreas [6, 8], kidneys [6, 8, 47], adrenal glands [6, 8], liver [8, 10, 21, 24, 26, 47], white matter of brain [6, 8, 24], gray matter of brain [8, 24], lenticular and caudate nuclei [6, 24], thalamus [24], cerebellar cortex [6, 8, 24]. In brain, greater copper levels were found in the white and gray matter of the temporal lobe, and in the thalamus (table 5). No reference in the literature was found concerning the copper contents of the red nucleus and substantia nigra in hepatolenticular degeneration; in cases 1 and 2 the copper levels were markedly increased in these mesencephalic nuclei.

No reference was found in the literature regarding the copper contents of the submaxillary glands and cerumen in this disease; in our cases the levels were increased as compared to normals [12, 14]. In hairs the copper content was low, a finding already observed by Rice and Goldstein [37], who stressed the absence of correlation between the copper concentration in hairs and the severeness of the disease. In nails the copper content fell in the normal range, thus confirming the results of Rice and Goldstein [37].

Copper metabolism and metabolic balances — Low blood copper and ceruloplasmin contents and increased copper concentration in urine and saliva were found in the three cases of Wilson's disease (table 7). Blood direct reacting copper was determined only in case 3 and showed a moderate increase. Cerebrospinal fluid copper content was found to be within the normal range [13], the total levels being close to the concentration of direct reacting copper. In A and C-bile the copper content was low, while in B-bile it was normal; in the mixture of the three fractions the copper level was low (table 7).

After administration of Versenate (case 1), dimercaprol (BAL) and especially $\beta\beta$dimethylcysteine (D-penicillamine) in case 3, a marked rise of copper excretion through urine was evidenced (table 6, figs. 3 and 4).

The metal binding agents promote this cupriuretic response in subjects with diseases other than hepatolenticular degeneration, and even in normals [23]. In patient A. D. (reg. HC-283812), showing a tremor in the extremities much alike the wilsonian hyperkinesia, but with high blood copper

	Periods (5 days)	Ingesta (by day)			Excreta (by day)						Balance (mg/day)		
		Diet (mg)			Feces (mg)			Urine mg()					
		Ca	P	Cu	Ca	P	Cu	Ca.	P	Cu	Ca	P	Cu
Routine diet	Control	802	951	2.250	119	150	1.595	636	1,024	0.488	+ 47	— 223	+ 0.167
	Control	780	908	2.180	276	126	1.500	540	667	0.445	— 36	+ 115	+ 0.235
Low copper diet	BAL	650	710	1.250	389	232	1.179	281	466	0.787	— 20	+ 12	— 0.716
	BAL	650	710	1.250	363	189	0.836	280	580	0.738	+ 7	— 59	— 0.324
	Control	650	710	1.250	201	289	0.812	267	419	0.426	+ 182	+ 2	+ 0.012
	Penicillamine 1.0 gm	650	710	1.250	292	158	0.980	347	567	1.450	+ 11	— 15	— 1.180
	Penicillamine 1.5 gm	650	710	1.250	284	324	0.782	634	527	1.786	— 268	— 141	— 1.318
	Penicillamine 2.0 gm	850	1.450	1.250	642	283	0.992	634	527	0.762	— 462	+ 640	— 0.504
	Penicillamine 2.0 gm	850	1.450	1.250	106	180	1.100	230	625	0.471	+ 514	+ 655	— 0.321
	Penicillamine 2.0 gm + Estrogen	850	1.450	1.250	235	199	0.351	391	730	2.000	+ 124	+ 521	— 1.101

Table 6 — Patient H.P. (case 3): copper, calcium and phosphorus balances.

	Blood Serum			Cerebrospinal fluid			Urine	Saliva	Bile			Slit-lamp examination
	Copper		Cerulo-plasmin	Copper		Cerulo-plasmin	Copper	Copper	Copper (μg/100 g)			
	Total (μg%)	Dir. R. (μg%)	(mg%)	Total (μg%)	Dir. R. (μg%)	(mg%)	(μg/day)	(μg%)	A	B	C	
A.O. (case 1)	78	—	3.5	4	—	—	1,640	366	—	—	—	K-F
M.L.O. (mother)	58	—	9.8	10	—	—	450	—	—	—	—	N
D.O. (sister)	138	—	12.6	—	—	—	174	—	—	—	—	N
H.O. (brother)	108	—	6.8	5	—	—	314	—	—	—	—	N
J.S. (case 2)	64 (2) 58-70	—	0.0	—	—	—	1,400	360	—	—	—	K-F
H.P. (case 3)	46 (11) 28-72	22 (7) 13-32	1.5 (11) 0.0-9.0	33 (2) 30-36	33 (2) 30-36	0.0 (2)	466 (10)	166 (3) 151-181	18	115	17	K-F
F.P. (father)	126	—	24.6	—	—	—	896	—	—	—	—	—
W.P. (son)	29	17	0.0	—	—	—	—	—	—	—	—	—
G.P. (son)	111	10	11.7	—	—	—	—	—	—	—	—	—
Al.P. (brother)	116	—	22.2	—	—	—	489	—	—	—	—	—
Ad.P. (sister)	72	—	6.4	—	—	—	615	—	—	—	—	K-F
J.P. (sister)	207	—	26.4	—	—	—	18	—	—	—	—	N
C.P. (sister)	88	—	29.5	—	—	—	190	—	—	—	—	N
Er.P. (sister)	103	—	17.8	—	—	—	88	—	—	—	—	N
R.M.P.L. (niece)	119	—	34.9	—	—	—	134	—	—	—	—	N
M.P.L. (niece)	96	—	28.9	—	—	—	263	—	—	—	—	N
M.L.P.L. (niece)	119	—	34.9	—	—	—	86	—	—	—	—	N
Ed.P. (niece)	—	—	—	—	—	—	72	—	—	—	—	—
J.I.P.L. (nephew)	150	—	34.9	—	—	—	117	—	—	—	—	N
S.P. (nephew)	—	—	—	—	—	—	53	—	—	—	—	—
M.P. (nephew)	—	—	—	—	—	—	125	—	—	—	—	—
Normal Values — Mean	108.1	—	29.9	42.6	—	—	374.0	31.7	109			—
Normal Values — Standard deviation	9.7	—	3.8	5.7	—	—	249.3	15.1	Range: 35-205			—

Table 7 — Study of copper metabolism in patients with Wilson's disease and in their relatives. (The figures into parentheses indicate the number of determinations, the result being the arithmetic mean. K-F: Kayser-Fleischer ring. N: normal.)

and ceruloplasmin contents and no Kayser-Fleischer ring, this effect of BAL was also marked (table 8).

D a t e (September 1962)	*Blood copper (µg/100 ml)*	*Urine copper (µg/day)*	*Treatment*
20	207	753	—
21	205	537	—
26	254	2,651	BAL
27	250	1,579	BAL
28	175	989	BAL
29	148	402	BAL

Table 8 — Patient A. D., with a non-wilsonian hyperkinetic syndrome: changes of copper concentration in blood and urine evoked by the treatment with BAL.

In case 3, the copper balance was positive in the control periods, becoming markedly negative after the use of metal binding agents, especially D-penicillamine; the association of estrogens evoked a new and strong negativation of the copper balance (table 6, fig. 3). As Goldstein et al. [23] already mentioned, the excretion of copper in urine is very pronounced at the onset of the treatment, but later it gradually falls, approaching the pretreatment range approximately one year afterwards, despite the continued use of the drug. In case 3 this fall of cupriuresis is also evident (fig. 4).

Regarding phosphorus balance, it was found that it was slightly negative in the control periods, becoming markedly negative 10 days after the onset of the treatment with penicillamine; this finding lead us to administer, during the subsequent treatment, a formula containing dibasic sodium phosphate.

Therapeutic results — In case 1 treatment with a chelating agent (Versenate) and cation exchanging resin was started few days before the death, which occurred unexpectedly after bronchopneumonia. In case 2 no specific therapy for Wilson's disease was instituted, since the patient was admitted in coma to an emergency ward and died ten days later. In case 3, however, the current therapeutic agents, including D-penicillamine, were used. Nevertheless, in spite of a markedly negative copper balance (table 6, fig. 3) the clinical improvement, other than moderate, appeared only about 2 months later, when estrogens were associated.

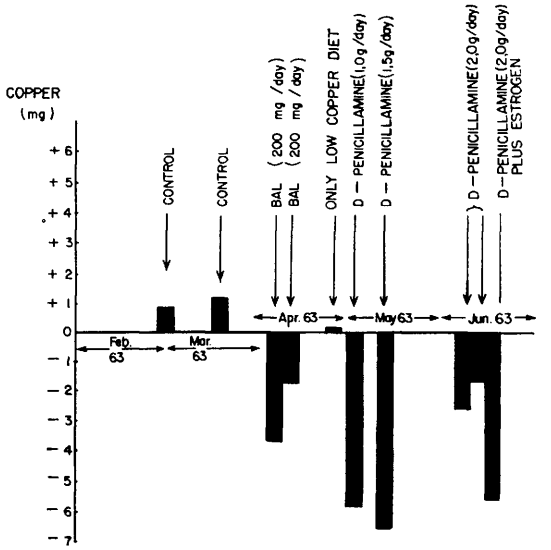

Fig. 3 — Patient H.P. (case 3). Metabolic balances of copper in the control periods and during the therapeutic courses with low copper diet, metal binding agents, and estrogens (periods of 5 days).

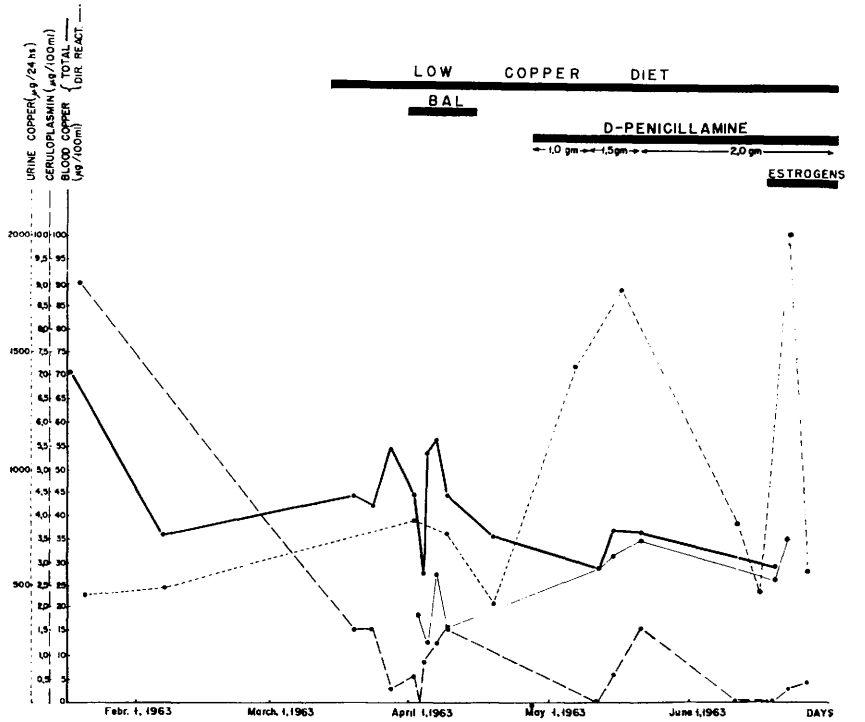

Fig. 4 — Patient H.P. (case 3). Blood and urine copper, and blood ceruloplasmin determinations before and during the therapeutic courses.

In spite of the still short therapeutic course with D-penicillamine, the benefits of which usually became apparent only months or years after its onset [23, 31], the partial failure noted in our case could be explained by the findings of Duckett et al. [15]; these authors, after determining the copper concentration in the tissues of a patient with Wilson's disease unsuccessfully submitted to treatment with penicillamine, draw the conclusion that the greatest copper depletion occurred in liver.

SUMMARY

After a review of the fundamental steps in the history of hepatolenticular degeneration, the authors report 3 cases of the disease. Two patients died, the post-mortem examination having confirmed the clinical and laboratorial diagnosis.

Among the neurologic particularities the presence, in one case, of flexion spasticity and cerebellar signs, besides the usual picture of Wilson's disease, is stressed. The Kayser-Fleischer corneal ring was always complete and bilateral. Among the unsteady features of the proteinogram of the cerebrospinal fluid, absence of pre-albumin, and lowering of α_1 and α_2 globulins were more frequently found. Low blood phosphorus and magnesium contents were found in our cases, seemingly for the first time in the literature.

Special attention was dedicated to the study of liver and kidney functions through the proper functional tests. The impairment of liver function was mild, in a striking disagreement with the degree of the histologic picture of post-necrotic cirrhosis. Regarding the kidney, in two cases increased urinary excretion of aminoacids was found, associated with a slightly lowered tubular excretion in one of them.

The genetic study could be more detailed in one of the cases, and included the search for Kayser-Fleischer corneal ring, the determination of copper in blood and urine, and of blood ceruloplasmin in the relatives, besides the cytogenetic study of the patient. Kayser-Fleischer ring was found in an otherwise asymptomatic sister, and disorders of copper metabolism were evidenced in her and some other relatives.

The study of copper contents in the tissues confirmed the data of the literature regarding the overload in pancreas, kidneys, adrenal glands, liver, white and gray matter of the brain, thalamus, cerebellar cortex, and in the lenticular and caudate nuclei, as well as normal levels in the nails and low contents in the hairs. Presumably for the first time, an increase of copper concentration in the substantia nigra and red nucleus, as well as in the submaxillary gland and cerumen, was found. In the cerebrospinal fluid the total copper content was very close to the direct reacting copper level, and fell into the normal range. In bile our data agree with the references in the literature, the copper content being normal in total bile and in B-fraction, and low in A and C-bile.

Low blood copper and ceruloplasmin contents and increased copper concentration in urine and saliva were found in the three cases. Blood direct reacting copper was determined only in one case and showed a moderate increase.

The study of the metabolic balance showed a positive result for copper in the control periods, and a slight negative result for phosphorus. After the use of BAL, and especially of D-penicillamine, the copper balance became negative and the phosphorus balance turned out markedly negative.

Low copper diet, cation exchanging resins or potassium sulphide were associated with the metal binding agents in different therapeutic schemes. The clinical improvement, although noticeable in case 3 after the use of D-penicillamine and estrogens, did not agree with the gratifying biochemical results.

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RESUMO

Degeneração hepatolenticular: estudo clínico e bioquímico de três casos.

Após reverem os marcos fundamentais da história da moléstia de Wilson, os autores relatam 3 casos desta afecção. Dois pacientes faleceram, tendo o exame necroscópico confirmado o diagnóstico clínico-laboratorial.

Entre as particularidades do quadro neurológico, sobressaía, em um caso, a existência de espasticidade em flexão e de sinais cerebelares. O anel corneano de Kayser-Fleischer apresentou-se sempre completo e bilateral. Entre os caracteres inconstantes do proteinograma do líquido cefalorraqueano são salientadas a ausência de pré-albumina e os baixos níveis de globulinas α_1 e α_2. Provàvelmente pela primeira vez, foi observada redução dos valôres de fósforo e magnésio no sôro sangüíneo.

O estudo das funções hepática e renal foi realizado por meio dos testes convencionais, tendo-se verificado que o comprometimento do fígado era discreto, em nítida desproporção com o grau de lesão histológica do órgão (cirrose pós-necrótica). Em relação aos rins, foi comprovada hiperaminacidúria em dois casos, associada a discreta diminuição da excreção tubular, em um dêles.

A análise genética pôde ser mais aprofundada em um dos casos, incluindo a pesquisa, nos seus familiares, do anel de Kayser-Fleischer, a determinação dos valôres de cobre no sangue e urina, e da ceruloplasminemia, além do estudo citogenético do paciente. O anel corneano foi comprovado em uma irmã assintomática, ocorrendo nela e em outros parentes nítidas alterações do metabolismo do cobre.

O estudo do conteúdo de cobre nos tecidos confirmou os dados da literatura quanto ao acúmulo dêsse metal no pâncreas, rins, supra-renais, fígado, substâncias branca e cinzenta do cérebro, tálamo, córtex cerebelar e núcleo lentículo-caudado, assim como níveis normais nas unhas e baixos nos cabelos. Verificação provàvelmente inédita foi o achado de acúmulo de cobre na substância negra e núcleo rubro, assim como nas glândulas submandibulares e no cerume. No líquido cefalorraqueano os valôres do cobre total se encontravam dentro dos limites normais e muito próximos dos do cobre de reação direta. Na bile, de acôrdo com as referências da literatura, observamos concentração de cobre normal na bile total e na fração B, e valôres diminuídos nas biles A e C.

Em todos os casos verificou-se diminuição da concentração de cobre e ceruloplasmina no sangue e elevação dos valôres na urina e saliva. O cobre de reação direta do sôro, determinado em um caso, mostrou-se em nível relativamente alto.

O estudo dos balanços metabólicos demonstrou positividade para o cobre nos períodos de contrôle, e discreta negatividade do balanço do fósforo. Após o emprêgo do BAL, e particularmente da D-penicilamina, o balanço do cobre se negativou e o do fósforo tornou-se mais acentuadamente negativo.

Dieta hipocúprica, resinas permutadoras de catíons ou sulfeto de potássio foram associados aos agentes complexantes. A melhora clínica, embora nítida no caso 3 após o uso de D-penicilamina e estrógenos, não correspondeu ao excelente resultado bioquímico.

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