INVESTIGATION OF THE AMINOACIDURIA IN WILSON'S DISEASE (HEPATOLENTICULAR DEGENERATION): DEMONSTRATION OF A DEFECT IN RENAL FUNCTION [1]

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Kinnier Wilson, in his paper entitled "Progressive Lenticular Degeneration: A Familial Nervous Disease Associated With Cirrhosis of the Liver" (1), established in 1912 as a nosologic entity the condition now referred to as Wilson's disease, or hepatolenticular degeneration. Although many subsequent advances have been made in knowledge of the clinical forms and morphologic features of this disease, there has been a paucity of information concerning metabolic abnormalities which are presumed to be present in some form in a disease with such widespread manifestations. Recently, Uzman, and Denny-Brown (2) described a patient with hepatolenticular degeneration who had increased quantities of amino acids in the urine as shown by one-dimensional paper partition chromatograms and chemical determination of the total amino nitrogen, alanine, and glutamic and aspartic acids in 24-hour urine specimens. The patient had no demonstrated renal disease, and only minimal evidence of liver disease. The authors suggested that the aminoaciduria might represent either a failure of deamination of amino acids by an impaired liver, or an underlying defect in the metabolism of amino acids resulting in increased excretion by a normal kidney.

The experiments to be described were undertaken to elucidate the mechanism of the excessive loss of amino acids in Wilson's disease with respect to the site and type of disorder.

MATERIALS AND METHODS

Alpha amino nitrogen was determined by the gasometric ninhydrin methods described by Hamilton and Van Slyke for plasma (3) and by Van Slyke, MacFadyen, and Hamilton for urine (4). All determinations were done in duplicate on separate plasma filtrates, and unless otherwise indicated the values refer to "unbound" alpha amino nitrogen. The ten "essential" amino acids and glutamic acid in urine were determined quantitatively according to the microbiological assay procedures of Stokes and his associates (5). One-dimensional paper partition chromatograms of amino acids in urine were done by the method of Consden, Gordon, and Martin (6) as adapted by Dent (7) for biological fluids. Venous blood sugar was determined according to Folin and Wu (8). The presence of sugar in the urine was confirmed and quantitated, after the routine Benedict's test, by the carmelization method of Somogyi (9). The serum and urine calcium (10) and phosphorus (11) were done by standard procedures. Standard methods also were used for determination of the serum bilirubin (12), cephalin cholesterol flocculation (13), and thymol turbidity (14).

The 24-hour urines were collected with 10 cc. of a 5:1 mixture of glacial acetic acid and toluol, while shorter collections were taken with 2 cc. of this preservative. The preservative was shown to have no effect on the alpha amino nitrogen determinations. Urines were stored at 4° C. for alpha amino nitrogen determinations, and at −20° C. for microbiological assay of individual amino acids. Protein-free plasma filtrates for alpha amino nitrogen determinations were prepared with 5 per cent tungstic acid and one-third normal sulfuric acid and were stored at −20° C. No deterioration was found after storage for two months.

The normal values for blood and urine alpha amino nitrogen and for individual amino acids in the urine were obtained from studies on house officers and technicians who were in good health.

CASE SUMMARIES

Studies were carried out on six patients with hepatolenticular degeneration. Four of these patients were referred to us by Dr. D. Denny-Brown of the Neurological Unit of the Boston City Hospital; one by Dr. J. C. Bullard of the Cushing Veterans Administration Hospital; and one by Dr. J. D. Crawford of the Massachusetts General Hospital.

J. G., A. G., I. S. These cases, males aged 30, 38, and 37, are reported in detail in previous papers (2, 15, 16) and their neurological findings had not changed essentially

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since those reports. All represented rather mild forms of Wilson's disease. At the time of the present study J. G. and A. G. presented no clinical evidence of hepatic dysfunction, while I. S. had a liver which was palpable 4 cm. below the costal margin with a firm, smooth, non-tender edge.

Routine laboratory studies were unremarkable except that several pre-breakfast urine specimens in each of these patients contained substances reducing Benedict's reagent.

M. R. This patient, also previously reported (15), was a 23-year-old female. A diagnosis of Wilson's disease had been made three years previously. Her disease progressed rapidly and she died of the ensuing complications of bulbar paralysis during the period of this study. When she entered the hospital she was severely malnourished and dehydrated, with pneumonitis at both lung bases and a temperature of 101° F. Despite the severity of her disease she still had no clinical evidence of hepatic involvement.

Except for an elevated white blood count and moderate numbers of white blood cells in the urine, her routine laboratory studies were normal.

All four of the above patients had classical neurological signs and Kayser-Fleischer rings, confirmed by slit-lamp examination. A. G. and J. G. were brothers, while M. R. had two brothers who had previously died of hepatolenticular degeneration.

The last two cases are presented in more detail since they have not previously been reported.

R. K. was a 21-year-old white man whose first symptoms may have been present at the age of 15, when his parents noted that his movements, speech, and changes of facial expression were performed with unusual slowness. There was no history of hepatic disease. When he was 18 he was given a medical discharge from the Navy, with a tentative diagnosis of early schizophrenia. About four months after his discharge, the patient first became aware of the presence of tremors, first in the right arm, but soon involving all four limbs. Six months later they were of such severity that he was unable to feed or dress himself. In addition he began to drool saliva and experienced some difficulty in swallowing. At the age of 20 he was studied at another hospital where the diagnosis of Wilson's disease was made and a liver biopsy was done.

Two siblings of the patient, aged 18 and 24, were examined and showed no evidence of Wilson's disease. The family background also was negative.

Upon admission to the hospital for the present study the patient had the typical open-mouthed grin and drooling of severe hepatolenticular degeneration. Coarse tremors were severe and present almost constantly, and upon attempting finely coordinated movements his whole body broke forth in a wild confusion of shaking. He walked with an unsteady, wide-based, rolling gait, keeping his arms closely pressed against his sides to control their motion. He was quite euphoric and had no insight into the severity of his illness.

Examination further revealed normal deep and superficial reflexes, abnormally slow palatal reflex, slight nystagmus of upward gaze, and a plastic rigidity of the limbs upon passive movement. Kayser-Fleischer rings were present. The liver was not palpable and seemed abnormally small to percussion. The tip of the spleen was felt.

Routine laboratory studies of blood and urine were normal.

J. A. The last patient in the series was a 13-year-old boy whose first deviation from apparent good health was noted three years previously. At that time he sought medical aid because of a febrile illness, and his physician noted that the liver was palpable 3 cm. and the spleen enlarged 5 cm. below the costal margins. No unusual neurological findings were observed. However, approximately one year later there was an insidious onset of dysphagia, difficult deglutition, and a fine tremor of the hands and arms, together with emotional instability. Definite Kayser-Fleischer rings were present at that time and the diagnosis of Wilson's disease was made. Hepatosplenomegaly persisted. The boy continued to be well nourished. During the ensuing year and one-half, however, there was a gradual and progressive increase in the severity of his neurological involvement, so that by the time the present studies were performed the boy was completely invalided and bedridden.

None of the other members of his family, including two siblings, were similarly afflicted.

The physical examination revealed a well-developed but undernourished boy with open-mouthed, mask-like facies, permitting on occasion a slow smile. He swallowed liquids with great difficulty, and continually drooled saliva. Marked emotional lability was present. Although unable to speak, the boy had quickly mastered a crude sign language which permitted him to communicate his basic wants to the nursing staff.

With assistance, he staggered with an unsteady, wide-based gait. Coarse tremors were present in all extremities and in the eyelids and tongue. The reflexes were normal. Pronounced Kayser-Fleischer rings were readily seen in both corneas, and there were early lenticular cataracts.

The liver and spleen were easily palpable and unchanged in size since first observed as an incidental finding three years previously. In addition, there was moderate palmar erythema, a rare "spider" angiomata, and definite bilateral gynecomastia. He was not jaundiced.

Examination of the blood revealed a mild anemia and leukopenia, but was otherwise normal. The routine urinalysis was within the limits of normal.

The first three cases described, J. G., A. G., and I. S., represent the type of Wilson's disease often called pseudosclerosis (17), with onset later in life, a long course, and little or no rigidity or dystonia. Cases R. K. and J. A. are typical of the progressive lenticular degeneration described by Wilson, while M. R. probably is a combination of the two forms.

HEPATIC STUDIES

An investigation of amino acid metabolism must involve consideration of the role of the liver, and

TABLE I

Liver function tests of patients with Wilson's disease

	Total serum bilirubin *mgm./100 cc.*	Cephalin cholesterol flocculation 0 to 4+	Thymol turbidity *cc. $BaSO_4$*	Thymol flocculation 0 to 4+	Bromsulphalein retention* *per cent*	Serum colloidal gold
Normal range	Below 1.0	0 to 2+	Below 1.7	0 to 1+	0 to 4	0 × 10
J. G.	0.7, 0.7, 0.6	1+, 2+, 2+, 4+	3.1, 3.8, 2.2	3+, 4+, 2+	2	
I. S.	0.4		3.7	3+	3	
A. G.	0.4, 0.7	1+, 2+, 3+	2.2, 1.3	0, 0	2	4444333322
M. R.	0.4, 0.9	1+	1.0, 1.1	0, 0	15 (fever)†	
R. K.	0.5, 0.9	2+, 1+	0.9, 1.2	1+, 0	5	5555433321
J. A.	2.1, 3.1, 1.0	4+, 4+, 4+	3.2	3+, 4+	40, 36	0000111111

* At 45 minutes after intravenous injection of 5 mgm. dye per kilogram of body weight.
† Because of fever due to infection this value is of doubtful significance (18).

an attempt was made to evaluate the state of liver function in each of the patients in this study. Five of the six patients denied symptoms referable to the liver either preceding or during their disease. Of these five patients, one had an enlarged liver, and another had a palpable spleen and an abnormally small liver to percussion. The sixth patient, J. A., demonstrated clinical and laboratory evidence of liver disease which anteceded the neurological manifestations of Wilson's disease. The results of liver function tests done on all patients at intervals during the study are shown in Table I. Although all patients have one or more abnormal values recorded, in only one case, J. A., could the dysfunction be considered consistent and severe.

Liver punch biopsies obtained from R. K., J. G., and A. G. were reported as follows: [4]

R. K. Specimen fixed in Zenker's solution. Well-preserved liver cells with granular acidophilic cytoplasm. The nuclei have loose chromatin and prominent nucleoli. Occasional giant cells and binucleate cells are seen but neither mitoses nor foci of necrosis are found. Occasional cells contain small clear vacuoles. There is one focal collection of collagenous fibrous tissue in which are enmeshed bile ducts and a few small round cells. The portal areas are otherwise unremarkable. No pigment is seen in the liver cells.

J. G. Specimen fixed in 10 per cent aqueous formalin. Sections essentially similar to those from R. K. except that there is no focal area of fibrosis and many of the parenchymal cells contain a granular yellow-brown pigment in their cytoplasm. This pigment is not acid-fast, therefore not ceroid, and does not react as hemosiderin or hemofuscin with specific stains.

A. G. Specimen fixed in 10 per cent aqueous formalin. The sections are similar to those described above, except that there is more pronounced vacuolization of the liver cells and the pigment is perhaps more prominent than in the sections from J. G. In addition, many of the liver cell nuclei are vacuolated.

The significance of these findings is difficult to estimate. There is no evidence of diffuse hepatic fibrosis or portal cirrhosis in any of the biopsies. The focus of fibrosis in the first biopsy may indicate a scarring of the post-necrotic or healed acute yellow atrophy type of cirrhosis, but more likely is just a subcapsular area of increased fibrous tissue.

The presence of binucleate cells and giant cells is thought by many to be evidence of reaction of the liver to noxious stimuli. However, no mitoses were seen nor were there foci of necrosis, so that the significance of these changes is questionable.

The vacuoles seen in the parenchymal cells most likely represent fat droplets, but only in the biopsy from A. G. were they present in definitely abnormal numbers. Their pattern was suggestive of ordinary fatty metamorphosis.

The pigment seen in the formalin-fixed sections is probably a lipofuscin of the type called Abnützung or "waste" pigment, usually increased in cells of the heart and liver in so-called brown atrophy. An increase of a similar pigment is seen in the biopsies of livers of patients hospitalized as a result of an alcoholic spree (19). The interpre-

[4] The authors gratefully acknowledge the assistance of Edgar Taft, M.D., First Assistant in Pathology, Mallory Institute of Pathology, Boston City Hospital, in the pathological interpretation of these specimens.

TABLE II

Comparison of plasma and urine alpha amino nitrogen values in normal subjects and in patients with Wilson's disease

	Normal			Wilson's disease		
	Number determinations	Number subjects	Average value (range)	Number determinations	Number patients	Average value (range)
Urine alpha amino nitrogen Diet *Ad Libitum* *mgm./24 hours*	20	15	164 (118 to 204)	36	6	390 (204 to 640)
Urine alpha amino nitrogen Fasting *mgm./one hour*	20	17	6.3 (3.5 to 13.0)	23	5	18.1 (6.4 to 37.2)
Plasma alpha amino nitrogen Fasting *mgm./100 cc.*	31	19	3.9 (3.1 to 4.7)	27	6	4.3 (3.3 to 5.7)

tation of increased pigment in the biopsies described above is difficult even to hypothesize.

EXPERIMENTS AND RESULTS

Alpha Amino Nitrogen Excretion. The free alpha amino nitrogen excreted in the urine in 24 hours by normal subjects averaged 164 mgm. compared with 390 mgm. excreted by patients with Wilson's disease (Figure 1, Table II). Although the range of values in the patients was large, there was no overlapping of values from patients and normals in the present sampling. Only one of the 20 determinations of alpha amino nitrogen in normal subjects exceeded 200 mgm. daily. In contrast, all of the 36 determinations in the patients with Wilson's disease exceeded this value; only three were below 250 mgm. daily, while 28 of the 36 determinations exceeded 300 mgm. daily.

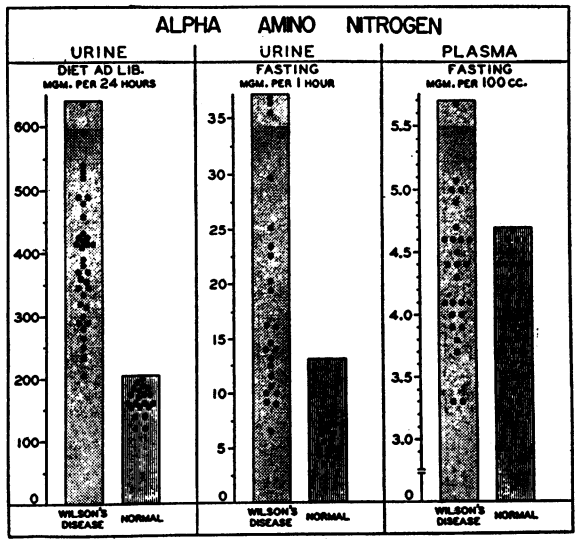

FIG. 1

This excessive excretion of amino acids, about two and one-half times the normal, was independent of variations in urine volume (Figure 2). Wide variations in the total urinary nitrogen excretion, induced by protein intakes ranging from approximately 30 grams to 180 grams daily, also had no significant effect on the alpha amino nitrogen excretion (Figure 3). This finding is at variance with the experience of Dent (20) with two patients with the Fanconi syndrome, in whom the ratio of total nitrogen of the urine to amino nitrogen was believed to provide a more significant index of the degree of aminoaciduria than did the amino nitrogen excretion alone.

The total alpha amino nitrogen after acid hydrolysis of the urine was determined in seven 24-hour collections and averaged three times (range two to ten times) the free alpha amino nitrogen. This proportion is comparable to that found in normal urine (21), and indicates that the increased excretion of free amino acids did not occur at the expense of the excretion of combined amino acids.

Excretion of 10 Essential Amino Acids. Preliminary one-dimensional partition chromatograms on 24-hour urine specimens of the patients were in agreement with those reported by Uzman and Denny-Brown (2), indicating that probably all the amino acids normally found in urine were present in increased amount. This method, however, is unreliable in that moderate, although defi-

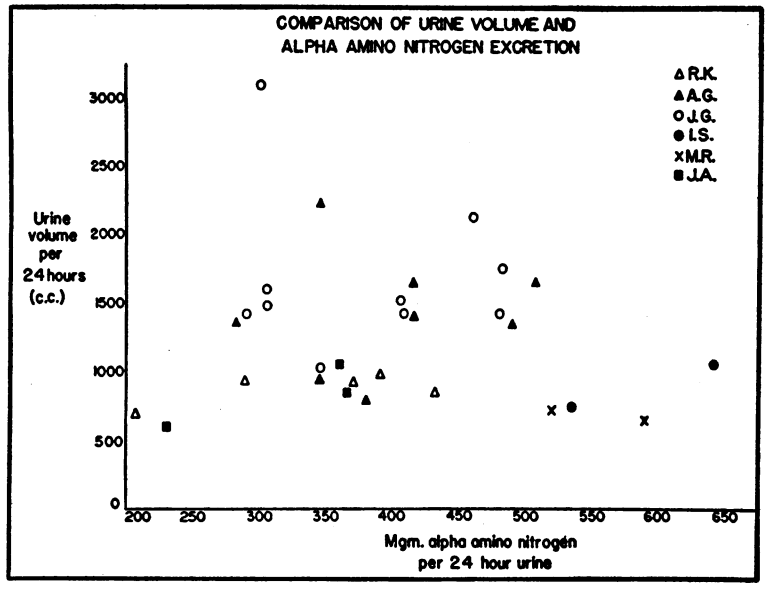

FIG. 2

nite elevations of amino acid excretion may not be clearly distinguished from the normal. In addition, the technique has not been sufficiently quantitative to allow accurate estimates of the excretion of individual amino acids. To compare the patients' pattern of excretion of individual amino acids with that of the normal, the 24-hour excretion of the 10 "essential" amino acids was determined microbiologically in patients with Wilson's disease and in normals. The 24-hour excretion patterns shown in Figure 4 are the averages of 20 urine collections from four patients with Wilson's disease and eight collections from eight normals. The normal values shown have been previously reported (22) and are similar to those reported by others employing microbiological procedures (23–25). All 10 amino acids were present in the urines of the patients in greater than normal amounts. Although the excretion pattern is roughly the same in both groups, the patients' excretion varied from less than twice the normal amount of isoleucine to more than 12 times the

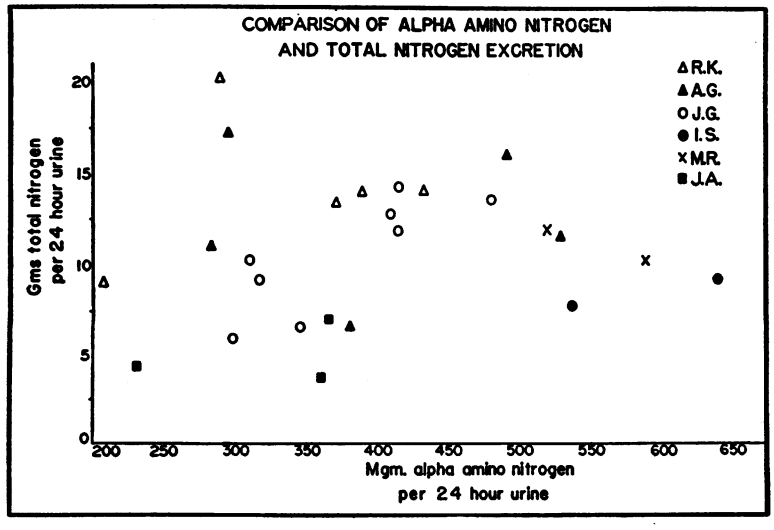

FIG. 3

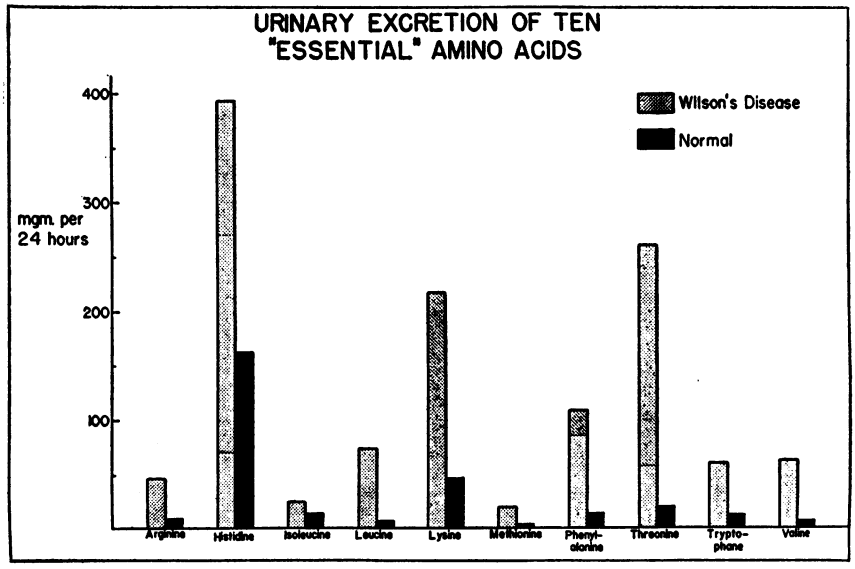

FIG. 4

normal amount of threonine. Methionine, the only sulphur containing amino acid measured, averaged four and one-half times the normal. The 10 amino acids measured comprised 24 per cent of the total amino acids excreted by the normals and 37 per cent of that excreted by the patients.[5]

Amino Acid Excretion by Patients With Laënnec's Cirrhosis of the Liver. A review of the literature revealed little data on amino acid excretion by patients with only moderate liver disease, comparable in degree to that found in the patients with Wilson's disease. The excretion of tryptophane, histidine, and cystine in the urine of patients with liver disease was found by Frankl, Martin, and Dunn (26) to be variable, most values being within the normal range, and as many abnormal values being below as above normal. The reports of excessive aminoaciduria in acute yellow atrophy may fairly be attributed to the elevated plasma amino acid concentration associated with massive destruction of the liver (27, 28) and are not considered pertinent to the present study.

To determine whether the aminoaciduria was a distinctive feature of Wilson's disease or was in some manner a reflection of hepatic dysfunction, a group of six patients with Laënnec's cirrhosis in a "stabilized" state were studied. Five of the six had been severe chronic alcoholics, while the etiology of the cirrhosis in one patient was undetermined. All had had evidence of severe hepatic failure within six months previous to the study. Two of the patients were still reaccumulating ascites at the time of the study, while the others still presented evidence of considerable hepatic derangement clinically and by liver function studies.

Eight 24-hour urine specimens from the six patients with cirrhosis of the liver contained an average of 158 mgm. of alpha amino nitrogen, and no excretion value exceeded 260 mgm. daily. In contrast, 33 out of 36 specimens from patients with Wilson's disease were above this upper limit. Furthermore, the average daily excretion of the 10 "essential" amino acids in the urine of seven additional patients with severe liver disease (five cirrhosis, one hemochromatosis, and one subacute yellow atrophy) did not differ markedly from normal subjects, although there was considerable variation among patients in the quantity of individual amino acids excreted (29). It would appear then that the patients with Wilson's disease form a group distinct from those with hepatic cirrhosis alone.

Amino Acid Excretion Fasting and During 24 Hours Receiving Ad Libitum Diets. To deter-

[5] The total of the amino acids excreted was calculated from the alpha amino nitrogen excretion, assuming alpha amino nitrogen to be 80 per cent of total nitrogen, and total nitrogen to be 16 per cent of amino acids.

mine to what extent the diet may have contributed to the excessive loss of amino acids occurring in patients with Wilson's disease, the excretion of alpha amino nitrogen was determined both in normals and in the patients while fasting. Two-hour urine specimens obtained from fasting normal subjects averaged 6.3 mgm. of alpha amino nitrogen per hour. Similar determinations from patients with Wilson's disease gave an average excretion of 18.1 mgm. of alpha amino nitrogen per hour fasting, approximately three times the normal (Figure 1, Table II). The range of values in the patients was large, due to a number of very high values. However, only two of the normal values exceeded 9.0 mgm. of alpha amino nitrogen per hour, while all but one of the 23 determinations in Wilson's disease exceeded this figure.

Multiplying these average values for fasting hourly excretion of alpha amino nitrogen by 24 and comparing with the average 24-hour excretion values cited above, the fasting excretion rate contributed 92 per cent of the total daily excretion in the normal, and 111 per cent of the total daily amino nitrogen excretion in the patients (Figure 5). The fasting excretion rate in the normals consistently accounted for less than the total amino nitrogen excreted on the same day, while in the patients there was considerable variability, with the fasting excretion rate accounting for from 50 per cent to 230 per cent of the total alpha amino nitrogen excretion for that day. Despite this high degree of variability in the patients, the average values do indicate that any post-

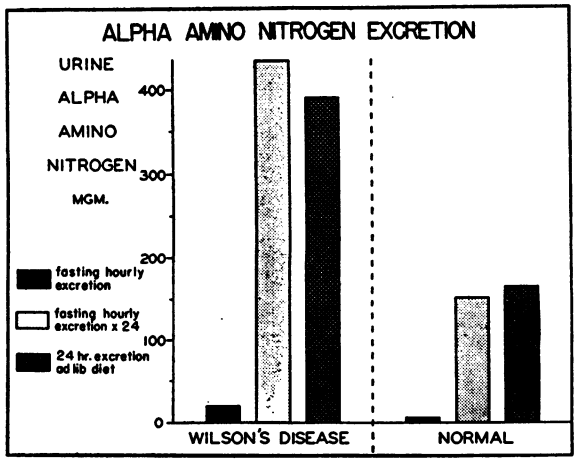

FIG. 5

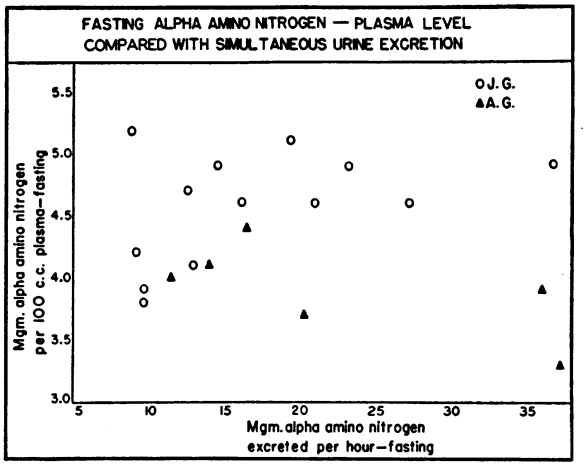

FIG. 6

prandial rise in plasma amino acid concentration contributes little, if anything, to the excessive amino acid excretion in Wilson's disease.

Fasting Plasma Alpha Amino Nitrogen Concentration. Determinations of plasma alpha amino nitrogen from normal fasting adults yielded an average value of 3.9 mgm. per 100 cc. and ranged from 3.1 to 4.7 mgm. per cent. The fasting alpha amino nitrogen of plasma from the patients with Wilson's disease averaged 4.3 mgm. per 100 cc. with a range of from 3.3 to 5.7 mgm. per cent. The range of values occurring in the patients was larger than that found for the normal series (Figure 1, Table II), although only five of the determinations in the patients were above the upper limit of normal. The patients on whom multiple determinations were done all had some values well within the normal range. Although both the plasma concentration and urinary excretion of amino nitrogen in patients with Wilson's disease were elevated, the two were not related as cause and effect. Figure 1 shows that although the urinary amino nitrogen was constantly and markedly elevated, the plasma amino nitrogen was usually well within the normal range, indicating that the occasionally increased plasma level did not correlate with the increased urinary excretion of amino acids.

Amino Acid "Clearance" Tests. In an attempt to define further any correlation between the plasma concentration of amino acids and the rate of excretion of amino acids, two-hour urine specimens (6 to 8 a.m.) were collected after a 10-hour

fast. At the mid-point of this two-hour period, blood was drawn and alpha amino nitrogen content of blood and urine was determined. The results of multiple determinations in two of the patients with Wilson's disease are plotted in Figure 6. It is seen that there is no correlation between the plasma concentration of alpha amino nitrogen and the quantity of amino acids excreted in the urine. Although the glomerular filtration rate was not determined, there was no reason to believe that it changed markedly from day to day in any of the patients. If this assumption be correct, there is further evidence that the plasma concentration of amino acids was not an important factor in the mechanism for the increased renal excretion of amino acids.

Ingestion of Individual Amino Acids. Gutman and Alexander (30) demonstrated that when one gram of glycine per 10 pounds of body weight is taken orally by normal persons, the plasma alpha amino nitrogen rises to approximately 9 mgm. per 100 cc. in one hour, with a gradual fall thereafter. One patient with Wilson's disease, given this dose of glycine, had a normal response. The fasting plasma alpha amino nitrogen was 4.7 mgm. per 100 cc. and one hour after glycine it was 10.1 mgm. per 100 cc., returning to the fasting level in four hours. Urines were collected during a two-hour period before ingestion of glycine, from ingestion to two hours after, and from two to four hours after ingestion. The alpha amino nitrogen content of these specimens was 25.3, 231, and 84.0 mgm., respectively. The 10 "essential" amino acids in these specimens were determined and it was found that their combined weight totaled 69.9 mgm. in the first, 161 mgm. in the second, and 118 mgm. in the third specimen. The small increase in the quantity of essential amino acids excreted after the ingestion of glycine could account for only a fraction of the alpha amino nitrogen excreted. Therefore, although glycine was not measured, it is reasonable to assume that the rise in alpha amino nitrogen excretion was largely due to the excretion of glycine.

The ingestion of glutamic acid by animals and man has been shown to induce only a slight rise or even a fall in plasma amino nitrogen, a rise in plasma glutamic acid, and a fall in plasma glutamine and other amino acid fractions (31–33). Because this effect of glutamic acid differs from that of glycine, it seemed likely that some different aspects of amino acid metabolism would be tested by the administration of glutamic acid. Tests were done in two subjects with Wilson's disease and one normal control. The l-glutamic acid was dissolved in water and neutralized with sodium bicarbonate, and was taken orally in single or multiple doses totaling from 25 to 40 grams.

TABLE III

Changes in plasma alpha amino nitrogen and in urine alpha amino nitrogen, 10 "essential" amino acids, and glutamic acid after ingestion of l-glutamic acid by a normal subject and by patients with Wilson's disease

	Plasma alpha amino nitrogen *mgm./100 cc.*					Urine alpha amino nitrogen (AN), total of the 10 "essential" amino acids (Ess), and l-glutamic acid (GA) *mgm./2 hrs.*		
	Control 0 hr.	Time after ingestion				Control −2 to 0 hr.	Time after ingestion	
		1 hr.	2 hr.	3 hr.	4 hr.		0 to 2 hr.	2 to 4 hr.
Control subject*	4.6	5.3	4.3	4.6	4.6	25.9 24.7 2.0	100 36.7 1065	20.3 (AN) 24.3 (Ess) 6.8 (GA)
Patient J. G.†	4.6	4.6	4.1	—	4.6	54.3 128 18.1	129 100 801	146 (AN) 54.9 (Ess) 1280 (GA)
Patient A. G.‡	5.0	5.9	5.5	5.4	4.6	87.2 234 21.6	161 124 1020	78.7 (AN) 120 (Ess) 334 (GA)

* Ingested 25 grams l-glutamic acid at 0 hr.; vomited at 1½ hr.
† Ingested 15 grams l-glutamic acid at 0 hr., 15 grams at 1 hr., and 10 grams at 2 hr.; vomited at 2½ hr.
‡ Ingested 15 grams l-glutamic acid at 0 hr., and 15 grams at 1 hr.; vomited at 1½ hr.

TABLE IV

*Changes in plasma and in urine alpha amino nitrogen after infusion of 500 cc. of 10 per cent solution of amino acids in normal subjects and in patients with Wilson's disease**

	Infusion rate *mgm. N/ kilo./min.*	Plasma alpha amino nitrogen *mgm./100 cc.*				Urine alpha amino nitrogen		
		Control fasting	Time after infusion			Control fasting *mgm./1 hr.*	0 to 4 hours post-infusion	
			5 min.	1 hr.	4 hr.		Excess *mgm./4 hr.*†	Per cent excreted‡
Normal subjects	4.2 (1.9 to 6.6)	3.7 (3.3 to 4.2)	20.1 (12.5 to 24.7)	7.1 (5.8 to 8.9)	4.1 (3.8 to 4.2)	4.8 (4.0 to 5.7)	513 (440 to 730)	9.5 (7.3 to 12.2)
Wilson's disease	4.1 (3.3 to 4.7)	4.7 (3.3 to 5.8)	23.0 (20.1 to 27.6)	7.6 (3.9 to 12.6)	4.9 (3.4 to 6.3)	16.4 (6.4 to 37.2)	597 (295 to 927)	9.9 (4.9 to 15.4)

* Values are the average and range (in parentheses) of six determinations from six normal subjects (21), and of six determinations from four patients with Wilson's disease.
† Mgm. of alpha amino nitrogen in excess of that excreted while fasting.
‡ Per cent of administered alpha amino nitrogen excreted in urine.

Each subject vomited approximately one-half hour after the last dose. The results of these experiments are shown in Table III. The marked increase of glutamic acid in the urines after its ingestion demonstrates that the vomiting experienced by all the subjects did not prevent the absorption of large amounts of glutamic acid. Measurement of the 10 "essential" amino acids in the urine before and after glutamic acid ingestion revealed that their total amount remained approximately the same in the normal individual, but decreased considerably in both patients. Essentially all of the increase of urinary alpha amino nitrogen may be accounted for by the increased excretion of glutamic acid. Although a large proportion of the glutamic acid was absorbed in each instance, the plasma alpha amino nitrogen concentration rose only slightly in the control and in one of the patients, and fell slightly in the other patient. A partial explanation for this phenomenon may be that an elevated plasma glutamic acid concentration is associated with a lowering of the plasma concentration of other amino acids. The results indicate no essential difference in the patients compared to the normal, and are in general accord with those obtained from the animal studies.

Amino Acid "Tolerance" Test. Because certain of the evidence thus far indicated a lowered renal threshold for amino acids, the following "tolerance" test was done. Five hundred cc. of a 10 per cent solution of amino acids [6] (a complete acid hydrolysate of casein supplemented with tryptophane) were infused intravenously in fasting subjects. Urines were collected during a two-hour period before infusion, and from the start of infusion to four hours after infusion. Bloods were drawn before infusion and five minutes, one hour, and four hours after infusion. Alpha amino nitrogen determinations were done on all specimens. The results are shown in Table IV. The patients' average values are the result of six tests in four persons and the control values are the average of six tests in six normal subjects and have been previously reported (22). The infusion rate, expressed as mgm. of nitrogen per kilogram of body weight per minute, averaged 4.2 in the normals, and 4.1 in the patients. There was little variation in the infusion rates among the patients, while the infusion rates among the normals varied from 1.9 to 6.6. There was, however, little correlation in this small series between infusion rate and amino acid loss during the post-infusion period.

The plasma concentration of alpha amino nitrogen at each period averaged slightly higher in the patients than in the normals. However, the rate of rise and fall of the plasma amino acid concentration is nearly identical for both groups, and at the end of four hours the plasma amino acid level was 111 per cent of the fasting level in the normals, and 104 per cent of the fasting level in the patients.

The hourly fasting urinary excretion rate aver-

[6] Developed and supplied by Merck & Co., Inc., Rahway, New Jersey.

aged 4.8 mgm. of alpha amino nitrogen per hour in the normals and 16.4 mgm. in the patients. Thus the patients' values averaged approximately three times the normal and were similar to the larger series cited above. The values in the patients, although variable, were consistently elevated. The urinary loss of amino acids due to the infusion averaged 513 mgm. of alpha amino nitrogen in the normals and 597 mgm. in the patients. These values represent average infusion losses of 9.5 and 9.9 per cent of the alpha amino nitrogen administered to normals and to patients, respectively (Table IV). Two of the values in the patients were below while one was above any of the normals. This observation, together with the small difference in the average values, excludes any significant difference between the two groups. This is in marked contrast to the differences noted in the fasting excretion and 24-hour excretion. It would appear, then, that patients with Wilson's disease do not have a lowered renal threshold for amino acids administered intravenously in large amounts.

Miscellaneous Studies. The possibility that the aminoaciduria might be similar in nature to that occurring in the Fanconi syndrome (20, 34) was considered, particularly since liver lesions have been reported in association with this disease. Determinations of the serum carbon dioxide combining power, inorganic phosphorus, calcium, and alkaline phosphatase in three patients were all normal. Bone X-rays in two patients revealed no evidence of osteomalacia. Five patients excreted an acid urine and showed no evidence clinically of acidosis. Sulkowitch tests of their urines were negative. However, the final patient referred for study (J. A.) previously had exhibited evidence of deranged calcium and phosphorus metabolism. Thus, the serum values averaged for calcium 8.3 mgm. per cent (range 7.6 to 9.2 for eight determinations), for phosphorus 2.8 mgm. per cent (range 1.5 to 4.1 for 11 determinations), and for alkaline phosphatase 14.7 Bodansky units (range 9.1 to 23.2 for eight determinations). X-ray films of the knees and wrists taken three years ago did not reveal any evidence of rachitic changes, although subsequent films taken one and one-half years later demonstrated a moderate degree of decalcification of all of the long bones.[7] Upon the present admission, the serum calcium was 11.3 mgm. per cent, phosphorus 4.0 mgm. per cent, and alkaline phosphatase 5.4 Bodansky units. While ingesting primarily a milk diet, a representative day's urine contained 3.80 grams total nitrogen, 2.25 grams urea nitrogen, 0.32 gram ammonia nitrogen, 0.36 gram alpha amino nitrogen, 0.97 gram inorganic phosphorus, and 0.65 gram calcium per 24 hours. The urine was alkaline (pH 7.5) and contained no acetone, diacetic acid, or sugar. The blood carbon dioxide combining power was 52.0 volumes per cent, nonprotein nitrogen 17.6 mgm. per cent, fasting blood sugar 91.0 mgm. per cent, and serum alpha amino nitrogen 4.34 mgm. per cent.

Oral glucose tolerance tests were done in five patients, administering 100 grams of glucose and determining venous blood and urine glucose at half-hour intervals for two and one-half hours after glucose ingestion. None of the patients had abnormal blood glucose curves. Three of the five spilled glucose in the urine when the venous blood sugar was below 115 mgm. per cent, in the fasting state, and two still spilled glucose at blood levels under 95 mgm. per cent. Two patients, R. K. and J. A., had no glucose in the urine when the venous blood glucose was 145 and 170 mgm. per cent, respectively. Although the venous blood sugar is not an accurate index of the arterial blood sugar (35), the fact that three patients had sugar in the urine in the fasting state with normal venous blood sugars indicates, for these patients, a decreased renal threshold to glucose. In one of the two patients who showed no sugar in the urine it cannot be stated whether or not the renal threshold may have been lowered, since the venous blood sugar was not raised above 145 mgm. per cent. Although it was noted by Dent in his study of the Fanconi syndrome that the aminoaciduria was proportional to the glycosuria (20), no such correlation existed in the patients with Wilson's disease.

DISCUSSION

The data demonstrate that six patients with hepatolenticular degeneration all had an increased

[7] The authors wish to thank Dr. Alan Butler, Pediatrics Department, Massachusetts General Hospital, Boston, for permission to publish these laboratory and X-ray findings.

daily excretion of amino acids, averaging two and one-half times the normal. This confirms the work of Uzman and Denny-Brown (2). There was a large day-to-day variability in the degree of aminoaciduria which could not be explained by changes in the protein content of the diet as reflected by the total urine nitrogen, nor by changes in urine volume or plasma amino acid concentration. In addition, the patients with Wilson's disease had on the average a slightly higher fasting plasma alpha amino nitrogen concentration than did the normals. However, these two findings, an increased urine and plasma alpha amino nitrogen, were statistically unrelated, the former being a constant finding and the latter an inconstant one. Experimental attempts to demonstrate a correlation between the aminoaciduria and the plasma amino nitrogen concentration were unsuccessful. There would appear to be no similarity between the aminoaciduria occurring here and that found in acute yellow atrophy of the liver in which the plasma amino acid concentration may be greatly elevated (27, 28).

All of the patients with Wilson's disease had some indication of liver disease, although in only one (J. A.) could the derangement of function be considered severe. There was no correlation, in these patients, between the extent of the aminoaciduria and the severity of the liver disease. In contrast to this group, patients with moderate or severe cirrhosis of the liver did not have the increased excretion of amino acids seen in Wilson's disease. To attribute the aminoaciduria to the hepatic involvement occurring in Wilson's disease, it is necessary to assume that the variety of liver abnormality is distinctly different from that of ordinary cirrhosis. Even then it is difficult to conceive of an hepatic defect causing excessive amino acid excretion except via an elevation of the plasma amino nitrogen concentration, which was not the *modus operandi* in these cases.

Further data are presented showing that the aminoaciduria does not represent a defect in the metabolism of amino acids by liver or muscle. If there were a failure of cellular absorption or deamination, one would expect that after a rapid infusion of amino acids the plasma amino acid concentration would rise much higher and return to fasting levels more slowly than in the normal. This was not the case.

The results after oral feeding of glycine and glutamic acid indicate that, at least for these two amino acids, there is no discernible defect in intermediary amino acid metabolism. Although the two amino acids may be metabolized differently, the two patients with Wilson's disease studied seemed capable of absorbing them from the gastrointestinal tract, and disposing of them from the blood stream in a normal fashion.

The demonstration by microbiological assay that all 10 amino acids determined were excreted in increased amounts with a pattern similar, although not identical, to the normal "pattern," excludes any specific metabolic defects such as occur in cystinuria, for example. Other than massive destruction of hepatic or muscle tissue, for which there was no evidence, the aminoaciduria would seem inexplicable on the basis of a disturbance of intermediary metabolism of amino acids. Thus, with the exception of the unexplained inconstant elevation of plasma amino nitrogen, one is led by simple exclusion to incriminate the kidney as the site of the abnormality. Renal glycosuria in three of the patients further confirms the existence of an anomaly of renal function.

The occurrence of excessive amino acid loss in the fasting state, unrelated to the plasma amino acid concentration, would indicate a lowered renal threshold for amino acids. If "lowered threshold" is interpreted to mean a reduction of the level of tubular reabsorption beyond which no further material is reabsorbed, then several aspects of the data remain unexplained. Since the amino nitrogen excretion is elevated even in the fasting state it would be expected, if this were due to a lowered threshold, that a considerable proportion of the daily amino nitrogen loss would have been incurred during the post-prandial elevations of plasma amino acid concentration. This was not the case, since approximately 100 per cent of the daily amino acid loss was accounted for by the rate of amino acid loss in the fasting state. The "tolerance" tests further demonstrated that although the patients excreted approximately three times as much alpha amino nitrogen in the fasting state as did the normals, the rise in the excretion after an infusion of amino acids was approximately the same for both groups. Any constant functional or anatomical renal defect in the patients should have resulted in a similar threefold rise in

excretion after infusion. A simple explanation would be available if the maximal rate of tubular reabsorption of amino acids (Tm) were reached during infusion, but it is most unlikely that the Tm for any of the amino acids was reached by rapid infusion of an amino acid mixture (36). Further elucidation of this problem probably must await clearance data on patients with Wilson's disease.

The finding of renal glycosuria in three patients and of deranged calcium and phosphorus metabolism in one patient forces a comparison with the Fanconi syndrome in which aminoaciduria is associated with glycosuria, phosphaturia, and a tendency to acidosis and osteomalacia, and in which cirrhosis or necrosis of the liver is a frequent finding (20, 34). Although only one of the patients with Wilson's disease appeared to have an abnormality of acid-base balance or of calcium and phosphorus metabolism, these cannot be entirely excluded in the other patients on the basis of the present study since balance experiments were not done.[8] The similarity of the two diseases with respect to aminoaciduria, glycosuria, and hepatic disease, raises the possibility that a renal disorder is the common denominator and would seem to provide some support for a theory postulating a defect of renal function as the primary disorder of Wilson's disease. The existence of a renal abnormality resulting in excessive loss of at least amino acids and glucose also raises the possibility that a loss of other substances essential to brain, liver, and corneal nutrition results finally in the syndrome of Wilson's disease.

The possible nutritional significance of the increased loss of amino acids in Wilson's disease merits consideration. Although the aminoaciduria is striking when compared with the normal, the average loss of 400 mgm. of alpha amino nitrogen per day is equivalent to about 3.2 grams of protein—a small portion of the usual daily protein intake. There was no evidence of any nutritional deficiency, except in the two patients in whom the severity of the neurological involvement eventually prevented an adequate food intake. Since no one essential amino acid was lost in very excessive amount, there is no evidence of a deficiency of a single amino acid necessary for brain or liver nutrition, although an excessive loss of some other essential nutrient is not excluded.

The case material included patients from each of the sub-classifications of Wilson's disease—the progressive lenticular degeneration of Wilson and the pseudo-sclerosis of Westphal—and both types had aminoaciduria of similar nature and degree. Thus there is further rationale, if any were needed, for the classification of these diseases under the same heading.

Two siblings of patient R. K., one a girl 18 years old, and the other a 24-year-old male, were examined with the aim of detecting a familial tendency to aminoaciduria in the absence of other signs of Wilson's disease. Neither subject had any clinical evidence of the disease and liver function tests were normal. Twenty-four hour urine samples from the two siblings contained normal amounts of alpha amino nitrogen, 170 mgm. daily in the female and 158 mgm. daily in the male. It would be of interest to perform this test on other siblings of patients with Wilson's disease with the aim of determining whether the aminoaciduria was present as a functional defect prior to the occurrence of any other evidence of the disease.

SUMMARY

Studies on six patients with Wilson's disease (hepatolenticular degeneration) yielded the following results:

1. The average daily excretion of alpha amino nitrogen in the urine was consistently elevated, averaging 390 mgm. compared to a normal average of 164 mgm. and an average of 158 mgm. in patients with severe cirrhosis of the liver.

2. The alpha amino nitrogen excretion during *ad libitum* feeding could be accounted for by the fasting rate of excretion, which averaged 18.1 mgm. per hour compared to a normal average of 6.3 mgm. per hour.

3. The rapid infusion of amino acids did not result in a significantly greater loss of amino acids in the patients compared to that of the normals.

4. Although the plasma alpha amino nitrogen concentration of the patients averaged slightly

[8] It is entirely possible, of course, that the laboratory and X-ray alterations observed in patient J. A. were incidental to his Wilson's disease. Furthermore, it cannot be stated with any degree of certainty that these findings resulted from a defect in the renal tubules, as is the case in the Fanconi syndrome (33), particularly since the patient did not exhibit renal glycosuria.

above the normal, this did not explain their aminoaciduria. There was no demonstrable defect of intermediary amino acid metabolism.

5. The occurrence in patients with Wilson's disease of an excessive amino acid excretion in the fasting state would indicate a lowered renal threshold for amino acids. However, a greater than normal amino acid loss following the ingestion of protein foods or injection of protein hydrolysates was not observed.

6. Renal glycosuria was demonstrated in three patients. Another patient presented evidence of deranged calcium and phosphorus metabolism and osteomalacia.

7. The kidney is believed to be the site of the defect resulting in excessive amino acid loss, but the type of abnormality is not explained.

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BIBLIOGRAPHY

1. Wilson, S. A. K., Progressive lenticular degeneration: a familial nervous disease associated with cirrhosis of the liver. Brain, 1912, **34**, 295.
2. Uzman, L., and Denny-Brown, D., Amino-aciduria in hepato-lenticular degeneration (Wilson's disease). Am. J. Med. Sci., 1948, **215**, 599.
3. Hamilton, P. B., and Van Slyke, D. D., The gasometric determination of free amino acids in blood filtrates by the ninhydrin-carbon dioxide method. J. Biol. Chem., 1943, **150**, 231.
4. Van Slyke, D. D., MacFadyen, D. A., and Hamilton, P. B., The gasometric determination of amino acids in urine by the ninhydrin-carbon dioxide method. J. Biol. Chem., 1943, **150**, 251.
5. Stokes, J. L., Gunness, M., Dwyer, I. M., and Caswell, M. C., Microbiological methods for the determination of amino acids. II. A uniform assay for the ten essential amino acids. J. Biol. Chem., 1945, **160**, 35.
6. Consden, R., Gordon, A. H., and Martin, A. J. P., Qualitative analysis of proteins: a partition chromatographic method using paper. Biochem. J., 1944, **38**, 224.
7. Dent, C. E., Detection of amino acids in urine and other fluids. Lancet, 1946, **2**, 637.
8. Folin, O., and Wu, H., A system of blood analysis. Supplement I. A simplified and improved method for determination of sugar. J. Biol. Chem., 1920, **41**, 367.
9. Somogyi, M., A rapid method for the estimation of urine sugar. J. Lab. & Clin. Med., 1941, **26**, 1220.
10. Laboratory Manual of Field Methods for Biochemical Assessment of Metabolic and Nutritional Condition. Harvard Fatigue Laboratory, Boston, pp. 62–65, 1945.
11. Fiske, C. H., and Subbarow, Y., The colorimetric determination of phosphorus. J. Biol. Chem., 1925, **66**, 375.
12. Ducci, H., and Watson, C. J., The quantitative determination of the serum bilirubin with special reference to the prompt-reacting and the chloroform-soluble types. J. Lab. & Clin. Med., 1945, **30**, 293.
13. Hanger, F. M., Serological differentiation of obstructive from hepatogenous jaundice by flocculation of cephalin-cholesterol emulsions. J. Clin. Invest., 1939, **18**, 261.
14. Ley, A. B., Lewis, J. H., and Davidson, C. S., The quantitative determination of the thymol turbidity reaction of serum. J. Lab. & Clin. Med., 1946, **31**, 910.
15. Homburger, F., and Kozol H. L., Hepatolenticular degeneration. J.A.M.A., 1946, **130**, 6.
16. Homburger, F., Hepatolenticular degeneration. II. Nutritional factors: observations on methionine and high-protein diets. New Eng. J. Med., 1946, **234**, 683.
17. Denny-Brown, D., Diseases of the basal ganglia and subthalamic nuclei, *in:* The Oxford Medicine, edited by Christian, H. A. D. Appleton-Century Co., Inc., New York, 1945 Revision, Vol. VI, Chapter XI, p. 261.
18. Hicks, M. H., Holt, H. P., Guerrant, J. L., and Leavell, B. S., The effect of spontaneous and artificially induced fever on liver function. J. Clin. Invest., 1948, **27**, 580.
19. Chalmers, T. C., Murphy, T. L., and Taft, E. B., The incidence, character and course of liver disease in chronic alcoholics as determined by needle biopsy. J. Clin. Invest., 1948, **27**, 528.
20. Dent, C. E., The amnio-aciduria in Fanconi syndrome. A study making extensive use of techniques based on paper partition chromatography. Biochem. J., 1947, **41**, 240.
21. Eckhardt, R. D., and Davidson, C. S., Urinary excretion of amino acids by a normal adult receiving diets of varied protein content. J. Biol. Chem., 1949, **177**, 687.
22. Eckhardt, R. D., and Davidson, C. S., Urinary excretion of amino acids following the rapid injection of a solution of amino acids in man. J. Clin. Invest., 1948, **27**, 727.
23. Steele, B. F., Sauberlich, H. E., Reynolds, M. S., and Baumann, C. A., Amino acids in the urine of human subjects fed eggs or soy beans. J. Nutrition, 1947, **33**, 209.

24. Dunn, M. S., Camien, M. N., Shankman, S., and Block, H., Urinary excretion of twelve amino acids by normal male and female subjects measured microbiologically. Arch. Biochem., 1947, **13**, 207.
25. Woodson, H. W., Hier, S. W., Solomon, J. D., and Bergeim, O., Urinary excretion of amino acids by human subjects on normal diets. J. Biol. Chem., 1948, **172**, 613.
26. Frankl, W., Martin, H. E., and Dunn, M. S., The apparent concentration of free tryptophan, histidine and cystine in pathological human urine measured microbiologically. Arch. Biochem., 1947, **13**, 103.
27. Stadie, W. C., and Van Slyke, D. D., The effect of acute yellow atrophy on metabolism and on the composition of the liver. Arch. Int. Med., 1920, **25**, 693.
28. Rabinowitch, I. M., Biochemical findings in a rare case of acute yellow atrophy of the liver. With particular reference to the origin of urea in the body. J. Biol. Chem., 1929, **83**, 333.
29. Eckhardt, R. D., Cooper, A. M., Faloon, W. W., and Davidson, C. S., The urinary excretion of amino acids in man. Tr. New York Acad. Sc., 1948, **10**, 284.
30. Gutman, G. E., and Alexander, B., Studies of amino acid metabolism. I. Blood glycine and alanine and their relationship to the total amino acids in normal subjects. J. Biol. Chem., 1947, **168**, 527. *Also,* Alexander, B., Amino acids in plasma. A correction. J. Biol. Chem., 1947, **171**, 821.
31. Christensen, H. N., Streicher, J. A., and Elbinger, R. L., Effects of feeding individual amino acids upon the distribution of other amino acids between cells and extracellular fluid. J. Biol. Chem., 1948, **172**, 515.
32. Seth, T. N., and Luck, J. M., The relation between the metabolism and the specific dynamic action of amino acids. Biochem. J., 1925, **19**, 366.
33. Bessman, S. P., Magnes, J., Schwerin, P., and Waelsch, H., The absorption of glutamic acid and glutamine. J. Biol. Chem., 1948, **175**, 817.
34. McCune, D. J., Mason, H. H., and Clarke, H. T., Progress in pediatrics. Intractable hypophosphatemic rickets with renal glycosuria and acidosis (Fanconi syndrome); report of case in which increased urinary organic acids were detected and identified, with review of literature. Am. J. Dis. Child., 1943, **65**, 81.
35. Mosenthal, H. O., Symposium on diabetes. Interpretation of glucose tolerance tests. M. Clin. North America, 1947, **31**, 299.
36. Wright, L. D., Renal clearances of essential amino acids. Tr. New York Acad. Sc., 1948, **10**, 271.

ERRATUM

On page 1458 of the November 1949 issue, in the article entitled, "Protamine (Salmine) Sulphate, Heparin, and Blood Coagulation," by A. Frank Portmann and William D. Holden, item No. 7 of the Summary should read: Protamine does not neutralize the serum heparin cofactor.