AMINO ACID METABOLISM IN INFECTIOUS HEPATITIS [1]

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Although Mann (1) clearly demonstrated the key role of the liver in the deamination process nearly three decades ago, it is only since the development of such techniques as the quantitative measurements of free alpha amino acids in the plasma and urine, and their separation by means of either paper and starch column chromatography or microbiological assay that more definitive studies on the metabolism of amino acids in diseases of the liver can be carried out.

Using microbiological methods, Frankl, Martin, and Dunn (2) first demonstrated considerable variations in the excretion of cystine, tryptophane, and histidine in patients with various types of liver disease. Dunn, Akawaie, Yeh, and Martin estimated that approximately 20 per cent of the patients with liver disease showed increased excretion of amino acids (3). In studies on patients with cirrhosis of the liver, Gabuzda, Eckhardt, and Davidson (4) found only minor disturbances of urine amino acid excretion, the most constant abnormality consisting of increased excretion of methionine and tryptophane, and a decreased excretion of isoleucine. Kinsell, Harper, Barton, Hutchin, and Hess (5) have reported an impaired methionine tolerance in patients with liver disease as compared with normal controls, but Wheeler and György (6) were unable to confirm this.

More recently, Dent and Walshe (7) and Walshe (8, 9) have suggested that because of the enormous reserve of the liver, a study of the total amino nitrogen concentration would not reveal any significant abnormality until the liver is extensively damaged. Using paper partition chromatography techniques, they found gross aminoaciduria in patients with acute yellow atrophy of the liver and hepatic coma, but only minor changes in patients with hepatitis and cirrhosis.

The present study was undertaken to study the changes in amino acid metabolism in infectious hepatitis by: 1) Measuring simultaneously the total alpha amino nitrogen in the plasma and urine, and 2) analyzing the changes of amino acid pattern in the urine by means of paper partition chromatography.

MATERIALS AND METHODS

A total of 18 children with acute infectious hepatitis was studied. The diagnosis was made in every case on the basis of malaise, anorexia, vomiting, fever, abdominal pain, dark urine, light stools, icterus of the skin and sclerae, and an enlarged and tender liver. Liver function tests including cephalin-cholesterol flocculation (10), thymol turbidity (11), thymol flocculation (12), one minute and total serum bilirubin (13, 14) were positive in each patient when first seen. In addition to these tests, increased bile and urobilinogen were demonstrated in the urine and infectious mononucleosis was ruled out by blood smears and heterophil-antibody agglutination. None of the patients had received injections or transfusions within six months prior to the onset of symptoms and contact with infectious hepatitis through the family or school was established in about a third of the cases. Although most of the children were moderately ill during the acute phase of the disease, none developed coma, and all recovered uneventfully.

Since Eckhardt and Davidson (15) and Silber, Seeler, and Howe (16) have previously shown that the urinary excretion of amino acids was only slightly altered by marked changes in protein intake, the patients were placed on a regular diet without restriction during the periods of study. Similarly, although no attempt was made to regulate fluid intake, except in one instance, the 24-hour urinary output was about average for the age and weight of the children studied.

The 24-hour urine collections were preserved with thymol and refrigerated. The urine alpha amino nitrogen was determined by the gasometric ninhydrin-CO_2 method by Van Slyke, MacFadyen, and Hamilton (17). The blood for plasma alpha amino nitrogen was taken two to three hours after the previous meal and determined by the gasometric ninhydrin-CO_2 method of Hamilton and Van Slyke (18).

The amino acids in the 24-hour urine collections were separated by the two dimensional paper partition chromatography using phenol and butanol-acetic acid as

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solvents as described by Consden, Gordon, and Martin (19), and modified by Dent (20, 21). The urine was desalted and concentrated to one-fourth the original volume and 15 μl. of this was analyzed. None of the specimens was hydrolyzed. Each of the amino acids was identified by superimposing with known amino acids and by the use of specific reagents when available (22). The results were tabulated semi-quantitatively by grading against an arbitrary standard, judged under constant lighting conditions in strengths from 1 to 10 units (8).

RESULTS

Plasma and Urine Alpha Amino Nitrogen Measurements

In normal controls

The plasma alpha amino nitrogen was determined in 16 normal children as shown in Table I.

TABLE I

Plasma alpha amino nitrogen levels in normal children

No. subjects:	16
Age of subjects:	3 wks.–8 yrs.
Plasma: *mgm./100 ml.*	
Range:	1.8–5.0
Mean:	3.4
S.D.:	0.9

The values ranged from 1.8 mgm. per cent to 5.0 mgm. per cent with a mean of 3.5 and a S.D. ± 1.0 mgm. per cent. These values are in close agreement with the normal range described by Hamilton and Van Slyke (18) and do not appear to vary with the sex or age of the subject tested.

The urine alpha amino nitrogen was determined in 24-hour collections from 17 children as shown in Table II. The values in the children varied from 0.69 to 1.01 mgm. per pound body weight per 24 hours with a mean of 0.85 and a S.D. ± 0.10 mgm. This is in keeping with the range of approximately 1 mgm. per pound body weight per 24 hours reported by Childs (23) and 2 mgm. per kilogram body weight per 24 hours reported by Jonxis (24) for normal children.

TABLE II

Twenty-four-hour urine alpha amino nitrogen excretion in normal children

No. subjects:	17
Age of subjects:	8 wks.–15 yrs.
Wt. of subjects:	11 lbs.–113 lbs.
Urine volume:	95 cc.–1,960 cc.
Urine: *mgm./lb./24 hr.*	
Range:	0.69–1.01
Mean:	0.85
S.D.:	0.09

In patients with acute infectious hepatitis

The plasma and urine alpha amino nitrogen values during the acute phase of infectious hepatitis are shown in Table III. One-third of the patients showed a moderate increase of urinary alpha amino nitrogen with an output of 50 per cent or more above that of the controls. Another third of the patients showed borderline changes with an output which varied between 20 and 50 per cent above normal. These changes are not marked, but are sufficiently above the normal range to be noteworthy. The remaining third of the patients showed normal urinary output of alpha amino nitrogen.

Simultaneous determinations of plasma alpha amino nitrogen also showed borderline increases. In the group of patients with moderate amino-aciduria, all five of the cases had values of more than 5.0 mgm. per cent and three of these above 5.5 mgm. per cent. In the borderline group, three of the six cases showed values of more than 5.0 mgm. per cent, but all were below 5.5 mgm. per cent. In the group exhibiting no amino-aciduria, the plasma alpha amino nitrogen in all five of the patients tested was found to be within the normal range. These determinations appear to be relatively unaffected by the sex, age, body weight, or urinary volume of the patients. Furthermore, the amino-aciduria did not correlate with the severity of disease clinically nor with the interval between the onset of symptoms and collection of samples.

In patients following recovery from infectious hepatitis

All six of the patients with moderate amino-aciduria, and three of the six patients with borderline amino-aciduria were restudied 5 to 18 weeks following the onset of symptoms. By then, none of them showed any signs of disease and the liver function tests had all returned to normal values. It can be seen from Table III that in the moderate amino-aciduria group, the plasma alpha amino nitrogen was within the normal range in all except Case 6, where some increase persisted. Simi-

TABLE III

*Plasma and urine alpha amino nitrogen determinations in patients with infectious hepatitis**

Sex	Age (*years*)	Wt. (*lbs.*)	Plasma amino N (*mgm./100 cc.*)		Urine amino nitrogen (*Volume cc.*)		(*mgm./24 hrs.*)		(*mgm./lb./24 hrs.*)	
			Acute phase	Follow-up	Acute phase	Follow-up	Acute phase	Follow-up	Acute phase	Follow-up
Moderate amino aciduria (Increase of more than 50%)										
M	7	58	5.2	3.9	1,660	930	120.0	58.0	2.07	1.00
M	10	75	5.2	3.4	2,900	1,900	145.5	76.0	1.94	1.00
M	9	55	5.7	2.5	620	960	95.4	38.2	1.73	0.70
M	10	78	—	4.3	600	950	123.6	73.3	1.58	0.94
F	2	27	6.0	2.9	490	550	37.7	35.0	1.40	1.29
M	10½	75	5.6	2.7	770	850	103.0	71.2	1.38	0.95
Average			5.5	3.3					1.70	0.98
Borderline amino aciduria (20% to 50% above normal)										
M	7	86	2.7	3.9	680	400	103.0	60.0	1.20	0.70
M	7	42	5.3	—	480	—	48.0	—	1.15	—
F	6	46	3.4	3.8	680	440	52.3	35.4	1.14	0.77
M	10	68	5.3	—	580	—	72.5	—	1.07	—
F	9	64	2.5	3.6	520	290	67.6	30.0†	1.05	0.47†
M	10	72	5.1	—	740	—	78.0	—	1.04	—
Average			2.9	3.8					1.11	0.65
No amino aciduria (Normal values)										
F	8	58	—	—	660	—	52.7	—	0.91	—
F	10	70	2.6	—	610	—	61.0	—	0.87	—
M	8	60	3.4	—	540	—	48.0	—	0.80	—
M	7	54	2.2	—	880	—	41.4	—	0.77	—
M	6	48	2.8	—	520	—	36.4	—	0.76	—
F	6	48	3.3	—	620	—	32.9	—	0.69	—
Average			2.9						0.80	

* Values include those during the acute phase of the disease and in follow-up studies after convalescence.
† Part of specimen accidentally lost.

larly, in the group with borderline amino-aciduria, plasma and urine alpha amino nitrogen was within normal limits in all three of the patients restudied.

Amino Acid Pattern by Paper Partition Chromatography

In normal controls

The distribution of specific amino acids analyzed in 24-hour collections of urine from six adults and nine children, who acted as normal controls is shown in Table IV. Alanine, glutamine, glycine, histidine, and serine were prominent in most cases. This agrees with the studies in normal individuals carried out by Woolf (25) and Dent (26) except for a greater prominence of taurine in the latter's cases and a more frequent appearance of histidine in the present group. These differences could be attributed to: 1) Loss of amino acids through desalting; 2) differences in the solvents used; and 3) studies based on 24-hour urine collections instead of single morning specimens.

In patients with acute infectious hepatitis

The distribution of amino acids in the 18 patients with infectious hepatitis is shown in Table V. Of the amino acids usually present in the urine, alanine, glycine, glutamine, and serine showed increases. In addition, many of the essential amino acids were seen in the urine of a number of these patients. These included moderate increases of isoleucine/leucine, lysine, and

TABLE IV

*Distribution of amino acids by paper partition chromatography in the urine of normal controls**

AMINO ACIDS	Rf Phenol	Rf But-Acet	ADULTS 22M	22M	28M	26M	40F	22M	CHILDREN 6M	12F	3M	6M	3M	7M	3M	8M	6F	MEAN
ALANINE	.62	.38	2		2	4	3	2	1	1	2	2	2	2		2	2	1.7
ARGININE	.85	.23								1					2			0.2
ASPARAGINE	.49	.30																
ASPARTIC ACID	.20	.23																
CYSTINE	.42	.17						4								4		0.5
GLUTAMIC ACID	.30	.34						4										0.2
GLUTAMINE	.57	.22	2	2	3	2			2	2	2					2	2	1.3
GLYCINE	.45	.36	5	4	5	5	4	3	4	4	4	4	4	4	4	3	3	4.0
HISTIDINE	.74	.21	2	1	4	2	3	3	3	3	4		4		4		2	2.3
HYDROXY-L-PROLINE	.51	.36																
ISOLEUCINE/LEUCINE	.79	.64																
LYSINE	.72	.15																
METHIONINE	.77	.57																
METHYLHISTIDINE	—	—																
PHENYLALANINE	.91	.60																
PROLINE	.40	.42																
SERINE	.41	.25	2	2	2		1		2		2		3			4	2	1.3
TAURINE	.47	.25																
THREONINE	.51	.30			1									2		2		0.3
TRYPTOPHANE	.76	.59										2						0.1
TYROSINE	.55	.59			2						1						2	0.3
VALINE	.81	.52	1		2		2										2	0.5
L AMINO ISO-BUTYRIC	.66	.40	1						2	3	1	3	1				2	0.8
Total No. Amino Acids			6	4	8	4	5	5	6	7	6	4	5	3	4	6	7	
Total No. Units			15	9	21	13	13	16	14	14	16	11	14	8	10	17	17	

Mean 14 units

*Average value for entire group to nearest 0.1 unit.

* Age and sex of subjects given at top of each column.

methionine, and slight increases of arginine, phenylalanine, threonine, and valine. A slight increase of tyrosine was also noted. This is in general agreement with the findings of Walshe (9) except for the absence of taurine and only a slight increase of cystine. The authors have been unable properly to identify *B*-amino-iso-butyric acid (27) and hence this substance cannot be evaluated in the present study.

There is a close correlation between the amino acid pattern and the total alpha amino nitrogen in the urine. In the group with moderate amino-aciduria, the excretion averaged 26 units. All except Case 2 showed higher levels than that of the controls. In that patient, the urinary volume was so large that the concentration of the amino acids was greatly diluted and hence they were not so prominent. In the group with borderline amino-aciduria, the amino acid excretion averaged 21 units, which is still higher than in the controls. In the so-called normal group, the amino acid excretion averaged 17 units, which represents only a slight increase over the controls. In individual patients, several of the amino acids were much more prominent than among the controls.

In patients following recovery from acute hepatitis

The distribution of amino acids in the nine patients following recovery from infectious hepatitis is shown in Table VI. Except for a continued increase of glutamine in most of the patients, the amino acids appear to have returned to normal levels.

TABLE V

Distribution of amino acids by paper partition chromatography in the urine of patients with acute infectious hepatitis

AMINO ACIDS	CASES																		MEAN*
	MODERATE AMINO-ACIDURIA						BORDERLINE AMINO-ACIDURIA						NO AMINO-ACIDURIA						
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	
ALANINE	4	2	2	3	2	4	4	2	4	4	2	3	3	3	2	5	2	6	3.2
ARGININE			2	2				2		1		1	1						0.6
ASPARAGINE																			
ASPARTIC ACID													2			1			0.2
CYSTINE			2	4	4							2							0.7
GLUTAMIC ACID									1	1						2			0.2
GLUTAMINE	4	2	4	4	4	4	4	4		4	2		1		2		4		2.4
GLYCINE	6	5	6	7	6	6	6	6	7	6	6	6	4	6	4	3	4	4	5.4
HISTIDINE	4		4		2	4			1		4		1	3	4			2	1.6
HYDROXY-L-PROLINE																			
ISO LEUCINE/LEUCINE	4		2	2		2	2			2	1	1	2	1	2				1.2
LYSINE				3	2							4							0.6
METHIONINE			1	1	2					1	1	2	3		2				0.7
METHYLHISTIDINE																			
PHENYLALANINE	2	1				2		2										1	0.4
PROLINE																			
SERINE	6	1	6	3	2				6		2		2	2	2		2		1.9
TAURINE																			
THREONINE						1			4		2	1							0.6
TRYPTOPHANE	2					1							2						0.3
TYROSINE			1	3	1		2				2	1			1			1	0.7
VALINE			2					4		2	1				1			2	0.8
L AMINO ISO-BUTYRIC		1							1	1	1			1		4	2	2	0.7
Total No. Amino Acids	8	6	11	10	9	8	5	6	7	9	11	9	10	6	9	5	5	7	
Total No. Units	32	12	32	32	25	24	18	20	24	22	24	21	21	16	20	15	14	18	
	Mean 26 units						Mean 21 units						Mean 17 units						

TABLE VI

Distribution of amino acids by paper partition chromatography in the urine following recovery from infectious hepatitis

	CASE NO.									
	1	2	3	4	5	6	7	9	11	Mean
ALANINE	3	2		2	2	3	2	3	3	2.2
ARGININE							1			0.1
ASPARAGINE										
ASPARTIC ACID										
CYSTINE										
GLUTAMIC ACID										
GLUTAMINE	4	1	1	2	3	2	2	1	3	2.1
GLYCINE	6	3	2	4	6	5	5	6	5	4.6
HISTIDINE										
HYDROXY-L-PROLINE			2		1		2	2	1	0.9
ISO LEUCINE/LEUCINE										
LYSINE						2		1		0.3
METHIONINE										
METHYLHISTIDINE										
PHENYLALANINE										
PROLINE										
SERINE	3				2	2		1		0.9
TAURINE										
THREONINE	1									0.1
TRYPTOPHANE	1				1	1	1			0.5
TYROSINE					2	2	1	1	1	0.8
VALINE										
L AMINO ISO-BUTYRIC	2			1		1			1	0.5
Total No. Amino Acids	7	3	3	4	7	8	7	7	6	
Total No. Units	20	6	5	9	17	18	14	15	14	
	Mean 13 units									

DISCUSSION

At least two different mechanisms for amino-aciduria have been postulated: 1) It occurs as a result of increased amino acid levels in the plasma which exceeds the normal renal threshold and results in an overflow into the urine. The amino-aciduria associated with progressive muscular dystrophy, cystinosis, phenylketonuria, idiopathic hypoproteinemia with edema in infancy, and severe liver disease has been attributed to this type of mechanism; 2) it can also occur as a result of dysfunction of the renal tubules, which causes poor reabsorption and increased excretion of amino acids. This can be an inherent congenital defect as in deToni-Fanconi syndrome, cystinuria, Wilson's Disease, and rickets, or a transient defect as a result of toxic action noted in lead, uranium, nitrobenzene, "lysol" poisoning, and the ingestion of galactose in galactosemia.

Bollman, Mann, and Magath (28) have shown that the removal of the liver in dogs results in complete cessation of deamination. This was demonstrated by the recovery of amino acids in the blood, urine, and tissues in such animals approximately equal to the anticipated formation of urea had the animals been normal. If amino acids were injected into these animals, the entire amount of amino acid nitrogen could be recovered unchanged many hours after administration. Although infectious hepatitis has generally been regarded as a mild disease, the present data suggest that it may cause sufficient damage to interfere with deamination. The amino acid disturbances follow essentially the same, though a much milder pattern, than that following hepatectomy. If instead of measuring only the total alpha amino nitrogen, we had measured each of the individual amino acids separately, the changes in some would probably have been more striking since the total alpha amino nitrogen reflects not only the increase in certain of the amino acids, but other amino acids which either remain unchanged or actually are decreased in this disease.

The data also provide an opportunity to speculate on the mechanism for overflow of plasma amino acids into the urine. Silber, Seeler, and Howe (16) have previously shown that rapid infusion of amino acid mixture resulted in a large increase of plasma alpha amino nitrogen, but only moderate or slight increase of urine alpha amino nitrogen. However, since these infusions were run over a relatively short period of time and produced changes in total blood volume, the conditions for study were somewhat different from those in a group of patients with elevations of alpha amino nitrogen due to disease. Although the increase of both plasma and urine alpha amino nitrogen in this study were slight, they tend to confirm Silber's findings, and show that in this type of amino-aciduria, the changes in the urine tend to be slight and subtle, and are quite different from the gross changes which occur when a tubular defect of the kidneys exist.

Although it would be tempting to attribute all of the amino acid changes in infectious hepatitis to failure of deamination, a separate renal tubular defect cannot be completely eliminated on the basis of the present data (29). Pitts (30) has shown that the quantitative characteristics of the reabsorptive processes are different for each of the amino acids and that certain amino acids may interfere with the absorption of others. To rule out definitely a tubular defect, therefore, one must not only have quantitative measurements of each of the amino acids, but also understand their action in combination with others. There is some evidence, however, to show that the clearance of the amino acids in infectious hepatitis may not be abnormal. In the first place, Pitts (31) has shown that with the rise of plasma glycine concentration, the rate of increased reabsorption fails to increase in proportion to the amount filtered and consequently excretion may become appreciable. This appears to occur with glycine, alanine, glutamine, and serine in normal individuals, and could occur with some of the other essential amino acids when their concentrations are increased in the plasma. Secondly, Walshe (9) has shown that in severe liver disease, methionine is greatly increased, alanine, glutamine, serine, arginine, cystine, tyrosine, lysine, and phenylalanine are moderately increased. In the present study, the urinary excretion of alanine, glycine, glutamine, and leucine/isoleucine was markedly increased, lysine, methionine, and serine were moderately increased, and arginine, phenylalanine, threonine, valine, and tyrosine were slightly increased. In load experiments in normal children, Jonxis (32) has found

that serine, threonine, glycine, alanine, and histidine are not as well reabsorbed as arginine, tryptophane, tyrosine, and phenylalanine are. The remaining amino acids lie between the two groups. Taking this into consideration, it would appear that except for cystine, most of the increases of the amino acids in the urine could be accounted for by the changes in the plasma in liver disease.

During recent years, high protein diets and supplements such as choline and methionine have been recommended as therapy for acute hepatitis. The present data would seem to suggest that such therapy is unimportant during the acute phase of the disease since there is no actual shortage of amino acids, but only an inability of the liver to deaminate them. Increasing the load of the amino acids either by mouth or parentally will only result in increased excretion.

SUMMARY

1. The changes in amino acid metabolism have been studied in 19 children with infectious hepatitis.

2. Measurements of the total urine alpha amino nitrogen by the gasometric ninhydrin-CO_2 method showed that one-third of the cases had a moderate amino-aciduria, another third had a borderline amino-aciduria, and the remaining third showed normal amino acid excretion.

3. Plasma alpha amino nitrogen studied by the same method was high in all of the patients with moderate amino-aciduria and in some of those with borderline amino-aciduria. All were within normal limits in those without increased amino acid excretion.

4. Both the urine and plasma alpha amino nitrogen returned to normal levels upon recovery.

5. Analysis of the specific amino acids by paper partition chromatography showed a generalized amino-aciduria in all cases with most of the increase among essential amino acids.

6. These changes in amino acid metabolism can be attributed to disturbances in deamination. Further investigation is needed to determine whether or not an additional renal defect exists.

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