Original Communications

Tryptophan and Nicotinic Acid Metabolism in Patients with Cirrhosis of the Liver

GEORGE J. GABUZDA, M.D.* AND CHARLES S. DAVIDSON, M.D.†

METABOLISM of tryptophan in man yields two biologically important substances. Serotonin (5-hydroxytryptamine) is produced from this amino acid with 5-hydroxytryptophan as an intermediate; 5-hydroxyindolacetic acid is the main excretory product in the normal subject.[1] Patients with hepatic cirrhosis excrete in the urine normal or low normal amounts of endogenous 5-hydroxyindolacetic acid.[2-4] Increased quantities of this derivative are excreted, however, when 5-hydroxytryptophan is given intravenously.[2] Tryptophan also is metabolized to nicotinic acid, with N^1-methylnicotinamide and the 6-pyridone derivative as major normal urinary excretory products.[1] This report concerns the metabolism of tryptophan by the latter pathway in patients with cirrhosis and in control subjects.

MATERIAL AND METHODS

The urinary excretion of N^1-methylnicotinamide was determined for ten men with obvious clinical and laboratory evidences of cirrhosis and nine control subjects without hepatic disease. Four patients with cirrhosis of the liver were paired with similarly undernourished control subjects. These paired patients were compared as to the urinary excretions of N^1-methylnicotinamide, niacin, tryptophan and nitrogen before, during and after the feeding of tryptophan and niacinamide. The studies in each pair were carried out simultaneously and under identical conditions of environment and food intake. The substances mentioned were determined in one of the control subjects (McN) only during an initial control period. An additional patient with cirrhosis (Gri) was studied separately.

The patients and control subjects were maintained on a metabolism ward. Food intakes for each pair of patients studied and for the patients studied separately were constant in protein and energy contents from day to day. Each patient had consumed an adequate and nutritious diet for several weeks prior to the institution of the study and the constant dietary regimen was maintained for at least six days before serial determinations of the urinary constituents were initiated. The diets provided during the study were adequate in protein, calorie, vitamin and mineral contents. Except as indicated, vitamins or other dietary supplements were not prescribed.

The paired studies were planned to compare five successive metabolic periods, sufficient in duration to ensure equilibration on each regimen employed, as follows: (1) control period, (2) DL-tryptophan oral supplement, (3) control period, (4) niacin oral supplement, and (5) control period. The durations of the periods were identical for each member of a pair. These are shown in Table I. It was possible to complete only the first three periods for one pair of subjects because in the patient with cirrhosis gastrointestinal bleeding developed which demanded termination of the constant regimen. The other patients with cirrhosis demonstrated clinical and

From the Thorndike Memorial Laboratory, Second and Fourth (Harvard) Medical Services, Boston City Hospital, and the Department of Medicine, Harvard Medical School, Boston, Massachusetts.

* Associate Professor of Medicine, Western Reserve University, School of Medicine, Department of Medicine at Cleveland Metropolitan General Hospital, Cleveland, Ohio; † Associate Professor of Medicine, Harvard Medical School; Associate Director, Second and Fourth Medical Services (Harvard), Boston City Hospital, Boston Massachusetts.

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TABLE I
Duration (Days) of Control and Experimental Periods

Patient	Diagnosis	Diet Alone	Diet and Tryptophan	Diet Alone	Diet and Niacinamide	Diet Alone
May	Control	6	6	6	6	6
Ahe	Cirrhosis	6	6	6	6	6
Rya	Control	9	6	6	6	6
Swa	Cirrhosis	9	6	6	6	6
Bel	Control	10	12	10	...	...
Her	Cirrhosis	10	12	10	...	...
McN	Control	9	...	...	...	...
Mar	Cirrhosis	9	9	12	9	9
Gri	Cirrhosis	9	6	6	6	6

laboratory evidences of gradual improvement of their liver disease during the course of these studies. DL-tryptophan (6 gm. daily) was mixed in red currant jelly and given orally. This quantity of amino acid added 0.8 gm. to the daily total nitrogen intake. Niacinamide (15 or 100 mg. daily) was administered in nonenteric-coated tablets. There was no clinical intolerance to the administration of these substances.

Total twenty-four hour urines were collected and preserved with a 5:1 mixture of toluol and glacial acetic acid, and refrigeration. After the completion of each day's collection, the volume was recorded and suitable aliquots stored in brown glass containers in the deep freeze at minus 20°C. for subsequent analysis. Total nitrogen content of urine was determined by micro Kjeldahl analysis. Urinary N^1-methylnicotinamide was measured by the fluorometric method of Huff and Perlzweig.[5] Tryptophan and niacin (total unmethylated nicotinic acid) in urine were determined microbiologically, the former employing Streptococcus faecalis (ATCC No. 9790)[6] and the latter Lactobacillus arabinosus (ATCC No. 17-5)[7] as the test organism. This streptococcus grows in the presence of L- but not D-tryptophan.

RESULTS

The quantities of N^1-methylnicotinamide excreted in the urine by ten patients with cirrhosis of the liver and nine subjects without liver disease are shown in Table II. The patients with cirrhosis of the liver excreted in the urine approximately two and a half times as much "endogenous" N^1-methylnicotinamide as did the control subjects.

The plan of study and the data collected for one pair of patients are shown in Figure 1. The increases in the urinary excretions of tryptophan and N^1-methylnicotinamide which occurred promptly following the administration of tryptophan are illustrated. The excretions of these constituents returned to control levels when tryptophan feeding was discontinued. With niacinamide administration, the urinary excretion of tryptophan was unchanged, but N^1-methylnicotinamide content

TABLE II
Urinary Excretion of N^1-Methylnicotinamide

Normal		Cirrhosis	
Patient	mg./day*	Patient	mg./day*
Bel	3.7	Her	9.2
McN	3.6	Mar	10.5
May	5.8	Ahe	10.0
Rya	2.5	Swa	8.5
Bli	6.2	Gri	9.2
Ear	7.4	Kel	13.2
Tur	6.9	Fie	13.0
Col	2.8	Klu	18.5
Odo	2.6	Wel	10.1
		Hay	10.8
Range	2.5–7.4		8.5–18.5
Mean	4.6		11.3
Standard deviation of mean	±2.0		±3.0
Standard error of mean	±0.7		±1.0
		p = <0.001	

* Each individual value represents the average of six to twelve consecutive daily determinations for each subject.

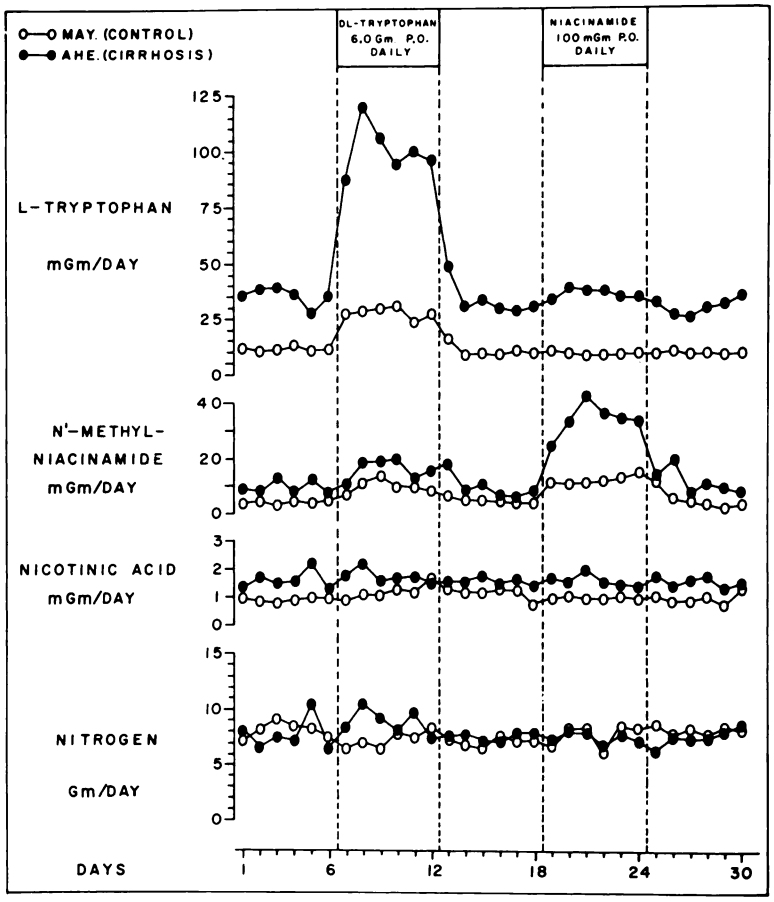

Fig. 1. Metabolism of DL-tryptophan and niacinamide in a patient with cirrhosis (●—●) and an undernourished control subject (○—○).

promptly increased. This increase following 100 mg. daily of niacinamide given orally was about twofold that resulting from feeding 6.0 gm. of tryptophan daily. The urinary excretions of niacin and of nitrogen were unaltered by the administration of either of these substances. This patient with liver disease excreted considerably more tryptophan than his paired control subject during control periods and when tryptophan was fed. This was not, however, a consistent finding in all patients studied, as indicated subsequently.

The data for each patient as shown in Tables III, IV, V and VI are derived from daily observations like those illustrated in Figure 1.

Urinary Excretion of L-Tryptophan (Table III)

The amounts of tryptophan excreted in the urine by the patients with cirrhosis did not differ consistently from those excreted by the control subjects during the initial control periods. During tryptophan administration, the rate of excretion of this amino acid increased promptly in every instance. No further increases followed the initial changes in excretion rates. Three of the five patients with cirrhosis of the liver excreted quantities of the administered tryptophan in the urine comparable to the control subjects, whereas the remaining two patients excreted considerably more. Following cessation of tryptophan feeding, values for excretion of this amino acid returned to control levels within two days. Only small amounts of the administered L-tryptophan were detected in the urine (0.5 to 0.7 per cent of total dose for control subjects, and 0.5 to 2.5 per cent for patients with cirrhosis). The oral administration of 100

TABLE III
Urinary Excretion of L-Tryptophan (mg./day)

Patient	Diagnosis	Diet Alone	Diet plus DL-tryptophan (6.0 gm. orally daily)	Diet Alone	Diet plus Niacinamide* (orally daily)	Diet Alone
May	Control	11.5	28.2	10.0	10.5	10.4
Ahe	Cirrhosis	35.2	99.9	34.3	36.3	32.5
Rya	Control	17.3	38.1	18.2	15.4	15.9
Swa	Cirrhosis	14.4	27.6	16.2	15.5	14.1
Bel	Control	7.4	21.0	7.8	...	...
Her	Cirrhosis	21.7	102.5	29.3	...	...
McN	Control	22.1	...	...	...	...
Mar	Cirrhosis	17.4	50.0	15.8	13.7	15.1
Gri	Cirrhosis	18.9	68.7	36.5	24.0	24.6

* Niacinamide added: May, Ahe, Rya, Swa, and Gri 100 mg.; Mar 15 mg.

TABLE IV
Urinary Excretion of N^1-Methylnicotinamide (mg./day)

Patient	Diagnosis	Diet Alone	Diet plus DL-Tryptophan (6.0 gm. orally daily)	Diet Alone	Diet plus Niacinamide* (orally daily)	Diet Alone
May	Control	5.3	10.0	5.2	12.6	6.3
Ahe	Cirrhosis	10.0	16.4	9.9	34.7	9.5
Rya	Control	3.8	6.2	5.1	16.1	7.0
Swa	Cirrhosis	8.5	16.0	11.8	28.2	8.8
Bel	Control	3.7	10.9	4.7	...	...
Her	Cirrhosis	9.2	46.2	15.4	...	...
McN	Control	3.6	...	...	...	...
Mar	Cirrhosis	10.5	19.6	10.8	10.7	7.5
Gri	Cirrhosis	9.2	22.1	11.1	26.7	8.2

* See footnote Table III.

mg. of niacin daily to three patients with cirrhosis and to two control subjects, and of 15 mg. daily to an additional patient with cirrhosis, had no effect on the urinary excretion of tryptophan.

Urinary Excretion of N^1-Methylnicotinamide (Tables II and IV)

The patients with cirrhosis consistently excreted more N^1-methylnicotinamide in the urine during the initial control periods than the control subjects. The urinary excretion of N^1-methylnicotinamide increased when tryptophan was fed. The magnitude of increase was comparable for the two groups with one exception. A patient with severe, active cirrhosis (Her) excreted abnormally large quantities of N^1-methylnicotinamide in the urine when tryptophan was given.

When 100 mg. of niacinamide was fed, all patients demonstrated prompt increases in the urinary excretion of N^1-methylnicotinamide. This increase was not noted in one patient (Mar), who was fed a smaller dose (15 mg.). The excretion of this metabolite was usually greater when niacinamide was given than with tryptophan for both groups of subjects. When the administration of niacinamide was dis-

TABLE V
Urinary Excretion of Nicotinic Acid (mg./day)

Patient	Diagnosis	Diet Alone	Diet plus DL-tryptophan (6.0 gm. orally daily)	Diet Alone	Diet plus Niacinamide* (orally daily)	Diet Alone
May	Control	0.99	1.22	1.18	1.04	1.03
Ahe	Cirrhosis	1.64	1.79	1.60	1.63	1.61
Rya	Control	1.02	0.84	0.90	1.21	0.98
Swa	Cirrhosis	1.24	1.14	1.22	1.51	1.47
Bel	Control	1.05	1.23	1.22	...	...
Her	Cirrhosis	1.26	1.65	1.35	...	...
McN	Control	0.90	...	...	...	...
Mar	Cirrhosis	1.70	1.38	1.63	1.22	0.99
Gri	Cirrhosis	1.11	1.07	1.08	0.93	0.78

* See footnote Table III.

TABLE VI
Urinary Excretion of Nitrogen (gm./day)

Patient†	Diagnosis	Diet Alone	Diet plus DL-tryptophan (6.0 gm. orally daily)	Diet Alone	Diet plus Niacinamide* (orally daily)	Diet Alone
May	Control	8.1	7.3	7.2	7.9	8.1
Ahe	Cirrhosis	7.7	8.9	7.6	7.5	7.7
Rya	Control	7.8	6.9	8.0	7.5	7.4
Swa	Cirrhosis	8.9	8.4	10.4	8.8	9.5
Bel	Control	7.2	7.7	7.3	...	...
Her	Cirrhosis	6.0	7.2	7.1	...	...
McN	Control	9.6	...	...	...	...
Mar	Cirrhosis	10.8	10.4	10.3	9.4	9.1
Gri	Cirrhosis	9.1	9.8	10.9	11.5	10.2

* See footnote Table III.
† Dietary nitrogen intakes: May and Ahe 12.3 gm./day; Rya and Swa 15.5 gm./day; Bel and Her 12.3 gm./day; McN and Mar 12.3 gm./day; Gri 15.5 gm./day.

continued, the levels of N^1-methylnicotinamide in the urine returned to control values within two days.

Urinary Excretion of Nicotinic Acid (Table V)

Urinary excretions of nicotinic acid during initial control periods were higher in four of the patients with liver disease than in their paired control subjects. This difference was maintained throughout all study periods for the paired subjects. The urinary excretions of nicotinic acid remained relatively constant throughout each patient study, and were not altered by the administration of tryptophan or of niacinamide.

Urinary Excretion of Nitrogen (Table VI)

The urinary excretions of nitrogen during initial control periods were comparable in the two groups of patients. The average values remained relatively constant for each patient study and were not influenced by the administration of tryptophan or of niacinamide. Although the data are not presented, the nitrogen content of the stools was determined for each study period for each patient. These likewise were constant and uninfluenced by the

experimental regimens. Positive nitrogen balances were demonstrated throughout each patient study. These balances attest to the comparable degrees of protein undernutrition for each pair of patients studied.

COMMENTS

Patients with cirrhosis excrete in the urine larger than normal quantities of "endogenous" N^1-methylnicotinamide, an excretory metabolite of niacin. Abnormal metabolism of niacin might thus account for the increased excretion of the N^1-methyl derivative in these patients. Because tryptophan is a niacin precursor in mammalian organisms including man,[8-13] a finding confirmed by the present study, an abnormality in the metabolism of this amino acid might account for the increased excretion of N^1-methylnicotinamide by patients with cirrhosis. Increased excretion of N^1-methylnicotinamide did result from tryptophan feeding in this study. However, since the increase was of the same magnitude for the undernourished control subjects as for the patients, an abnormality in tryptophan metabolism cannot be implicated to account for the increased excretion of this substance by patients with cirrhosis. Other possible sources of this metabolite must be considered, either in other metabolic pathways in the body or possibly arising from the action of gastrointestinal bacteria.

Another possibility is that patients with liver disease may excrete other compounds in the urine which are determined as N^1-methylnicotinamide. One such is indole which arises from tryptophan in the gut and which may be excreted in increased amounts by patients with liver disease. Indole has been shown by Schweigert[14] to enhance the apparent N^1-methylnicotinamide content of rat urine. The enhanced excretion of N^1-methylnicotinamide after niacinamide administration cannot be accounted for in this way, however, as this vitamin is not changed to indole. Also, if a gastrointestinal factor accounted for the increases in N^1-methylnicotinamide excretion in the patients with cirrhosis, one would expect these patients to demonstrate excretions of this substance in excess of those occurring in the control subjects after tryptophan feeding. This was not the case.

Although the studies reported here failed to define a metabolic defect to account for the increased urinary excretion of N^1-methylnicotinamide by patients with liver disease, several other points can be made. The patients with hepatic cirrhosis did demonstrate ability, comparable to that noted in undernourished subjects, to convert tryptophan to nicotinic acid as indicated by the increased urinary excretion of N^1-methylnicotinamide accompanying the administration of this amino acid. The patients with liver disease also demonstrated no limitation of ability to convert orally administered niacinamide to the methylated derivative. In fact, there is some suggestion that the patients with liver disease converted a larger percentage of the 100 mg. dose than did the undernourished subjects.

The small but consistently increased excretion of nicotinic acid by the patients with cirrhosis as compared to the control subjects remains unexplained. Wasting of dietary niacin (preformed or derived from tryptophan) or enhanced conversion of tryptophan to niacin cannot be incriminated, as feeding tryptophan or niacin did not augment niacin excretions. Others have similarly reported that increases in the excretion of nonmethylated nicotinic acid do not result from oral administration of tryptophan or niacin in man.[8,10]

An increased urinary excretion of tryptophan is characteristic of the mild aminoaciduria that occurs in some patients with cirrhosis of the liver.[15] Several of the patients studied here demonstrated a similar tryptophanuria which became more pronounced when tryptophan was fed. On the other hand, several of the patients with liver disease demonstrated urinary excretions of tryptophan before and during tryptophan administration that were comparable to control values. Feeding of tryptophan or niacinamide did not result in decreases in the urinary excretion of nitrogen. Utilization of dietary protein, therefore, was not limited by the tryptophanuria and nicotinic aciduria noted in the patients with cirrhosis of the liver.

SUMMARY AND CONCLUSION

Patients with cirrhosis of the liver associated with alcoholism excrete approximately two and a half times more N^1-methylnicotinamide in the urine than control subjects fed comparable diets.

Five male patients with cirrhosis were compared to undernourished control subjects with respect to certain aspects of niacin and tryptophan metabolism. Three patients with cirrhosis were studied in paired experiments, each being observed simultaneously with an undernourished control subject who was maintained on an identical dietary and environmental regimen.

The urinary excretions of N^1-methylnicotinamide and of nicotinic acid were greater for the patients with cirrhosis of the liver than for the undernourished control subjects. The excretion of nicotinic acid was not altered by tryptophan or niacinamide feeding. The excretion of N^1-methylnicotinamide increased further when tryptophan or niacin was given orally. The magnitude of these increases was comparable for patients with cirrhosis of the liver and undernourished control subjects. Patients with cirrhosis of the liver adequately convert orally administered niacinamide and tryptophan to N^1-methylnicotinamide.

Urinary excretions of nitrogen were unaltered by the oral administration of tryptophan or of niacinamide in the patients with hepatic disease and in the undernourished control subjects. Thus, for these patients, utilization of dietary nitrogen was not limited by unavailability of these nutrients.

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