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URINARY UROCANIC ACID IN MAN: THE IDENTIFICATION OF UROCANIC ACID AND THE COMPARATIVE EXCRETIONS OF UROCANIC ACID AND N-FORMIMINOGLUTAMIC ACID AFTER ORAL HISTIDINE IN PATIENTS WITH LIVER DISEASE

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The amino acid L-histidine is obtained by man primarily from dietary protein. The essential need for this amino acid, however, has not been demonstrated conclusively (1). Previous studies have indicated that the major pathways of utilization of L-histidine are by way of incorporation into body protein, excretion into the urine, and metabolism to glutamic acid. It is believed that the pertinent mammalian pathway follows the reactions outlined in Figure 1 (2–4). Prior to our initial report (5) of the compounds shown in Figure 1, histidine, α-formiminoglutamic acid (FiGlu), and glutamic acid had been definitively identified in the urine of man. Urocanic acid (UA) had been identified in the urine of small mammals (2) and tentatively identified chromatographically in the urine of man (6). Imidazolonepropionic acid has not been identified in urine as yet, although a metabolic oxidation product derived from this intermediate, hydantoin-5-propionic acid, has been identified in rat urine (7). Since our original report, characterizing the occurrence of UA in the urine of man (5), McIsaac and Page (8) have described the identification of UA in the urine of a patient with hepatic coma, and similarly UA has been identified by Whitehead and Arnstein in a patient with kwashiorkor (9).

FiGlu excretion has been studied in recent years in patients with folic acid deficiency (10–12) and treated with folic acid antagonists (13). It has been found that such patients, particularly when given loading doses of histidine, excrete increased amounts of this metabolite in the urine. Small amounts of folic acid correct this defect. The role of vitamin B_{12} (14) and the behavior of patients with pernicious anemia after histidine loading have not been completely defined. Several patients with vitamin B_{12} deficiency, apparently uncomplicated by folic acid deficiency, have had increased amounts of FiGlu in the urine with (15) or without (16) histidine loading, with an accentuation of the defect in two patients given folic acid (15). However, an increased FiGlu excretion after histidine loading does not always occur in uncomplicated vitamin B_{12} deficiency (10). It has been reported by Carter, Schaffner and Heller (17) that FiGlu excretion in patients with hepatic cirrhosis is increased after histidine loading. Our studies were initiated on a malnourished patient with a macrocytic anemia, who had been referred to the National Institutes of Health. This patient was given a loading dose of histidine and excreted large amounts of UA, a phenomenon previously observed in the urine of rats fed ethionine (18). The specific identification of UA and the excretion of UA and FiGlu in normal patients, patients with variable diagnoses, and patients with chronic alcoholism and liver disease are the subject of this paper.

METHODS

The 25 subjects comprising this study were: *1*) normal subjects (group A, nos. 1–6) who were laboratory personnel employed at the Clinical Center, National Institutes of Health; *2*) patients (group B, nos. 7–16) who had no history or definite evidence of primary hepatocellular disease; and *3*) patients (group C, nos. 17–25) who had varying types of diffuse cellular liver disease, primarily as a consequence of chronic alcoholism. The clinical diagnoses and pertinent laboratory data are summarized in Table I.

Method for obtaining urine specimens for analyses. Except as indicated, the patients were eating a regular

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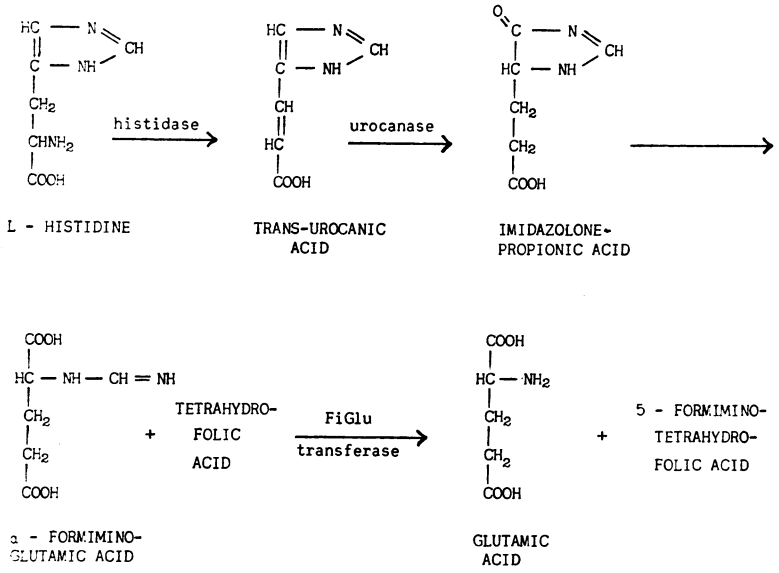

FIG. 1. HISTIDINE DEGRADATION (SCHEMATIC).

hospital diet without vitamin supplementation at the time of study. A 24-hour control urine was collected, 5 ml of toluene added, and this refrigerated at 4° C to preserve the FiGlu and UA. After collecting the control urine and at the beginning of the second 24-hour specimen, L-histidine (Sigma Chemical Company, St. Louis, Mo.), 50 mg per kg, was administered orally (maximal dose 3.0 g). If the specimens were not to be analyzed on the day after collection they were frozen until the time of analysis. Frozen samples were suitable for analysis for at least 2 weeks.

Determination of urocanic acid and formiminoglutamic acid. A procedure previously described for the determination of FiGlu and UA, the metabolites of histidine, in rat urine (18) has now been modified and applied to the determination of these metabolites in human urine. The method involves the treatment of aliquants of urine with an active extract of chicken liver and subsequent microbiological assay of the reaction mixture. The net reaction is the conversion of ring carbon-2 of UA or the formimino-carbon of FiGlu into the N-5 formyl group of citrovorum factor (N^5-formyl tetrahydrofolic acid).

The application of the method to human urine is now described. Two aliquants, an untreated sample and an autoclaved sample, of each specimen were assayed. The autoclaved samples were prepared as follows. To 1 ml of urine, 1 drop of 1.0 M potassium hydroxide was added and the urine autoclaved at 15 pounds per square inch for 30 minutes. One drop of 1.0 M hydrochloric acid was then added to the autoclaved samples. This procedure destroys the FiGlu (19) but leaves the UA intact.

The dialyzed extract of chicken liver was that previously described (20). Because variability exists in the activity of commercially frozen chicken livers from which extracts were prepared, each lot of chicken liver was evaluated before use. Extracts were tested in a volume of 0.25 ml and in the presence of 0.5 ml of supplement (containing 0.55 μmole of folic acid, 200 μmoles of monobasic potassium phosphate adjusted to pH 6.0 with potassium hydroxide, 20 μmoles of citric acid adjusted to pH 6.0, 20 μmoles of magnesium sulfate·$7H_2O$, and 0.12 μmoles of triphosphopyridine nucleotide in each ml). Those lots were used which effected the quantitative conversion of the ring carbon-2 of 1 μg of UA·$2H_2O$ into a formyl group of tetrahydrofolic acid in 2 hours at 37° C. For routine analysis, 0.5 ml of the active dialyzed liver extract, 0.5 ml of the above supplement and suitable volumes (0.01 to 0.2 ml) of both autoclaved and untreated urine were incubated at 37° C.[1] After 2 hours, 0.25 ml of ascorbate solution (100 mg per ml ascorbic acid adjusted to pH 6.0 with potassium hydroxide) was added and the samples autoclaved for 30 minutes. The samples together with blanks (containing all ingredients except sample) were then assayed for citrovorum factor by conventional microbiological procedures. Correction was made for the blank values. The value found for the heated specimen was taken to represent the value for UA. The difference in values found for the unheated specimen (total formylating activity) and autoclaved specimen represented the FiGlu value.

This method for the determination of these histidine metabolites is valid when applied to rat urine (18). The portion of the procedure concerned with the estimation of UA in human urine has not been tested extensively, but in this study it has been demonstrated that, if urine specimens show the presence of UA by the above

[1] Since no inhibitors of folic acid reductase were present in the urine specimens employed, the preincubation period of liver extract and reducing supplement was omitted (21).

TABLE I

Summary of laboratory data

Subj. no.	Diagnosis	Age	Sex	Race	Liver*	BSP	C.F.	T.T.	T.P.	A/G	Alk. p'ase	SGOT	Pro.	Bilirubin 1 min/ total	Chol.	BUN	Hb.	Hct.	RBC
		yrs				%		*unit*	*g/100 ml*		*units*		%	*mg/100 ml*			*g/100 ml*	%	
1	Normal control	24	M	W	0												14.6		
2	Normal control	30	M	W	0														
3	Normal control	34	M	W	0														
4	Normal control	30	M	W	0	2	0	3	6.8	3.7/3.1			100				14.0		
5	Normal control	27	F	W	0				6.6	3.6/3.0						11	12.3	38	N
6	Normal control	3	F	W	0				7.0	4.0/3.0	20					12	11.2	34	N
7	Chronic lymphocytic leukemia	62	M	W	1			2	6.4	3.8/2.6	19			0.20/0.8		18	11.6		
8	Melanosarcoma	45	M	W	10	9					8	22		0.05/0.4	126	16	11.1	36	N
9	Polycythemia vera, myelofibrosis	71	F	W	2											16	7.1	25	N
10	Blood loss anemia from uterine fibromyomata	43	F	N	0												3.7	17	Mic/H
11	Primary amyloidosis, diabetes mellitus	52	F	W	0				5.5	2.8/2.7	6		100	0.04/0.2	196		9.9	32	N
12	Sickle cell anemia	5	F	N	7		0	3	7.4	3.7/3.7	11	59		0.37/2.1	98	8	6.4	22	N
13	Chronic renal disease	40	F	W	2											40	7.9	28	N
14	Mild malnutrition	82	F	N	5		0	2	5.6	3.0/2.6	12	18			117	13	8.9	27	N
15	Malnutrition, carcinoid?	39	M	W	7	3	0		6.8	3.3/3.5	9		100	0.07/0.4	149	12	12.0	38	N
16	Malnutrition, carcinoid	51	M	W	9	4	0		5.7	2.5/3.2	18		100	0.04/0.2	168	12	12.0	40	N
17a†	Chronic alcoholism, megaloblastic anemia	45	F	N	3	44	2+	6	5.0	1.5/3.5	11	67	100	0.32/0.6	140	11	6.8	23	Mac
17b	Malnutrition				0	3	0	5	8.6	4.6/4.0	13	14	100	0.05/0.3	180	12	13.7	42	N
18	Idiopathic steatorrhea, gastrointestinal bleeding	51	F	W	0	0		9	5.4	1.9/3.5	8		15			12	8.5	36	Mic
19a	Laennec's cirrhosis, esophageal varices	50	M	W	7	44	4+	5	6.5	3.3/3.2	27	26	23	7.0/12	191	8	7.0	22	N
19b					8	22	2+	2	6.6	2.9/3.7	14	14	50	0.40/1.5	203	10	12.2	38	N
20	Laennec's cirrhosis, ascites	44	F	W	6	13	0	6	7.7	3.4/4.3	6	30	80	0.05/0.4		7	13.4	41	N
21	Laennec's cirrhosis, portal hypertension, diabetes mellitus	43	M	N	6	46	3+	12	6.7	4.0/2.7	10	150	65	1.1/3.0	250	15		45	N
22	Chronic alcoholism	37	F	N	2	3	0	1	7.3	3.8/3.5	8	34	100	0.06/0.4	220	12	13.9	44	N
23	Chronic alcoholism, rheumatoid arthritis	60	M	N	1	7	0	2	7.3	3.4/3.9	7			0.04/0.4	282		14.1	43	N
24	Serum hepatitis	34	M	W	2	50	2+	9	6.5	4.4/2.1	18	340	90	4.6/12	372	14	15.0	45	N
25	Postnecrotic cirrhosis Sickle cell anemia	8	F	N	8		3+	10	8.5	2.6/5.9	37	200	80	2.6/5.9			7.8	24	

* Palpable below right costal margin in cm, BSP—bromsulphalein retention at 45 minutes, C.F.—cephalin flocculation, T.T.—thymol turbidity, T.P.—total serum protein, A/G—serum albumin/serum globulin, Alk. p'ase—alkaline phosphatase (King-Armstrong units), SGOT—serum glutamic oxalacetic transaminase, Chol.—cholesterol, Pro. time—prothrombin time, BUN—blood urea nitrogen, Hb.—hemoglobin, Hct.—hematocrit, RBC—erythrocyte appearance (N—normocytic, Mac—macrocytic, Mic—microcytic, H—hypochromic).

† a—initial values, b—representative of later values.

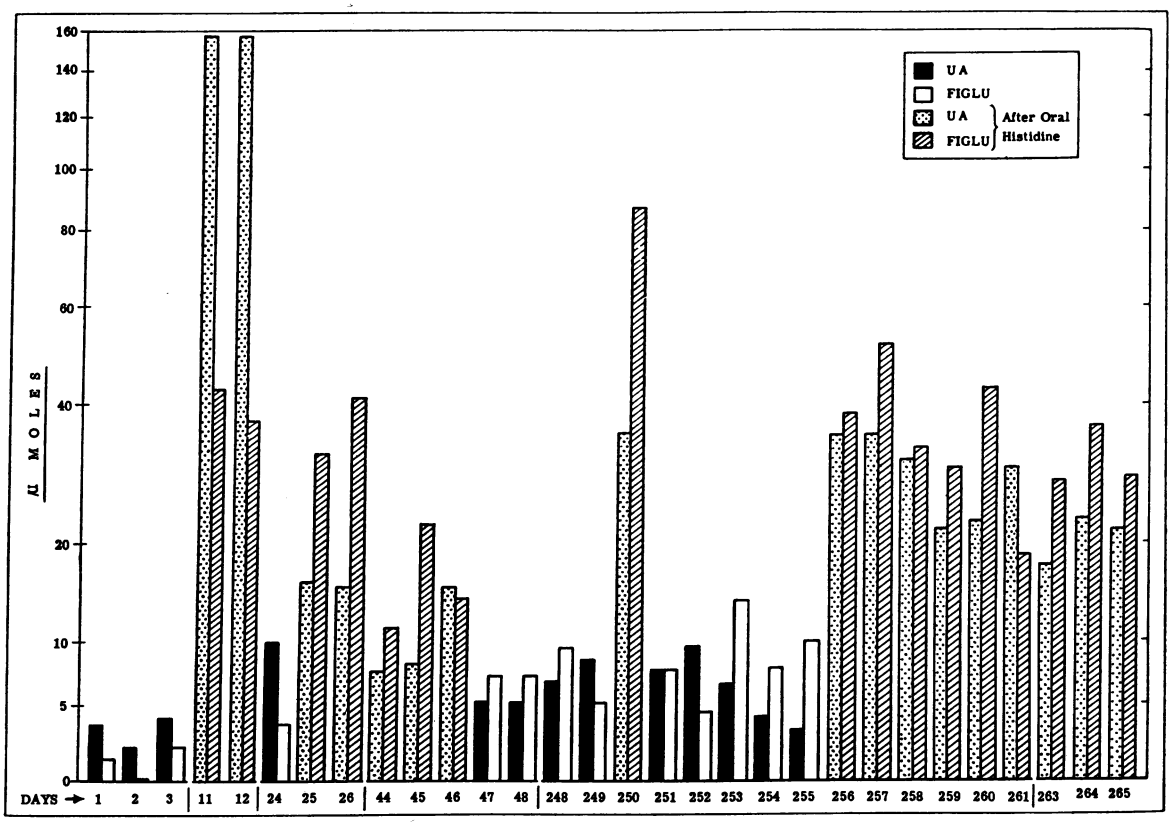

FIG. 2. SUBJECT 17; DAILY EXCRETION OF FIGLU AND UA WITH AND WITHOUT ORAL HISTIDINE (3.0 G) PLOTTED AS MICROMOLES PER 24-HOUR URINE. Approximate upper limits of UA and FiGlu without histidine loading are, respectively, 15 and 25 μmoles; comparable values after histidine are 20 and 35 μmoles.

assay procedure, the presence of UA can be confirmed by isolation or chromatography. It is conceivable that other formyl donors could be present in urine specimens and that these could complicate results in future studies.

When metabolites were present in concentrations of 10 μmoles or less per L, the results of assays of the same sample on different days showed differences less than 50 per cent. When these concentrations were in the order of 20 μmoles per L or more, the differences were less than 25 per cent.

Case reports. The findings in two case reports are pertinent to the development of our conclusions relating to the excretion of FiGlu and UA in man.

The first patient (Subject 17) was a 45-year-old negro female who was admitted May 9, 1959, for evaluation of a macrocytic anemia. At the time of admission she complained of burning feet of 3 weeks' duration. Since age 20 the patient had drunk large quantities of alcohol. From 1953 until 1956 she was hospitalized for pulmonary tuberculosis and discharged improved, after a right lobectomy. During the 3 years prior to admission she had continued to drink and had eaten irregularly. She had progressively lost weight from approximately 120 to 77 pounds in the previous year. For several weeks before admission she had noted extreme weakness and had fainted several times; just before coming to the hospital she found it difficult to walk because of burning feet and weakness. Except for rather frequent episodes of nausea she had no gastrointestinal symptoms or other history of significance. Her physical examination at the time of admission revealed her to be emaciated but in no acute distress. Her temperature was 38° C, pulse 100 per minute, respiration 20 per minute, and blood pressure 90/60 mm Hg. Findings in the right chest were consistent with her history of healed tuberculosis. The liver was palpable 3 cm below the right costal margin. The spleen was not palpable. The neurological examination revealed a decrease in vibration sense and hypesthesia of a stocking distribution extending to the midthigh bilaterally.

Additional pertinent laboratory data included: bone marrow-megaloblastoid erythroid hyperplasia not considered diagnostic of pernicious anemia; erythrocyte indices (mean cell hemoglobin 35 μμg, mean cell volume 118 μ^3, mean cell hemoglobin concentration 30 per cent); total leukocyte count 3,800 per mm^3 (polymorphonuclear leukocytes 33 per cent, lymphocytes 56 per cent, monocytes 11 per cent); reticulocytes 9.6 per cent (after 3 days of a regular diet); serum vitamin B_{12} 1,550 μμg per

ml (normal 300 to 700 $\mu\mu$g per ml) ; serum iron 43 μg per 100 ml; total iron-binding capacity 79 μg per 100 ml.

On the day after admission the patient was restricted to a 1,600 calorie diet consisting of folic acid-deficient casein (70 g), corn oil, and water. She remained on this diet for 2 weeks, during which the studies of histidine metabolism were performed. During this period the reticulocyte count varied from 9 to 12 per cent, then declined during the last few day of the folic acid-deficient diet, falling on the last day to 1.5 per cent. After this 2-week period the patient was placed on a regular diet. There was a rapid rise in the unsaturated iron-binding capacity to 226 μg per 100 ml after 3 months' hospitalization. During this period serum iron fell to levels of 12 μg per 100 ml. Initially the hemoglobin rose from 6.8 to 8.4 g per 100 ml 6 weeks after admission, and there-

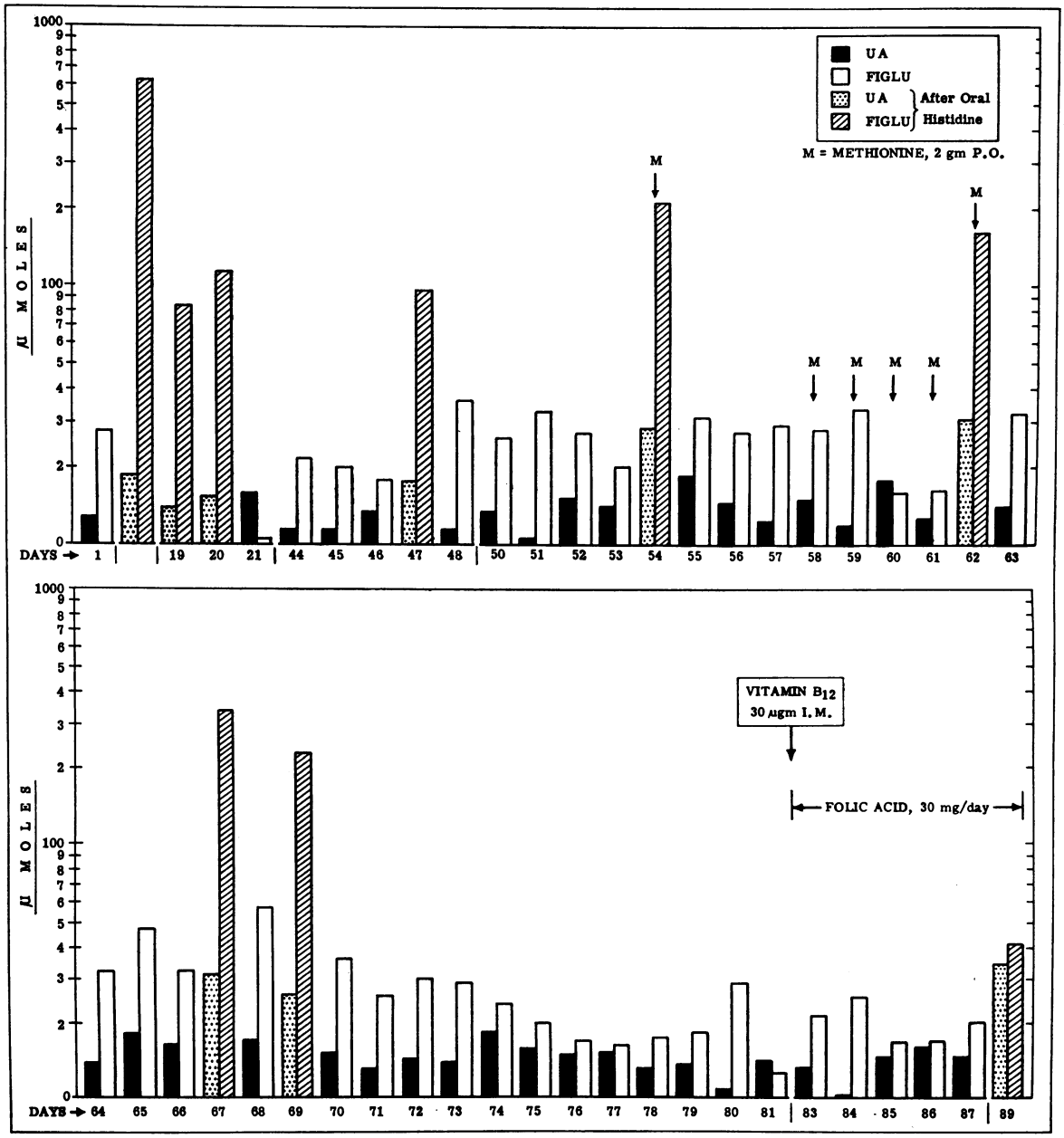

FIG. 3. SUBJECT 19; DAILY EXCRETION OF FIGLU AND UA WITH AND WITHOUT ORAL HISTIDINE (3.0 G) PLOTTED AS MICROMOLES PER 24-HOUR URINE. Approximate upper limits of UA and FiGlu without histidine loading are, respectively, 15 and 25 μmoles; comparable values after histidine are 20 and 35 μmoles.

after fell to 7.1 g per 100 ml. The mean cell volume reached a low of 88 μ^3. The patient's hematological indices and serum iron rapidly responded to oral ferrous sulfate therapy which was begun 3 months after admission. At 8 months the values were: hemoglobin 13.7 g per 100 ml; hematocrit 42 mm; erythrocyte count 4.6 million per mm^3; serum iron 92 μg per 100 ml; and total iron-binding capacity 306 μg per 100 ml. In addition, the serum proteins slowly rose to 4.6 g per 100 ml albumin and 4.0 g per 100 ml globulin. Within a 4-week period after institution of a regular diet, the sulfobromophthalein retention had fallen from an initial 44 per cent to 3 per cent. During the 8-month period of study the patient stated that she did not drink alcoholic beverages and that she ate regularly. Her weight rose from 77 to 113 pounds. The FiGlu and UA excretion data are presented in Figure 2.

The final pertinent diagnoses were: chronic alcoholism complicated by malnutrition, nutritional megaloblastic anemia, severe nutritional liver dysfunction, peripheral neuropathy, and iron deficiency.

The second patient (Subject 19), a 50-year-old white male, was admitted October 8, 1959, with complaints of nervousness, nausea, and weakness following prolonged alcoholic intake. He stated that he had been an alcoholic since the age of 17. He had previously been treated medically for complications of his liver dysfunction, including jaundice, fluid retention, and bleeding esophageal varices. At the time of admission the patient appeared chronically ill. Pertinent findings included an enlarged, tender, smooth liver, 7 cm below the right costal margin in the midclavicular line. The spleen was just palpable below the left costal margin. Jaundice was visible. There was minimal stocking hypesthesia of both feet. The pertinent laboratory data are seen in Table I.

While on a regular hospital diet the patient's excretion of FiGlu and UA was followed with and without oral loading with histidine. Attempts were made to vary his response to histidine loading by adding methionine and folic acid (Figure 3). He slowly improved and was discharged. Shortly thereafter he bled from his esophageal varices and a portocaval anastamosis was performed at another hospital. Six weeks after surgery he again became jaundiced and was told that he had developed serum hepatitis. He gave no history of alcohol intake during this period. He was readmitted to the Clinical Center on September 28, 1960, for re-evaluation of his liver function and response to histidine loading (see Figure 3). The liver remained tender and firm and was palpable 8 cm below the right costal margin in the midclavicular line. The spleen was not palpable. Slight hypesthesia of the feet was present. The laboratory data are summarized below the previous data in Table I.

The final pertinent diagnoses were: Laennec's cirrhosis, portal hypertension, esophageal varices, and chronic alcoholism.

Isolation of urocanic acid from urine of Subject 17. Urine was collected for 24 hours after the ingestion of a loading dose of histidine and placed in storage at −10° C. The urine was collected on day 12 (Figure 2). The total volume was 634 ml, of which 350 ml was available for the attempt at isolation.

An aliquant of urine containing 83 μmoles of UA (biological assay) was passed through a column (3.5 × 20.0 cm) of Dowex-50H. The resin was washed with 700 ml of H_2O and 1 L of 1.1 N sulfuric acid, and the activity was eluted with 1.95 N sulfuric acid. Fractions of 100 ml were collected, and the activity in each was determined by bioassay in samples freed of sulfate with barium carbonate. Fractions 11 through 18, containing 63.8 μmoles of UA, were combined and freed of sulfate with barium carbonate and barium hydroxide. The sulfate-free solution was passed through 15 ml of Dowex-50H packed in a 100-ml buret. The resin was washed with 100 ml of 0.55 N sulfuric acid followed by 100 ml of 1.1 N sulfuric acid. A gradient was developed by passing 2.78 N sulfuric acid through 90 ml of H_2O, and 10-ml fractions were collected. Each fraction was examined spectrophotometrically (ultraviolet) for UA. The active fractions (30 through 40) were combined, freed of sulfate with barium carbonate and adjusted to pH 7.0 with barium hydroxide.

The combined fractions were passed through 9.5 ml Dowex-1-acetate contained in a 100-ml buret. Normal acetic acid passed through a head of 45 ml of water was used to elute the UA from the resin. Five-ml fractions were collected and examined spectrophotometrically. The UA (32 μmoles; 6.3 mg as dihydrate) was found in fractions 7 through 11. These fractions were combined, concentrated to 1.0 ml, and stored for 24 hours at −10° C. Upon thawing, a white crystalline precipitate settled out. It was collected on a sintered glass funnel, and dried *in vacuo*. The yield was 4.0 mg.

The ultraviolet absorption spectra of the isolated sample were consistent with those of UA. In 0.1 M phosphate buffer, pH 7.2, maximal absorption occurred at 278 mμ; in 0.01 M hydrochloric acid at 268 mμ; in 0.01 M potassium hydroxide at 278 mμ; and in 6 M potassium hydroxide at 308 mμ. Calculated as the dihydrate of UA, the molecular extinction coefficient was found to be 17,400 (0.01 M phosphate buffer, pH 7.2 at 278 mμ). Mehler and Tabor (22) have reported 18,800 at 277 mμ. The isolated sample was then recrystallized from H_2O and melting-point determinations made with a Kofler microapparatus. The following melting points were found: synthetic UA·$2H_2O$,[2] 230 to 231° C, isolated material 229 to 230° C. The addition of the isolated material to the synthetic compound gave no depression of the melting point.

The infrared absorption spectra (Nujol mull) of the recrystallized isolated material and synthetic UA·$2H_2O$ were identical (Figure 4). On Schleicher and Schuell paper (no. 598) the isolated material and synthetic sample moved at essentially the same rate in three solvent systems: *1*) η-butanol: η-propanol: 1.0 M hydrochloric acid (2:1:1), synthetic Rf 0.53, isolated 0.54; *2*) η-butanol: H_2O: 1.0 M acetic acid (4:1:5), synthetic Rf 0.51,

[2] California Corporation for Biochemical Research, Los Angeles, Calif.

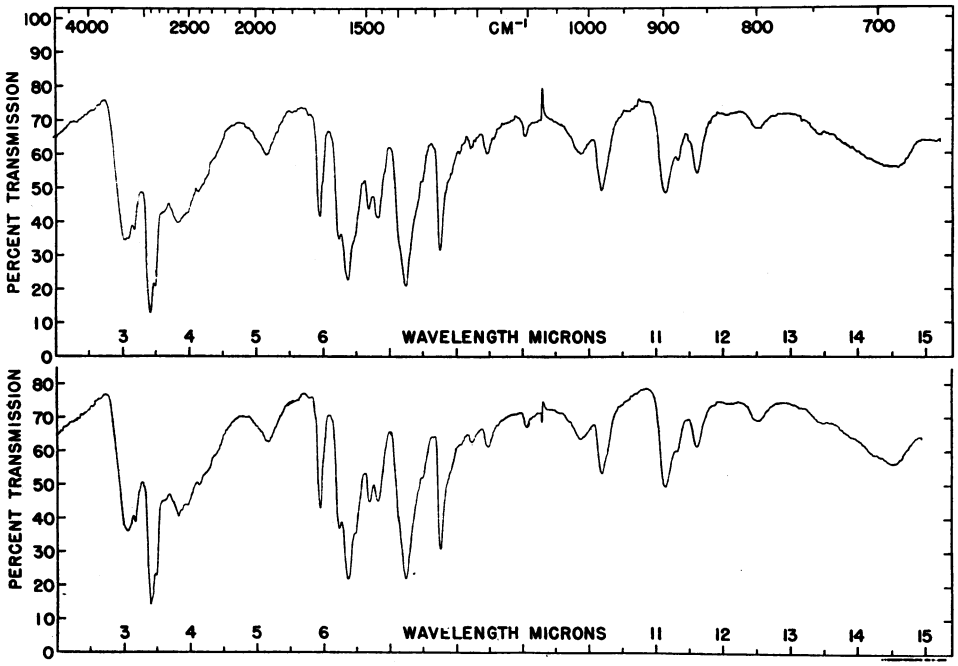

FIG. 4. SUBJECT 17; INFRARED ABSORPTION SPECTRA. Upper curve is the isolated material, lower curve is that obtained with synthetic UA·$2H_2O$.

isolated 0.51; and *3*) η-butanol: H_2O: ammonium hydroxide (28 M ammonia) (4:3:1), synthetic Rf 0.13, isolated 0.12.

Demonstration by chromatography of the presence of UA in urine. Subject 18 excreted 65 μmoles of UA (bioassay) in a 24-hour, 3,000-ml urine sample. An aliquot of urine, 22 ml, was passed through 9.5 ml of Dowex-1-acetate. The resin was washed with 15 ml of H_2O and eluted with 40 ml of 0.5 M acetic acid. The eluate was concentrated to dryness *in vacuo,* the residual solids extracted with 1 ml H_2O, and the extract stored 24 hours at −10° C. After thawing, the insoluble materials were rejected by filtration. The filtrate was acidified with 2 drops of 2.0 M hydrochloric acid and extracted five times with 10-ml portions of butanol. The butanol extracts were combined, evaporated to dryness, and the residue dissolved in 1 ml H_2O. The final extract, synthetic UA dihydrate, and a mixture of the two were chromatographed on Schleicher and Schuell no. 470 paper for 18 hours at 23° C. The spots were detected by spraying the dried paper with diazotized sulfanilic acid. The results are shown in Table II.

TABLE II

R_f values of isolated material from Subject 18 compared with synthetic UA

Solvent system	A Synthetic urocanic acid	B Isolated material	Mixture of A and B
BuOH, PrOH, HCl	0.48 (0.22)*	0.48 (0.21)	0.48 (0.22)
BuOH, H_2O, HAc	0.54	0.53	0.54
BuOH, H_2O, NH_4OH	0.12	0.10	0.11

* Minor spot seen in BuOH, PrOH, HCl.

RESULTS

The excretion of UA and FiGlu in the three groups of patients during control periods and after oral histidine is seen in Table III. The UA and FiGlu excretion designated as 17a, 17b, 19a, and 19b correspond in time to the same periods so designated in Table I. The mean levels of excretion and their standard errors are seen in Table IV. A statistical examination of the results obtained in these subjects was of interest (23). The grouping of the patients was performed before we knew the urinary content of UA and FiGlu.

The patients in group B, with an assortment of diseases, had little evidence of liver dysfunction (Table I). The excretion of UA, FiGlu, and the combined total of these components in groups A and B did not differ significantly during the control period, after loading with histidine, or expressed as a change in excretion due to histidine. The baseline excretion of UA, 5.2 ± 1.9 μmoles

TABLE III

Individual urine content of FiGlu and UA before and after oral histidine

Group	Subj. No.	Control*			Histidine			Difference		
		UA	FiGlu	Total†	UA	FiGlu	Total	UA	FiGlu	Total
A	1	0	0	0	12	0	12	12	0	12
	2	0	4	4	6	38	44	6	34	40
	3	12	12	24	21	32	53	9	20	29
	4	5	10	15	11	25	36	6	15	21
	5	5	10	15	14	21	35	9	11	20
	6	5	7	12	6	12	18	1	5	6
B	7	15	22	37	9	38	47	−6	16	10
	8	3	11	14	3	26	29	0	15	15
	9	0	0	0	0	8	8	0	8	8
	10	3	8	11	23	51	74	20	43	63
	11	0	0	0	0	28	28	0	28	28
	12	3	9	12	7	23	30	4	14	18
	13	0	0	0	0	4	4	0	4	4
	14	3	2	5	3	6	9	0	4	4
	15	8	26	34						
	16	17	23	40						
C	17a	4	2	6	158	42	200	154	40	194
	17b	7	9	16	36	88	124	29	79	108
	18	16	3	19	64	19	83	48	16	64
	19a	5	27	31	17	639	656	12	612	624
	19b	9	19	28	34	41	75	25	22	47
	20	8	24	32	25	46	71	17	22	39
	21	28	149	177	76	490	566	48	341	389
	22	0	65	65	0	167	167	0	102	102
	23	1	20	21	10	54	64	9	34	43
	24	19	12	31	19	34	53	0	22	22
	25	17	39	56	31	187	218	14	148	162

* All values given micromoles per 24-hour urine.
† Sum of FiGlu and UA.

per day, was not significantly greater than that of the normal controls, 4.5 ± 1.8 μmoles per day, $p > 0.35$ (one-tailed t test). The mean excretion of FiGlu during the control period by group B patients was 10.1 ± 3.2 μmoles per day, compared with 7.2 ± 1.8 for the group A patients, $p < 0.10$. It is noteworthy that two of the three patients in this group with significantly elevated FiGlu excretion had clinically evident malnutrition. After administration of histidine, the mean excretion of UA among the normal group was 11.7 ± 2.3 μmoles per day in contrast to a mean excretion of only 5.6 ± 2.8 in group B, $p < 0.10$. This difference is in the opposite direction from the expected value. The mean excretions of FiGlu after histidine administration in groups A and B were nearly equal, 21.3 ± 5.6 and 23 ± 5.8 μmoles per day, respectively, $p > 0.7$.

The nine patients in group C had laboratory evidence of significant liver dysfunction (Table I). Jaundice was present in four patients, impaired sulfobromophthalein excretion in five, abnormal cephalin flocculation in five, and elevated serum glutamic oxalacetic transaminase values in four

TABLE IV

Means and standard errors of the urine content of FiGlu and UA before and after oral histidine in the three groups of patients

Group	Control*			After histidine			Difference		
	UA	FiGlu	Total†	UA	FiGlu	Total	UA	FiGlu	Total
A	4.5±1.8	7.2± 1.8	11.7± 3.5	11.7± 2.3	21.3± 5.6	33.0± 6.3	7.2± 1.5	14.2± 4.9	21.3± 5.0
B	5.2±1.9	10.1± 3.2	15.3± 5.0	5.6± 2.8	23.0± 5.8	28.6± 8.3	2.2± 2.6	16.5± 5.7	18.8± 6.9
C	10.9±3.2	37.9±15.3	48.8±17.1	44.4±16.4	186 ±75.0	231 ±75.0	33.6±16.2	149 ±67.0	182 ±67.0

* All values given as micromoles per 24-hour urine.
† Sum of FiGlu and UA.

patients. Depression of serum albumin levels with reversal of the albumin:globulin ratio occurred in six patients and prothrombin concentrations below 90 per cent in five patients. Eight of the nine patients in group C excreted in the control period either UA or FiGlu in excess of the largest amount of corresponding histidine metabolite in group A. The mean UA excretion during the control period was 10.9 ± 3.2 μmoles per day, which was significantly greater than that of group A ($p < 0.05$) but not group B ($p < 0.10$). Similarly, the mean excretion of FiGlu in the control period in group C, 37.9 ± 15.3 μmoles per day, was significantly greater than that in group A ($p < 0.05$) but not group B ($p < 0.10$). In six of the patients in group C, FiGlu was excreted in excess of UA (Table III) during the control period. In five of these, FiGlu was still the major metabolite excreted after histidine loading. Thus histidine loading did not alter the relative rates of UA and FiGlu excretion, despite the fact that it greatly enhanced the excretion of both metabolites. In only Patient 17 was the loading with histidine necessary to bring out a defect in both UA and FiGlu excretion. In this patient the excretion of UA after histidine was the highest in the series, 158 μmoles per day, and UA excretion was approximately four times that of FiGlu when the patient was first studied. After clinical improvement, UA excretion after histidine loading fell below that of FiGlu whereas FiGlu excretion increased; but both were still abnormally elevated with histidine loading. In Patient 18 the excretion of UA was minimally elevated during the control period (16 μmoles per day), and upon histidine loading the UA excretion rose to 64 μmoles per day, whereas FiGlu excretion remained normal, increasing from 3 to 19 μmoles with histidine administration. In this and the previous patient the clinical status suggested a concomitant nutritional deficiency, including that of folic acid and iron. These were the only two instances in which UA excretion exceeded that of FiGlu after a histidine load. In the remainder of the subjects the control period output of either UA, or FiGlu, or both exceeded the mean normal excretion by more than 2 SD.

After histidine loading (Table IV) group C excreted significantly greater amounts of UA or FiGlu than either group A or B ($p < 0.05$). The difference in excretions due to a histidine load was similarly increased in group C ($p < 0.05$). When the combined excretions of UA and FiGlu after histidine or the combined difference due to histidine in group C are compared with group A or B, a significant increase is again noted ($p < 0.025$).

The two patients described in detail (17 and 19) were studied at length. Their excretions of UA and FiGlu are seen in Figures 2 and 3. Subject 17 showed a rapid increase in her ability to metabolize UA. This coincided with her clinical improvement during hospitalization while she was on a regular hospital diet. On days 44 to 46 the patient's ability to respond normally to an oral histidine load was almost complete. The daily excretion of UA and FiGlu was followed for 18 days. An indication of the consistency of the data obtained from the urinary excretion of these metabolites with and without the administration of oral histidine is seen in Figure 2.

Subject 19 (Figure 3) had severe Laennec's cirrhosis. The impairment in degradation of FiGlu was severe, however; there was only slight impairment in the ability of this patient to metabolize UA. During a prolonged evaluation there was gradual improvement in the ability to metabolize both of these products. Methionine had no apparent effect on the patient's ability to metabolize FiGlu or UA. After what was considered maximal clinical improvement, 30 μg vitamin B_{12} was given intramuscularly, followed by 30 mg oral folic acid in divided doses for 7 days. The ability to metabolize an oral dose of histidine seemed to increase slightly. The UA excretion was unchanged. Subjects 21, 22, and 24 showed a similar pattern of improvement during hospitalization while on a regular diet, with a decrease in FiGlu excretion after an oral histidine load.

Subject 24, who had equivocally elevated UA excretion before histidine, had severe liver dysfunction due to serum hepatitis but was not symptomatically ill and maintained a good appetite with an excellent food intake. He responded to oral histidine with less than 65 μmoles combined excretion of FiGlu and UA. While such a result is not recognizably abnormal, his response fell from 53 to 36 μmoles per 24-hour urine 16 days later, at the time that laboratory evidence of liver dysfunction was rapidly abating.

In Figure 5 the UA and FiGlu excretion rates after histidine are plotted in four time-periods. Most of the UA and FiGlu appear during the first 12 hours as a result of the oral histidine load given at the beginning of the urine collection. It is interesting to note that the peak of UA excretion occurs before that of FiGlu. One would expect this result from a knowledge of the metabolic pathway of histidine as outlined in Figure 1. Actually, most of the increased excretion of these metabolites seems to occur during the first 6 hours after an oral histidine load of 3.0 g.

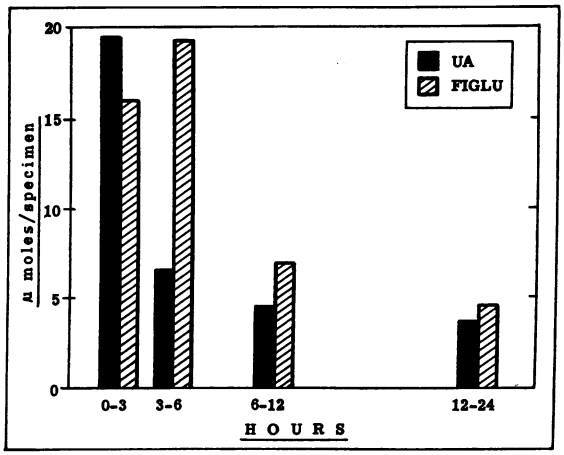

FIG. 5. SUBJECT 17; FIGLU AND UA EXCRETION RATES AFTER ADMINISTRATION OF 3.0 G OF HISTIDINE PLOTTED AS MICROMOLES PER URINE SPECIMEN. The data were obtained on day 256 of follow-up (see Figure 2).

DISCUSSION

The fact that urocanic acid is an intermediate in the mammalian degradation of histidine (Figure 1) seems well substantiated (2). The existence of a similar pathway has been presumed in man. While the excretion of α-formiminoglutamic acid, another metabolite of histidine, is known as a breakdown product of histidine in man and is known to be increased after oral histidine loading of patients who have folic acid deficiency (11, 15), UA had not been definitively identified before the initial report of this work (5). The isolation and identification of UA, presented herein, leaves no doubt of its presence in man, documenting further the existence in man of the degradative pathway of histidine metabolism shown in Figure 1. In this sequence of reactions the ring carbon-2 of histidine becomes the formimino group of FiGlu via UA. The formimino group is then transferred to tetrahydrofolic acid, a reaction catalyzed by formiminoglutamic acid transferase, ultimately to enter the pool of metabolically active one-carbon fragments as a derivative of N-10-formyltetrahydrofolic acid. The excretion of FiGlu in folic acid deficiency (11, 15), pernicious anemia (15, 16), or folic acid antimetabolite therapy (13, 21) is believed to be due to a lack of tetrahydrofolic acid receptor for the formimino group.

Liver disease was simultaneously reported in abstracts by Carter and colleagues (17), and by the present authors (5) as an additional clinical condition in which FiGlu excretion is increased after an oral histidine load. It should be noted that Daft (24) had previously indicated that FiGlu excretion might serve as an index of liver function. In the present study, laboratory evidence of liver disease correlates well with an impaired ability to metabolize histidine. In view of the known relationship between folic acid deficiency and FiGlu excretion, and previous observations indicating that some cirrhotic patients may be deficient in folic acid (24–26), it is reasonable to conclude that folic acid deficiency may also play a role in the excretion of FiGlu in the patients reported herein. That two patients in group B who excreted larger amounts of FiGlu were undernourished, and the response to an adequate diet in five patients (nos. 17, 19, 21, 22, and 24) would bear this out. Nevertheless, the fact that restoration of adequate nutrition did not result in a complete return to normal of FiGlu excretion in patients in this study or in that of Jandl and Lear, and Carter and colleagues (25, 26) suggests that liver dysfunction of sufficient degree per se may interfere with the formiminoglutamic acid transferase reaction. Although the lack of marked FiGlu excretion despite profoundly abnormal liver function tests in serum hepatitis also suggests that malnutrition may play a significant role in the abnormal excretion of FiGlu in cirrhosis, the basic differences in the two pathologic processes and the chronicity of cirrhosis make this conclusion tenuous at best.

The low UA excretion in the patients of group B after histidine loading was unexpected and unexplained. The most likely explanation is that it is due to chance, in view of the small number of patients. Another possibility is that the group

as a whole was in negative nitrogen balance, and an increased fraction of the administered histidine was utilized anabolically. If this were so, one would expect FiGlu excretion to be lower also, which was not the case.

In view of the observations in rats that methionine abolishes the abnormal FiGlu excretion due to folic acid or cyanocobalamin deficiency, and increases the utilization of histidine ring carbon-2 (14, 27), methionine was fed to Subject 19. Since the excretion of FiGlu did not change significantly even after histidine administration, one might present this as evidence against an increased FiGlu excretion caused by deficiency of folic acid or vitamin B_{12}. Such reasoning is highly speculative, however, since the cause of the "methionine effect" is poorly understood.

The interesting role played by an increased UA excretion in patients subjected to a metabolic load of histidine concurrent with liver dysfunction and probable folic acid deficiency is difficult to reconcile in view of the absence of known effects of folic acid on this portion of the metabolic pathway. If this were due to a buildup of metabolic products along the pathway as a consequence of folic acid deficiency, then a predominance of FiGlu would be expected. This did not occur in the patients who had a marked UA excretion (nos. 17 and 18). Two independent observations of UA excretion in patients with severe liver dysfunction have been recently reported (8, 9). In the first, a patient in hepatic coma is reported to have UA in the urine. No other clinical information is given. In the second, a series of patients with kwashiorkor is reported. Such patients have severe protein depletion and an apparent secondary liver dysfunction. No hematological findings are reported. One might also expect a folic acid deficiency to be present. The question arises: Does UA excretion represent a lack of the enzyme urocanase due to liver disease or is there some as yet unknown cofactor necessary for the metabolism of this product? Again, the multiplicity of etiologic and pathologic factors in cirrhosis and kwashiorkor makes answering these questions difficult. The observation that the UA excretion decreased, but not to normal levels, in Patients 17 and 19 suggests that both factors—liver damage and specific cofactor deficiency—may be involved. The improvement in both UA and FiGlu excretion might well be a result simply of reversal of fatty infiltration which accompanied better nutrition and abstinence from alcohol.

The presence of multiple variables operating in this series of patients indicates that the mechanism underlying these defects in histidine metabolism will require correlation of the excretion of these metabolic products with liver function and biopsy studies in a wider variety of liver diseases if, indeed, the mechanism can be elucidated in man at all. In the meantime, the correlations and responses to therapy shown in this small series offer some hope that excretion of urocanic acid and formiminoglutamic acid as parameters of liver function may have some prognostic, if not diagnostic, value.

SUMMARY

Urocanic acid (UA) has been definitively identified as a urinary metabolite in man. Methodologies for the quantification of UA and α-formiminoglutamic acid (FiGlu) in human urine are described. Both UA and FiGlu excretion are increased after oral histidine loading in normal control subjects and in patients with hepatocellular disease. The response to loading in patients with hepatocellular disease is exaggerated. The complexity of the pathophysiological process in patients with liver disease is such that conclusions relative to the roles of folic acid deficiency, hepatocellular damage, and decreased enzyme activities in increasing UA and FiGlu excretions are speculative. The daily excretions of UA and FiGlu with and without oral histidine are reported in two subjects in detail.

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