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Research Article

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Evidence that Histidine is an Essential Amino Acid in Normal and Chronically Uremic Man

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ABSTRACT The requirement for dietary histidine was investigated in four normal and three chronically uremic men. Subjects lived in a metabolic unit where they were fed three isonitrogenous diets in the following order: a 40-g protein diet (28±SD 8 days), a semisynthetic amino acid diet deficient in histidine (35±2 days), and an amino acid diet which contained histidine (31±5 days). With ingestion of the histidine-deficient diet, nitrogen balance gradually became negative, and serum albumin decreased in six subjects. Plasma histidine fell by 82±6%; muscle histidine decreased by 62± 19%; the hematocrit fell by 25±9%; and serum iron rose. Subjects felt unwell, and in five cases a skin lesion consisting of fine scales, dry skin, and mild erythema developed. After administration of the histidine-repletion diet, nitrogen balance became positive in six subjects; serum albumin increased in five cases; plasma and muscle histidine rose; serum iron fell abruptly; a reticulocytosis ensued; and the hematocrit rose. The clinical symptoms and skin lesions disappeared. These observations indicate that histidine is an essential amino acid in normal and chronically uremic man. The absence of dietary histidine is associated with failure of normal erythropoiesis.

INTRODUCTION

Although histidine is considered to be an essential amino acid for the human infant and several species of animals (1–4), studies have failed to establish a need for exogenous histidine in older children and adult humans (5–10). However, the demonstration that histidine is not an essential amino acid in these latter subjects has been largely based on nitrogen balance studies which were often of short duration. Recently, two laboratories have reported that histidine is an essential amino acid in adults suffering from chronic renal failure (11, 12). Because of these latter observations, we decided to reassess the need for dietary histidine and to compare the metabolic response to deletion of histidine from the diet in normal and chronically uremic men. The results indicate that histidine is an essential amino acid for both the normal and chronically uremic adult male. The metabolic response to ingestion of a histidine-deficient diet in the normal and chronically uremic man is similar.

METHODS

Subjects. Seven subjects, four adult men with no evidence of renal disease and three clinically stable patients with advanced chronic renal failure, were studied in a metabolic unit. Characteristics of each subject are given in Table I. The chronically uremic patients had been azotemic (serum creatinine > 4.0 mg/100 ml) for a mean period of 27 mo (range, 10–37 mo).

Diets. Subjects were fed, initially, a diet consisting of ordinary foods which provided 40 g of primarily high quality protein for 28±SD 8 days. They then received for 35±2 days an essentially isonitrogenous, semisynthetic diet in which approximately 92% of the nitrogen was provided as free L-amino acids in the proportions present in egg, but which contained only 60 mg of histidine. Finally, for 31±5 days, six subjects received a diet identical in calorie and mineral content to the second diet. This third diet contained 1,210 mg of histidine, and the content of each of the other amino acids was reduced proportionally so that the nitrogen content of this diet was the same as the histidine-deficient diet. In the seventh subject, a chronically uremic patient, the third diet provided only 590 mg/day of histidine. He ingested this diet for 4 days, left the metabolic unit for 3 days, and on his return was fed his original 40-g protein diet for 11 days.

The 40-g protein diet provided on an average 30 g/day as either egg or beef protein, 7.4 g/day of egg protein (range,

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TABLE I

Clinical Characteristics of Seven Subjects Receiving 40-g Protein, Histidine-Deficient, and Histidine-Repletion Diets

Subject	Age	Race*	Height	Weight‡	Creatinine clearance‡	Urea clearance‡	Medical problems
	yr		*cm*	*kg*	*ml/min*		
1	48	N	183	85.2	154	—	Idiopathic hypertrophic subaortic stenosis.
2	38	C	183	90.7	145	—	None.
3	39	C	190	97.9	140	—	Low back strain, mild.
4	24	C	168	56.2	115		Chronic bronchitis, mild. Intermittent asthma, in remission.
5	55	N	166	81.6	19.7	6.7	Nephrosclerosis. Essential hypertension, well controlled. Cerebral vascular accident 9 mo before study. Residual mild right hemiparesis.
6	54	C	183	72.6	8.9	4.5	Polycystic kidneys. Right nephrectomy 19 yr before study. Recurrent cerebral vascular accidents 8–18 yr before study with mild-moderate impairment of intellect but no paresis.
7	43	C	190	88.6	21.8	10.8	Interstitial nephritis. Hypertension, mild.

* N, Negro; C, Caucasian.

‡ Data at onset of study.

6.4–9.6), and 22.8 g/day of beef protein (range, 22.3–23.5). The amino acid diets provided 48–52 g of free crystalline L-amino acids. The amino acid content of the three diets, calculated from standard food tables (13) for the ordinary foodstuffs and confirmed by ion exchange chromatography for the amino acid mixtures, is shown in Table II.

The energy and mineral content of the diets is given in Table III. In the 40-g protein diet, calories were provided by a combination of ordinary foods high in calories and bread made from wheatstarch. In the amino acid diets, energy was provided primarily from high-calorie, low-nitrogen foodstuffs such as wheatstarch bread, margarine fat, and flavored drinks or puddings containing sucrose or a fat and carbohydrate supplement (Controlyte, Doyle Pharmaceutical Co., Minneapolis, Minn). The 40-g protein diet was usually supplemented with calcium lactate. Supplements of sodium, potassium, calcium, phosphorus, and magnesium were also given with the amino acid diets. A duplicate diet containing these supplemental salts was analyzed for mineral content every 2–4 wk (Table III). Subject 4 also received a daily supplement containing zinc, 15 mg; manganese, 4 mg; iodide, 0.1 mg; and copper, 2 mg, with the amino acid diets, and one-third this amount with the 40-g protein diet. In each subject both the 40-g protein and amino acid diets were supplemented with a multivitamin preparation containing vitamin A, 1.5 mg; vitamin D, 10 μg; thiamine hydrochloride, 1 mg; riboflavin, 1 mg; ascorbic acid, 60 mg; niacinamide, 10 mg; pyridoxine hydrochloride, 0.5 mg; *d*-pantothenyl alcohol, 3 mg; and cyanocobalamin, 3 μg. In addition, subjects received folic acid, 2 mg/day; and injections of vitamin K, 10 mg every 10 days, and vitamin B_{12}, 500–1,000 μg, at the end of the 40-g protein diet. Iron, 120 mg/day, was given as ferrous sulfate syrup divided in three doses and taken with meals throughout the study in subjects 2, 3, 4 and 7 and during the latter part of study in case 1.

Subjects were fed three meals per day and an evening snack. Amino acids and supplements of calcium, phosphorus, magnesium, and potassium were administered with each feeding.

Nitrogen balance and laboratory methods. Nitrogen balance studies were conducted according to previously described techniques (15). Stool collection periods, marked with brilliant blue, were generally of 5 days duration (range, 4–7). Duplicate diets prepared once weekly, vomited and rejected food, urine collected daily, and urine and feces pooled during each period were analyzed for nitrogen content. Nitrogen balances were calculated by subtracting fecal and urinary losses from net intake, i.e., the content in diet minus that in vomited and refused food. Balances were adjusted each period for changes in body urea content (15), but not for losses from skin, respiration, or blood sampling.

Blood was drawn between 8:00 a.m. and 8:30 a.m., after an 8–9-h fast, several times per week. The volume removed from each subject averaged 16±6 ml/day. At the end of study with each diet, a needle biopsy of the gastrocnemius muscle (16) was performed between 8:00 a.m. and 8:30 a.m. in fasting subjects for amino acid analysis. Amino acids were determined with a Beckman 121 Hp Amino Acid Analyzer (Beckman Instruments, Inc., Palo Alto, Calif.). Nitrogen was determined by the macro-Kjeldahl technique, as modified by Meeker and Wagner (17). Albumin was measured by the bromcresol green method (18) modified for use with the SMA 12 (Technicon Instruments Corp., Tarrytown, N. Y.). and standardized by protein electrophoresis. Urea, creatinine, phosphorous, iron and iron binding capacity were analyzed with an AutoAnalyzer (Technicon Instruments Corp.). Glutamic oxaloacetic transaminase, lactic acid dehydrogenase, alkaline phosphatase, and bilirubin were analyzed with the SMA 12. Sodium and potassium were determined by flame photometry with an internal lithium standard. Calcium and magnesium were determined by atomic absorption spectrophotometry (19). Hematocrits were measured in duplicate or triplicate with the use of capillary tubes or a Coulter counter, Coulter Electronics Inc., Hialeah, Fla. leukocytes

TABLE II

*Amino Acid Content of 40-g Protein, Histidine-Deficient, and Histidine-Repletion Diets**

L-amino acid	40 g protein	Histidine deficient		Histidine repletion‡	
		Foods	Plus crystalline a.a.	Foods	Plus crystalline a.a.
			g/day		
Essential					
Isoleucine	1.91±0.08§	0.07±0.00	3.32±0.10	0.07±0.02	3.25±0.11
Leucine	3.03±0.13	0.14±0.02	4.43±0.13	0.12±0.04	4.34±0.14
Lysine‖	2.93±0.11	0.11±0.01	3.24±0.10	0.10±0.03	3.16±0.11
Methionine	1.01±0.04	0.03±0.00	1.56±0.05	0.04±0.00	1.54±0.04
Phenylalanine	1.77±0.08	0.06±0.00	2.89±0.08	0.07±0.01	2.83±0.08
Threonine	1.71±0.08	0.07±0.00	2.50±0.08	0.07±0.01	2.45±0.08
Tryptophan	0.49±0.02	0.03±0.00	0.83±0.02	0.02±0.00	0.81±0.03
Valine	2.08±0.09	0.09±0.01	3.71±0.12	0.09±0.01	3.64±0.12
Half-cystine¶	0.54±0.04	0.02±0.00	1.17±0.04	0.03±0.00	1.15±0.03
Tyrosine¶	1.40±0.06	0.06±0.01	2.16±0.07	0.06±0.01	2.12±0.07
Nonessential					
Alanine	2.07±0.17	0.09±0.02	2.70±0.08	0.09±0.02	2.65±0.08
Arginine	2.27±0.10	0.07±0.00	3.27±0.10	0.09±0.03	3.23±0.09
Aspartic acid	3.64±0.32	0.19±0.04	3.62±0.10	0.22±0.06	3.58±0.09
Glutamic acid	5.86±0.19	0.35±0.05	6.39±0.20	0.41±0.10	6.32±0.18
Glycine	1.71±0.06	0.06±0.00	1.79±0.06	0.06±0.01	1.76±0.05
Histidine	1.12±0.05	0.06±0.01	0.06±0.01	0.06±0.01	1.21±0.04
Proline	1.64±0.08	0.11±0.01	2.19±0.07	0.11±0.02	2.14±0.07
Serine	1.88±0.11	0.09±0.00	4.19±0.13	0.10±0.01	4.12±0.12
Total	37.06	1.70	50.02	1.81	50.30

* Amino acid content of foods was calculated from standard tables (13); the content of the amino acid mixtures was confirmed by ion exchange chromatography.
‡ Data from subject 7, who received a 40-g protein diet, are omitted.
§ Mean±SD.
‖ Provided in the amino acid mixtures as lysine acetate; the numbers indicate the amount of lysine present in the nonsalt form.
¶ Semiessential amino acids.

TABLE III

*Calorie and Mineral Content of 40-g Protein, Histidine-Deficient, and Histidine-Repletion Diets**

	40 g protein	Histidine deficient	Histidine repletion
Calories, *kcal/day*	3,230±460‡	3,260±400	3,170±340
kcal/kg/day	39.8±3.5	40.4±5.4	39.4±5.7
Sodium, *mg/day*	2,800±1,380	2,540±870	2,680±1,610
Potassium, *mg/day*	2,090±320	1,980±190	2,030±220
Calcium, *mg/day*	830± (180–1,430)	980 (240–1,430)	1,030 (240–1,450)
Phosphorus, *mg/day*	700±60	920±120	900±110
Magnesium, *mg/day*	180±20	200±40	200±30

* Calorie intake was calculated from food tables (14); the mineral content of the diets was determined by chemical analysis (see text).
‡ The calorie and mineral content is represented as mean±SD except for calcium which is depicted as mean and range.

were counted with a Coulter counter; reticulocyte counts were performed with the new methylene blue N method (20); and platelets were counted manually. Statistical analyses were performed with the Student's *t* test, the paired *t* test, and by continuous linear regression analysis (21).

This study was approved by the VA Wadsworth Hospital Center Research and Education Committee, and conducted only after informed consent was obtained from each patient.

RESULTS

Nitrogen balance, serum albumin, and body weight. Nitrogen balance was often negative during the first few days of ingestion of the 40-g protein diet (Table IV), which may have been due to a higher protein intake before the study. However, balance became neutral or positive in each case and remained so except for case 5 who developed a mild respiratory infection during the last few days with this diet. When the histidine-deficient diet was instituted, nitrogen balance tended to become progressively less positive, and after 5–30 days it became negative in each subject. After histidine was added to the diet, balance became positive within 24 h in five of seven subjects and remained positive for the duration of study.

When case 2 ingested the histidine-repletion diet, nitrogen balance became transiently more positive, but remained negative throughout the study with this diet. Nitrogen balance also remained negative in case 7 during the first 4 days of this diet which provided only 590 mg/day of histidine. He was eating erratically during this period, and after day 8, when he was pre-

TABLE IV

Nitrogen Balance Data

	Normal subjects				Uremic subjects		
	1	2	3	4	5	6	7
	g nitrogen/day				*g nitrogen/day*		
40-g protein diet							
Intake	7.07	6.58	6.60	6.57	6.54	6.76	6.38
Corrected balance*	(5)‡ −0.71	(5) −3.09	(5) −1.81	(6) −0.61	(5) +0.03	(5) +0.66	(5) −0.78
	(6) +0.36	(5) +0.46	(5) −0.09	(5) +0.14	(5) +0.04	(5) −0.09	(5) −1.09
	(4) +1.31	(5) +0.71	(5) +0.34	(5) +0.98	(5) +0.94	(5) −0.42	(5) −0.57
	(4) +0.84	(5) +0.55	(5) +0.21	(5) +0.96	(5) −0.48	(5) +0.29	(5) +0.89
		(5) +1.17	(5) −0.23	(6) +0.92		(4) +0.05	(5) +1.00
		(5) +0.56	(6) 0.00	(4) +0.91			
		(5) +1.33		(3) +0.21			
		(7) +1.78					
Histidine-deficient diet							
Intake	6.80	6.50	6.54	6.32	7.09	6.64	6.48
Corrected balance*	(5) +0.16	(5) +0.27	(5) +1.01	(6) +0.11	(5) +0.26	(5) −0.06	(5) +0.92
	(5) +0.45	(5) −1.09	(5) +1.14	(6) +0.39	(5) +0.99	(5) −0.54	(5) +0.88
	(5) +0.13	(5) −0.21	(5) +1.59	(6) +0.14	(5) +0.46	(5) +0.21	(5) +0.21
	(5) +0.25	(5) −1.14	(5) +0.03	(6) +0.79	(5) −0.45	(5) +0.06	(5) +0.27
	(5) −0.08	(5) −1.05	(5) +0.19	(6) +0.22	(5) +0.05	(5) −0.02	(5) −0.08
	(5) −0.22	(3) −1.47	(5) −0.55	(6) −0.31	(5) −0.67	(5) +0.12	(6) −0.35
	(6) −0.90	(7) −1.32	(4) −0.29		(5) −0.47	(6) −0.39	
			(3) −0.46				
Histidine-repletion diet							
Intake	6.82	6.50	6.52	6.46	7.01	6.77	6.19
Corrected balance*	(5) +0.54	(5) −0.34	(5) +1.69	(6) +2.27	(5) +2.65	(5) +2.62	(4) −0.64§
	(5) +0.02	(5) −0.88	(5) +2.18	(6) +2.52	(5) +1.78	(5) +2.02	(4) —‖
	(5) +0.12	(5) −0.55	(5) +2.19	(6) +1.74	(5) +2.21	(5) +1.39	(3) +0.44¶
	(5) +0.46	(5) −0.87	(5) +1.64	(6) +1.91	(5) +1.49	(5) +1.22	(4) +0.89¶
	(5) +0.84	(5) −1.49	(5) +1.25		(5) +1.32	(6) +0.92	(3) +1.12¶
	(5) +0.18	(5) −1.78	(5) +0.59		(5) +1.87	(3) +1.35	
	(5) +0.47	(5) −1.82					

* Nitrogen balance is adjusted for changes in body urea content but not for unmeasured losses (see text).

‡ Parentheses indicate the number of days in each collection period.

§ The diet during this period provided only 590 mg of L-histidine/day (see text).

‖ Subject left hospital for 3 days and ate an unselected diet (see text).

¶ Subject ingested a 40-g protein diet (see text).

scribed 40 g of protein and ate his entire diet each day, balance became positive.

Nitrogen balance was calculated for each subject during the last 7–12 days of study with each diet when equilibration had been achieved. Mean nitrogen balance during this period was $+0.65\pm0.59$ g/day, -0.48 ± 0.44 g/day, and $+0.70\pm1.21$ g/day with the 40-g protein, histidine-deficient, and histidine-repletion diets, respectively (40-g protein vs. histidine-deficient diets, $P < 0.02$; histidine-deficient vs. histidine-repletion diets, $P < 0.01$).

In the three uremic subjects, serum urea nitrogen decreased during the 40-g protein diet, rose during the histidine-deficient diet, and fell with the histidine-repletion diet (Fig. 1). In addition, the urinary excretion of urea and total nitrogen increased in all seven subjects during the histidine-deficient diet and decreased with the histidine-repletion diet. Indeed, the fall in urinary excretion of both urea and total nitrogen occurred within 24 h of initiating the histidine-repletion diet in six of seven subjects. These changes cannot be attributed to alterations in renal function as the creatinine and urea clearances did not change in any subject during the study.

Serum albumin did not change during ingestion of the 40-g protein diet (Fig. 2). Albumin levels decreased in six subjects and were unchanged in one with the histidine-deficient diet, and rose in five subjects and were unchanged in two with the histidine-repletion diet. Mean serum albumin levels at the end of study with the three diets were 4.3 ± 0.4, 3.9 ± 0.4, and 4.3 ± 0.4 g/100 ml, respectively (histidine-deficient vs. 40-g protein or histidine-repletion diets, $P < 0.10$).

Mean body weight increased to 83.6 ± 14.2 kg with the 40-g protein diet ($P < 0.01$), was unchanged with the histidine-deficient diet (84.2 ± 14.8 kg, P: NS), and rose slightly to 85.5 ± 15.5 kg with the histidine-repletion diet ($P = 0.05$). The magnitude of the change in weight was similar in the normal and uremic subjects.

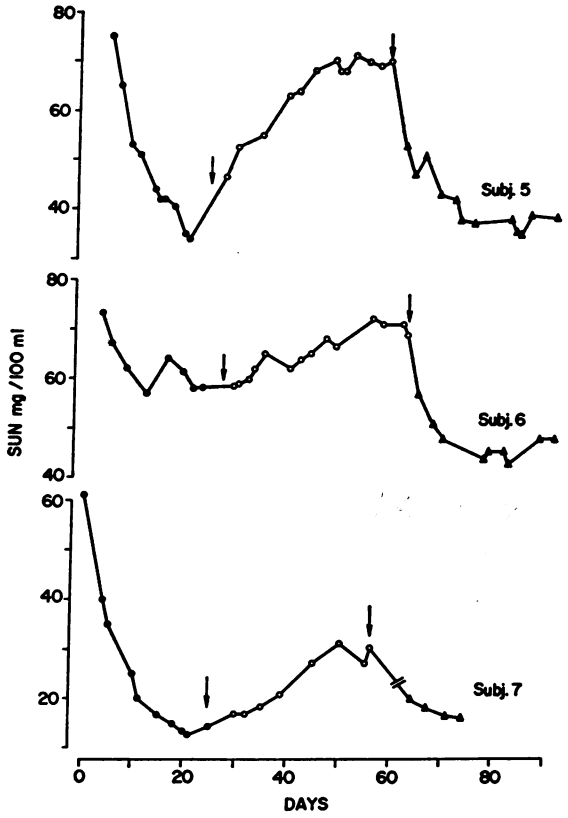

FIGURE 1 Changes in serum urea nitrogen (SUN) in three chronically uremic men fed 40-g protein (closed circles), histidine-deficient (open circles), and histidine-repletion diets (closed triangles). For each case, the first arrow indicates the time of onset of the histidine-depletion diet, and the second arrow, the beginning of the histidine-repletion diet. The break in the data of subject 7 indicates a period of 3 days during which he did not participate in the study (see text).

TABLE V

Plasma Histidine Levels during Ingestion of 40-g Protein, Histidine-Deficient, and Histidine-Repletion Diets

	40 g protein		Histidine deficient		Histidine repletion	
	Initial	Final	Initial	Final	Initial	Final
	μmol/liter		*μmol/liter*		*μmol/liter*	
Normal						
1	66 (4)*	73 (19)	34 (1)	10 (36)	24 (2)	57 (32)
2	69 (0)	60 (42)	30 (1)	10 (32)	21 (2)	74 (34)
3	84 (0)	68 (31)	28 (1)	10 (34)	10 (4)	70 (31)
4	70 (0)	72 (33)	14 (2)	12 (36)	16 (2)	23 (23)
Chronically uremic						
5	96 (1)	76 (23)	24 (5)	12 (35)	37 (3)	72 (33)
6	82 (2)	57 (27)	17 (2)	15 (35)	42 (5)	91 (27)
7	62 (7)	68 (24)	37 (1)	14 (29)	20 (1)	40 (17)
Mean	76	68	26	12	24	61
SD	12	6.9	8.5	2.0	11	23

* Parentheses indicate number of days subject had received the diet at the time of blood sampling.

Plasma and muscle histidine. The postabsorptive plasma histidine levels at the onset of the study were similar in the uremic and control subjects (Fig. 3, Table V). With the 40-g protein diet, mean plasma histidine levels fell slightly but not significantly. However, there was an inverse correlation between the initial plasma histidine values and the magnitude of its fall during this period ($r = -0.85$, $P < 0.02$). Within 23 h of initiation of the histidine-deficient diet, plasma histidine fell by $52\pm6\%$. Plasma histidine levels continued to decrease, and by the end of this diet, histidine levels had fallen to $17\pm2\%$ of the concentrations present with the 40-g protein diet. After the onset of the histidine-repletion diet, plasma histidine began to in-

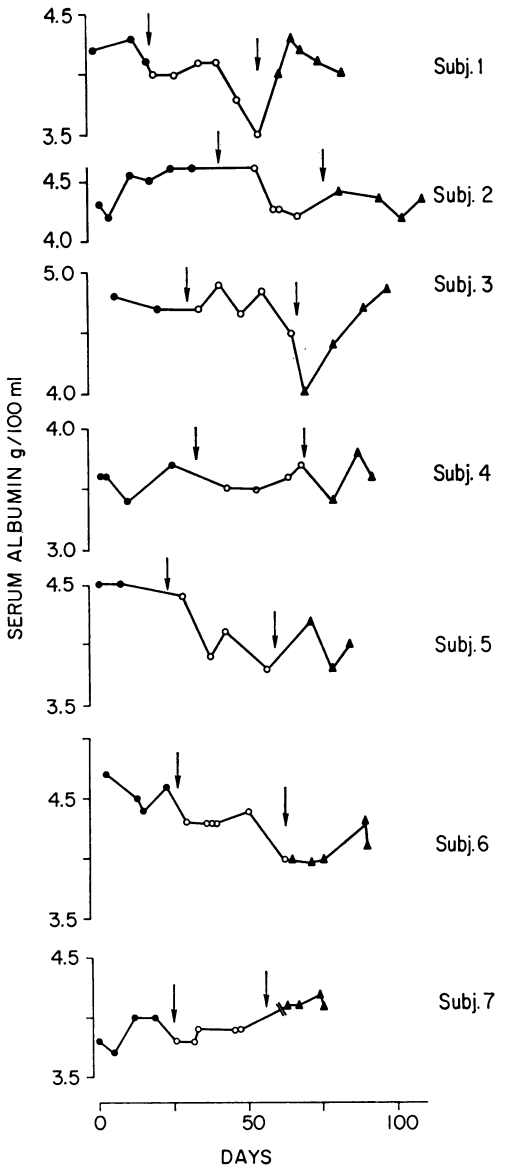

FIGURE 2 Serum albumin levels in four control men (subjects 1–4) and three chronically uremic patients (subjects 5–7) during ingestion of 40-g protein, histidine-deficient, and histidine-repletion diets. Symbols are defined in the legend for Fig. 1.

crease within 2–5 days in subjects 1, 2, 5, and 6, but in subjects 3, 4, and 7 there was no clear rise in plasma histidine until after 8–16 days (Fig. 3, Table V). By the termination of this diet, plasma histidine had increased to the levels present with the 40-g protein diet in five subjects and was still reduced in cases 4 and 7, who received the histidine-repletion diet for only 23 and 18 days, respectively.

Muscle histidine also decreased markedly with ingestion of the histidine-deficient diet, to 38±19% of the levels present with the 40-g protein diet (Table VI). With the histidine-repletion diet, muscle histidine increased to control levels. There was a direct correlation between the changes in muscle histidine with the three diets and the coincident changes in plasma histidine concentrations ($r = 0.80$, $P < 0.001$).

Hematology. Serial hematocrits, reticulocyte counts, and serum iron levels during the course of study are shown in Fig. 4. The hematocrit was 45.5±2.5% in the normal subjects and 38.8±5.8% in the uremic patients at the onset of study. With the 40-g protein diet, the hematocrit fell slightly to 41.6±3.1% in the normal subjects and 35.2±5.7% in the uremic patients. After starting the histidine-deficient diet, the hematocrit decreased more rapidly, and this fall was most marked during the last 3–9 days of this diet. At its termination, the hematocrit was 32.8±2.9% in the normal subjects and 23.9±4.8% in the uremic patients, a decrease of 24.8±9.0% when compared to the 40-g protein diet ($P < 0.001$).

After initiation of the histidine-repletion diet, the hematocrit often continued to decrease for 1–4 days; it then stabilized for 1–6 days; and then gradually and progressively rose. At the end of this diet, the hematocrit had increased to 38.8±4.1% in normal subjects and 31.2±5.1% in uremic patients. Moreover, in subjects 2, 3, 4, 6, and 7 the hematocrit was still rising when this diet was terminated. In subject 1, there was a transient decrease in hematocrit associated with decreasing serum iron levels. Iron therapy was started, and the hematocrit again rose.

The reticulocyte counts were relatively constant during the 40-g protein diet and were 1.5±0.9% and 1.4 ±0.5% at the beginning and end of this diet (Fig. 4). At the end of the histidine-deficient diet, the mean reticulocyte count was 1.8±0.7%. 2–6 days after instituting the histidine-repletion diet, the reticulocyte count began to increase and rose to a maximum of 5.2% (range, 3.3–8.6). Data concerning reticulocyte counts and serum iron levels for cases 5 and 6 are limited because the relationship between anemia and the histidine deficiency was not recognized early in the study.

During the 40-g protein diet, serum iron levels did not change and were 80±18 and 91±35 μg/100 ml at its onset and termination (Fig. 4). However, in each observed case, serum iron rose during administration of the histidine-deficient diet to a mean of 148±29 μg/100 ml ($P < 0.01$). With institution of the histidine-repletion diet, serum iron decreased abruptly to 106±41 μg/100 ml at 24 h and 57±14 μg/100 ml at 48 h. Serum iron then remained at approximately this level until the end of study, a value significantly less than with the histidine-deficient diet ($P < 0.001$). Iron binding

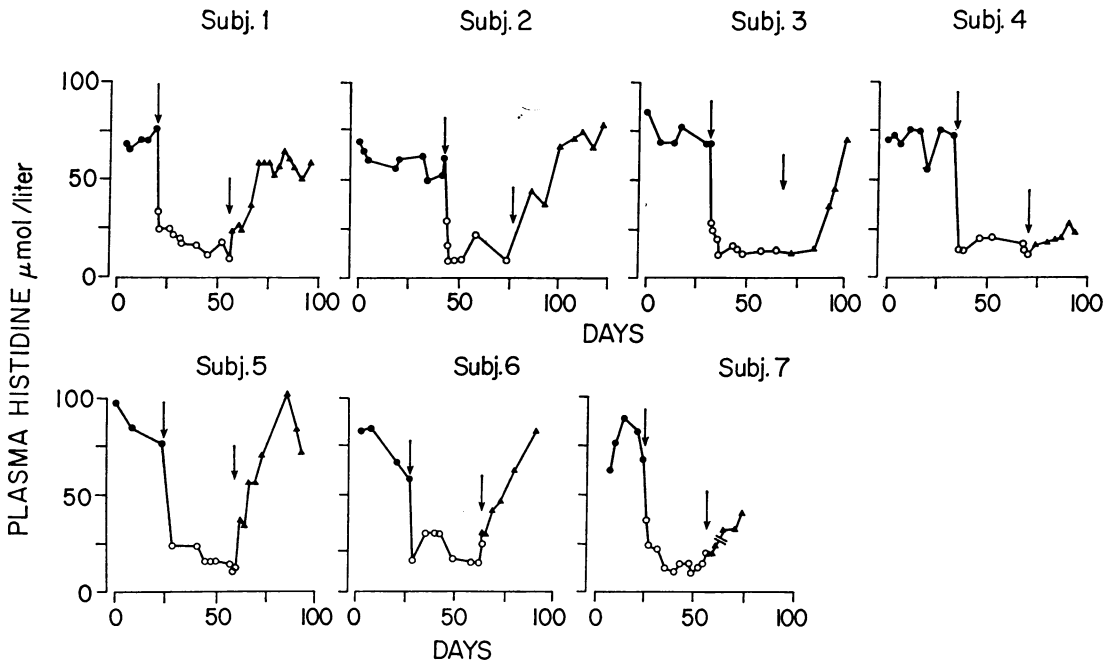

FIGURE 3 Plasma histidine levels in four control men and three chronically uremic patients during ingestion of 40-g protein, histidine-deficient, and histidine-repletion diets. Symbols are defined in the legend for Fig. 1.

capacity decreased from 349±70 to 288±66 μg/100 ml during the 40-g protein diet ($P < 0.01$), fell insignificantly to 272±30 μg/100 ml with the histidine-deficient diet, and rose to 334±60 μg/100 ml with the histidine-repletion diet ($P < 0.05$).

Platelet and leukocyte counts and serum glutamic oxaloacetic transaminase, lactic acid dehydrogenase, alkaline phosphatase, and bilirubin were not different with the 40-g protein, histidine-deficient, and histidine-repletion diets.

Clinical syndrome. A clinical syndrome of varying intensity developed in each case during ingestion of the histidine-deficient diet. Symptoms usually began subtly and progressed with time. Their precise times of onset and disappearance were often hard to identify. A sense of fatigue and lack of energy were generally the first symptoms to appear, and near the end of this diet subjects generally spent their days lying down or sitting at the bedside. Anorexia and nausea were also frequent findings, and three subjects had one or two episodes of vomiting. At the end of study with the histidine-deficient diet, a sense of depression or sadness was not uncommon. Irritability and occasionally hostility and anxiety also occurred. Four subjects complained of a slight loss of memory for recent events and decreased ability to concentrate or think clearly. They tended to forget within a day or two conversations they had or letters they had mailed.

Five subjects developed a dry, scaly skin eruption. The scales were fine, and the surrounding skin was often mildly erythematous. The lesion usually began either on the anterior legs in the shin area or on the volar surface of the hands and fingers. It was observed less commonly in a circumferential pattern on the abdomen and lower thorax and on the thighs, face, and arms. In addition, the skin on the volar aspects of the fingers and hands became dry and wrinkled and looked as if it had been soaked excessively in water.

After institution of the histidine-repletion diet, symptoms did not improve immediately and sometimes progressed for an additional 1–4 days. Improvement was very gradual, initially, and was usually first noted between 5 and 12 days after starting the latter diet. Despite definite improvement in every symptom with this diet, each subject reported that he did not feel completely normal until after the study was terminated and he was eating his ordinary food.

DISCUSSION

The findings of negative nitrogen balance, markedly decreased plasma and muscle histidine levels, lowered serum albumin concentrations, anemia, and clinical symptoms with the histidine-free diet, and the reversal of this syndrome with the addition of only histidine to the diet suggest that histidine is an essential amino acid for both normal and chronically uremic adults. It

TABLE VI

Muscle Histidine Levels after Ingestion of 40-g Protein, Histidine-Deficient, and Histidine-Repletion Diets

Subjects	40 g protein	Histidine deficient	Histidine repletion
		μmol/kg wet weight	
Normal			
1	210	120	170
2	150	90	180
3	190	—	230
4	312	89	162
Chronically uremic			
5	420	80	210
6	290	80	310
7	—	100	—
Mean	262	93	210
SD	99	15	55

Muscle histidine levels, 40-g protein diet vs. histidine-deficient diet, $P < 0.01$.

Muscle histidine levels, histidine-deficient diet vs. histidine-repletion diet, $P < 0.001$.

is pertinent that nitrogen balance was not adjusted for unmeasured losses of approximately 0.5 g/day from respiration, growth of integumentary structures, sweat, and blood sampling (22). With correction for these losses, the magnitude of the negative nitrogen balance with the histidine-deficient diet becomes even greater, while balances with the 40-g protein and histidine-repletion diets would be neutral.

The observation that nitrogen balance became negative with the histidine-deficient diet is in contrast to the reports of Rose et al. who found that nitrogen balance remained neutral or positive in young college-age men receiving diets devoid of histidine (5, 7–9); however, their studies were often of short duration. Albanese, Holt, Frankston, and Irby also found neutral nitrogen balance in three normal men studied for 36 days with a histidine-deficient diet, although each subject experienced progressive weight loss (6). Moreover, the histidine content of their diets can be calculated from food tables to exceed 200 mg/day (13), and two subjects received a dietary supplement of histidine for 2–4 days during the study. Since in the present study nitrogen balance only became negative after ingestion of the histidine-free diet for 5–30 days, it is not surprising that balance remained positive in these previous studies. The earlier investigations concerning the essentiality of histidine were largely limited to nitrogen balance techniques, and it is of interest that recently Anderson and Linkswiler observed a decrease in plasma histidine levels in healthy adults ingesting histidine-free diets (23). Moreover, several investigators have found that nitrogen balance is not maintained in healthy subjects fed diets containing the eight essential amino acids and one or more nonessential amino acids, but not histidine (24–26). These results were observed whether the essential amino acids were fed in amounts equal to or greater than the minimum requirements defined by Rose. Weller, Calloway, and Margen have suggested that this failure to achieve nitrogen balance may be due to the lack of dietary histidine (26).

The failure to attain positive nitrogen balance after initiation of the histidine-repletion diet in subject 2 is puzzling. He ingested 37.5 kcal/kg per day with this diet, which was somewhat lower than average, and it is possible that his energy requirement was greater than the other men. An inadequate energy intake may also explain the unusually rapid development of negative nitrogen balance with the histidine-deficient diet in this case. The amount of calories/kilogram body weight which could be offered to the subjects was limited by the difficulty with ingesting amino acid diets for relatively long periods of time, the anorexia associated with the histidine-deficient diet, and the large size of some subjects. Thus subjects received 39.9±5.3 kcal/kg per day with the amino acid diets, which is less than that used by Rose in his studies with college students who led a more active life (27). The adequacy of energy intake in the other six subjects in the present study was evidenced by their positive nitrogen balance at the onset of the histidine-deficient diet and during ingestion of the histidine-repletion diet.

It is not clear why nitrogen balance did not become negative until 15–30 days after ingestion of the histidine-deficient diet in most cases. Although plasma histidine levels fell rapidly, it is possible that the histidine pools at sites where protein is synthesized did not become critically depleted until much later. Nasset and Gatewood have suggested that preservation of histidine pools may occur through the release of histidine from breakdown of peptides and proteins, such as hemoglobin which is particularly rich in histidine (1). A decrease in hemoglobin formation probably began shortly after beginning the histidine-deficient diet (Fig. 4, *videinfra*), and this may have made more histidine available for other anabolic processes. Other adaptive responses that might have preserved critical histidine pools were a reduced rate of degradation (28) and decreased urinary excretion of histidine. After initiation of the histidine-repletion diet, both the rate of oxidation and the urinary excretion of histidine rose.

The mechanisms by which the lack of histidine caused the clinical syndrome with the histidine-free diet are obscure. Rose pointed out that anorexia, fatigue, and nervous irritability occurred in healthy subjects fed

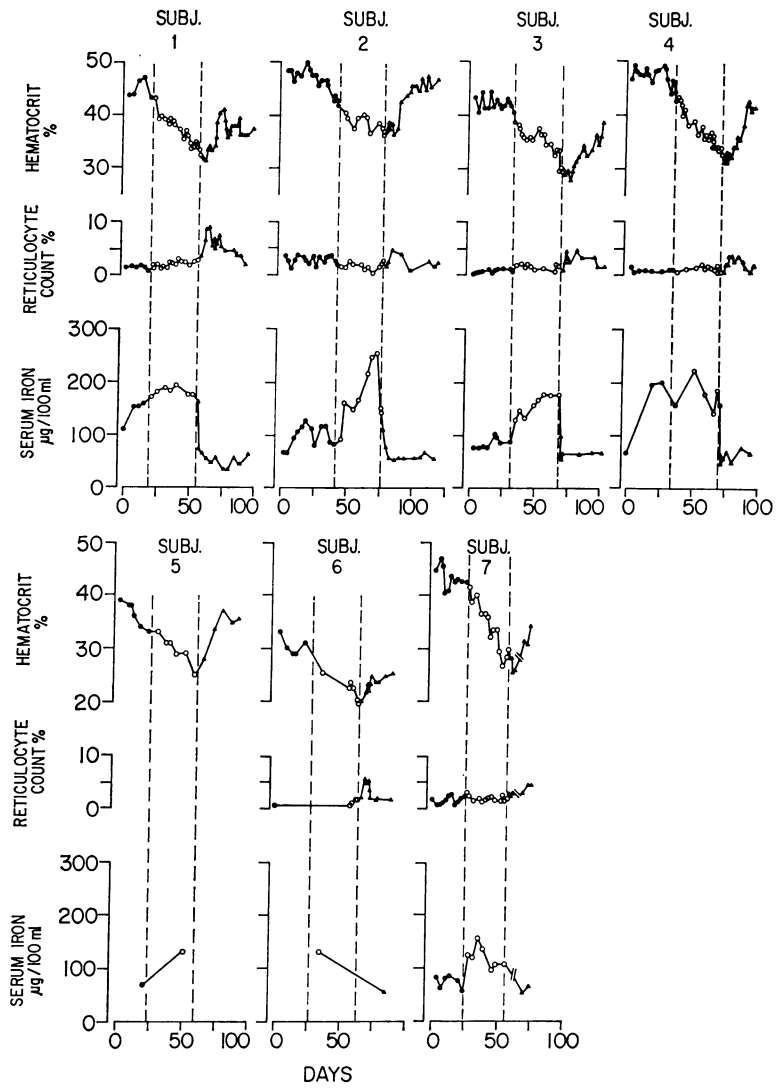

FIGURE 4 Serial hematocrits, reticulocyte counts, and serum iron levels in four control men and three chronically uremic patients during ingestion of 40-g protein, histidine-deficient, and histidine-repletion diets. For each case, the first and second broken vertical lines indicate the times of onset of the histidine-depletion and histidine-repletion diets, respectively. Other symbols are defined in the legend for Fig. 1.

diets deficient in any essential amino acid (27). Hence, the malaise, anorexia, and mental changes may represent a nonspecific reaction to the unavailability of any amino acid or nutrient for metabolic processes. Although subjects felt better after histidine was added to the diet, they did not feel completely normal until they had begun eating their ordinary food. This observation may indicate that the histidine-repletion diet was deficient in certain essential, possibly unknown, nutrients.

The skin eruption observed in several subjects in the present study has not been described with deficiencies of other essential amino acids. It is of interest that Snyderman, Boyer, Roitman, Holt, and Prose noted that infants fed histidine-deficient diets developed a skin lesion similar to that observed in the present study (2). They suggested that the eruption may be due to low histamine levels in skin, and this lesion may therefore represent a specific response to histidine deficiency. However, it is also possible that the failure to observe this lesion with diets deficient in other essential amino acids may reflect the shorter periods for which these latter diets have generally been studied.

The results of the present study clearly indicate that histidine deficiency can promote or intensify anemia.

Moreover, histidine deficiency appears to act by decreasing erythropoiesis. The observation that serum iron rose and the reticulocyte count remained low with the histidine-deficient diet (Fig. 4), and that serum iron fell and the reticulocyte count increased shortly after beginning histidine repletion supports this thesis. These findings confirm earlier observations that histidine may affect hemoglobin metabolism. Nasset and Gatewood have shown that adult rats fed histidine-deficient diets become anemic (1). In one of Rose's subjects ingesting a histidine-deficient diet, a slight decrease in the hemoglobin concentration was observed (7). In addition, Pinals, Harris, Frizzell, and Gerber have reported that in patients with rheumatoid arthritis, ingestion of supplemental histidine may improve anemia (29). Experimental evidence also supports the contention that histidine specifically promotes hemoglobin production. Sebrell and McDaniel have shown that dietary histidine is a particularly important amino acid for hemoglobin synthesis in weanling rats (30). Also, Giordano, De Santo, Rinaldi, De Pascale, and Pluvio have shown that the administration of histidine to uremic patients who were ingesting histidine-free diets increases [^{14}C]leucine uptake by reticulocytes (12). The mechanism by which histidine enhances erythropoiesis is not known.

The possibility that bleeding contributed to the anemia was excluded by periodic stool examinations for blood. Vitamin deficiency anemia also appears unlikely since subjects received vitamin supplements including folic acid and B_{12} throughout the study. Blood sampling could have added to the anemia and may have caused the slight fall in hematocrit with the 40-g protein diet. However, the volume of blood drawn each week was constant throughout the study with the three diets. Thus, it is not likely that the more rapid fall in the hematocrit with the histidine-depletion diet or its rise with the histidine-repletion diet could be explained by blood sampling.

It is of interest that the serum levels of glutamic oxaloacetic transaminase, lactic acid dehydrogenase, alkaline phosphatase, and bilirubin did not rise during ingestion of any of the three diets. These findings are in contrast to the observations of Kofrányi, Jekat, Brand, Hackenberg, and Hess who found elevated serum glutamic oxaloacetic transaminase and glutamic pyruvic transaminase in healthy subjects ingesting diets providing the eight classical essential amino acids in the proportions found in egg and potato protein and various sources of nonessential nitrogen (31). When the same diets were supplemented with histidine and arginine, the serum transaminases remained normal. The findings from the present study may indicate that the elevated transaminases found by Kofrányi and coworkers were not caused solely by the removal of histidine from the diet.

The observation that the response to the histidine-deficient and histidine-repletion diets were similar in the normal and uremic subjects provides some evidence that the metabolism of histidine is not altered in renal failure. In this regard, histidine may differ from such amino acids as phenylalanine or citrulline which are reported to be metabolized abnormally in uremia (32–34). However, Fürst has recently reported that after the administration of an ^{15}N tracer, histidine labeled with ^{15}N could be recovered from normal subjects but not from uremic patients (35).

The results of this study indicate that histidine is an essential amino acid for the healthy adult as it is for the patient with renal failure. It may therefore be of value to assess the daily dietary requirements for histidine and to determine whether the dietary intake of certain populations may be deficient in this amino acid. The foregoing observations also suggest that there may be value in ascertaining whether other types of anemia may respond to dietary supplementation with histidine.

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