Capacity of Human Subjects to Utilize Keto Analogues of Valine and Phenylalanine

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ABSTRACT Three adult human subjects were maintained for 7 days (period I) on a protein-free formula diet containing the minimum daily requirements of the eight essential amino acids plus 40 g glycine. During the last 5 days of this period, the average daily nitrogen balances for the three subjects were + 0.52, + 0.71, and + 0.30 g, respectively. During the next 7 days (period II), valine was withdrawn from the diet, and the glycine ration increased by an equimolar amount. During the last 5 days of period II, average daily nitrogen balances declined to − 1.82, − 1.61, and − 1.87 g, respectively. In the final period of 7 days (period III), the keto analogue of valine, α-ketoisovaleric acid, was added to the diet in a quantity equimolar to the minimum daily requirement of valine. During the last 5 days of this period, average daily nitrogen balances improved to − 0.02, − 0.18, and − 0.83 g, respectively. Analogous experiments in three subjects involved the withdrawal from the diet of phenylalanine (period II) and replacement by its keto analogue, phenylpyruvic acid (period III). The average daily nitrogen balances were as follows: period I: + 1.04, + 0.96, and +0.53 g; period II: − 1.45, − 1.83, and − 1.94 g; period III: + 0.07, + 0.11, and − 0.52 g.

The data demonstrate that man can convert α-ketoisovaleric acid and phenylpyruvic acid to the corresponding essential amino acids, valine and phenylalanine. The efficiency of these conversions is considerably less than 100%.

INTRODUCTION

In order to maintain nitrogen balance, normal adult human subjects require 250–1100 mg daily of each of eight essential amino acids in the L-configuration (threonine, valine, methionine, isoleucine, leucine, phenylalanine, lysine, and tryptophan) (Table I), plus about 2.6 g nitrogen supplied in the form of 15–30 g of glycine or other nonessential amino acids (1, 2). With the latter 2.6 g of nitrogen, and exogenous or endogenous carbon sources, the subject synthesizes the proper complements of nonessential amino acids. The requirement for "nonessential" nitrogen can be partially satisfied by ammonium salts or urea, a portion of which is converted to NH_3 and CO_2 by gastrointestinal microorganisms (2–8). It is believed that the uremic subject, in order to maintain nitrogen balance, requires the normal amounts of essential amino acids but less than the customary ration of nonessential nitrogen, because of his ability to draw upon the large pool of endogenous nitrogen circulating as urea (9).

In the diet of the growing rat, the essential amino acids can be replaced by their alpha keto acid analogues (Table II) without reduction in the animal's rate of growth (10–15). In this species, apparently, it is the carbon skeletons of these amino acids which are essential (i.e. which cannot be synthesized). Given the carbon skeleton, the rat by amination or transamination converts it to the essential amino acid. Transaminases capable of these conversions have been demonstrated in mammalian liver, kidney, and muscle (16, 17). So efficient is this process in the rat that the growth rate of young animals is not impaired by complete replacement of any of the essential amino acids (with the possible exception of arginine [18, 19], essential to the rat but not to man) by a molar equivalent quantity of the keto analogue (10–15).

If man also is capable of such conversions, it would be possible to meet the requirements for protein synthesis

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TABLE I

Minimum Daily Requirements (in Milligrams) of Adult Male Subjects for the Essential Amino Acids (1)

L-Threonine	500
L-Valine	800
L-Methionine	1100
L-Isoleucine	700
L-Leucine	1100
L-Phenylalanine	1100
L-Lysine	800
L-Tryptophane	250

in uremic subjects with a virtually nitrogen-free diet[1] containing the carbon skeletons of the eight essential amino acids in amounts equimolar to the daily requirements shown in Table I (18). Whether man possesses this metabolic capacity has not been previously investigated. The present study was undertaken, therefore, to learn whether human subjects are capable of utilizing the keto analogues of two of the essential amino acids, valine and phenylalanine.

METHODS

Four individuals, weighing 40–55 kg, who were undergoing daily physical therapy for chronic neurologic disability, were the subjects. Cases A (male, 50 yr), B (female, 43 yr), and C (male, 47 yr) had multiple sclerosis; case D (male, 52 yr) had transverse myelitis with residual paraparesis. The neurologic disease was inactive in each subject at the time of the study; hepatic, renal, and endocrine status were normal; body weight was in the range −10% to +10% of ideal body weight, calculated from the Metropolitan Life Insurance Tables (21).

Each experiment was conducted with an essentially protein-free formula diet (see below) and three successive 7-day periods. The first six experiments were designed as follows. During period I, the subject ingested the formula diet containing the eight essential amino acids in the quantities shown in Table I, plus 40 g glycine as the source of nitrogen for endogenous synthesis of nonessential amino acids; the effectiveness of such a regimen for maintaining nitrogen balance in human subjects had been demonstrated by Rose and Wixom (2). During period II, either valine or phenylalanine was removed from the formula, and an equimolar amount of glycine was added to maintain the nitrogen intake unchanged. During period III, an equimolar amount of the sodium salt of the keto analogue of L-valine (α-ketoisovaleric acid) or of L-phenylalanine (phenylpyruvic acid) was added. In the last two experiments, the design was: period I, complete diet minus valine (or phenylalanine) plus α-ketoisovaleric acid (or phenylpyruvic acid); period II, complete diet minus valine (or phenylalanine); period III, complete diet.

The "protein-free" formula diet[2] was made up as follows. Canned fruits and sucrose supplied the carbohydrate and butter was used for fat content. Cookies made from butter, sucrose, cornstarch, and water were added to the formula to prevent separation of the mixture. Ingredients were homogenized in an electric blender with enough water to give a final volume of 1000 ml. Flavor was varied by alternating between apricot, peach, pineapple, and strawberry as the fruit component. Each patient's daily formula was based on 35 cal/kg per day of ideal body weight, and had the following composition: volume, 1000 ml; 1.5–3 cal/ml; 104–160 g fat; 154–308 g carbohydrate; 190–220 mg nitrogen; 55–70 mg calcium; 50–70 mg phosphorus; 19–26 mg sodium; 482–528 mg potassium. Patients on the formula diet received the following mineral and vitamin supplements daily: calcium, 700–900 mg; phosphorus, 800–1100 mg; magnesium, 300–400 mg; sodium, 3000–4000 mg; potassium, 2000–2500 mg; chloride, 4000–6000 mg; iron, 8–15 mg; zinc, 12–18 mg; copper, 2–3 mg; iodine, 150–200 μg; manganese, 407 mg; and one Multicebrin capsule. The purified amino acids (all of the L-configuration) were purchased from Nutritional Biochemicals Corp.; α-ketoisovaleric acid and phenylpyruvic acid were obtained from Mann Research Labs. Each day's ration of amino acids and α-keto acid was added as a powder to 1000 ml of freshly prepared formula at 7:30 a.m. and dissolved by stirring. The mixture, which was stored at 5°C, was consumed by the patient in 200-ml portions at 8 a.m., 11 a.m., 2 p.m., 5 p.m., and 8 p.m.

Nitrogen balance of the subject was measured by the techniques of Reifenstein, Albright, and Wells (22). Successive 24-hr urine collections, pooled 5-day stool collections (obtained during the last 5 days of each period), and the daily diet were analyzed for nitrogen (23). The first 2 days of each 7-day period were considered to represent equilibration of the patient with the new diet,[3] and nitrogen balances were calculated from the data of the last 5 days of each period.

Concentrations of valine and phenylalanine in heparinized plasma were measured according to Perry and Hansen (24); a Beckman 120C analyzer was used. In experiments 1–8, fasting plasma was analyzed; in experiments 7 and 8, 2-hr postprandial plasmas (obtained at 10 a.m.) were also studied.

RESULTS

Experiments with valine and α-ketoisovaleric acid. The daily data for experiment 1 (case A) are given in Table III. During days 3–7 of period I (the first 2 days representing transition from the previous diet), the daily nitrogen balance averaged + 0.52 g. Since loss of nitrogen through the skin, which amounts to 0.5–1.0 g daily (25), was not included in the calculation of nitrogen

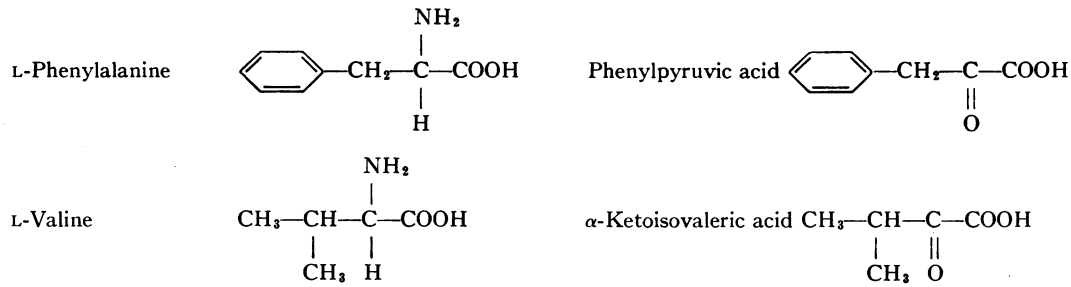
[1] Such a diet would theoretically be capable of maintaining nitrogen balance in the *uremic* subject if one considered the daily reduction in quantity of endogenous urea nitrogen to be the "nitrogen intake." The *normal* subject, while consuming a nitrogen-free diet, excretes daily 3–4 g nitrogen in the urine and 0.5–1.0 g nitrogen through the skin (20), and does not have a significant quantity of endogenous urea nitrogen available for protein synthesis. Therefore in normal individuals, a nitrogen-free diet based on keto acid analogues of essential amino acids would lead to daily negative nitrogen balance of 3.5–5 g.

[2] The nitrogen content indicated about 1.4 g protein in the daily formula.

[3] The data of Rose, Wixom, Lockhart, and Lambert (1), and our own measurements of nitrogen balance during days 1 and 2 of each period, which were made in every experiment although omitted from the tables, show that equilibration was achieved by day 3.

TABLE II

Structures of Phenylalanine, Valine, and Their α-Keto Analogues



balance, the actual balance was close to zero. Fasting plasma valine concentrations averaged 184 μmoles/liter, within the normal range (175–210 μmoles/liter) for this laboratory. During period II (valine removed from the diet with equimolar increase in glycine), the daily nitrogen balance became negative, averaging − 1.82 g for the last 5 days of the period ($P < 0.001$ for the difference between average nitrogen balance during period II and that during period I), and plasma valine concentration fell below the normal range to an average value of 114 μmoles/liter ($P < 0.01$ for the difference between this value and the corresponding value of period I). Plasma phenylalanine values, contrastingly, did not change significantly. Addition of α-ketoisovaleric acid

TABLE III

*Experiment 1 (Case A): Effect of Withdrawal of Valine, with Subsequent Replacement by α-Ketoisovaleric Acid, on the Nitrogen Balance and Fasting Plasma Concentrations of Valine and Phenylalanine**

Period	Day	N output		N balance	Fasting plasma concentrations		Diet
		Urine	Feces		Valine	Phenylalanine	
		g	g	g	μmoles/liter	μmoles/liter	
I	3	7.54	0.51	+0.35			
	4	6.98	0.51	+0.91			Complete; nine amino acids
	5	7.25	0.51	+0.64	166	56	
	6	7.98	0.51	−0.09	200	48	
	7	7.11	0.51	+0.78	185	65	
	Average ±SE			+0.52 ±0.18	184 ±10	56 ±5	
II	3	9.09	0.64	−1.33			
	4	9.79	0.64	−2.03			
	5	9.86	0.64	−2.10	128	67	No valine; eight amino acids
	6	10.20	0.64	−2.44	94	35	
	7	8.94	0.64	−1.18	120	60	
	Average ±SE			−1.82 ±0.25	114 ±11	54 ±10	
	P (II–I)‡			$0.001 > P$	$0.01 > P > 0.005$	$0.90 > P > 0.80$	
III	3	7.79	0.56	+0.05			
	4	7.39	0.56	+0.45			
	5	8.64	0.56	−0.80	150	49	α-Ketoisovaleric acid; eight amino acids
	6	8.33	0.56	−0.49	141	53	
	7	7.15	0.56	+0.69	159	45	
	Average ±SE			−0.02 ±0.28	150 ±5	59 ±4	
	P (III–II)			$0.001 > P$	$0.05 > P > 0.025$	$0.70 > P > 0.60$	
	P (III–I)			$0.20 > P > 0.10$	$0.05 > P > 0.025$	$0.40 > P > 0.30$	

* Nitrogen intake was 8.40 g daily.

‡ P value for difference between avearge daily N balance (or average plasma amino acid concentration) during period II and the corresponding average value during period I.

TABLE IV

Experiments 2–6: Effect of Substituting α-Ketoisovaleric Acid for Valine, or Phenylpyruvic Acid for Phenylalanine, on the Daily Nitrogen Balance and Fasting Plasma Concentrations of Valine and Phenylalanine

	N balance	Fasting plasma concentrations	
		Valine	Phenylalanine
	g	*μmoles/liter*	
Expt. 2			
(Case A)			
Period I (complete diet)	+1.04 ±0.15	190 ±16	51 ±10
Period II (complete diet − phenylalanine)	−1.45 ±0.29	180 ±14	49 ±7
Period III (complete diet − phenylalanine + phenylpyruvic acid)	+0.07 ±0.20	188 ±14	41 ±8
P values Period II − Period I	$0.001 > P$	$0.70 > P > 0.60$	$0.90 > P > 0.80$
Period III − Period II	$0.001 > P$	$0.80 > P > 0.70$	$0.50 > P > 0.40$
Period III − Period I	$0.005 > P > 0.001$	$0.40 > P > 0.30$	$0.50 > P > 0.40$
Expt. 3			
(Case B)			
Period I (complete diet)	+0.71 ±0.16	175 ±15	40 ±8
Period II (complete diet − valine)	−1.61 ±0.20	100 ±14	55 ±9
Period III (complete diet − valine + α-ketoisovaleric acid)	−0.18 ±0.19	158 ±11	50 ±7
P values Period II − Period I	$0.001 > P$	$0.02 > P > 0.01$	$0.30 > P > 0.25$
Period III − Period II	$0.001 > P$	$0.025 > P > 0.02$	$0.70 > P > 0.60$
Period III − Period I	$0.01 > P > 0.005$	$0.50 > P > 0.40$	$0.40 > P > 0.30$
Expt. 4			
(Case B)			
Period I (complete diet)	+0.96 ±0.16	201 ±22	58 ±7
Period II (complete diet − phenylalanine)	−1.73 ±0.20	190 ±18	50 ±8
Period III (complete diet − phenylalanine + phenylpyruvic acid)	+0.11 ±0.15	181 ±15	54 ±7
P values Period II − Period I	$0.001 > P$	$0.80 > P > 0.70$	$0.50 > P > 0.40$
Period III − Period II	$0.001 > P$	$0.80 > P > 0.70$	$0.80 > P > 0.70$
Period III − Period I	$0.005 > P > 0.001$	$0.50 > P > 0.40$	$0.80 > P > 0.70$
Expt. 5			
(Case C)			
Period I (complete diet)	+0.30 ±0.15	185 ±10	41 ±6
Period II (complete diet − valine)	−1.87 ±0.25	105 ±11	45 ±5
Period III (complete diet − valine + α-ketoisovaleric acid)	−0.83 ±0.21	140 ±6	43 ±9
P values Period II − Period I	$0.001 > P$	$0.005 > P > 0.001$	$0.70 > P > 0.60$
Period III − Period II	0.01	$0.05 > P > 0.025$	$0.90 > P > 0.80$
Period III − Period I	$0.005 > P > 0.001$	$0.01 > P > 0.005$	$0.90 > P > 0.80$
Expt. 6			
(Case D)			
Period I (complete diet)	+0.53 ±0.14	192 ±20	61 ±7
Period II (complete diet − phenylalanine)	−1.94 ±0.26	183 ±21	50 ±9
Period III (complete diet − phenylalanine +phenylpyruvic acid)	−0.52 ±0.21	180 ±11	53 ±6
P values Period II − Period I	$0.001 > P$	$0.80 P > 0.70$	$0.40 > P > 0.30$
Period III − Period II	$0.005 > P > 0.001$	$P > 0.90$	$0.80 > P > 0.70$
Period III − Period I	$0.005 > P > 0.001$	$0.70 > P > 0.60$	$0.50 > P > 0.40$

Values represent average ±SE (n = 5 for N balance, n = 3 for amino acid concentration).

TABLE V

Experiments 7 and 8: Effects of Dietary Variations on Daily Nitrogen Balance and on Fasting and 2-hr Postprandial Plasma Amino Acid Concentrations

	N balance	Plasma valine		Plasma phenylalanine	
		Fasting	Postprandial	Fasting	Postprandial
	g	*μmoles/liter*		*μmoles/liter*	
Expt. 7					
(Case B)					
Period I (complete diet − valine +α-ketoisovaleric acid)	−0.28 ±0.11	165 ±17	150 ±12	45 ±10	51 ±6
Period II (complete diet − valine)	−2.07 ±0.22	94 ±11	105 ±11	58 ±9	47 ±8
Period III (complete diet)	+0.65 ±0.21	195 ±18	211 ±20	50 ±7	56 ±10
P values Period II − Period III	0.001 > *P*	0.005 > *P* > 0.001	0.01 > *P* > 0.005	0.50 > *P* > 0.40	0.60 > *P* > 0.50
Period I − Period II	0.001 > *P*	0.02 > *P* > 0.01	0.05 > *P* > 0.025	0.40 > *P* > 0.30	0.80 > *P* > 0.70
Period I − Period III	0.005 > *P* > 0.001	0.30 > *P* > 0.25	0.05 > *P* > 0.025	0.70 > *P* > 0.60	0.70 > *P* > 0.60
Expt. 8					
(Case D)					
Period I (complete diet − phenylalanine + phenylpyruvic acid)	+0.04 ±0.10	174 ±24	183 ±20	41 ±10	45 ±7
Period II (complete diet − phenylalanine)	−1.70 ±0.30	190 ±19	185 ±16	56 ±6	40 ±11
Period III (complete diet)	+1.30 ±0.09	183 ±15	171 ±19	48 ±5	50 ±8
P values Period II − Period III	0.001 > *P*	0.80 > *P* > 0.70	0.60 > *P* > 0.50	0.40 > *P* > 0.30	0.50 > *P* > 0.40
Period I − Period II	0.001 > *P*	0.70 > *P* > 0.60	*P* > 0.90	0.30 > *P* > 0.25	0.80 > *P* > 0.70
Period I − Period III	0.001 > *P*	0.80 > *P* > 0.70	0.70 > *P* > 0.60	0.60 > *P* > 0.50	0.70 > *P* > 0.60

Values represent average ±SE (n = 5 for N balance, n = 3 for amino acid concentrations).

to the diet during period III raised the average daily nitrogen balance to −0.02 g (a 77% correction of the decline in nitrogen balance during period II) and also caused a partial return towards normal of the plasma valine concentration (a 52% correction). These changes in nitrogen balance and in plasma valine level during period III constituted significant ($P < 0.05$) improvements over the values of period II; the plasma valine level, however, was still significantly ($P < 0.05$) lower than during period I.

Identical experiments (Table IV) with α-ketoisovaleric acid in case B (experiment 3) and in case C (experiment 5) gave results closely similar to those detailed above for case A (experiment 1). In cases B and C, α-ketoisovaleric acid corrected 60% and 48% of the decrement in nitrogen balance, and 77% and 44% of the decrement in plasma valine concentration, which had followed the withdrawal of valine.

Experiments with phenylalanine and phenylpyruvic acid. Three experiments (Nos. 2, 4, and 6) were done in three different subjects (cases A, B, and D) with similar results. In each experiment, withdrawal of phenylalanine from the diet caused the daily nitrogen balance to decline from the range + 0.53 to + 1.04 g into the range − 1.45 to − 1.94 g. Nevertheless, plasma concentrations of phenylalanine remained within the normal range (40–60 μmoles/liter). Addition of phenylpyruvic acid to the diet caused a 58–68% correction of the decrement in nitrogen balance which had followed the withdrawal of phenylalanine; plasma phenylalanine concentration did not change significantly.

Inversion of periods I and III; postprandial amino acid levels. Although the experiments above indicated utilization of α-ketoisovaleric acid and phenylpyruvic acid, two points merited further study. (*a*) 3 wk of formula diet could affect the intestinal flora, with resulting alterations in the extent of bacterial transformation of dietary amino acids and ketoacids. This possibility could be investigated by changing the order in which the essential amino acid, its keto analogue, and the complete diet minus either nutrient, were administered to the patient. (*b*) The failure of fasting concentration of plasma phenylalanine to fall during ingestion of a phenylalanine-free diet, and to rise during the phenylpyruvic acid period, was not consistent with the fall and rise in nitrogen balance during these intervals. Possibly, however, postprandial amino acid levels might have provided such a correlation, since nutritional studies (26, 27) indicate that much of the utilization of dietary amino acids for protein synthesis occurs within a few hours after each meal.

Accordingly two more experiments were carried out. In experiment 7, complete diet minus valine plus α-ketoisovaleric acid was administered in period I, complete diet minus valine in period II, and complete diet in period III. Plasma amino acid concentrations were measured both in the fasting state and at 10 a.m., 2 hr after the subject had ingested ⅓ of the day's ration. Confirming experiments 1, 3, and 5, nitrogen balance during

periods I, II, and III averaged −0.28, −2.07, and +0.65 g/day (Table V). Fasting plasma valine concentration again was reduced by removal of valine from the diet (period II compared to period III) and partially restored by addition of α-ketoisovaleric acid (period I compared to period III). 2-hr postprandial valine concentrations did not differ significantly from the fasting values. In experiment 8, similar studies were done with phenylalanine and phenylpyruvic acid. The effects of the dietary manipulations on nitrogen balance and on fasting plasma amino acid concentrations confirmed the results of experiments 2, 4, and 6. Postprandial phenylalanine concentrations, like the fasting values, were not significantly affected by the dietary content of phenylalanine and phenylpyruvic acid.

DISCUSSION

The essential nature of an amino acid can be demonstrated in at least two different ways (28): (*a*) cessation of growth, in the young growing subject, when the amino acid is removed from an otherwise adequate diet; and (*b*) development of negative nitrogen balance, when the amino acid is removed from such a diet. In addition, (*c*) reduction in the plasma concentration of an essential (but not of a nonessential) amino acid might be expected to follow removal of the amino acid from the diet (29). Each technique also, in theory, provides a means of determining whether a possible precursor can be metabolically converted into a particular essential amino acid.

With the first technique, the amino acid requirements of young rats (28) and of human infants (30) were defined, and the capacity of young rats to utilize α-keto acid analogues in place of essential amino acids was demonstrated (10–15). The second technique (employed here) has been used by Rose and coworkers (1) to determine the daily requirements of adult human subjects for the essential amino acids. The present data on the degree of negative nitrogen balance which follows withdrawal of valine or phenylalanine from the diet of adult subjects are in general agreement with those of Rose et al. The third technique, also attempted here, has not yet been properly evaluated as a test for the essential nature of an amino acid or for the effectiveness of a possible precursor. Swendseid et al. reported that removal of valine from the diet of human subjects caused a sharp drop in fasting plasma valine level (29), but that removal of phenylalanine did not have the corresponding effect (31). Our limited data on this point seem to be in agreement. Evidently, endogenous sources of preformed phenylalanine are sufficient to maintain normal fasting plasma levels of this amino acid when it is absent from the diet, but such sources are not adequate for maintenance of a normal fasting concentration of valine. The fasting amino acid levels, therefore, do not in every case reflect deficiency of an essential amino acid or correction of the deficiency by a precursor substance.

Equimolar substitution of α-ketoisovaleric acid for valine led to 44–77% correction of the decrements in nitrogen balance and in plasma valine concentration which had been caused by withdrawal of valine from the diet. Equimolar substitution of phenylpyruvic acid for phenylalanine corrected 58–68% of the decline in nitrogen balance which had followed withdrawal of phenylalanine. Thus adult man, like the growing rat, can utilize the keto analogues of these two amino acids in place of the corresponding essential amino acids. But the conversion of α-ketoisovaleric acid to valine, and of phenylpyruvic acid to phenylalanine is not complete, since considerably less than 100% correction was achieved by an equimolar replacement.[4] When this incomplete correction by α-ketoisovaleric acid in man is compared with the full restoration of growth in corresponding experiments with young rats (11), it would seem that the rat utilizes this keto acid more efficiently than man. The 80–100% corrective effective of phenylpyruvic acid in the growing rat (11) suggests a more highly efficient utilization by the rat of this keto acid as well.

Inefficient utilization of keto acid analogues could result from incomplete gastrointestinal absorption, from degradation of a portion of the ingested ration by intestinal microbes, or from degradation of a portion of the absorbed ketoacid by catabolic reactions in liver and other organs, thereby preventing complete conversion by transamination to the amino acid.

Quantitative interpretation of the present data on the capacity of man to utilize keto acids, however, may be limited by the use of abnormal subjects in these experiments. While it seems unlikely that the apparently inactive neurological disease of these patients would influence their intermediary metabolism of amino acids, it will be desirable to repeat these experiments in normal individuals before drawing quantitative conclusions about the human capacity to utilize keto acids.

Our data suggest that in the case of at least two of the eight essential amino acids, the nitrogen-free keto analogues should be effective in maintaining the nitrogen balance of uremic patients,[5] as proposed by

[4] Since we do not know the quantitative relationship between degree of negative nitrogen balance and amount of essential amino acid available (between the limits 0 and the minimum daily requirement), we cannot estimate from the per cent correction of decrement in nitrogen balance, what per cent of the ingested keto acid analogue was converted to the corresponding essential amino acid.

[5] A protein-free diet containing the keto acid analogues would theoretically be more effective in lowering the blood urea concentration of uremic subjects than a protein-free diet based on the corresponding essential amino acids (9). But the former diet would have no advantage over the latter

Richards, Metcalfe-Gibson, Ward, Wrong, and Houghton (32), although the keto acid would probably need to be substituted for the amino acid in a greater than equimolar quantity. How much greater than equimolar this substitution needs to be in the case of valine and phenylalanine, and to what extent these relationships apply to the six other essential amino acids, are questions for future study.

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in correcting the acidosis resulting from accumulated end-products of metabolism of aromatic and sulfur-containing amino acids. Another potential application of the protein-free diet containing the keto acid analogues is in treatment of hepatic patients with ammonia intoxication, provided efficient amination or transamination of the keto acids can be performed in the diseased liver and/or extrahepatic tissues of these subjects.