JCI The Journal of Clinical Investigation

The Effect of Keto-analogues of Essential Amino Acids in Severe Chronic Uremia

Mackenzie Walser, A. Will Coulter, Shrikant Dighe, Frank R. Crantz

J Clin Invest. 1973;52(3):678-690. https://doi.org/10.1172/JCI107229.

Research Article

Alpha keto-analogues of valine, leucine, isoleucine, methionine, phenylalanine, and (in one instance) tryptophan and histidine, along with the remaining essential amino acids, were administered orally to 10 patients with severe chronic uremia fed a diet low in protein but adequate in calories. Ketoacid dosage varied from 6 to 14 g daily, as sodium or calcium salts. Net nitrogen intake, calculated as intake minus urinary protein nitrogen, averaged 1.8 g/day. The urea space was either estimated or measured with [^{14}C]urea and daily changes in the body urea pool were calculated. Urea appearance was measured as the sum of urea excretion and the change in urea pool. If these ketoacids were converted to amino acids and utilized for protein synthesis, a fall in urea nitrogen appearance should occur. In five subjects, ketoacids were given for 15-18 days and then withdrawn. Urea nitrogen appearance increased 1.55 g/day on withdrawing ketoacids, and corrected nitrogen balance decreased by 1.73 g/day. In two other subjects ketoacid administration was followed, on two occasions each, by a period of administration of nine essential amino acids. In three of these four instances, urea appearance rose significantly with amino acids. In four patients studied at high blood urea levels, ketoacid treatment was relatively ineffective; two of these patients responded more favorably when studied again after peritoneal dialysis. One of these […]

The Effect of Keto-analogues of Essential Amino Acids in Severe Chronic Uremia

MACKENZIE WALSER, A. WILL COULTER, SHRIKANT DIGHE, and FRANK R. CRANTZ

From the Department of Pharmacology and Experimental Therapeutics and the Department of Medicine, The Johns Hopkins University School of Medicine, Baltimore, Maryland 21205

ABSTRACT Alpha keto-analogues of valine, leucine, isoleucine, methionine, phenylalanine, and (in one instance) tryptophan and histidine, along with the remaining essential amino acids, were administered orally to 10 patients with severe chronic uremia fed a diet low in protein but adequate in calories. Ketoacid dosage varied from 6 to 14 g daily, as sodium or calcium salts. Net nitrogen intake, calculated as intake minus urinary protein nitrogen, averaged 1.8 g/day. The urea space was either estimated or measured with [^{14}C]urea and daily changes in the body urea pool were calculated. Urea appearance was measured as the sum of urea excretion and the change in urea pool. If these ketoacids were converted to amino acids and utilized for protein synthesis, a fall in urea nitrogen appearance should occur. In five subjects, ketoacids were given for 15–18 days and then withdrawn. Urea nitrogen appearance increased 1.55 g/day on withdrawing ketoacids, and corrected nitrogen balance decreased by 1.73 g/day. In two other subjects ketoacid administration was followed, on two occasions each, by a period of administration of nine essential amino acids. In three of these four instances, urea appearance rose significantly with amino acids. In four patients studied at high blood urea levels, ketoacid treatment was relatively ineffective; two of these patients responded more favorably when studied again after peritoneal dialysis. One of these improved enough clinically to be managed as an out-patient for short intervals, despite virtual anuria. No accumulation of ketoacids in plasma or urine could be detected, and no toxicity was identified.

Presented previously at: 1971, Fifth Annual Meeting of the American Society of Nephrology, Washington, D. C. 87. (Abstr.); and 1972, Fifth International Congress of Nephrology, Mexico City. 981. (Abstr.).

Received for publication 15 August 1972 and in revised form 3 November 1972.

INTRODUCTION

In 1967, Richards, Metcalfe-Gibson, Ward, Wrong, and Houghton (1), suggested that alpha keto-analogues of the essential amino acids might be useful in the treatment of uremia. This proposal was based on several earlier observations: (*a*) urea is continually degraded to ammonia and carbon dioxide by intestinal urease in normal subjects (2, 3), as well as in patients with uremia (3–7); (*b*) uremic patients respond favorably to restriction of dietary protein (8–10); (*c*) urea or ammonia can be utilized for protein synthesis (1, 11–18), particularly when dietary protein is restricted (1, 11, 12, 15, 17); and (*d*) alpha keto-analogues of most of the essential amino acids can promote growth in rats fed diets devoid of the corresponding amino acids (19–26). Thus, urea nitrogen might be reutilized for conversion of ketoacids to essential amino acids, lowering blood urea concentration and promoting protein synthesis simultaneously. Because of the scarcity of these ketoacids this proposal has not been tested, but Richards and his associates (27, 28), as well as other workers (29–30), have shown that two ketoacids, the analogues of valine and phenylalanine, can replace valine or phenylalanine in the diet of normal (27, 29, 30) as well as uremic (27) individuals, though not with complete efficiency.

The present study was undertaken to evaluate the efficacy, safety, and feasibility of administering these ketoacids as supportive therapy in the management of severe chronic uremia. Seven potentially useful keto-analogues are known, but because of difficulties in obtaining two of them, most of these studies were conducted using only five, the analogues of valine, leucine, isoleucine, methionine, and phenylalanine. Apparently, only two (the analogues of valine and phenylalanine) have previously been administered to human subjects.

TABLE I

Presenting Clinical Data

Subject	G. E.	J. S.	R. G.	L. W.	M. S.	E. W.	C. T.	J. F.	J. W.	I. C.
Age	67	21	33	52	49	62	32	62	55	63
Sex	F	M	M	F	F	F	M	M	M	F
Diagnosis*	1	2	3	1	4	5	4	1	6	6
Urea clearance, *ml/min*	0.6	1.5	5.5	0.9	1.1	0.3	0.4	0.5	2.2	1.4
Creatinine clearance, *ml/min*	0.9	2.9	10.1	1.6	2.0	0.5	0.6	0.8	4.4	2.1
Serum urea N, *mg/100 ml*	121	150	59	137	131	172	174	153	174	120
Serum creatinine, *mg/100 ml*	17	22	11	14	18	29	23	23	10	17
Hematocrit, %	21.7	15.4	23.1	23.6	19.0	21.0	19.4	20.2	25.0	18.0
Serum albumin, *g/100 ml*	3.6	3.1	2.6	2.9	3.1	1.3	2.6	2.7	2.4	2.4
Proteinuria, *g/day*	1	3	8	4	3	2	2	2	3	6
Calcium, *mg/100 ml*	6.5	8.6	5.7	7.0	6.8	5.4	7.6	8.6	9.1	5.7
Phosphorus, *mg/100 ml*	6.1	7.8	7.4	5.2	6.9	8.5	10.1	5.8	4.5	5.1
Uric acid, *mg/100 ml*	10.0	12.0		9.6	10.0	13.0	16.0	9.2	8.3	8.4
Weight, *kg*	45	60	88	60	55	87	57	80	51	59
Total days ketoacid Rx	15	15	18	17	18	25	8	61	29	39

* 1, arteriolar nephrosclerosis; 2, familial glomerulonephritis (Alport's disease); 3, membranous glomerulonephritis; 4, chronic glomerulonephritis, type undetermined; 5, unknown; 6, chronic pyelonephritis.

METHODS

10 patients with chronic uremia were selected for study. Clinical data on these subjects at the onset of study are summarized in Table I. They fall into three categories with respect to severity: In the least severe category was only subject R. G. who had a urea clearance of 5 ml/min. Dietary management with no supplements has proven adequate to maintain him in good health for 9 mo after the study. The second category included six patients (G. E., J. S., L. W., M. S., J. W., and I. C.) who had more severe renal failure, with urea clearances between 0.6 and 2.2 ml/min. They could be maintained on diet alone only with continued symptoms and progressive weight loss; without strict dietary control they soon developed advanced uremia, and required dialysis. In the third category were four patients (C. T., E. W., J. F., and J. W.) all of whom were virtually anuric (urea clearance < 0.5 ml/min). Patient J. W. was studied in both the second category and third category at different stages of his illness.

Metabolic balance studies were conducted in the Clinical Research Center of The Johns Hopkins Hospital. Low protein diets were developed by the ward dietician to suit each subject's individual tastes and his requirements for calories, fluid, sodium, and potassium. The level of protein intake for each patient was selected on the basis of the severity of renal failure, and the amount of proteinuria. The principal caloric component of the diet was Cal-Power, a partially hydrolyzed starch ("liquid glucose," U.S.P.) deionized by passage through ion-exchange resins. Other special low protein foods included bread made from Paygel-P flour, Aproten rusks, Aproten spaghetti, and Aproten semolina cereal.[1] Fat was provided as butter. The diet used in the anuric subjects contained 4–6 g protein. One such diet is illustrated in Table II.[2]

As soon as possible, the diet for each subject was finalized. Nitrogen content was calculated from tables and was verified by analysis of a single day's diet, homogenized in water. A constant diet was not provided, an imposition we felt to be unjustified in these severely ill and often anorexic subjects. As illustrated below, nitrogen balance, under the conditions of these studies, is dominated by changes in the urea nitrogen pool. No correction was made for occasional vomiting which occurred before breakfast, in view of the fact that the protein content of gastric juice is low, approximately 1.8 g/liter (31). Patient I. C. vomited frequently (17 times during 39 balance study days) after breakfast. On these days, the

[1] Cal-Power, Paygel-P, and Aproten products were generously supplied by General Mills Inc., Minneapolis, Minn., through the courtesy of Mr. Fred Hafner.

[2] All subjects received daily vitamin supplements, including 50 mg pyridoxine.

TABLE II

Diet Employed in Subject J. F., Containing 4 g Protein and 2500 cal

Breakfast
- Cal-Power, 80 ml
- Ginger ale, 40 ml
- Paygel-P bread, 2 slices
- Butter
- Jelly
- Applesauce or canned pears

Midmorning, midafternoon, and bedtime
- Cal-Power, 80 ml
- Ginger ale, 40 ml
- Paygel-P bread, 1 slice
- Butter
- Jelly

Lunch and dinner
- Cal-Power, 80 ml
- Ginger ale, 40 ml
- Paygel-P bread, 2 slices
- Mayonnaise
- Sliced tomato or lettuce
- Lemon pudding

nitrogen intake of her breakfast (0.35 g) was not included in calculated intake of nitrogen. When vomiting occurred later in the day in any subject, the balance data for that day were omitted. Calculated caloric intake was in all cases over 2100 cal.

Evaluation of the results of nitrogen balance data. Conventional nitrogen balance calculations are not well suited to the study of patients such as these. In order to take into account changes in the body urea pool and urinary loss of protein, modifications are required. For the purposes of this study, body nitrogen is considered as consisting of two pools: one containing protein, nucleic acids, and other nitrogenous components of protoplasm; and a second consisting of urea, creatinine, uric acid, and ammonia, referred to collectively as nonprotein nitrogen. The first pool cannot be measured in an intact subject. The second is dominated by urea nitrogen in these subjects and is only slightly greater than the urea nitrogen pool itself; daily changes in this pool are closely approximated by daily changes in the urea nitrogen pool.

The rate of appearance of nonprotein nitrogen, C, is given by the sum of nonprotein nitrogen excretion in urine and stool and change in the urea pool. Urinary nonprotein nitrogen is defined as total nitrogen minus urinary protein nitrogen, P.

Fecal nitrogen is considered to be nonprotein nitrogen. Loss of nitrogen through the skin is ignored. Change in the body protein nitrogen pool, i.e., corrected nitrogen balance, b_N, is given by dietary nitrogen, D, minus urinary protein nitrogen minus nonprotein nitrogen appearance:

$$b_N = D - P - C. \quad (1)$$

This analysis differs from the usual balance, defined as intake minus output, in two ways: intake is redefined as net intake, and output includes not only external loss but also accumulation.

The rate of appearance of urea nitrogen, U, is calculated as urinary urea nitrogen excretion plus change in the urea nitrogen pool. This quantity is equal to the difference between the rate of urea nitrogen production, A, and the rate of urea nitrogen breakdown, M:

$$U = A - M. \quad (2)$$

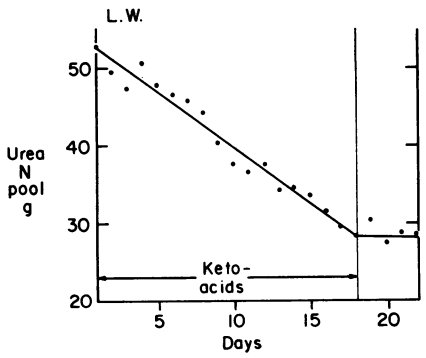

FIGURE 1 Daily urea nitrogen pool during and after keto-acid administration in subject L. W. The pool is calculated as SUN times (weight minus 21.88 kg.) The latter quantity, nonurea space, was calculated from the measured urea space (34.12 liters) and body weight (56.0 kg) on day 20, and was assumed to be constant. Straight lines were drawn visually through the points. The slopes of these lines give daily change in urea nitrogen pool.

An increase in M (urea breakdown) may lead to an equivalent increase in A (urea nitrogen production), if all of the resulting ammonia nitrogen is resynthesized into urea. On the other hand, if all of the ammonia is utilized for protein synthesis, A is not changed and hence U decreases. Thus urea nitrogen appearance is also equal to the difference between urea nitrogen production from sources other than portal ammonia and the rate of utilization of ammonia nitrogen for protein synthesis.

The hypothesis to be examined here is that ketoacids promote utilization of ammonia derived from ureolysis. If this is correct, nonprotein nitrogen and urea nitrogen appearance should both diminish on adding ketoacids to the diet. The magnitude of the change should be a measure of the extent of ammonia utilization induced by ketoacids, providing that other factors remain constant.

An advantage of using urea nitrogen appearance to evaluate the results (in addition to nitrogen balance) is that dietary nitrogen is more difficult to measure accurately than is urea nitrogen appearance, in these patients, for reasons noted above. During severe protein restriction in normal persons, or in uremic patients, nonprotein nitrogen appearance is little affected by changes in dietary nitrogen in the range of 0–4 g/day (32, 33). Thus nitrogen balance is highly dependent on the choice of protein intake. Selecting the optimal dietary nitrogen for these patients is difficult, particularly since the balance data do not become available promptly. Hence the values of nitrogen balance observed in this study are in large measure a reflection of our choice of protein intake rather than the success of therapy. On the other hand, the value of urea nitrogen appearance is a more appropriate measure of efficacy. The ability of patients with far advanced uremia to survive without dialysis may be dependent upon reducing this quantity. For example, at a urea clearance of 1 ml/min (the average clearance seen in our patients), 3 g of urea nitrogen appearing per day would eventuate in a serum urea nitrogen concentration (SUN)[3] of over 200 mg/100 ml.

In 6 of the 10 patients, urea space was measured by injecting 10 μCi [^{14}C]urea intravenously and obtaining blood samples 2 h later and then daily for several days. The disappearance of [^{14}C]urea specific activity was a simple exponential function, as reported previously (2). The extrapolated concentration of [^{14}C]urea at the time of injection, expressed as microcuries per milliliter of plasma water, divided into 10 μCi gives the urea space in liters or kilograms. No correction for excretion during the period of equilibration is necessary in these patients. In the other four patients, urea space was assumed to be 60% of initial body weight. Body weight minus urea space gives "nonurea space" in kilograms. Nonurea space was assumed to remain constant. Urea space on any day could therefore be calculated as measured body weight minus nonurea space. Urea nitrogen pool each day was obtained as the product of urea space and urea nitrogen concentration in serum water. Water content of serum or plasma was taken as 95%. For each subject a graph was made of daily urea nitrogen pool and straight lines were drawn visually through the resulting points (Figs. 1–3). The slope of these lines then gives the daily change in the urea nitrogen pool. The purpose of this procedure was to minimize the

[3] *Abbreviations used in this paper:* keto-his, imidazolepyruvic acid; keto-ile, α-keto-β-methylvaleric acid; keto-leu, α-ketoisocaproic acid; keto-met, α-keto-β-methiolbutyric acid; keto-phe, phenylpyruvic acid; keto-trp, indolepyruvic acid; keto-val, α-ketoisovaleric acid; SUN, serum urea nitrogen concentration.

fluctuation in calculated nitrogen balance which would otherwise be caused by small changes in SUN and to obtain the best estimate of the changes in urea pool.

Continuous 5-day (or, occasionally 2-day) stool collections were obtained in metal cans, diluted with water, and homogenized by ultrasonication. Continuous 24-h urine collections were obtained, using 10 ml 6 N HCl as a preservative.

Urea, creatinine, and electrolytes in serum and urine were measured daily in the clinical chemistry laboratory by automated methods. Urine protein was measured daily by the method of Lowry, Rosebrough, Farr, and Randall (34). In some subjects, plasma and urine keto-acids were measured[4] periodically.

Total nitrogen in urine, homogenized diet, and stool was measured with the Coleman Nitrogen Analyzer (Coleman Instruments Div., Perkin Elmer Corp., Maywood, Ill). Recovery of nitrogen from standard samples of egg albumin, urea, and creatinine, averaged 100%, 98.5%, and 99.9%, respectively. Creatinine standards were measured daily. A comparison was made in every 24 h urine of the urinary nitrogen calculated as the sum of measured values of protein times 0.16, urea nitrogen, creatinine nitrogen, and uric acid nitrogen with the analytical value for total nitrogen. The ratio of the former quantity to the latter was averaged for each subject. The average ratios varied between subjects from 0.89 to 1.05, with a mean of 0.98. The standard deviations varied from 0.05 to 0.12 with a mean of 0.08.

Ammonia was measured in a few urine samples and found to be a negligibly small fraction of total nitrogen. This is to be expected in these individuals because of the small fraction of renal function remaining.

The ketoacids employed were α-ketoisovaleric acid (keto-val), α-ketoisocaproic acid (keto-leu), α-keto-β-methylvaleric acid (keto-ile), α-keto-β-methiolbutyric acid (keto-met), phenylpyruvic acid (keto-phe), indolepyruvic acid (keto-trp), and imidazolepyruvic acid (keto-his).

The first four compounds were synthesized in our laboratory by modifications of the procedure of Weygand, Steglich, and Tanner (35). Phenylpyruvic acid was obtained from Koch-Light Laboratories, Ltd., Colnbrook, Buckinghamshire, England. Keto-trp and keto-his were synthesized by Cyclo Chemical Co., Los Angeles, Calif. All seven ketoacids were crystallized as sodium salts. In the last four studies, the sodium salts of the first five compounds were converted to calcium salts. The identity of the compounds was established by elemental analysis, thin-layer chromatography, infrared spectroscopy, high resolution mass spectroscopy, and nuclear magnetic resonance spectroscopy. Keto-ile exists in two stereoisometric forms. In two patients (L. W. and R. G.), a racemic mixture was employed, obtained from Cyclo Chemical Co. In the other eight, pure levorotary α-keto-β-methylvaleric acid was used, made in our laboratory, with an optical rotation of −32°; this was checked on each lot.

Reagent grade L-amino acids were obtained from Sigma Chemical Co., St. Louis, Mo. Their purity and identity were verified by ion-exchange chromatography on the Technicon Amino Acid Analyzer (Technicon Instruments Corp., Tarrytown, N. Y.) and by determining optical rotation.

The drugs were administered in three or four divided doses, each of which contained nine compounds: valine (or keto-val), leucine (or keto-leu), isoleucine (or keto-ile), methionine (or keto-met), phenylalanine (or keto-phe), histidine (or keto-his), tryptophan (or keto-trp), lysine, and threonine. These were placed in gelatin capsules (size 00).

[4] Walser, M., P. Lund, N. Ruderman, and H. A. Krebs. Unpublished data.

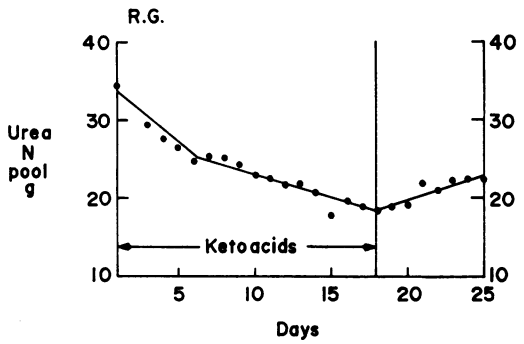

FIGURE 2 Daily urea nitrogen pool during and after ketoacid administration in subject R. G. Withdrawal of ketoacids arrests the fall in urea nitrogen pool.

The nitrogen content of the empty gelatin capsules was measured and taken into account in calculating nitrogen balance. Histidine was included because recent work indicates that it is an essential amino acid in patients with uremia, although it is not essential in normal adults (36–38).

In the first two cases, dosage of ketoacids was increased gradually over a period of several days to the final value. In the other patients, full dosage was given from the onset of therapy (Table III).

Keto-his and keto-trp were used only in the first subject, G. E.; further supplies of these compounds could not be obtained.

Dosage of the branched chain compounds (keto-val, keto-leu, and keto-ile) was increased in later studies when results of experiments on perfused rat liver (39) became available, showing that these ketoacids undergo more degradation relative to transamination than do keto-met or keto-phe.

Acute studies of ketoacid toxicity in mice and chronic toxicity studies in dogs on a protein-free diet, to be reported elsewhere, preceded these studies. Approval was obtained from the appropriate institutional committees and an Investigational New Drug Authorization was obtained from the Food and Drug Administration. Informed consent was obtained from the patients.

RESULTS

Nitrogen metabolism

Comparison of ketoacid administration with a subsequent control period. In five subjects the mixture of ketoacids and amino acids was given for 15–18 days followed by a control period of several days. The con-

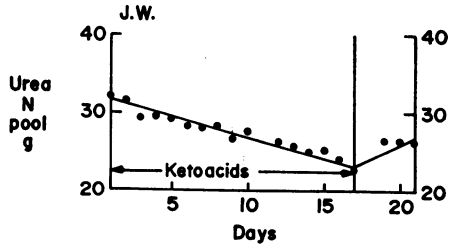

FIGURE 3 Daily urea nitrogen pool during ketoacid administration and during a subsequent period of amino acid administration in subject J. W.

TABLE III

Dosage of Individual Keto- and Amino Acids Employed in each Period in 10 Patients

Subject	Period	"Rose units"* per day of keto (K) or amino (A) acids								
		Val	Leu	Ile	Met	Phe	Trp	His	Lys	Thr
G. E	KA	1K	1K	1K	1K	1K	1K	1K	1A	1A
J. S.	KA	1.5K	1.5K	1.5K	1.5K	1.5K	1.5A	1.5A	1.5A	1.5A
R. G.	KA	1.5K	1.5K	1.5K‡	1.5K	1.5K	1.5A	1.5A	1.5A	1.5A
L. W.	KA	1K	1K	1K‡	1K	1K	1A	1A	1A	1A
M. S.	KA	1K	1K	1A§	1K	1K	1A	1A	1A	1A
E. W.	KA	1K	1K	1A§	1.5K	1.5K	1A	1A	1A	1A
C. T.	KA	1.7K	1.7K	1.7K	1.7K	1.7K	1.7A	1.7A	1.7A	1.7A
J. F.	KA_1	3.3K	3.3K	3.3K	1.7K	1.7K	1.3A	1.3A	1.3A	1.3A
	KA_2	3K	3K	3K	1.5K	1.5K	1A	1A	1A	1A
	KA_3	3K	3K	3K	1.5K	1.5K	1A	1A	1A	1A
J. W.	KA_1	3K	3K	3K	1.5K	1.5K	1A	1A	1A	1A
	AA_1	2A	1.5A	2A	1.5A	1.5A	1A	1A	1A	1A
	KA_2	3K	4K	3K	1.5K	1.5K	1A	1A	1A	1A
	AA_2	2A	1.5A	2A	1.5A	1.5A	1A	1A	1A	1A
I. C.	KA_1	3K	3K	3K	1.5A	1.5A	1A	1A	1A	1A
	AA_1	2A	1.5A	2A	1.5A	1.5A	1A	1A	1A	1A
	KA_2	3K	4K	3K	1.5K	1.5K	1A	1A	1A	1A
	AA_2	2A	1.5A	2A	1.5A	1.5A	1A	1A	1A	1A

* 1 "Rose unit" of amino acid (or keto-analogue) was defined as 6.90 mmol valine, 8.48 mmol leucine, 5.79 mmol isoleucine, 7.42 mmol methionine, 6.12 mmol phenylalanine, 3.50 mmol histidine, 4.38 mmol lysine, 1.23 mmol tryptophane, and 4.20 mmol threonine (51).

‡ Racemic α-keto-β-methylvaleric acid; other subjects received *S*-(—)-α-keto-β-methylvaleric acid.

§ Isoleucine given instead of keto-analogue owing to unavailability of the latter.

trol period was placed at the end because of the well-known adaptation which occurs in normal or obese subjects during prolonged protein restriction or starvation (40–43). Placing the control period at the end tends to bias the results against demonstrating an effect of ketoacids on nitrogen conservation, or an effect of these compounds on adaptation that persists after they are withdrawn. However, the opposite sequence would have introduced the opposite bias.

The results are shown in Table IV. Net nitrogen intake (intake minus urine protein nitrogen), including dietary nitrogen, nitrogen content of amino or keto-acids (keto-his and keto-trp in subject G. E.), and nitrogen content of the gelatin capsules themselves, averaged 1.81 g/day. During the control period which followed, net nitrogen intake averaged 1.79 g/day. Negative nitrogen balance was observed in all of the subjects during the control periods. During the last half of the period of ketoacid administration nitrogen balance was positive in G. E. and M. S., neutral in J. S. and I. W., and negative in R. G. As indicated above, this variable response is chiefly a reflection of our varying success in selecting the appropriate nitrogen intake. Of greater importance is the fact that the change in nitrogen balance on withdrawing ketoacids was invariably significant, averaging — 1.73 g/day.

During the last week of ketoacid administration the rate of appearance of urea nitrogen in urine and in body water averaged only 0.64 g/day. In G. E. the rate of appearance was on the average negative; i.e., the quantity of urea appearing in the urine was less than the quantity disappearing from urea space, though not significantly so. G. E. was the only patient who received seven ketoacids. In every case, withdrawal of ketoacids was followed by a significant increase in urea nitrogen appearance, averaging +1.55 g/day.

The clinical significance of these changes in urea appearance is illustrated by relating them to the simultaneously measured urea clearance, also shown in the table. The SUN level required to excrete the urea appearing every day can be calculated as appearance rate divided by clearance (expressed in liters per day). This "steady-state SUN" would be attained eventually if urea appearance rate and clearance remained constant. The results of this calculation are shown in Fig. 4. For subject R. G., whose renal function was several times greater than the other patients', no important increase in SUN is predicted. For the others, considerably greater changes are predicted. In J. S., for example, the steady-state SUN on ketoacids is calculated to be 19 mg/100 ml, while after withdrawing them it is 132 mg/100 ml. Any higher nitrogen intake in the

TABLE IV

Nitrogen Metabolism in Uremic Patients during and after Administration of Ketoacids (KA)

Subject	Days	Rx	Net N intake	Urea N appearance	N balance	Urea clearance
			g/day	*g/day*	*g/day*	*ml/min*
G. E.	1–8	KA*	0.99±0.27	1.07±0.49		0.61±0.06
	9–15	KA	1.68±0.11	−0.37±0.23	+0.87±0.26	0.69±0.14
	16–20	None	1.18±0.04	1.30±0.32	−0.95±0.38	1.58±0.25
	Change‡		−0.50±0.12§	+1.67±0.39§	−1.82±0.46§	+0.89±0.29§
J. S.	1–8	KA*	2.47±0.17	2.32±0.16		1.64±0.06
	9–15	KA	1.84±0.30	0.04±0.11	+0.36±0.22	1.44±0.07
	16–20	None	1.53±0.29	3.09±0.05	−2.95±0.34	1.62±0.04
	Change‡		−0.31±0.42	+3.05±0.12§	−3.31±0.40§	+0.18±0.08‖
R. G.	1–7	KA	0.95±0.27	2.60±0.84	−2.96±0.52	5.35±0.30
	8–18	KA	3.04±0.14	2.44±0.11	−0.90±0.26	5.82±0.74
	19–23	None	2.89±0.11	3.18±0.29	−1.88±0.37	5.04±0.56
	Change‡		−0.15±0.18	+0.74±0.31§	−0.98±0.45‖	−0.78±1.31
L. W.	1–9	KA	1.18±0.19	0.58±0.12	−0.62±0.28	1.05±0.12
	10–17	KA	1.66±0.06	0.48±0.04	+0.03±0.12	1.34±0.08
	18–21	None	1.44±0.16	1.63±0.01	−0.91±0.25	1.17±0.13
	Change‡		−0.22±0.17	+1.15±0.04§	−0.94±0.28§	−0.17±0.15
M. S.	1–9	KA	2.09±0.17	0.95±0.06	−0.04±0.26	1.09±0.02
	10–18	KA	2.23±0.04	0.61±0.10	+0.89±0.15	1.10±0.04
	19–24	None	1.68±0.07	1.73±0.07	−0.72±0.08	1.19±0.06
	Change‡		−0.55±0.08§	+1.12±0.12§	−1.61±0.17§	+0.09±0.09

* Ketoacid dosage increased gradually to final value during this period.

‡ Difference between second period of ketoacid therapy and final control period.

§ Difference is significant ($P < 0.02$).

‖ Difference is significant ($P < 0.05$).

control periods could only have resulted in higher SUN levels.

All five of these patients improved clinically while on ketoacids. No change in their clinical status could be detected during the brief control period which followed.

Comparison of ketoacid administration with a subsequent period of administration of essential amino acids. In two patients (J. W. and I. C.) periods of ketoacid administration were followed by periods of administration of essential amino acids. The results are summarized in Table V. Subject J. W. was studied twice: once when he still had appreciable renal function (2–3 ml/min urea clearance) and later when he was becoming virtually anuric owing to progression of his disease (arteriolar nephrosclerosis). In the first study, changing from ketoacids to amino acids, at constant nitrogen intake, had little or no effect on urea appearance or nitrogen balance. However, in the second study, urea nitrogen appearance was essentially zero during 17 days of ketoacid treatment but increased by 0.97±0.06 g on changing to amino acids. Changes in urea nitrogen pool during the study are illustrated in Fig. 3. At the very low urea clearance J. W. exhibited at the end of the study (0.17±0.03 ml/min), a SUN of 339 mg/100 ml would have eventuated if urea appearance had remained constant while receiving amino acids.

In subject I. C., studied on two occasions, changing from ketoacids to amino acids was associated with small but significant increases in urea appearance. The predicted changes in steady-state SUN are from 53 to 76 mg/100 ml in the first study and from 79 mg to 128 mg/100 ml in the second.

The average change in urea nitrogen appearance on switching to amino acids was +0.50 g/day for the four studies.

Ketoacid administration in the presence of high blood urea. Four subjects who had high SUN's were given ketoacids without subsequent control periods. Nitrogen balance was negative in all four (Table VI).

In E. W., who was already preterminal (Table I), urea nitrogen appearance was nearly eliminated for 25 days but because of her markedly reduced urea clearance little fall in SUN occurred. Stool nitrogen ex-

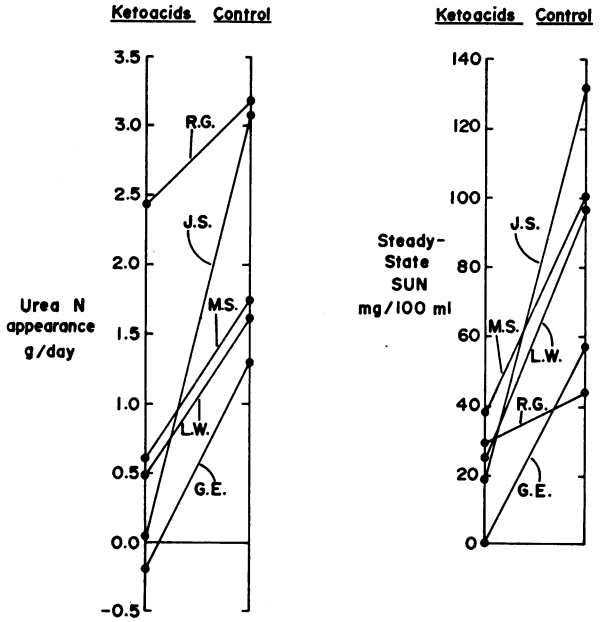

FIGURE 4 Effect of withdrawing ketoacids on urea nitrogen appearance and steady-state SUN. Urea nitrogen appearance is calculated as the sum of urinary urea nitrogen excretion and the change in urea nitrogen pool. Steady-state SUN is calculated as urea clearance divided by urea nitrogen appearance, and represents the SUN that would eventuate if these quantities remained constant. In each patient, withdrawal of ketoacids is associated with a rise in urea appearance and in the steady-state SUN.

ceeded nitrogen intake because of diarrhea. She initially improved clinically but then became weaker, developed pseudomonas septicemia, and died.

Subject C. T. showed no change in his clinical state during 1 wk of treatment, and SUN rose slightly. He was accepted for chronic dialysis and transplantation and has subsequently done well.

J. F. also had diarrhea, presumably caused by uremic enteritis. He showed neither improvement nor worsening but treatment was discontinued because he developed chest pain and dyspnea. He was later dialyzed peritoneally and treated again; the results are presented below.

Subject J. W. showed strongly negative nitrogen balance and rising SUN. Stool guaiacs were found to be strongly positive, owing to a gastric ulcer. The increased protein intake produced by this blood in the gastrointestinal tract probably caused the excessive nitrogen catabolism. He was also subjected to peritoneal dialysis and later treated with ketoacids again. The results have been presented in Table V.

These data suggest that ketoacid administration may be of no avail if uremia is too severe initially. Two of these four subjects responded more favorably when given ketoacids again after dialysis. A possible explanation may be the so-called "catabolic" effect of uremia (44), but gastrointestinal complications clearly played a role.

TABLE V

Nitrogen Metabolism during Administration of Ketoacids (KA) and during Subsequent Periods of Administration of Essential Amino Acids (AA)

Subject	Days	Rx	Net N intake	Urea N appearance	N balance	Urea clearance
			g/day	*g/day*	*g/day*	*ml/min*
J. W.	10–15	KA	4.69±0.28	2.03±0.19	+1.35±0.26	2.10±0.24
	16–20	AA	4.45±0.08	1.75±0.05	+1.14±0.06	2.47±0.10
	Change		−0.24±0.29	−0.28±0.20	−0.21±0.27	+0.37±0.26
	55–60	KA	1.18±0.24	0.11±0.07	−0.18±0.18	0.49±0.09
	61–65	KA	1.55±0.14	−0.09±0.07	+0.96±0.19	0.32±0.05
	66–71	KA	1.57±0.19	−0.14±0.05	+0.82±0.25	0.26±0.04
	72–77	AA	2.53±0.20	0.83±0.03	+0.47±0.21	0.17±0.03
	Change		0.96±0.28*	+0.97±0.06*	−0.35±0.33	−0.09±0.05
I. C.	21–26	KA	2.64±0.38	0.92±0.14	+0.95±0.36	1.21±0.09
	27–35	AA	2.65±0.30	1.75±0.26	+0.34±0.38	1.60±0.19
	Change		+0.01±0.48	+0.83±0.29*	−0.61±0.52	+0.39±0.21
	41–51	KA	2.87±0.26	1.24±0.12	+0.59±0.27	1.87±0.10
	52–61	KA	4.62±0.17	2.06±0.10	1.67±0.28	1.82±0.06
	62–66	AA	4.62±0.20	2.52±0.17	1.33±0.21	1.37±0.12
	Change		0.00±0.27	+0.46±0.19‡	−0.34±0.35	−0.45±0.13*

* Change is significant ($P < 0.02$).
‡ Change is significant ($P \simeq 0.03$).

TABLE VI

Nitrogen Metabolism in Four Patients during Administration of Ketoacids in the Presence of High Blood Urea Levels

Subject	Serum urea N	Days of ketoacid therapy	Net N intake	Urea N appearance	N balance	Urea clearance
	mg/100 ml		*g/day*	*g/day*	*g/day*	*ml/min*
E. W.	168	25	1.04	0.03	−1.74	0.32
			±0.21	±0.20	±0.13	±0.02
C. T.	204	8	1.52	0.93	−1.56	0.42
			±0.11	±0.40	±0.47	±0.02
J. F.	156	15	0.89	1.13	−2.16	0.52
			±0.10	±0.15	±0.57	±0.04
J. W.	174	5	1.57	2.55	−5.43	1.47
			±0.15	±0.23	±0.26	±0.10

Response of virtually anuric subjects to ketoacids. Subject J. W., as noted above, was maintained on ketoacids with no urea accumulation as his urea clearance fell from 0.49 to 0.17 ml/min over a period of several weeks (Table V).

In subject J. F., peritoneal dialysis was performed on days 31 and 36, resulting in a reduction of SUN from 206 to 65 mg/100 ml and serum creatinine from 32.4 to 14.4 mg/100 ml. He was then maintained on ketoacids for 26 days during which SUN rose only to 86 mg/100 ml while creatinine rose to 29.6 mg/100 ml (Fig. 5). Urine output during this period fell from 200 ml/day to zero. Nevertheless, he improved clinically and was discharged on ketoacids on day 43, returning for weekly clinical visits. He was oriented, alert, and free of vomiting during this period, consuming a diet containing 4 g protein (Table II). Total nitrogen intake was approximately 1.4 g/day. He was readmitted on day 58 because of development of mental confusion and symptoms of uremic pleuritis. After another dialysis he was again discharged, anuric, on day 70. He did well for 1 wk at home, with little rise in SUN as creatinine rose to 31.8 mg/100 ml. Ketoacid administration was then discontinued, and he died 10 days later with SUN 150 mg/100 ml, creatinine 42.3 mg/100 ml.

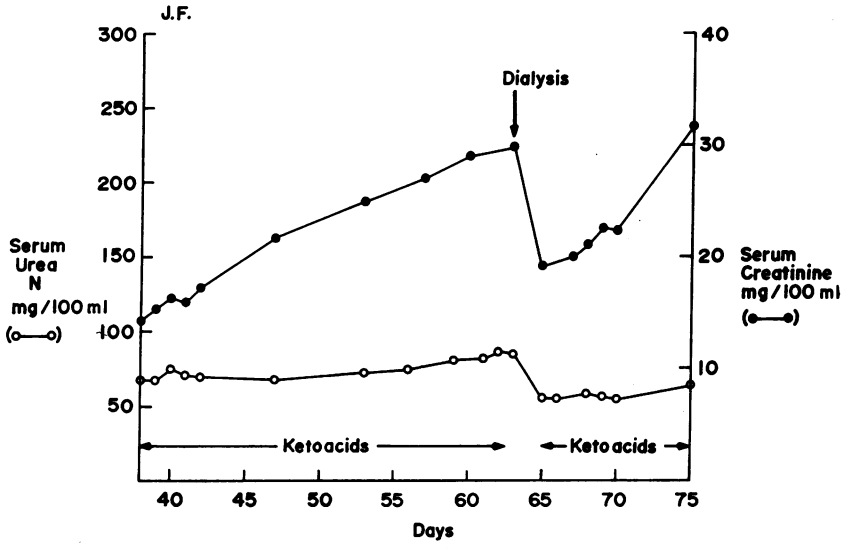

FIGURE 5 Course of patient J. F. during a month of ketoacid treatment. Urine output fell from 200 ml/day to zero during this period, as serum creatinine rose steadily. Nevertheless, SUN rose only 20 mg/100 ml and clinical improvement was sufficient to warrant out-patient management from days 40 to 53 and days 70 to 75. Dialysis was required on day 64 because of mental confusion and uremic pleuritis.

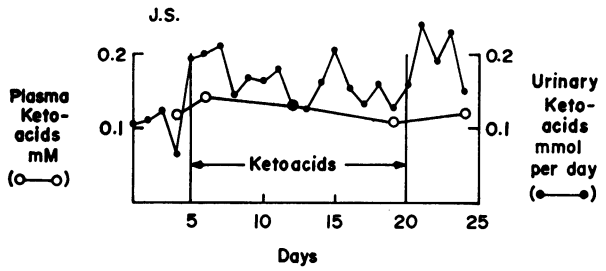

FIGURE 6 Plasma and urine ketoacids before, during, and after a period of ketoacid administration. No accumulation of ketoacids in plasma or urine during treatment is seen.

Although accurate balance observations were not made during most of this time, it is clear that the rate of urea appearance was markedly retarded. Clinical benefit was obtained, though obviously of a temporary nature.

Ketoacid accumulation. In five patients, fasting plasma and 24-h urine samples were analyzed for total ketoacids during and after treatment. No change could be detected (Fig. 6). The analytical method employed measures pyruvate and alpha-ketoglutarate as well as the compounds administered, and is therefore useful only to detect large changes. For example, in one patient with branched chain ketoaciduria, studied through the courtesy of Dr. Neil Holtzman, plasma ketoacids were 2 mM before treatment.

TABLE VII

Effect of Ketoacid Administration on Relative Clearances of Creatinine (Cr), Urea, and Uric Acid in Uremic Patients

Subject	Rx	C_{urea}/C_{Cr}	C_{urate}/C_{Cr}	Serum urate
				mg/100 ml
G. E.	KA	0.71±0.02	0.59±0.04	12.6
	None	0.80±0.02*	0.58±0.03	7.7
J. S.	KA	0.57±0.01	0.36±0.01	9.9
	None	0.57±0.02	0.35±0.01	10.1
L. W.	KA	0.58±0.01	0.46±0.02	8.9
	None	0.61±0.03	0.52±0.02	9.3
M. S.	KA	0.57±0.02	0.52±0.03	9.3
	None	0.66±0.02*	0.58±0.01*	10.2
J. W.	KA	0.50±0.02	0.21±0.01	7.0
	AA	0.50±0.01	0.19±0.01	7.4
	KA	0.46±0.01	0.40±0.01	7.5
	AA	0.50±0.04	0.56±0.05*	7.5
I. C.	KA	0.62±0.02	0.70±0.03	7.3
	AA	0.66±0.02	0.49±0.03‡	7.6
	KA	0.64±0.02	0.54±0.02	6.2
	AA	0.65±0.03	0.62±0.03*	6.5
E. W.	KA	0.65±0.03	0.81±0.08	13.6
C. T.	KA	0.64±0.02	0.50±0.04	13.8
J. F.	KA	0.62±0.01	0.61±0.04	10.2

* Increase is significant ($P < 0.02$).
‡ Decrease is significant ($P < 0.01$).

Effect of ketoacid administration on renal function. Table IV shows urea clearance during and after ketoacid administration in five patients. In two patients, significant increases occurred after withdrawing ketoacids. In one of them (G. E.), this increase was over 100%; in the other it was too small to be clinically significant. The explanation of the large increase in G. E. is not apparent. Urea clearance measured during a 4 day period which preceded the period of ketoacid administration in this patient was 0.49±0.08 ml/min; hence, ketoacid administration was not associated with any reduction in filtration rate.

Table V shows the progressive fall in urea clearance that occurred in patient J. W. The greatest fall, from 2.47 to 0.49 ml/min, occurred during an interval when he was receiving neither ketoacids nor amino acids. Progression during ketoacid administration clearly occurred; the most probable explanation is his underlying disease. J. F. also progressed from a urea clearance of 0.52 ml/min to anuria during 2 mo of intermittent ketoacid administration. Again there seems to be little reason to attribute this fall to the ketoacids.

The ratio of urea and uric acid clearances to creatinine clearance was measured daily in all subjects. The results are shown in Table VII. Very constant urea clearance ratios were observed, both within and between patients. In two patients, a small but statistically significant increase in urea: creatinine clearance ratio followed withdrawal of ketoacids. These observations would suggest that ketoacids can affect the tubular transport of urea, creatinine chromogens, or both. However, the changes are very small.

Urate: creatinine clearance ratio was significantly increased in two instances on withdrawing ketoacids, but was significantly decreased in two others. Here acid-base balance, water reabsorption, and other factors may have come into play.

Proteinuria, measured daily in all subjects, was unaffected by ketoacids. Creatininuria was also unaffected by ketoacids. However, a progressive fall occurred in creatinine output in J. W., from 650 to 110 mg/day, during the 77 day study. The rate of accumulation of creatinine in body fluids, calculated on the basis of a creatinine space of 40% body weight, did not amount to more than 70 mg/day. Thus creatinine appearance rate fell by at least 70%.

Other observations. Periodic measurements were also made during these studies of electrolytes, hematocrit, red blood cell count, white blood cell count and differential, serum total protein, albumin, magnesium, calcium, phosphorus, uric acid, transaminases, lactic dehydrogenase, creatinine phosphokinase, alkaline phos-

phatase, bilirubun, and prothrombin time. No effect of ketoacids on any of these could be discerned. Serum albumin and prothrombin time tended to rise when nitrogen balance was positive and to fall when it was negative. No improvement in anemia could be detected; several subjects required transfusions. However, blood was drawn nearly every day during the studies; this loss may well have contributed to the anemia.

Control of acidosis was usually easier during ketoacid administration. This is to be expected since sodium or calcium salts were used.

Undetermined anions, calculated as serum sodium minus chloride minus bicarbonate, were normal in subjects doing well clinically and were not increased by ketoacids. In patients doing poorly (such as those in Table VI) this quantity was high.

DISCUSSION

These observations appear to establish that the oral administration of alpha keto-analogues of essential amino acids can diminish the rate at which urea appears in the urine and in the body fluids of patients with severe chronic uremia. In three of four comparisons, these compounds were more effective in this respect than were the corresponding amino acids.

Because of the severe degree of impairment of glomerular filtration rate in these patients, reduction in urea appearance rate would be expected to bring about a substantial reduction in the degree of azotemia during long-term therapy. In one subject, SUN rose only 20 mg/100 ml during a month of virtual anuria.

The significance of urea in the clinical syndrome of uremia remains controversial (45). Thus an agent capable of reducing blood urea may or may not be beneficial, if this is its only action. But if, in addition, it can promote nitrogen anabolism by directing ammonia derived from intestinal ureolysis to protein synthesis, clinical improvement will almost certainly result.

The reduction in urea appearance rate can be explained in two ways: first, the intrinsic mechanisms for nitrogen conservation and reutilization may have been strengthened; second, the compounds may have been converted to amino acids.

The requirements of the organism for nitrogen depend chiefly on two processes: external losses of nitrogen, and internal destruction by catabolism of the carbon skeletons of the essential amino acids. If nitrogen derived from catabolism were wholly retained and if keto-analogues of essential amino acids, formed during transamination or oxidative deamination, were quantitatively converted back to amino acids, the requirement for exogenous protein would apparently cease. The administration of ketoacids might facilitate nitrogen conservation directly by restoring carbon skeletons lost by degradation. But they might also alter metabolic pathways so that endogenous ketoacids are more efficiently reutilized, either by means of enzyme induction or other mechanisms.

The present data do not establish whether the improvement in nitrogen balance was a consequence of altered metabolic pathways or a direct conversion of the administered compounds to essential amino acids.

The suggestion has been made that patients with uremia can conserve nitrogen more efficiently than normal persons, based upon the observation of positive nitrogen balance on protein intakes below minimal requirements for normal subjects (1, 8–10). The average rate of nonprotein nitrogen appearance in our patients during control periods was about 3 g/day, a value not distinctly below that seen in normal individuals (38).

Richards et al. (1) have suggested that the alleged ability of uremics to conserve nitrogen is attributable to accelerated rates of release of ammonia into the portal circulation, derived from intestinal ureolysis.

This hypothesis remains unproven. Furthermore, as shown here, increased blood urea does not facilitate nitrogen conservation; if anything the opposite is the case. Although it is widely held that urea breakdown is increased in absolute terms in uremic patients, the available data (2–7), considered *in toto,* do not establish this conclusion unequivocally.

In order to explore this possibility further, patient M.S. was readmitted for study a month after discontinuing ketoacids. During 4 days of observation on a net intake of 3.73±0.05 g nitrogen per day, urea nitrogen appearance was 1.90±0.12 g/day and nitrogen balance 0.74±0.17 g/day. This rate of urea nitrogen appearance is no greater than was seen immediately after discontinuing ketoacids, 1.73±0.07 g/day (Table IV). Thus no evidence could be obtained for an effect of ketoacids on nitrogen conservation persisting into the control period (unless this effect were still manifest a month later).

When ketoacid administration was followed by a period without supplements (Table IV), the average increase in urea nitrogen appearance, 1.55 g/day, was nearly equal to the average decrease in nitrogen balance, 1.73 g/day. Both quantities indicate the amount of nitrogen anabolism that can be attributed to ketoacids. The quantity of ketoacids given these subjects would have required only 0.57 g/day of nitrogen (on the average) to be converted to amino acids (calculated from Table III). Thus another 1 g/day (approximately) of nitrogen has become incorporated into protein from endogenous sources or from the diet. This is to be expected, since the five amino acids whose analogues were employed in most of these patients comprise only about one-third of the total amino acid residues in proteins such as hemoglobin or albumin (46).

Since there is no reason to expect that ketoacids should alter the rate at which urea is broken down in the gut (except in so far as the gradual fall in SUN might do so secondarily) it can probably be safely assumed that the reduction in urea appearance is entirely caused by a reduction in urea production. This fall, in turn, presumably reflects the altered fate of ammonia derived from urea breakdown: during ketoacid administration, about 1.6 g/day of the ammonia nitrogen is utilized for protein synthesis; afterwards, this amount is resynthesized into urea.

The efficiency of conversion of ketoacids to amino acids can only be surmised from these data. However, the comparison of ketoacids with amino acid supplements (Table V) gives more information. The average change in urea nitrogen appearance in these four studies is + 0.50 g/day, and the average change in nitrogen balance is − 0.38 g/day. The amount of nitrogen required to convert the administered ketoacids to amino acids averages 1.23 g/day. If the only ammonia utilization induced was that required to aminate the ketoacids themselves, the efficiency of amination is calculated to be 0.50/1.23 or 41%, and therefore 59% of the administered compounds was degraded. If any additional ammonia utilization was induced, the estimated efficiency of amination would be lower.

A possible limitation of this method of treatment could occur if SUN fell so far that ammonia release became inadequate. Measurements of ureolysis made in six of the patients studied here, to be reported elsewhere, show that the quantity of urea nitrogen broken down was in every case greater than the calculated rate at which ammonia nitrogen was utilized. Thus this problem did not arise. If it did arise on continued treatment, microencapsulated urease (47), administered by mouth, could be used to increase intestinal ureolysis. In theory, external loss of urea could in this way be reduced to any desired level.

The risks of this mode of therapy seem low. All of these compounds are presumably normal constituents of the body, even though few have been isolated (48). Their toxicity is unknown. However, in branched chain ketoaciduria ("maple syrup urine disease"), accumulation of keto-val, keto-leu, and keto-ile is believed by some workers to be responsible for the impaired mental development and convulsions which characterize this disorder (49). No accumulation approaching the levels seen in this disorder could be detected in our patients.

Most of these studies were conducted using only four or five of the seven potentially useful compounds. Dosage was varied from one subject to another. Hence the potentialities of this mode of therapy have yet to be fully explored. Techniques for economically synthesizing keto-trp and keto-his have yet to be developed. The analogues of lysine and threonine, though known chemically (48), have rarely been studied biologically (50). From earlier data (48) it would appear that these two amino acids do not participate in transamination reactions, and that therefore their keto-analogues would not become aminated. However, this question may deserve to be reexamined in animals on low protein diets.

The acceptability to patients of a low protein diet and of taking gelatin capsules every day is doubtless variable. None of our patients complained vigorously about the capsules, but some rebelled at the monotonous diet. The low protein foods now available are palatable, but certainly do not provide an interesting fare day after day.

Further study of ketoacid treatment as a substitute for, or in conjunction with intermittent dialysis, is indicated.

ACKNOWLEDGMENTS

We are greatly indebted to Mrs. Elizabeth Chandler for planning the diets, and to Mrs. Sylvia Butler, Mrs. Valerie Hammond, Mrs. Patricia Margulies, and Mr. Kang Sun Lee, for technical assistance.

This work was supported by a Research Grant (AM 15603), an Undergraduate Training Grant (HE 00260), and a Clinical Center Grant (RR 35) from the National Institutes of Health.

REFERENCES

1. Richards, P., A. Metcalfe-Gibson, E. E. Ward, O. Wrong, and B. J. Houghton. 1967. Utilisation of ammonia nitrogen for protein synthesis in man, and the effect of protein restriction and uraemia. *Lancet.* **2**: 845.
2. Walser, M., and L. J. Bodenlos. 1959. Urea metabolism in man. *J. Clin. Invest.* **38**: 1617.
3. Jones, E. A., R. A. Smallwood, A. Craigie, and V. M. Rosenoer. 1969. The enterohepatic circulation of urea nitrogen. *Clin. Sci. (Oxf.).* **37**: 825.
4. Robson, A. M., R. Ashcroft, B. A. Clarkson, D. B. Horn, and D. N. S. Kerr. 1964. Diet and anabolic steroids in acute renal failure. *In* Acute Renal Failure. S. Shaldon and G. C. Cook, editors. F. A. Davis Company, Philadelphia. 111.
5. Deane, N., W. Desir, and T. Umeda. 1968. The production and extrarenal metabolism of urea in patients with chronic renal failure treated with diet and dialysis. *In* Dialysis and Renal Transplantation: Proceedings of the Fourth Conference of the European Dialysis and Transplant Association. D. N. S. Kerr, editor. Excerpta Medica Foundation, New York. 245.
6. Scholz, A. 1968. Investigations on the distribution and turnover rate of ^{14}C-urea and tritiated water in renal failure. *In* Dialysis and Renal Transplantation: Proceedings of the Fourth Conference of the European Dialysis and Transplant Association. D. N. S. Kerr, editor. Excerpta Medica Foundation, New York. 240.
7. Walser, M. 1970. Use of isotopic urea to study the distribution and degradation of urea in man. *In* Urea and the Kidney. B. Schmidt-Nielsen, editor. Excerpta Medica Foundation, Publishers, Amsterdam. 421.
8. Giordano, C. 1963. Use of exogenous and endogenous urea for protein synthesis in normal and uremic subjects. *J. Lab. Clin. Med.* **62**: 231.

9. Giovannetti, S., and Q. Maggiore. 1964. A low-nitrogen diet with proteins of high biological value for severe chronic uraemia. *Lancet.* **1**: 1000.
10. Berlyne, G. M., and A. G. Hocken. 1968. The dietary treatment of chronic renal failure. *In* Nutrition and Renal Disease. G. M. Berlyne, editor. The Williams & Wilkins Company, Baltimore. 38.
11. Underhill, F. P., and S. Goldschmidt. 1913. Studies on the metabolism of ammonium salts. III. The utilization of ammonium salts with a non-nitrogenous diet. *J. Biol. Chem.* **15**: 341.
12. Rose, W. C., and E. E. Dekker. 1956. Urea as a source of nitrogen for the biosynthesis of amino acids. *J. Biol. Chem.* **223**: 107.
13. Wrong, O. 1967. The metabolism of urea and ammonia in the healthy and uraemic colon. *Med. J. Aust.* **2**: 281.
14. Fürst, P., B. Josephson, G. Maschio, and E. Vinnars. 1969. Nitrogen balance after intravenous and oral administration of ammonium salts to man. *J. Appl. Physiol.* **26**: 13.
15. Read, W. W. C., D. S. McLaren, M. Tchalian, and S. Nassar. 1969. Studies with ^{15}N-labelled ammonia and urea in the malnourished child. *J. Clin. Invest.* **48**: 1143.
16. Nicholson, J. F. 1970. Metabolism of ingested ammonium-^{15}N by premature infants. *Pediatr. Res.* **4**: 398.
17. Tripathy, K., S. Klahr, and H. Lotero. 1970. Utilization of exogenous urea nitrogen in malnourished adults. *Metab. (Clin. Exp.).* **19**: 253.
18. Gallina, D. L., and J. M. Dominguez. 1971. Human utilization of urea nitrogen in low calorie diets. *J. Nutr.* **101**: 1029.
19. Harrow, B., and C. P. Sherwin. 1926. Synthesis of amino acids in the animal body. IV. Synthesis of histidine. *J. Biol. Chem.* **70**: 683.
20. Berg, C. P., W. C. Rose, and C. S. Marvel. 1929. Tryptophane and growth. III. 3-indolepropionic acid and 3-indolepyruvic acid as supplementing agents in diets deficient in tryptophane. *J. Biol. Chem.* **85**: 219.
21. Jackson, R. W. 1929. Indole derivatives in connection with a diet deficient in tryptophane. II. *J. Biol. Chem.* **84**: 1.
22. Cahill, W. M.. and G. M. Rudolph. 1942. The replaceability of *dl*-methionine in the diet of the rat with its α-keto-acid analogue. *J. Biol. Chem.* **145**: 201.
23. Bubl, E. C., and J. S. Butts. 1949. The utilization of some phenylpyruvic acids for growth in the rat. *J. Biol. Chem.* **180**: 839.
24. Meister, A. 1951. Studies on *d*- and *l*-α-keto-β-methylvaleric acids. *J Biol. Chem.* **190**: 269.
25. Wretlind, K. A. J. 1953. Replacement of valine in the diet by α-ketoisovaleric acid. *Acta Physiol. Scand.* **27**: 183.
26. Wood, J. L., and S. L. Cooley. 1954. Substitution of α-keto acids for five amino acids essential for growth of the rat. *Proc. Soc. Exp. Biol. Med.* **85**: 409.
27. Richards, P., B. J. Houghton, C. L. Brown, and E. Thompson. 1971. Synthesis of phenylalanine and valine by healthy and uraemic men. *Lancet.* **2**: 128.
28. Giordano, C., C. De Pascale, M. E. Phillips, N. G. De Santo, P. Fürst, C. L. Brown, B. J. Houghton, and P. Richards. 1972. Utilisation of keto-acid analogues of valine and phenylalanine in health and uraemia. *Lancet.* **1**: 178.
29. Rudman, D. 1971. Capacity of human subjects to utilize keto analogues of valine and phenylalanine. *J. Clin. Invest.* **50**: 90.
30. Gallina, D. L., J. M. Domínguez, J. C. Hoschoian, and J. R. Barrio. 1971. Maintenance of nitrogen balance in a young woman by substitution of α-ketoisovaleric acid for valine. *J. Nutr.* **101**: 1165.
31. Norpoth, L. 1948. Über stickstoffhalfige, insbesondere eiweissartige Substanzen in Mageninhalt. *Klin. Wochenschr.* **26**: 406.
32. Hegsted, D. M. 1968. Normal protein requirements. *In* Nutrition in Renal Disease. G. M. Berlyne, editor. The Williams & Wilkins Company, Baltimore. 1.
33. Calloway, D. H., and S. Margen. 1971. Variation in endogenous nitrogen excretion and dietary nitrogen utilization as determinants of human protein requirement. *J. Nutr.* **101**: 205.
34. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193**: 265.
35. Weygand, F., W. Steglich, and H. Tanner. 1962. Eine neue Methode zur Umwandlung von alpha-Aminosaüren in alpha-Ketosäuren. *Justus Liebigs Ann. Chem.* **658**: 128.
36. De Santo, N. G., C. De Pascale, R. Esposito, C. Balestrieri, and C. Giordano. 1968. Effetti dell'is tidina sulla sintesi di emoglobina in vitro nell'uremia. *Biochim. Appl.* **15**: 556.
37. Bergström, J., P. Fürst, B. Josephson, and L-O. Norée. 1970. Improvement of nitrogen balance in a uremic patient by the addition of histidine to essential amino acid solutions given intravenously. *Life Sci. Part II Biochem. Gen. Mol. Biol.* **9**: 787.
38. Irwin, M. I., and D. M. Hegsted. 1971. A conspectus of research on amino acid requirements of man. *J. Nutr.* **101**: 539.
39. Walser, M., P. Lund, N. Ruderman, A. W. Coulter, and H. A. Krebs. 1972. Formation of essential amino acids from their α-keto analogues by perfused liver and muscle. *Clin. Res.* **20**: 634. (Abstr.)
40. Hegsted, D. M. 1964. Protein requirements. *In* Mammalian Protein Metabolism. H. N. Munro and J. B. Allison, editors. Academic Press, Inc., New York. **2**: 135.
41. Young, V. R., and N. S. Scrimshaw. 1968. Endogenous nitrogen metabolism and plasma free amino acids in young adults given a "protein-free" diet. *Br. J. Nutr.* **22**: 9.
42. Adibi, S. A., E. D. Livi, and P. M. Amin. 1971. Alteration in the urinary excretion rate of amino acids and nitrogen by dietary means in obese and normal human subjects. *J. Lab. Clin. Med.* **77**: 278.
43. Owen, O. E., P. Felig, A. P. Morgan, J. Warren, and G. F Cahill, Jr. 1969. Liver and kidney metabolism during prolonged starvation. *J. Clin. Invest.* **48**: 574.
44. Lacy, W. W. 1969. Effect of acute uremia on amino acid uptake and urea production by perfused rat liver. *Am. J. Physiol.* **216**: 1300.
45. Johnson, W. J., W. H. Hagge, R. D. Waggoner, R. P. Dinapoli, and J. W. Rosevar. 1972. Effects of urea loading in patients with far-advanced renal failure. *Mayo Clin. Proc.* **47**: 21.
46. Fruton, J. S., and S. Simmonds. 1958. General Biochemistry. John Wiley & Sons, Inc., New York. 2nd edition. 125.

47. Chang, T. M. S., F. C. MacIntosh, and S. G. Mason. 1966. Semipermeable aqueous microcapsules. I. Preparation and properties. *Canad. J. Physiol. Pharmacol.* **44**: 115.
48. Meister, A. 1965. Biochemistry of the Amino Acids. Academic Press, Inc., New York.
49. Gaull, G. E 1969. Pathogenesis of maple-syrup-urine disease: observations during dietary management and treatment of coma by peritoneal dialysis. *Biochem. Med.* **3**: 130.
50. Pond, W. G., L. H. Breuer, Jr., J. K. Loosli, and R. G. Warner. 1964. Effects of the α-hydroxy analogues of isoleucine, lysine, threonine, and tryptophan and the α-keto analogue of tryptophan and the level of the corresponding amino acids on growth of rats. *J. Nutr.* **83**: 85.
51. Rose, W. C., R. L. Wixom, H. B. Lockhardt, and G. F. Lambert. 1955. The amino acid requirements of man. XV. The valine requirement; summary and final observations. *J. Biol. Chem.* **217**: 987.