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O P Ganda, … , R S Morrison, G F Cahill Jr

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Research Article

Arterial blood concentrations of insulin, glucagon, and various substrates were determined in six anephric subjects in the postabsorptive state and immediately after hemodialysis. Plasma glucose and serum insulin concentrations were normal, and declined during dialysis. Plasma glucagon was elevated and remained unchanged. There was moderate hypertriglyceridemia before dialysis, but this decreased significantly after administration of heparin just before the start of dialysis, and at the end of dialysis was lowered further into the normal range. Comparison of postabsorptive whole blood concentrations of amino acids with those in normal, healthy adults revealed striking differences. Glutamine, proline, citrulline, glycine and both 1- and 3-methyl-histidines were increased, while serine, glutamate, tyrosine, lysine, and branched-chain amino acids were decreased. The glycine/serine ratio was elevated to 300% and tyrosine/phenylalanine ratio was lowered to 60% of normal. To investigate the potential role of blood cells in amino acid transport, the distribution of individual amino acids in plasma and blood cell compartments was studied. Despite a markedly diminished blood cell mass (mean hematocrit, 20.6 +/- 1.4%), there was no significant decrease in the fraction of most amino acids present in the cell compartment, and this was explained by increases of several amino acids in cellular water. None were decreased. Furthermore, during dialysis, whole blood and plasma amino acids declined by approximately 30% and 40%, respectively, whereas no […]

Hormone-Fuel Concentrations in Anephric Subjects

EFFECT OF HEMODIALYSIS (WITH SPECIAL REFERENCE TO AMINO ACIDS)

OM P. GANDA, THOMAS T. AOKI, J. STUART SOELDNER, ROBERT S. MORRISON, and GEORGE F. CAHILL, JR.

From the Elliott P. Joslin Research Laboratory, Department of Medicine, Harvard Medical School and Peter Bent Brigham Hospital, Boston, Massachusetts 02215 and Department of Medicine, Lemuel Shattuck Hospital, Boston, Massachusetts 02130

ABSTRACT Arterial blood concentrations of insulin, glucagon, and various substrates were determined in six anephric subjects in the postabsorptive state and immediately after hemodialysis. Plasma glucose and serum insulin concentrations were normal, and declined during dialysis. Plasma glucagon was elevated and remained unchanged. There was moderate hypertriglyceridemia before dialysis, but this decreased significantly after administration of heparin just before the start of dialysis, and at the end of dialysis was lowered further into the normal range.

Comparison of postabsorptive whole blood concentrations of amino acids with those in normal, healthy adults revealed striking differences. Glutamine, proline, citrulline, glycine and both 1- and 3-methyl-histidines were increased, while serine, glutamate, tyrosine, lysine, and branched-chain amino acids were decreased. The glycine/serine ratio was elevated to 300% and tyrosine/phenylalanine ratio was lowered to 60% of normal.

To investigate the potential role of blood cells in amino acid transport, the distribution of individual amino acids in plasma and blood cell compartments was studied. Despite a markedly diminished blood cell mass (mean hematocrit, 20.6±1.4%), there was no significant decrease in the fraction of most amino acids present in the cell compartment, and this was explained by increases of several amino acids in cellular water. None were decreased. Furthermore, during dialysis, whole blood and plasma amino acids declined by approximately 30% and 40%, respectively, whereas no significant change was observed in the cell compartment. Alanine was the only amino acid whose concentration declined in the cells as well as in plasma.

The results indicate (*a*) significant alterations in the concentrations of hormones and substrates in patients on chronic, intermittent hemodialysis; (*b*) removal of amino acids during hemodialysis, predominantly from the plasma compartment, with no significant change in cell content; and (*c*) a redistribution of amino acids in plasma and blood cell compartments with increased gradients of most of the amino acids per unit cell water, by mechanism(s) as yet undetermined.

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INTRODUCTION

A wide variety of metabolic disturbances are known to take place in patients with chronic renal disease (1–3). Defects in the regulation of insulin (4), glucagon (5), and growth hormone (6), as well as alterations in the metabolism of carbohydrates (7), proteins (8), and lipids (9) have been observed. While some of these abnormalities are, to a variable degree, reversible by chronic treatment with dialysis, the immediate effects of this "therapeutic" intervention on the concentrations of hormones and substrates have not received much attention. This is of particular relevance to amino acids which, due to their relatively small molecular weight, are susceptible to being "washed out" during several hours of each dialysis period (10–13).

Recently, a dynamic role of blood cells in amino acid transport has been emphasized (14–16), and alterations in the distribution of amino acids between blood cells and plasma have been found in several clinical disorders, including protein-calorie malnutrition (17). In stud-

TABLE I

Clinical Data of the Patients

Patient	Age	Sex	Wt	Hct pre/post	BUN pre/post	Creat pre/post	K pre/post	Alb	Diagnosis	Date dialysis started	Date of nephrectomy	C	P	Diet fat	Cal	Protein intake
	yr		*kg*	%	*mg/100 ml*	*mg/100 ml*	*meq/liter*	*g/100 ml*					*g/day*		*day*$^{-1}$	*g/kg*
H. J.	46,	F	51	13/13	75/19	12.8/4.8	6.2/3.3	3.6	CGN	7/69	9/69	200	55	75	1,700	1.08
D. P.	39,	M	69	22/25	95/30	15.4/6.5	6.1/3.5	3.5	PRD	3/69	4/70	200	80	125	2,245	1.16
J. B.	29,	M	64	16/18	69/29	14.4/7.3	5.5/3.6	3.3	CGN	8/71	1/73	180	85	105	2,005	1.33
E. M.	28,	F	48	26/27	42/9	8.7/3.6	4.5/3.4	4.5	CGN	8/70	10/70	290	70	90	2,250	1.46
S. B.	57,	F	58	19/23	75/18	13.5/5.1	5.3/3.4	4.5	PRD	4/67	8/72	120	60	85	1,485	1.03
D. H.	37,	M	55	21/24	51/12	12.0/4.0	4.9/3.0	3.0	Severe NSC	12/73	1/74	250	90	100	2,260	1.64

Hct, hematocrit; BUN, blood urea nitrogen; Creat, serum creatinine; K, serum potassium; Alb, serum albumin; pre/post, predialysis/postdialysis; C, carbohydrate; P, protein; Cal, approx. total calories per day; CGN, chronic glomerulonephritis; PRD, polycystic renal disease; NSC, nephrosclerosis.
Studies were performed between 7/74 and 9/74.

ies so far available on uremic patients, only plasma concentrations of amino acids were determined (10, 11, 18–24). Therefore, the potential role, if any, of the blood cell compartment in amino acid transport in such patients remains uncertain.

In this study, the serum or plasma concentrations of insulin, glucagon, and various substrates in blood, including glucose, pyruvate, lactate, lipids, and individual amino acids (whole blood and plasma) were determined immediately before and after dialysis in a group of anephric subjects maintained on chronic, intermittent hemodialysis. The distribution of amino acids in plasma and blood cell compartments was analyzed and the acute alterations induced by dialysis were examined.

METHODS

Six surgically nephrectomized subjects, three men and three women, undergoing chronic intermittent hemodialysis at the Lemuel Shattuck Hospital, Boston, Mass., and the Lakeville Hospital, Lakeville, Mass., were studied. The clinical data are summarized in Table I. The subjects were on the dialysis program for periods varying between 1 and 7 yr and all had been bilaterally nephrectomized 8 mo–4½ yr previously. The nutritional status of each subject was carefully evaluated by a research dietician by assessing the diet consumed over several weeks before study. As shown in Table I, each patient was on a balanced, eucaloric diet, containing 1.08–1.64 g protein/kg body wt/day. Approximately three-fourths of the protein intake was of high biologic value.

All patients had chronic anemia, the mean hematocrit being 20.6±1.4%, mean±SEM (range 13–26%); none had hypoalbuminemia. There was no history of diabetes mellitus in any of the subjects. Medications included multivitamins, folic acid, and Amphojel (Wyeth Laboratories, Div. of American Home Products, Philadelphia, Pa.). J. B. was also taking fluoxymesterone, 5 mg daily.

Each subject underwent hemodialysis (capillary-type coil kidney apparatus; EX-21) for 6–7 h three times a week. The dialysis fluid was delivered at 97–99°F at a rate of 500 ml/min and contained: 130 meq/liter sodium, 2.0 meq/liter potassium, 3.0 meq/liter calcium, 1.5 meq/liter magnesium, 101.5 meq/liter chloride, and 35 meq/liter acetate, without glucose. Each subject received systemic heparin during dialysis in the usual dosage, i.e. 3,000 U as a bolus at the beginning, followed by 3,000–5,000 U/h for the duration of dialysis.

Informed written consent was obtained from each subject. On the day of study, the patients were asked to arrive in the dialysis unit in the postabsorptive state after a 12-h overnight fast. Food was withheld until the end of dialysis. Blood, plasma, and serum samples for glucose, insulin, glucagon, cholesterol, triglycerides, pyruvate, lactate, α-ketoglutarate, ammonia, and amino acids were obtained from the arterial end of the pre-existing arteriovenous shunt for dialysis in each subject 5–10 min before starting dialysis and within 5 min after the end of dialysis. Samples for cholesterol and triglyceride determinations were also obtained in each subject 5–6 min after the initial rapid heparin administration, before starting dialysis.

Preparation and analyses of the samples for whole blood and plasma amino acids, whole blood lactate, and pyruvate have been described elsewhere (15, 25). Plasma glucose was determined by a β-glucose-oxidase technique with the Beckman glucose analyzer (Beckman Instruments, Fullerton, Calif.), serum immunoreactive insulin (IRI) [1] by a double antibody technique (26), plasma immunoreactive glucagon (IRG) by a modification (27) of the method (28) employing 30K antibody, kindly supplied by Dr. Roger Unger (Dallas, Texas), blood ammonia by a modified enzymatic assay (29), blood α-ketoglutarate by the enzymatic method of Bergmeyer and Bernt (30), and plasma cholesterol and triglycerides by Technicon II dual-channel Auto-Analyzer (Technicon Instruments Corp., Tarrytown, N. Y.) (31).

A macrohematocrit was obtained in duplicate on each blood sample. From the whole blood and plasma concentrations of each amino acid, the content in plasma compartment in a liter of whole blood was calculated as (100 − hematocrit) (0.01) (micromoles per liter of plasma). The amino acid content in the blood cells was then calculated in a given sample: cell content = whole blood concentration − plasma content.

The individual amino acid concentrations in whole blood and their fractional distribution in plasma and blood cells in the patients were compared with the data previously ob-

[1] *Abbreviations used in this paper:* IRG, immunoreactive glucagon; IRI, immunoreactive insulin.

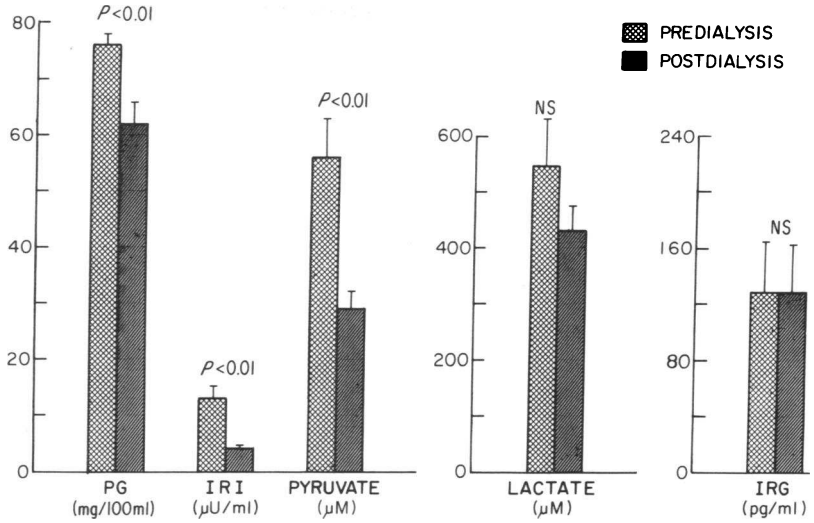

FIGURE 1 Arterial plasma glucose (PG), serum IRI, plasma IRG, blood pyruvate, and blood lactate immediately before and after dialysis in anephric subjects on chronic, intermittent hemodialysis ($n = 6$). Mean±SEM, NS, not significant.

tained in this laboratory in normal, adult subjects in the postabsorptive state (*32*) by the same technique of analysis. The hematocrits in the normal subjects varied in a close range between 43 and 46%. Finally, the individual amino acid concentrations in the blood cell-water compartment were calculated for each patient, by assuming 65% of the erythrocytes as water (*33*), and compared with those similarly derived for the normal subjects.

Statistical analyses were performed by employing Student's t tests (*34*) and the Wilcoxon rank sum test (*35*) as appropriate. The results are expressed as mean±SEM.

RESULTS

Glucose, IRI, IRG, pyruvate, and lactate (*Fig. 1*). The fasting plasma glucose was 76±2.0 mg/100 ml and IRI 13±2.0 μU/ml (normal postabsorptive concentration in this laboratory (*26*), 8.4±0.35 μU/ml, $n = 75$, mean±SEM). The mean IRG was considerably elevated at 127±36.0 pg/ml ($P < 0.02$ compared to the normal postabsorptive concentration in this laboratory; 64±8.3 pg/ml, $n = 18$). One of the patients (J. B.) had no detectable glucagon in the plasma after corrections for blank value (*27*). If this subject is excluded, the mean of the remaining five subjects was greater than the normals to a more significant degree (152±31.4, $P < 0.001$). The pyruvate and lactate concentrations were normal at 56±7.0 μM and 545±83 μM, respectively. At the end of dialysis, there was a significant decline in the concentration of plasma glucose ($P < 0.01$), IRI ($P < 0.01$), and pyruvate ($P < 0.01$). There was no significant change in IRG and lactate.

The mean α-ketoglutarate (16±2.0 μM) and ammonia (77±7.0 μM) levels were normal and did not change after dialysis (18±1.0 μM and 76±10 μM, respectively).

Plasma cholesterol and triglycerides (*Fig. 2*). Fig. 2 depicts the triglyceride levels before and 5 min after initiation of heparin administration and at the end of dialysis. The mean cholesterol was 184±20 mg/100 ml and

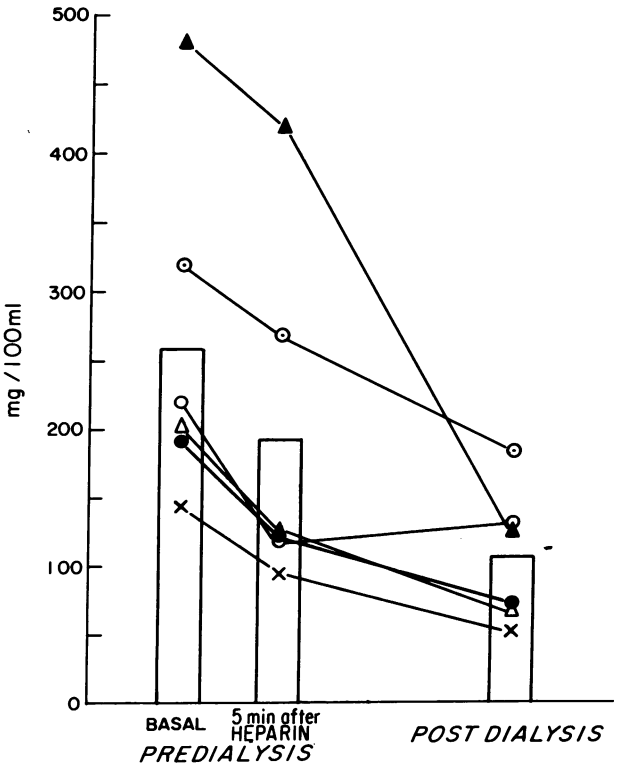

FIGURE 2 Arterial plasma triglyceride concentrations immediately before and after dialysis in anephric subjects on chronic, intermittent hemodialysis. The bars represent mean levels. Each symbol represents an individual subject.

TABLE II

Arterial Amino-Acid Concentrations (Mean±SEM) in Anephric Subjects (n = 6), Compared with Normal Subjects (n = 8)

	Whole blood concentration				Plasma content				Blood-cell content		
	Anephrics	Normals	P		Anephrics	Normals	P		Anephrics	Normals	P
Decreased				Decreased				Decreased			
Serine	104±13	176±11	<0.01	α-Aminobutyrate	10±2	16±1	<0.02	Serine	38±6	95±9	<0.001
Glutamate	112±15	152±6	<0.05	Valine	105±11	136±7	<0.05	Glutamate	80±9	121±5	<0.01
Valine	151±12	230±12	<0.001	Leucine	49±5	76±7	<0.02	Valine	46±14	94±10	<0.02
Isoluecine	42±3	60±4	<0.01	Tyrosine	19±2	30±2	<0.01	Leucine	27±10	51±5	<0.05
Leucine	76±9	127±9	<0.01					Tyrosine	8±3	25±2	<0.001
Tyrosine	28±4	56±4	<0.001								
Lysine	145±17	187±8	<0.05								
Increased				Increased				Increased			
Glutamine	888±98	650±22	<0.05	Threonine	127±12	82±11	<0.02	Citrulline	37±10	18±3	<0.05
Proline	302±36	179±8	<0.01	Asparate	38±3	ND		1-CH_3-Histidine	19±9	ND	*
Citrulline	110±6	36±4	<0.001	Glutamine	623±20	301±13	<0.001	3-CH_3-Histidine	7±3	ND	*
Glycine	607±83	321±17	<0.01	Proline	210±42	105±8	<0.02				
1-CH_3-Histidine	51±12	ND	*	Citrulline	72±6	19±2	<0.001				
3-CH_3-Histidine	19±2	ND	*	Glycine	376±69	124±9	<0.01				
				Alanine	220±20	142±14	<0.01				
				1-CH_3-Histidine	33±9	ND	*				
				3-CH_3-Histidine	12±2	ND	*				
				Arginine	62±7	41±3	<0.001				
Unchanged				Unchanged				Unchanged			
Taurine	148±27	154±19	NS	Taurine	32±6	24±1	NS	α-Aminobutyrate	4±3	9±2	NS
Aspartate	132±19	130±11	NS	Serine	66±10	81±8	NS	Taurine	116±24	130±19	NS
Threonine	206±36	139±11	NS	Glutamate	33±7	31±2	NS	Aspartate	94±17	130±11	NS
Alanine	339±19	279±20	NS	Isoleucine	27±4	36±4	NS	Threonine	79±27	57±6	NS
Phenylalanine	40±5	49±2	NS	Phenylalanine	27±1	29±2	NS	Glutamine	265±73	349±28	NS
Ornithine	99±18	95±7	NS	Ornithine	47±13	33±2	NS	Proline	92±21	74±6	NS
Histidine	84±10	90±4	NS	Lysine	88±9	106±9	NS	Glycine	232±41	197±13	NS
Arginine	92±17	57±6	NS	Histidine	48±6	51±4	NS	Alanine	120±17	137±10	NS
α-Aminobutyrate	14±4	24±3	NS					Isoleucine	15±5	25±2	NS
								Phenylalanine	12±5	20±2	NS
								Ornithine	52±9	63±7	NS
								Lysine	57±15	81±5	NS
								Histidine	36±8	39±4	NS
								Arginine	29±13	17±3	NS

All values are in micromoles per liter of whole blood. Plasma and cell contents were as described in text. Anephric subjects were on chronic intermittent hemodialysis. ND, not detectable.

* A significant increase, because not detectable in normal subjects.

did not change significantly after the priming injection of heparin (175±24 mg/100 ml) or at the end of dialysis (171±18 mg/100 ml). The triglycerides, on the other hand, were elevated at 259±50 mg/100 ml (range, 143–480 mg/100 ml) and declined to 191±52 mg/100 ml ($P < 0.001$,, paired t test) 5 min after the priming injection of heparin. There was a further decline of triglycerides to 105±20 mg/100 ml by the end of dialysis, but this value was not significantly different when compared to the level achieved 5 min after initial heparin administration.

Amino acids. The whole blood concentrations of free amino acids in the postabsorptive state and the fractional contents of the respective amino acids in the plasma and cell compartments were compared with the data obtained on postabsorptive normal subjects. These comparisons are presented in Table II. The sum of each amino acid in plasma and cell compartment equals the whole blood concentration per liter for that amino acid.

As indicated in the table, whole blood concentrations of glutamine, proline, glycine, citrulline, 1-methyl-histidine, and 3-methyl-histidine were significantly increased. The contents of only the last three amino acids were significantly elevated in the blood cell compartment, whereas the plasma compartment had increases in threonine, aspartate, alanine, and arginine in addition to those found increased in whole blood. In contrast, the concentrations of serine, glutamate, valine, isoleucine, leucine, tyrosine, and lysine in whole blood were significantly decreased. In cells, serine, glutamate, valine, leucine, and tyrosine, and in plasma, valine, leucine, tyrosine, and α-aminobutyrate were found to be decreased. The rest of the amino acids in whole blood and the respective plasma and cell contents were unchanged.

Table III depicts the changes in the mean whole blood concentrations of individual amino acids and their plasma and cell contents in response to dialysis. Most of the amino acids were strikingly decreased in whole

blood and plasma. The notable exceptions were glutamine, which was elevated before dialysis and remained unchanged in whole blood, and isoleucine and leucine, the last being increased after dialysis, particularly in the plasma compartment ($P < 0.02$). In contrast, in the blood cell compartment, none of the amino acids showed a significant change, with two exceptions. Glutamate increased and this increase was also noted in whole blood, and alanine showed a significant decrease, like its decrease in plasma and whole blood. Overall, there was approximately a 30% decrease ($P < 0.01$) in total amino acid concentration in whole blood and a 40% decrease ($P < 0.01$) in plasma content in response to dialysis, while the content in cell compartment revealed no significant change.

Table IV presents the blood cell-water concentration of individual amino acids and compares these with those of normal subjects in whom the blood cell-water concentration was similarly calculated. In view of the wide variability in the concentrations in patients, the significance of differences between the two groups were determined by the Wilcoxon rank sum test (35). As apparent from the table, the calculated concentrations in the water compartment of cells were significantly elevated for 10 of the 22 amino acids when compared to normals, and remained elevated for most of these amino acids even after dialysis. The concentrations of the other amino acids were not significantly different, and none of the amino acids was decreased. When pre- and postdialysis concentrations were compared, only alanine revealed a significant decrease; the remainder of the amino acids were unaltered.

DISCUSSION

Patients with chronic renal disease have been found to manifest a diverse variety of disturbances in hormones and substrates involving carbohydrate, protein, and lipid metabolism (1–9). The present study was designed to evaluate the blood concentrations of hormones and substrates in nephrectomized subjects and to assess the immediate response to hemodialysis. Although all subjects were undergoing dialysis three times a week for a variable length of time, abnormalities of several parameters were observed in the postabsorptive state, less than 48 h after their last dialysis.

Fasting plasma glucose and insulin concentrations were normal and within a narrow range, whereas others (7) have observed that the glucose-insulin interrelationships can still be abnormal in response to alimentation in these subjects. Food was withheld during the dialysis on the day of the study and there was no glucose in the dialysis fluid. After dialysis, all subjects showed a modest decline in the plasma glucose concentration, perhaps reflecting limited glucogenic reserves. A diminished glucose production rate and alanine turnover may exist in chronic renal insufficiency (36). Since alanine

TABLE III

Arterial Concentrations (Mean±SEM) of Amino Acids Immediately Before and After Dialysis in Anephric Subjects (n = 6)

	Whole blood concentration			Plasma content			Blood-cell content		
	Pre-dialysis	Post-dialysis	*P*	Pre-dialysis	Post-dialysis	*P*	Pre-dialysis	Post-dialysis	*P*
Taurine	148±27	143±30	NS	32±6	20±3	NS	116±24	123±28	NS
Aspartate	132±19	102±16	<0.01	38±3	14±1	<0.001	94±17	88±16	NS
Threonine	206±36	136±23	<0.01	127±12	75±11	<0.001	79±27	61±12	NS
Serine	104±13	81±6	NS	66±10	38±3	<0.02	38±6	43±5	NS
Glutamine	888±98	841±37	NS	623±20	529±20	<0.02	265±73	312±33	NS
Glutamate	112±15	124±17	<0.05	33±7	32±7	NS	80±9	91±12	<0.05
Proline	302±36	160±13	<0.01	210±42	92±10	<0.05	92±21	68±9	NS
Citrulline	110±6	60±4	<0.001	72±6	26±3	<0.01	37±10	34±4	NS
Glycine	607±83	335±41	<0.01	376±69	176±25	<0.02	232±41	158±30	NS
Alanine	339±19	135±11	<0.001	220±20	64±4	<0.001	120±17	70±8	<0.05
α-Aminobutyrate	14±4	11±3	NS	10±2	7±2	NS	4±3	5±2	NS
Valine	151±12	122±14	<0.001	105±11	73±7	<0.02	46±14	49±9	NS
Isoleucine	42±3	48±6	NS	27±4	31±2	NS	15±5	17±4	NS
Leucine	76±9	97±14	NS	49±5	61±6	<0.02	27±10	36±9	NS
Tyrosine	28±4	21±2	<0.05	19±2	12±1	<0.01	8±3	9±1	NS
Phenylalanine	40±5	35±3	NS	27±1	21±1	<0.001	12±5	14±3	NS
Ornithine	99±18	70±14	<0.02	47±13	27±6	NS	52±9	43±9	NS
Lysine	145±17	116±15	<0.01	88±9	60±8	<0.02	57±15	56±8	NS
1-Mehyl-histidine	51±12	32±6	<0.05	33±9	17±3	NS	19±9	15±3	NS
Histidine	84±10	63±7	<0.01	48±6	33±2	<0.05	36±8	30±5	NS
3-Methyl-histidine	19±2	8±1	<0.001	12±2	5±1	<0.05	7±3	3±1	NS
Arginine	92±17	69±14	<0.01	62±7	39±8	<0.01	29±13	30±7	NS
Total	3,789±172	2,731±118	<0.01	2,324±187	1,431±70	<0.01	1,465±148	1,299±91	NS

All values are in micromoles per liter of whole blood. Plasma and cell contents were as described in text.

is a principal gluconeogenic precursor (37), its limited availability could account for the inadequancy of hepatic glucose production in replenishing glucose loss in dialysate. In this regard, it is of interest that during dialysis, whole blood alanine levels declined by 60% (Table III). In fact, alanine was the only amino acid that declined not only in the plasma compartment but in blood cells as well (Tables III and IV). The plasma insulin concentration also fell by the end of dialysis, probably as an appropriate response to the decrease in plasma glucose (Fig. 1). The significant decline in pyruvate would be in keeping with the decreasing alanine levels during dialysis (37).

Elevated glucagon concentrations have been reported in chronic renal disease, even after several weeks of dialysis (5), and our results are in agreement with this observation. Kidney is the primary site of glucagon degradation (38) and this may account for its accumulation in patients with uremia (39), if there is either a lack or a derangement of homeostatic feedback control. In view of this, it was not surprising that the mean glucagon levels did not change after dialysis.

Endogenous hypertriglyceridemia is characteristic of azotemic states (9, 40, 41)). Five of the six patients in the present study had increased triglyceride levels with normal cholesterol levels. Of interest was the prompt decrease in triglycerides in response to heparin (Fig. 2). While excessive hepatic synthesis contributes to increased triglyceride levels in such patients (40), a diminished clearance from plasma, as indicated by a subnormal postheparin lipolytic activity (PHLA), has also been observed (41, 42). During dialysis, PHLA has been found to be unchanged (40) or significantly improved (41). In this study, a rapid decline in plasma triglyceride concentrations after initial heparin administration, before the start of dialysis, would suggest an immediate activation of lipoprotein lipase by the large dose of heparin, rather than a hypotriglyceridemic effect of dialysis itself. Since triglyceride levels do return to predialysis levels in less than 48 h after dialysis, diminished basal lipoprotein lipase activity is probably an important underlying mechanism of endogenous hypertriglyceridemia seen with azotemia.

The crucial role of kidney in nitrogen homeostasis

TABLE IV

Amino Acid Concentrations (Mean±SEM) in Blood Cell-Water in Anephric Subjects (n = 6) Compared with Normal Subjects (n = 8)

		Anephric subjects				
	Normal subjects	Predialysis	*P* (vs. normals)	Postdialysis	*P* (vs. normals)	(Before vs. after dialysis)
	μM	*μM*		*μM*		
Taurine	444±65	943±214	<0.02	854±175	<0.05	NS
Aspartate	444±37	781±153	NS	651±120	NS	NS
Threonine	195±20	694±249	NS	461±108	NS	NS
Serine	325±30	323±72	NS	313±35	NS	NS
Glutamine	1,193±95	2,045±493	NS	2,315±350	<0.01	NS
Proline	253±20	721±216	<0.05	506±73	<0.05	NS
Glutamate	414±20	625±39	<0.001	639±35	<0.001	NS
Citrulline	61±10	313±88	<0.001	247±26	<0.001	NS
Glycine	673±45	1,888±372	<0.01	1,163±218	<0.05	NS
Alanine	468±34	968±136	<0.01	515±62	NS	<0.01
α-Aminobutyrate	31±7	38±26	NS	38±15	NS	NS
Valine	321±34	393±132	NS	374±58	NS	NS
Isoleucine	85±7	127±49	NS	119±36	NS	NS
Leucine	174±17	234±96	NS	247±65	NS	NS
Tyrosine	85±7	72±26	NS	65±9	NS	NS
Phenylalanine	68±7	109±46	NS	103±17	NS	NS
Ornithine	215±24	456±115	<0.05	335±82	NS	NS
Lysine	277±17	505±154	NS	429±85	NS	NS
1-Methyl-histidine	ND	170±76	*	107±21	*	NS
Histidine	133±17	318±85	<0.05	232±45	<0.05	NS
3-Methyl-histidine	ND	65±28	*	25±5	*	NS
Arginine	58±10	259±124	NS	236±63	<0.02	NS

ND, not detectable.

* Significant increase because not detected in normal subjects.

TABLE V

Mean Ratios of Tyrosine:Phenylalanine and Glycine:Serine in Patients with Chronic Azotemia

Authors (reference)		Glycine:Serine		Tyrosine:Phenylalanine	
		Patients	Normals	Patients	Normals
Muting and Dishuk (18)*	Serum	2.5	1.6	1.50	1.2
Gulyassy et al. (21)*	Plasma	2.0	1.4	0.62	1.1
Condon and Asatoor (22)*	Plasma	Serine not reported		0.75	1.0
Held et al. (24)‡	Plasma	2.3	1.6	0.65	1.1
This study§	Whole blood	5.9	1.8	0.70	1.1
	Plasma	5.7	1.5	0.70	1.0
	Blood cells	6.1	2.1	0.75	1.2

* Patients not on dialysis.

‡ Patients on chronic intermittent hemodialysis.

§ Patients anephric; on chronic intermittent hemodialysis.

has been emphasized (43, 44). Alterations in the plasma levels of several amino acids have been noted in patients with chronic renal failure (18–24). In this study, we examined the arterial concentrations of amino acids in both whole blood and plasma to evaluate the role of human blood cells in amino acid transport (15, 16). First, the comparison of the whole blood amino acid pattern with that of normal subjects revealed several interesting differences. The concentrations of glutamine, proline, citrulline, glycine, and 1- and 3-methyl-histidine were markedly elevated. These amino acids are primarily extracted by kidney in normal man (43, 44). Therefore, their increase in anephric subjects suggests a lack of removal from the circulation. Earlier work of Owen and Robinson (45) and of Pitts et al. (46) indicated that the uptake of glycine by the kidney results in its stoichiometric conversion to serine, and that kidney is the primary source for serine production in the body. In this regard, it is of great interest that in the present study, the increase of whole blood concentration of glycine was accompanied by a striking diminution of serine (Table II). The glycine-to-serine ratio was found to be approximately 6.0, as compared to less than 2 in normal subjects (Table V). A similar but modest increase in the ratio of glycine to serine was calculated from the plasma data in previous reports of nonnephrectomized paitents with renal failure (Table V).

Besides serine, the whole blood concentrations of branched-chain amino acids and of tyrosine, glutamate, and lysine were significantly diminished. Since branched-chain amino acids are mainly metabolized by nonhepatic tissues (47), their decreased blood concentration and that of lysine may reflect a reduced rate of protein catabolism (48), although the site(s) of the diminished proteolysis have not been characterized. Whereas similar decreases in branched-chain amino acids and lysine have been observed in clinical states of protein-calorie malnutrition (49) and kwashiorkor (50), the patients in this study were on a eucaloric, balanced diet, containing 1.1–1.6 g protein/kg per day (Table I), approximately three-fourths of which were of high biologic value. Ginn et al. (10) have shown that an intake of 0.75 g/kg per day of high biologic value protein is adequate to maintain nitrogen balance in patients on hemodialysis. None of the patients showed any clinical evidence of muscle wasting or edema and all had normal serum albumin levels. Gross malnutrition in these subjects is therefore unlikely. The decrease in tyrosine concentration with decreased tyrosine-to-phenylalanine ratio has also been observed in uremia by other investigators (Table V). Although phenylalanine hydroxylase has been found in liver and pancreas, as well as in kidney (51), the correlation between reduced phenylalanine-to-tyrosine ratios with diminishing renal function (52) suggests an impotrant contribution by kidney in tyrosine synthesis in man. Furthermore, a significantly delayed disappearance of exogenously administered phenylalanine has recently been shown in patients with chronic renal failure (53).

Since most of the alterations in whole blood concentrations of amino acids were reflected by alterations in plasma (Table II), especially for those amino acids that increased, the cell water concentrations of individual amino acids were calculated.[2] As indicated in Table IV, when compared to normal subjects, the cell water concentrations of several of the amino acids were signifi-

[2] These calculations were done assuming 65% of erythrocytes as water, based on the studies of McMenamy et al. in normal man (33). While the water content of erythrocytes of uremic subjects may not necessarily be the same, the differences in amino acid concentrations in the cell water compartment in the two groups of subjects in this study were striking and will most probably remain significant.

cantly increased. The unaltered, fractional content of most of the amino acids in the cell compartment, therefore, reflects the diminished blood cell mass, the mean hematocrit being only 20%. However, the mechanism of the increase in the concentrations of the various amino acids per unit cell water remains uncertain. Moreover, during dialysis, there was a significant decrease (about 30%) in the total amino acid concentration in whole blood, indicating a modest α-amino nitrogen depletion, since others have calculated that total nitrogen loss during 6–8 h of hemodialysis is usually of the order of 1–3 g (10–13). This was, again, due to a 40% decrease in the plasma compartment, the blood cell compartment revealing no significant depletion, whether the fraction in cells or the absolute concentration in cell water was considered (Tables III, IV). It is of interest, however, that an increased uptake (in vitro) of certain amino acids in blood cells and increased ratios of erythrocyte to plasma amino acids have also been documented in protein-calorie malnutrition and other diverse situations (17), and suggest alterations in the active transport across the cell membrane and accumulation inside the cell.

Finally, it is noteworthy that the whole blood concentrations of certain amino acids, including glutamine, leucine, and isoleucine, remained unchanged during dialysis and that of glutamate, paradoxically, increased (Table III). The mechanism of the lack of decline in the concentration of these amino acids remains to be determined. One possibility is a selective removal during dialysis due to differences in molecular configuration and charge. However, our preliminary observations (unpublished) indicate substantial quantities of these amino acids in the dialysate. Moreover, previous studies (8–11) have reported significant losses of most of the amino acids (including glutamine and glutamate) in the dialysate, particularly when a capillary-type kidney, as in this study, is employed (10) and when a glucose-free dialysis fluid is used (11). Therefore, a more likely explanation is that during dialysis the release of certain amino acids from muscle or other tissues (e.g. liver) must accelerate to maintain their unaltered blood levels. An augmented hepatic release of glutamine has indeed been shown in dogs during dialysis, with or without induced metabolic acidosis (54). Further studies on the effect of hemodialysis on the amino acid release from muscle and liver in man appear warranted to characterize the regulation of nitrogen homeostasis in renal disease.

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