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Research Article

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Internal Redistribution of Tissue Protein Synthesis in Uremia

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ABSTRACT Tissue composition and in vivo tissue protein synthesis were altered by acute uremia, induced in rats by bilateral nephrectomy. Net protein synthesis (anabolism minus catabolism) was increased in liver and heart and decreased in skeletal muscle, as judged from changes in total organ weight, ratios of protein: DNA and RNA: DNA, and leucine-^{14}C incorporation into trichloroacetic acid (TCA)-insoluble, nucleic acid–free material. Concentration of free lysine, a major constituent of histones and ribonucleoproteins, also was increased in liver and decreased in skeletal muscle, a finding suggesting lysine shifted from muscle to liver in association with the changes in protein synthesis. Acute uremia also altered tissue levels of other amino acids. Hepatic concentrations and liver: blood concentration ratios tended to be increased for the essential, but not for the nonessential amino acids. Moreover, the phenylalanine: tyrosine concentration ratio, which reflects activity of the enzyme phenylalanine hydroxylase, was increased in blood, muscle, and liver. These findings indicate uremia selectively alters tissue composition and protein synthesis in different organs and may modify intermediary metabolism of some individual amino acids.

INTRODUCTION

Liver homogenates from uremic rats incorporate increased quantities of leucine-^{14}C into trichloroacetic acid (TCA)-insoluble material in vitro (1). Moreover, uptake of several labeled amino acids and amino acid incorporation into urea are increased in perfused liver preparations from uremic rats (2). Skeletal muscle may react differently. Here, decreased activity is suggested by the finding that protein synthesis by muscle ribosome preparations from normal rats is inhibited by compounds removed from uremic patients during dialysis (3).

The present investigation was undertaken to determine if, as suggested by the above findings, uremia exerts different effects on protein metabolism in different organs, and to establish whether or not the in vitro changes reflect alterations which occur in the intact animal. Our results presented below, indicate that in vivo tissue protein synthesis is increased in the heart and liver and decreased in skeletal muscle of acutely uremic rats.

METHODS

Male Sprague-Dawley rats, weighing approximately 220 g, were anesthetized and subjected to either bilateral nephrectomy or a sham operation. In the sham procedure both kidneys were exteriorized through flank incisions and were then returned to the abdominal cavity after the capsules had been removed. Some rats were anesthetized with ether and others with intraperitoneally administered pentobarbital to determine the effect of the anesthetic agent per se. Results for the two anesthetic groups were combined after they were found to be similar.

Before surgery the rats were given standard chow and tap water. Postoperatively they were fasted, and water was provided ad lib. Water intake was limited to 6–10 ml/24 hr in a separate group of nephrectomized rats to be certain the changes observed with uremia did not reflect overhydration from continued water ingestion in the face of anuria. Results of tissue analyses in these rats were indistinguishable from those in nephrectomized rats permitted water ad lib. but are not included in the tabulated data.

To eliminate possible changes due to diurnal and day to day variation, control and experimental rats were included on each study day, all were exsanguinated between 9:00 a.m. and 12 noon, and control and experimental animals were sacrificed alternately.

On the day of study, 48 hr postoperatively, approximately 4 μc of uniformly labeled L-leucine (^{14}C, 248 mc/mmole) were injected into a tail vein.[1] 2 hr later the rats were

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[1] The exact quantity of isotope administered to each rat was determined from syringe weights taken before and after

anesthetized and sacrificed by exsanguination from the abdominal aorta. Whole livers and hearts and portions of skeletal muscle were removed, blotted, weighed, and immediately placed in ice-cold distilled water. Sufficient water was added to make a 10 or 20% solution, and the tissues were homogenized with a Virtis Omni-Mixer (The Virtis Co., Inc., Gardiner, N. Y.). Proteins and nucleic acids were precipitated from the homogenates by addition of TCA to a final concentration of 10%. The precipitates were washed twice with 10% TCA and twice with ether and dissolved in 0.3 normal potassium hydroxide (KOH) solution for 18 hr at 37°C. Aliquots of this solution were taken for analysis of deoxyribonucleic acid (DNA) by the diphenylamine (4) reaction and total protein by the biuret reaction. Protein in the range encountered in the rat tissues did not alter DNA measurements when added to solutions containing DNA in known amounts. After the aliquots had been removed, TCA was added to reprecipitate DNA and protein. The supernatant, containing ribonucleic acid (RNA) which had been hydrolyzed by the KOH was used to measure RNA content by the orcinol (4) reaction. This maneuver was required because, in initial attempts to validate the methodology, protein and DNA both altered results of the orcinol reaction.

Leucine-^{14}C activity in TCA-insoluble, nucleic acid–free material was measured in other aliquots of the same tissue homogenates. These also were treated with TCA and ether, but, in addition, were heated in TCA for 15 min at 90°C. This procedure hydrolyzes nucleic acids and releases into the acid-soluble fraction any leucine-^{14}C present in amino acid–RNA complexes (5). The final precipitates were dissolved in hyamine hydroxide and counted by standard liquid scintillation techniques by use of the channels ratio method to correct for quenching of individual samples. All determinations were performed in duplicate with separate aliquots of the tissue homogenates.

Free amino acid contents of liver, muscle, and blood plasma also were measured. For this, samples were prepared by precipitating the proteins with 20% sulfosalicylic acid solution. The supernatant fraction was applied directly to an ion-exchange column (Beckman automated amino acid analyzer).

RESULTS

The nephrectomized rats were severely uremic when sacrificed 48 hr postoperatively. Blood urea nitrogen (BUN) concentrations were 221–361 mg/100 ml as compared with 15–26 mg/100 ml in the sham-operated controls. Within the nephrectomized group, results did not correlate with the degree of increase in BUN.

Hepatic DNA concentration was reduced in the uremic rats, whether expressed in relation to wet or fat-free dry liver weight. Mean values (μg/mg wet wt) in the uremic and control animals were 1.20 ±0.06[2] and 1.72 ±0.04, respectively ($P < 0.001$). Evidence suggesting the low concentrations were caused by increased degradation of DNA by constituents of uremic liver tissue, or altered hepatic DNA metabolism was sought but not found. Sections of liver tissue taken from uremic and control rats and examined by light microscopy did not reveal evidence of altered nuclear structure or polypoidy formation.[3] When DNA content of uremic and control liver homogenates was measured before and after known amounts of DNA were added, recoveries of the added DNA from uremic and control tissues were comparable. DNA levels were also measured in serial samples from uremic and control rat livers which were left in the rat for periods of 2 hr after exsanguination. Hepatic DNA concentrations and ratios of protein: DNA (mg:mg) and RNA:DNA (μg:μg) did not change significantly with time. Finally, total hepatic DNA content (liver weight times DNA concentration) was comparable in the uremic (9.7 ±0.3 mg) and control rats (10.4 ±0.5 mg, $P > 0.20$). These findings indicate the low hepatic DNA concentrations in uremia reflected dilution by proteins and other components of

TABLE I

Tissue Composition and Leucine-^{14}C Incorporation in Acutely Uremic Rats

	Sham-operated controls (BUN 15–26 mg/100 ml)				Nephrectomized (BUN 221–361 mg/100 ml)		
	n	Mean	SEM	P	n	Mean	SEM
Heart							
Organ weight, mg*	9	553	10	<0.001	11	686	16
Total DNA, μg	9	820	60	>0.80	11	810	50
μg RNA/μg DNA	9	1.8	0.1	<0.001	11	2.6	0.2
mg protein/mg DNA	9	127	8	<0.05	11	153	8
^{14}C cpm/mg protein	9	43.6	6.8	<0.01	11	74.6	7.4
^{14}C cpm/μg DNA	9	5.6	0.9	<0.005	11	11.3	1.2
Liver							
Organ weight, g*	18	6.2	0.2	<0.001	26	7.3	0.2
Total DNA, mg	18	10.4	0.5	>0.20	26	9.7	0.3
μg RNA/μg DNA	7	5.5	0.1	<0.001	13	7.1	0.3
mg protein/mg DNA	7	114	0.3	<0.005	13	140	6
^{14}C cpm/mg protein	7	212	9	<0.01	12	272	16
^{14}C cpm/μg DNA	7	24.4	1	<0.001	12	36.3	2
Muscle							
μg RNA/μg DNA	13	5.2	0.3	>0.10	18	4.7	0.2
mg protein/g tissue	13	191	5	<0.005	18	169	3
mg protein/mg DNA	13	60.3	4.6	<0.05	18	49.6	1.6
^{14}C cpm/mg protein	8	24.3	1.8	<0.005	9	17	0.9
^{14}C cpm/μg DNA	8	13.6	0.9	<0.001	9	8.2	0.5

* Similar differences were obtained when organ weights were related to body weight.

quantitative injection and from ^{14}C activity of a standard leucine solution made up from each batch of leucine used. From these data the amount of isotope injected was mathematically adjusted to unity for each animal. This correction factor was applied to the leucine incorporation values to cancel out small variations in the quantity of isotope injected into individual rats. In practice, this manipulation altered the data very little and did not change any results of tests for statistical significance.

[2] All data are presented as means ±SEM.

[3] Histologic studies were kindly performed by Dr. Richard Stenger, Department of Pathology, Case Western Reserve University School of Medicine.

TABLE II
Amino Acid Levels in

	Muscle*			Liver*		
	Sham	Uremic	*P*	Sham	Uremic	*P*
	μmoles/g of protein			*μmoles/g of protein*		
Essential amino acids						
Threonine	2.7 ±0.2	1.5 ±0.2	<0.005	4.9 ±1.5	2.8 ±0.6	NS
Valine	1.4 ±0.03	1.3 ±0.1	NS	1.2 ±0.1	1.4 ±0.2	NS
Isoleucine	0.9 ±0.1	0.8 ±0.1	NS	0.7 ±0.1	0.7 ±0.1	NS
Leucine	1.2 ±0.1	1.2 ±0.1	NS	1.4 ±0.1	1.5 ±0.2	NS
Phenylalanine	0.6 ±0.03	0.6 ±0.03	NS	0.43 ±0.02	0.54 ±0.04	<0.05
Arginine	1.6 ±0.03	0.7 ±0.05	<0.001	Not measurable in liver		
Lysine	4.2 ±0.1	2.1 ±0.2	<0.001	4.4 ±0.3	7.5 ±1.2	<0.05
Histidine	1.2 ±0.1	1.5 ±0.2	NS	2.6 ±0.2	3.7 ±0.2	<0.01
Methionine	0.5 ±0.04	0.5 ±0.06	NS	0.2 ±0.02	0.4 ±0.02	>0.001
Nonessential amino acids						
Serine	3.8 ±0.3	1.7 ±0.2	<0.001	2.8 ±0.3	1.2 ±0.1	<0.001
Proline	2.0 ±0.2	1.4 ±0.1	<0.05	Not measurable in liver		
Tyrosine	0.7 ±0.03	0.49 ±0.03	<0.001	0.44 ±0.03	0.39 ±0.05	NS
Glycine	19 ±2.0	10 ±1.0	<0.01	17 ±0.3	12 ±3.0	NS
Alanine	14 ±1.0	8.0 ±2.0	<0.02	12 ±2.0	7.0 ±2.0	NS
Ornithine	0.3 ±0.1	0.2 ±0.01	NS	1.5 ±0.1	1.9 ±0.3	<0.025
Glutamine	12 ±3.0	8.0 ±2.0	NS	21 ±3.0	10 ±1.0	<0.01
Glutamic acid	7.7 ±0.8	4.8 ±0.4	<0.01	23 ±3.0	10 ±1.0	<0.001
Aspartic acid	2.0 ±0.3	1.5 ±0.1	NS	6.3 ±0.6	2.8 ±0.3	<0.001

* Similar differences between uremic and control rats were obtained when amino acid concentrations were related to tissue DNA instead of to protein content.

liver tissue rather than altered metabolism of DNA per se. Therefore DNA content was considered a valid standard to which other tissue constituents may be related in uremia.

Livers from the uremic rats demonstrated increased leucine-^{14}C activity in TCA-insoluble, nucleic acid–free material (Table I). Hepatic protein specific activity (^{14}C cpm/mg of protein) averaged 272 ±16 in the nephrectomized and 212 ±9 in the control rats ($P <$ 0.01). ^{14}C cpm/μg of DNA averaged 36 ±2 and 24 ±1 ($P < 0.001$). These increased incorporation values only suggest protein synthesis was increased, because leucine precursor pool specific activity at the site of protein synthesis could not be measured. However, alterations in hepatic tissue composition, which paralleled the incorporation values, indicated net protein synthesis (anabolism minus catabolism) was increased. Increases with uremia were demonstrated for total liver weight (means 7.3 ±0.2 and 6.2 ±0.2 gram, $P < 0.001$), and the ratios of protein: DNA (means 140 ±6 and 114 ±3, $P < 0.005$) and RNA: DNA (means 7.1 ±0.3 and 5.5 ±0.1, $P < 0.001$).

Heart muscle from uremic animals demonstrated changes in leucine-^{14}C incorporation and tissue composition which resembled the alterations in uremic liver, a finding indicating net cardiac protein synthesis also was increased. Leucine-^{14}C incorporation was increased whether expressed as protein specific activity (74.6 ±7.4 and 43.6 ±6.8 cpm/mg of protein, $P < 0.01$) or related to DNA content (11.3 ±1.2 and 5.6 ±0.9 cpm/μg of DNA, $P < 0.005$). Uremic heart also showed increases in total organ weight (means 686 ±16 and 553 ±10 mg, $P < 0.001$, and ratios of protein: DNA (means 153 ±8 and 127 ±8, $P <$0.05) and RNA: DNA (means 2.6 ±0.2 and 1.8 ±0.1, $P <$0.001).

Skeletal muscle from uremic animals demonstrated changes opposite from those in liver and heart, a finding indicating net muscle protein synthesis was diminished, Mean protein specific activities (cpm/mg of protein) in the uremic and control rats were 17 ±0.9 and 24.3 ±1.8, $P < 0.005$, and the ratios of ^{14}C cpm/μg of DNA were 8.2 ±0.5 and 13.6 ±0.09 $P < 0.001$. The ratio of RNA: DNA was slightly but not significantly decreased (means 4.7 ±0.2 and 5.2 ±0.3, $P > 0.10$). However, protein content per gram of tissue (means 169 mg ±3 and 191 ±5, $P < 0.005$) and the ratio of protein: DNA (means 49.6 ±1.6 and 60.3 ±4.6, $P < 0.05$) were diminished.

Of the amino acids measured (Table II), tissue lysine levels were unique in that concentrations were increased in liver and decreased in muscle, a finding suggesting a

	Blood plasma			Liver : blood		
	Sham	Uremic	*P*	Sham	Uremic	*P*
		μmoles/100 ml			%	
Essential amino acids						
Threonine	23.1 ±0.7	10.2 ±0.6	<0.001	16 ±6	22 ±5	NS
Valine	23.0 ±0.9	13.0 ±0.8	<0.001	5 ±0.4	11 ±1	<0.01
Isoleucine	11.2 ±0.5	6.6 ±0.3	<0.001	6 ±0.4	13 ±2	<0.005
Leucine	20.7 ±0.8	12.0 ±0.7	<0.001	7 ±0.4	12 ±2	<0.005
Phenylalanine	8.2 ±0.3	6.3 ±0.2	<0.001	5 ±0.2	8 ±1	<0.005
Arginine	14.9 ±0.8	6.2 ±0.3	<0.001	Not measurable in liver		
Lysine	44.2 ±1.6	28.5 ±2.1	<0.001	10 ±0.6	26 ±4	<0.005
Histidine	6.9 ±0.3	12.4 ±0.7	<0.001	38 ±3	30 ±2	<0.05
Methionine	5.6 ±0.2	4.1 ±0.3	<0.005	4 ±0.5	9 ±1	<0.01
Nonessential amino acids						
Serine	25.4 ±0.7	8.4 ±0.5	<0.001	12 ±1	16 ±1	<0.05
Proline	13.2 ±0.4	9.4 ±0.7	<0.001	Not measurable in liver		
Tyrosine	8.6 ±0.3	3.7 ±0.1	<0.001	5 ±0.4	10 ±1	<0.005
Glycine	38.0 ±1.5	20.2 ±1.7	<0.001	47 ±2	56 ±12	NS
Alanine	31.6 ±1.8	16.0 ±1.6	<0.001	37 ±6	39 ±8	NS
Ornithine	4.7 ±0.2	2.3 ±0.2	<0.001	32 ±2	82 ±11	<0.001
Glutamine	48.0 ±4.1	46.7 ±4.9	NS	49 ±4	23 ±3	<0.001
Glutamic acid	27.9 ±1.4	18.8 ±1.7	<0.001	85 ±9	46 ±6	<0.005
Aspartic acid	Not measurable in blood			Not measurable in blood		

relationship to the changes in protein synthesis. Blood and tissue levels of other amino acids also were altered in uremia, and essential and nonessential amino acids appeared to have been affected differently. Each amino acid, with the exception of histidine and glutamine, had a decreased concentration in blood. Liver contents of most essential amino acids were normal or increased, whereas concentrations of several nonessentials were decreased. Moreover, liver : blood concentration ratios were increased for six of eight essential amino acids but only for serine, tyrosine and ornithine among the nonessentials. Histidine had a decreased liver : blood ratio despite an increased concentration in liver, because the concentration in blood also was high. Finally, the phenylalanine : tyrosine concentration ratio, which is inversely related to activity of the enzyme phenylalanine hydroxylase (6), was increased in uremic liver, muscle, and blood (Table III).

TABLE III

Phenylalanine: Tyrosine Ratios in Control and Acutely Uremic Rats*

	Liver	Muscle	Blood
Uremic Rats	1.41 ±0.09	1.32 ±0.05	1.68 ±0.12
Control Rats	0.99 ±0.03	0.87 ±0.02	0.96 ±0.02
P	<0.005	<0.001	<0.001

* Means ±SEM.

DISCUSSION

Results of the present study demonstrate that in vivo protein synthesis is selectively altered in various tissues of acutely uremic rats. Organ weights and tissue protein contents indicated that net protein synthesis (anabolism minus catabolism) was increased in uremic liver and heart and decreased in uremic skeletal muscle. Thus uremia, like a number of other conditions (i.e., protein depletion [7, 8], steroid administration [9]) may be associated with mobilization of "labile protein reserves" (10) and internal redistribution of nitrogen among various tissues. The cause of this redistribution is still conjectural. It could have been due, in part, to adrenal steroid hormone release (9) which probably was greater in the uremic rats.

The changes in RNA content and leucine-^{14}C incorporation which paralleled the organ weights and protein contents suggest that these alterations resulted from changes in rates of tissue protein synthesis per se rather

than to degradation. However, this conclusion cannot be drawn with certainty because specific activity of the leucine pool at the site of protein synthesis cannot be measured directly in single injection isotope studies. Our findings of unchanged total free leucine levels in muscle and liver suggest that precursor pool size and specific activity were not significantly altered by uremia. Moreover, previous results in vitro have demonstrated increased leucine-^{14}C incorporation by uremic rat liver not attributable to precursor pool specific activity artefact (1). Nevertheless, this aspect of the present study was not definitive, because separate amino acid pools with varying specific activities may exist at different sites within tissue (11–13), and after a single injection of leucine-^{14}C intracellular specific activity may vary with time and be impossible to quantitate. Other experiments, utilizing prolonged constant rate infusions of labeled amino acid to achieve an equilibrium state, are required to establish whether the observed changes in net protein synthesis resulted from altered rates of protein synthesis or degradation.

Changes in tissue free lysine levels (increased in uremic liver, decreased in uremic muscle) paralleled the changes in protein synthesis. Lysine is a major constituent of nuclear histones and cellular ribonucleoproteins (14), which are important regulators of tissue protein synthesis. Soon after partial hepatectomy, when the rate of hepatic protein synthesis accelerates, lysine content of liver tissue increases (15, 16). One might speculate that the present changes in uremia were analogous. If so, they would indicate that lysine was mobilized from muscle to liver in association with the increased hepatic protein synthesis.

To exclude the possibility that the increased hepatic lysine levels might reflect dissolution of lysine-rich histones, DNA template activity was measured (17) by use of chromatin isolated from three control and three uremic rat livers.[4] Rate of RNA synthesis was measured in the presence of isolated chromatin, DNA-dependent RNA polymerase purified from *Escherichia coli*, and required RNA precursors adenosine triphosphate (ATP), guanosine triphosphate (GTP), uridine triphosphate (UTP) and tritium-labeled cytidine triphosphate (CTP-^{3}H). Template activity in the uremic and control tissues was indistinguishable, a finding suggesting the increased hepatic protein synthesis in uremia could not be attributed to histone dissolution opening up new DNA transcription sites. Adequacy of this assay system to detect changes in template activity is indicated by results of similar experiments in which chromatin isolated from regenerating rat liver demonstrated increased activity.[5]

[4] These measurement were made by Dr. Donald D. Anthony, Department of Pharmacology, Case Western Reserve University School of Medicine.

[5] Anthony, D. D., Unpublished observations.

Tissue and blood levels of other amino acids were grossly and generally altered, but not all to the same degree. Hepatic levels were well maintained for most of the essential, but not for the nonessential amino acids. Moreover, blood and tissue phenylalanine: tyrosine concentration ratios were increased, as they are in conditions associated with decreased activity of the enzyme phenylalanine hydroxylase (6, 18, 19) and in some patients with renal disease (20). Interpretation of these changes in individual amino acid levels must, at this point, be conjectural. However, their nonuniform nature suggests uremia may modify activity of enzymes regulating utilization and (or) synthesis of a variety of individual compounds. Such a change has been postulated previously to explain altered metabolism of vitamin D by patients with renal disease (21).

Results of the present study thus demonstrate that acute uremia is associated with widespread alterations in protein and amino acid metabolism which differ in various individual tissues. Such changes must be considered in the interpretation of studies on nitrogen metabolism in uremia.

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