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 \(\mu\)c of uniformly labeled L-leucine (\(^{14}C\), 248 mc/mnmole) were injected into a tail vein.\(^1\) 2 hr later the rats were

\(^1\) 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 imme-
diately 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 dis-
solved 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 diphenyl-
amine (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 contain-
ing DNA in known amounts. After the aliquots had been
removed, TCA was added to reprecipitate DNA and pro-
tein. 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-14C 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-14C present in amino acid-
RNA complexes (5). The final precipitates were dissolved
in hydroxide and counted by standard liquid scintilla-
tion techniques by use of the channels ratio method to
correct for quenching of individual samples. All determina-
tions 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% sulfosalicyclic 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
weight) in the uremic and control animals were 1.20 ± 0.06
and 1.72 ± 0.04, respectively (P < 0.001). Evidence sug-

ggst that 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 poly-

ogy formation. 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 indi-
cate the low hepatic DNA concentrations in uremia
reflected dilution by proteins and other components of

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**TABLE I**

<table>
<thead>
<tr>
<th>Tissue Composition and Leucine-14C Incorporation in Acutely Uremic Rats</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sham-operated</strong></td>
</tr>
<tr>
<td><strong>controls</strong></td>
</tr>
<tr>
<td>Heart</td>
</tr>
<tr>
<td>Organ weight, mg*</td>
</tr>
<tr>
<td>Total DNA, µg</td>
</tr>
<tr>
<td>µg RNA/µg DNA</td>
</tr>
<tr>
<td>mg protein/µg DNA</td>
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<tr>
<td>14C cpm/µg protein</td>
</tr>
<tr>
<td>14C cpm/µg DNA</td>
</tr>
<tr>
<td>Liver</td>
</tr>
<tr>
<td>Organ weight, g*</td>
</tr>
<tr>
<td>Total DNA, mg</td>
</tr>
<tr>
<td>µg RNA/µg DNA</td>
</tr>
<tr>
<td>mg protein/µg DNA</td>
</tr>
<tr>
<td>14C cpm/µg protein</td>
</tr>
<tr>
<td>14C cpm/µg DNA</td>
</tr>
<tr>
<td>Muscle</td>
</tr>
<tr>
<td>µg RNA/µg DNA</td>
</tr>
<tr>
<td>mg protein/µg tissue</td>
</tr>
<tr>
<td>mg protein/µg DNA</td>
</tr>
<tr>
<td>14C cpm/µg protein</td>
</tr>
<tr>
<td>14C cpm/µg DNA</td>
</tr>
</tbody>
</table>

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

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* All data are presented as means ± SEM.
* Histologic studies were kindly performed by Dr. Richard Stenger, Department of Pathology, Case Western Reserve University School of Medicine.

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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 \(\text{mean} \, 7.3 \pm 0.2 \text{ and } 6.2 \pm 0.2 \text{ gram, } P < 0.001\), and the ratios of protein: DNA \(\text{mean} \, 140 \pm 6 \text{ and } 114 \pm 3, P < 0.005\) and RNA:DNA \(\text{mean} \, 7.1 \pm 0.3 \text{ and } 5.5 \pm 0.1, P < 0.001\).

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 \(74.6 \pm 7.4\) and \(43.6 \pm 6.8 \text{ cpm/mg of protein, } P < 0.01\) or related to DNA content \(11.3 \pm 1.2\) and \(5.6 \pm 0.9 \text{ cpm/µg of DNA, } P < 0.005\). Uremic heart also showed increases in total organ weight \(\text{mean} \, 686 \pm 16 \text{ and } 553 \pm 10 \text{ mg, } P < 0.001\), and ratios of protein:DNA \(\text{mean} \, 153 \pm 8 \text{ and } 127 \pm 8, P < 0.005\) and RNA:DNA \(\text{mean} \, 2.6 \pm 0.2 \text{ and } 1.8 \pm 0.1, P < 0.001\).

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 \(\text{cpm/mg of protein}\) in the uremic and control rats were \(17 \pm 0.9 \text{ and } 24.3 \pm 1.8, P < 0.005\), and the ratios of \(^{14}\)C cpm/µg of DNA were \(8.2 \pm 0.5 \text{ and } 13.6 \pm 0.09 P < 0.001\). The ratio of RNA: DNA was slightly but not significantly decreased \(4.7 \pm 0.2 \text{ and } 5.2 \pm 0.3, P > 0.10\). However, protein content per gram of tissue \(\text{means} \, 169 \pm 3 \text{ and } 191 \pm 5, P < 0.005\) and the ratio of protein:DNA \(\text{mean} \, 49.6 \pm 1.6 \text{ and } 60.3 \pm 4.6, P < 0.05\) 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

<table>
<thead>
<tr>
<th>TABLE II</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Amino Acid Levels in</strong></td>
</tr>
<tr>
<td><strong>Muscle</strong></td>
</tr>
<tr>
<td><strong>Sham</strong></td>
</tr>
<tr>
<td>---</td>
</tr>
<tr>
<td><strong>Essential amino acids</strong></td>
</tr>
<tr>
<td>Threonine</td>
</tr>
<tr>
<td>Valine</td>
</tr>
<tr>
<td>Isoleucine</td>
</tr>
<tr>
<td>Leucine</td>
</tr>
<tr>
<td>Phenylalanine</td>
</tr>
<tr>
<td>Arginine</td>
</tr>
<tr>
<td>Lysine</td>
</tr>
<tr>
<td>Histidine</td>
</tr>
<tr>
<td>Methionine</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Essential amino acids</th>
<th>Blood plasma</th>
<th>Liver: blood</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sham</td>
<td>Uremic</td>
</tr>
<tr>
<td></td>
<td>µmoles/100 ml</td>
<td></td>
</tr>
<tr>
<td>Threonine</td>
<td>23.1 ± 0.7</td>
<td>10.2 ± 0.6</td>
</tr>
<tr>
<td>Valine</td>
<td>23.0 ± 0.9</td>
<td>13.0 ± 0.8</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>11.2 ± 0.5</td>
<td>6.6 ± 0.3</td>
</tr>
<tr>
<td>Leucine</td>
<td>20.7 ± 0.8</td>
<td>12.0 ± 0.7</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>8.2 ± 0.3</td>
<td>6.3 ± 0.2</td>
</tr>
<tr>
<td>Arginine</td>
<td>14.9 ± 0.8</td>
<td>6.2 ± 0.3</td>
</tr>
<tr>
<td>Lysine</td>
<td>44.2 ± 1.6</td>
<td>28.5 ± 2.1</td>
</tr>
<tr>
<td>Histidine</td>
<td>6.9 ± 0.3</td>
<td>12.4 ± 0.7</td>
</tr>
<tr>
<td>Methionine</td>
<td>5.6 ± 0.2</td>
<td>4.1 ± 0.3</td>
</tr>
</tbody>
</table>

Nonessential amino acids

<table>
<thead>
<tr>
<th></th>
<th>Sham</th>
<th>Uremic</th>
<th>P</th>
<th>Sham</th>
<th>Uremic</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serine</td>
<td>25.4 ± 0.7</td>
<td>8.4 ± 0.5</td>
<td>&lt; 0.001</td>
<td>12 ± 1</td>
<td>16 ± 1</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>Proline</td>
<td>13.2 ± 0.4</td>
<td>9.4 ± 0.7</td>
<td>&lt; 0.001</td>
<td>Not measurable in liver</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tyrosine</td>
<td>8.6 ± 0.3</td>
<td>3.7 ± 0.1</td>
<td>&lt; 0.001</td>
<td>5 ± 0.4</td>
<td>10 ± 1</td>
<td>&lt; 0.005</td>
</tr>
<tr>
<td>Glycine</td>
<td>38.0 ± 1.5</td>
<td>20.2 ± 1.7</td>
<td>&lt; 0.001</td>
<td>47 ± 2</td>
<td>56 ± 12</td>
<td>NS</td>
</tr>
<tr>
<td>Alanine</td>
<td>31.6 ± 1.8</td>
<td>16.0 ± 1.6</td>
<td>&lt; 0.001</td>
<td>43 ± 6</td>
<td>39 ± 8</td>
<td>NS</td>
</tr>
<tr>
<td>Ornithine</td>
<td>4.7 ± 0.2</td>
<td>2.3 ± 0.2</td>
<td>&lt; 0.001</td>
<td>32 ± 3</td>
<td>82 ± 11</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Glutamine</td>
<td>48.0 ± 4.1</td>
<td>46.7 ± 4.9</td>
<td>NS</td>
<td>49 ± 4</td>
<td>23 ± 3</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Glutamic acid</td>
<td>27.9 ± 1.4</td>
<td>18.8 ± 1.7</td>
<td>&lt; 0.001</td>
<td>85 ± 9</td>
<td>46 ± 6</td>
<td>&lt; 0.005</td>
</tr>
<tr>
<td>Aspartic acid</td>
<td>Not measurable in blood</td>
<td>Not measurable in blood</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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).

**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-4°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.

TABLE III

**Phenylalanine: Tyrosine Ratios in Control and Acutely Uremic Rats**

<table>
<thead>
<tr>
<th></th>
<th>Liver</th>
<th>Muscle</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uremic Rats</td>
<td>1.41 ± 0.09</td>
<td>1.32 ± 0.05</td>
</tr>
<tr>
<td>Control Rats</td>
<td>0.99 ± 0.03</td>
<td>0.87 ± 0.02</td>
</tr>
<tr>
<td>P</td>
<td>&lt; 0.005</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

* Means ± SEM.
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-14C incorporation by uremic rat liver
not attributable to precursor pool specific activity arte-
fact (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-14C intracellular specific activity may vary
with time and be impossible to quantify. Other experi-
ments, 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 con-
stituent of nuclear histones and cellular ribonucleopro-
tins (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 his-
tones, DNA template activity was measured (17) by use
of chromatin isolated from three control and three
uremic rat livers.5 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-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.8

5 These measurement were made by Dr. Donald D.
Anthony, Department of Pharmacology, Case Western Re-
serve University School of Medicine.

8 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 con-
centration ratios were increased, as they are in condi-
tions 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 regu-
larizing 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 vari-
ous individual tissues. Such changes must be considered
in the interpretation of studies on nitrogen metabolism
in uremia.

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histologic examinations of liver and to Dr. Donald D.
Anthony for measuring template activity. Doctors George J.
Gabuzda and David Goldthwait provided invaluable assistance
and advice during these studies.

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