Proposed Mechanism for Urea Nitrogen Re-Utilization: Relationship between Urea and Proposed Guanidine Cycles

To the Editor:
In our recent publication (1), we pointed out that we were unable to form guanidinosuccinate by transamidination. This is strongly supported by the fact that patients with genetic absence of arginase (EC 3.5.3.1) are capable of transamidination from arginine to glycine, thus forming guanidinoacetate and then creatine. These patients do not excrete guanidinosuccinate even in the presence of very high arginine concentrations (2,3). The guanidino carbon of guanidinosuccinate can be shown to come from the side chain of arginine; this could only occur if guanidinosuccinate were formed from urea, resulting from the cleavage of arginine (4). We therefore directed our attention to urea and its metabolites as a source for the guanidine derivatives.

In the eluates from the chromatogram obtained after incubation of viable liver cells with arginine and aspartate, we noted that the urea (substance reacting with diacetylmonoimine reagent) generated from arginine, was always a double peak (1). The eluate corresponding to the second peak was identified as primarily urea, because it was hydrolyzed by urease (EC 3.5.1.5). A substantial portion of the first peak was unaffected by urease.

We also found that hydroxyurea was not hydrolyzed by urease, even when tested in the sensitive reaction for NH3 formation by reaction with oxaloacetate and reduction by NADH (5). In addition, eluate corresponding to the first peak reduced ferricyanide to form Prussian Blue. This is also a property of hydroxyurea. The portion of eluate corresponding to the second peak did not reduce ferricyanide.

On thin-layer chromatography by a procedure similar to that for amino acids (6), but with dimethylaminobenzaldehyde sprayed as the detecting reagent, urea has the higher mobility and stains a bright yellow, while hydroxyurea stains orange. This orange color and mobility of hydroxyurea were noted with the eluate containing the first of the aforementioned double peaks.

On the basis of these data we suspected that this first peak was hydroxyurea. For further confirmation, we instituted studies to see whether urea could be oxidized by the microsomal hydroxyating system. In these experiments, we compared the rate of formation of hydroxyurea from urea to the rate of hydroxylation of guanidine and hexobarbital (7). Table 1 lists the data obtained in this study.

When authentic hydroxyurea was heated at 100 °C and pH 7.4 in a sealed tube, half was hydrolyzed in 1 h. The products were identified as hydroxylamine and carbamate. This suggests a mechanism for hydroxylamine formation in humans, and suggests that this might be the link to the formation of the guanidine compounds from urea. In aqueous solution, at pH 7.4, hydroxylamine reacts instantly with esters to form the hydroxamic acids. This property has been used for assay of cholinesterase (EC 3.1.1.7) and triglycerides in serum, because hydroxamic acids give a reddish color with ferric salts (8,9). We applied this reaction to the methyl ester of homoserine to form the hydroxamic acid, which we could readily isolate as the potassium salt (m.p. 129–131 °C) if the reaction was done in methanol. This type of structure tends to form the anhydride on acidification. In this case, the product would be the anhydride of canaline. In the biological system, the homoserine would be in the form of the ester with coenzyme A, and reaction with hydroxylamine would be nonenzymatic. However, ring closure

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**Fig. 1. Reutilization of urea nitrogen**

Urea is oxidized to hydroxyurea. Hydrolysis yields carbamate and hydroxylamine. The carbamate reacts with ATP to form carbamylphosphate. This enters both cycles to form citrulline and ureidohomoserine, respectively. Condensation with aspartate forms argininosuccinate and canavaninesuccinate. Argininosuccinate is acted upon by a lysate to form fumarate and arginine. The canavaninesuccinate can be reduced to form guanidinosuccinate and homoserine, a reaction which is not reversible, or it can continue in the cycle to form fumarate and canavanine by the action of a lysate. Canavanine can be reduced to form homoserine and guanidine; this reaction is irreversible. Canavanine can react with glycine to form guanidinoacetate and then creatine by methylation. In this reaction canaline is formed and completes the cycle. In the case of arginine, transamidination to glycine forms ornithine, which completes the cycle.

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Rate of hydroxylation (μmol/min/mg protein)</th>
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</thead>
<tbody>
<tr>
<td>Hexobarbital (40)</td>
<td>9.8 ± 1.76</td>
</tr>
<tr>
<td>Urea (40)</td>
<td>18.3 ± 6.01</td>
</tr>
<tr>
<td>Guanidine (40)</td>
<td>12.0 ± 2.34</td>
</tr>
</tbody>
</table>

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**Table 1. Comparison of the Rate of Hydroxylation of Urea, Guanidine, and Hexobarbital by a Rat Liver Microsome Preparation**

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**References**


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and subsequent hydrolysis to canaline are probably enzymatic reactions and need to be studied. These reactions are depicted below.

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\begin{align*}
\text{HOMOSERINE} & \quad \text{METHYL HYDROXAMIC ESTER ACID} \\
\end{align*}
\]

This proposed mechanism affords a logical and simple method of forming the \(-\text{C-O-N-}\) linkage of canaline. In earlier studies, a cyclic sequence of reactions leading to the formation of guanidinosuccinate was formulated (9–11). The in vivo validity of every step in this cycle was documented with human tissue. It remained only to demonstrate a system for entering the cycle. The studies reported above suggest such a system.

The homoserine required is readily available in the human, being derived from methionine by demethylation. Oxidation of the \(-\text{SH}\) group of homocysteine to sulfate by oxymethoglobin takes place with molybdenum as a cofactor. Hydrolysis yields sulfate and homoserine (12).

Figure 1 summarizes the observations reported above. In the urea cycle, arginine from the diet supplies a structure for removing the nitrogen. In the guanidine cycle, homoserine (derived from methionine in the diet) performs a similar function. In both cases, a basic structure (ornithine and canaline) is regenerated. Both cycles serve to generate creatine required for metabolism. According to this scheme, when nitrogen is in short supply any urea generated can be recycled by oxidation and hydrolysis to carbamate and hydroxylamine, thus permitting a continuous supply of creatine phosphate. In starvation or during hibernation, we suggest that the guanidine cycle would be activated.

In the scheme of Figure 1 both guanidine and guanidinosuccinate can be formed as overflow products when the guanidine cycle is overloaded, which would explain the observation that patients with genetic arginase deficiency and accumulation of arginine do not excrete guanidinosuccinate because urea is in short supply. It also provides a reasonable explanation for the formation of guanidine, which is found in human urine in increased amounts in uremia. It explains why guanidinosuccinate does not appear in the urine in significant amounts except in uremia and why there is no quantitative correlation between guanidinosuccinate concentration in the urine and urea concentration in the serum (13).

The question arises as to the primary function of the cycles shown in Figure 1.

It seems that the major objective of both cycles is to generate creatine for continued muscular activity. When nitrogen is in short supply, this can be accomplished by reutilization of any nitrogen not used in the formation of creatine by the urea cycle. This is done by channeling the nitrogen into the guanidine cycle where urea is not formed. When there is an oversupply of nitrogen, then urea is an overflow product of the urea cycle and the guanidine cycle is muted.

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References

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Protein Measurement of Standards for Serum Ferritin Assays, and Comparison of Liver and Spleen Ferritins as Standards

To the Editor:

In 1976 we showed that results for protein analyses of purified ferritin were markedly affected by the iron content of the preparations when the biuret reaction or ultraviolet absorbance at 210 or 280 nm was used (1). Because there is no such interference with the method of Lowry et al. (2), the method used most often to estimate the protein content in the standard ferritin preparations for radioimmunoassays, it seemed best to

![Fig. 1. Method of Lowry et al. (2). Absorbance vs. protein content, liver and spleen ferritin and bovine albumin](image-url)

Protein content of all preparations was determined by Kjeldahl analysis