THE METABOLISM OF PARENTERALLY ADMINISTERED AMINO ACIDS

III. AMMONIA FORMATION

BY HENRY KAMIN* AND PHILIP HANDLER

(From the Department of Biochemistry and Nutrition, Duke University School of Medicine, Durham, North Carolina)

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Studies previously reported (1-3), involving the constant intravenous infusion of a number of amino acids, singly and in combination, have permitted the authors to obtain data concerning a number of physiological and biochemical phenomena associated with amino acid metabolism. The present report deals with the effect of amino acid infusion upon the renal excretion of ammonia. In this phase of metabolism, as in others previously reported, it has been found that the infusion of the dicarboxylic amino acids, glutamic and aspartic acids, and their amides, glutamine and asparagine, have yielded the data of greatest potential interest.

Present concepts of the mechanism of ammonia formation by the kidney are based primarily upon two observations: those of Van Slyke and his associates (4), who found a renal arteriovenous drop in plasma glutamine and amino acid concentration associated with formation of ammonia by the kidney, and those of Lotspeich and Pitts (5), who found an increase in urinary ammonia excretion during the infusion of a number of amino acids to acidotic dogs. Thus, it is generally believed that, under conditions of acidosis, about two-thirds of urinary ammonia is derived from plasma glutamine and the other third from plasma amino acids.

EXPERIMENTAL

Female, mongrel dogs, maintained on a stock diet, and fasted for 18 to 24 hours prior to experiment, were employed. The animals were those reported in previous publications from this laboratory, and the present data were obtained concomitantly in the course of the earlier experiments. The experimental and analytical techniques employed have been reported in detail (1-3). The dogs were maintained under light sodium pentobarbital anesthesia and urine samples were collected by means of an indwelling catheter. After a control period, during which the dogs received either no fluid or 0.5 per cent sodium chloride intravenously to induce

* Postdoctorate Research Fellow, National Institutes of Health, United States Public Health Service.
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diuresis, the amino acid infusion was begun and maintained at a constant rate for several hours. The amino acids were administered at rates varying from 0.5 to 4.0 mg. of N per kilo per minute in different experiments.

Ammonia was determined by the Conway technique (6). When glutamine was infused, the urine ammonia N was corrected for the spontaneous decomposition of urine glutamine in the course of the Conway analysis, which was found in separate experiments to be between 4 and 6 per cent of the glutamine amide N present for a diffusion period of 2$\frac{1}{2}$ hours at room temperature.

The experiments reported here varied in one particular from those of Lotspeich and Pitts (5). Whereas these workers rendered their animals acidotic prior to experiment by administration of ammonium chloride, this procedure was not routinely followed in the present studies. Rather, variations in the acid-base status of the experimental animals were produced by the direct effect of the amino acid infusion itself, and this status varied with the nature of the amino acid infused, its rate of metabolism, and its degree of neutralization. These factors have been discussed previously (2). In this manner ammonia formation as related to a number of physiological variables, such as plasma bicarbonate concentration, urine pH, and plasma amino acid concentration was studied. To have controlled, in an orderly fashion, all of these variables, for all of the amino acids infused, would have involved a prohibitive number of experiments; thus, the data are not altogether complete and, in many cases, are suggestive rather than conclusive.

Results

The data are presented in Table I. The rates of ammonia formation recorded were obtained after periods of 2 to 4 hours. It will be noted that all of the amino acids studied have been of the naturally occurring configuration; in addition, ammonia formation from $\alpha$-alanine has been studied to provide comparison with the natural isomer.

The variables which may conceivably affect ammonia excretion include the following: the nature of the amino acid infused, the concentration of that amino acid in plasma, urine pH, urine flow, plasma bicarbonate, and plasma pH. The latter was not measured, while variations in ammonia formation associated with variations in rate of urine flow were found to be insignificant compared with changes brought about by other variables. Within the limits of plasma amino acid concentration encountered in this study, all considerably above the physiological range, small differences in concentration appeared to be of secondary importance; in several instances, the rate of ammonia formation could be correlated with plasma $\alpha$-amino nitrogen, but the data are insufficient for proper evaluation.
The two factors which most affected ammonia formation were the nature of the amino acid infused and the plasma bicarbonate concentration. In

### Table I

**Effect of Amino Acid Infusion upon Ammonia Excretion**

<table>
<thead>
<tr>
<th>Amino acid administered</th>
<th>Dog No.</th>
<th>Increment, plasma α-amino N (mg. per cent)</th>
<th>Plasma HCO₃⁻ (m. eq. per l.)</th>
<th>Urine pH</th>
<th>Urine NH₃-N (γ per kg. per min.)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Controls</strong></td>
<td>19 dogs</td>
<td>0</td>
<td>(14.7-25.0)</td>
<td>6.84</td>
<td>4.14</td>
</tr>
<tr>
<td><strong>Enzymatic casein</strong></td>
<td>99-AGM</td>
<td>6.38</td>
<td>(6.3-7.8)</td>
<td>2.0-6.9</td>
<td></td>
</tr>
<tr>
<td>hydrolysate</td>
<td>203-AGM</td>
<td>6.78</td>
<td></td>
<td>110</td>
<td></td>
</tr>
<tr>
<td><strong>L-Glutamic acid</strong></td>
<td>62-GLA</td>
<td>6.68</td>
<td></td>
<td>66</td>
<td></td>
</tr>
<tr>
<td></td>
<td>63-GLA</td>
<td>6.58</td>
<td></td>
<td>69</td>
<td></td>
</tr>
<tr>
<td></td>
<td>64-NaG</td>
<td>6.72</td>
<td></td>
<td>20.9</td>
<td></td>
</tr>
<tr>
<td></td>
<td>61-NaG</td>
<td>6.58</td>
<td></td>
<td>7.3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>65-NaG</td>
<td>6.52</td>
<td></td>
<td>66</td>
<td></td>
</tr>
<tr>
<td><strong>L-Aspartic acid</strong></td>
<td>69-As</td>
<td>6.52</td>
<td></td>
<td>69</td>
<td></td>
</tr>
<tr>
<td></td>
<td>86-As</td>
<td>6.53</td>
<td></td>
<td>7.3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>70-NA</td>
<td>6.58</td>
<td></td>
<td>69</td>
<td></td>
</tr>
<tr>
<td><strong>L-Asparagine</strong></td>
<td>72-ȦN</td>
<td>6.70</td>
<td></td>
<td>117</td>
<td></td>
</tr>
<tr>
<td></td>
<td>73-APN</td>
<td>6.72</td>
<td></td>
<td>60</td>
<td></td>
</tr>
<tr>
<td><strong>L-Glutamine</strong></td>
<td>74-GAM</td>
<td>6.73</td>
<td></td>
<td>128</td>
<td></td>
</tr>
<tr>
<td></td>
<td>75-GAM</td>
<td>6.80</td>
<td></td>
<td>183</td>
<td></td>
</tr>
<tr>
<td><strong>Glycine</strong></td>
<td>94-G</td>
<td>6.90</td>
<td></td>
<td>66</td>
<td></td>
</tr>
<tr>
<td></td>
<td>93-G</td>
<td>6.70</td>
<td></td>
<td>57</td>
<td></td>
</tr>
<tr>
<td><strong>L-Alanine</strong></td>
<td>85-AL</td>
<td>6.70</td>
<td></td>
<td>148</td>
<td></td>
</tr>
<tr>
<td></td>
<td>200-AL</td>
<td>6.70</td>
<td></td>
<td>106</td>
<td></td>
</tr>
<tr>
<td><strong>n-L-Alanine</strong></td>
<td>85-AL</td>
<td>6.70</td>
<td></td>
<td>106</td>
<td></td>
</tr>
<tr>
<td></td>
<td>201-AL</td>
<td>6.70</td>
<td></td>
<td>20.1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>202-AL</td>
<td>6.70</td>
<td></td>
<td>7.28</td>
<td></td>
</tr>
<tr>
<td><strong>L-Leucine</strong></td>
<td>98-L</td>
<td>6.70</td>
<td></td>
<td>28.0</td>
<td></td>
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<tr>
<td><strong>L-Methionine</strong></td>
<td>87-M</td>
<td>6.52</td>
<td></td>
<td>55</td>
<td></td>
</tr>
<tr>
<td></td>
<td>88-M</td>
<td>6.84</td>
<td></td>
<td>43.0</td>
<td></td>
</tr>
<tr>
<td><strong>L-Cysteine</strong></td>
<td>91-Cy</td>
<td>6.79</td>
<td></td>
<td>18.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>90-Cy</td>
<td>6.79</td>
<td></td>
<td>48.0</td>
<td></td>
</tr>
<tr>
<td><strong>L-Lysine</strong></td>
<td>84-Ly</td>
<td>6.77</td>
<td></td>
<td>3.8</td>
<td></td>
</tr>
<tr>
<td></td>
<td>83-Ly</td>
<td>6.77</td>
<td></td>
<td>2.0</td>
<td></td>
</tr>
<tr>
<td><strong>L-Histidine</strong></td>
<td>80-H</td>
<td>6.42</td>
<td></td>
<td>63</td>
<td></td>
</tr>
<tr>
<td></td>
<td>81-H</td>
<td>6.30</td>
<td></td>
<td>121</td>
<td></td>
</tr>
<tr>
<td></td>
<td>95-H</td>
<td>6.30</td>
<td></td>
<td>25.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>96-H</td>
<td>7.12</td>
<td></td>
<td>35.0</td>
<td></td>
</tr>
<tr>
<td><strong>L-Arginine</strong></td>
<td>204-Ar</td>
<td>7.78</td>
<td></td>
<td>4.6</td>
<td></td>
</tr>
<tr>
<td></td>
<td>82-Ar</td>
<td>7.84</td>
<td></td>
<td>4.3</td>
<td></td>
</tr>
</tbody>
</table>

general, rates of ammonia formation were higher in dogs with low plasma bicarbonate concentration. This relationship is most apparent in dogs
receiving l-aspartic acid, and a similar situation may obtain for l-histidine. It is important to note, however, that a low plasma bicarbonate concentration is not a prerequisite for high rates of ammonia formation. Thus, Dog 202-AL, receiving dL-alanine, excreted 77 γ of ammonia N per kilo per minute in the presence of a plasma bicarbonate concentration of 37 m.eq. per liter. It is also of interest that large quantities of ammonia can be excreted in the absence of an acid urine; thus, one of the highest rates of ammonia excretion was observed in Dog 85-AL receiving l-alanine, in urine of pH 7.28, while the highest ammonia excretion rate observed in the entire study was that of Dog 75-GAM, which excreted 183 γ of ammonia N per kilo per minute in urine of pH 6.9.

The amino acids studied by Lotspeich and Pitts (5) were glycine, dL-

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>M.eq. Na</th>
<th>M.eq. amino acid</th>
<th>No. of dogs</th>
<th>Final plasma α-amino N mg. per cent</th>
<th>Amino acid excreted per cent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glutamic</td>
<td>0.61</td>
<td>0.76</td>
<td>1.0-1.1</td>
<td>0.71</td>
<td>0.77</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>3</td>
<td>2</td>
<td>72 (56-90)</td>
<td>47 (35-65)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1</td>
<td>34 (32-35)</td>
<td>38 (38-38)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.77</td>
<td>34</td>
<td>34</td>
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<td></td>
<td></td>
<td></td>
<td>1.00</td>
<td>35</td>
<td>35</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1.79</td>
<td>35</td>
<td>40</td>
</tr>
</tbody>
</table>

Amino acids infused for 4 hours at 0.1 m.eq. per kilo per minute. Sodium added as sodium bicarbonate.

alanine, l-leucine, dL-aspartic acid, l-glutamic acid, l-lysine, and l-arginine, as well as a casein hydrolysate. All of these amino acids (with the exception of dL-aspartic acid, in place of which the natural isomer was used) have also been infused in the present study. The results, in general, were similar to those of Lotspeich and Pitts. In addition, the effect of infusion of l-glutamine, l-aspartic acid, l-alanine (as well as dL-alanine), l-asparagine, l-methionine, and l-cysteine has been studied.

The present studies, which supplement those of Lotspeich and Pitts, permit a classification of amino acids according to their ability to produce ammonia formation. In view of the variation in plasma bicarbonate concentration among the various animals, it is impossible to arrange these amino acids in definite order of potency. However, they may be arranged into groups. Thus, it is apparent that casein hydrolysate, glutamine, asparagine, l- and dL-alanine, and possibly histidine are among the most potent ammonia formers; aspartic acid, glycine, leucine, methionine, and
cysteine occupy an intermediate position. Glutamic acid, lysine, and arginine appear to be completely devoid of activity.

It should be noted that, in the course of administering glutamic and aspartic acids at varying degrees of neutralization, a relationship between the amount of sodium administered and of glutamic acid excreted was observed. Thus, when sodium was made available, more glutamic acid was excreted and less retained in plasma. This relationship is indicated in Table II. It can also be noted that this relationship does not hold for aspartic acid.

The reason for this difference in behavior between glutamic and aspartic acids is not immediately apparent. However, it is possible that the permeability of kidney cells to sodium may be linked to glutamic acid metabolism in a manner analogous to the potassium-glutamic acid relationship observed in brain and retina (7).

**DISCUSSION**

There are significant qualitative differences between the ammonia-forming response of the kidney to ammonium chloride acidosis and to the infusion of amino acids. The infusion of amino acids causes ammonia formation even in the absence of acidosis, and the response is rapid. Ammonia formation resulting from the ingestion of ammonium chloride, on the other hand, is a delayed response, and requires a prolonged period of acidosis (8). Since profound acidosis resulting from the infusion of unneutralized glutamic acid failed to elicit ammonia formation during the course of these experiments, it appears likely that the effects of this amino acid may be similar to those of ammonium chloride. The reason for this delayed (or absent) response is not apparent, since plasma already contains the known necessary precursors in approximately the same concentrations as when the kidney ammonia-forming response is maximal (4, 5). These differences between the ammonia-forming response to ammonium chloride and to amino acid infusion suggest that different mechanisms may have been operating under each of these conditions. Were this so, it would be difficult to interpret the results of Lotspeich and Pitts, since both of these conditions were present in their experiments. In the present studies, the formation of ammonia during the infusion of amino acids cannot be unreservedly included in the evidence for the amino acid origin of a portion of the urinary ammonia in acidosis. The best evidence for this origin is still the renal arteriovenous difference in plasma amino acid concentration observed by Van Slyke et al. (4).

Regardless of the relevance of data such as the present, and those of Lotspeich and Pitts, to mechanisms of ammonia formation in ammonium chloride acidosis, it remains of value to examine possible mechanisms whereby α-amino nitrogen could be converted to ammonia nitrogen by
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the kidney. Lotspeich and Pitts have postulated, on the basis of the correlation within their series between ammonia-forming ability of the respective amino acid and its susceptibility to oxidative deamination by renal amino acid oxidases, that these oxidases are concerned in the living animal with the synthesis of ammonia by the kidney. The data obtained from the additional amino acids infused in the present study fail to extend the correlation upon which this hypothesis is based.

Thus, L-aspartic acid, which is not deaminated by kidney L-amino acid oxidase (9), leads to the formation of considerable quantities of ammonia. Furthermore, L-alanine and L-histidine, upon which the action of L-amino acid oxidase is but minimal, are among the most potent ammonia formers studied. Moreover, attempts to correlate ammonia formation in the dog with specificity of L-amino acid oxidase are probably academic, since the dog was one of the species in which Blanchard et al. (9) were unable to demonstrate L-amino acid oxidase activity. Since the presence of asparaginase in kidney has been both affirmed (10, 11) and denied (12), it is not possible to state whether there is a correlation between the high rate of ammonia formation from asparagine and the presence of a potent asparaginase in kidney.

The failure to correlate ammonia formation with susceptibility of the amino acid to deamination by known amino acid oxidases does not, of course, mean that amino acid oxidases are not involved in the formation of ammonia by the kidney. It may merely mean that the oxidases studied in vitro are not the ones actually operating in vivo. However, at the present time, it becomes increasingly difficult to conceive that an L-amino acid can be metabolized by first being oxidatively deaminated to the keto acid and ammonia either by a series of specific oxidases or by a single oxidase with varying specificity. The general picture of amino acid metabolism which appears to be developing is one which involves amino group (or ammonia) transfer rather than the formation of free ammonia as the first step in the intermediate metabolism of amino acids. In liver, this "transfer" concept is supported by the isotopic experiments of Hirs and Rittenberg (13) who demonstrated that α-amino nitrogen from several amino acids did not equilibrate with free ammonia during the process of urea synthesis. Similarly, Kamin and Handler (2) found that the infusion of a mixture of D,L-amino acids (upon which D-amino acid oxidase may be expected to operate) led to the formation of free plasma ammonia, whereas the infusion of the natural forms failed to do so.

In kidney, this problem is still obscure, and directly pertinent experimental data are lacking. However, the recent demonstration of the wide scope of activity of renal transaminases (14) suggests that transfer mechanisms may also play a dominant rôle in kidney. These transaminases
of themselves cannot account for ammonia formation in the kidney, since
the end-product of their action is glutamic acid, which does not cause
ammonia formation. It is possible, however, that these transaminases
furnish the kidney with mechanisms which permit this organ to metabolize
amino acids without the formation of ammonia.

The formation of urinary ammonia from plasma glutamine is well es-
tablished (4), and the high order of activity of kidney glutaminase pro-
vides a rational mechanism for its formation. It would be of great
interest to determine whether amino acids, in kidney, could transfer their
α-amino N directly to form the amide group of glutamine. Should this
process occur, this mechanism would provide a pathway whereby amino
acids could yield ammonia via the same mechanism as plasma glutamine;
under such conditions all rather than two-thirds of urinary ammonia
would arise from glutamine. The data of Schoenheimer and his associ-
ates (15), indicating a high rate of turnover of protein amide nitrogen
upon the administration of isotopic amino acids, suggest that these mech-
anisms may actually exist.

Were this true, one could then picture the process of renal ammonia
formation from amino acids as involving the channeling of α-amino nitro-
gen into either of two alternative pathways: a "glutamine" pathway,
causing the formation of ammonia, or (should the kidney require the
carbon skeleton of the amino acid under circumstances in which ammonia
formation is disadvantageous) into a "glutamic acid" pathway, preventing
ammonia formation. Thus, the regulation of renal ammonia formation
could occur at a single locus, perhaps by a pH-sensitive mechanism, direct
or humoral, which would determine into which metabolic pathway the
amino acid is to be directed.

**SUMMARY**

Dogs were infused at a constant rate with various amino acids, and
ammonia excretion was measured at different concentrations of plasma
bicarbonate. In certain cases, high rates of ammonia formation were
observed in the absence of acidosis and in the presence of relatively high
urine pH. The amino acids causing the highest rates of ammonia forma-
tion were L-glutamine, L-asparagine, L- and DL-alanine, L-histidine, and
casein hydrolysate; L-aspartic acid, glycine, L-leucine, L-methionine, and
L-cysteine occupied an intermediate position. L-Glutamic acid, L-lysine,
and L-arginine appeared to be completely devoid of activity.

The rate of excretion of glutamic (but not aspartic) acid varied with
the degree of neutralization of that amino acid.

Implications of these findings upon theories of ammonia formation by
the kidney are discussed.
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The authors wish to thank Dr. Jesse P. Greenstein for generous gifts of the L-alanine and L-methionine used in this study.

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