GLUTAMINE, GLUTAMIC ACID, AMMONIA ADMINISTRATION, AND TISSUE GLUTAMINE*

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Despite the recent revival of interest in amide-nitrogen metabolism in animal tissues, only limited information is available on the effect of glutamine administration on the glutamine content of blood and tissues. Bessman et al. (1) found that glutamine was not converted to glutamic acid during intestinal absorption. More recently, this same group of workers (2) has studied the uptake of glutamic acid and glutamine by brain and other tissues in the rat and mouse. Berenbom and White (3) recently reported that intravenously administered isotopic ammonium glutamate (N¹⁵H₄C₆H₅O₂N) and glutamine similarly labeled in the amide group were both almost quantitatively converted to urea by the rat.

Various aspects of amide-nitrogen metabolism in animal tissues have been under investigation in this laboratory for several years. In studies of the mechanism of glutamine formation, the experiments reported here were performed to determine the effect of oral and parenteral administration of glutamine and its possible precursors on the concentration of this compound in certain tissues.

EXPERIMENTAL

Materials and Methods—Male rats of the Sprague-Dawley strain weighing 250 to 300 gm. were used as the experimental animals in all these studies. Except as otherwise indicated, the animals received the following basal ration: casein 21 per cent, sucrose 70, cottonseed oil 5, salts (4) 4, thiamine 0.4 mg. per 100 gm., riboflavin 0.6, pyridoxine 0.3, calcium pantothenate 2.0, nicotinic acid 2.0, folic acid 0.1, inositol 2.0, p-aminobenzoic acid 2.0, and choline chloride 100. In addition, vitamins A and D in the form of cod liver oil and α-tocopherol were administered by dropper twice each week. In all cases food and water were supplied ad libitum.

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† Deceased, July 29, 1950.
GLUTAMINE CONTENT OF TISSUES

Following a period in which the animals were allowed to become adjusted to the partially purified ration, the experimental diets were fed for a period of 10 days. For oral administration, glutamic acid or ammonia replaced an equivalent amount of casein nitrogen in the diet. For parenteral administration, glutamine, glutamic acid, and glutamic acid plus ammonium carbonate were dissolved in normal saline, the solutions were adjusted to pH 7.4, and injected intraperitoneally. Control groups were sham-injected. All animals were killed by exsanguination under light ether anesthesia and the tissues rapidly removed, frozen, and stored at $-14^\circ$ until analyzed. 15 minutes elapsed between injection and the death of the animal.

The glutamine was determined in tissues by nesslerization of the ammonia liberated after incubation with a beef-kidney glutaminase preparation, according to the general procedure of Archibald (5). Preparation of the tissues for enzymatic hydrolysis of the amide group involved homogenization of a weighed sample with 15 ml. of 0.04 N potassium cyanide, dilution to 20 ml., and centrifugation for 10 minutes. Aliquots of the supernatant liquid were then taken for analysis.

Effect of Oral Administration of Glutamine and Related Compounds—The data in Table I show that feeding 1.9 gm. of added glutamine per kilo of ration caused small and usually insignificant increases of glutamine in most tissues. An increase of about 20 per cent was detected when twice this concentration was fed. Calculations based upon an average daily consumption of 15 gm. of ration showed that at this higher level each animal was receiving 57 mg. of glutamine in addition to that present in the casein. The increased glutamine in the carcass and organs, therefore, accounted for approximately half of the additional daily dietary intake. Since there was detectable retention in the tissues even at such relatively low levels of feeding, it appears that glutamine was metabolized somewhat less rapidly than certain other amino acids. It likewise seems evident

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Glutamine, mg. per cent per group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver</td>
<td>98 102 115</td>
</tr>
<tr>
<td>Lung</td>
<td>82 93 107</td>
</tr>
<tr>
<td>Kidney</td>
<td>106 109 121</td>
</tr>
<tr>
<td>Heart</td>
<td>205 219 253</td>
</tr>
<tr>
<td>Spleen</td>
<td>167 188 199</td>
</tr>
<tr>
<td>Muscle</td>
<td>187 190 203</td>
</tr>
<tr>
<td>Glutamine ingested, gm. per kg. ration</td>
<td>0 1.9 3.8</td>
</tr>
</tbody>
</table>
that extensive hydrolysis of glutamine to glutamic acid and ammonia did not occur in the gastrointestinal tract. That intestinal glutaminases apparently are not present in the rat was shown by a series of experiments involving the incubation of glutamine with rat intestinal contents and preparations of rat intestinal mucosa and pancreas. The results of these tests (Table II) suggest that practically no deamidation of glutamine occurred prior to absorption. The intracellular glutaminases of liver, kidney, and nervous tissue are implicated as the principal systems involved in the splitting of the amide group.

Addition of free glutamic acid to the ration in relatively small amounts was without marked effect on the tissue glutamine level (Tables III and IV). However, in some tissues, notably brain, there was an appreciable decline. This failure of administered glutamic acid to cause an elevation in tissue glutamine is in accord with the short time observations of Bessman et al. (1). These workers found that the intral ileal injection of glutamic acid did not cause any consistent change in the glutamine level of plasma. Similar results were obtained in human beings following oral administration of glutamate.

One of the most obvious mechanisms for the synthesis of glutamine is a reversal of the deamidation reaction. Studies were therefore made on the effect of oral administration of ammonia, glutamic acid, and ammonia plus glutamic acid (Table IV). When ammonium carbonate replaced an equivalent amount of dietary nitrogen, a slight though consistent increase

**Table II**

<table>
<thead>
<tr>
<th>Deamidation of L-Glutamine by Aqueous Extracts of Rat Intestinal Content, Intestinal Mucosa, and Pancreas</th>
</tr>
</thead>
<tbody>
<tr>
<td>Extract of</td>
</tr>
<tr>
<td>------------</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Intestinal content</td>
</tr>
<tr>
<td>&quot;mucosa&quot;</td>
</tr>
<tr>
<td>Pancreas</td>
</tr>
<tr>
<td>Liver</td>
</tr>
</tbody>
</table>

* The reaction mixture contained 1 ml. of veronal-acetate buffer (pH 6.8), 1 ml. of 0.014 M L-glutamine, 1 ml. of 0.03 M pyruvate (pH 7.0), and 0.5 ml. of the appropriate extract equivalent to 166 mg. of tissue or 333 mg. of intestinal content; incubated for 4 hours at 37°.

† The reaction mixture contained 2 ml. of veronal-acetate buffer with 0.02 M phosphate added (pH 8.0), 1 ml. of 0.014 M L-glutamine, and 0.5 ml. of the appropriate extract equivalent to 166 mg. of tissue or 333 mg. of intestinal content; incubated for 4 hours at 37°.
in tissue glutamine was observed. Statistical treatment of the data by analysis of variance (6) failed to reveal significant differences between experimental and control groups in glutamine content of any tissue, al-

**Table III**

*Effect of Ingested Glutamic Acid upon Rat Tissue Glutamine*

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Glutamine, mg. per cent</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>7 rats</td>
</tr>
<tr>
<td>Liver</td>
<td>102</td>
</tr>
<tr>
<td>Lung</td>
<td>70</td>
</tr>
<tr>
<td>Kidney</td>
<td>109</td>
</tr>
<tr>
<td>Heart</td>
<td>215</td>
</tr>
<tr>
<td>Spleen</td>
<td>158</td>
</tr>
<tr>
<td>Muscle</td>
<td>185</td>
</tr>
</tbody>
</table>

Glutamic acid ingested, gm. per kg. ration...

|          | 0 | 2.4 | 4.8 |

**Table IV**

*Effect of Ingested Glutamic Acid and Ammonia upon Rat Tissue Glutamine*

The values are given in mg. per cent.

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Glutamine, mg. per cent</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Basal ration</td>
</tr>
<tr>
<td></td>
<td>5 rats</td>
</tr>
<tr>
<td>Liver</td>
<td>95</td>
</tr>
<tr>
<td>Lung</td>
<td>82</td>
</tr>
<tr>
<td>Kidney</td>
<td>108</td>
</tr>
<tr>
<td>Heart</td>
<td>211</td>
</tr>
<tr>
<td>Spleen</td>
<td>157</td>
</tr>
<tr>
<td>Muscle</td>
<td>171</td>
</tr>
<tr>
<td>Brain</td>
<td>79</td>
</tr>
</tbody>
</table>

Glutamic acid ingested, gm. per kg. ration...

|          | 0 | 0   | 4.8  | 4.8  |

Ammonium carbonate ingested, gm. per kg. ration...

|          | 0 | 1.58 | 0 | 1.58 |

though in the case of kidney and heart the $F$ value approached that required for significance at the 5 per cent level of probability. It is possible that with larger numbers of animals this positive effect of oral administration of ammonia might be more conclusively demonstrated. The untimely death of the senior author makes such investigation impossible at this time.
The simultaneous ingestion of glutamic acid and ammonia produced the most striking response, amounting to an increase of about 50 per cent in all the tissues analyzed. It is not obvious why the ingestion of a much larger quantity of glutamic acid combined in the dietary protein was so much less effective than free glutamic acid. It may be that absorption of ammonia preceded the release of glutamic acid from casein by proteolysis. Sapirstein (7) has shown that glutamic acid increases the resistance of dogs to convulsions produced by the injection of ammonium chloride. In the light of the findings of this study, it might be suggested that this protective effect was due to the more rapid formation of glutamine in the presence of glutamic acid. These observations also tend to support the recent work of Bartlett and coworkers (8), indicating that ammonia storage may be one of the functions of glutamine.

Effect of Parenteral Administration of Glutamine and Related Compounds—In view of the results obtained with the oral administration of glutamine, glutamic acid, and ammonia, and because of the greater precision permitted by parenteral administration, the concentration of glutamine in tissues was measured following the intraperitoneal injection of these materials. In the case of glutamine, 160 mg. of amide-nitrogen were administered per kilo of body weight; glutamic acid and ammonia were given in equivalent amounts on a nitrogen basis. This quantity of amino acid nitrogen has been found by Friedberg and Greenberg (9) to produce readily detectable changes in the concentration of various amino acids in several tissues of the rat.

Rapid removal of the administered glutamine from the site of injection was shown by the marked increases in glutamine concentration found in all tissues examined (Table V). The concentration increased nearly 2-fold in all tissues except heart and skeletal muscle, which showed a more moderate increase. These observations are of interest when compared with the results of Friedberg and Greenberg (9), who showed that intravenously administered amino acids were concentrated most actively by liver and kidney, less so by skeletal muscles, and not at all by brain. Similar findings with respect to glutamic acid were reported by Schwerin, Bessman, and Waelsch (2), but these workers noted a tendency for an increase in the glutamine content of brain following the intravenous administration of glutamine to both rats and mice.

The injection of an amount of ammonia nitrogen equivalent to 160 mg. of amide-nitrogen per kilo was without effect upon the glutamine level of any of the tissues examined (Table VI). In view of the positive correlation between tissue glutamine and oral ingestion of ammonium carbonate, it appears that either insufficient time for glutamine synthesis had elapsed prior to sacrificing the animals or suitable precursors were not
### Table V

**Effect of Intraperitoneally Injected Glutamine upon Tissue Glutamine Levels in Eight Rats**

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Glutamine, mg. per cent</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Saline control</td>
</tr>
<tr>
<td>Liver</td>
<td>95</td>
</tr>
<tr>
<td>Lung</td>
<td>87</td>
</tr>
<tr>
<td>Kidney</td>
<td>115</td>
</tr>
<tr>
<td>Heart</td>
<td>208</td>
</tr>
<tr>
<td>Spleen</td>
<td>151</td>
</tr>
<tr>
<td>Muscle</td>
<td>172</td>
</tr>
<tr>
<td>Brain</td>
<td>82</td>
</tr>
</tbody>
</table>

* The values represent concentration in tissues of animals injected intraperitoneally with 2.5 ml. of 0.9 per cent sodium chloride solution and killed 15 minutes after injection.

† Animals injected intraperitoneally with 160 mg. per kilo of amide nitrogen in the form of glutamine dissolved in 0.9 per cent sodium chloride solution and adjusted to pH 7.4. Animals killed 15 minutes after administration of the glutamine.

### Table VI

**Effect of Intraperitoneal Injection of Glutamic Acid and Ammonia upon Rat Tissue Glutamine from Six Rats**

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Glutamine, mg. per cent</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Saline control</td>
</tr>
<tr>
<td>Liver</td>
<td>97</td>
</tr>
<tr>
<td>Lung</td>
<td>76</td>
</tr>
<tr>
<td>Kidney</td>
<td>115</td>
</tr>
<tr>
<td>Heart</td>
<td>213</td>
</tr>
<tr>
<td>Spleen</td>
<td>150</td>
</tr>
<tr>
<td>Muscle</td>
<td>183</td>
</tr>
<tr>
<td>Brain</td>
<td>78</td>
</tr>
</tbody>
</table>

* The values represent concentration in tissues of animals injected intraperitoneally with 2.5 ml. of 0.9 per cent sodium chloride solution and killed 15 minutes after injection.

† Animals injected intraperitoneally with 160 mg. per kilo, body weight, of either ammonia nitrogen, glutamic acid nitrogen, or both dissolved in 0.9 per cent sodium chloride solution and adjusted to pH 7.4. Animals killed 15 minutes after injection. Available in sufficient quantity. Administration of glutamic acid alone did not affect the glutamine content of any of the tissues examined with the possible exception of brain, which showed a slight decrease. The simultaneous administration of both glutamic acid and ammonia, however, produced a significant increase in the glutamine content of all the tissues.
examined except the brain, which again was refractory. This may well be due to the previously mentioned failure of glutamic acid readily to enter the brain cells.

The rapid accumulation of glutamine in certain tissues in the presence of both obvious precursors suggests that a major mechanism for glutamine synthesis may be the condensation of glutamic acid and ammonia. It has been shown by Speck (10) that glutamine synthesis is readily accomplished by pigeon liver preparations in the presence of ammonia, glutamate, adenosinetriphosphate, and Mg++. These data suggest that this reaction may be common to other tissues of the body and that transformation of free ammonia to the amide form is an important mechanism for storage of ammonia. That it might suitably serve such a metabolic function is suggested by the rapidity with which it is formed and the ease with which it is picked up by the tissues.

**SUMMARY**

Supplementation of the ration of rats with glutamine for a period of 10 days resulted in an increase of the glutamine content of liver, kidney, heart, lung, spleen, and skeletal muscle. Increased intake of glutamic acid was without effect. The feeding of ammonium carbonate resulted in small but consistent increases in tissue glutamine content, including brain tissue. Simultaneous feeding of ammonia and glutamate resulted in significantly increased glutamine levels.

Intraperitoneally injected glutamine was rapidly taken up by various tissues, as evidenced by sharply increased concentrations in kidney, liver, lung, spleen, and brain, and a lesser increase in muscle. Similar injections of ammonia or glutamate were without effect. Simultaneous parenteral administration of ammonia and glutamic acid produced a marked rise in glutamine in all the tissues examined except brain. Glutamine formation is suggested as a possible mechanism for the storage of ammonia.

**BIBLIOGRAPHY**

GLUTAMINE, GLUTAMIC ACID, AMMONIA ADMINISTRATION, AND TISSUE GLUTAMINE
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