THE ABSORPTION OF GLUTAMIC ACID AND GLUTAMINE*

By S. P. BESSMAN,† J. MAGNES,‡ PAULA SCHWERIN, and
HEINRICH WAELSCH

(From the Departments of Biochemistry, New York State Psychiatric Institute and the
College of Physicians and Surgeons, Columbia University, New York)

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The occurrence of free glutamine in plant tissue extracts has been recognized for a considerable time, but its presence in the protein-free filtrates of mammalian tissues was established only recently (1). The functional significance of the free amide in tissues cannot be appreciated without a knowledge of its quantitative relationship to glutamic acid and of the biological mechanisms regulating the interconversions of the two compounds. A study of these questions was made possible by the development of a chemical micromethod for the determination of glutamic acid and glutamine, each in the presence of a large excess of the other (2, 3). The method was applied in studies of the absorption of glutamic acid, glutamine, and glutathione from the gut of the cat by analysis of the portal blood, and of the concentration changes of glutamic acid and glutamine in peripheral blood after oral administration of glutamic acid to human subjects. The analytical procedure was simplified and extended to permit the complete removal of glutathione. This modification became necessary not only for the use of the method in the experiments in which intestinal absorption of glutathione was studied, but also for its application to tissue analysis, which will be the subject of subsequent reports.

EXPERIMENTAL

Determination of Glutamic Acid and Glutamine—In the previous study on
the glutamic acid and glutamine content of blood plasma and serum the
glutamine concentration was calculated as the difference between total
 glutamic acid determined after acid hydrolysis in one sample and free
 glutamic acid determined in another. The method has been simplified,
with a saving of material, by the direct determination of glutamine in the
filtrate from the adsorption column. The filtrate (2 ml.) of the solution
containing glutamic acid and glutamine and the wash water (2 ml.) were

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† Fellow of the Children's Hospital Research Foundation, Washington, D. C.
‡ On leave from the Department of Physiology, The Hebrew University, Jerusalem.
collected in a 10 ml. volumetric flask and hydrolyzed with 2 ml. of 6 N HCl for 1 hour. The hydrolysate was neutralized and diluted to 10 ml. as described previously. 2 ml. of the neutralized solution were taken for the glutamine determination. In pure solution and in the absence of asparagine the glutamic acid may be determined directly without further adsorption on a second column. Under such conditions the hydrolyzed solution was neutralized, 5 mM of acetic acid were added, and the solution was diluted to 10 ml. with water. 4 ml. of this solution were treated with ninhydrin. In all determinations on tissue filtrates the hydrolyzed solution, containing glutamic acid originating from glutamine, was passed through a second column. The recovery of glutamine alone or in solutions containing glutamic acid and amide, as obtained in the above procedure, amounted to 95 to 105 per cent.

Removal of Glutathione—In glutathione-containing solutions, cysteine and glutamic acid, equivalent to approximately 20 per cent of the tripeptide when expressed as glutamic acid, were liberated under the conditions employed for glutamine hydrolysis. The removal of glutathione by precipitation with metal salts (copper, cadmium, lead) led to considerable losses of glutamic acid. A nearly complete removal of glutathione or cysteine without loss of glutamic acid was accomplished by adsorbing the sulphydryl compound on lead carbonate introduced on top of the aluminum oxide column. 1 mg. of lead carbonate (2PbCO3·Pb(OH)2) suspended in 0.5 ml. of water was superimposed on the aluminum oxide column under gentle suction. By this modification of the column, glutathione and any cysteine were removed to the extent of at least 99 per cent (Table I). The last two glutathione experiments show that glutathione does not interfere with the direct determination of glutamine after glutamic acid adsorption. Glutathione in the amounts known to occur in mammalian tissue (4) can be successfully eliminated by this procedure.

Determination and Removal of Keto Acids—In the course of the investigation it became desirable to determine the keto acids formed after the intraintestinal administration of glutamic acid and glutamine. It was noted that high concentrations of keto acids, equivalent to more than 5 mg. of pyruvic acid per 100 ml. of plasma, lowered the glutamic acid recovery after acid hydrolysis of samples for the determination of total glutamic acid (glutamic acid plus glutamine) by the original method (3). No interference was experienced when the filtrates from glutamic acid adsorption, containing only glutamine, were submitted to acid hydrolysis, since most of the keto acid was retained in the column. If total glutamic acid is to be determined by the original procedure, the keto acids may be removed as the 2,4-dinitrophenylhydrazones, and they may be estimated by the same procedure.
In the experiments with blood plasma filtrates, 2 ml. of 0.1 per cent 2,4-dinitrophenylhydrazone in 2 N HCl were added to 1 ml. of the trichloroacetic acid filtrate. After 25 minutes the hydrazone was extracted with eight 4 ml. portions of benzene. The hydrazone was extracted from the benzene solution with three 2 ml. portions of a 10 per cent solution of sodium bicarbonate, the color was developed by the addition of 5 ml. of 2 N NaOH to 5 ml. of the extract, and read after 5 minutes in the Coleman junior spectrophotometer at wave-lengths 420 and 520 m\(\mu\) in order to determine ketoglutaric acid and pyruvic acid. Sodium pyruvate served as the standard (5, 6).

For the determination of the total glutamic acid content after the removal of keto acids, 1 ml. of the extracted trichloroacetic acid filtrate was hydrolyzed with 0.5 ml. of 6 N HCl at 100° for 1 hour, neutralized, and diluted to 5 ml. with water. 2 ml. aliquots were taken for the duplicate determinations.

Absorption Experiments. Cats—A cannula was introduced into the trachea of a cat (4 to 5 kilos) under diallylbarbituric acid\(^1\) anesthesia, and heparin\(^1\) was injected intravenously. After ligation of the gastrosplenic vein, a 2-way cannula with a side arm was introduced into the portal vein. Thus the portal blood flow was not obstructed. A blood sample (8 ml.) was removed before the injection into the small intestine of 5 ml. of saline

\(^1\) We are indebted to Ciba Pharmaceutical Products, Inc., for a gift of diallylbarbituric acid and to Roche-Organon for a generous supply of heparin.

### Table I

Determination of Glutamic Acid and Glutamine in Presence of SH Compounds

<table>
<thead>
<tr>
<th>SH compound added</th>
<th>In sample</th>
<th>Found</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Glutamic acid</td>
<td>Glutamine</td>
</tr>
<tr>
<td>Glutathione</td>
<td>604 (\gamma)</td>
<td>41.4</td>
</tr>
<tr>
<td></td>
<td>1808 (\gamma)</td>
<td>37.3</td>
</tr>
<tr>
<td></td>
<td>3012 (\gamma)</td>
<td>37.3</td>
</tr>
<tr>
<td></td>
<td>564 (\gamma)</td>
<td>37.3</td>
</tr>
<tr>
<td></td>
<td>600 (\gamma)</td>
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</tr>
<tr>
<td></td>
<td>1800 (\gamma)</td>
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</tr>
<tr>
<td></td>
<td>2400 (\gamma)</td>
<td>37.3</td>
</tr>
<tr>
<td></td>
<td>1114 (\gamma)</td>
<td>20.8</td>
</tr>
<tr>
<td></td>
<td>2228 (\gamma)</td>
<td>41.6</td>
</tr>
<tr>
<td>Cysteine</td>
<td>21.2 (\gamma)</td>
<td>41.4</td>
</tr>
</tbody>
</table>

* Corrected for a content of 92.5 per cent of glutamine.
† Corrected for retention of 1 per cent of glutamine as glutamic acid (2).
containing glutamic acid, glutamine, or glutathione, adjusted to pH 7.3. Two further blood samples were taken 15 and 30 minutes later. The cell volume was determined in all blood samples.

*Man*—Venous blood was taken from human subjects at least 18 hours after the last meal. Two further blood samples were removed 1 and 2 hours after the intake of 1 gm. of glutamic acid per 10 kilos of body weight. The whole amount of glutamic acid was suspended in 100 ml. of water.

**RESULTS AND DISCUSSION**

The analysis of the blood of the portal vein after the intraintestinal administration of glutamic acid or glutamine showed that both compounds passed the intestinal wall without any significant interconversion (Table II). A considerable increase of the administered compound occurred 15 minutes after administration, with only a small change in the level of the other. Simultaneously with the large increase in the glutamic acid concentration in the plasma after the administration of this amino acid (Cats 1 and 2) there was found, after 15 minutes, a decrease in the glutamine values. The increase of the glutamine concentration at 30 minutes may be interpreted as a release from the tissues of glutamine either formed from the administered glutamic acid or mobilized as a result of the increased glutamic acid concentration. Both phenomena, the decrease of the glutamine concentration and the following increase, have also been found in the peripheral blood plasma of human subjects after the ingestion of glutamic acid. The effect of an increased glutamic acid concentration in plasma in decreasing

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**Table II**

Concentration of Free Glutamic Acid and Glutamine in Blood Plasma of Portal Vein after Intraintestinal Administration of Glutamic Acid, Glutamine, and Glutathione

Values expressed as mg. per 100 ml. of plasma.*

<table>
<thead>
<tr>
<th>Time after administration (min.)</th>
<th>Cat 1</th>
<th>Cat 2</th>
<th>Cat 3</th>
<th>Cat 4</th>
<th>Cat 5</th>
<th>Cat 6</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Acid</td>
<td>Amide</td>
<td>Acid</td>
<td>Amide</td>
<td>Acid</td>
<td>Amide</td>
</tr>
<tr>
<td>0</td>
<td>2.8</td>
<td>10.7</td>
<td>1.7</td>
<td>10.4</td>
<td>2.5</td>
<td>6.4</td>
</tr>
<tr>
<td></td>
<td>100 mg. glutamic acid administered</td>
<td>2.5</td>
<td>6.4</td>
<td>1.6</td>
<td>5.9</td>
<td>1.9</td>
</tr>
<tr>
<td>15</td>
<td>12.9</td>
<td>4.2</td>
<td>6.6</td>
<td>8.3</td>
<td>3.9</td>
<td>12.7</td>
</tr>
<tr>
<td>30</td>
<td>14.8</td>
<td>13.0</td>
<td>1.0</td>
<td>15.5</td>
<td>3.1</td>
<td>25.8</td>
</tr>
</tbody>
</table>

* Per cent blood cells: Cat 1, 44, 36, 35; Cat 6, 49, 42, 41; Cats 2 to 5, change in blood cell volume less than 3 per cent.
the glutamine level appears to be part of a general mechanism since a lowering of the concentrations of glutamine, glycine, and residual amino nitrogen has been found under similar conditions in dogs (7).

In the glutamine experiments, there was found a small increase of glutamic acid, which is apparently not related to the glutamine concentration, and may therefore be due to a release of glutamic acid originating from the increased glutamine concentration in the tissue.

Glutamic acid and glutamine are apparently not converted into each other during the passage through the intestinal wall, and there seems to be no extensive deamination during this process. Only insignificant increases in the keto acid concentration were found and the optical absorption ratios of the hydrazones at 420 and 520 mµ varied between 1.2 and 1.4, a result which indicates that the relative concentration of ketoglutaric acid did not change significantly, either during the absorption of glutamine or the parent amino acid.

After the intraintestinal administration of an equivalent amount of glutathione, there was no change in the glutamic acid or glutamine concentration of the plasma of portal blood during the experimental period comparable with that found during the absorption of the amino acid and its amide. It has been pointed out in the experimental part that glutathione, if not removed, contributed to the glutamine fraction after acid hydrolysis about 20 per cent of its concentration in glutamic acid equivalents. Values obtained for glutamine in the glutathione experiments with and without the modification developed for the removal of glutathione agreed within the error of the method. It appears therefore that the tripeptide, if absorbed during the experimental period, is taken up rapidly by the cells or that it is not metabolized during passage through the intestinal wall to glutamic acid or glutamine to any considerable degree.

The direct evidence (8, 9) for the occurrence of glutamine and asparagine in proteins is based on the isolation of the amides from enzymatic hydrolysates. If changes in the glutamine content of the food proteins due to storage and preparation of the food are disregarded for the present, our experiments suggest the possibility that glutamic acid and glutamine are absorbed in about the ratio in which they occur in the original protein. Depending on the composition of the proteins ingested, varying amounts of the two compounds will therefore be absorbed and the organism, by enzymatic mechanisms, will have to adjust the amounts to the specific ratios of the tissues.

The oral administration of glutamic acid to human subjects led always to an increase of varying degree in the glutamic acid concentration of the peripheral blood (Table III). Two types of responses to the elevation of the blood glutamic acid may be distinguished. A small increase of the
glutamic acid concentration appeared to be accompanied by a considerable increase in the glutamine concentration, which may be interpreted as the return of the amidated amino acid from the tissue or a mobilization of tissue glutamine. A high glutamic acid concentration in the blood appeared to lead to a considerable decrease in the glutamine values 1 hour after the administration. In the experiments with cats, both the decrease and the increase in glutamine concentration were found in the portal blood of the same animal as a response to an elevated glutamic acid concentration. These findings are an additional demonstration of the influence of the blood concentration of one amino acid on that of another. These experiments do not permit any prediction as to the glutamic acid and glutamine levels in blood to be expected if glutamic acid is administered in combination with other amino acids, as in hydrolysates or whole protein.

Beneficial effects of the oral administration of glutamic acid to epileptics and mental defectives have been reported (10, 11). It had been difficult to understand why a daily administration of only 10 to 20 gm. of glutamic acid could have any effect in view of the large amounts of glutamic acid ordinarily ingested with protein. The absorption experiments with the cat show that glutamine and glutamic acid may pass the intestinal tract without interconversion. Therefore, depending on the glutamine-glutamic acid ratio in the protein, a small amount of additional glutamic acid may considerably increase the relative intake of this amino acid. Our experiments with human subjects show that the ingestion of 2 or 3 times the therapeutic amount of glutamic acid leads to a significant increase in the blood glutamic acid level and to alterations in the metabolism of glutamine.

**SUMMARY**

A simplified modification of the method for the determination of glutamic acid and glutamine is described. Glutamine and glutamic acid are ab-

### TABLE III

<table>
<thead>
<tr>
<th>Time after administration</th>
<th>Subject 1</th>
<th>Subject 2</th>
<th>Subject 3</th>
<th>Subject 4</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Acid</td>
<td>Amide</td>
<td>Acid</td>
<td>Amide</td>
</tr>
<tr>
<td>hrs.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>0.6</td>
<td>8.4</td>
<td>0.6</td>
<td>10.6</td>
</tr>
<tr>
<td>1</td>
<td>1.0</td>
<td>10.3</td>
<td>1.2</td>
<td>14.0</td>
</tr>
<tr>
<td>2</td>
<td>1.0</td>
<td>8.9</td>
<td>0.8</td>
<td>11.3</td>
</tr>
</tbody>
</table>
sorbed from the gut of the cat without interconversion. The elevated glutamic acid level in the portal blood is accompanied by a decrease in the glutamine level, followed by an increase. The oral administration of glutamic acid to human subjects leads to an increase of the glutamic acid level in the peripheral blood, with a simultaneous decrease or increase in the glutamine concentration.

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S. P. Bessman, J. Magnes, Paula Schwerin and Heinrich Waelsch


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