TRANSAMINATION FROM GLUTAMINE TO \(\alpha\)-KETO ACIDS*

BY ALTON MEISTER AND SARAH V. TICE

(From the National Cancer Institute, National Institutes of Health, Bethesda, Maryland)

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The striking activation of the deamidation of glutamine in rat liver extracts by pyruvic acid was first reported by Greenstein and Carter (2) and has been the subject of several subsequent investigations (3-7). This phenomenon was observed with pyruvic, phenylpyruvic, and \(\alpha\)-ketoisocapric acids, but not with pyruvoylglycine, phenylpyruvoylglycine, lactic acid, or \(\gamma\)-ketovaleric acid. The enzyme which catalyzes the \(\alpha\)-keto acid activation of glutamine deamidation was separated from phosphate-activated glutaminase and partially purified by Errera (6). As an approach to an understanding of the mechanism of this interesting keto acid effect, it seemed of importance to investigate the specificity requirements of the system with respect to the keto acid. In the course of these studies, an apparently new type of transamination reaction has been observed, involving the transfer of the \(\alpha\)-amino group of glutamine to a number of \(\alpha\)-keto acids. The results of an investigation of this reaction, which is associated with the deamidation of glutamine, form the basis of this report.

EXPERIMENTAL

Materials—L-Glutamine was obtained from the Nutritional Biochemicals Corporation. When boiled with 2 N hydrochloric acid for 1 minute, 95 per cent of the theoretical amide nitrogen was recovered. Synthetic L-glutamine, prepared according to Bergmann et al. (8), and L-glutamine, labeled with N\(^{15}\) in the amide group (9), were donated by Dr. M. Berenborn. The normal \(\alpha\)-keto acids were obtained and characterized as described previously (10). Sodium \(\alpha\)-ketoisocaproate, L-isoglutamine, acetyl-DL-glutamic acid, D-alloisoleucine, and the L isomers of methionine, glutamic acid, leucine, alanine, \(\alpha\)-aminobutyric acid, and tyrosine were donated by Dr. J. P. Greenstein. L-Norvaline and L-\(\alpha\)-aminocaproic acid were prepared by enzymatic resolution of the corresponding racemic amino acids (11, 12). L-Glutamic acid, L-phenylalanine, and \(\alpha\)-ketoglutaric acid were obtained from the Nutritional Biochemicals Corporation.

Phenylpyruvic acid was synthesized by the method of Herbst and Shemin (13). \(\alpha\)-Ketoisovaleric acid was prepared by refluxing 2-phenyl-173

* A preliminary report of this work has appeared (1).
4-isopropylidene-5-oxazolone (14) with 3 N hydrochloric acid for 5 hours. The keto acid was extracted with ether and purified by vacuum distillation (analysis, calculated, C 51.68, H 6.94; found, C 51.61, H 7.00). The crystalline sodium salt was prepared by neutralization with sodium hydroxide followed by addition of an excess of acetone (Na, calculated 16.65; found, 16.55). Barium α-keto-γ-methylbutyrate was prepared according to Cahill and Rudolph (15), and the barium removed quantitatively with sulfuric acid before use.

The d-α-keto-β-methylvaleric acid was prepared from d-alloisoleucine by the action of d-amino acid oxidase in the following manner: A mixture of 40 gm. of d-alloisoleucine dissolved in 1800 cc. of water and 3100 cc. of an aqueous extract of hog kidney acetone powder was adjusted to pH 8.2 with sodium hydroxide and stirred for 7 hours at 37°. A stream of oxygen was bubbled into the solution throughout incubation. The mixture was adjusted to pH 4.0 with hydrochloric acid and heated at 70° for 10 minutes with norit. The solution was filtered, concentrated in vacuo to 800 cc., and acidified to Congo red paper with concentrated hydrochloric acid. The keto acid was extracted with ether and purified by vacuum distillation. The crystalline sodium salt was prepared as described for sodium α-ketoisovalerate (Na, calculated, 15.12; found, 15.08). The sodium salt of d-α-keto-β-methylvaleric acid, which has the same configuration about the β-carbon as L-isoleucine, gave a specific optical rotation of +31.4° (0.7 per cent aqueous solution). The L isomer of the keto acid was prepared as the sodium salt in a similar manner from DL-isoleucine (Merek, lot No. 40761) (Na, calculated, 15.12; found, 15.18). The specific rotation of this salt was −32.6° (1 per cent aqueous solution), and the specific rotation of the 2,4-dinitrophenylhydrazone of the keto acid was −16.5° (2 per cent solution in ethanol). Greenstein, Levintow, Baker, and White (personal communication) observed rotations of −16.9° and +16.8°, respectively, for the 2,4-dinitrophenylhydrazones of the keto acids obtained from pure samples of d-isoleucine and d-alloisoleucine by the action of d-amino acid oxidase.

Oxalacetic acid was obtained by the method of Krampitz and Werkman (16). The α,γ-diketo acids, β,δ-diketocaproic acid and its δ-lactone, and dehydroacetic acid were synthesized and characterized (17, 18). β-Acetylacrylic acid was prepared according to Wolff (19) and purified by sublimation in vacuo. The β-keto acids were synthesized as the ethyl esters by the procedure of Fischer et al. (20) and saponified and extracted as described by Davies (21). The solutions of the sodium salts of the β-keto acids thus obtained were standardized by the aniline-citrate procedure (22). Ethyl acetooacetate, γ-ketovaleric acid, methyl ethyl ketone, methyl

1 The microanalyses were performed by Mr. R. J. Koegel.
propyl ketone, and acetylacetone were Eastman products. Pyruvamide was synthesized according to Anker (23). The \( \gamma \)-methyl and \( \gamma \)-ethyl amides of \( L \)-glutamic acid and \( L \)-pyrrolidonecarboxylic acid were prepared according to Lichtenstein (24).

Carbamyl-\( L \)-glutamic acid was prepared as follows: 9 gm. of potassium cyanate (Eastman Kodak Company) were added to 100 cc. of a 1 M solution of \( L \)-glutamic acid neutralized to pH 6.8 with potassium hydroxide. After standing at room temperature (22-28\(^\circ\)) for 48 hours, the mixture was cooled in ice and acidified to Congo red paper with concentrated hydrochloric acid. The product, which crystallized on scratching, was recrystallized from hot water. The melting point was 169\(^\circ\) (corrected), which is somewhat higher than that given in the literature (25) (analysis, calculated, C 37.88, H 5.30, N 14.73; found, C 37.98, H 5.33, N 14.70). The \( \gamma \)-ethyl ester of \( L \)-glutamic acid was prepared according to Bergmann and Zervas (26). The pyridoxal phosphate was a gift from Dr. W. W. Umbreit.

**Methods**

Ammonia was determined as described by Greenstein and Leuthardt (27). \( \alpha \)-Keto acids were determined by the method of Lu (28) and with crystalline lactic dehydrogenase and reduced diphosphopyridine nucleotide (DPNH\(_2\)) as previously described (10). The \( \beta \)-keto acids were determined by the procedure of Edson (22). The \( \alpha,\gamma \)-diketo and \( \beta,\delta \)-diketo acids were estimated by the spectrophotometric procedures previously reported (17, 18, 29). \( \alpha \)-Ketoglutarate was determined in the presence of pyruvate, \( \alpha \)-ketobutyrate, \( \alpha \)-ketovalerate, or phenyl pyruvate in the following manner: A suitable aliquot of the reaction mixture was treated with an excess of crystalline lactic dehydrogenase and DPNH\(_2\) in order to reduce the monocarboxylic \( \alpha \)-keto acid. When reduction was complete (as judged by the absence of further decrease in the absorption of DPNH\(_2\)), 4 cc. of a 0.1 per cent solution of 2,4-dinitrophenylhydrazine in 2 N hydrochloric acid were added, and the mixture was allowed to stand for 15 minutes. Extractions with ethyl acetate and sodium carbonate were carried out according to Lu (28) and the colors compared with those of standards in a Coleman spectrophotometer at 600 \( \mu \mu \).

Glutamic acid and glutamine were determined by quantitative decarboxylation with *Clostridium welchi* (strain SR 12) suspensions according to Krebs (30). The reported values are based upon the quantity of carbon dioxide formed and are not corrected for ammonia formation as described by Krebs. Alanine, phenylalanine, and leucine were determined by microbiological methods with *Leuconostoc citrovorum* and *Leuconostoc mesenteroides* (31, 32).

Paper chromatograms were used for the detection and identification of
amino acids. The following solvents were employed: phenol saturated with water in an atmosphere of hydrocyanic acid and ammonia; 77 per cent ethanol; formic acid (15 per cent), tertiary butanol (70 per cent), and water (15 per cent); and lutidine mixture (33). Authentic samples of the various amino acids and the experimental samples were run on the same set of paper strips.

Sprague-Dawley and Osborne-Mendel rats of both sexes, fed ad libitum and weighing 150 to 350 gm., were employed. Liver homogenates were prepared with 3 volumes of ice-cold distilled water in a small Waring blendor and centrifuged at 20,000 \( \times g \) for 1 hour before use. The enzyme preparation used in these studies was prepared essentially according to Errera (6). The ethanol concentration of a homogenate of 300 gm. of liver was adjusted to 20 per cent by the addition of cold \((-10^\circ \) ethanol. After standing at \(-8^\circ \) for 12 hours, the precipitate was removed by centrifugation, and the supernatant adjusted to pH 5.3 with 0.2 M acetic acid. After standing at \(-8^\circ \) for 2 to 3 hours, the precipitate was collected by centrifugation and suspended in 150 cc. of cold water. The suspension was stored at 0\(^\circ \) for 2 to 3 hours, centrifuged, and the supernatant adjusted to pH 6.5 and an ethanol concentration of 35 per cent. The solution was allowed to stand at \(-8^\circ \) for 12 hours and the precipitate was centrifuged and dissolved in 80 to 120 cc. of cold water, the insoluble residue being removed by centrifugation. The preparation, which contained about 1 mg. of protein nitrogen per cc., yielded no ammonia from glutamine in the absence of \( \alpha \)-keto acids and was 30 to 50 times more active than the original homogenate (on the basis of protein nitrogen) in catalyzing the deamidation of glutamine in the presence of pyruvate. When stored at 0\(^\circ \), the preparation was stable for 4 or 5 days, followed by a rapid decrease in activity. Further purification, as described by Errera, led to a preparation which was about 20 per cent more active but which was otherwise similar to that described above.

Results

Effect of Keto Acids on Deamidation of Glutamine

The effect of a wide variety of keto acids on the deamidation of glutamine by rat liver extracts was studied under the conditions employed by Greensteln and Price (7). Only acids possessing an \( \alpha \)-keto group produced significant acceleration of the deamidation of glutamine (Table I). The...

\(^2\) The authors are indebted to Dr. H. A. Sober for carrying out the chromatographic procedures.

\(^3\) The effect of various keto acids on the deamidation of asparagine was similar to that observed with glutamine in that only \( \alpha \)-keto acids produced acceleration. Studies on the keto acid activation of asparagine deamidation will be reported at a later date.
mean value for eleven experiments with pyruvic acid was 8.88 μM of ammonia (range 7.67 to 10.4). This value and those given in Table I for phenylpyruvic and α-ketoisocaproic acids are in essential agreement with those of previous investigators (2-7). The following keto acids produced no significant increase in glutamine deamidation: α-ketoisovaleric, d- and l-α-keto-β-methylvaleric, the normal β-keto acids from acetoacetic to β-ketoundecylic, β,δ-diketocaproic, triacetic lactone, dehydroacetic, β-acety lacrylic, and γ-ketovaleric. Acetylace tone, acetone, methyl ethyl ketone, and methyl propyl ketone were also inactive. Pyruvamide did not increase the deamidation of glutamine nor was this compound enzymatically deamidated. The pH optimum for glutamine deamidation in

TABLE I

<table>
<thead>
<tr>
<th>Keto acid</th>
<th>Relative value</th>
<th>Keto acid</th>
<th>Relative value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pyruvic</td>
<td>(100)</td>
<td>Oxalacetic</td>
<td>99</td>
</tr>
<tr>
<td>None</td>
<td>10</td>
<td>α,γ-Diketovaleric</td>
<td>91</td>
</tr>
<tr>
<td>α-Ketobutyric</td>
<td>98</td>
<td>α,γ-Diketocaprio</td>
<td>92</td>
</tr>
<tr>
<td>α-Ketovaleric</td>
<td>101</td>
<td>α,γ-Diketoheptanoic</td>
<td>87</td>
</tr>
<tr>
<td>α-Ketocaproic</td>
<td>100</td>
<td>α,γ-Diketoacetoic</td>
<td>95</td>
</tr>
<tr>
<td>α-Ketoheptanoic</td>
<td>93</td>
<td>α,γ-Diketononanoic</td>
<td>73</td>
</tr>
<tr>
<td>α-Ketoocaproic</td>
<td>80</td>
<td>α,γ-Diketocapric</td>
<td>72</td>
</tr>
<tr>
<td>α-Ketononanoic</td>
<td>51</td>
<td>α,γ-Diketoundecylic</td>
<td>43</td>
</tr>
<tr>
<td>α-Ketoisocaproic</td>
<td>67</td>
<td>α,γ-Diketo-δ-methylcaproic</td>
<td>41</td>
</tr>
<tr>
<td>Phenylpyruvic</td>
<td>64</td>
<td>α,γ-Diketo-γ-phenylbutyric</td>
<td>65</td>
</tr>
<tr>
<td>p-Hydroxyphenylpyruvic</td>
<td>64</td>
<td>α-Ketogluutaric</td>
<td>40</td>
</tr>
</tbody>
</table>

* The reaction mixtures contained initially keto acid (28 μM), glutamine (14 μM), 0.05 M veronal buffer at pH 7.1, and 1 cc. of extract in a volume of 4 cc.; incubated at 37° for 4 hours. The relative values are expressed as per cent of the value for pyruvic acid. Inactive keto acids (relative values 3 to 13) are listed in the text.

the presence of α-ketoglutaric and phenylpyruvic acids was found to be 8.0 to 8.5 (Fig. 1). The optima for glutamine deamidation with pyruvate and α-ketoisocaproate were reported to be pH 7.0 and 8.8, respectively (7).

From 90 to 95 per cent of the added β-keto acids was recovered at the end of the incubation period. However, in the case of the α,γ-diketo acids, only 5 to 50 per cent of the added amount was recovered, and corresponding quantities of pyruvic acid were formed. Although α,γ-diketo acids are rapidly hydrolyzed to pyruvic acid by liver extracts (17), Errera's preparation was found to possess only slight hydrolytic activity towards diketo acids. When α,γ-diketo acids were tested with this preparation, little glutamine deamidation occurred and no appreciable breakdown of diketo acid was observed (Table II). These results are com-
Fig. 1. pH-activity curves. Curves 1 and 2, deamidation of glutamine in the absence and presence of α-ketoglutaric acid, respectively; conditions as given in Table I. Curve 3, reaction mixtures contained glutamine (10 μM), phenylpyruvic acid (10 μM), 1 cc. of purified enzyme, and 0.05 M veronal-acetate buffer, in a volume of 5 cc.; incubated for 2 hours. ●, disappearance of glutamine; ○, formation of ammonia.

TABLE II
Deamidation of Glutamine and Keto Acid Recovery with Purified Enzyme Preparation*

<table>
<thead>
<tr>
<th>Keto acid added</th>
<th>NH₃ formed</th>
<th>Keto acid found</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Pyruvic (glutamine omitted)</td>
<td>2.44</td>
<td>27.4†</td>
</tr>
<tr>
<td>&quot; (δ-lactone)</td>
<td>0.04</td>
<td>27.2</td>
</tr>
<tr>
<td>β,δ-Diketocaproic</td>
<td>0</td>
<td>27.4</td>
</tr>
<tr>
<td>α,γ-Diketovaleric</td>
<td>0.63</td>
<td>25.2</td>
</tr>
<tr>
<td>α,γ-Diketocaproie</td>
<td>0.72</td>
<td>25.2</td>
</tr>
<tr>
<td>Oxalacetic</td>
<td>0.27</td>
<td>26.4</td>
</tr>
<tr>
<td>α-Ketoglutaric</td>
<td>1.20</td>
<td>27.6</td>
</tr>
</tbody>
</table>

* The reaction mixture contained initially 28 μM of keto acid, 14 μM of glutamine, veronal buffer at pH 7.1, and 1 cc. of enzyme in a volume of 4 cc. Incubated ½ hour at 37°.
† Determined with crystalline lactic dehydrogenase and DPNH₂.

Compatible with the findings obtained with primary rat hepatoma. Although pyruvate activated the deamidation of glutamine in extracts of hepatoma, α,γ-diketovalerate did not, and, since the neoplastic tissue was found to possess only a weak hydrolytic activity towards α,γ-diketo acids, it was
concluded that the diketo acid itself was inactive in the glutamine system (17). The effect of oxalacetic acid on glutamine deamidation by the purified enzyme preparation was about 10 per cent of the value obtained with the homogenate. Almost all of the added oxalacetate was recovered in these experiments, whereas none was recovered in experiments with liver extracts. Since β,δ-diketocaproic acid and its stable δ-lactone are metabolized to acetoacetate and acetate by liver homogenates (18, 34, 35), these keto acids were also tested with the purified preparation. No appreciable deamidation of glutamine occurred nor were these compounds hydrolyzed (Table II).

**Transamination from Glutamine to α-Keto Acids with Purified Enzyme**

It has been reported that 80 to 90 per cent of the pyruvate added to a digest of rat liver extract with glutamine could be recovered at the end of the incubation period. The procedure was based on the color intensity of an alkaline solution of the 2,4-dinitrophenylhydrazone (2-7). Employing the colorimetric procedure for the determination of keto acids, we have confirmed these findings for pyruvate and have obtained similar results with α-ketobutyrate and α-ketovalerate.

In several experiments with the purified enzyme preparation, pyruvate analyses were carried out with crystalline lactic dehydrogenase and reduced DPN. Although this enzyme system is not specific for pyruvate, it reduces certain keto acids such as oxalacetic and α-ketoglutaric acids at extremely low rates (10). Employing this technique for pyruvate determination, we observed that pyruvate disappeared in amounts approximately equimolar with ammonia formation. Furthermore, when the incubated samples were treated with an excess of lactic dehydrogenase and DPNH₂, and subsequently with 2,4-dinitrophenylhydrazine, an alkalinsoluble 2,4-dinitrophenylhydrazone was obtained which possessed an absorption curve similar to that of α-ketoglutaric acid with a maximum at 4200 A. In addition, the formation of appreciable amounts of alanine in these mixtures was demonstrated by means of paper chromatography. Transamination was further demonstrated by quantitative determinations of glutamine, alanine, pyruvate, and α-ketoglutarate. Similar results were obtained with α-ketoisocaproic, α-ketovaleric, α-ketobutyric, and phenylpyruvic acids. The values for keto acid and glutamine disappearance and those for the formation of amino acid, α-ketoglutarate, and ammonia are given in Table III. Within experimental error, the disappearance of keto acid and glutamine in these experiments was associated with equimolar formation of α-ketoglutarate, amino acid, and ammonia. Good agreement between the values for ammonia formation and those for glutamine disappearance were obtained at various values of pH (Fig. 1).
The $\alpha$-ketoglutarate formed was isolated as the 2,4-dinitrophenylhydrazone in the following manner: A mixture of 3 mM each of phenylpyruvic acid and glutamine, dissolved in 150 cc. of 0.1 M veronal buffer at pH 8.2, was incubated with 280 cc. of purified enzyme at 37° for 6 hours. The solution was deproteinized with trichloroacetic acid and treated with 650 mg. of 2,4-dinitrophenylhydrazine dissolved in 75 cc. of 2 N hydrochloric acid. After standing at 5° for 16 hours, the dinitrophenylhydrazone was filtered and recrystallized from hot water. The dried product weighed 486 mg. and had a melting point of 218° (uncorrected) (analysis, N, calculated, 17.18; found, 17.20). The melting point of an authentic sample and a mixed melting point were 218–219° and 218°, respectively.

No deamidation of glutamine or transamination was observed with $\alpha$-ketoisovaleric acid or $d$- and $l$-$\alpha$-keto-$\beta$-methylvaleric acids with the purified enzyme preparation. Although deamidation of glutamine occurred with $\alpha$-ketoglutarate, there was no change in the concentration of the keto acid. Studies with carbon-labeled $\alpha$-ketoglutaric acid or glutamine would be necessary to demonstrate transamination in this system.

### Table III

<table>
<thead>
<tr>
<th>Keto acid added</th>
<th>Incubation period</th>
<th>Keto acid disappearance</th>
<th>Amino acid formation</th>
<th>Glutamine disappearance</th>
<th>$\alpha$-Ketoglutarate formation</th>
<th>Ammonia formation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>min.</td>
<td>$\mu$M</td>
<td>$\mu$M</td>
<td>$\mu$M</td>
<td>$\mu$M</td>
<td>$\mu$M</td>
</tr>
<tr>
<td>Pyruvic</td>
<td>60</td>
<td>3.30</td>
<td>3.2†</td>
<td>3.20</td>
<td>3.32</td>
<td>3.18</td>
</tr>
<tr>
<td></td>
<td>120</td>
<td>5.00</td>
<td>4.2†</td>
<td>4.74</td>
<td>5.06</td>
<td>4.76</td>
</tr>
<tr>
<td>$\alpha$-Ketoisocaproic</td>
<td>90</td>
<td>3.56</td>
<td>2.8‡</td>
<td>3.05</td>
<td>4.00</td>
<td>3.10</td>
</tr>
<tr>
<td></td>
<td>180</td>
<td>5.10</td>
<td>4.0‡</td>
<td>4.13</td>
<td>5.10</td>
<td>4.26</td>
</tr>
<tr>
<td>Phenylpyruvic</td>
<td>90</td>
<td>3.56</td>
<td>3.4§</td>
<td>3.80</td>
<td>3.15</td>
<td>3.20</td>
</tr>
<tr>
<td></td>
<td>180</td>
<td>5.10</td>
<td>4.7§</td>
<td>5.10</td>
<td>5.00</td>
<td>4.90</td>
</tr>
<tr>
<td>$\alpha$-Ketobutyric</td>
<td>60</td>
<td>3.40</td>
<td>3.52</td>
<td>3.52</td>
<td>3.52</td>
<td>3.22</td>
</tr>
<tr>
<td></td>
<td>120</td>
<td>5.16</td>
<td>5.22</td>
<td>4.78</td>
<td>4.78</td>
<td>4.88</td>
</tr>
<tr>
<td>$\alpha$-Ketovaleric</td>
<td>90</td>
<td>4.52</td>
<td>4.60</td>
<td>4.60</td>
<td>4.60</td>
<td>4.21</td>
</tr>
<tr>
<td></td>
<td>180</td>
<td>6.78</td>
<td>6.80</td>
<td>7.01</td>
<td>7.01</td>
<td>7.14</td>
</tr>
</tbody>
</table>

* The reaction mixtures contained initially 0.005 M keto acid, 0.0025 M L-glutamine, 0.05 M veronal-acetate buffer at pH 7.1, and 1 cc. of enzyme in a final volume of 4 cc.; incubated at 37°.
† Alanine.
‡ Leucine.
§ Phenylalanine.

**Studies with $N^{15}$-Labeled Glutamine**

Previous work has indicated that the increase in ammonia formation from glutamine due to the presence of pyruvate could be accounted for...
by a corresponding decrease in the amide nitrogen group of glutamine, as determined by acid hydrolysis (2). However, in view of the possibility that the amide group of glutamine might serve as a precursor of the \( \alpha \)-amino groups formed in this reaction, glutamine labeled with \( ^{15}N \) in the amide group was employed. The ammonia formed in experiments with added pyruvate and phenyl pyruvate contained the same isotope concentration as did the amide group of the glutamine\(^{4}\) (Table IV). Within experimental error, all of the isotope was recovered as ammonia. These experiments therefore unequivocally demonstrate that the ammonia formed in the presence of added pyruvate or phenyl pyruvate arises from the amide group of glutamine and suggest that the \( \alpha \)-amino group of the amino acid formed is derived from the amino group of glutamine.

### Table IV

**Experiments with Glutamine Containing \( ^{15}N \) in Amide Group**

<table>
<thead>
<tr>
<th>Keto acid</th>
<th>Incubation period</th>
<th>NH(_3) found</th>
<th>( ^{15}N ) concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( \text{hrs.} )</td>
<td>mg.</td>
<td>atom per cent excess</td>
</tr>
<tr>
<td>Pyruvate*</td>
<td>2</td>
<td>0.119</td>
<td>1.03</td>
</tr>
<tr>
<td>&quot;</td>
<td>4</td>
<td>0.170</td>
<td>1.04</td>
</tr>
<tr>
<td>Phenylpyruvate(^{+})</td>
<td>2</td>
<td>0.125</td>
<td>1.38</td>
</tr>
<tr>
<td>&quot;</td>
<td>4</td>
<td>0.174</td>
<td>1.40</td>
</tr>
</tbody>
</table>

\(^*\) The reaction mixtures contained initially sodium pyruvate (20 \( \mu M \)), glutamine (10 \( \mu M \)), 0.05 M veronal buffer at pH 7.1, and 2 cc. of enzyme in a volume of 5 cc. The amide group of the glutamine contained 1.02 atom per cent excess of \( ^{15}N \).

\(^{+}\) The reaction mixtures contained initially phenyl pyruvate (10 \( \mu M \)), glutamine (10 \( \mu M \)), veronal buffer at pH 8.1, and 2 cc. of enzyme in a volume of 5 cc. The amide group of glutamine contained 1.43 atom per cent excess of \( ^{15}N \).

### Comparison of Glutamine with Glutamate in Transamination

The results thus far described could be explained on the basis of a two-step reaction involving an \( \alpha \)-keto acid-catalyzed deamidation of glutamine yielding ammonia and glutamic acid, followed by transamination between glutamate and the \( \alpha \)-keto acid. However, previous studies on transamination in pigeon muscle have shown that, although glutamic acid transaminates rapidly with pyruvate, the rates with other keto acids are relatively low (36). In contrast, the deamidation of glutamine, as well as the associated transamination reaction, occurs with a number of \( \alpha \)-keto acids at rates of about the same order of magnitude. It was of importance, therefore, to compare transamination from glutamine with that from glutamic acid to various \( \alpha \)-keto acids. The results of such a comparison

\( ^{4}\) The authors wish to thank Dr. J. White for the isotope analyses.
indicate that transamination from glutamic acid to α-keto acids occurs at very low rates except in the case of pyruvic and α-ketobutyric acids (Table V). Under the same conditions, appreciable transamination takes place between glutamine and all of the α-keto acids studied except α-ketoisovaleric and α-keto-β-methylvaleric acids. The formation of alanine, α-aminobutyric acid, norvaline, leucine, tyrosine, methionine, and phenylalanine was demonstrated in the experiments described in Table V by paper chromatography. Under similar conditions, the formation of glutamic acid from various amino acids and α-ketoglutaric acid was found to occur at a very low rate except in the case of alanine and α-aminobutyric acid. When 10 μM each of α-ketoglutarate and L-amino acid were incubated under the conditions described in Table V, 0.5 to 1.5 μM of glutamate were formed in 4 hours with norvaline, α-aminocaproic acid, methionine, phenylalanine, tyrosine, and leucine, while the formation of 5 μM of glutamate was observed in 15 and 60 minutes with alanine and α-aminobutyric acid, respectively.

Study of the time-course of the glutamate-pyruvate and glutamine-pyruvate reactions indicates that the former reaction occurs more rapidly than the latter (Fig. 2). Similar results were obtained with α-ketobutyrate. On the other hand, in the case of phenyl pyruvate, as well as the remaining α-keto acids, the reaction was slower with glutamate. Although the curves for the disappearance of glutamine (as determined by de-
carboxylation with *C. welchi* suspensions) and for ammonia formation were about the same for the first half of the reaction, there was a relative increase in the ammonia formation towards the end of the reaction (Fig. 2). Since by the method employed for glutamine determination, glutamine cannot be distinguished from glutamate, these findings suggested that glutamic acid was formed by transamination between the α-keto-glutarate and amino acid formed. This hypothesis was supported by the finding of glutamic acid by paper chromatography and is compatible with

![Fig. 2. Time-course of the reaction. Reaction mixtures contained initially keto acid (20 μM), glutamic acid or glutamine (10 μM), 0.05 M Verona1 buffer (pH 7.2), and 2 cc. of enzyme in a volume of 5 cc. Glutamine plus keto acid, Curve 1, ammonia formation; Curve 2, glutamine disappearance. Glutamate plus keto acid, Curve 3, glutamate disappearance.](image)

the results of experiments on the formation of glutamate from α-keto-glutarate and various amino acids described above.

The low rate of transamination between glutamic acid and phenyl pyruvate (and α-ketovalerate) was not increased by addition of pyridoxal phosphate or ammonium chloride. Similar data were obtained when the glutamate was introduced slowly through a hypodermic needle over a 4 hour period by means of a Klett compensator connected to a tuberculin syringe.

Because impurities in glutamine isolated from natural sources have sometimes been responsible for anomalous results (37), synthetic L-glutamine was tested with several of the active α-keto acids. The results with the synthetic and isolated samples of glutamine were identical.
Effect of Other Glutamic Acid Derivatives

Acetylglutamic acid and isoglutamine were only as active as glutamic acid in transaminating with α-ketovalerate and phenyl pyruvate, although these compounds were hydrolyzed to completion during incubation with the enzyme preparation. The rates of hydrolysis of these compounds to glutamic acid were not increased by addition of α-keto acids. The effectiveness of other glutamic acid derivatives, viz. pyrrolidonecarboxylic acid, carbamylglutamic acid, and the γ-ethyl ester of glutamic acid in transaminating with α-ketovalerate and phenyl pyruvate, was of the same low order of magnitude as that of glutamate. Furthermore, the γ-ethyl ester of glutamic acid did not transaminate with pyruvate. The ester was not hydrolyzed in this system, as determined by the rate of decarboxylation by C. welchii suspensions.

It was found that the γ-methylamide of glutamic acid was considerably more active than glutamic acid in transaminating with α-ketovaleric and phenylpyruvic acids, by measuring the rate of decrease of the concentration of the keto acids. In an experiment with 10 μM of the methylamide, 20 μM of α-ketovalerate, and 2 cc. of enzyme, in veronal buffer at pH 7.1, 6 μM of keto acid disappeared in 3 hours. Under the same conditions, 9.5 μM and 0.5 μM of keto acid disappeared with glutamine and glutamic acid, respectively. The formation of a steam-volatile base in the experiments with the methylamide suggests that methylamine was formed. In contrast to the results with the methylamide, the γ-ethylamide of glutamic acid and glutathione were approximately as inactive as glutamic acid.

DISCUSSION

The present evidence suggests that α-keto acid-catalyzed deamidation of glutamine and transamination are associated. With the purified enzyme, neither reaction occurs in the absence of certain α-keto acids, and both deamidation and transamination occur at the same relative rates at various pH values. It has thus far not been possible to separate α-keto acid-catalyzed deamidation from transamination. It is noteworthy that α-ketoisovaleric and α-keto-β-methylvaleric acids are inactive in both reactions. The inactivity of these keto acids is of interest because, in contrast to the keto acids which were active in this system, these acids possess only 1 hydrogen atom on the β-carbon. Since glutamate reacted much more slowly than did glutamine, with all the active α-keto acids except pyruvic and α-ketobutyric acids, it appears unlikely that glutamic acid is an intermediate in this reaction. Thus, simple hydrolysis of glutama-

C. welchii suspensions decarboxylate L-glutamic acid approximately 40 times faster than the γ-ethyl ester of L-glutamic acid. L-Pyrrolidonecarboxylic acid is not decarboxylated under these conditions.
mine to glutamic acid and ammonia is probably not the initial step of the reaction. It is possible that transamination and deamidation occur simultaneously, or that transamination precedes deamidation. The latter mechanism would involve the intermediate formation of α-ketoglutaramic acid and subsequent deamidation of this compound to yield α-ketoglutarate and ammonia.

It seems probable that the mechanism of the reaction between glutamine and α-keto acids is similar for all of the active α-keto acids. That transamination to pyruvate and α-ketobutyrate was more rapid with glutamate than with glutamine may be ascribed to the presence of glutamic-alanine transaminase in the purified enzyme preparation. The purified glutamic-alanine and glutamic-aspartic transaminases of muscle have been reported to be inactive with glutamine (38). It has been found in collaboration with Dr. H. P. Morris that livers of vitamin B6-deficient rats and mice exhibit no decrease in the glutamine-pyruvate and glutamine-phenyl pyruvate deamidation reactions, although a considerable loss in glutamic-alanine transaminase activity was observed (39).

The system responsible for catalysis of the glutamine-α-keto acid reaction is probably not identical with either of the two classical transaminases.

It has been stated that glutamine cannot enter into transamination reactions unless previously split by hydrolytic enzymes (40). The present data indicate that glutamine may transaminate with certain α-keto acids without prior hydrolysis to glutamate. Elucidation of the mechanism of this reaction and its significance in the intermediary metabolism of amino acids must await the results of further investigation.

SUMMARY

1. Deamidation of glutamine by rat liver extracts and by a purified liver preparation was increased by a number of α-keto acids. Oxalacetate, α-ketoisovalerate, d- and l-α-keto-β-methylvalerate, pyruvamide, and acids possessing β-, γ-, or δ-keto groups were inactive. α,γ-Diketo acids were active only if hydrolyzed to pyruvic acid.

2. Transamination from glutamine to α-keto acids was demonstrated by isolation of the α-ketoglutaric acid formed as the 2,4-dinitrophenylhydrazone, identification and quantitative determination of the amino acid formed, and determinations of the disappearance of glutamine and α-keto acid.

3. With glutamine labeled with N15 in the amide group, and pyruvate or phenyl pyruvate, all of the isotope was recovered as ammonia, demonstrating unequivocally that the ammonia formed arises from the amide group of glutamine.

*These experiments will be described in a subsequent report.
4. Transamination from glutamate to α-ketovalerate, α-ketocaproat, α-ketoisocaproat, phenyl pyruvate, p-hydroxyphenyl pyruvate, and α-keto-γ-methylbutyrate occurred at a much slower rate than did transamination from glutamine to these keto acids. With pyruvate and α-ketobutyrate, transamination occurred more rapidly with glutamate, probably owing to the presence of glutamic-alanine transaminase in the enzyme preparation.

5. Other glutamic acid derivatives were no more active than glutamic acid itself in transamination with α-ketovalerate and phenyl pyruvate. However, the γ-methylamide of glutamic acid was appreciably more effective than glutamic acid.

6. The data indicate that transamination between glutamine and certain α-keto acids occurs in this system without prior hydrolysis of glutamine to glutamic acid and ammonia.

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