THE CONVERSION OF CITRULLINE TO ARGinine
(TRANSIMINATION) BY TISSUE SLICES
AND HOMOGENATES*

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The conversion of citrulline to arginine by a transamination reaction involving glutamic acid and metabolically related compounds has been reported to take place in kidney slices by Borsook and Dubnoff (1). These authors found that liver slices did not catalyze the reaction and further that the breakdown of cell structure of kidney tissue, such as by homogenization, resulted in a loss of activity.

In the course of an investigation on the mechanism of urea synthesis in liver, some evidence for the transamination reaction was obtained in homogenates fortified with adenosine triphosphate (ATP), magnesium ions, and cytochrome c. These findings, which are reported in a separate publication (2), led to a study of the transamination reaction in kidney and other tissue homogenates. Experiments reported in this paper deal with (1) the conditions required for demonstrating the transamination reaction in tissue homogenates and (2) the relative rates of this reaction in slices and homogenates of kidney and liver.

EXPERIMENTAL

Preparation of Tissues

Slices—Livers and kidneys of adult white rats were removed shortly after the animals had been decapitated, bled, and chilled on ice, and the slices were cut immediately thereafter. For liver studies, three slices, totaling approximately 12 to 15 mg. in dry weight, were used in each flask. In kidney studies, two slices, approximately 7 to 10 mg. in dry weight, were used.

Homogenates—Tissues were removed from adult rats treated in the same manner as described above, and chilled on ice before being cut into small pieces to facilitate homogenization. Homogenization was carried out in the apparatus of Potter and Elvehjem (3). The cold homogenate, after straining through four layers of cheese-cloth, was added to Warburg flasks containing cold medium and substrates. All operations were carried out in a 1°C cold room to insure maintenance of low temperatures. The homogenate

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239
which was prepared with 2 volumes of buffer (0.0128 M sodium phosphate, pH 7.4, 0.123 M sodium chloride, 0.005 M potassium chloride, 0.0033 M magnesium sulfate) was diluted to the final tissue concentration desired. Liver homogenates, representing 10 to 15 per cent wet weight, were found to contain 2 to 3 mg. of N per ml.; 20 to 25 per cent kidney homogenate contained 4 to 5 mg. of N per ml. Aliquots of the homogenates were taken for analysis of the total nitrogen, on which basis the results are expressed.

A representative homogenate of liver tissue was examined for cytolysis. Macroscopically, it was an opaque homogeneous preparation containing no visibly large particles. A portion of the homogenate was centrifuged and the centrifugate, which represented 10 to 15 per cent of the total volume, was fixed with 10 per cent formaldehyde, embedded in paraffin, sectioned, and stained. Histological examination revealed an occasional clump of cells. It was estimated that about 5 per cent of the homogenate consisted of unruptured cells.

Potter (4) has suggested that the preparation of tissue homogenate in water increases the degree of cytolysis. When this was done and the water homogenate was compared with a buffer homogenate, it was found that the water preparation had 75 per cent of the activity of the buffer homogenate for liver and about 85 per cent for kidney. It is possible that the lower activity of the water homogenates was due to a delay in restoring the isotonicity of the medium.

Incubation

All incubations were carried out in Warburg flasks at 38° for a period of 40 minutes, unless otherwise specified. All flasks were charged with pure tank oxygen just prior to introduction into the bath. For anaerobic experiments tank nitrogen was used with an oxygen-carbon dioxide-absorbing mixture of Van Slyke (5) in the center wells of the flasks.

Substrates and Cofactors

Tissue Slice Experiments—Each flask contained the following components in the main compartment: dl-citrulline, glutamic acid or other imino group donor, and tissue slices in a total volume of 3.5 ml. made up with buffer, pH 7.4 (see “Homogenates” under “Preparation of tissues”). At the end of the incubation period, the slices were removed, rinsed in water, and placed in tared cups for drying and weighing. The contents of the flasks were then analyzed for either urea or arginine or both, as described under “Analytical methods.”

Homogenate Experiments—Incubation mixtures consisted of dl-citrulline, glutamic acid, ATP, cytochrome c, homogenate and buffer, pH 7.4, to make a final volume of 3.0 or 3.5 ml., as indicated. In most experiments with
liver homogenates, 3 to 10 units of purified calf liver arginase were added to the incubation mixture to insure complete conversion of arginine to urea. However, control experiments indicated no need for the arginase addition. At the end of the incubation period, the contents of the flasks were analyzed for either urea or arginine or both. Liver incubation mixtures were always analyzed for urea, and arginine when specified. On the assumption that the urea formed by liver preparations was derived from a quantitative hydrolysis of the arginine formed, the results are presented as mg. of arginine formed. Kidney incubation mixtures were always analyzed for arginine, and occasionally for urea, as indicated.

Preparations Used—The dl-citrulline used throughout this study was obtained from the Amino Acid Manufactures, University of California, Los Angeles.

\( l(+)-\)glutamic acid was a commercial preparation.

ATP was prepared from rabbit muscle following magnesium and barbiturate anesthesia (6). This preparation was 79 per cent pure on the basis of its easily hydrolyzed phosphorus (7 minutes at 100° in \( 1 \) N HCl).

Cytochrome \( c \) was prepared by the method of Keilin and Hartree (7).

\( l(+)-\)Ornithine hydrochloride was prepared according to Hunter (8).

Glutamine was obtained from Dr. H. B. Vickery, and cozymase (80 per cent pure) from Dr. G. A. LePage. The authors are indebted to these investigators for these generous gifts.

\( \alpha \)-Ketoglutaric acid was prepared by synthesis from ethyl oxalate and ethyl succinate.

Succinamic acid was prepared by the hydrolysis of succinimide.

Arginase was prepared by the method of Hunter and Downs (9).

Analytical Methods

Determination of Urea—Urea was determined by the method of Krebs and Henseleit (10), with a 1 per cent solution of purified urease (The Arlington Chemical Company). Solutions obtained from slices were brought to pH 5.0 with 0.3 ml. of \( 3 \) N acetate buffer, and the urea content determined manometrically. Solutions from homogenates were centrifuged after the addition of the acetate buffer to remove the precipitated protein, and an aliquot of the clear supernatant was taken for the urea determination.

Determination of Arginine—All solutions with or without prior urease treatment were treated with 1 ml. of 10 per cent trichloroacetic acid, centrifuged, and an aliquot taken from the clear supernatant. The method employed was essentially that of Dubnoff (11). Samples for colorimetric analysis were prepared as follows: To a 1 ml. aliquot of the deproteinized incubation mixture were added 9 ml. of distilled water and 1 ml.
of the naphthol-urea reagent, followed by 0.5 ml. of sodium hypobromite solution. For the blank, 10 ml. of distilled water plus the reagents were used.

Results

Effect of ATP—The requirements of ATP by liver and kidney homogenates are shown in Fig. 1. In the absence of ATP only a negligible amount of arginine was formed. This was particularly noticeable in studies with liver, in which almost a maximum activity was seen at one-half the ATP concentration (8 × 10⁻⁴ M) required for a maximum response with kidney homogenate. However, even with increased amounts of ATP the synthesis by kidney of arginine did not at any time exceed 40 per cent of the activity of liver.

Effect of Magnesium Ions—When increasing concentrations of magnesium ions were used with liver homogenate, a maximum activity was noted at about 3 × 10⁻³ M concentration (Table I). Stoner and Green (12) have reported that magnesium ions inhibit the deamination and dephosphorylation of ATP. This in all probability is the basis for the activation noted in the present experiments. Magnesium ions in optimum concentration were used throughout the experiments. The effect of magnesium ions on kidney homogenate was not investigated.

Effect of Cytochrome c—Cytochrome c was included throughout the study, although evidence for its requirement has not been consistent. In experi-
ments where stimulation has been noted, this has been as high as 100 per cent. Borsook and Dubnoff (1) have indicated that cytochrome c plays a rôle in the oxidation of the hypothetical citrulline-glutamic acid intermediate.

Comparison of Kidney Slices and Homogenate—Fig. 2 shows the relative rates of arginine formation by kidney slices and homogenate. The data obtained with kidney slices confirm Borsook and Dubnoff's findings (1). These investigators found approximately 0.027 mg. of arginine produced per mg. of dry weight of tissue per hour, or, on the basis of 12.63 per cent nitrogen (determined experimentally) in dry kidney tissue, 0.216 mg. of arginine per mg. of tissue nitrogen per hour. In the present experiments, values of the order of 0.265 mg. of arginine were found at the end of 1 hour incubation. Attempts to demonstrate arginine formation in kidney ho-

<table>
<thead>
<tr>
<th>Magnesium ion concentration</th>
<th>Arginine formed per mg. tissue N</th>
</tr>
</thead>
<tbody>
<tr>
<td>$3.33 \times 10^{-4}$ M</td>
<td>0.1113</td>
</tr>
<tr>
<td>$1.33 \times 10^{-3}$ &quot;</td>
<td>0.1305</td>
</tr>
<tr>
<td>$2.67 \times 10^{-3}$ &quot;</td>
<td>0.1780</td>
</tr>
<tr>
<td>$6.67 \times 10^{-2}$ &quot;</td>
<td>0.0673</td>
</tr>
<tr>
<td>$1.00 \times 10^{-1}$ &quot;</td>
<td>0.0708</td>
</tr>
</tbody>
</table>

mogenate brought to light a system only about 40 per cent as active as that in intact slices. This system, however, showed a greater specificity as to the source of the imino group donator, namely glutamic acid, and a dependence on the presence of ATP.

Comparison of Liver Slices and Homogenate—In contrast to kidney slices and homogenate, liver homogenate was found to contain an unusually active system having about 80 per cent greater activity than kidney slices at 1 hour, and a 500 per cent greater activity than kidney homogenate and liver slices. The requirements of the liver homogenate for the necessary components, ATP (Fig. 1), citrulline (Fig. 3), and glutamic acid (Fig. 4), were of much smaller magnitude than that required by kidney homogenate. Liver slices, on the other hand, showed a very low activity.

Comparison of Liver and Kidney Preparations. Oxygen—The require-
ment of the transamination system for oxygen as first shown with kidney slices by Borsook and Dubnoff (1) has been confirmed in the present studies. Under an anaerobic atmosphere, neither slices nor homogenates of both liver and kidney synthesized arginine.

**Fig. 2.** Comparison of the rates of transamination by different tissue preparations. Tissue concentration per flask: liver homogenate 1.46 mg. of N; liver slices 15 to 20 mg. dry weight; kidney homogenate 1.38 mg. of N; kidney slices 7 to 9 mg. dry weight. Substrate and cofactor final concentration as follows: liver homogenate and slices, dl-citrulline and glutamic acid, each at $5.72 \times 10^{-8}$ M, adenosine triphosphate $8.57 \times 10^{-4}$ M, cytochrome c $4.72 \times 10^{-1}$ M; following variations employed with kidney slices and homogenate: homogenate, adenosine triphosphate $1.14 \times 10^{-3}$ M; slices, glutamic acid $8.57 \times 10^{-8}$ M.

**Fig. 3.** Effect of citrulline concentration on transamination by liver and kidney homogenates. Tissue concentration per flask: liver 1.461 mg. of N, kidney 1.572 mg. of N. Substrate concentration is the same as that given under Fig. 2 for liver homogenate. *dL*-Citrulline was used as the substrate in twice the concentration given as the I(+) form on the abscissa.

Citrulline—The relative utilization of citrulline by both liver and kidney homogenates is shown in Fig. 3. Without citrulline no appreciable amounts of arginine were formed. The optimum citrulline concentration for kidney was found to be approximately $6 \times 10^{-3}$ M. In the case of liver a rapid
rise in arginine formation resulted from an increase in citrulline concentration to approximately $1.5 \times 10^{-3}$ M; beyond this point the synthesis of arginine was less strikingly influenced by the citrulline concentration. At optimum concentrations of citrulline for both kidney and liver homogenates, the former had only 40 per cent of the activity of the latter.

Transimination with Compounds Similar to Glutamic Acid—Borsook and Dubnoff (1) found that kidney slices showed a considerable utilization of compounds metabolically related to glutamic acid. Glutamine and aspartic acid were equally as effective as glutamic acid, and asparagine, glutathione, lysine, proline, and ornithine about half as effective. It was found in the present study with homogenates that substrate specificity was more limited (Table II). Thus with both homogenates glutamine was the only compound other than glutamic acid showing an appreciable effect. The activity of glutamine was to be expected in view of the active glutaminase system in both tissues. Aspartic acid and asparagine were less than one-fourth as active as glutamic acid. The failure in the utilization of ammonium chloride by kidney homogenate is in agreement with the work on slices. In contrast to liver slices, liver homogenate failed to utilize ammonium chloride (7.3 per cent as effective as glutamic acid), which appears to be metabolized more rapidly than glutamic acid in the synthesis of urea by slices (2). $\alpha$-Ketoglutaric acid plus ammonium chloride showed only 20 per cent the activity of glutamic acid with kidney homogenates. Borsook and Dubnoff found a 46 to 80 per cent activity with this combination with kidney slices. Liver homogenate was less efficient than kidney with these substrates. Ornithine, which was 42 per cent as active as glutamic acid in

### Table II

<table>
<thead>
<tr>
<th>Substance in addition to citrulline</th>
<th>Kidney, relative rate</th>
<th>Liver, relative rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glutamic acid</td>
<td>00.0</td>
<td>100.0</td>
</tr>
<tr>
<td>Glutamine</td>
<td>84.5</td>
<td>71.9</td>
</tr>
<tr>
<td>Aspartic acid</td>
<td>15.8</td>
<td>22.8</td>
</tr>
<tr>
<td>Asparaginse</td>
<td>23.2</td>
<td>19.0</td>
</tr>
<tr>
<td>$\alpha$-Ketoglutaric acid + ammonium chloride</td>
<td>19.4</td>
<td>5.8</td>
</tr>
<tr>
<td>Ammonium chloride</td>
<td>0.0</td>
<td>7.3</td>
</tr>
<tr>
<td>Ornithine</td>
<td>2.2</td>
<td>0.0</td>
</tr>
<tr>
<td>Lysine</td>
<td>19.1</td>
<td></td>
</tr>
<tr>
<td>Succinamic acid</td>
<td></td>
<td>27.5</td>
</tr>
<tr>
<td>Lactic acid + ammonium chloride</td>
<td></td>
<td>2.5</td>
</tr>
</tbody>
</table>
kidney slices, yielded no arginine with either kidney or liver homogenates. Apparently the mechanism for converting ornithine and other potential imino group donators to glutamic acid (13) is lost when kidney tissue is homogenized. Succinamic acid with liver homogenate showed a relative effectiveness of 27.5 per cent. The basis for the activity of this compound

![Graph](http://www.jbc.org/)

**Fig. 4.** Effect of glutamic acid concentration on transimination by liver and kidney homogenates. Tissue concentration per flask: liver 1.461 mg. of N; kidney 2.22 mg. of N. Substrate concentrations are the same as those given under Fig. 2 for liver homogenate.

![Graph](http://www.jbc.org/)

**Fig. 5.** Effect of tissue concentration on transimination by liver and kidney homogenates. The substrate concentrations are the same as those given under Fig. 2 for liver homogenate.

other than by metabolic conversion to an α-amino compound is obscure. Lactic acid and ammonium chloride, reported by Krebs and Henseleit (10) to increase the amount of urea (or arginine) produced by liver slices over that of ammonium chloride used alone, was ineffective with homogenate.

**Glutamic Acid**—The requirements for glutamic acid by the two tissue
homogenates are shown in Fig. 4. Increasing the glutamic acid concentration beyond \(3 \times 10^{-3} \text{ M}\) resulted in no further increase in arginine formation by the liver, while kidney evidently required a concentration greater than \(12 \times 10^{-3} \text{ M}\). Even at this concentration, however, the activity of kidney was only one-third of that observed in liver.

**Cofactors**—The possibility of necessary cofactors was investigated. A boiled aqueous rat liver extract was added to the system without any significant effect. Pyridoxal and pyridoxamine were also tried with negative results. The addition of coenzyme I resulted in some stimulation with low tissue concentrations.

**Tissue Concentration**—Increasing the amounts of tissue (Fig. 5) brought about a remarkable increase in arginine formation by liver, which was not paralleled by kidney. With a higher level of liver tissue per cup, the blank values rose proportionately. At a tissue level of 1 to 1.5 mg. of N per flask, however, blank values were practically zero, and consequently this level was used throughout the study. With kidney homogenates, on the other hand, increasing the tissue concentration resulted in no striking increases in arginine synthesis. Activity at a level of 4 mg. of tissue N per flask was only about 10 per cent that of liver.

**Arginine Formation by Various Rat Tissue Homogenates**—Data from Table III indicate that liver and kidney homogenates were the only tissues showing any appreciable arginine formation from citrulline and glutamic acid. Both urea and arginine were determined in all instances. The relative arginase activities of different tissues have been reported to be as follows (10): liver 2000, kidney 67, testes and heart 0. It would be expected that any arginine formed by liver would be rapidly and quantitatively

### Table III

**Arginine Formation by Different Tissue Homogenates**

The substrate and cofactor final concentration are the same as in Table IV.

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Tissue N per flask (I)</th>
<th>Urea formed per mg. tissue N (II)</th>
<th>Urea equivalents expressed as arginine per mg. tissue N (calculated from column III) (IV)</th>
<th>Arginine found per mg. tissue N (V)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver</td>
<td>2.750</td>
<td>60.5</td>
<td>0.471</td>
<td>0.011</td>
</tr>
<tr>
<td>Kidney</td>
<td>2.686</td>
<td>4.01</td>
<td>0.031</td>
<td>0.091</td>
</tr>
<tr>
<td>Heart</td>
<td>1.497</td>
<td>0.69</td>
<td>0.005</td>
<td>0.013</td>
</tr>
<tr>
<td>Testes</td>
<td>3.465</td>
<td>-0.04</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Brain</td>
<td>1.810</td>
<td>-0.41</td>
<td>0.0</td>
<td>0.017</td>
</tr>
</tbody>
</table>
converted to urea. This was borne out in the present experiment in which it is to be noted that whereas 60.5 microliters of urea (corresponding to 0.471 mg. of arginine) were found, the amount of arginine present was negligible. With kidney homogenate, on the other hand, arginine accumulated as such, only a small portion being converted to urea.

Effect of Inhibitors—Inhibition by cyanide and arsenite at a final concentration of 0.001 M was reported by Borsook and Dubnoff to be about 95 per cent complete in the case of kidney slices (1). With liver homogenate cyanide and arsenite inhibited 86 and 99 per cent respectively (Table IV). Fluoride and iodoacetate have been reported to inhibit the regeneration of ATP and phosphorylation mechanisms (14). The inhibition observed in the present study (fluoride 98 per cent, iodoacetate 74 per cent) might be explainable on this basis. While inhibition by cyanide and arsenite at a final concentration of 0.001 M was reported by Borsook and Dubnoff to be about 95 per cent complete in the case of kidney slices (1). With liver homogenate cyanide and arsenite inhibited 86 and 99 per cent respectively (Table IV). Fluoride and iodoacetate have been reported to inhibit the regeneration of ATP and phosphorylation mechanisms (14). The inhibition observed in the present study (fluoride 98 per cent, iodoacetate 74 per cent) might be explainable on this basis. While inhibition by

Table IV
Effect of Inhibitors on Liver Homogenate

<table>
<thead>
<tr>
<th>Inhibitors</th>
<th>Final concentration (molarity)</th>
<th>Per cent inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium arsenite</td>
<td>0.001</td>
<td>99.3</td>
</tr>
<tr>
<td>&quot; fluoride</td>
<td>0.01</td>
<td>98.4</td>
</tr>
<tr>
<td>&quot; malonate</td>
<td>0.0057</td>
<td>90.2</td>
</tr>
<tr>
<td>Potassium cyanide</td>
<td>0.001</td>
<td>85.8</td>
</tr>
<tr>
<td>Sodium iodoacetate</td>
<td>0.01</td>
<td>74.4</td>
</tr>
<tr>
<td>&quot; azide</td>
<td>0.001</td>
<td>23.8</td>
</tr>
<tr>
<td>Calcium ions</td>
<td>0.001</td>
<td>90.0</td>
</tr>
</tbody>
</table>

malonate at lower concentrations of the order of 0.001 M has been ascribed to inhibition of succinoxidase activity, concentrations of 0.01 M and higher are thought to influence other systems. The mechanism of malonate inhibition in the transimination system reported here is not apparent. Inhibition of the cytochrome system by azide has been described by Keilin and Hartree (15), who reported its action as being less complete than that with cyanide. The transimination system in this study showed 24 per cent inhibition with azide, and 86 per cent with cyanide.

In addition to the inhibitors named above, calcium ions were also found to inhibit the system. This inhibition was not relieved by the addition of 0.001 M magnesium ions over that already present in the buffer nor by an increase in ATP concentration to $1.7 \times 10^{-3}$ M.
It appears from these results that the transimination reaction is in some manner intimately associated with a high energy phosphate donator system. At least the system is active in the presence of ATP when cells are disrupted. This effect is more striking in the case of liver which actually shows a more active transimination system with homogenates than with slices. On the other hand, kidney homogenates, while active, never equaled slices in their transimination activity. This may be accounted for in part by the possibility that the optimum conditions which were established in the case of liver were not optimum when applied to kidney homogenates. Thus it is possible that components other than, or in addition to, those found active in the case of liver are necessary for kidney. In addition, the presence of more active phosphatases in kidney preparations as compared with liver may also be responsible for the lower activity on the basis of more rapid ATP breakdown.

The significance of the transimination reaction in kidney as a mechanism for arginine synthesis has been pointed out by Borsook and Dubnoff (1). As pointed out by Krebs (16), this reaction may account for a significant amount of urea synthesis by transfer of arginine formed by transimination in the kidney to the liver, where it would be broken down to ornithine and urea by arginase.

The most striking finding of the present experiments, however, is that liver homogenates are capable of carrying out the transimination reaction at an extremely rapid rate even exceeding that of kidney slices. The importance of this reaction in urea synthesis by liver is apparent. The details of this reaction in liver are discussed in a separate publication (2).

It would appear from the present study that the transimination reaction is limited chiefly to liver and kidney. Apparently, only the lack of a highly active arginase system in kidney prevents that organ from contributing a significant amount of urea to the total metabolic urea of the body.

SUMMARY

1. The conversion of citrulline to arginine by transimination with glutamic acid in kidney slices and homogenates was studied. The relative activity of the reaction by these preparations was as follows: liver homogenate > kidney slices > kidney homogenate > liver slices.

2. Homogenized tissue preparations of liver and kidney required the following substrates and cofactors: adenosine triphosphate, citrulline, glutamic acid, cytochrome c, magnesium ions, and an oxygen atmosphere. Liver in every case required smaller concentrations of the substrates and
showed a greater activity than kidney homogenate. The significance of these findings is discussed.

3. Aside from glutamic acid, glutamine was the only compound showing any appreciable activity in the transimination system.

4. Brain, testes, and heart homogenates showed no transimination activity.

BIBLIOGRAPHY

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