THE CONVERSION OF ORNITHINE TO CITRULLINE
BY RAT LIVER HOMOGENATES*

BY PHILIP P. COHEN AND MIKA HAYANO
(From the Laboratory of Physiological Chemistry, University of Wisconsin, Madison)

(Received for publication, June 23, 1947)

The conversion of ornithine to citrulline as an obligatory step in the synthesis of urea in mammalian liver was first postulated by Krebs and Henseleit (1). This reaction was later investigated in detail by Gornall and Hunter (2) using rat liver slices. The use of liver slices for the study of this reaction is unsatisfactory in that citrulline, once formed from ornithine, is rapidly converted through arginine to urea. The use of homogenized liver preparations has eliminated this difficulty in part because of more adequate control of the conditions necessary for the various steps in the cycle.

It was found early in this study that components and conditions necessary for the synthesis of citrulline from ornithine were such as to catalyze the disappearance of citrulline at an appreciable rate when magnesium ions were present (3). The absence of magnesium ions, however, resulted in an accumulation of citrulline. This means of stopping the urea cycle at citrulline provided a convenient system for the study of the optimum conditions necessary for the conversion of ornithine to citrulline.

Srb and Horowitz, as a result of studies on Neurospora mutants (4), have offered evidence for the existence of two distinct enzymatically catalyzed steps in the conversion of ornithine to citrulline, the first of these being the introduction of carbon dioxide, and the second, the introduction of ammonia. In the present work, this synthesis has been studied as a unit reaction. Experiments reported in this paper deal particularly with the formation of citrulline in the stepwise synthesis of urea from ornithine by rat liver homogenates.

Preparations

Preparation of Homogenates—A detailed description for the preparation of homogenates has been published previously (3). 0.5 ml. of a 20 per cent rat liver homogenate prepared in isotonic KCl was used in each incubation flask throughout the study, unless otherwise specified. This amount of homogenate contained between 2.3 and 2.8 mg. of tissue nitrogen as determined by micro-Kjeldahl analysis.

* Aided in part by a grant from the Wisconsin Alumni Research Foundation.
CONVERSION OF ORNITHINE TO CITRULLINE

Incubation—All incubations were carried out in Warburg flasks at 38°C, for a period of 1 hour, unless otherwise specified. All flasks were gassed with a 5 per cent CO₂-95 per cent O₂ mixture previous to introduction into the bath. This procedure resulted in relatively uniform results from experiment to experiment. Further, the gaseous CO₂ present per flask (approximately 1000 microliters) was found to be adequate for the usual length of incubation, as shown by experiments in which regassing at frequent intervals yielded values no higher than those in which the gas had been introduced only once prior to the incubation.

Incubation mixtures giving optimum results consisted of DL-ornithine hydrochloride (at concentrations calculated on the basis of the L isomer only), L-glutamic acid, ammonium chloride, adenylic acid, liver homogenate, potassium phosphate buffer, pH 7.15, and bicarbonate ions at a concentration sufficient to bring the pH to 7.15 after equilibration with 5 per cent CO₂ in the gas phase. The total incubation volume was 3.0 ml.

Analytical—At the end of the incubation period, 0.3 ml. of 3 M acetate buffer, pH 5.0, was added to stop the reaction. In most experiments, 1.0 ml. of incubation mixture was then pipetted into 1.0 ml. of 10 per cent trichloroacetic acid and the precipitated protein centrifuged down. 0.5 ml. of the clear supernatant was taken for analysis of citrulline by the method of Archibald (5) with slight alterations. In these analyses, the previous destruction of urea by urease was eliminated since the amount of urea formed was negligible. When the amount of urea was measurable, citrulline was determined after a prior treatment with urease and trichloroacetic acid. The period of color development was lengthened to 15 minutes.

For samples to be analyzed for urea, the incubation mixture was centrifuged after the addition of acetate buffer and an aliquot of the supernatant taken for analysis by the method of Krebs and Henseleit (1).

All results are reported in microliters of product, citrulline or urea, or both, per mg. of tissue nitrogen. Approximate Q₉₅ values can be obtained by dividing the microliters of end-product per mg. of N by 10, assuming that 10 per cent of the dry weight of liver is nitrogen (6).

Preparations Used—DL-Ornithine hydrochloride and DL-citrulline were obtained from the Amino Acid Manufactures, University of California, Los Angeles.

Adenosine triphosphate (ATP) was prepared from rabbit muscle (7) as the hydrated barium salt. Adenylic acid was prepared from ATP by the method of Kerr (8). An initial sample of the acid was obtained from Dr. G. A. LePage. The authors are indebted to him for this gift.

α-Ketoglutaric acid and oxalacetic acid were synthetic products (7).

Glutamine samples were obtained from the following sources: Bios Laboratories, Sample A; Dr. F. M. Strong and Dr. R. H. Burris, Samples B
and C respectively; Dr. P. B. Hamilton, Sample D (Lederle), Sample E (synthesized by Dr. J. S. Fruton), and Sample F (Drug Products Company); Dr. J. P. Greenstein, Sample G (Lederle) and isoglutamine. Dr. P. B. Hamilton kindly furnished the assay values for Samples D, E, and F, 82, 97, and 85 per cent purity, respectively. The authors are indebted to the generosity of the above in providing these samples.

All other reagents employed were commercial products.

5 per cent CO₂-95 per cent O₂ and 5 per cent CO₂-95 per cent N₂ gas mixtures were commercial preparations. The same tanks were used throughout the study to insure uniformity of results.

Results

Substrate Concentrations—A study of the effect of varying the amount of ornithine revealed the optimum concentration to be 0.0033 M when 2.3 to 2.8 mg. of homogenate nitrogen were used per flask (Fig. 1). The synthesis of citrulline increased linearly to the optimum level. Further addition of ornithine had no influence on the quantity converted. At 0.0033 final molarity, 50 to 60 per cent of the ornithine was usually converted in 1 hour. This concentration is high compared to the amount of ornithine sufficient to maintain the operation of the complete urea cycle, in which the amino acid is continually regenerated. Preliminary work with homogenates showed that 0.0003 final molarity was as effective with the complete cycle as with any higher concentration.

The effect of increasing concentrations of ammonium chloride is shown...
in Fig. 2. In the presence of 0.033 to 0.067 M glutamic acid, 0.0067 M ammonium chloride was apparently sufficient to saturate the system.

![Graph showing the effect of glutamic acid concentration on citrulline formation.](image)

**TABLE I**

Percentage of Citrulline Synthesis with Glutamine

<table>
<thead>
<tr>
<th></th>
<th>Relative rate</th>
<th>per cent</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Controls</strong></td>
<td></td>
<td>100.0</td>
</tr>
<tr>
<td>Glutamic acid 3.3 X 10^-2 M, ammonium chloride 6.7 X 10^-3 M</td>
<td>95.4</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glutamine Samples A, B, and D</td>
<td>0.7</td>
<td></td>
</tr>
<tr>
<td>Sample C</td>
<td>31.7</td>
<td></td>
</tr>
<tr>
<td>&quot; E</td>
<td>27.4</td>
<td></td>
</tr>
<tr>
<td>&quot; F</td>
<td>56.2</td>
<td></td>
</tr>
<tr>
<td>&quot; G</td>
<td>55.2</td>
<td></td>
</tr>
<tr>
<td>Isoglutamine</td>
<td>3.9</td>
<td></td>
</tr>
</tbody>
</table>

The optimum glutamic acid concentration was far in excess of the ammonium chloride required (Fig. 3). A ratio of glutamic acid to ammonium chloride to ornithine of 15 to 20:2:1 was found to yield optimum values. The peak of the glutamic acid curve was found to vary within 0.015 to 0.067 M concentration from liver to liver, the rising portion of the curve being displaced proportionately. Both glutamic acid and ammonia were
essential, only 15 per cent of the optimum yield being obtained when glutamic acid at 0.067 M was incubated without ammonium chloride, and 2 to 4 per cent when ammonium chloride at 0.0067 to 0.053 M was used alone. Experiments were carried out at two levels of glutamic acid, 10 and 20 times the concentration of ornithine. While the yield of citrulline increased another 20 per cent at the higher concentration, the trend of the results was identical. Typical data and curves were selected to represent the effect of different substrates on the system in the present paper.

The reason for the requirement of both ammonia and glutamic acid, and particularly of the manifold excess of the latter, is not as yet apparent.

Table II

Citrulline Synthesis with Compounds Other Than Glutamic Acid

<table>
<thead>
<tr>
<th>Relative rate</th>
<th>Relative rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Compounds</td>
<td>per cent</td>
</tr>
<tr>
<td>Glutamic acid</td>
<td>100.0</td>
</tr>
<tr>
<td>Aspartic</td>
<td>2.4</td>
</tr>
<tr>
<td>Asparagine</td>
<td>2.9</td>
</tr>
<tr>
<td>α-Ketoglutaric acid</td>
<td>21.1-7.8</td>
</tr>
<tr>
<td>Oxalacetic</td>
<td>3.5</td>
</tr>
<tr>
<td>Malic</td>
<td>26.7-26.2</td>
</tr>
</tbody>
</table>

Various workers in the field have suggested the importance of glutamine in the synthesis of urea (9, 10). The possibility that glutamine was being synthesized from the glutamic acid and ammonia and entering into the reaction as a more specific donor of ammonia or the carbamino group of citrulline was explored. Experiments were devised in which glutamine replaced ammonium chloride and glutamic acid (Table I). The different samples tested showed a range of activity of 0 to 56 per cent of the control value. A synthetic product (Sample E), assaying 97 per cent pure, was 27.4 per cent as active as glutamic acid plus ammonium chloride. Isoglutamine gave a value of 4 per cent compared with the control.

Replacement of Glutamic Acid—Of a series of compounds tested in place of glutamic acid none showed more than 35 per cent of the activity of glutamic acid (Table II). Previous work with liver slices had shown a stimulation of urea synthesis by many of the components listed in a system in-
volving added ornithine and ammonia (1, 2, 10). However, in the case of homogenates, glutamic acid appears to have a relatively specific effect. The analogue, aspartic acid, and asparagine, its half amide, were inactive. As can be seen from Table II, the main components of the citric acid cycle showed values ranging from 3.5 per cent activity with oxalacetic acid to 35 per cent with fumaric acid. A few of these compounds were tested through a varying range of ammonium chloride concentration (0.0067 to 0.033 M). α-Ketoglutaric acid, malic acid, and fumaric acid showed a decrease in activity with an increase in ammonia. Glucose activity, on the other hand, increased with the ammonia concentration.

The presence of other compounds in addition to a suboptimum concentration of glutamic acid (0.013 M) was in most cases no more effective than

<table>
<thead>
<tr>
<th>Glutamic acid 0.013 M</th>
<th>Citrulline per mg. tissue N (microliters)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.026</td>
<td>37.8</td>
</tr>
<tr>
<td>0.013 + fumaric acid 0.013 M</td>
<td>47.1</td>
</tr>
<tr>
<td>0.013 + sucoinic acid 0.013 M</td>
<td>34.6</td>
</tr>
<tr>
<td>0.013 + α-ketoglutaric acid 0.013 M</td>
<td>33.2</td>
</tr>
<tr>
<td>0.013 + oxalacetic acid 0.013 M</td>
<td>41.4</td>
</tr>
<tr>
<td>0.013 + lactic acid 0.013 M</td>
<td>43.6</td>
</tr>
<tr>
<td>0.013 + citric 0.013 M</td>
<td>38.8</td>
</tr>
<tr>
<td>0.013</td>
<td>39.2</td>
</tr>
</tbody>
</table>

Fig. 4. Effect of adenylic acid and ATP concentrations on the formation of citrulline. Final substrate concentrations, glutamic acid 3.3 × 10^{-2} M, ornithine 3.3 × 10^{-3} M, ammonium chloride 6.7 × 10^{-2} M. Tissue concentration per flask 3.24 mg. of N.
glutamic acid alone (Table III). Fumaric acid and succinic acid decreased the yield of citrulline, but citric acid and lactic acid had no significant effect. While α-ketoglutaric acid and oxalacetic acid increased citrulline formation, they were only 38.7 and 62.4 per cent effective as an equal additional quantity of glutamic acid.

**ATP and Adenylic Acid**—Both ATP and adenylic acid stimulated the synthesis of citrulline (Fig. 4). Results obtained with ATP varied from liver to liver within 50 per cent of the values shown. In all probability its effect in this system is dependent on its prior hydrolysis to the more active adenylic acid. The fact that the latter reagent gave more consistent results at higher levels would indicate that it, per se or through the formation of some intermediate other than ATP, is the more active component in this synthesis. 3 micromoles of adenylic acid were used per flask throughout the study.

**Cytochrome c**—Since cytochrome c stimulated the synthesis only 7 per cent at the relatively high concentration of 0.000035 M, it was not used in subsequent experiments.

**Oxygen and Carbon Dioxide**—No synthesis of citrulline from ornithine occurred anaerobically. Bicarbonate ions and gaseous CO₂ were used in all experiments throughout the study. The elimination of either added bicarbonate or gaseous CO₂ from the incubation flask resulted in about 50 per cent as much conversion as with the complete system.

---

**Fig. 5**

*Effect of potassium ion concentration on the formation of citrulline. Final substrate concentrations, glutamic acid $6.7 \times 10^{-2}$ M, ornithine $3.3 \times 10^{-3}$ M, ammonium chloride $6.7 \times 10^{-3}$ M, adenylic acid $1 \times 10^{-3}$ M. Tissue concentration per flask 3.22 mg. of N.*

**Fig. 6**

*Effect of phosphate ion concentration on the formation of citrulline. Final substrate concentrations, glutamic acid $6.7 \times 10^{-2}$ M, ornithine $3.3 \times 10^{-3}$ M, ammonium chloride $6.7 \times 10^{-3}$ M, adenylic acid $1 \times 10^{-3}$ M. Tissue concentration per flask 3.01 mg. of N.*
Kidney Homogenate—No synthesis of citrulline was noted when kidney homogenate was incubated under the conditions found optimum for liver homogenate.

Inorganic Ions—Stimulation by potassium ions increased linearly to the maximum possible concentration (Fig. 5). 250 per cent as much activity was seen when all the sodium ions, except that added as sodium bicarbonate, had been replaced by potassium ions. Since commercial potassium bicarbonate contains magnesium ions as an impurity, it was not used in this study.

![Graph 7](image1)

**Fig. 7.** Effect of magnesium ion concentration on the formation of citrulline and urea. Final substrate concentrations, glutamic acid $3.3 \times 10^{-2} \text{ M}$, ornithine $3.3 \times 10^{-3} \text{ M}$, ammonium chloride $6.7 \times 10^{-3} \text{ M}$, adenylic acid $1 \times 10^{-3} \text{ M}$. Tissue concentration per flask 3.45 mg. of N.

**Fig. 8.** Effect of pH on the formation of citrulline. Final substrate concentrations, glutamic acid $3.3 \times 10^{-2} \text{ M}$, ornithine $3.3 \times 10^{-3} \text{ M}$, ammonium chloride $6.7 \times 10^{-3} \text{ M}$, adenylic acid $1 \times 10^{-3} \text{ M}$. Tissue concentration per flask 2.8 mg. of N.

Stimulation was also noted with phosphate ions (Fig. 6). Essentially no synthesis occurred in the absence of these ions. Maximum activity is reached at relatively low concentrations, 0.002 to 0.015 M. This latter molarity, added in the form of phosphate buffer, was used throughout the study. Higher concentrations inhibited the synthesis. The influence of phosphate ions suggests a specific effect, since concentrations of 0.002 M phosphate are too low to exert a significant buffering action in this system.

The effect of magnesium ions on the synthesis of citrulline and urea is seen in Fig. 7. The absence of these ions resulted in the accumulation of citrulline. With an increase in concentration to 0.0015 M, more of the citrulline formed was converted to urea. Higher concentrations appear to inhibit both reactions. In previous work (3), the effect of magnesium ions
on the conversion of citrulline to arginine was interpreted on the basis of its reported influence on ATP breakdown (11). Data presented here point to a more specific rôle of magnesium ions in that reaction. The possibility that citrulline accumulated as a result of stopping the urea cycle at arginine was investigated, since arginase requires covalent ions for activation. No arginine was found. Moreover, in the absence of any added ion, 0.0033 M arginine was found to be converted almost completely to urea in an hour by liver homogenate.

![Graph 1](image1.png)

**Fig. 9** Effect of incubation time on the formation of citrulline. Final substrate concentrations, glutamic acid $3.3 \times 10^{-2}$ M, ornithine $3.3 \times 10^{-3}$ M, ammonium chloride $6.7 \times 10^{-2}$ M, adenylic acid $1 \times 10^{-3}$ M. Tissue concentration per flask 2.53 mg. of N.

![Graph 2](image2.png)

**Fig. 10** Effect of tissue concentration on the formation of citrulline and urea. Final substrate concentrations, glutamic acid $3.3 \times 10^{-2}$ M, ornithine $3.3 \times 10^{-3}$ M, ammonium chloride $6.7 \times 10^{-2}$ M, adenylic acid $1 \times 10^{-3}$ M. Tissue concentration per flask 2.81 mg. of N.

Manganese ions below 0.001 M exerted approximately the same effect as magnesium ions.

**Effect of pH**—The range between pH 7.1 to 7.2 was optimum for the synthesis of citrulline (Fig. 8). Both bicarbonate-carbon dioxide and phosphate buffers were used to maintain pH. The concentrations of bicarbonate to be added to the media were calculated from the Henderson-Hasselbalch equation on the basis of a 5 per cent CO$_2$ content in the gas phase. At the optimum pH of 7.15, the concentration of the bicarbonate added to the media was 0.008 M.

**Incubation Time**—Production of citrulline usually slowed down abruptly after 50 to 60 minutes of incubation (Fig. 9). At this time, approximately 50 to 60 per cent of the ornithine added was found to be converted. The
reason for this abrupt decrease in the rate of synthesis is not clear. No citrulline disappeared within that time, nor was there any significant amount of urea formed. The possibility exists that ornithine is being removed in this system by some other reaction.

_Tissue Concentration—_In the absence of magnesium ions, citrulline accumulated in incubation flasks containing from 0.5 to 4 mg. of tissue nitrogen (Fig. 10). The conversion of citrulline to urea in this range of tissue concentration was very small. As the tissue concentration was increased, less citrulline and more urea were formed. The shape of these curves suggests two possibilities: (1) that either the presence of urea inhibits the formation of citrulline, or (2) that a protein-bivalent ion complex catalyzes the disappearance of the amino acid. The former possibility was eliminated through experiments which showed that urea had no inhibiting effect. The existence of a magnesium-activated enzyme catalyzing the conversion of citrulline to arginine, on the other hand, is not unlikely. The fact that there is little citrulline converted at low tissue concentrations in the absence of magnesium ions indicates that the ion-protein complex exists in favor of the inactive dissociated form. Thus an increase in homogenate nitrogen per flask correspondingly increases the concentration of the combined form to a point at which the rate of citrulline conversion exceeds the rate of its formation. In the presence of magnesium ions no citrulline accumulated at any tissue nitrogen level.

**Table IV**

_Effect of Inhibitors on Synthesis of Citrulline_

Final substrate concentrations: glutamic acid $6.7 \times 10^{-2} \text{ M};$ ornithine $3.3 \times 10^{-3} \text{ M};$ ammonium chloride $6.7 \times 10^{-3} \text{ M};$ adenylie acid $1 \times 10^{-5} \text{ M}.$ Tissue concentration per flask, 2.81 mg. of N.

<table>
<thead>
<tr>
<th>Inhibitors</th>
<th>Final molarity</th>
<th>Per cent inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arsenite</td>
<td>0.001</td>
<td>97.0</td>
</tr>
<tr>
<td>Cyanide</td>
<td>0.001</td>
<td>96.1</td>
</tr>
<tr>
<td>Iodoacetate</td>
<td>0.01</td>
<td>72.5</td>
</tr>
<tr>
<td>Azide</td>
<td>0.001</td>
<td>14.8</td>
</tr>
<tr>
<td>Malonate</td>
<td>0.003</td>
<td>15.7</td>
</tr>
<tr>
<td>&quot;</td>
<td>0.0057</td>
<td>58.8</td>
</tr>
<tr>
<td>Calcium ions</td>
<td>0.001</td>
<td>98.6</td>
</tr>
</tbody>
</table>

Inhibitors—Of a group of various inhibitors tried (Table IV), arsenite, cyanide, and calcium ions inhibited almost completely at 0.001 M. Since these substances are known to affect oxidative mechanisms, their effect in this system was not unexpected.

Of interest was the finding that fluoride stimulated the synthesis of
citrulline in concentrations up to 0.007 M (Fig. 11). 0.05 M was required for almost complete inhibition. In previous work, fluoride was found to inhibit the conversion of citrulline to arginine almost completely at 0.01 M (3). This differential inhibition by fluoride of the two reactions can also be used to advantage in studying the synthesis of citrulline.

The magnitude of inhibition seen with the other substances listed in Table IV is similar to that observed with the transimination reaction with the exception of malonate which is less effective in this system. Studies on

![Fig. 11](http://www.jbc.org/)  
Fig. 11. Effect of fluoride concentration on the formation of citrulline. Final substrate concentrations, glutamic acid $6.7 \times 10^{-2}$ M, ornithine $3.3 \times 10^{-3}$ M, ammonium chloride $6.7 \times 10^{-3}$ M, adenylic acid $1 \times 10^{-3}$ M. Tissue concentration per flask 3.22 mg. of N.

![Fig. 12](http://www.jbc.org/)  
Fig. 12. Effect of caffeine and theophylline on the formation of citrulline. Final substrate concentrations, glutamic acid $6.7 \times 10^{-2}$ M, ornithine $3.3 \times 10^{-3}$ M, ammonium chloride $6.7 \times 10^{-3}$ M, adenylic acid $1 \times 10^{-3}$ M. Tissue concentration per flask 2.11 mg. of N.

caffeine inhibition of urea synthesis have been carried out by Bernheim and Bernheim (12). They found that caffeine and like derivatives inhibited the disappearance of ammonia in liver and kidney slices and the synthesis of urea in liver slices and in man in vivo. A study of a series of purines revealed that caffeine and theophylline inhibited the synthesis of citrulline from ornithine and not at all the transimination reaction. Inhibition by theophylline was more marked than by caffeine (Fig. 12).

**DISCUSSION**

On the basis of the present data and those previously reported (3, 13), it is apparent that the enzymatic steps in the synthesis of urea by mammalian liver can be studied in properly fortified cell-free systems. The
CONVERSION OF ORNITHINE TO CITRULLINE

need for coupling with some energy-yielding mechanism is indicated from these studies. The greater effectiveness of adenylic acid over that of ATP in the conversion of ornithine to citrulline would suggest either a specific effect of adenylic acid or the mediation of an energy-coupling reaction which does not involve ATP as such.

The apparently obligatory rôle of glutamic acid in both the conversion of ornithine to citrulline and of citrulline to arginine is of particular interest. This key rôle of glutamic acid in the urea cycle again supports the unique position of this amino acid in intermediary metabolism. The recent finding of Krebs, Eggleston, and Hems (14) that liver homogenates are capable of rapidly forming glutamic acid from α-ketoglutaric acid and ammonia under anaerobic conditions provides a mechanism for supplying glutamic acid from the intermediary metabolic pool of nitrogen compounds for the transamination reaction as well as for the conversion of ornithine to citrulline. It is of interest to point out that any amino acid capable of transamination with α-ketoglutaric acid could contribute its nitrogen to urea without having to undergo primary oxidative deamination.

While the rôle of glutamic acid in the transamination reaction is clear, its rôle in the conversion of ornithine to citrulline is obscure. The need for concentrations of glutamic acid 10 to 20 times that of ornithine or ammonia does not permit any simple stoichiometric relationship. Further, the requirement for ammonium ions in the presence of high glutamic acid concentrations makes the possibility of direct amino group transfer from glutamic acid unlikely. The possible rôle of glutamine in this system would seem to be excluded from the data presented. Another possibility is that glutamic acid is required as a cometabolite for an energy-yielding system coupled with the synthesis of citrulline from ornithine. This is somewhat difficult to accept in view of the specificity for glutamic acid in this system. More direct information on this point is anticipated from projected studies with isotopically labeled glutamic acid.

It should be pointed out that the system reported here capable of synthesizing citrulline from ornithine, carbon dioxide, and ammonia has been considered as a single enzymatic step. It is highly probable that at least two reactions are involved in this synthesis and consequently an analysis of the rôle of the various reactants will be difficult until these enzymatic steps are resolved.

SUMMARY

1. The conversion of ornithine to citrulline has been demonstrated to occur in rat liver homogenates in the presence of glutamic acid, ammonium ions, adenylic acid or adenosine triphosphate, phosphate, and bicarbonate-carbon dioxide buffers. ATP was found to be 50 per cent as effective as adenylic acid.
2. Components of the citric acid cycle and other compounds similar to glutamic acid showed only up to 56 per cent as much activity as glutamic acid when substituted in part or completely for the acid.

3. The formation of citrulline is stimulated by phosphate and potassium ions.

4. The reaction proceeded maximally at a pH of 7.15.

5. In the presence of magnesium ions and high homogenate tissue concentrations, the citrulline formed was converted to urea.

6. Fluoride stimulated the synthesis up to 0.007 M concentration; complete inhibition occurred at 0.05 M. Caffeine and theophylline progressively inhibited the reaction with increasing concentrations.

7. The significance of these findings in relation to the Krebs-Henseleit urea cycle is discussed.

BIBLIOGRAPHY

THE CONVERSION OF ORNITHINE TO CITRULLINE BY RAT LIVER HOMOGENATES
Philip P. Cohen and Mika Hayano


Access the most updated version of this article at http://www.jbc.org/content/170/2/687.citation

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC’s e-mail alerts

This article cites 0 references, 0 of which can be accessed free at http://www.jbc.org/content/170/2/687.citation.full.html#ref-list-1