The Formation of Ornithine from Proline in Animal Tissues

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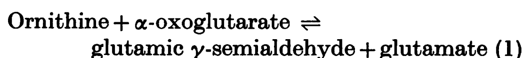
(*Received* 30 *January* 1967)

1. Homogenates of liver or kidney from rat, mouse, dog and guinea pig formed ornithine from proline but not from glutamate. Rat kidney was most active in this reaction and was used for further studies. 2. The overall reaction was found to be catalysed by proline oxidase to yield glutamic γ-semialdehyde, followed by transamination of this product with glutamate as catalysed by ornithine–keto acid aminotransferase. 3. The unfavourable equilibrium of the ornithine–keto acid aminotransferase reaction was overcome chiefly by glutamate dehydrogenase in the tissue, which removed the α-oxoglutarate produced, by reduction with endogenous ammonia and NADH. 4. Aspartate aminotransferase in these preparations also aided in the removal of α-oxoglutarate. In this case the overall reaction was driven also by the rapid decarboxylation of oxaloacetate. 5. No evidence could be found for a pathway of ornithine synthesis involving acylated intermediates as has been observed in some micro-organisms. 6. The rate of ornithine synthesis in homogenates of several rat tissues paralleled the activity of ornithine–keto acid aminotransferase in these tissues, indicating that this enzyme was rate-determining for the synthesis. 7. The possible influence of these reactions on urea synthesis is discussed.

Arginine is not essential for nitrogen balance in the adult man, dog, rat or mouse (Rose, 1938, 1949; Rose & Rice, 1939; Bauer & Berg, 1943; Totter & Berg, 1939; Almquist, Mecchi, Kratzer & Grau, 1942) and partially dispensable in growing rats (Rose, 1938). Even in the last-named animals the total arginine increase in tissues is two to three times greater than can be accounted for by arginine in the diet (Scull & Rose, 1930). It is evident that arginine can be synthesized in all of these animals. This amino acid is almost certainly derived from ornithine via citrulline; no other pathway for its formation has been described in animal tissues (Ratner, 1954). Nutritional studies and experiments with isotopes have provided evidence that ornithine in turn can be formed from either glutamate or proline (Stetten & Schoenheimer, 1944; Womack & Rose, 1949; Sallach, Koeppe & Rose, 1951). However, direct conversion of glutamate or proline into ornithine has not been demonstrated. This conversion conceivably could take place by reversal of the reaction catalysed by ornithine–keto acid aminotransferase (reaction 1).



$$\text{Ornithine} + \alpha\text{-oxoglutarate} \rightleftharpoons \text{glutamic } \gamma\text{-semialdehyde} + \text{glutamate} \quad (1)$$

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Enzyme systems have been described that yield glutamic γ-semialdehyde either from glutamate (Vogel & Davis, 1952; Strecker, 1957) or from proline (Johnson & Strecker, 1962). However, the free energy for reaction (1) is highly unfavourable for ornithine formation (Strecker, 1965), and indeed another more favourable pathway involving acetylated intermediates has been demonstrated in micro-organisms (Vogel, 1955).

The study reported in the present paper was undertaken to investigate possible routes for biosynthesis of ornithine from glutamate or proline in animal tissues. Evidence is provided that ornithine can be formed from proline by oxidation of the latter to glutamic γ-semialdehyde followed by transamination of this product with glutamate. The unfavourable equilibrium is overcome by removal of α-oxoglutarate in the glutamate dehydrogenase reaction and possibly by transamination with aspartate.

MATERIALS AND METHODS

Enzyme preparation. Rats weighing 200–250g. were decapitated, the required tissues removed and chilled, minced with fine scissors and ground in 0·25M-sucrose solutions, with either a manual (Dounce, Witter, Monty, Pate & Cottone, 1955) or a motor-driven all-glass homogenizer. The suspensions were diluted with 0·25M-sucrose solution to a final concentration of 20% (w/v). Subcellular

fractions were prepared according to Schneider & Hogeboom (1950). For incubations, particulate fractions were suspended to the original volume in 0·25M-sucrose solution. The particulate fractions were disrupted by sonic treatment for 5min. with a Raytheon Sonic Oscillator (model S102A), and the soluble phase was separated from the residue by centrifugation at 105000g for 1hr. For some experiments the resulting pellet was resuspended in 0·25M-sucrose solution to the original volume.

Enzyme assays. Tissue preparations were incubated together with the components presented with each Table or Figure, for 2hr. at 37°, with shaking. Anaerobic incubations were conducted in Thunberg-type flasks which were alternately evacuated and flushed with nitrogen several times. The reaction was terminated by the addition of perchloric acid to a final concentration of 3%. The denatured protein was removed by centrifugation, the supernatant solution carefully neutralized with 6N-KOH or 2M-K_2CO_3 and the potassium perchlorate removed by centrifugation. The supernatant solution was used for the analytical methods described below. Protein was determined by the method of Lowry, Rosebrough, Farr & Randall (1951). Representative values for the protein concentration of the preparations used in the experiments were as follows: kidney homogenate, 24·0mg./ml.; kidney fraction M, 15·8mg./ml.; kidney fraction A, 8·6mg./ml. and kidney fraction B, 8·8mg./ml.

Alanine aminotransferase (EC 2.6.1.2) and aspartate aminotransferase (EC 2.6.1.1) were determined spectrophotometrically according to the respective procedures of Segal, Beattie & Hopper (1962) and Sizer & Jenkins (1962). Ornithine–keto acid aminotransferase (EC 2.6.1.13) was determined as described by Strecker (1965). Glutaminase (EC 3.5.1.2) activity was calculated from the rate of ammonia formation in a system containing the tissue preparation, 0·06M-potassium phosphate solution, pH7·5, and 7mM-L-glutamine in a total volume of 3ml. The mixture was incubated at 37° with shaking and portions were removed at suitable intervals.

Analytical methods. For electrophoretic identification and determination of amino acids, the supernatant solutions after deproteinization were frozen and evaporated to dryness *in vacuo*. The residue was dissolved in 0·2ml. of water. Usually 10μl. portions were applied to strips of Whatman no. 1 paper, 3cm. × 30cm., and the amino acids separated by electrophoresis in a hanging-strip cell (Beckman Instruments Inc., Fullerton, Calif., U.S.A.) at 300mA for 2hr.; the buffer solution was 0·1M-pyridine–0·25M-acetate, pH4·3 (Durrum, 1950). The amino acids were located by treatment with 0·2% ninhydrin in acetone, and identified by comparison with the migration rates of samples of authentic amino acids subjected to electrophoresis under the same conditions at the same time. The strips were scanned for radioactivity with a windowless, gas-flow, Geiger detector; counts were recorded with the aid of a ratemeter and a strip chart recorder (Vanguard Instrument Co., La Grange, Ill., U.S.A.)

For quantitative determination of ornithine, formed on incubation, the supernatant solution after deproteinization was applied to a column (5cm. long × 1cm. diam.) of Dowex 50 (X8; 200–400 mesh; NH_4^+ form). The column was eluted first with 30ml. of 0·1M-ammonium formate, which removed proline and glutamic γ-semialdehyde, followed by 30ml. of 0·3M-ammonium formate, which removed ornithine. Usually a 1ml. portion of this second eluate was treated with ninhydrin by the method of Chinard (1952). In experiments with [^{14}C]proline, suitable portions of the solution containing radioactive ornithine were dissolved in Bray's (1960) solvent and radioactivity was measured in a scintillation spectrometer (Packard Instrument Co. Inc., Downers Grove, Ill., U.S.A.). Glutamic γ-semialdehyde (Δ^1-pyrroline-5-carboxylate) was determined by reaction with o-aminobenzaldehyde (Strecker, 1957), millimolar extinction coefficient 2·71 being used for the reaction product (Strecker, 1965). For determination of glutamate formed during incubation, the supernatant solution after deproteinization was applied to a column (11cm. long × 1cm. diam.) of Dowex 1 (X8; 200–400 mesh; acetate form). The column was washed with 25ml. of water, the glutamic acid eluted with 25ml. of 0·5M-acetic acid and its concentration determined by reaction with ninhydrin (Rosen, 1957). Ammonia was determined by the microdiffusion method of Conway (1957) on untreated samples of incubation mixture with 1% boric acid as absorbent. Pyruvate and oxaloacetate were determined enzymically with lactate dehydrogenase and malate dehydrogenase respectively. The assay medium contained 0·1M-potassium phosphate, pH7·5, 1·5mM-NADH, 0·1 unit of enzyme and a suitable portion of the deproteinized supernatant solution. The concentration of the keto acid was calculated from the decrease of extinction at 340mμ by using millimolar extinction coefficient $6{\cdot}22 \times 10^3$ for NADH (Horecker & Kornberg, 1948). α-Oxoglutarate was measured by the method of Friedemann & Haugen (1943). Readings were corrected for the extinction contributions due to pyruvate and oxaloacetate as calculated from the enzymic determinations.

Materials. Δ^1-Pyrroline-5-carboxylate (glutamic γ-semialdehyde) was prepared as described by Strecker (1960*a*). L-[U-^{14}C]Glutamic acid obtained from various commercial sources was purified as described by Niwaguchi, Motohashi & Strecker (1965). L-[^{14}C]Proline was obtained from New England Nuclear Corp., Boston, Mass., U.S.A. Crystalline lactate dehydrogenase (EC 1.1.1.27) was from Worthington Biochemical Corp., Freehold, N.J., U.S.A., crystalline malate dehydrogenase (EC 1.1.1.37) was generously supplied by Dr S. Englard, alanine aminotransferase and oxaloacetate were from C. F. Boehringer und Soehne G.m.b.H., Mannheim, Germany, and α-oxoglutarate was from Sigma Chemical Co., St Louis, Mo., U.S.A. All other chemical compounds were of the highest purity commercially available.

RESULTS

Formation of ornithine from proline stimulated by glutamate, glutamine or aspartate. L-[U-^{14}C]Proline incubated with homogenates of rat liver or kidney gave rise to ^{14}C-labelled ornithine and ^{14}C-labelled glutamate, demonstrable by paper electrophoresis. Brain homogenates were somewhat less active, whereas little or no conversion of proline was seen under these conditions with spleen or heart. Homogenates of mouse liver or kidney and to a lesser extent of dog or guinea-pig kidney also formed ornithine from proline. The livers of the last two animal species were relatively inactive in effecting this conversion. Incubation of L-[U-^{14}C]-glutamate with homogenates of these tissues did

not yield any proline, ornithine or glutamic γ-semialdehyde. The products formed from glutamate were mainly α-oxoglutarate and aspartate together with other tricarboxylic acid metabolites. A search for acylated amino acids or for other *N*-substituted glutamic acid derivatives revealed only pyrrolidone-carboxylic acid. The data dealing with this reaction have been reported (Niwaguchi *et al.* 1965).

The formation of ornithine from proline requires donation of a further nitrogen atom since the latter amino acid contains only one nitrogen atom. To identify the nitrogen donor, rat kidney, the tissue most active in converting proline into ornithine, was incubated with L-[U-^{14}C]proline and various amino acids or amines. The products of the incubation were separated by paper electrophoresis and the ^{14}C-labelled ornithine was determined semi-quantitatively by scanning with a Geiger detector as described in the Materials and Methods section. Glutamine, glutamate and aspartate each increased the yield of ornithine over that obtained from proline alone. γ-Aminobutyrate, valine and leucine inhibited the formation of ornithine, and other amino acids were inactive. Some representative results are shown in Fig. 1.

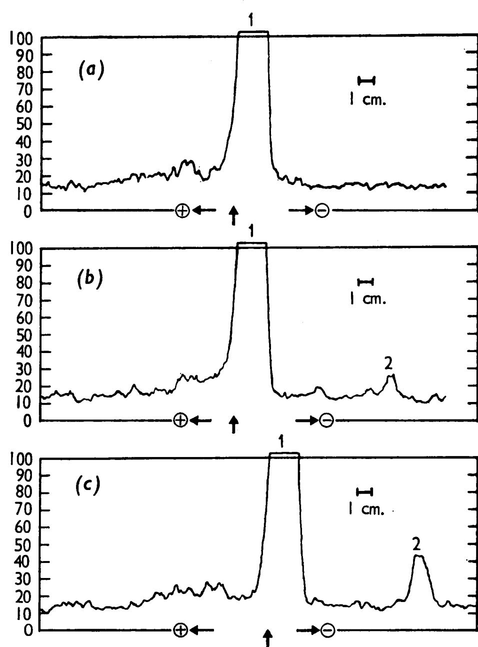

Fig. 1. Scanning of radioactivity of products formed during incubation of kidney homogenates with L-[^{14}C]proline (uniformly labelled) and separated by electrophoresis on paper strips at pH 4·3. Kidney homogenate (1·0 ml.) was incubated with 150 μmoles of potassium phosphate, pH 7·5, 90 μmoles of L-[^{14}C]proline ($3{\cdot}7\times10^5$ counts/min.), and (*a*) 100 μmoles of L-valine, (*b*) no further additions or (*c*) 100 μmoles of L-glutamate, in a total volume of 3·0 ml. The components represented by the numbered peaks are 1, proline, 2, ornithine.

The homogenate was separated by centrifugation into the four subcellular fractions described by Schneider & Hogeboom (1950), and each fraction tested for conversion of proline into ornithine by the paper-electrophoretic technique for detection and determination of ornithine. The mitochondrial fraction was most active, thereby suggesting the involvement of proline oxidase and ornithine–keto acid aminotransferase (and the common intermediate Δ^1-pyrroline-5-carboxylate), both known to be mitochondrial enzymes (Johnson & Strecker, 1962; Peraino & Pitot, 1963; Strecker, 1965). Glutamine and glutamate were stimulatory also with this fraction, but aspartate less so. NAD^+ inhibited the production of ornithine and at the same time increased the production of glutamate, thus further implicating Δ^1-pyrroline-5-carboxylate, since this compound is oxidized to g utamate by an NAD-requiring dehydrogenase present in mitochondria (Strecker, 1960*b*).

Because of the difficulty of measuring ornithine quantitatively by electrophoresis, a procedure was developed for separation of ornithine from the reaction products and determination either by radioactivity or by reaction with ninhydrin. This procedure, which is described in the Materials and Methods section, was used for the subsequent experiments.

The data in Table 1 confirm the essential points noted in the experiments with paper electrophoresis. The stimulation of ornithine formation by added glutamate or aspartate was compared by using the homogenate of whole kidney and the fraction sedimented at 10000 *g* for 10 min. (fraction M). Although this fraction contains both nuclei and red blood cells, it behaved with respect to ornithine formation no differently from a more homogeneous mitochondrial fraction and was used for many experiments because of convenience of preparation. It can be seen that the amount of ornithine formed when glutamate was added was about the same either with the whole homogenate or fraction M. However, the yield of ornithine from proline alone, or from proline plus aspartate, was less with fraction M, and the net increase of ornithine, brought about by adding aspartate, was also less. These results suggested that components present in the supernatant or microsomal compartments aided synthesis of ornithine from either proline or proline plus aspartate but that these components had no effect when glutamate was available. All of the remaining naturally occurring animal amino acids were also tested; of these only glutamine stimulated the synthesis of ornithine and the inhibition by

Table 1. *Effect of aspartate and glutamate on ornithine formation*

L-[^{14}C]Proline (60μmoles; $3\cdot7\times10^5$ counts/min.), 100 μmoles of L-aspartate, 100μmoles of L-glutamate, 150μmoles of potassium phosphate, pH 7·5, and 2·0 ml. of tissue suspension were incubated in a total volume of 3·6 ml. and assayed as described in the Materials and Methods section.

Tissue preparation	Additions	Ornithine formed (counts/min.)
Homogenate	Proline	2480
	Proline + aspartate	4240
	Proline + glutamate	5880
Fraction M	Proline	860
	Proline + aspartate	1760
	Proline + glutamate	6200

Table 2. *Stimulation of ornithine synthesis by supernatant fraction, glutamate or glutamine*

L-Proline (90μmoles) was incubated with 1·0 ml. of fraction M, 200μmoles of potassium phosphate, pH 7·5, and, where indicated, 1·0 ml. of high-speed supernatant fraction, 100μmoles of aspartate and 5μmoles of glutamate or glutamine in a total volume of 3·0 ml. Other conditions were as previously indicated.

	Ornithine (μmoles)	
Additions	Expt. A	Expt. B
None	0·48	0·54
Supernatant	0·93	1·08
Aspartate	0·72	0·90
Supernatant + aspartate	—	1·76
Glutamate	1·23	—
Glutamate + aspartate	2·28	—
Glutamine	—	1·44
Glutamine + aspartate	—	2·64

leucine and valine, previously noted, was confirmed.

Recombination of fraction M with the high-speed (105000*g* for 1 hr.) supernatant fraction increased the yield of ornithine from proline alone and from proline plus aspartate. In the latter case the stimulation obtained was more than additive (Table 2, Expt. B). The stimulatory factor was stable to heat (100° for 5 min.) and by fractionation of the high-speed supernatant on a column of Dowex 1 (acetate form) was shown to be glutamate. As seen in Table 1, glutamate alone had been shown to stimulate conversion of proline into ornithine and it was now found that the combination of glutamate plus aspartate increased the yield of ornithine more than would be expected from two independent effects (Table 2, Expt. A). Glutamine, either alone or together with aspartate, was somewhat more effective than glutamate. The greater stimulation by glutamine, compared with glutamate, was observed at all concentrations (2–30 mM) tested and for varying periods of incubation from 15 min. to 2 hr. The promotion of ornithine synthesis by glutamine seemed to depend on its rapid decomposition to glutamate and ammonia, catalysed by glutaminases in the preparation. The rate of formation of ammonia from glutamine added to the preparations used for these experiments was found to be 0·15μmole/mg. of protein of fraction M/min. This rate was more than adequate to decompose all of the added glutamine to glutamate after a few minutes of incubation. That at least part of this effect is due to phosphate-activated glutaminase (Greenstein, 1948) was shown by a 30–35% decrease in stimulation by glutamine if borate was substituted for phosphate in the buffer system used in these experiments. Activity with glutamate alone or with glutamate plus aspartate was not affected by borate.

Identification of enzymes responsible for ornithine synthesis. The mitochondria-containing fraction (M) used for these experiments was resolved partially into subfractions by fragmenting the particles either by freezing and thawing or by sonic treatment. Two fractions, the residual particles (A) and a soluble phase (B), were separated by centrifugation at 105000*g* for 1 hr. Fractions M, A and B were tested for capacity to form ornithine from either proline or Δ^1-pyrroline-5-carboxylate. With fraction M both substrates were equally effective. However, fractions A and B together were required to convert proline into ornithine, whereas fraction B alone effectively catalysed the conversion of Δ^1-pyrroline-5-carboxylate into ornithine (Table 3). Fraction A was shown to contain proline oxidase and to oxidize proline to Δ^1-pyrroline-5-carboxylate. A preparation of proline oxidase from liver mitochondria as described by Johnson & Strecker (1962) together with cytochrome *c* fully substituted for fraction A from kidney. Cytochrome *c* was always added in all further experiments with proline and fraction A.

The soluble fraction B contained most of the ornithine–keto acid aminotransferase activity originally present in fraction M. However, the content of this enzyme was not solely responsible for ornithine synthesis. Incubation of purified ornithine–keto acid aminotransferase (Strecker, 1965), at the same concentration as found in fraction B, together with Δ^1-pyrroline-5-carboxylate and glutamate resulted in forming only the small quantity of ornithine expected from the equilibrium constant of the reaction. Aspartate also had no effect in the system with the purified enzyme. It thus appeared that if ornithine–keto acid aminotransferase indeed catalysed the synthesis of ornithine, some other system was present in the crude preparations which

Table 3. *Products formed from proline or glutamic semialdehyde by different enzyme fractions*

The reaction mixture contained, in 6·0ml.: 400 μmoles of potassium phosphate, pH 7·5, 1·0ml. of each enzyme fraction as indicated, 60 μmoles of L-proline or 60 μmoles of DL-glutamic γ-semialdehyde (Δ^1-pyrroline-5-carboxylate) (GSA), 40 μmoles of L-glutamate, 0·06 μmole of cytochrome *c* and 8 μg. of pyridoxal phosphate. The incubations and assays are described in the Materials and Methods section.

Enzyme preparation	Reaction mixture	Glutamic γ-semialdehyde (μmoles)	Pyruvate (μmoles)	α-Oxoglutarate (μmoles)	Ornithine (μmoles)
Fraction A+B	Proline+glutamate	4·63	0·40	1·34	3·12
	Proline+glutamate +aspartate (7mM)	3·13	2·50	0·68	4·89
	Proline+glutamate +aspartate (0·7mM)	4·16	0·67	1·34	3·36
	GSA+glutamate	—	—	—	3·40
	GSA+glutamate+aspartate (7mM)	—	—	—	4·90
Fraction B	GSA+glutamate	—	0·30	2·30	3·56
	GSA+glutamate+aspartate (7mM)	—	2·28	1·30	5·36

could overcome the unfavourable equilibrium, for example by removing α-oxoglutarate. The stimulation by aspartate and the presence of aspartate aminotransferase in fraction B suggested that this enzyme could function for this purpose. Determination of the amount of α-oxoglutarate that accumulated during the reaction with aspartate revealed that indeed it was less than the quantity of ornithine formed. However, this was also true when glutamate alone was added to drive the reaction. Furthermore, oxaloacetate, the expected product of the aspartate aminotransferase reaction, was not found. This last result was explained by establishing that oxaloacetate was rapidly decarboxylated to pyruvate in these preparations (cf. Corwin, 1959) and indeed more pyruvate was found to be formed in the presence of aspartate than in its absence. Moreover the increase in ornithine synthesis brought about by aspartate was accompanied by a similar increase in pyruvate formation. The data on the products formed with fractions A and B with proline as substrate and fraction B only with Δ^1-pyrroline-5-carboxylate as substrate are presented in Table 3.

Surprisingly, alanine, which might be expected also to remove α-oxoglutarate by transamination, had little or no effect on ornithine formation. Determination of the relative activities of aspartate and alanine aminotransferases revealed that the former enzyme was present at 10 times the active concentration of the latter; the specific activities were 0·34 μmole/mg. of protein and 0·038 μmole/mg. of protein respectively. The relatively low activity of alanine aminotransferase together with the relatively high K_m of α-oxoglutarate for this enzyme as compared with that of aspartate aminotransferase (Velick & Vavra, 1962) would appear to explain the ineffectiveness of alanine. This conclusion was supported by finding that the addition of purified alanine aminotransferase and alanine to an incubation mixture increased the yield of ornithine about 30%.

As seen in Table 3, the sum of the amounts of α-oxoglutarate and pyruvate accumulated was always less than ornithine formed. This discrepancy was more pronounced when aspartate was not added, suggesting an alternative pathway for removing α-oxoglutarate. Examination of the fate of α-oxoglutarate incubated with these preparations revealed that much of its disappearance was accounted for by glutamate formed. This removal of α-oxoglutarate was increased by adding ammonia or NADH, indicating that glutamate dehydrogenase was responsible (Table 4). The small increase of pyruvate seen in Table 4 was probably due to decarboxylation of oxaloacetate formed by transamination of α-oxoglutarate with endogenous aspartate. It seems that very little α-oxoglutarate was utilized by oxidative mechanisms since arsenite inhibited very little. Anaerobiosis stimulated ornithine synthesis from Δ^1-pyrroline-5-carboxylate. This result could be attributed to increased removal of α-oxoglutarate (Table 5).

The evidence obtained thus indicates that ornithine can be synthesized from glutamic γ-semialdehyde arising from proline in a reaction catalysed by ornithine–keto acid aminotransferase. Although equilibrium of the latter reaction is unfavourable, it proceeds through coupling with glutamate dehydrogenase and aspartate aminotransferase. These last two enzymes are present in rat tissues in considerably higher activities than ornithine–keto acid aminotransferase and it might be expected that ornithine transamination is rate-

Table 4. *Utilization of α-oxoglutarate*

The reaction mixtures contained, in 6·0 ml.: 400 μmoles of potassium phosphate, pH 7·5, 2 ml. of fraction M and, where indicated, 12 μmoles of α-oxoglutarate, 6 mg. of NADH, 10 μmoles of NH_4Cl and 6 μmoles of sodium arsenite. Expts. 1 and 2 were separate experiments conducted aerobically and anaerobically respectively.

Expt.	Additions	Δ(α-Oxoglutarate) (μmoles)	Δ(Pyruvate) (μmole)	Δ(Glutamate) (μmoles)
1	None	0	+0·10	+0·20
	α-Oxoglutarate	−4·00	+0·45	+3·40
	α-Oxoglutarate + arsenite	−3·70	+0·57	—
2	α-Oxoglutarate	−3·60	+0·68	—
	α-Oxoglutarate + NH_4Cl	−5·12	+0·62	—
	α-Oxoglutarate + NADH	−9·16	+0·10	—

Table 5. *Effect of anaerobiosis on ornithine synthesis and utilization of α-oxoglutarate*

The reaction mixtures contained, in 3·0 ml.: 200 μmoles of potassium phosphate, pH 7·5, 1·0 ml. of fraction M, 30 μmoles of DL-glutamic γ-semialdehyde and, where indicated, 10 μmoles of glutamate or 100 μmoles of L-aspartate. α-Oxoglutarate (5 μmoles) was present initially in Expt. 1. Anaerobiosis was obtained as described in the Materials and Methods section.

Expt. 1 α-Oxoglutarate removal (μmoles)			Expt. 2 Ornithine synthesis (μmoles)	
Aerobic	Anaerobic	Additions	Aerobic	Anaerobic
1·76	2·66	Glutamate	0·90	1·53
		Glutamate + aspartate	2·25	2·97

Table 6. *Comparison of ornithine–keto acid aminotransferase and ornithine synthesis from proline*

The incubation mixtures contained in 3·0 ml.: 200 μmoles of potassium phosphate, pH 7·5, 1·0 ml. of tissue homogenate, 30 μmoles of L-proline, 20 μmoles of L-glutamate and, where mentioned, 20 μmoles of L-aspartate. The incubations and assays are described in the Materials and Methods section.

Tissue	Activity of ornithine–keto acid aminotransferase* (μmoles/g./hr.)	Ornithine synthesized (μmoles/g./2 hr.) − Aspartate	+ Aspartate
Kidney	127 ± 6	11·8	18·0
Liver	73 ± 8	7·3	11·0
Brain	11 ± 2	1·0	1·6
Heart	13 ± 3	1·4	2·5
Spleen	10 ± 3	0·9	1·3

* From Peraino & Pitot (1963).

limiting. That this supposition is probably correct was indicated by comparing in several rat tissues the rate of formation of ornithine, with and without aspartate present, with the activities of ornithine–keto acid aminotransferase in these tissues as reported by Peraino & Pitot (1963). As shown in Table 6, ornithine formation and ornithine–keto acid aminotransferase activity did indeed parallel one another in the tissues studied.

DISCUSSION

The biosynthesis and degradation of many metabolites have been shown to proceed over separate pathways although in many instances common intermediates are involved. For example, the interconversion of glutamate and proline, via the intermediate glutamic γ-semialdehyde, has been shown to be catalysed by different enzyme systems for the forward and reverse directions (Strecker, 1960*b*).

Ornithine is also interconvertible with both glutamate and proline, with glutamic γ-semialdehyde as a likely intermediate (Stetten, 1955), and an enzyme system has been described in microorganisms that could synthesize ornithine irreversibly via acetylated intermediates from glutamate (Vogel, 1955). The comparative irreversibility of the ornithine–keto acid aminotransferase reaction (Strecker, 1965) would then suggest that this reaction functions only for degradation of ornithine to glutamic γ-semialdehyde, which in turn could be metabolized to either glutamate (Strecker, 1960*b*) or proline (Meister, Radhakrishnan & Buckley, 1957; Yura & Vogel, 1959; Smith & Greenberg, 1957; Peisach & Strecker, 1962). Both pathways indeed have been described in yeast, *Neurospora* and *Bacillus subtilis* (Scher & Vogel, 1957; DeDeken, 1963; Fincham, 1953; Vogel & Vogel, 1963; DeHauwer, Lavalle & Wiame, 1962), and in these organisms synthesis and degradation could proceed by different pathways. In some organisms, however, only one pathway appears to exist. For example, *Escherichia coli* and other Gram-negative organisms are reported to be lacking ornithine–keto acid aminotransferase (Scher & Vogel, 1957), and the pathway for ornithine

Glutamate

↓ ?

Proline ⟶ glutamic γ-semialdehyde ⟶ ornithine

Glutamine ⟶ glutamate; Glutamine ⟶ NH_3 (+NADH)

glutamate ⇄ α-oxoglutarate (aspartate or NH_3 (+NADH))

ornithine ↓ CO_2, NH_3

urea ⟵ arginine ⟵ citrulline (aspartate)

arginine ⟶ ornithine

Scheme 1.

formation using acetylated intermediates has not been found in animals. The lack of ornithine–keto acid aminotransferase in *E. coli* is in accord with the observations that ornithine and proline are not interconvertible in this organism (Abelson, Bolton, Britten, Cowie & Roberts, 1953; Abelson, 1954; Abelson, Bolton & Aldous, 1952). The lack, in animals, of the pathway involving acetylated intermediates in ornithine synthesis would mean that ornithine (and arginine) formation from proline must depend on reversing the ornithine–keto acid transaminase reaction by coupling to glutamate dehydrogenase or to asparate aminotransferase or both as reported in this paper. Presumably, the formation of ornithine from glutamate via glutamic γ-semialdehyde would depend on these same enzyme systems. However, our preparations did not convert glutamate into ornithine and the direct formation of glutamic γ-semialdehyde from glutamate has not been demonstrated in animal tissue. The stimulation of ornithine synthesis by ammonia acting through glutamate dehydrogenase seems especially advantageous since excess of ammonia in the animals could have a twofold effect on increasing urea synthesis, directly by forming carbamoyl phosphate and indirectly by increasing ornithine concentration. At the same time the formation of glutamate would remove toxic ammonia. It is interesting that urea is not formed in organisms with the acetylornithine pathway. These considerations are summarized in Scheme 1.

The greater effectiveness of glutamine as compared with glutamate in stimulating ornithine synthesis could depend on the formation of ammonia as well as glutamate from this compound and could be related to the stimulatory effect of glutamine on urea synthesis (for review, see Ratner, 1954).

Finally, the stimulatory effect of aspartate acting through aspartate aminotransferase would suggest that aspartate in addition to its role in the step from citrulline to arginine may increase urea formation by elevating the amount of ornithine.

A Fulbright Travel Grant is gratefully acknowledged by A.D.S. M.B. is on leave from Laboratory of Organic and Biological Chemistry, Hebrew University of Jerusalem, Israel. H.J.S. is the recipient of a U.S. Public Health Service research career programme award (1-K6-GM-2487) from the National Institute of General Medical Studies. The work was supported by grants from the American Cancer Society (P-352) and from the National Science Foundation (GB-243).

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