The Anaerobic Interconversion of Ornithine and Citrulline by *Streptococcus faecalis*

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A variety of micro-organisms are capable of converting citrulline into ammonia, ornithine and carbon dioxide. This reaction has been found in *Streptococcus faecalis* (Akamatsu & Sekine, 1951; Knivett, 1952*a*; Oginsky & Gehrig, 1953; Slade, 1953), in *Strep. lactis* (Korzenovsky & Werkman, 1953) and in *Clostridium perfringens* (Schmidt, Logan & Tytell, 1952). The breakdown of citrulline by cell-free extracts of *Strep. faecalis* is accompanied by the phosphorylation of adenosine diphosphate (ADP) to adenosine triphosphate (ATP) (Knivett, 1953, 1954*a, b*; Slade, 1954). If arsenate is present, citrulline breakdown by cell-free extracts occurs without the addition of phosphate and ADP. Preliminary investigations in Cambridge (Knivett, 1952*b*) showed that citrulline breakdown also occurred if arsenate or ADP were replaced by a washed suspension of *Escherichia coli* containing ornithine decarboxylase (Gale, 1946). The present paper is a more detailed study of this effect. The breakdown of citrulline by an extract of *Strep. faecalis* with arsenate or phosphate and ADP is inhibited by L-ornithine. The inhibition by ornithine can be overcome by the addition of ornithine decarboxylase. That the breakdown of citrulline is reversible is demonstrated by the formation of citrulline from ornithine, ammonia and carbon dioxide in the presence of ATP.

METHODS

The organism, growth medium and preparation of suspensions were as previously described (Knivett, 1954*a*).

Estimation of ornithine. A strain of *Esch. coli* (National Collection of Industrial Bacteria no. 8571) was used as a source of ornithine decarboxylase. The organism was cultured on agar slopes containing 1.0% (w/v) 'Oxoid' Tryptone (a commercial casein digest marketed by Oxo Ltd., London) and 1.0% (w/v) glucose. Cells for experimental purposes were cultured on this medium for 18 hr. at 30°. The ornithine decarboxylase activity of *Esch. coli* was considerably increased when the growth medium was supplemented with ornithine, but ornithine was too expensive for use in growth media. The arginine-rich medium used for the growth of *Strep. faecalis* provided a readily available source of ornithine, since during the growth of *Strep. faecalis* arginine is converted into ornithine, and after removal of the cells, this spent medium contains about 1% ornithine.

Growth medium *A* was used to prepare cells with an active ornithine decarboxylase. This medium contained 1.0% (w/v) 'Oxoid' Tryptone, 2.0% (w/v) glucose (sterilized separately) and 10% (v/v) of ornithine-containing spent medium. The medium was adjusted to pH 6.5 before autoclaving, and incubated for 18–24 hr. at 30°. Growth medium *B* was similar to medium *A* but contained no added ornithine, and was adjusted to pH 8.5 before autoclaving. Growth medium *C* contained 0.5% (w/v) NH_4Cl ; 0.1% (w/v) NH_4NO_3 ; 0.1% (w/v) KH_2PO_4 ; 0.3% (w/v) K_2HPO_4 ; 0.15% (w/v) L-asparagine; 0.001% (w/v) $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$; 0.05% (w/v) $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$; 0.01% (w/v) $\text{MnSO}_4 \cdot 4\text{H}_2\text{O}$; 2.0% (w/v) glucose and 1.5% (w/v) agar, adjusted to pH 7.0 before autoclaving.

Preparation of cell-free extracts. A Mickle Sonic Disintegrator (Mickle, 1948) was used to prepare the *Strep. faecalis* extract (Table 1), and the *Esch. coli* extract (Table 2) as previously described (Knivett, 1954*a*). A Hughes (1951) press was used for the preparation of the other extracts (Knivett, 1954*b*). Most of the extracts were dialysed at 4° for 18–24 hr. against distilled water. The protein content of the extracts was measured by the method of Kalkar (1947). Dry weights of suspensions and extracts were checked by drying a known volume at 105° to constant weight.

Measurement of citrulline breakdown. The evolution of CO_2 during citrulline breakdown was followed in Warburg manometers at 37° and at pH 5.8. The gas phase was nitrogen. The results reported are representative of a number of experiments. The estimation of citrulline was as previously described (Knivett, 1954*b*), except that an equal number of standards and estimations were used.

Reagents. The barium salt of ATP was prepared from rabbit muscle by the method of Needham (1942), and then converted into the barium salt of ADP (Bailey, 1942). The sodium salt of ADP was prepared as required (Knivett, 1954*a*), and the solution (0.025*M*) stored at -14°. The barium salt of ATP was prepared from rabbit muscle according to the directions of LePage (1949). Pyridoxal phosphate (Roche Products Ltd.) solution (50–100 $\mu\text{g./ml.}$) was freshly prepared.

RESULTS

The effect of ornithine decarboxylase

An attempt was made to follow the formation of ornithine from citrulline by measuring the carbon dioxide produced (*a*) by a cell-free extract of *Strep. faecalis* with added ADP, and (*b*) by the same mixture with the addition of sufficient ornithine decarboxylase (a washed suspension of *Esch. coli*) to

ensure that ornithine was decarboxylated rapidly and did not accumulate. Citrulline was not attacked either by washed cells of *Esch. coli* or by a cell-free extract of *Strep. faecalis* in the absence of adenosine polyphosphates. However, when citrulline was added to a mixture of washed cells of *Esch. coli* and *Strep. faecalis* extract without the addition of adenosine polyphosphates, 2 moles of carbon dioxide were released/mole of citrulline added, i.e. double the amount released with ADP. A strain of *Esch. coli* (National Collection of Type Cultures no. 7020) with no ornithine decarboxylase showed only feeble activity (Knivett, 1952b).

The effect of phosphate

When phosphate buffer was replaced by sodium phthalate buffer, the production of carbon dioxide was very slow (45 μ l./hr.), but upon the addition of 0.033M sodium phosphate buffer, the rate of carbon dioxide was increased (330 μ l./hr.). Potassium acetate and methylglyoxaline buffers behaved in the same manner as sodium phthalate buffer, indicating that the effect observed with phthalate was not due to an inhibition by the latter. The rate of production of carbon dioxide was followed in the presence of varying quantities of added phosphate (Table 1). After a small initial release of gas upon the addition of the *Esch. coli* cells from the side compartment, carbon dioxide was liberated at a rate which remained constant for more than an hour. Table 1 shows that phosphate was essential for carbon dioxide formation, and that at low concentrations the rate of release of gas was proportional to the concentration of phosphate.

The effect of an extract of Escherichia coli

Esch. coli, deficient in ornithine decarboxylase (Gale, 1946), was prepared by growing the cells either in a chemically defined medium (medium C) or in a medium containing no added ornithine and the pH of which was 8.5 (medium B). The production of carbon dioxide from citrulline in the presence of an extract of *Strep. faecalis* and phosphate buffer was related to, but not directly proportional to, the ornithine decarboxylase activity of the cells (Table 2). A cell-free extract of these cells was prepared by shaking the cells with glass beads in a Mickle Sonic Disintegrator (Mickle, 1948) and then removing the cell debris by centrifuging. Pyridoxal phosphate was added to stimulate ornithine decarboxylase activity (Gale, 1946). There was a significant liberation of carbon dioxide from citrulline even by an extract containing no ornithine decarboxylase if an extract of *Strep. faecalis* and phosphate buffer were present.

Table 1. *The effect of phosphate upon the rate of release of carbon dioxide from L-citrulline by a cell-free extract of Streptococcus faecalis and a washed suspension of Escherichia coli*

Main compartment contents: 1.0 ml. 0.06M L-citrulline in 2M sodium phthalate buffer, pH 5.6; 1.0 ml. cell-free extract of *Strep. faecalis* (10.1 mg. dry wt. of a Mickle preparation/ml.), and 0.5 ml. Na_2HPO_4 (6.48 mM) or water. Side compartment contents: 1.0 ml. washed suspension of *Esch. coli* (21 mg. dry wt. of cells/ml.). Gas phase air; temp. 37°.

Phosphate concentration (mM)	Rate of CO_2 release (μ moles/hr.)
0	1.80
1.7	3.45
3.4	5.55
6.8	7.80

Table 2. *The effect of ornithine decarboxylase upon citrulline breakdown in the presence of a cell-free extract of Streptococcus faecalis*

Main compartment contents: 0.1 ml. cell-free extract of *Strep. faecalis* (7.8 mg. protein/ml.), 0.2 ml. 2M sodium phosphate buffer, pH 5.8; 0.05 ml. 0.12M- MgCl_2 .

Expt. 1. 0.5 ml. washed suspension of *Esch. coli* (7.15 mg. dry wt. of cells/ml.) grown in media leading to different ornithine decarboxylase activities (see below), and water to 1.1 ml. Side bulb contents: 0.1 ml. 2M sodium phosphate buffer, pH 5.8, and 0.3 ml. 0.025M L-ornithine dihydrochloride or 0.025M L-citrulline or water.

Expts. 2 and 3: 0.5 ml. cell-free extract (Mickle preparation) of *Esch. coli* prepared from cells A and C, 0.1 ml. pyridoxal phosphate (approx. 50 μ g./ml.), and water to 1.1 ml. Side bulb contents: 0.1 ml. 2M sodium phosphate buffer, pH 5.8, and 0.3 ml. 0.3M L-citrulline or 0.025M L-ornithine dihydrochloride or water.

Expt. 3: extracts A and C were stored at -14° for 2 weeks, and only 0.06 ml. *Strep. faecalis* extract was used per cup. Gas phase nitrogen; temp. 37°; period of incubation 1 hr. Growth media for *Esch. coli*: cells A—nutrient broth with added ornithine. Cells B—nutrient broth without added ornithine. Cells C—agar plates containing a chemically defined medium. For full details see text.

Expt. no.	<i>Esch. coli</i> preparation	CO_2 released (μ l./hr./mg. dry wt. <i>Esch. coli</i> cells or extract)		
		Control	Citrulline	Ornithine
1	Cells A	4.0	19.5	246.0*
	Cells B	1.5	10.0	18.0*
	Cells C	2.5	3.0	3.0*
2	Extract A	3.5	35.0	44.0
	Extract C	0.5	9.0	1.0
3	Extract A	3.5	18.0	23.0
	Extract C	0.5	9.0	0.0

* Calculated from CO_2 released in first 10 min. after addition of ornithine.

Table 3. *The effect of pyridoxal phosphate and heat treatment upon the activity of a dialysed extract of Escherichia coli*

Main compartment contents: 0.5 ml. dialysed cell-free extract of *Esch. coli* (20 mg. dry wt./ml.); 0.1 or 0.2 ml. cell-free extract of *Strep. faecalis* (7.8 mg. protein/ml.); 0.3 ml. 2M sodium phosphate buffer, pH 5.8; 0.05 ml. 0.12M-MgCl₂; and water to 1.2 ml. Additions: 0.1 ml. pyridoxal phosphate (approx. 50 µg./ml.). Side compartment contents: 0.3 ml. 0.025M L-citrulline or L-ornithine dihydrochloride. Gas phase nitrogen; temp. 37°; period of incubation 1 hr.

Substrate	<i>Strep. faecalis</i> extract (ml.)	<i>Esch. coli</i> extract (ml.)	Pyridoxal phosphate (ml.)	CO ₂ released (µmoles/hr.)
Ornithine	0.1	0.5	0	2.0
Ornithine	0.1	0.5	0.1	3.6
Citrulline	0.1	0.5	0	6.8
Citrulline	0.1	0.5	0.1	8.2
Citrulline	0.2	0.5	0.1	9.6
Citrulline	0.1	0.5*	0.1	5.1
Citrulline	0.1	0.5†	0.1	0.6

* *Esch. coli* extract heated at 60° for 10 min.

† *Esch. coli* extract boiled for 10 min.

Fig. 1. The effect of hydroxylamine upon the breakdown of citrulline. Main compartment: 0.05 ml. 0.12M-MgCl₂; 0.15 ml. dialysed extract of *Strep. faecalis* (11.1 mg. protein/ml.); 0.5 ml. dialysed extract of *Esch. coli* (16.6 mg. protein/ml.); 0.2 ml. 2M sodium phosphate buffer, pH 5.8; either 0.1 ml. pyridoxal phosphate (approx. 50 µg./ml.) or 0.1 ml. 0.014M-NH₂OH, HCl. Side compartment no. 1: 0.3 ml. 0.025M L-citrulline; 0.1 ml. 2M sodium phosphate buffer, pH 5.8; 0.1 ml. water. Side compartment no. 2: 2N-HCl. Gas phase nitrogen; temp. 37°. At intervals, the reaction was stopped by the addition of acid from the side compartment. The samples were also mixed with 5.5 ml. 6% (w/v) trichloroacetic acid and centrifuged. ○, CO₂; ●, citrulline; —, control; ---, +NH₂OH.

The activity of dialysed extracts of Esch. coli. The rate of release of carbon dioxide from citrulline by the combined extracts of *Strep. faecalis* and *Esch. coli* was increased in the presence of magnesium chloride, which was thereafter always added. Heating the dialysed extract of *Esch. coli* at 60° for 10 min. reduced the rate of release of carbon dioxide from citrulline by 38%, whilst boiling almost completely destroyed the activity (Table 3). In the presence of added pyridoxal phosphate (the

Fig. 2. Non-competitive inhibition by hydroxylamine of the release of CO₂ from citrulline in the presence of phosphate. Main compartment: 0.5 ml. dialysed cell-free extract of *Esch. coli* (11.4 mg. protein/ml.); 0.1 ml. dialysed cell-free extract of *Strep. faecalis* (11.1 mg. protein/ml.); 0.15 ml. M sodium succinate buffer, pH 5.6; 0.05 ml. 0.12M-MgCl₂; 0.1 ml. pyridoxal phosphate (approx. 50 µg./ml.) or 0.1 ml. 1.4 mM-NH₂OH, HCl. Side compartment: 0.3 ml. 0.3M L-citrulline; 0.3 ml. (0.0-1M) sodium phosphate buffer, pH 5.6, or water. Gas phase nitrogen; temp. 37°. CO₂ release was measured at 5 min. intervals after tipping, and the velocity calculated from the steady gas output in the first 20 min. ○, control; ●, +hydroxylamine.

prosthetic group of ornithine decarboxylase—Gale, 1946), the rate of production of carbon dioxide from citrulline or from ornithine was increased.

Inhibition by hydroxylamine

The rate of production of carbon dioxide from citrulline was measured in the presence of sufficient hydroxylamine to inhibit ornithine decarboxylase.

completely (Gale, 1946). The rate of citrulline removal was inhibited 50 % (Fig. 1) in the presence of hydroxylamine and only 1 mole of carbon dioxide released/mole of citrulline removed. The inhibition by hydroxylamine was investigated further, since hydroxylamine does not inhibit citrulline breakdown in the presence of phosphate and ADP (Knivett, 1952b).

The rate of carbon dioxide production was measured over a range of phosphate concentrations, with sufficient hydroxylamine to inhibit ornithine decarboxylase completely. The reciprocal of the initial velocity (v) was plotted against the reciprocal of phosphate concentrations (s) according to the method of Lineweaver & Burk (1934) and the line of closest fit calculated by the method of least squares (Fig. 2). The calculated maximum velocity (μ moles $\text{CO}_2/\text{hr.}/\text{ml.}$) was 1.3 in the presence of the inhibitor and 4.6 in its absence, whilst the Michaelis constant

(K_m) was 1.76 mM phosphate in each case. In the presence of hydroxylamine and phosphate buffer (0.4M) the Michaelis constant for citrulline was 0.01M.

The effect of varying amounts of the two bacterial extracts upon the rate of production of carbon dioxide from citrulline is shown in Table 4. Concentrations of *Strep. faecalis* extract above 0.1 ml./cup had little effect upon the rate of release of carbon dioxide. However, the rate was increased by addition of greater amounts of *Esch. coli* extract. The rate of carbon dioxide release was increased several fold when arsenate buffer replaced phosphate.

Table 5 illustrates the effect of citrulline concentration upon the rate of formation of carbon dioxide in the presence of sufficient hydroxylamine to inhibit ornithine decarboxylase completely. For higher citrulline concentrations, the difference

Table 4. *The release of carbon dioxide from citrulline by varying concentrations of dialysed extracts of Streptococcus faecalis and Escherichia coli*

Main compartment contents: varying quantities of dialysed extracts of *Strep. faecalis* (11.1 mg. protein/ml.) and *Esch. coli* (12.3 mg. protein/ml.); 0.3 ml. 2M sodium phosphate or arsenate buffer, pH 5.8; 0.1 ml. pyridoxal phosphate (approx. 100 $\mu\text{g.}/\text{ml.}$) or 0.1 ml. 1.4 mM- NH_4OH , HCl; and water to 1.45 ml. Side bulb contents: 0.3 ml. 0.3M L-citrulline or 0.025M L-ornithine dihydrochloride. Gas phase nitrogen; temp. 37°.

<i>Strep. faecalis</i> extract (ml.)	<i>Esch. coli</i> extract (ml.)	CO ₂ released (μ moles/hr.)	
		Control	+NH ₄ OH
0.1	0	0.2	—
0.1	0.25	7.8	2.4
0.2	0.25	8.7	—
0.3	0.25	9.7	2.7
0.1	0.50	12.6	—
0.3	0.50	15.8	4.5

<i>Strep. faecalis</i> extract (ml.)	<i>Esch. coli</i> extract (ml.)	Substrate	Buffer	CO ₂ released (μ moles/15 min.)
0.1	0.25	Citrulline	Arsenate	21.0
0.2	0.25	Ornithine	Phosphate	3.7
0.2	0.25	Ornithine	Phosphate	1.2*

* Pyridoxal phosphate omitted.

Table 5. *The effect of citrulline concentration upon the rate of release of carbon dioxide in the presence and absence of hydroxylamine*

Main compartment contents: 0.25 ml. dialysed extract of *Esch. coli* (12.3 mg. protein/ml.); 0.2 ml. dialysed extract of *Strep. faecalis* (11.1 mg. protein/ml.); 0.05 ml. 0.12M- MgCl_2 ; 0.3 ml. 2M sodium phosphate buffer, pH 5.8; 0.1 ml. pyridoxal phosphate (approx. 50 $\mu\text{g.}/\text{ml.}$) or 0.1 ml. 1.4 mM- NH_4OH , HCl; water to 1.2 ml. Side bulb contents: 0.3 ml. L-citrulline (0.0375–0.3M) or 0.025M L-ornithine dihydrochloride. Gas phase nitrogen; temp. 37°; period of incubation 2 hr.

Citrulline (mM)	Ornithine (mM)	CO ₂ released (μ moles)		Difference in CO ₂ released owing to NH ₄ OH (μ moles)
		Control	+NH ₄ OH	
0	0	0.1	—	—
7.5	0	6.6	1.8	4.8
15.0	0	8.5	2.3	6.2
30.0	0	9.1	3.1	6.0
60.0	0	9.6	3.7	5.9
0	5	6.0	0.1	—

Table 6. *The effect of ornithine and putrescine on the rate of carbon dioxide formation from citrulline*

All cups contained: 0.15 ml. 0.3 M L-citrulline; 0.05 ml. 0.12 M-MgCl₂; 0.4 ml. L-ornithine dihydrochloride or DL-ornithine dibromide or putrescine; 0.02 or 0.1 ml. dialysed extract of *Strep. faecalis* (11.1 mg. protein/ml.). Other additions—(a) Arsenate system: 0.6 ml. M sodium arsenate buffer, pH 5.8. (b) Phosphate and ADP system: 0.3 ml. 2 M sodium phosphate buffer, pH 5.8, and 0.25 ml. 0.025 M-ADP. (c) Phosphate, NH₂OH and *Esch. coli* system: 0.3 ml. 2 M sodium phosphate buffer, pH 5.8; 0.5 ml. dialysed extract of *Esch. coli* (16.6 mg. protein/ml.) and 0.1 ml. 1.4 M-NH₂OH, HCl. (d) Phosphate, and *Esch. coli* system: as (c) except 0.1 ml. pyridoxal phosphate (approx. 100 µg./ml.) instead of hydroxylamine. Water to 1.5 ml. Gas phase nitrogen; temp. 37°.

Test system ...	Arsenate	Phosphate + ADP	Phosphate, NH ₂ OH and <i>Esch. coli</i> extract	Phosphate and <i>Esch. coli</i> extract
Initial L-ornithine concentration (mM)	Inhibition of rate of CO ₂ formation (%)			
0	0	0	0	—
5	15	18	20	—
10	36	38	38	—
20	50	57	56	—
40	60	76	69	—
80	82	88	85	—
Initial DL-ornithine concentration (mM)				
10	22	—	—	—
20	35	—	—	—
Initial putrescine concentration (mM)				
15	10	0	0	65

between the rates of the inhibited and uninhibited reactions was constant and was almost equal to the ornithine decarboxylase activity of the system. A number of *Esch. coli* extracts were tested in this manner, and in no case was the decrease of the rate of production of carbon dioxide with hydroxylamine greater than the ornithine decarboxylase activity of the extract.

The effect of ornithine and putrescine on citrulline breakdown

The addition of hydroxylamine prevented the decarboxylation of ornithine to putrescine and led to an accumulation of ornithine. To test whether the decreased rate of citrulline breakdown in the presence of hydroxylamine (Fig. 1) was due to an inhibition by ornithine, the effect of ornithine concentration on citrulline breakdown was tested with either arsenate or phosphate and ADP or phosphate, hydroxylamine, and *Esch. coli* extract. Sufficient hydroxylamine was added to produce complete inhibition of ornithine decarboxylase activity during the experiment. In all three cases, approximately 50 % inhibition of citrulline (30 mM) breakdown was observed when L-ornithine (20 mM) was present (Table 6). DL-Ornithine was about half as effective an inhibitor as L-ornithine. Putrescine

Table 7. *The anaerobic synthesis of citrulline from ornithine by a cell-free extract of Streptococcus faecalis*

Side bulb contents: 0.1 ml. 0.18 M L-ornithine dihydrochloride, 0.1 ml. 0.18 M-NH₄Cl and 0.1 ml. 0.03 M ATP (or ADP or AMP), previously adjusted to pH 7.0 with HCl or NaOH; 0.05 ml. 0.12 M-MgCl₂; 0.3 ml. 0.24 M-KHCO₃. Main compartment contents: 0.2 ml. dialysed extract of *Strep. faecalis* (8.1 mg. protein/ml.), and water to 0.85 ml. temp. 37°. Gas phase: 5% CO₂, 95% H₂ or N₂ (when KHCO₃ omitted). The reaction was stopped by the addition of 3.0 ml. 5% (w/v) trichloroacetic acid and 0.5 ml. of the filtrate removed for the estimation of citrulline.

Conditions	Time of incubation (min.)	Citrulline formed (µmoles/cup)
Complete system	0	0.0
Complete system	5	1.2
Complete system	15	2.0
Complete system	45	2.4
Complete system	70	2.8
KHCO ₃ and CO ₂ omitted	60	0.0
0.3 ml. 0.06 M-KHCO ₃ in place of 0.3 ml. M-KHCO ₃	60	0.2
Ornithine omitted	60	0.0
NH ₄ Cl omitted	60	0.0
Extract omitted	60	0.0
ATP omitted	60	0.0
ATP replaced by ADP	60	1.3
ATP replaced by AMP	60	0.0

(15 mm) had no significant inhibitory effect on citrulline breakdown, except when an *Esch. coli* extract with no hydroxylamine was present.

The anaerobic synthesis of citrulline from ornithine

As L-ornithine inhibited the conversion of L-citrulline into L-ornithine, it seemed likely that the inhibition by ornithine might be due to a reversal of citrulline breakdown. Accordingly, attempts were made to demonstrate synthesis of citrulline from ornithine by the bacterial extract under anaerobic conditions. The results obtained are shown in Table 7. Ornithine, ammonia, carbon dioxide and ATP were all essential for citrulline synthesis, and no significant amount of citrulline was detectable if any of these components were absent. Some citrulline was synthesized when ATP was replaced by ADP, but this was probably due to the action of adenylate kinase (myokinase) in the extract. Adenosine monophosphate (AMP) could not replace ATP.

DISCUSSION

The inhibition by ornithine of citrulline breakdown can be overcome by the addition of ornithine decarboxylase (*Esch. coli* extract), in which case the rate of citrulline breakdown is determined by the activity of the ornithine decarboxylase in the extract and is stimulated by the addition of pyridoxal phosphate. Inhibitors of ornithine decarboxylase have no effect upon citrulline breakdown with arsenate or phosphate and ADP, but only with the *Esch. coli* extract and phosphate.

Hydroxylamine inhibits the release of carbon dioxide from citrulline noncompetitively, because it inhibits only the release of carbon dioxide from ornithine and has no effect on the formation of carbon dioxide from citrulline.

Phosphate is essential for the breakdown of citrulline, whether an active ornithine decarboxylase is present or not in the *Esch. coli* extract. The factor in the extract responsible for citrulline breakdown in the absence of ornithine decarboxylase was not identified, but in view of its thermolabile properties it may well be an enzyme. The rate of carbon dioxide output usually remained constant for more than an hour, even when limiting concentrations of phosphate were present. This suggests that the concentration of phosphate remained constant and was not appreciably reduced by the formation of a phosphorylated intermediate. Furthermore, any phosphorylated intermediate can only be present in trace amounts and be rapidly decomposed so that no accumulation can occur. It seems likely that the apparent inhibition by ornithine is due to a shift in the equilibrium towards citrulline.

Gale (1945) found that in the absence of arginine, growth of the strain of *Strep. faecalis* used in this work took place in the presence of ornithine; the rate of growth and the total crop depended upon the concentration of carbon dioxide in the medium. He suggested that under these conditions, ornithine was converted into arginine, but he was unable to detect any disappearance of ornithine.

The conversion of ornithine into arginine has been studied with 'biochemical' mutants of *Neurospora crassa* (Srb & Horowitz, 1944), *Penicillium notatum chrysogenum* (Bonner, 1946) and *Escherichia coli* (Davis, 1950). Their results indicate that citrulline is an intermediate in the synthesis of arginine from ornithine.

SUMMARY

1. Citrulline is decomposed by dialysed cell-free extracts of *Streptococcus faecalis* without the addition of adenosine diphosphate or arsenate if a dialysed cell-free preparation of *Escherichia coli* containing ornithine decarboxylase is present.

2. The rate of release of carbon dioxide is dependent upon the concentrations of citrulline and of phosphate, and is stimulated by the addition of pyridoxal phosphate and of magnesium ions.

3. Hydroxylamine, sufficient to inhibit ornithine decarboxylase, inhibits the rate of removal of citrulline by 50 % and only 1 mole of carbon dioxide is released/mole of citrulline removed.

4. Citrulline breakdown is inhibited in the presence of L-ornithine or to a lesser extent by DL-ornithine. Putrescine does not inhibit directly the breakdown of citrulline.

5. Formation of citrulline by an extract of *Strep. faecalis* occurs, under anaerobic conditions, in the presence of ornithine, ammonia, carbon dioxide and adenosine triphosphate.

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The Synthesis of Serine and *Leuconostoc citrovorum* Factor by Cell Suspensions of *Streptococcus faecalis* R

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The evidence that growth factors of the folic acid group are required for some step in the biosynthesis of serine by various micro-organisms has been reviewed by Shive (1951), Woods (1952) and Lascelles, Cross & Woods (1954). It is of an indirect nature and springs from observations with growing cultures that serine is a component of certain mixtures of amino acids and nucleic acid derivatives which can replace the requirement for *p*-aminobenzoic acid or folic acid† either for growth or for overcoming inhibition of growth by sulphonamides (Winkler & de Haan, 1948; Lampen, Jones & Roepke, 1949; Holland & Meinke, 1949; Snell, 1951; Lascelles *et al.* 1954).

Glycine replaces serine for the growth of mutant strains of *Neurospora* and *Escherichia coli* (Tatum, 1949; Roepke, Libby & Small, 1944; Wright, 1951) and, under certain conditions, of *Leuconostoc mesenteroides* P 60 (*Streptococcus equinus* P 60) (Lascelles *et al.* 1954); glycine may therefore be an intermediate in serine formation, as it may be also in animal tissues (see below). After growth of *Torulopsis utilis* on a medium containing [carboxy-¹⁴C]-glycine the bulk of the isotope was found in the glycine, serine and proline fractions of the hydrolysed cell-protein (Ehrensward, Sperber, Saluste, Reio & Stjernholm, 1947).

Growth experiments with micro-organisms also provide evidence, again indirect, that folic acid is required for the conversion of glycine into serine. With *Ln. mesenteroides* P 60 there was an increased

requirement for *p*-aminobenzoic acid or *N*⁵-formyl-5:6:7:8-tetrahydropteroylglutamic acid (leucovorin) when the organism grew with high concentrations of glycine in the absence of serine (Lascelles *et al.* 1954). Folic acid would not replace serine for the growth of *Lactobacillus bifidus* unless glycine were present (Nepple, Wright & Skeggs, 1951) while, with the protozoan *Tetrahymena pyriformis* W, Kidder & Dewey (1953) found that growth occurred on a medium containing glycine but no serine only if high concentrations of folic acid were added; increased thioctic acid (protogen) was also required.

Pyridoxal is required for growth of *Ln. mesenteroides* P 60 on media containing glycine but no serine (Lascelles *et al.* 1954); it may therefore also take part in the synthesis of the latter amino acid from the former. A function of vitamin B₆ at some stage of serine formation had also been indicated by replacement experiments with growing cultures of *Streptococcus faecalis*, *Lactobacillus arabinosus* and *Ln. mesenteroides* (Lyman, Moseley, Wood, Butler & Hale, 1947; Steele, Sauberlich, Reynolds & Baumann, 1949).

In the experiments with growing cultures summarized above, with few exceptions, synthesis of serine was not actually demonstrated but inferred from the production of new cell material (assumed to contain serine) on media not containing this amino acid. Lascelles *et al.* (1954), however, showed that cells of *Ln. mesenteroides* grown with glycine contained a normal content of serine, and Ehrensward *et al.* (1947) also have this confirmation with *Torulopsis utilis*. In most cases the growth media contained many other substances besides those discussed; some of these or their metabolic

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† The term folic acid will be used throughout in general reference to the group as a whole; individual members will be named specifically.