Short Communications

Carbon dioxide fixation in the synthesis of aspartic acid by a strain of *Staphylococcus aureus*

During investigations on the pathway of glucose metabolism in cells of *Staphylococcus aureus* (strain Duncan), which will be described in detail in a further paper, the distribution of carbon from [^{14}C]glucose among the compounds present in hot-water extracts of the organism was determined. Glucose, generally labelled with ^{14}C (final specific activity, 1 μC/μmole), was added to cultures growing exponentially in peptone–glucose medium[1]; after 1 h hot-water extracts of the cells were prepared and the compounds present were separated by two-dimensional paper chromatography using the solvent systems phenol–water (500 g/112 ml) followed by *n*-butanol–acetic acid–water (7:2:5, v/v/v). The radioactive compounds were located by radioautography and identified initially by reference to the position of known markers. Among the many compounds which became labelled, radioactivity was found in a compound whose position corresponded exactly with that of aspartic acid; no radioactivity was detectable in the positions of glutamic acid or of any of the intermediates of the tricarboxylic acid cycle. These results, together with others which show that production of $^{14}CO_2$ from [6-^{14}C]glucose by this organism is very small in comparison with that from [1-^{14}C]glucose and glucose generally labelled with ^{14}C, suggest that the tricarboxylic acid or glyoxalate cycles do not operate in this organism to any significant extent.

The most likely alternative route for incorporation of carbon from glucose into aspartic acid appeared to be that *via* the formation of oxaloacetate from pyruvate, involving fixation of CO_2; such a mechanism has been shown to occur in a number of species of *Lactobacilli*[2–4] and in the minute *Streptococci*[5]. It was found that exponentially-growing cells of *S. aureus* (strain Duncan) incorporate radioactivity from

TABLE I

INCORPORATION OF RADIOACTIVITY FROM $^{14}CO_2$ BY GROWING ORGANISMS

3-ml cultures of *S. aureus* (strain Duncan) were grown in PG medium[1] in Warburg manometer vessels. When the bacterial density reached about 0.2 mg/ml, $^{14}CO_2$ (initial specific activity, 7,500 counts/min/μmole) was liberated from $Ba^{14}CO_3$ in the side arm by addition of 1 *N* H_2SO_4 from the hollow stopper. After 2 h the organisms were harvested and fractionated[1]; the (protein + cell wall) fraction was hydrolysed (6 *N* HCl, 105°, 15 h) and this hydrolysate and the hot-water extract were separated by paper chromatography[1]. The values in the table are averages from 2 cultures.

Fraction	*Radioactivity (counts/min/ml culture)*	*% of total radioactivity in aspartic acid*
Hot-water extract (100°, 10 min)	2600	93.2
Soluble in hot trichloroacetic acid (90°, 6 min)	44	—
Protein + cell wall mucopeptide	4250	95.5

$^{14}CO_2$, most of the radioactivity appearing in the hot-water-extractable and protein fractions. Over 90 % of the radioactivity in both of these fractions was found to be present in aspartic acid, identified by its chromatographic behaviour and also by its sensitivity to the specific aspartic decarboxylase of *Nocardia globerula*[6] (Table I). In the hot-water-extractable fraction, the only other component which became radioactive corresponded in position with oxaloacetic acid; in the protein fraction a weakly radioactive spot appeared in the area of the chromatogram occupied by glycine and serine. When the [^{14}C]aspartic acid, isolated from the protein fraction by preparative paper chromatography, was degraded using aspartic decarboxylase[6], 92 % of the radioactivity was released as $^{14}CO_2$, showing that it was present originally in the γ-carboxyl position of L-aspartic acid.

We investigated if this incorporation of $^{14}CO_2$ represented net synthesis of aspartic acid, or an exchange reaction, *via* oxaloacetate, with aspartic acid present in the growth medium. For this purpose, a larger quantity of [γ-^{14}C]aspartic acid was prepared by incorporation of $^{14}CO_2$ into growing cells, and purified by paper chromatography. The production of $^{14}CO_2$ from this [γ-^{14}C]aspartic acid was compared with the incorporation of $^{14}CO_2$, using suspensions of non-growing organisms to prevent removal of aspartic acid into cell protein. Table II shows that under these conditions

TABLE II

REVERSIBILITY OF INCORPORATION OF $^{14}CO_2$ INTO ASPARTIC ACID

Growing organisms were harvested and incubated in Warburg flasks at a concentration of about 5 mg/ml in defined growth medium[7] containing chloramphenicol (200 μg/ml). In one flask (A) the aspartic acid included [γ-^{14}C]aspartic acid prepared by $^{14}CO_2$ incorporation. In the other flask (B), $^{14}CO_2$ was liberated from $Ba^{14}CO_3$. After 2 h 0.5 ml *N* H_2SO_4 was tipped into both flasks and CO_2-free 7 *N* KOH was introduced through the hollow stopper into the side arm of flask A. After a further hour the absorbed CO_2 in flask A was precipitated as $BaCO_3$, washed and counted. The initial specific activity of the CO_2 in flask B was estimated using a second identical flask.

Reaction		*Specific activity of precursor (counts/min/μmole)*	*Total counts/min in product*
[γ-^{14}C]aspartic acid $\rightarrow$ $^{14}CO_2$	(A)	10,150	770
$^{14}CO_2$ $\rightarrow$ [γ-^{14}C]aspartic acid	(B)	1,510*	508

* Initial specific activity; dilution with respiratory CO_2 would reduce this figure finally to at least 1 % of this value. The value for the amount of CO_2 fixed is therefore a minimal one.

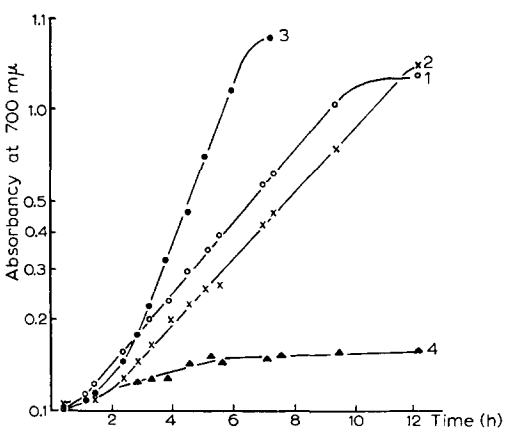

Fig. 1. Growth of *S. aureus* (strain Duncan) in defined medium[7] with aspartic acid (200 μg/ml) (cultures 1 and 3) or omitting aspartic acid (cultures 2 and 4). Cultures 1 and 2 were aerated slowly with air; a rapid stream of CO_2-free air (passed successively through 7 *N* KOH, 1 *N* $Ba(OH)_2$ and distilled water) was passed through cultures 3 and 4.

the amount of $^{14}CO_2$ produced from [γ-^{14}C]aspartic acid is very small compared with the incorporation of $^{14}CO_2$; the incorporation therefore involves a net fixation of CO_2.

This conclusion is further supported by observations on the interrelations between CO_2 and aspartic acid in the growth of this organism (Fig. 1). When a defined medium[7] was used, with or without aeration, there was no difference in the growth rate when aspartic acid was omitted from the medium. When precautions were taken to exclude CO_2 from the culture medium, and a stream of CO_2-free air was passed through the culture, the growth rate and the final cell density in the absence of aspartic acid were virtually zero, indicating that fixation of CO_2 is the main pathway of synthesis of aspartic acid in this organism under these conditions.

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The catalytic effect of glutamic acid derivatives in urea synthesis

From the experiments of JONES *et al.*[1] it seems most probable that the first step in the synthesis of urea in mammalian liver leads to the formation of carbamyl phosphate. This step is followed by a reaction of carbamyl phosphate with ornithine to form citrulline. The earlier work, emanating from the laboratory of COHEN, has demonstrated convincingly that the first step requires the presence of a cofactor with catalytic properties which was identified as acetylglutamic acid[2], though related derivatives of this compound also exerted catalytic activities[3]. The enzymes responsible for this reaction[4] (see below) were recently extracted from frog liver[5,6]:

$$2\ ATP + NH_3 + CO_2 \xrightarrow{\text{acetylglutamic acid}} 2\ ADP + \text{carbamyl phosphate} + H_3PO_4 \quad (1)$$

It can be assumed that initially acetylglutamic acid is involved in a reaction with one or more of the substrates. From this the question arises which of the three substrates would react first and which of the molecular groups of the cofactor would take part in this reaction. As a working hypothesis it was thought that the carboxyl groups of acetylglutamic acid would in the first place react with either NH_3 or with ATP whereby acid amides and phosphoric acid anhydrides respectively would be formed. It was therefore decided to synthesize various amide and anhydride derivatives of acetylglutamic acid and of related compounds and to test such compounds for their catalytic activities in the above reaction.

The references given below refer to known methods used for the synthesis of

Abbreviations: ATP, ADP, adenosine tri- and diphosphate.