by the formation of amino acyl-RNA. A method is described for the separation of amino acyl-RNA from enzyme reaction mixtures by chromatography on DEAE-cellulose.

3. The pyrophosphate-exchange reaction catalysed by the synthetases from the protein-body preparation showed an apparent inhibition with added ATP. The pyrophosphate-exchange catalysed by the enzymes from the supernatant preparation was almost completely dependent on ATP and stimulated by the addition of ATP. The formation of amino acyl-RNA by enzyme preparations from both the protein-body and supernatant preparations was stimulated by the addition of ATP.

4. An enzyme fraction obtained from the protein-body preparation after treatment with ultrasonic vibration catalysed the incorporation of amino acids into amino acyl-RNA and soluble protein.

5. The localization of enzymes and RNA associated with the initial stages of protein synthesis in the two preparations from wheat endosperm, and the significance of these findings in relation to the independent synthesis of storage and cytoplasmic proteins of wheat endosperm, is discussed.

Grateful acknowledgement is made of the financial support from the Wheat Industry Research Fund. We thank Mr. D. Morton and Miss M. Ryder for their able technical assistance.

REFERENCES


Biochem. J. (1964), 91, 546

Photoassimilation of Acetate and the Biosynthesis of Amino Acids by Chlorobium thiosulphatophilum

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The green sulphur bacteria Chlorobium limicola and Chlorobium thiosulphatophilum, unlike other photosynthetic bacteria, are both strict anaerobes and strict autotrophs. With the exception of the green organism isolated from the symbiotic aggregate Chlorochromatium by Mechsner (1957) and Chloropseudomonas ethylicum isolated by Shaposhnikov, Kondratieva & Fedorov (1960), growth of the green sulphur bacteria has not been obtained in media containing organic compounds in place of reduced compounds of sulphur. However, Larsen (1953) found that washed suspensions of C. thiosulphatophilum carboxylated propionate to succinate, and Sadler & Stanier (1960) found that Chlorobium limicola grew in the presence of low concentrations of some simple organic compounds, added to the standard basal medium for autotrophic growth, and that this resulted in a greater final cell yield. Acetate was the most effective of the organic compounds tested in promoting increased cell yield and chlorophyll formation. It was shown that acetate was assimilated by growing cultures only if both bicarbonate and a source of reducing power such as sulphide were available.

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Under these conditions the gross distribution of acetate carbon atoms in the cell fractions was virtually the same as that found with bicarbonate carbon atoms. The sulphide requirement for acetate assimilation suggested that C. limicola had a very limited ability to oxidize acetate.

The photoassimilation of acetate in resting cell suspensions of the photoheterotroph *Rhodospirillum rubrum* has shown that glutamate is an early product and that it cannot be formed by the conventional pathway involving citrate and other intermediates of the tricarboxylic acid cycle (Hoare, 1963). The investigations of Cutinelli, Ehrensvard, Reio, Saluste & Stjernholm (1951) also showed that acetate was assimilated into a number of amino acids, and the isotope distribution in alanine and aspartic acid was not in accord with that established in *Escherichia coli* and other aerobic micro-organisms.

Investigations were therefore carried out with another closely related species of green sulphur bacterium, *C. thiosulphatophilum* (Larsen, 1952), with the following main objectives: to see whether the observations of Sadler & Stanier (1960) with *C. limicola* also applied to *C. thiosulphatophilum*, to extend the observations to resting cell suspensions, since the bicarbonate requirement for the photoassimilation differed from *Rhodospirillum rubrum* in which acetate is photoassimilated in the absence of bicarbonate to form poly-β-hydroxybutyrate, to establish, in growing cultures, whether acetate was incorporated into any of the amino acids of the cell proteins of *C. thiosulphatophilum* and, if so, to determine whether the acetate was incorporated into the amino acids in a specific manner. The experiments of Cutinelli et al. (1951) with *Rhodosporillum rubrum*, those of Tomlinson (1954a, b) with *Clostridium kluyveri*, and the more recent studies with *Ruminococcus flavefaciens* (Allison, Bryant & Doetsch, 1962; Allison & Bryant, 1963) all indicate that the biosynthesis of some amino acids in anaerobes proceeds by mechanisms which differ from those so thoroughly established in *E. coli* and often assumed to occur in most, if not all, other micro-organisms. A preliminary account of some of the results has been reported (Gibson & Hoare, 1963).

**MATERIALS AND METHODS**

**Growth and maintenance of micro-organisms.** Two strains of *Chlorobium thiosulphatophilum* NCIB nos. 8346 and 8327 were obtained from Dr J. Lascelles. One of the strains 8327 (OC) had also been maintained continuously in Sheffield for 8 years but appeared to differ slightly in growth rate from the strain freshly supplied in 1961; this strain was used for most of the experiments. Organisms were maintained in stab cultures of Larsen’s thiosulphate medium (Larsen, 1952) kept in the light at 26–29° and were transferred monthly. Liquid cultures were grown in glass-stoppered bottles completely filled with thiosulphate medium with the addition of sodium acetate where indicated. Some irregularities in growth were encountered occasionally when transferring from stab cultures to liquid cultures. Only cultures growing normally and of a bright deep-green colour were used as inocula for growth curves and for manometric experiments. In all cases the chemicals used for making media were A.R. grade and the sodium thiosulphate was recrystallized three times; both thiosulphate and sulphide solutions were made up fresh each time media were prepared. *Rhodospirillum rubrum* strain S1 was grown photosynthetically under semi-anaerobic conditions on ‘malate medium’ (Hoare, 1963). *Rhodobacter microbium vanilliell* was grown anaerobically on the medium of Duchow & Douglas (1949) with acetate in place of ethanol. *Nocardia globerula* NCIB no. 6376 was maintained and grown as described by Crawford (1958).

**Radioactive compounds.** Sodium [14C]carbonate, sodium [1-14C]acetate and sodium [2-14C]acetate were obtained from The Radiochemical Centre, Amersham, Bucks.

**Procedure for growth experiments.** Graded amounts of sterile sodium acetate solution were pipetted into duplicate sterile 25 ml. glass-stoppered bottles, which were then completely filled with inoculated thiosulphate medium. The bottles were incubated in a light-cabinet at 26–29° and at intervals one of each pair was opened and a sample removed for the estimation of extinction at 600 mμ with a Unicam SP. 600 spectrophotometer and a blue filter. The sample was then poured back into the bottle, which was topped up with a few drops of fresh medium, stopped again and returned to the light-cabinet. The highly selective nature of the medium minimizes the risk of contamination. Furthermore, the growths in the duplicate bottles, the extinctions of which were measured at only two points during growth, were in good agreement with the bottles used for determinations of growth curves.

**Manometric experiments.** Since pronounced losses in photosynthetic activity were produced by washing the cells harvested at all stages of growth the cells were harvested by centrifuging for 5 min. at 15000 g and were then suspended in a medium (medium A) containing: K₂HPO₄, 0·1%; MgCl₂·6H₂O, 0·05%; CaCl₂, 0·01%. The medium was gassed with H₂ + CO₂ (95:5) for 10 min. and was adjusted to pH 7·0 with NaOH. The rate of photosynthesis with thiosulphate as hydrogen donor varied greatly from suspension to suspension, but with H₂ consistently high rates were obtained. Warburg flasks contained 2–4 mg. dry wt. of cells (determined spectrophotometrically from the extinction at 660 mμ and calculated from a calibration curve prepared with a cell suspension of known dry weight) in 1·8 ml. of medium A with 0·2 ml. of 4 N·H₂SO₄ in the side bulb. The acid served both to give a measure of the bound bicarbonate and to inactivate the cells at the end of the experiment. The flasks were gassed with H₂ + CO₂ (95:5) and were incubated at 30°. The experiment was started by switching on the lights of the photosynthesis bath at ‘zero time’.

**Isolation of poly-β-hydroxybutyrate.** The procedure of Stanier, Doudoroff, Kunisawa & Contopoulou (1959) was used. The ready isolation of white amorphous material soluble in hot chloroform was taken as presumptive evi-
dence for the presence of poly-β-hydroxybutyrate in *Rhodospirillum rubrum* and in *Rhodobacter sphaeroides*. The procedure of Law & Slepecky (1961) was used in an attempt to determine poly-β-hydroxybutyrate in the indigestible residues from *C. thiosphaerophilum*.

**Experiments with isotopes**

(1) **Isotope-incorporation experiments with resting cell suspensions.** Experiments were carried out in Warburg flasks as in the manometric experiments described above. The radioactive substrate was added to the cell suspension immediately before assembly of the manometers. At the end of some of the experiments in which radioactive acetate was used, the CO₂ in the reaction vessels was flushed out for 40 min. with CO₂-free air and was trapped in 3·5 ml. of CO₂-free 2N-NaOH and plated as BaCO₃. The radioactivity incorporated into the cells was determined as follows. The cells were first washed twice by suspending in 1% acetic acid (5 ml.) and centrifuging, followed by resuspension in water and centrifuging. The cells (3 mg. dry wt.) were then suspended in water and filtered through Oxoid membrane filters and allowed to dry in air. Radioactivity was then estimated directly with a thin-end-window tube (General Electric Co. Ltd., type 2 B 2) connected to a Panax D657 Scaler; the membrane filters were held flat with a cardboard ‘mask’ with a circular hole in the centre and the end-window tube was placed centrally on top of the mask. In some of the earlier experiments the membrane filters were trimmed and stuck to filter-paper disks on metal blocks, in a similar way to that described by Sadler & Stanier (1960), and radioactivity was then measured with a Packard model 200 A windowless gas-flow counter connected to a Baird-Atomic model 1035 scaler with 98% ‘minimum stench’ propane (Shellmead and B.P. Gases Ltd.) as the gas phase.

(2) **Isotope-incorporation experiments with growing cultures.** Larson’s basal thiosulphate medium with the addition of sodium acetate to a final concentration of 2·5 mM was used in completely filled glass-stoppered bottles of about 140 ml. capacity. The bottles were inoculated with a ‘healthy’ green culture of *C. thiosphaerophilum* grown in liquid medium. The bottles were incubated in a light-cabinet at 26–29° and when growth had started, as observed visually, a sample was removed for the determination of the extinction at 600 μm and radioactive acetate (50 μC) was added. The cultures were then incubated for 18–24 hr. and the extinction of the cell suspension was measured at the end of the growth period. The cells were harvested by centrifuging for 30 min. at 20000g and were washed once by suspending in 25 ml. of water and centrifuging for 30 min. at 20000g. The washed cells from two of the experiments (series II and III with [2-14C]acetate and [1-14C]acetate respectively) were fractionated by the procedure of Roberts, Cowie, Abelson, Bolton & Britten (1955). In the first experiment (series I) a slightly modified procedure was used, to examine the radioactive products of the ‘water-soluble’ fraction by two-dimensional chromatography under conditions similar to those used with *Rhodospirillum rubrum* (Hoare, 1963). The cells were extracted twice with 25 ml. of ethanol–water–acetic acid (750: 250: 1, by vol.). The extract was evaporated to dryness by rotary evaporation at 30° to reduce the volume to 1–2 ml., followed by freeze-drying. The freeze-dried residue was then suspended in 2 ml. of water, left in a deep-freeze overnight, thawed out and centrifuged. The clear pigment-free supernatant comprised the ‘water-soluble fraction’, which is presumed to be comparable in composition with the cold 5% trichloroacetic acid fraction obtained by the procedure of Roberts et al. (1955). The pigmented residue from the freeze-dried material was dissolved in 10 ml. of ethanol, giving a dark-green solution comparable with the ‘alcohol-soluble fraction’ of Roberts et al. (1955). The residual material was then further fractionated by the procedure of Roberts et al. (1955) by successive extractions with ethanol–water–ether (3:1:4, by vol.) and hot 5% (w/v) trichloroacetic acid. The final cell-residue fraction was hydrolysed for 8 hr. in 6N-HCl by boiling under a reflux condenser. The hydrolysate was evaporated to dryness on a boiling-water bath in a stream of air, the residue was dissolved in 10 ml. of water and samples were taken for total amino nitrogen estimation (Yemm & Cocking, 1954) and for radioactivity determinations. Radioactivity determinations on the various cell fractions were made by plating small samples at infinite thinness on stainless-steel planchets and counting with an end-window tube.

**Separation and isolation of amino acids**

Since the total amino acid nitrogen in the hydrolysates of the cell residues was comparatively low (160, 250 and 140 μg.atoms in series I, II and III respectively), amino acid separations were carried out on a microscale by paper-chromatographic and paper-electrophoretic techniques. Whatman 3MM papers were used throughout. A primary separation of amino acids was effected by applying samples as a line 20 cm. from the anode end of a 60 cm. strip of paper which was subjected to electrophoresis for 90 min. at 1800v in a pyridine–acetic acid buffer, pH 3·6 (Dixon, Kaufman & Neurath, 1958). This resolved the mixture into aspartic acid and glutamic acid, which were well separated and moved towards the anode, the ‘neutral amino acids’, remaining on or close to the line of application, and the basic amino acids, which moved towards the cathode. The basic amino acids were partly resolved: arginine was separated from histidine and lysine. Radioactive bands revealed by radioautography were eluted from the paper strips with water. The basic amino acids were then resolved by descending paper chromatography in butan-2-ol–aq. 3% NH₃ soln. (5:2, v/v) (Hausman, 1952). In this way histidine was separated from lysine. The neutral amino acids were then partially separated by descending paper chromatography for 32–36 hr. in propan-1-ol–water (4:1, v/v) (Kemble & McPherson, 1954). Diaminopimelic acid and phenylalanine were completely separated from other amino acids. Glycine and serine overlapped and in some cases alanine was not completely separated from threonine. These pairs of amino acids were then separated by paper electrophoresis in pyridine–formic acid buffer, pH 2·2 (Richmond & Hartley, 1959). The amino acids were applied as a line 20 cm. from the anode and the paper was subjected to electrophoresis for 90–100 min. at 1800v.

Other amino acids which overlapped (proline with tyrosine, valve with methionine and leucine with isoleucine) were resolved by descending chromatography for 36–40 hr. in 2-methylpropan-1-ol–ethyl methyl ketone–water (7:5:3, by vol.) (Kemble & McPherson, 1954). Each
isolated amino acid was finally purified by paper chromato-
graphy in the butan-1-ol–propionic acid solvent system of
Benson et al. (1950).

Preparation of amino acid solutions for
degradations

All eluates from paper chromatograms and paper-
electrophoresis strips were concentrated to dryness in vacuo
with P2O5 as desiccant. The final eluates containing resolved
amino acids were made up to a standard volume (2 or 4 ml)
with water to form a ‘master solution’. Samples were then
taken for amino acid estimation by the procedure of Yemm
& Cocking (1954), with corresponding standard amino acid
solutions, and for the different degradation procedures.

Radioactive degradation procedures

Combustion of organic compounds was carried out by
the procedure of Sakami (1955a) with the ‘combustion
reagents for general use’ of Van Slyke, Plazin & Weissiger
(1951). Decarboxylation of amino acids with chloramine-T
was carried out essentially as described by Kemble &
McPherson (1954). Warburg flasks contained the amino acid
solution (generally 10 μmoles) in the side bulb, the main
compartment contained 1-5 ml of fresh 10% (w/v) 
chloramine-T, 0.1 ml of 10% formaldehyde and 0.5 ml of
m-citrate buffer, pH 2-5. The reaction was carried out at
30°. The evolved CO2 was passed into 3-5 ml. of 2N NaOH
in a gas bubbler (20 min.), the alkali was made up to 10 ml.
with the addition of 1-2 ml of 0.48M Na2CO3 carrier, and
1 ml. samples were taken for plating as BaCO3 and assay
of radioactivity.

Degradation of alanine was carried out by a modification
of the procedure of Knight (1962), suggested by H.J. Somer-
ville (personal communication). The decarboxylation with
ninhidrin was carried out in the apparatus described by
Knight but the apparatus used for trapping acetaldehyde
contained 8 ml. of 5M CrO3 in 25% (v/v) H2SO4 in place of
the sodium bisulphite. In this way the acetaldehyde produced
as a result of the reaction between alanine and
ninhidrin was oxidized directly to acetic acid. When the
reaction was complete and the evolved CO2 had been
trapped, the contents of the bead tower containing acetic
acid and excess of chromic acid reagent were washed out
into a round-bottomed flask and the acetic acid was
recovered by steam-distillation in the apparatus of Eslenden
& Gibson (1954) and purified by ion-exchange chromatogra-
phy on Amberlite CG-50 (Seki, 1958).

Aspartic acid was degraded by a modification of the
procedure of Knight (1962), washed cell suspensions of
Nocardia globreula being used for the decarboxylation of
aspartic acid (Krebs & Bellamy, 1960). The alanine
recovered from the reaction products as described by
Knight (1962) was then further degraded as described
above.

Several procedures were used for the partial and complete
degradation of glutamic acid. For the complete and
unequivocal degradation of glutamic acid the procedure of
Moshbach, Phares & Carson (1951) was used with minor
modifications to adapt it to a semi-microscale. The reaction
was carried out in a pear-shaped vessel of 50 ml. capacity.
The vessel contained 2-3 ml. of glutamate soln. (100 μmoles
of glutamate) adjusted to pH 6.5-7.0 with NaOH
(Koeppe & Hill, 1955). The solution was gassed for 10 min.
with a gentle stream of O2-free N2. A solution (1-2 ml) containing 100 μmoles of chloramine-T (freshly prepared)
was then added drop by drop. Gassing with O2-free N2
was continued throughout the reaction. Five minutes after
the addition of the chloramine-T, the temperature was raised
to 50° for 40 min. Potassium hydroxide (10%, w/v), 1 ml.,
freshly distilled diethyle glycol, 3 ml., and 90% (w/v)
hydrazine hydrate, 0-5 ml., were then added, and the mixture
was heated under a reflux water condenser for 75 min. The
condenser was removed and the solution heated to remove
water. An air condenser was then attached and the tempera-
ture of the reaction mixture was slowly raised to 160° and
maintained between 160° and 170° for 75 min. The reaction
mixture on cooling was transferred to a Kutscher–Steudel
exactor and extracted for 2 hr. with peroxide-free ether
(100 ml.). The ether phase was discarded and the aqueous
phase was acidified and steam-distilled from the lactic acid
apparatus of Eslenden & Gibson (1954). The distillate (200 ml.)
was titrated with standard alkali, concentrated to dryness
and the butyric acid recovered by chromatography on
Amberlite CG-50 (Seki, 1958). Total acid recovered in the
steam-distillate was about 100 μmoles but consisted of a
mixture of acetate and butyrate. The recovery of butyrate
after ion-exchange chromatography was 30-50 μmoles. The
butyrate was then degraded stepwise by standard pro-
cedures (Sakami, 1955b).

The terminal carboxyl group (C-5) was removed by a
direct Schmidt reaction on glutamic acid. This procedure
was used by Cutinelli et al. (1951). The degradation of
glutamate to succinate, followed by the decarboxylation
of succinate to propionate, was carried out by the microscale

Phenylalanine was partially degraded to benzoic acid by
the procedure of Gilvarg & Bloch (1962) with minor modifi-
cations. The reaction was carried out on a semi-microscale.
A 150 ml. round-bottomed flask with two necks contained
phenylalanine (1 m-mole) dissolved in 25% (w/v) H2SO4
(15 ml.). The vessel was immersed in an ice bath and finely
pulverized K2Cr2O7 (4 g.) was added. A reflux water
condenser was attached and a gassing inlet tube was
inserted in the other neck. A tube of CaO-NaOH was
attached to the gassing tube and the top of the condenser
was connected to a trap containing 1% (w/v) of 2,4-dinitro-
phenylhydrazine in 2N HCl (5 ml.). The trap retained small
amounts of benzoic acid that were formed during the
oxidation of the phenylalanine. The apparatus was then
flushed with CO2-free N2 for 30 min. A bubbler containing
CO2-free 2N NaOH (5 ml.) was then attached to the
assembly and the reaction vessel was immersed in a water
bath. Gas (CO2-free N2) was passed through the reaction
vessel at a slow and steady rate (2-3 bubbles/sec.) through-
out the reaction. The temperature of the water bath was
raised slowly to boiling point and the vessel was shaken
periodically. Oxidation generally started before the water
in the bath reached boiling point. After 2 hr. at 100° the
reaction vessel was removed from the bath and CO2-free
N2 was bubbled through for a further 50 min. Yields of CO2
were invariably greater than theoretical and although
samples were plated and counted the results are considered
to be only qualitatively valid in comparisons of incorpora-
tion patterns from [1-14C] and [2-14C-acetate.

Benzoic acid was recovered from the reaction mixture by
two extractions with 50 ml. of light petroleum (b.p.
presence of taken for from a water sulphatophilum 550 those 30 40°; Schmidt crystallized from hot water. Samples of benzoic acid were taken for total combustion and for decarboxylation by the Schmidt reaction. The Schmidt reaction was carried out on 600 μmoles of benzoic acid under conditions identical with those used for the decarboxylation of fatty acids and yields of CO₂ were 95–100%. Aniline was recovered from the reaction products by ether extraction from alkaline solution. Ether was removed by evaporation in a stream of air and the oily drop of aniline was acetylated by the addition of acetic anhydride (0.2 ml.) and a drop of conc. H₂SO₄. The mixture was left overnight at 37°. About 3 ml. of water and a small amount of decoloring charcoal were added and the mixture was boiled and filtered hot. Acetanilide crystallized from the filtrate and was dried and subjected to combustion.

RESULTS

Growth of Chlorobium thiosulphatophilum in the presence of acetate. Preliminary experiments showed that the addition of 5 mM-sodium acetate to the basal thiosulphate medium increased the cell yield of *C. thiosulphatophilum* in a similar way to that found with *C. limicola* by Sadler & Stanier (1960). The increase in cell yield with *C. thiosulphatophilum* strain 8346 was less than that with the two cultures of strain 8327 (Table 1). Growth curves were similar for all strains and showed that acetate (final concn. 1–5 mM) increased the rate of growth as well as the final cell yield (Fig. 1). Acetate concentrations greater than 10 mM reduced first the rate of growth and finally the cell yield (below yields of cultures without acetate).

Influence of acetate on photosynthesis by resting cell suspensions. In manometric experiments with hydrogen gas as a source of reducing power, the rate of gas uptake was not affected by the presence of acetate (5–10 mM) whether the cells were grown on thiosulphate or thiosulphate with acetate. The manometric technique may not be sufficiently sensitive to show any influence of acetate on carbon dioxide uptake. In experiments with Na₂¹⁴CO₃ there was a progressive fixation of radioactivity by resting cell suspensions in the presence and absence of acetate (Table 2). Resting cell suspensions grown with or without acetate also assimilated sodium [¹⁴C]acetate (Table 3). The presence of light, a source of reducing power and of bicarbonate were essential. Radioactivity was not

### Table 1. Effect of acetate on growth of Chlorobium thiosulphatophilum

<table>
<thead>
<tr>
<th>Conc. of acetate (mM)</th>
<th>Final cell yield (E at 600 μμ)</th>
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<tbody>
<tr>
<td></td>
<td>Strain 8327 (OC)</td>
</tr>
<tr>
<td>0</td>
<td>638</td>
</tr>
<tr>
<td>1</td>
<td>780</td>
</tr>
<tr>
<td>2</td>
<td>960</td>
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<tr>
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<tr>
<td>15</td>
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</tr>
<tr>
<td>0</td>
<td>750</td>
</tr>
<tr>
<td>2</td>
<td>120</td>
</tr>
<tr>
<td>5</td>
<td>190</td>
</tr>
<tr>
<td>10</td>
<td>240</td>
</tr>
</tbody>
</table>

![Fig. 1. Effect of acetate concentration on the growth of *C. thiosulphatophilum* 8327 (OC). Basal thiosulphate medium (25 ml.), with acetate added to the concentrations indicated, was inoculated and illuminated at 26–29°C; growth was followed by measuring extinction at 600 μμ. ○, No acetate present; ▲, 5 mM-acetate; ■, 10 mM-acetate.](image)

### Table 2. Effect of acetate on the assimilation of sodium [¹⁴C]carbonate by resting cell suspensions of Chlorobium thiosulphatophilum

Warburg flasks contained 1 ml. of cell suspension (3 mg. dry wt./ml.), 0.8 ml. of medium A and 10 μl. of Na₂¹⁴CO₃ (10 μCi, 10 μmoles). Gas phase, H₂, Temp., 30°C. When acetate was present it was added in 10 μl. of 0.5M-sodium acetate. Reaction was initiated by illuminating the system. Organisms were filtered, washed and counted on Oxoid membrane filters as described in the Materials and Methods section.

<table>
<thead>
<tr>
<th>Incubation time (min.)</th>
<th>10⁻³ × Na₂¹⁴CO₃ incorporated (counts/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetate absent</td>
<td>Acetate present</td>
</tr>
<tr>
<td>0</td>
<td>Not determined</td>
</tr>
<tr>
<td>5</td>
<td>1.34</td>
</tr>
<tr>
<td>10</td>
<td>5.20</td>
</tr>
<tr>
<td>20</td>
<td>7.25</td>
</tr>
<tr>
<td>40</td>
<td>24.1</td>
</tr>
<tr>
<td>60</td>
<td>31.2</td>
</tr>
<tr>
<td>60 (dark)</td>
<td>0.06</td>
</tr>
</tbody>
</table>
detected in the carbon dioxide at the end of manometric experiments with either [1-\(^{14}\)C]- or [2-\(^{14}\)C]-acetate.

**Products of the photoassimilation of acetate by resting cell suspensions.** Stanier et al. (1959) found that washed suspensions of *Rhodospirillum rubrum* assimilated acetate in the absence of carbon dioxide to form poly-\(\beta\)-hydroxybutyrate. Since carbon dioxide was essential for the assimilation of acetate by resting cell suspensions of *C. thiocapsa* it seemed possible that the latter could not form poly-\(\beta\)-hydroxybutyrate. Accordingly cells of *C. thiocapsa* grown on thiosulfate were found subsequently with growing cultures (Table 4).

Acetate assimilation by growing cultures and its distribution in different cell fractions. Cultures were grown on the basal medium with acetate added to a final concentration of 2.5 mM. After several transfers on this medium, completely filled glass-stoppered bottles were inoculated and incubated in an illuminated cabinet at 25–30°. When growth had started, sodium [1-\(^{14}\)C]acetate or sodium [2-\(^{14}\)C]-acetate was added, and, after a period of 18–24 hr., when the culture gave an extinction of approx. 1.0, the culture was removed from the light-cabinet. A sample of the culture was assayed to determine the total radioactivity in the system. The cells were then removed by centrifuging for 30 min. at 20,000 \(g\) and a sample of the cell-free supernatant was assayed for radioactivity. The total counts in the cells were then determined by difference. The data and results for three separate experiments are given in Table 4. The results show that 80–90% of the radioactivity in the system was incorporated into the cells under the above growth conditions. An attempt was made in one of the experiments (series III, with sodium [1-\(^{14}\)C]acetate) to determine the total radioactivity in the bound bicarbonate (carbon dioxide) in the system as this would give an index of the extent of acetate oxidation by growing cultures. A portion (100 ml) of the freshly harvested cell-free culture medium was acidified in a closed system and the gas passed into 3.5 ml of 2\(\%\)-sodium hydroxide; the alkali was diluted to 10 ml and samples were taken for

<table>
<thead>
<tr>
<th>Incubation time (min.)</th>
<th>Grown with acetate</th>
<th>Grown without acetate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Gas phase</td>
<td>Gas phase</td>
</tr>
<tr>
<td></td>
<td>(H_2 + CO_2)</td>
<td>(H_2)</td>
</tr>
<tr>
<td>0</td>
<td>45</td>
<td>Not determined</td>
</tr>
<tr>
<td>5</td>
<td>413</td>
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<tr>
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<td>1063</td>
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<td>60</td>
<td>11527</td>
<td>25</td>
</tr>
<tr>
<td>(dark control)</td>
<td>137</td>
<td>Not determined</td>
</tr>
</tbody>
</table>

Not determined was used when the radioactivity was below the limit of detection.
radioactivity assay by plating as barium carbonate (total radioactivity $1.7 \times 10^4$ counts/min. corrected for self-absorption). This accounts for less than 0.1% of the radioactivity in the system and therefore confirms that C. thiosulphatophilum has a very limited capacity to oxidize acetate.

The distribution of radioactivity in different cell fractions was investigated on the freshly harvested cells. These were washed once by suspending in 25 ml. of water and centrifuging for 30 min. at 20,000g; this produced a clear pale-yellow supernatant. The washed cells were then fractionated by the procedure of Roberts et al. (1955) and the different cell fractions were assayed for radioactivity (Table 4). The total radioactivity in the cells estimated from the sum of that found in the different cell fractions was in all cases less than that estimated by difference between the activity of the whole culture and the culture supernatant. The reason for this discrepancy is not clear but may be due in part to a loss of radioactivity in the cell washings or there may have been some loss from the 'cell-residue' fraction during acid hydrolysis, since radioactivity measurements were made on samples of the hydrolysed material: the cell-wall material might be expected to contain acetyl groups (see review by Perkins, 1963), which would be lost during the acid hydrolysis. The distribution of radioactivity in the cell fractions is similar to that found by Sadler & Stanier (1960) with Chlorobium limicola. The 'cell residue', containing mainly proteins and cell-wall material, contained the highest proportion of the activity from both [1-14C]- and [2-14C]-acetate.

**Incorporation of acetate carbon atoms into the amino acids of the cell proteins.** The 'cell-residue' fractions were hydrolysed as described in the Materials and Methods section. Suitable samples of the hydrolysed material were then taken for assay of radioactivity, total amino acid content and for two-dimensional chromatography followed by radioautography. In all three series of experiments it was clear that acetate was incorporated into most of the amino acids. Individual amino acids were isolated as described in the Materials and Methods section. Samples of each amino acid were then taken for amino acid estimation and for total combustion to determine the approximate specific activities of the isolated amino acids (Table 5).

**Decarboxylation of isolated amino acids by reaction with chloramine-T.** To determine whether acetate is incorporated into the amino acids in a specific manner it would be undesirable to degrade each of the isolated radioactive amino acids completely and unequivocally. Since such degradations have not yet been devised for all the common amino acids, this approach was limited to a few amino acids for which unequivocal degradations had been established. A good indication of the incorporation of acetate carbon atoms into all the amino acids could be obtained very simply by decarboxylation with chloramine-T (Kemble & McPherson, 1954). Under these conditions it was found that, in addition to the neutral amino acids studied by Kemble & McPherson (1954), the basic amino acids arginine,

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**Table 4. Acetate assimilation by growing cultures and its intracellular distribution**

Stoppered bottles containing Larsen's basal medium (about 130 ml.) with 2.5 M-sodium acetate were inoculated and incubated in the light at 25°. Radioactive acetate (50 μc) was added when growth was established. Cell density (from E at 600 μp) was measured when radioactive acetate was added (initial cell density) and after 24 hr. (final cell density) when cells were harvested and fractionated. The 'total' figures represent the sum of counts in all cell fractions and do not correspond to the amount estimated by difference between the whole culture and the culture supernatant. For series I the extraction procedure was slightly different (see text). Radioactivity measurements are expressed as counts/min.

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>(1) Cold 5% trichloroacetic acid-soluble fraction</td>
<td>4-2 (5-6%)</td>
<td>2-7 (4-2%)</td>
<td>3-8 (5-1%)</td>
</tr>
<tr>
<td>(2) Ethanol-soluble fraction</td>
<td>15-0 (20-0%)</td>
<td>11-7 (18-2%)</td>
<td>25-7 (34-6%)</td>
</tr>
<tr>
<td>(3) Ethanol-ether-soluble fraction</td>
<td>2-9 (3-9%)</td>
<td>2-2 (3-4%)</td>
<td>4-3 (5-8%)</td>
</tr>
<tr>
<td>(4) Hot-5% trichloroacetic acid-soluble fraction</td>
<td>15-1 (20-0%)</td>
<td>8-6 (13-4%)</td>
<td>13-6 (18-3%)</td>
</tr>
<tr>
<td>(5) Cell residue ('protein')</td>
<td>37-9 (50-6%)</td>
<td>38-8 (61-0%)</td>
<td>26-9 (36-2%)</td>
</tr>
<tr>
<td>$10^8 \times$ Total</td>
<td>75-1 (100%)</td>
<td>64-0 (100%)</td>
<td>74-3 (100%)</td>
</tr>
</tbody>
</table>
histidine and lysine were quantitatively decarboxylated, glutamic acid was decarboxylated at the α-carboxyl (C-1) position, and both aspartic acid and diaminopimelic acid were decarboxylated at both the α-carboxyl and the terminal carboxyl position. The results are given in Table 6.

Degradations of alanine, aspartic acid, glutamic acid and phenylalanine. Alanine, aspartic acid and glutamic acid formed from both [1-14C]acetate and [2-14C]acetate were completely and unequivocally degraded by standard procedures. The results are given in Tables 7–10.

Table 5. Specific activities of isolated amino acids from 'cell-residue' hydrolysates of cells grown in the presence of sodium [1-14C]acetate and sodium [2-14C]acetate

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Amino N (μmoles)</th>
<th>10⁻³ × Total activity (counts/min.)</th>
<th>10⁻³ × Sp. activity (counts/μmole)</th>
<th>Amino N (μmoles)</th>
<th>10⁻³ × Total activity (counts/min.)</th>
<th>10⁻³ × Sp. activity (counts/μmole)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspartic acid</td>
<td>18-5</td>
<td>630</td>
<td>34-2</td>
<td>12-6</td>
<td>119-5</td>
<td>17-4</td>
</tr>
<tr>
<td>Glutamic acid</td>
<td>19-2</td>
<td>787</td>
<td>40-8</td>
<td>8-4</td>
<td>14-2</td>
<td>17-0</td>
</tr>
<tr>
<td>Diaminopimelic acid</td>
<td>1-2</td>
<td>64</td>
<td>53-3</td>
<td>0-9</td>
<td>36-5</td>
<td>40-0</td>
</tr>
<tr>
<td>Glycine</td>
<td>5-3</td>
<td>249</td>
<td>47-1</td>
<td>5-5</td>
<td>7-6</td>
<td>1-4</td>
</tr>
<tr>
<td>Serine</td>
<td>4-1</td>
<td>229</td>
<td>56-2</td>
<td>6-3</td>
<td>206</td>
<td>32-6</td>
</tr>
<tr>
<td>Alanine</td>
<td>11-3</td>
<td>447</td>
<td>39-6</td>
<td>21-6</td>
<td>285</td>
<td>13-2</td>
</tr>
<tr>
<td>Threonine</td>
<td>3-5</td>
<td>122</td>
<td>34-8</td>
<td>3-9</td>
<td>95</td>
<td>24-3</td>
</tr>
<tr>
<td>Proline</td>
<td>5-5</td>
<td>193</td>
<td>35-1</td>
<td>2-0</td>
<td>87</td>
<td>45-5</td>
</tr>
<tr>
<td>Valine</td>
<td>5-1</td>
<td>483</td>
<td>96-0</td>
<td>5-2</td>
<td>358</td>
<td>69-0</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>1-3</td>
<td>248</td>
<td>185-0</td>
<td>1-9</td>
<td>200</td>
<td>104-0</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>2-6</td>
<td>247</td>
<td>93-7</td>
<td>2-3</td>
<td>346</td>
<td>150-0</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>3-4</td>
<td>352</td>
<td>105-0</td>
<td>6-4</td>
<td>380</td>
<td>59-0</td>
</tr>
<tr>
<td>Leucine</td>
<td>4-9</td>
<td>447</td>
<td>91-5</td>
<td>4-3</td>
<td>497</td>
<td>115-0</td>
</tr>
<tr>
<td>Arginine</td>
<td>4-0</td>
<td>167</td>
<td>42-0</td>
<td>2-2</td>
<td>60-4</td>
<td>29-7</td>
</tr>
<tr>
<td>Histidine</td>
<td>0-8</td>
<td>130</td>
<td>174-0</td>
<td>1-6</td>
<td>150</td>
<td>110-0</td>
</tr>
<tr>
<td>Lysine</td>
<td>5-3</td>
<td>563</td>
<td>106-0</td>
<td>4-4</td>
<td>285</td>
<td>64-7</td>
</tr>
</tbody>
</table>

Table 6. Decarboxylation of amino acids formed from sodium [1-14C]acetate and sodium [2-14C]acetate

Samples of radioactive amino acids with the addition of carrier (10 μmoles) were decarboxylated in Warburg flasks according to the procedure of Kemble & McPherson (1954). All counts are corrected for self-absorption. Recoveries of CO₂ in the chloramine-T reactions were 98–103% of theoretical.
### Table 7. Degradation of alanine from cells grown in the presence of sodium [1-\(^{14}\)C]acetate and sodium [2-\(^{14}\)C]acetate

Samples of a solution of alanine derived from [1-\(^{14}\)C]- and [2-C]acetate diluted with added carrier L-alanine were degraded. Additional carrier acetate was added for the Schmidt-reaction step. All counts are as BaCO\textsubscript{3} and are corrected for self-absorption. Reaction steps: 1, ninhydrin reaction; 2, Schmidt reaction.

<table>
<thead>
<tr>
<th>Series</th>
<th>Source of (^{14})C</th>
<th>Reaction step</th>
<th>Reactant ((\mu)moles)</th>
<th>Carrier added ((\mu)moles)</th>
<th>Total counts</th>
<th>Sp. activity (counts/min./(\mu)mole)</th>
<th>Product ((\mu)moles)</th>
<th>Total counts</th>
<th>Corrected* sp. activity (counts/min./(\mu)mole)</th>
<th>Origin of carbon atoms</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>[1-(^{14})C]Acetate</td>
<td>1</td>
<td>Alanine (2-8)</td>
<td>100</td>
<td>111650</td>
<td>1084</td>
<td>CO\textsubscript{2} (100)</td>
<td>600</td>
<td>6-0</td>
<td>C-1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>Acetate (61)</td>
<td>700</td>
<td>64500</td>
<td>84-4</td>
<td>Acetate (67) &amp; CO\textsubscript{2} (537) &amp; CH\textsubscript{3}\textsubscript{2}NH\textsubscript{3} (700)</td>
<td>71700</td>
<td>1070 &amp; 38600 &amp; 925 &amp; 5330 &amp; 95 &amp; C-2 &amp; C-3</td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>[2-(^{14})C]Acetate</td>
<td>1</td>
<td>Alanine (4-0)</td>
<td>200</td>
<td>71250</td>
<td>349</td>
<td>CO\textsubscript{2} (177)</td>
<td>225</td>
<td>1-6</td>
<td>C-1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>Acetate (117)</td>
<td>800</td>
<td>38520</td>
<td>42-0</td>
<td>Acetate (130) &amp; CO\textsubscript{2} (703) &amp; CH\textsubscript{3}\textsubscript{2}NH\textsubscript{3} (762)</td>
<td>42800</td>
<td>329 &amp; 650 &amp; 7 &amp; 34150</td>
<td>349 &amp; C-2 &amp; C-3</td>
</tr>
</tbody>
</table>

* Corrected for additions of carrier.

### Table 8. Degradation of aspartic acid from cells grown in the presence of sodium [1-\(^{14}\)C]acetate and sodium [2-\(^{14}\)C]acetate

Samples of a solution of aspartic acid derived from [1-C\(^{14}\)]- and [2-\(^{14}\)C]-acetate diluted with added carrier L-aspartic acid were degraded. Additional carrier alanine was added for the ninhydrin reaction and further carrier acetate was added for the Schmidt reaction. Reaction steps: 1, decarboxylation with a washed-cell suspension of Nocardia globula; 2, ninhydrin reaction; 3, Schmidt reaction.

<table>
<thead>
<tr>
<th>Series</th>
<th>Source of (^{14})C</th>
<th>Reaction step</th>
<th>Reactant ((\mu)moles)</th>
<th>Carrier added ((\mu)moles)</th>
<th>Total counts</th>
<th>Sp. activity (counts/min./(\mu)mole)</th>
<th>Product ((\mu)moles)</th>
<th>Total counts</th>
<th>Corrected* sp. activity (counts/min./(\mu)mole)</th>
<th>Origin of carbon atoms</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>[1-(^{14})C]Acetate</td>
<td>1</td>
<td>Aspartic acid (40-7)</td>
<td>—</td>
<td>171200</td>
<td>4200</td>
<td>CO\textsubscript{2} (33-5) &amp; Alaniine (40) &amp; CO\textsubscript{2} (160) &amp; CH\textsubscript{3}\textsubscript{2}NH\textsubscript{3} (523)</td>
<td>150</td>
<td>4-5 &amp; 139400 &amp; 3485</td>
<td>C-4 &amp; C-1 &amp; C-2 &amp; C-3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>Alanine (35-6)</td>
<td>160</td>
<td>124066</td>
<td>634</td>
<td>CO\textsubscript{2} (41) &amp; CH\textsubscript{3}\textsubscript{2}NH\textsubscript{3} (523)</td>
<td>90540</td>
<td>3434 &amp; 61530</td>
<td>2960 &amp; 840 &amp; 654 &amp; C-2 &amp; C-3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3</td>
<td>Acetate (130-5)</td>
<td>800</td>
<td>74970</td>
<td>80-6</td>
<td>CO\textsubscript{2} (810) &amp; CH\textsubscript{3}\textsubscript{2}NH\textsubscript{3} (523)</td>
<td>8840</td>
<td>565 &amp; 34150</td>
<td>349 &amp; C-2 &amp; C-3</td>
</tr>
<tr>
<td>II</td>
<td>[2-(^{14})C]Acetate</td>
<td>1</td>
<td>Aspartic acid (22-4)</td>
<td>—</td>
<td>45500</td>
<td>2030</td>
<td>CO\textsubscript{2} (22) &amp; Alaniine (20) &amp; CO\textsubscript{2} (400) &amp; Acetate (167)</td>
<td>120</td>
<td>5-5 &amp; 34400</td>
<td>1720 &amp; 20170</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>Alanine (17-9)</td>
<td>200</td>
<td>29788</td>
<td>138</td>
<td>CO\textsubscript{2} (140) &amp; CO\textsubscript{2} (805) &amp; CH\textsubscript{3}\textsubscript{2}NH\textsubscript{3} (766)</td>
<td>34400</td>
<td>1720 &amp; 4260 &amp; 408 &amp; 10200</td>
<td>1024 &amp; C-2 &amp; C-3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3</td>
<td>Acetate (150)</td>
<td>800</td>
<td>18153</td>
<td>19-1</td>
<td>CO\textsubscript{2} (805) &amp; CH\textsubscript{3}\textsubscript{2}NH\textsubscript{3} (766)</td>
<td>20170</td>
<td>1452 &amp; 4260</td>
<td>408 &amp; 10200</td>
</tr>
</tbody>
</table>

* Corrected for additions of carrier.
Table 9. Degradation of glutamic acid from cells grown in the presence of sodium [2-14C]acetate

Samples of a solution of glutamic acid derived from [2-14C]acetate diluted with added carrier L-glutamic acid were degraded by a modification of the procedure of Mosbach et al. (1951). Additional carrier was added for the Schmidt reactions (steps 2-4).

<table>
<thead>
<tr>
<th>Reaction step</th>
<th>Reactant (µmoles)</th>
<th>Carrier added (µmoles)</th>
<th>Total counts</th>
<th>Sp. activity (counts/min./µmole)</th>
<th>Product (µmoles)</th>
<th>Total counts</th>
<th>Corrected sp. activity (counts/min./µmole)</th>
<th>Origin of carbon atoms</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Glutamate (21-7)</td>
<td>94</td>
<td>13900</td>
<td>120</td>
<td>Butyrate (42)</td>
<td>4800</td>
<td>562</td>
<td>C-5</td>
</tr>
<tr>
<td>2</td>
<td>Butyrate (40)</td>
<td>950</td>
<td>4560</td>
<td>4-6</td>
<td>CO2 (560)</td>
<td>Nil</td>
<td>Nil</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Propionate (433)</td>
<td>500</td>
<td>2133</td>
<td>2-28</td>
<td>Propionate (481)</td>
<td>2370</td>
<td>590</td>
<td>C-4</td>
</tr>
<tr>
<td>4</td>
<td>Acetate (540)</td>
<td>500</td>
<td>553</td>
<td>0-53</td>
<td>CO2 (600)</td>
<td>600</td>
<td>260</td>
<td>C-3</td>
</tr>
<tr>
<td>5</td>
<td>Glutamate (21-7)</td>
<td>—</td>
<td>13900</td>
<td>642</td>
<td>CH3-NH2 (960)</td>
<td>Nil</td>
<td>Nil</td>
<td>C-2</td>
</tr>
</tbody>
</table>

Table 10. Degradation of glutamic acid derived from sodium [1-14C]acetate

Glutamic acid was degraded by the procedure of Mosbach et al. (1951): (A) series I sample; (B) series III sample; (C) series I sample degraded via succinate (Hoare, 1963); (D) direct Schmidt reaction on series I sample. Specific activities of glutamate samples before addition of carrier were 5700 counts/min./µmole for series I and 4625 counts/min./µmole for series III.

<table>
<thead>
<tr>
<th>Reactant (µmoles)</th>
<th>Carrier added (µmoles)</th>
<th>Total counts</th>
<th>Sp. activity (counts/min./µmole)</th>
<th>Product (µmoles)</th>
<th>Total counts</th>
<th>Corrected* sp. activity (counts/min./µmole)</th>
<th>Origin of carbon atoms</th>
</tr>
</thead>
<tbody>
<tr>
<td>(A)</td>
<td>Glutamate (24)</td>
<td>80</td>
<td>138500</td>
<td>1330</td>
<td>Butyrate (42)</td>
<td>52400</td>
<td>5420</td>
</tr>
<tr>
<td></td>
<td>Butyrate (40)</td>
<td>900</td>
<td>49780</td>
<td>50-6</td>
<td>CO2 (500)</td>
<td>2220</td>
<td>450</td>
</tr>
<tr>
<td></td>
<td>Propionate (253)</td>
<td>750</td>
<td>8280</td>
<td>8-28</td>
<td>Propionate (270)</td>
<td>9220</td>
<td>3480</td>
</tr>
<tr>
<td></td>
<td>Acetate (440)</td>
<td>400</td>
<td>1980</td>
<td>2-36</td>
<td>Acetate (489)</td>
<td>2200</td>
<td>1830</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>CO2 (750)</td>
<td>1320</td>
<td>1310</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>CH3-NH2 (810)</td>
<td>205</td>
<td>194</td>
</tr>
<tr>
<td>(B)</td>
<td>Glutamate (34)</td>
<td>165</td>
<td>150110</td>
<td>755</td>
<td>Butyrate (74)</td>
<td>64200</td>
<td>4340</td>
</tr>
<tr>
<td></td>
<td>Butyrate (70)</td>
<td>800</td>
<td>60990</td>
<td>70-1</td>
<td>CO2 (900)</td>
<td>3014</td>
<td>243</td>
</tr>
<tr>
<td></td>
<td>Propionate (492)</td>
<td>300</td>
<td>24570</td>
<td>31-2</td>
<td>Propionate (547)</td>
<td>27300</td>
<td>3090</td>
</tr>
<tr>
<td></td>
<td>Acetate (100)</td>
<td>300</td>
<td>Not determined</td>
<td></td>
<td>CO2 (800)</td>
<td>12965</td>
<td>1901</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Acetate (100)</td>
<td>Not determined</td>
<td>Not determined</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>CH3-NH2 (378)</td>
<td>67</td>
<td>83 (Nil)</td>
</tr>
<tr>
<td>(C)</td>
<td>Glutamate (24)</td>
<td>—</td>
<td>138500</td>
<td>5700</td>
<td>CO2 (24)</td>
<td>300</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>Succinate (16)</td>
<td>—</td>
<td>91000</td>
<td>5670</td>
<td>CO2 (16)</td>
<td>8880</td>
<td>554</td>
</tr>
<tr>
<td></td>
<td>Propionate (10-5)</td>
<td>900</td>
<td>20520</td>
<td>22-6</td>
<td>Propionate (11-7)</td>
<td>22800</td>
<td>1949</td>
</tr>
<tr>
<td></td>
<td>Ethylamine (750)</td>
<td></td>
<td></td>
<td></td>
<td>CO2 (900)</td>
<td>2850</td>
<td>285</td>
</tr>
<tr>
<td>(D)</td>
<td>Glutamate (12)</td>
<td>900</td>
<td>69250</td>
<td>76</td>
<td>CO2 (857)</td>
<td>15730</td>
<td>1320</td>
</tr>
</tbody>
</table>

* Corrected for all additions of carrier.
Phenylalanine was partially degraded to determine whether the carbon atoms of acetate were incorporated into the aromatic ring system; the results are given in Table 11.

DISCUSSION

Although *C. thiosulphatophilum* is a strict autotroph, the cell yield can be increased by the addition of low concentrations of acetate to the culture medium and the growth response is similar to that found with the closely related *C. limicola* by Sadler & Stanier (1960). Acetate is assimilated by resting cell suspensions of *C. thiosulphatophilum* provided that bicarbonate and a source of reducing power (hydrogen gas) are present; this striking difference from *Rhodospirillum rubrum*, which assimilates acetate in the absence of bicarbonate to form poly-\(\beta\)-hydroxybutyrate, appears to be due to the inability of *C. thiosulphatophilum* to form this polymer. This may be a unique feature of the autotrophic green sulphur bacteria, since poly-\(\beta\)-hydroxybutyrate has been found in representative species of the other major groups of photosynthetic bacteria; namely *Chromatium okenii* (Schlegel, 1962; Schlegel & Gottschalk, 1962) and in *Rhodomicrobium vannielii*.

As with *C. limicola* it has been found that acetate is incorporated into all the major cell fractions of *C. thiosulphatophilum* (Table 4).

When *C. thiosulphatophilum* is grown in the presence of radioactive acetate, the acetate is incorporated into most of the amino acids of the cell proteins (Table 5). No clear indication of metabolic interrelationships between the different amino acids can be deduced from the data in Table 6. This is probably attributable to the fact that the carbon-atom skeletons of the amino acids are built up mainly from bicarbonate carbon atoms by autotrophic assimilation and acetate carbon atoms simply supplement this. Thus radioactivity incorporated into the isolated amino acids may represent the sum of two independent processes: autotrophic assimilation, presumably by the route established in green algae since all the enzymes of this mechanism have been shown to be present in *C. thiosulphatophilum* (Smillie, Rigopoulos & Kelly, 1962), and the assimilation of acetate by mechanisms not yet elucidated. The exceedingly low specific activity of glycine from cells grown in the presence of sodium [2-\(^{14}\)C]acetate suggests that there is very little incorporation of the methyl group (C-2) of acetate into glycine. The comparatively high specific activities of the aromatic amino acids suggests that acetate carbon atoms are incorporated into the aromatic ring and this is confirmed by the results of the partial degradation of phenylalanine (Table 11). The results of the

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**Table 11. Degradation of phenylalanine from cells grown in the presence of sodium [1-\(^{14}\)C]acetate and sodium [2-\(^{14}\)C]acetate.**

<table>
<thead>
<tr>
<th>Source of (^{14})C Acetate</th>
<th>Reaction step</th>
<th>Reactant (amoles)</th>
<th>Product (amoles)</th>
<th>Total counts</th>
<th>Total counts</th>
<th>Total counts</th>
<th>Total counts</th>
</tr>
</thead>
<tbody>
<tr>
<td>[1-(^{14})C] Acetate</td>
<td>1</td>
<td>Phenylalanine (1-88)</td>
<td>CO(_2) (690)</td>
<td>83,000</td>
<td>16,540</td>
<td>101,540</td>
<td>147,000</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>Phenylalanine (1-15)</td>
<td>CO(_2) (560)</td>
<td>86,500</td>
<td>12,540</td>
<td>99,040</td>
<td>112,000</td>
</tr>
<tr>
<td>[2-(^{14})C] Acetate</td>
<td>1</td>
<td>Phenylalanine (1-80)</td>
<td>CO(_2) (690)</td>
<td>64,000</td>
<td>8,540</td>
<td>72,540</td>
<td>88,540</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>Phenylalanine (1-80)</td>
<td>CO(_2) (560)</td>
<td>80,000</td>
<td>12,540</td>
<td>92,540</td>
<td>105,000</td>
</tr>
</tbody>
</table>

* Corrected for additions of carrier.
Table 12. Summary of isotope distributions in amino acids

<table>
<thead>
<tr>
<th>Amino Acid</th>
<th>Isotope Distribution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alanine</td>
<td>CH₃CH(NH₂)CO₂H m c b</td>
</tr>
<tr>
<td>Aspartic</td>
<td>HO₂CCH(NH₂)CH₂CO₂H b c m b</td>
</tr>
<tr>
<td>Glutamic</td>
<td>HO₂CCH(NH₂)CH₂CH₂CO₂H b (e) m(e) m(c) (e)</td>
</tr>
<tr>
<td>Glycine</td>
<td>HO₂CCH₂(NH₂) b c</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>CH₃CH(NH₂)CO₂H m and c m (e) b</td>
</tr>
</tbody>
</table>

Note: m, Carbon atoms derived from [2,14C]acetate; c, carbon atoms derived from [1,14C]acetate; b, carbon atoms presumed to be derived from bicarbonate since insignificant amounts of [1,14C]- or [2,14C]-acetate were incorporated.
repeated on a larger scale. Since only [1-14C]acetate is incorporated into glycine and the carboxyl group contained no radioactivity it can be inferred that the carboxyl group is derived from bicarbonate and the α-(methylene) carbon atom from the carboxyl group of acetate. 

The partial degradation of phenylalanine (Table 11) was done to determine whether acetate was incorporated into the aromatic ring system and the isolation of acetanilide with isolated activity from phenylalanine clearly demonstrates that both carbon atoms of acetate are incorporated. Unfortunately no conclusions can be drawn from the radioactivities found in carbon dioxide produced during the chromic acid oxidation of phenylalanine since the high recoveries suggest that some ring fission occurred. It is, however, clear from the decarboxylation of the benzoic acid samples that [1-14C]-acetate is not incorporated into the carboxyl group (which is the β-carbon atom of the side chain of phenylalanine), whereas [2-14C]acetate is incorporated into this position. A comparison of the specific activities of the benzoic acid samples with those of the phenylalanine samples from which they were derived further shows that [1-14C]acetate must have been incorporated into the α-carbon atom of the side chain whereas very little [2-14C]-acetate can have been incorporated. Since the decarboxylation of phenylalanine with chloramine-T (Table 6) showed that neither [1-14C]- nor [2-14C]-acetate was incorporated into the carboxyl group, it can be assumed that this is derived from bicarbonate. This then shows that the side chain of phenylalanine has the following isotope distribution:

\[
\begin{align*}
&\text{CH}_2\text{CH(NH}_2\text{)CO}_2\text{H} \\
&m & c & b
\end{align*}
\]  

(for m, c and b see Table 12). Assuming by analogy with E. coli (see review by Sprinson, 1960) that the aromatic amino acids are synthesized via shikimic acid, the side chain would be derived directly from phosphoenolpyruvate and the above isotope distribution would be in accord with that found in alanine and aspartic acid in C. thiosulphatophilum, and lends further support to the formation of a C₄ unit (pyruvate or phosphoenolpyruvate) with this characteristic isotope distribution. The earlier experiments on acetate and bicarbonate incorporation into the side chain of tyrosine in Rhodospirillum rubrum are also identical with the above isotope distribution (Ehrensvard & Reio, 1953).

Further elucidation of the pathways involved in the biosynthesis of amino acids in C. thiosulphatophilum and in other strict anaerobes is clearly desirable. The isotope-incorporation technique coupled with the complete and unequivocal degradation of the radioactive products is a valuable indicator of novel biosynthetic pathways but studies of the intermediates in the biosynthetic processes and of the enzymes concerned demand the application of other techniques. The steps in the formation of alanine with the specific labelling pattern now established in three anaerobic bacteria, C. thiosulphatophilum, Rhodospirillum rubrum and Clostridium kluyveri, remain completely unknown.

Since the green sulphur bacteria are regarded as strict autotrophs these experiments, and the earlier studies with C. limicola by Sadler & Stanier (1960) showing that autotrophic bacteria are able to assimilate organic compounds into a large number of cell constituents, suggest that similar experiments should be carried out with the chemoautotrophs, which may also be found capable of assimilating low concentrations of some simple organic substrates such as acetate. The limitations of autotrophs may reside not in their capacity to assimilate or metabolize organic compounds (most of which they synthesize from bicarbonate and therefore have to metabolize anyhow), but in their capacity to oxidize them and so derive reducing power (presumably in the form of NADPH) for biosynthetic reactions leading to cell growth.

**SUMMARY**

1. Growth of Chlorobium thiosulphatophilum was increased by the addition of low concentrations of acetate to the basal thiosulphate medium.

2. Uptake of radioactive acetate by resting cell suspensions required the presence of bicarbonate and a source of reducing power such as hydrogen gas.

3. Acetate was incorporated into all the major cell fractions in resting cell suspensions and in growing cultures.

4. Poly-β-hydroxybutyrate was not detected in C. thiosulphatophilum under conditions in which it was readily isolated from Rhodospirillum rubrum and Rhodomicrobium vannielii.

5. Both [1-14C]acetate and [2-14C]acetate were incorporated into most of the amino acids of the cell proteins.

6. Radioactive amino acids were isolated and decarboxylated; with the exception of leucine and isoleucine, very little radioactivity was found in the carboxyl groups.

7. Alanine, aspartic acid and glutamic acid were degraded completely. The isotope distributions in alanine and aspartic acid were similar to those found in Rhodospirillum rubrum and Clostridium kluyveri.

8. Phenylalanine was partially degraded and it was established that both [1-14C]acetate and [2-14C]acetate were incorporated into the aromatic ring.
The authors acknowledge the skilful technical assistance of Mr R. J. Watkinson throughout these investigations. This research was supported in part by the Rockefeller Foundation.

REFERENCES


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Metabolism of Nucleic Acids during Liver Maturation in the Neonatal Rat

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In a previous paper (Oliver, Ballard, Shield & Bentley, 1962) several biochemical aspects of normal liver growth in postnatal rats were reported. Changes observed in the concentration of liver nucleic acids during the first 25 days after birth suggested that rapid cell division preceded a later phase of cytoplasmic growth. Since these results appeared to be the reverse of the situation that occurs in regenerating adult rat liver (Hecht & Potter, 1956), observations have now been extended by a study of the rates of incorporation of labelled precursor (14C)orotic acid) into liver nucleic acids in vivo. In addition, it has been necessary to attempt a definition of the cell types that contribute to changes in nucleic acids, since the rat liver is known to be haematopoietic in early