The Assimilation of 1-C Compounds

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A wide variety of micro-organisms can grow on 1-C compounds as sole carbon source; the growth substrates range in oxidation level from carbon dioxide to methane. The organisms may be subdivided into three main classes (Table 1).

Group A is comprised of the photosynthetic and chemosynthetic autotrophs. The elucidation of the way in which cell constituents could be made *de novo* from carbon dioxide came principally from Calvin's laboratory in 1954 (Bassham *et al.* 1954). These authors suggested a cycle of reactions which may be termed the ribulose diphosphate cycle of carbon dioxide fixation. Variants on the basic theme of the cycle may permit synthesis of triose-, tetrose-, pentose-, or hexose phosphates from carbon dioxide (Elsden, 1962). The operation of the cycle has been well demonstrated in various photosynthetic tissues but there are still one or two areas of uncertainty, e.g. the considerable discrepancy between the activity of some of the cycle enzymes as measured *in vitro* and the rates of carbon dioxide fixation observed in intact cells (Peterkofsky & Racker, 1961). This may, however, only reflect a greater activity of the enzymes when organized within the chloroplast than when extracted.

Table 1. *Subdivision of micro-organisms capable of growth on 1-C compounds*

Group	Source of energy	Growth conditions	Carbon source
A	Inorganic oxidation, light	Aerobic or anaerobic	CO ₂
B	Organic oxidation	Aerobic	Reduced 1-C compounds
C	Organic dismutation	Anaerobic	CH ₃ OH, HCO ₂ H, CO

Following the discovery of the ribulose diphosphate cycle in 1954, its occurrence in a variety of non-photosynthetic autotrophs has been established (for review, see Quayle, 1961). Such work indicates that, so far, autotrophic growth may be equated with the ribulose diphosphate cycle, and that a cycle of this type is probably responsible for *de novo* synthesis of polycarbon compounds from carbon dioxide, whatever the energy source.

Less is known about the metabolism of micro-organisms capable of aerobic growth on reduced C₁ compounds. Some of the better authenticated species of such organisms are given in Table 2. There are so many similarities between the coloured organisms that they have been tentatively bracketed together as being related to *Pseudomonas methanica*, an organism re-isolated by Dworkin & Foster (1956), 50 years after its first isolation as *Bacillus methanicus* by Söhngen (1906). Of the remaining four organisms in Table 2, one of these, *P. aminovorans* described by den Dooren de Jong (1926), does not appear to be available now. Hence it is not possible to decide whether it might be related to *P. methanica*. The other three organisms in Table 2, however, are quite distinct.

Up to 1958, very little was known of the mode of biosynthesis of cell constituents from reduced 1-C growth substrates. Bhat & Barker (1948) and van Niel (1954) had pointed out the possibility that an organism growing on a highly oxidized 1-C substrate, such as formate, might utilize an autotrophic type of metabolism, in which the energy of oxidation of the substrate is coupled to the assimilation of carbon dioxide. In fact, several authors, e.g. Thimann (1955), have assumed that this takes place without there being any proof that it does. However, of all the organisms tested so far, only one has proved to be autotrophic, viz. formate-grown *Pseudomonas oxalaticus*. Isotopic work with whole cells and enzymic studies with cell-free extracts point to growth of this organism on formate as being a strictly autotrophic process in which the bulk of the carbon is assimilated by the ribulose diphosphate cycle of carbon dioxide fixation, the necessary energy being derived from oxidation of formate (Quayle & Keech, 1959*a, b*).

Table 2. *Some micro-organisms capable of growth on 1-C compounds*

Organism*	Characteristic features	Oxidation level of 1-C growth substrates
<i>Pseudomonas methanica</i> (over 30 strains)	<i>P. methanica</i> ? Mostly pink. Yellow, brown and colourless strains of some of them are known	Ranges from $\text{CH}_4 \rightarrow \text{HCO}_2\text{H}$. Some organisms are 1-C specific
<i>Pseudomonas</i> } <i>extorquens</i>		
<i>Flavobacterium</i> }		
<i>Pseudomonas</i> PRL-W 4		
<i>Pseudomonas</i> AM 1		
<i>Protaminobacter ruber</i>		
<i>P. alboflavus</i>		
<i>Hyphomicrobium vulgare</i>	Colourless. Slow growing, 'stalked' cells	CH_3OH , HCO_2H . 1-C specific
<i>Bacillus sphaericus</i>	Colourless	<i>N</i> -methyl urea. Not 1-C specific
<i>Pseudomonas aminovorans</i>	Colourless to yellow, prob- ably needs re-isolating	CH_3OH , HCO_2H . Not 1-C specific
<i>P. oxalaticus</i>	Colourless	HCO_2H . Not 1-C specific

* For bibliography, see Quayle (1961) and Peel & Quayle (1961).

It would appear to be energetically wasteful to assimilate carbon at the level of carbon dioxide and reduce it to the level of cellular material when the substrate itself presents the cell with reduced carbon. When the substrate is as highly reduced as methane, the wastage is very obvious. It is thus not surprising to find evidence now accumulating of the occurrence of a heterotrophic type of growth on reduced 1-C compounds in which a substantial portion of the carbon is taken in at a reduction level higher than that of carbon dioxide. The first direct evidence for this type of metabolism was the finding by Leadbetter & Foster (1958) that the specific radioactivity of cells of various strains of *Pseudomonas methanica* grown under an atmosphere of methane + air + $^{14}\text{CO}_2$ was always much less than that of the exogenous $^{14}\text{CO}_2$. This showed that the cells could not have been synthesized exclusively from exogenous carbon dioxide or from respiratory carbon dioxide in equilibrium with the exogenous carbon dioxide. Kaneda & Roxburgh (1959) incubated methanol-grown *Pseudomonas* strain PRL-W 4 with ^{14}C -methanol and $^{14}\text{CO}_2$ and found that

the first stable intermediate in methanol metabolism was serine. They concluded that the metabolism was non-autotrophic. Large, Peel & Quayle (1961) carried out a kinetic analysis of the course of entry of [^{14}C]-substrate and $^{14}\text{CO}_2$ into methanol- and formate-grown *Pseudomonas* AM1, and methanol-grown *Hyphomicrobium vulgare*. These studies showed that serine is a primary product of [^{14}C]-methanol or [^{14}C]-formate incorporation (and glycollate in *H. vulgare*) and that malate or aspartate is a primary product of $^{14}\text{CO}_2$ incorporation. With all tracers, glycine too, was an early labelled product; 50 % of the cell carbon was found to be exchangeable with exogenous carbon dioxide during growth of *Pseudomonas* AM1 on [^{14}C]-methanol. Leadbetter recently analysed the kinetics of entry of [^{14}C]-methylamine and $^{14}\text{CO}_2$ into a pink, methylamine-grown organism and found very similar results. Labelling patterns of glycine, serine, phosphoglycerate and malate have been determined in methanol-grown *Pseudomonas* AM1 incubated with [^{14}C]-methanol or $^{14}\text{CO}_2$ (Large, Peel & Quayle, 1962). On the basis of these data a scheme was proposed for a heterotrophic type of metabolism in which hydroxymethylation of glycine to give serine serves as a major pathway for synthesis of 3-C compounds from 2-C compounds. The crucial problem of the necessary synthesis of glycine, or its precursors, from 1-C units is unsolved.

Virtually nothing is known of the metabolism of the anaerobic organisms in Group C. Lack of knowledge is due largely to the difficulty of isolating and handling these bacteria.

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