MICROBIOLOGICAL REVIEWS, June 1978, p. 251-273
0146-0749/78/0042-0251$02.00/0
Copyright © 1978 American Society for Microbiology
Vol. 42, No. 2
Printed in U.S.A.

Evolutionary Aspects of Autotrophy

J. RODNEY QUAYLE[1]* AND THOMAS FERENCI[2]

Department of Microbiology, University of Sheffield, Sheffield S10 2TN, England,[1] and Universität Konstanz, Fachbereich Biologie, D-775 Konstanz, Postfach 7733, West Germany[2]

INTRODUCTION

The commencement of the writing of this review coincided with the first microbiological experiments conducted on the surface of Mars, within the Viking spacecraft. Thus, on the one hand scientists speculate on the sequence of events which led to the development of life on this planet a few thousand million years ago, while on the other hand, a man-made scoop measures out Martian soil into culture vessels in order to see whether life has ever developed or is likely to develop on another planet of the solar system more than 35 million miles away. The fascination of this arching of the intellect is familiar to the many kinds of scientist who have reflected on whence they came and has led to a literature which is as extensive, rich, and varied as the very primeval soup which it describes. Having drawn attention to this wide literature, it behoves us to justify the writing of a new article on the subject and, since it could embrace thousands of millions of years of biology, it is necessary to stake out a modest few Gy (1 Gy = 1 gigayear = 10^9 years) for more detailed consideration.

The period in the Earth's history with which we are mainly concerned is the shadowy area between 4 and 3 Gy ago and, more particularly, the period of evolution between the first heterotrophic organisms and the rise of the autotrophs. (In this review, an autotroph is defined as an organism which synthesizes all its cell constituents from carbon dioxide, irrespective of biochemical pathway. With such a definition, inability to synthesize a vitamin cofactor such as B_{12}, biotin, pantothenate, folic acid, etc., would not in itself exclude an organism from being considered an autotroph.) The development of autotrophy, gradual as it may have been, was a major evolutionary advance, inasmuch as it permitted the direct biological utilization of the carbon dioxide/carbonate reserves, thereby short-circuiting the slow, random process of chemical evolution of biologically usable molecules. Given that the earliest prebionts and earliest self-reproducing organisms used available, ready-made materials and therefore possessed

the biochemical pathways for utilizing such compounds, it is of obvious interest to consider how CO_2 fixation arose from earlier (heterotrophic) modes of metabolism.

The additional complexity of CO_2 fixation, in contrast to cellular synthesis from reduced carbon compounds such as sugars or formaldehyde, is that synthesis of cellular material (roughly at the redox level of carbohydrate) from CO_2 is, overall, an endergonic process. Thus, in order to develop the ability to utilize CO_2, not only are enzymes required for CO_2 fixation, but a parallel ability to generate and transfer chemical energy is also necessary. Clearly, this complexity of metabolism is likely to have been preceded by assimilation pathways that were not endergonic and therefore did not necessarily require the added machinery of energy transduction.

Research over the last 10 years by several groups has led to the recognition that there are several pathways by which microorganisms can synthesize cell constituents in net fashion from one-carbon (C_1) compounds at different reduction levels (3, 81, 87). Net syntheses of intermediary metabolites from C_1 compounds such as formaldehyde have been found which involve biosynthetic sequences that are actually exergonic. Some reduced C_1 assimilation sequences may turn out to have little evolutionary connection with autotrophy. However, at this stage they should all be scrutinized to see whether any of them could have served as templates upon which autotrophic metabolism was fashioned. We feel that it is timely to start such a scrutiny, and it is the purpose of this review to present in detail one particular sequence, viz., the ribulose monophosphate cycle of formaldehyde fixation (RuMP cycle) (101), as a possible autotrophic template.

THE PRIMITIVE ENVIRONMENT

The literature dealing with the probable nature of the primitive environment is very extensive and has been contributed to by chemists, physicists, geologists, and biologists. It would be outside the scope of this review and the competence of its authors to attempt to assess it. We shall instead present a brief outline of a model which appears to be broadly accepted. In such an outline, individual literature citation is not appropriate; instead, the reader is referred to a list of more recent books and reviews which we have found to be particularly helpful (12, 17, 32, 57, 69, 74, 77, 78, 79, 89, 93, 95). This list is not meant in any way to be comprehensive.

The Atmosphere

There is general agreement that the primary reducing atmosphere surrounding this planet during its formation from the solar nebula was lost and was replaced by a secondary atmosphere resulting from outgassing of the Earth's interior. This process is still visibly operating in the form of volcanic activity. It is a matter of debate as to the composition of the secondary atmosphere thus formed. One view is that the atmosphere was a completely reduced one consisting mainly of methane, ammonia, water, and perhaps some hydrogen; another view is that the atmosphere was neutral and consisted mainly of carbon dioxide, water, and nitrogen; other suggestions have included carbon monoxide as a further component. Although it is not possible at present to decide between these alternatives, it is nevertheless generally agreed that oxygen was absent. Some oxygen may have been formed from photolysis of water by ultraviolet radiation in the upper atmosphere, followed by escape of the resulting hydrogen from the atmosphere, but the contribution of this process towards the overall oxygen content of the Earth is considered to be very small (93). Rather, is it considered that the bulk of the oxygen arose from biological photosynthesis which started some 3.7 Gy ago (93), and there is fossil evidence which indicates the existence, 3 Gy ago, of organisms resembling the present day blue-green bacteria (cyanobacteria) (94, 95). Since an oxygen-producing photosynthetic autotroph is, biochemically speaking, a highly developed organism, it follows that it could only have appeared after a long period of evolution from simpler organisms, under anaerobic conditions.

The Primeval Soup

After the initial formation of the planet 4.5 Gy ago, its chemical constituents underwent a long period of chemical, abiogenic evolution (17, 32, 57, 69, 77, 78) in which a large array of chemical compounds was synthesized and "accumulated till the primitive oceans reached the consistency of hot dilute soup" (40)—the primeval soup. (Later considerations [32, 69] of the stability of many of the compounds of biological importance and their increased interaction in concentrated solution have favored the idea that the primeval soup was cold and locally concentrated rather than hot and dilute. Also, the idea of chemical evolution taking place in homogeneous solution is, of course, too simple; many of the reactions must have occurred in different kinds of phase interactions.) Many sources of energy were available for chemical evolution, e.g., solar radiation, lightning discharge, radioactive decay, heat energy, and shock waves generated by passage of meteorites through the atmosphere. The

different energy sources may have been useful for different chemical reactions, e.g., ultraviolet light may have been largely involved in dissociation of the components of the early atmosphere into active species which recombined into small molecules. Longer-wavelength light may have been useful in synthesis of larger molecules. Many of these sources of energy have been tested under laboratory conditions following the classic experiment in 1953 of Miller (66), who subjected mixtures of methane, ammonia, hydrogen, and water to prolonged electric discharge and isolated therefrom a wide variety of molecules, many of them familiar cell constituents. Provided no oxygen is present, carboxylic acids, aldehydes, sugars, amino acids, porphyrins, and purines are among the compounds that have been isolated from reaction mixtures simulating the primitive environment (12, 17, 32, 57, 89). It is a fact of the greatest importance that permutation of the components of putative primordial gas mixtures and use of different energy sources still results in the formation of mixtures of organic compounds in which biologically important compounds predominate rather than other conceivable organic compounds. Thus, having established the generality of formation of many biochemicals under a wide range of possible primitive conditions, it becomes less important to determine which of the permutations actually simulates most closely primitive Earth conditions (57). Of particular interest to this review is the prebiotic synthesis of formaldehyde and carbohydrates.

Formation of formaldehyde. The potential importance of formaldehyde in prebiotic evolution has long been recognized because of the ease with which it may be formed under simulated prebiotic conditions and the ease with which it condenses with itself to form carbohydrates. This potential importance has been underlined in spectacular fashion by the discovery of even interstellar "clouds" of formaldehyde in our galaxy by astronomic application of microwave spectroscopy (32, 69, 113). Although the concentration of formaldehyde is extremely low (ca. 10^3 molecules per cm^3), which corresponds to the conditions of an ultra-high vacuum, its presence, together with a surprising number of other candidates for prebiotic precursors such as carbon monoxide, hydrogen cyanide, cyanoacetylene, methyl alcohol, and formic acid, emphasizes that chemical evolution of a kind may take place in interstellar space, even to the extent of polysaccharide formation (47).

Formaldehyde was one of the earliest organic compounds to be synthesized under laboratory conditions simulating possible prebiotic conditions, e.g., its formation has been observed during electric discharge through mixtures of methane and water (61); irradiation by α-particles of aqueous solutions of carbon dioxide and ferrous ions (36); ultraviolet or γ-irradiation of carbon dioxide and water (37, 39); spark discharge through mixtures of methane, ammonia, water, and hydrogen (67, 68). Hence, there is no doubt that formaldehyde could have been formed in large quantities during the period of chemical evolution. Formaldehyde is a highly reactive compound and, once formed, it could enter into a wide variety of reactions (109). Its formation in the primitive atmosphere would have been accompanied to a greater or lesser extent by its destruction by ultraviolet radiation of wavelengths shorter than 370 nm (2). Once transported into the ocean, all but 0.01% would be hydrated and hence protected against further decomposition by ultraviolet radiation. In solution the formaldehyde could form many compounds, e.g., polymerization to polymers such as paraformaldehyde and metaformaldehyde under neutral or acid conditions, polymerization with ammonia to polymers such as hexamethylene-tetramine, reaction with thiols to form thiohemiacetals, reaction with cyanide to give glyconitrile ($HOCH_2CN$), self-condensation to monosaccharides, etc.

Walker, in his comprehensive account of formaldehyde chemistry (109), quotes a statement by Gault, which expresses well the unique chemical reactivity of this small molecule: "... Like those organisms which can multiply to give several successive generations in a few hours, formaldehyde can at times produce entire families of polymers, sugars, phenoplasts and aminoplasts in a few instants of time and all of these substances possess the original imprint of formaldehyde. For many years yet to come, legions of chemists will strive to augment and develop the branches of its genealogical tree."

Formation and occurrence of sugars. The self-condensation of formaldehyde is of special interest as a potential prebiotic synthesis of carbohydrate.

The work of Butlerow (14) in the last century first showed that formaldehyde condenses with itself under basic conditions to give a mixture of sugars. This reaction, termed the formose reaction, has since been extensively studied (for a detailed review, see 70). The reaction is autocatalytic, starting with a slow direct condensation to glycolaldehyde by a mechanism not yet clarified:

$$2CH_2O \rightarrow HOCH_2 \cdot CHO \qquad (1)$$

Once this is formed, rapid aldol condensations and tautomerizations occur to give straight-chain sugars, e.g.,

$$HCHO + HOCH_2 \cdot CHO \rightarrow HOCH_2 \cdot CHOH \cdot CHO \quad (2)$$

$$HOCH_2 \cdot CHOH \cdot CHO \rightleftharpoons HOCH_2 \cdot CO \cdot CH_2OH \quad (3)$$

$$HCHO + HOCH_2 \cdot CO \cdot CH_2OH \rightarrow HOCH_2 \cdot CHOH \cdot CO \cdot CH_2OH \quad (4)$$

$$HOCH_2 \cdot CHOH \cdot CO \cdot CH_2OH \rightleftharpoons HOCH_2 \cdot CHOH \cdot CHOH \cdot CHO \quad (5)$$

or branched chain sugars, e.g.,

$$HCHO + HOCH_2 \cdot CHOH \cdot CHO \rightarrow HOCH_2 \cdot C(CH_2OH)OH \cdot CHO \quad (6)$$

Crossed Cannizaro reactions leading to sugar alcohols can also occur, e.g.,

$$HOCH_2 \cdot CHOH \cdot CHO + HCHO + OH^- \rightarrow HOCH_2 \cdot CHOH \cdot CH_2OH + HCO_2^- \quad (7)$$

Aldoses which are formed may themselves react with activated methylene groups by aldol condensation to widen further the possible reaction products

$$R' \cdot CHO + HOCH_2 \cdot CO \cdot R'' \rightarrow R' \cdot CHOH \cdot CHOH \cdot CO \cdot R'' \quad (8)$$

where $R' = R'' = (CHOH)_nCH_2OH$. Addition of glycolaldehyde or other acyloins to a reaction mixture abolishes the initial induction period, suggesting that these compounds catalyze reaction 1.

The number of possible products is theoretically almost limitless, and an interesting discussion of this aspect of the formose reaction, described as a "semi-chaotic system," has been given by Cairns-Smith and Walker (15). In practice, however, there are limitations. Synthesis virtually stops at straight-chain sugars containing more than eight carbon atoms because, starting with C_5 sugars, furanose and pyranose ring formation removes the necessary activation of the α-hydroxymethylene group. Reactions similar to 6 and 7 are dead-end reactions because of removal of either the α-hydrogen atom or the carbonyl group responsible for its activation. Nevertheless, even with such limitations, over 30 sugar species have been detected in a reaction mixture using refined methods of analysis (70); included in the products are essentially all the common heptoses, hexoses, pentoses, tetroses, and trioses. The composition of the mixture of reaction products can be varied widely by alterations in temperature, pH, reaction time, and ratio of formaldehyde to metal hydroxide catalyst. This has been elegantly investigated by Weiss and his colleagues (70) in a type of "chemical chemostat" in which formaldehyde and calcium hydroxide were independently fed into a reaction vessel and the products were continually withdrawn and analyzed.

The role which the formose reaction may have played in prebiotic evolution is a source of much speculation. On the one hand, the circumstantial evidence for it being the primordial template for carbohydrate metabolism is compelling: (i) straight-chain sugars containing two to eight carbon atoms are the predominant carbohydrate currency of all living cells—the formose reaction can supply them all and does not supply larger ones; (ii) the pattern of reactions and products encountered in the overall formose reaction furnishes templates of many enzyme-catalyzed reactions of importance in intermediary carbohydrate metabolism, e.g., aldolases, transaldolases, isomerases, and epimerases; (iii) the single necessary starting compound for the formose reaction, formaldehyde, must have been continuously formed in large quantities throughout the period of chemical evolution. On the other hand, many questions remain unanswered as to how the process might have operated under primeval conditions, e.g., unrealistically high concentrations of formaldehyde and alkali are necessary for appreciable reaction to occur in the test tube (1, 46). Gabel and Ponnamperuma (35) investigated alternative experimental conditions and demonstrated formation of sugars in a more dilute formaldehyde solution (10^{-2} M) by boiling in the presence of alumina or kaolinite as catalysts. These conditions were designed to simulate those in a primordial hydrothermal spring. A similar experiment, with similar results, was performed by Reid and Orgel, (86) who used carbonate-apatite in place of alumina or kaolinite. Hence, it could be envisaged that continual formation of formaldehyde in cold primeval soup might have resulted in continual precipitation of paraformaldehyde, and this would represent a concentration process. At some future time a local heating, as in an eruption of hot springs, would result in depolymerization and generation of high local concentrations of formaldehyde for subsequent formose reaction. The difficulty then arises that under the conditions used by Reid and Orgel (86), the sugars once formed would soon decompose, leading these authors to question whether the formose reaction (at least under the conditions so far tested) could satisfactorily explain the prebiotic synthesis of sugars. This need not be an insuperable difficulty, even with the given experimental conditions, provided that a steady generation of carbohydrates was maintained over long periods of time. In biochemical evolution, it is not so much the accumulation of potential substrates which is important, but rather their continual presence (even at low

concentrations). A clear example of this is the gradual adaptation of the primitive anaerobes to oxygen, presumably at very low initial concentrations but continually generated by the cyanobacteria. It should also be noted that alkaline condensation is not the only way of making carbohydrates from formaldehyde: the formation of several sugars including pentoses, deoxypentoses, and hexoses has been observed after irradiation of solutions of formaldehyde with ultraviolet or γ-radiation (76).

The true answer to the role of the formose reaction in prebiotic synthesis of sugars will of course never be known, but the irony of now considering its use, 4 Gy later, as an industrial source of carbohydrates to alleviate world food shortages (70), should not be lost on the reader.

C_1 ASSIMILATION SEQUENCES

There are presently three assimilation sequences known which can account for the net synthesis of C_3 or C_4 skeletons from C_1 compounds: the ribulose diphosphate cycle of CO_2 fixation (RuDP cycle), the RuMP cycle, and the isocitrate lyase$^+$ (*icl*$^+$)-serine pathway. These have been the subject of several recent reviews or symposia (3, 9, 42, 63, 81, 82, 87, 99). There is also a variant of the serine pathway which does not involve isocitrate lyase; the biochemistry of this variant is not completely understood (82). These assimilation sequences cover a wide range of organisms which can grow on C_1 compounds as the sole carbon source, but the picture may not yet be complete; in particular, little is known about the biosynthetic metabolism of the C_1-utilizing methanogenic anaerobes. There is also renewed uncertainty about the pathway of carbon assimilation in autotrophically grown *Chlorobium thiosulphatophilum*. Evans et al. (29) proposed the operation in this organism of a cycle of net CO_2 fixation, the reductive carboxylic acid cycle, involving the reversal of several reactions of the tricarboxylic acid cycle. It was suggested that this cycle operated in addition to, or in place of, the RuDP cycle. The evidence for the former cycle has been questioned (10, 63, 81), but further experiments have recently been reported (13, 98) which indicate that the RuDP cycle is not functioning in the organism. Its absence would of course necessitate operation of an alternative autotrophic sequence.

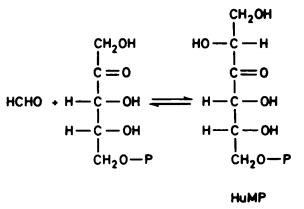
For the purpose of this review, only the RuDP and RuMP cycles will be considered in detail. Although the evolutionary relationships of the serine pathways will clearly be of great interest, they are not likely to be directly linked to the RuDP cycle in the way we believe the RuMP cycle to be.

Ribulose Diphosphate Cycle

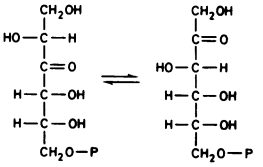
The RuDP cycle and its variants is established as the main (if not the only) sequence for aerobic and photosynthetic autotrophic CO_2 fixation (for recent reviews, see 52, 63, 108). The basic cycle is represented in Fig. 1. It is of considerable interest that during the early presentations of the cycle, an alternative rearrangement sequence involving transaldolase (*tal*), but not sedoheptulose diphosphatase (*sda*) (Fig. 2), was considered (58). Later work, principally by Bassham (7, 8), has shown that CO_2 fixation occurs in the chloroplast by the cycle shown in Fig. 1. The transaldolase is involved, not in CO_2 fixation, but in operation of the oxidative pentose phosphate cycle in the dark, thus providing the chloroplast with reduced nicotinamide adenine dinucleotide phosphate (NADPH) in the absence of photosynthesis. Although only one variant of the rearrangement reactions is involved in CO_2 fixation by chloroplasts, it will be seen later that both variants are encountered in the RuMP cycle.

Ribulose Monophosphate Cycle

The operation of the RuMP cycle can be divided into three phases. Phase I (the fixation phase) involves the reactions unique to the pathway, the condensation of formaldehyde with RuMP and the subsequent isomerization of the product, D-*arabino*-3-hexulose-6-phosphate (HuMP), to yield fructose-6-phosphate (FMP):



(9)



(10)

Phase II of the cycle (the cleavage phase) involves cleavage of the hexose phosphate skeleton to form the glyceraldehyde-3-phosphate (GAP) necessary for the rearrangement reactions of phase III and the production of a C_3 skeleton (dihydroxyacetone phosphate [DHAP] or pyruvate) that can be looked on as the primary net product of the cycle. Phase III (the rearrangement phase) contains the reactions

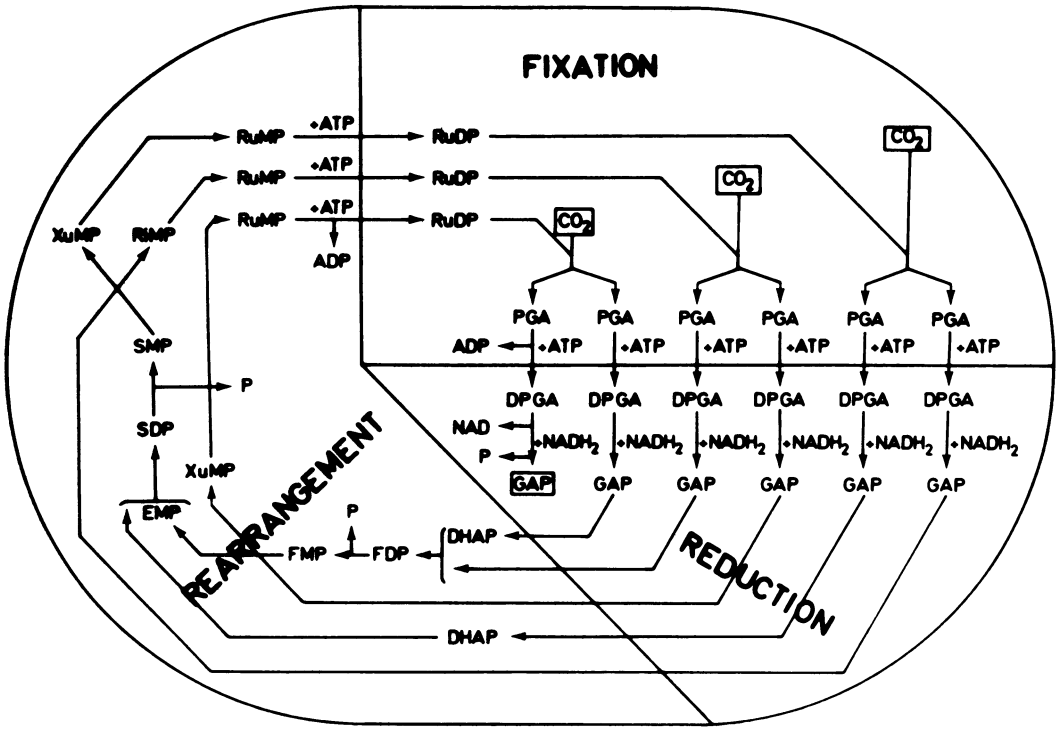


FIG. 1. *RuDP cycle (I). (The cycle involves sedoheptulose diphosphatase but not transaldolase.) Abbreviations throughout figures: DPGA, 1,3-diphosphoglycerate; EMP, erythrose-4-phosphate; SMP, sedoheptulose-7-phosphate; GMP, glucose-6-phosphate; XuMP, xylulose-5-phosphate; NuMP, nucleoside monophosphate; 6-PG, 6-phosphogluconate; Py, pyruvate.*

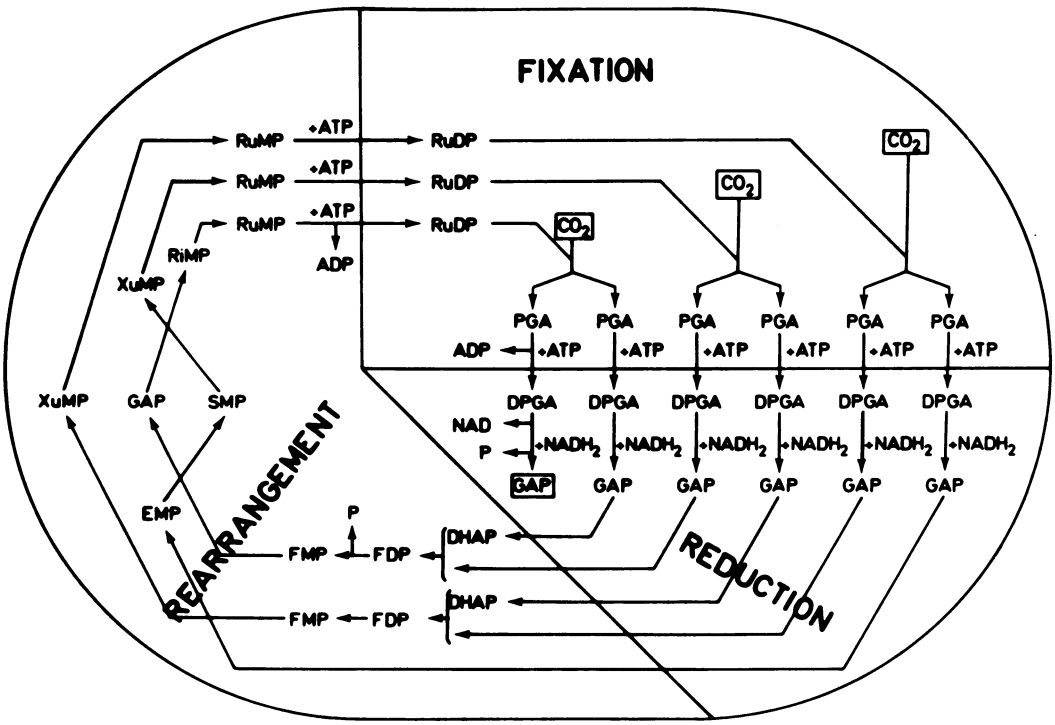


FIG. 2. *Hypothetical variant of the RuDP cycle (II). (The variant involves transaldolase but not sedoheptulose diphosphatase.)*

necessary for the regeneration of the acceptor (RuMP) needed for reaction 9, so completing the cycle of reactions. In phases II and III, alternative routes have been implicated, and no single set of reactions describes the cycle as it may operate in different organisms. Two possible rearrangement variants are shown in Fig. 3 and 4, which correspond to the two rearrangement variants of the RuDP cycle shown in Fig. 1 and 2.

Phase I (fixation). A considerable body of evidence is now consistent with the operation of reactions 9 and 10 in the assimilation of reduced C_1 compounds by methane- and methanol-grown *Pseudomonas methanica* and *Methylococcus capsulatus*. Although the evidence obtained before 1972 has been fully reviewed (81), only recent data pertinent to the operation of the pathway will be discussed in detail. On the basis of ^{14}C labeling experiments with intact cells, it was established that C_1 units from methane, methanol, and formaldehyde were rapidly incorporated into sugar phosphates, with hexoses being early labeled products (49, 56, 60). The incorporation of CO_2 as the major route of carbon assimilation could not explain the labeling patterns, and RuDP carboxylase was absent from extracts of *P. methanica*. However, crude cell extracts could catalyze the incorporation of formaldehyde in the presence of ribose-5-phosphate (RiMP), forming a hexose phosphate initially identified as allulose-6-phosphate (55). Subsequently, it was established that RuMP and not RiMP was the acceptor molecule in reaction 9 and RiMP was effective in unfractionated extracts only because of the presence of high levels of phosphoriboisomerase (53, 101). Similarly, the product of reaction 9 has been subsequently identified as the 6-phosphate of D-*erythro*-L-*glycero*-3-hexulose, a sugar hitherto unidentified as a biochemical intermediate (54, 101). One reason why HuMP may have been misidentified in the earlier literature is because it is an unstable molecule, undergoing spontaneous isomerization to (among other products) allulose-6-phosphate and FMP in neutral and alkaline solutions (50, 101). The half-life of HuMP in aqueous solution at 20°C is about 40 h at pH 7.0 and 30 min at pH 11.0 (101). In confirmation, study of the metabolism by cell extracts of chemically synthesized allulose-6-phosphate did not support any role for this compound as an intermediate in the assimilation pathway (101). As further proof, recent purification and study of the substrate specificity of the two enzymes catalyzing reactions 9 and 10 in *M. capsulatus* confirmed the identity of the acceptor as RuMP and the intermediate product as HuMP (30).

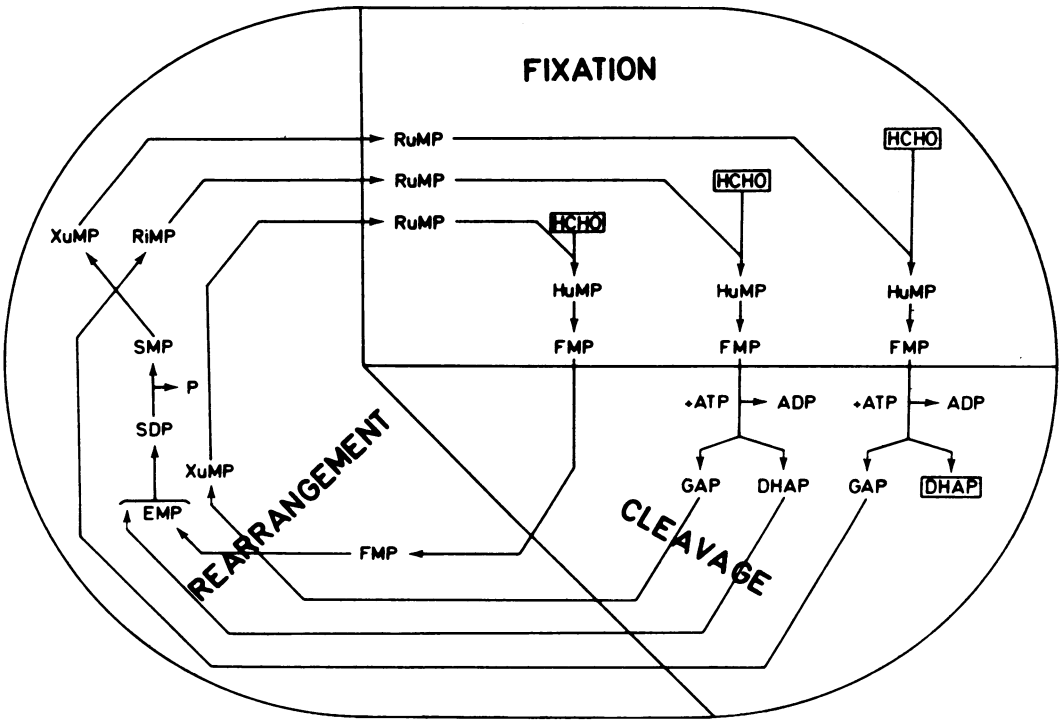


FIG. 3. *RuMP cycle (I). (This scheme involves sedoheptulose diphosphatase but not transaldolase.)*

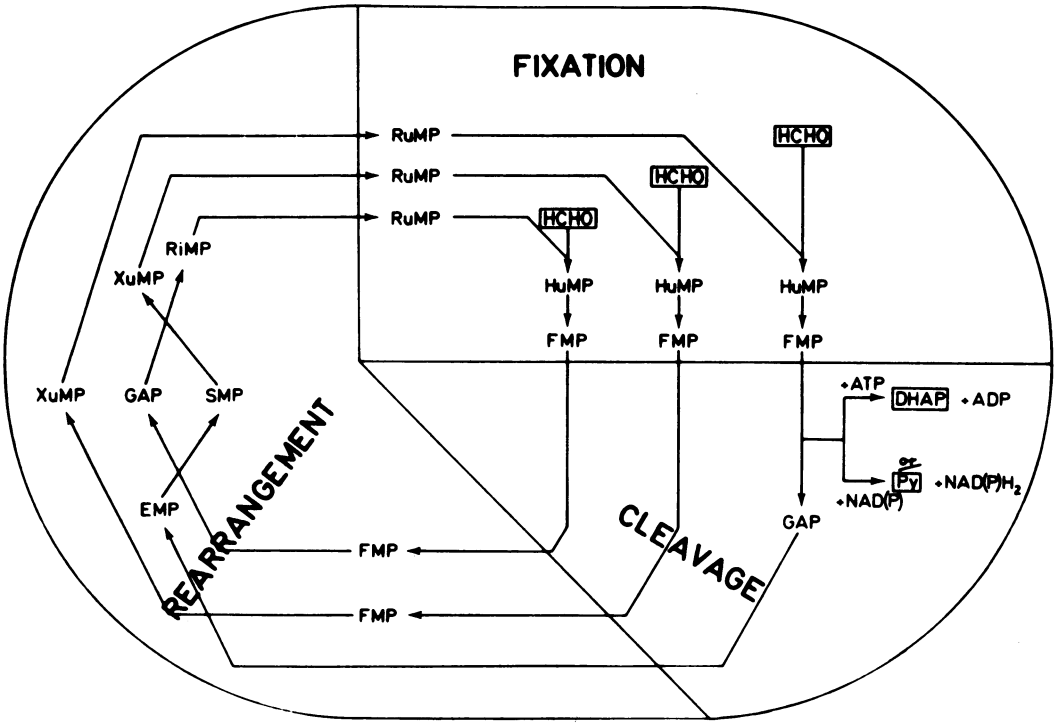


FIG. 4. *RuMP cycle (II). (This rearrangement variant involves transaldolase but not sedoheptulose diphosphatase. Two alternative modes of cleavage are shown involving FDP aldolase and KDPG aldolase, respectively; see Fig. 5.)*

The equilibrium constants of reactions 9 and 10 have been estimated following purification of the enzymes catalyzing these reactions. In the presence of HuMP synthase, the equilibrium concentrations of formaldehyde and HuMP suggested an equilibrium constant of 4.0×10^{-5} M $\pm$ 0.7×10^{-5} at pH 7.0 (30). Reaction 10 was found to favor FMP formation to such an extent that the equilibrium concentration of HuMP in the presence of phospho-3-hexuloisomerase could not be detected. Using an indirect estimation employing the Haldane relationship, a constant of 1.9×10^{-2} M was estimated for reaction 10 (30). The formation of FMP, which exists largely as a ring-stabilized fructofuranose in solutions (102), as distinct from HuMP, which exists in an acyclic form (101), may partly explain the position of equilibrium of reaction 10. The cumulative effect of the equilibrium constants of reactions 9 and 10 is such that the formation of FMP from formaldehyde and RuMP is a nearly irreversible process. This was indicated experimentally by the finding that FMP was formed almost quantitatively from formaldehyde and RuMP in the presence of purified RuMP synthase and phospho-3-hexuloisomerase (101).

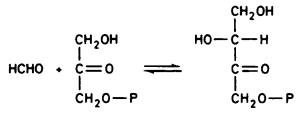
Enzymes catalyzing the condensation of formaldehyde with pentose phosphate have been purified or partially purified from methane-grown *M. capsulatus* (30), methanol-grown *Methylomonas* M15 (92), and *Methylomonas aminofaciens* 77a (50, 51) and separated from phosphohexuloisomerase in *Bacterium* 4B6 (25) (Table 1). It can be seen that the most striking differences in the properties of the enzymes from the different bacteria are those of intracellular distribution and molecular weight. *M. capsulatus* is an obligate methane or methanol utilizer, whereas the other organisms are obligate methanol utilizers. The HuMP synthase from *M. capsulatus* was purified from the methane-grown organism, and the differences in the enzyme from those in the other organisms may be related to the presence of a complex intracellular membrane assembly generally found in methane utilizers that contains enzymes responsible for oxidation of methane (88, 105). A detailed examination of the HuMP synthases from a wide range of methylotrophic bacteria may yield important information concerning their evolutionary connections, as has RuDP carboxylase with respect to the autotrophs (71). (In this review, a methylotroph is defined as an organism which

TABLE 1. *Comparison of HuMP synthases*

Source	Intracellular distribution	Final sp act (μmol/min per mg) (°C, pH)	Mol wt	No. of subunits	Apparent K_m (mM)			Reference
					$MgCl_2$	HCHO	RuMP	HuMP	
M. capsulatus (Texas)	Particulate	69.0 (37, 7.0)	310,000	6		0.49 (at 0.57 mM RuMP)	0.083 (at 4 mM HCHO)	0.075	30
Methylomonas M15	Soluble	66.5 (30, 7.5)	43,000	2	0.25	1.1	1.6		92
M. aminofaciens 77a	Soluble	53.0 (30, 7.4)	45,000–47,000	2	0.17	0.29 (at ∞ RuMP)	0.059 (at ∞ HCHO)	0.036	51

synthesizes all its cell constituents from either a carbon compound containing one or more carbon atoms but containing no carbon-carbon bonds or a mixture of such a compound and carbon dioxide. Some organisms have the metabolic versatility which allows them to be classified as methylotrophs or autotrophs, depending on the growth conditions.)

Reaction 9 is an aldol condensation, and the involvement of divalent metal in HuMP synthase activity suggests a resemblance of the enzymes to type 2 metal-containing aldolases (44). The condensation of formaldehyde as in reaction 9 is also analogous to the condensation of formaldehyde with DHAP forming L-erythrulose-1-phosphate catalyzed by fructose-1,6-diphosphate (FDP) aldolase from muscle (27, 63):



(11)

A similar reaction occurs in rat liver (21), Swiss chard (71), and erythritol-grown *Propionibacterium pentosaceum* (110).

There is evidence which has been taken to indicate that a reaction similar to 9 may occur in methanol-grown yeasts. This evidence has been reviewed by Sahm (90). Whole cell isotope studies by Fujii and co-workers (33, 34) have shown that isotope from [^{14}C]methanol is rapidly incorporated in phosphorylated derivatives of fructose, glucose, and mannose during growth of *Candida* N-16 on methanol. Several groups of workers (90) have reported low activities of RiMP-dependent fixation of formaldehyde by crude cell extracts of methanol-grown yeasts, which is stimulated by the presence of adenosine 5′-triphosphate (ATP). In his review, Sahm (90) refers to unpublished work done by his group in fractionation of this enzyme activity in extracts of methanol-grown *Candida boidinii*. He ascribes the activity to an unstable, Mg^{2+}- and ATP-stimulated HuMP synthase. However, confirmation of the operation of an RuMP cycle in C_1-grown yeasts awaits further work on short-term labeling patterns of sugar phosphates in whole cell studies and on the complete characterization of the two key enzymes of phase I of the cycle—HuMP synthase and HuMP isomerase. This is particularly important in view of the inability of Kato et al. (50) to detect HuMP isomerase in cell extracts of two methanol-utilizing yeasts.

Less has been published on HuMP isomerase, the enzyme catalyzing reaction 10, partly due, no doubt, to the substrate for it not being readily

available. It has been purified 150-fold from *M. capsulatus*, yielding a protein of molecular weight 67,000 (30). The enzyme was distinct from phosphoriboisomerase and phosphoglucoisomerase and, as far as tested, was specific for the interconversion of HuMP and FMP. In contrast to HuMP synthase, the enzyme was inhibited to some extent by all divalent metal ions tested, including Mg^{2+}.

Phase II (cleavage). The first formulation of the pentose phosphate cycle of formaldehyde assimilation proposed the glycolytic cleavage of FMP to triose phosphate via FDP and FDP-aldolase (55, 81). Subsequent surveys of the enzymes present in organisms that possess the RuMP cycle suggested that an Entner-Doudoroff type of cleavage may also be present or is an alternative to the glycolytic sequence in the cycle (6, 101; Fig. 5). In the methane-utilizing bacteria *P. methanica* and *M. capsulatus*, all the enzymes necessary for the cleavage of FMP by both routes are present (101) and there is no evidence yet as to whether both pathways operate in these two organisms or whether one is of more significance than the other. Babel and co-workers (5, 6, 43) have shown that methanol-grown *Pseudomonas* W6 contains only enzymes for Entner-Doudoroff cleavage; appreciable activities of phosphofructokinase and FDP-aldolase were not found.

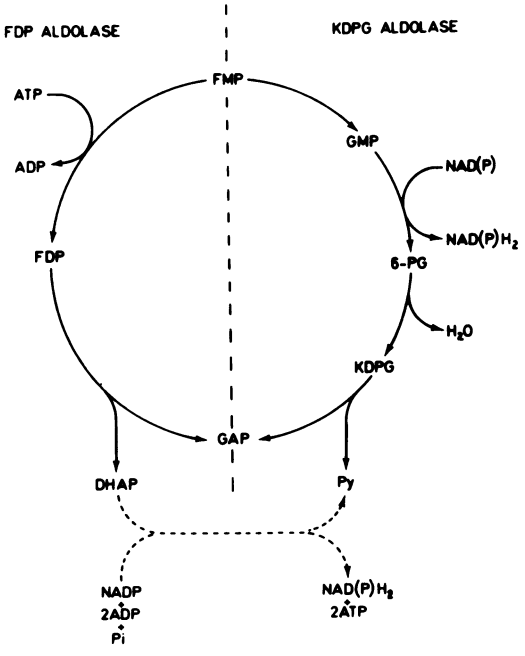


FIG. 5. *Alternative modes of cleavage of hexose phosphate. (The dotted line summarizes the conversion of dihydroxyacetone phosphate to pyruvate by established glycolytic steps.)*

Recent investigation of six isolates of non-methane-utilizing bacteria capable of growth on a variable range of substrates containing one-carbon units such as trimethylamine, dimethylamine, methylamine, and methanol established that RuMP cycle enzymes were present (23). In none of the six isolates were both cleavage routes present as in methane utilizers. Organisms S2A1 and PM6 (gram-positive, spore-forming, aerobic rods) possessed FDP-aldolase and phosphofructokinase, but not the Entner-Doudoroff enzymes. In contrast, the other four gram-negative isolates, 4B6, C2A1, W3A1, and W6A, did not contain the two key glycolytic enzymes, but possessed all the enzymes of the Entner-Doudoroff pathway.

Phase III (rearrangement). The problem of regenerating RuMP to act as acceptor for formaldehyde in the RuMP cycle is almost identical to the problem of regenerating pentose phosphate to act as acceptor in the RuDP cycle of CO_2 fixation. In the RuDP cycle, it has already been mentioned that two possible sets of rearrangement reactions can theoretically account for the formation of three pentose phosphates from one molecule of FMP and three molecules of triose phosphate or from two molecules of FMP and one molecule of triose phosphate. Pattern I involves sedoheptulose diphosphatase but not transaldolase (Fig. 1), whereas pattern II involves transaldolase but not sedoheptulose diphosphatase (Fig. 2). In formaldehyde assimilation, it appears that in methane-utilizing bacteria such as *P. methanica* and *M. capsulatus*, pattern II (Fig. 4) is used for the rearrangement reactions, as transaldolase is present in high activity in these organisms but sedoheptulose diphosphatase is absent (101). The same pattern II applies for organisms 4B6, C2A1, W6A, and W3A1 (23). In contrast, organisms PM6 and S2A1 have negligible transaldolase activity, but sedoheptulose diphosphatase is present, and hence pattern I is used for the rearrangement (Fig. 3). In pattern I, two molecules of FMP need to be cleaved to supply the three molecules of GAP necessary for the rearrangements formulated in Fig. 3. This compares with the necessity for cleaving one molecule of FMP in pattern II (Fig. 4).

Net synthesis of tetrose, pentose, and hexose skeletons. The basic RuMP cycle as expressed in Fig. 3 and 4 shows the net synthesis of triose phosphate or Py from three molecules of formaldehyde. If the triose phosphate is recycled, however, net synthesis of tetrose, pentose, and hexose phosphates from formaldehyde can be accomplished (Fig. 6). The RuMP cycle can therefore furnish the different carbohydrate

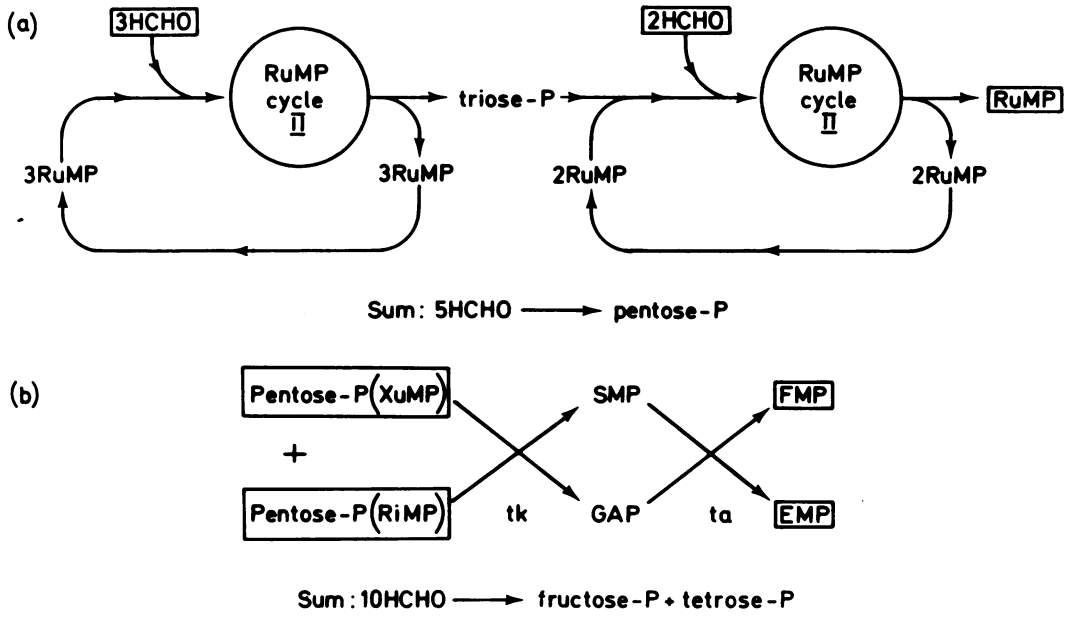


FIG. 6. *Net synthesis of tetrose, pentose, and hexose by the RuMP cycle.*

skeletons necessary for synthesis of cell walls, aromatic amino acids, nucleic acids, and polysaccharides. Such syntheses need not involve any enzymes additional to those implicated in the cycle shown in Fig. 4, except in the case of 2-keto-3-deoxy-6-phosphogluconate (KDPG) aldolase cleavage, where Py would need to be converted to triose phosphate.

Dissimilatory cycle of formaldehyde oxidation. In addition to the assimilatory pathways discussed above, a number of the enzymes involved in assimilation can further be assembled into a cycle of formaldehyde oxidation to carbon dioxide (23, 101; Fig. 7). The only additional enzyme necessary for such a dissimilatory cycle is phosphogluconate dehydrogenase, which has been detected in *P. methanica* and *M. capsulatus* (101), *Pseudomonas* W6 (6), and six trimethylamine-grown organisms (23). In organisms such as *P. methanica* and *M. capsulatus* that have alternative enzymes for the direct oxidation of formaldehyde and formate, the main role for such a cycle may be in the generation of NADPH, as none of the enzymes involved in methane oxidation to CO_2 is nicotinamide adenine dinucleotide phosphate (NADP) linked (31). The dissimilatory cycle probably has a direct role in energy generation in organisms such as PM6 and S2A1, which possess all the enzymes necessary for the oxidation cycle but lack formaldehyde- and formate-dehydrogenases (23). Consistent with such a role is the unusually high level of NADPH oxidase in these

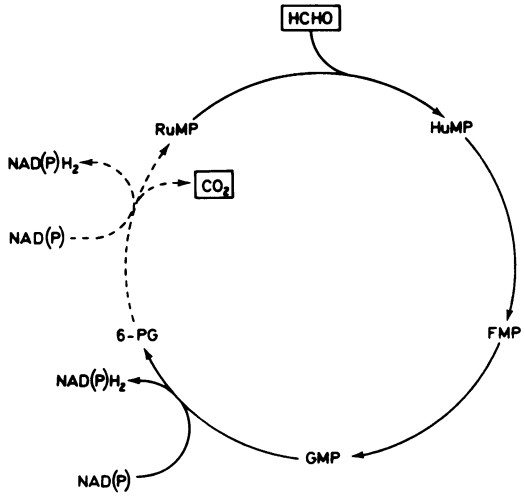


FIG. 7. *Dissimilatory cycle of formaldehyde oxidation. (The dotted line shows the single step that is additional to those which are also involved in the assimilatory cycle.)*

bacteria (22).

Energetics. The permutation of two modes of cleavage with two modes of rearrangement leads to four possible variants of the RuMP cycle, and each of these variants has a different energy budget; it is also interesting to compare these with those of the *icl*$^+$-serine pathway and RuDP cycle (Table 2). Comparison is made easier if triose phosphate and phosphoglyceric acid (PGA) are converted to pyruvate by established

glycolytic steps (Fig. 5) and account is taken of the resulting changes in NAD(P)H and ATP. The results of this normalizing process are shown in Table 3. It can be seen that the second and third variants of the RuMP cycle (*eda*$^+$, *tal*$^+$ and *fda*$^+$, *sda*$^+$, respectively) follow the equation:

$$3CH_2O + NAD(P) \rightarrow \text{pyruvate} + NAD(P)H_2 \quad (12)$$

and there is no net ATP change. However, the fourth variant (*fda*$^+$, *tal*$^+$) follows:

$$3CH_2O + NADP + ADP + P_i \rightarrow \text{pyruvate} + NAD(P)H_2 + ATP \quad (13)$$

where P_i is inorganic orthophosphate. This sequence then offers an exergonic synthesis of Py from formaldehyde, which would be unique among known C_1-assimilation sequences. With this variant, it would also be theoretically possible to construct a fermentation of formaldehyde to lactate, for example, which would be energy yielding:

$$3CH_2O + ADP + P_i \rightarrow \text{lactate} + ATP \quad (14)$$

Comparison of the energetics of the RuMP cycle with those of the *icl*$^+$-serine pathway and RuDP cycle shows in a striking fashion the energetic cost to an organism of using CO_2 rather than formaldehyde as a C_1 unit in a net assimilation process. The broad theoretical predictions are borne out in practice. Goldberg et al. (38) have measured the growth yields of eight species of bacteria able to grow on C_1 compounds. The molar growth yield values on methanol obtained for those bacteria using the RuMP cycle were higher by 17 to 44% than the yields of those using a serine pathway. Detailed theoretical studies of predicted growth yields of methylotrophs have been made by van Dijken and Harder (107) and Anthony (4).

Physiological significance of variants of the cycle. It may be predicted that the *eda*$^+$/*sda*$^+$ variant is unlikely to be encountered frequently, if at all, owing to its higher energy requirement. It is therefore not surprising that

TABLE 2. *Energy budgets for C_1-assimilation sequences*[a]

Cycle	Cleavage phase		Rearrangement phase		Reactants	Product	Energy change		
	FDP aldolase (*fda*)	KDPG aldolase (*eda*)	Transaldolase (*tal*)	SDPase (*sda*)			Δ $NAD(P)H_2$	Δ FPH_2	Δ ATP
RuMP	−	+	−	+	3HCHO	Pyruvate	+1	0	−3
	−	+	+	−	3HCHO	Pyruvate	+1	0	0
	+	−	−	+	3HCHO	Triose P	0	0	−2
	+	−	+	−	3HCHO	Triose P	0	0	−1
icl$^+$-Serine					2HCHO + $1CO_2$	P glycerate	−2	+1	−3
RuDP			−	+	$3CO_2$	Triose P	−6	0	−9
			+	−	$3CO_2$	Triose P	−6	0	−9

[a] *fda*, Fructose diphosphate aldolase; *eda*, 2-keto-3-deoxy-6-phosphogluconate aldolase; *tal*, transaldolase; *sda*, sedoheptulose diphosphatase; FP, flavoprotein of succinate dehydrogenase.

TABLE 3. *Energy budgets for C_1-assimilation sequences, normalized to pyruvate production*[a]

Cycle	Cleavage phase		Rearrangement phase		Reactants	Product	Energy change		
	FDP aldolase (*fda*)	KDPG aldolase (*eda*)	Transaldolase (*tal*)	SDPase (*sda*)			Δ $NAD(P)H_2$	Δ FPH_2	Δ ATP
RuMP	−	+	−	+	3HCHO		+1	0	−3
	−	+	+	−	3HCHO		+1	0	0
	+	−	−	+	3HCHO		+1	0	0
	+	−	+	−	3HCHO		+1	0	+1
						Pyruvate			
icl$^+$-Serine					2HCHO + $1CO_2$		−2	+1	−2
RuDP			−	+	$3CO_2$		−5	0	−7
			+	−	$3CO_2$		−5	0	−7

[a] See footnote *a* of Table 2.

no organisms have yet been reported which possess the appropriate enzyme profile. The necessary enzymes for operation of the remaining three variants have been reported in various organisms (5, 6, 23, 43, 101), but further detailed comparative work will have to be done before clear correlations emerge between the type of cycle and growth physiology (99). At this stage, however, the basic biochemistry is well enough understood for us to emphasize how the RuMP cycle in all its variations touches upon virtually every pathway of bacterial carbohydrate metabolism. By the use of formaldehyde as the sole input molecule, net biosyntheses are effected with minimum or zero input of energy, or even in some cases, output of energy. As outlined in the following sections, these are the properties which we believe place the cycle in a unique position with respect to biochemical evolution.

PRIMITIVE CARBOHYDRATE METABOLISM

The occurrence of sugars in the macromolecules (nucleic acids, polysaccharides, membranes, cell walls, etc.) and intermediary metabolites of all known cells attests to the importance of sugars in the earliest development of self-replicating organisms. The purpose of this section is to consider how sugars may have been metabolized at the primitive stages of biochemical evolution and how the gap between their availability from chemical evolution and from later biochemical synthesis may have been bridged.

As has been discussed in a previous section, the prebiotic synthesis of carbohydrates could have occurred through the formose reaction network. Two main factors may have exerted a selective pressure on the biochemical evolution of carbohydrate metabolism starting from this initial mixture. First, given that nucleic acids always provided the coding of their own replication and, in turn, of protein sequences, a growing demand for nucleic acid precursors would have occurred. This would have led to a demand for chemically formed nucleotides, and, in turn, pentoses, once catalysts capable of nucleoside phosphate synthesis had evolved. A second factor, given the endergonic nature of macromolecular synthesis, would have been the need to develop sources of energy for use by the primitive cell. As has often been pointed out, the substrate-linked phosphorylation steps of glycolysis have the simplicity of feature that suggests these reactions were perhaps among the earliest forms of biological energy transduction (12). Early evolution of carbohydrate fermentation would therefore also have placed a demand for carbohydrates pre-formed during chemical evolution.

Pentose Availability for Nucleic Acid Synthesis

The increasing demand for pentoses for nucleic acids would have resulted in the evolution of enzymes capable of accelerating the production of pentoses and pentose phosphates from readily available precursors. Such a process would be an example of retroevolution, as proposed by Horowitz (45). Likely precursors would have been sugars or sugar phosphates capable of rearranging to pentoses or pentose phosphates. The reactions encountered in the formose network are capable of interconverting a variety of sugars, including pentoses, and could have formed the templates of reactions which are now catalyzed by familiar enzymes—aldolases, transaldolases, epimerases, and isomerases. The essential chemical mechanisms of these interconversions are not significantly changed when sugars are replaced by sugar phosphates. The chronological relationships between interconversions of phosphorylated and non-phosphorylated sugars in primitive metabolism are not clear, and, indeed, it may be noted there are many enzymes known today which act on free sugars rather than on phosphates (72).

The stages in the evolution of phosphorylating systems which might have been involved in phosphorylation of sugars have been the subject of much speculation and experiment, detailed consideration of which lies outside the scope of this review. Brief mention might, however, be made that direct phosphorylation with inorganic phosphate in reactions of the type

$$\text{sugar} + H_3PO_4 \rightarrow \text{sugar—phosphate} + H_2O \quad (15)$$

$$2H_3PO_4 \rightarrow H_2O_3P—O—PO_3H_2 + H_2O \quad (16)$$

leads to the difficulty of suggesting plausible dehydrating agents capable of reacting in aqueous solution. A case has been made, to take one example, for considering the cyanide/carbodiimide grouping, much used in synthetic organic chemistry, as a prebiotic condensing agent (17, 41, 57). Hydrogen cyanide is abundantly formed when mixtures of methane, ammonia, water, and hydrogen are sparked, and it reacts with ammonia to give cyanamide, a tautomer of carbodiimide. Note might also be made of the early experiment by Ponnamperuma et al. (80), who demonstrated the formation of adenosine 5'-monophosphate (AMP), ADP, and ATP by subjecting a solution of adenine, ribose, and metaphosphate to ultraviolet irradiation.

Glycolytic Substrate-Linked Phosphorylations

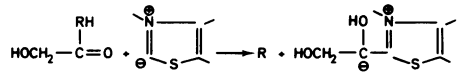
As primitive organisms developed, the supply

of chemically prepared monomers would have diminished, necessitating the elaboration of new biochemical syntheses. Endergonic biosyntheses needed to be coupled to exergonic reactions, for which purpose the pyrophosphate bond became the predominant biochemical energy currency. The simplest response to a need for pyrophosphate bond formation would probably have been the evolution of substrate-linked phosphorylation—alternative energy transduction mechanisms, such as electron transport or photosynthesis, coupled to phosphorylation, are structurally complex in comparison. In addition, the latter mechanisms require proton-impermeable membranes that might have been a handicap in terms of substrate permeability to heterotrophic organisms requiring access to a wide range of substrates. Although substrate-linked phosphorylation could theoretically have developed on any aldehyde-carboxylate conversion, ring formation in aldose phosphates containing more than four carbon atoms would have rendered them less suitable as templates as compared to GAP, which is necessarily acyclic and therefore aldehydic. Hence coupled dehydrogenation of GAP, as a source of pyrophosphate bond energy, may have provided another drain on primitive carbohydrate metabolism. Again, the resulting phosphoglycerate molecule could lead into further immensely important avenues of biochemical evolution, following its isomerization and dehydration to phosphopyruvate (PEP), as we shall see later.

Ribulose Monophosphate Cycle as a Primitive Pathway

In considering the next phase of evolution of carbohydrate metabolism, it may be asked what would have happened upon exhaustion of the pool of prebiotic sugars. It is conceivable that gluconeogenesis could have taken place by reversal of the glycolytic sequence. This process, however, would have required an input of ATP and also a supply of gluconeogenic substrates. This poses the question as to the likely source of such gluconeogenic substrates. We suggest, instead, that the earliest sugar shortage was overcome by evolution of a pathway of net sugar synthesis from formaldehyde similar to the present-day RuMP cycle.

The RuMP cycle has several features to recommend it as a primitive pathway. It is a versatile sequence of reactions. It can serve to synthesize the basic carbohydrate elements of living cells (Fig. 6). It can be assembled into a simple fermentation of formaldehyde to lactate which is ATP yielding (reaction 14). The cycle would have been dependent on a ready supply of formaldehyde, a condition which we have already noted probably existed at that time. Most of the essential chemical reactions involved in the cycle are those encountered in the formose network of reactions, and these (either at the level of free sugars or phosphorylated sugars) could have provided the templates for the development of enzyme catalysts. Indeed, with only one exception, from chemical reactions occurring during the overall formose reaction network, it would be possible to construct a network of reactions occurring with free sugars (Fig. 8) that would be very similar to a RuMP cycle (Fig. 4). (Hexulose synthesis from formaldehyde and ribulose would merely be one of the many possible aldol condensations involving formaldehyde.) The one exception would be the reaction catalyzed by transketolase, whose mechanism is somewhat different to that of an aldolase reaction in that a nucleophilic, rather than electrophilic, center has to be generated at the carbonyl group of the ketol donor (85). This is accomplished by dipolar ion stabilization with thiamine pyrophosphate:



(17)

For these reasons we believe that the RuMP cycle may have constituted a core of primitive carbohydrate metabolism which enabled the early anaerobes to be weaned from complete dependence on the ever-thinning primeval soup.

In looking at the contemporary RuMP cycle, two special points may be made in aside. First, it may seem curious that the first step of the formose network, reaction 1, which is an autocatalytic reaction, has not been developed as an enzymically catalyzed reaction. In fact, $C_1 + C_1$ condensations have, so far, rarely been found to operate in biosynthetic metabolism. Rather, cyclic mechanisms are found in which a multicarbon C_1 acceptor is regenerated; the RuMP, RuDP, and serine pathways are all cases in point. Second, one is continually struck by the existence of chemical templates for reactions of this cycle, e.g., the isomerization of HuMP to FMP is catalyzed by a highly efficient isomerase (30), yet, due to the acyclic nature of HuMP, HuMP isomerizes under alkaline conditions, without enzyme, to a mixture of compounds including FMP (101).

THE ADVENT OF CARBON DIOXIDE FIXATION

The basic assumption which underlies ideas on the evolution of primitive cells is that their chemistry and biochemistry were slowly built up

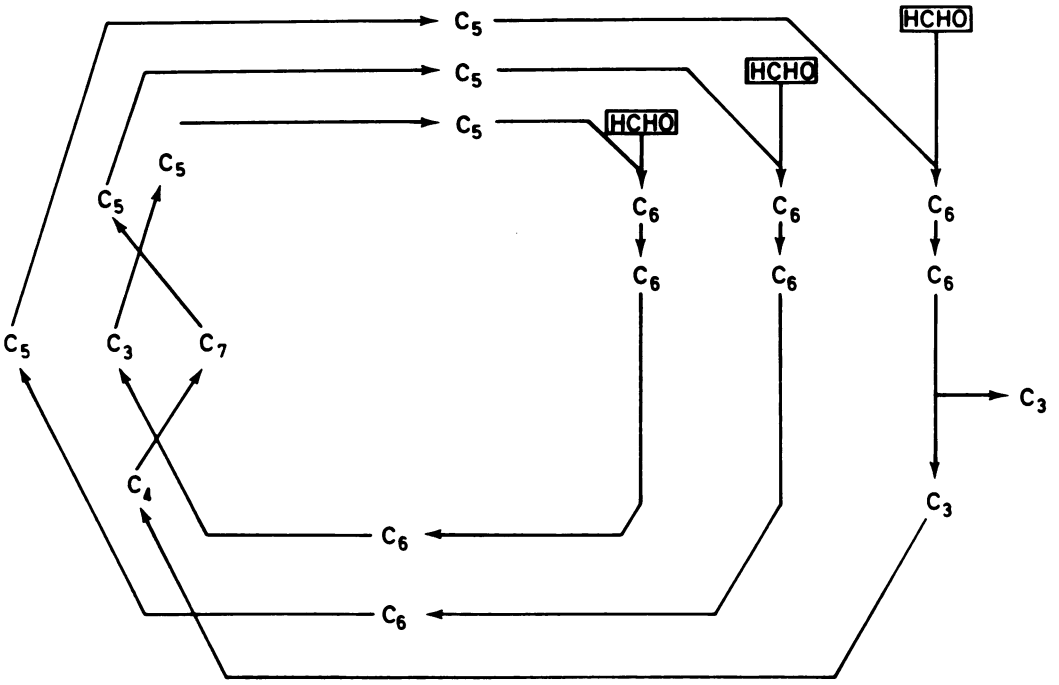
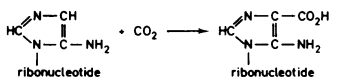


FIG. 8. *Network derived from chemical reactions involved in the formose reaction.*

from the wide variety of chemicals synthesized abiogenically in the primeval soup. As these chemicals were depleted, the primitive cells acquired the ability to replace them with molecules whose formation was catalyzed by enzyme prototypes. This would lead to the first retroevolutionary steps being taken, as outlined by Horowitz (45). Exergonic chemical reactions which proceeded spontaneously, albeit slowly, would surely have been among the first reactions for which catalysts were developed. Catalysis of endergonic reactions would require the extra sophistication of concurrent energy input or coupling to preexisting exergonic reactions. The previous section has developed the theme of how a central core of carbohydrate metabolism might have sprung from chemical reactions of formaldehyde. Further avenues of biochemical evolution lay open to primitive organisms by their acquiring the ability to synthesize additional carbon skeletons from these carbohydrates. An enormous reservoir of carbon in the form of carbonates and CO_2 existed from early times, and use of CO_2 as a biochemical building block, joining it onto other carbon atoms, would short-circuit some of the random chemical processes which replenished the primeval soup with biochemically useful intermediates. It seems reasonable to suppose that the first carboxylation reactions to be so used were exergonic, and hence we should look at contemporary biochemistry and note which carboxylations fall into this category.

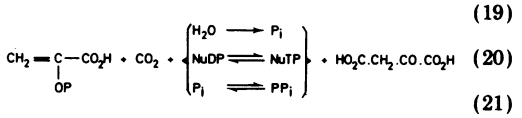
Spontaneous Carboxylations

There are many ways of classifying enzymic carboxylation reactions depending on the particular feature which is being stressed, e.g., cofactor involvement, reaction mechanism, physiological function, etc. Perhaps the most useful from our point of view is that adopted by Calvin and Pon in a penetrating article on carboxylation and decarboxylation reactions published 18 years ago (18). Carboxylation reactions involving C—C bond formation can be divided into those having energy requirements in the form of ATP or a reducing agent and those having no apparent extra energy requirement. Apart from RuDP carboxylase, there are two spontaneous carboxylation reactions which are enzymically catalyzed without additional energy requirements: (i) carboxylation of an aminoimidazole ribonucleotide involved in purine biosynthesis



(18)

and (ii) carboxylation of PEP. This reaction can be subdivided into three types, depending on the acceptor for P_i (106):

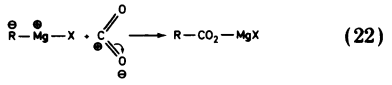


(19)

(20)

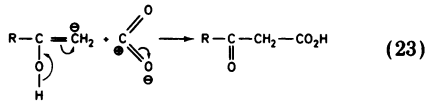
(21)

Reactions 19, 20, and 21 are catalyzed by enzymes which are known, respectively, as PEP carboxylase, PEP carboxykinase, and PEP carboxytransphosphorylase, where Nu equals nucleoside. It will be noted that reactions 18 to 21 involve the carboxylation of an enol derivative (amine or phosphate). This is in accord with the mechanism of carboxylation reactions encountered in organic chemistry in which CO_2 reacts as an electrophilic reagent. The nucleophilic acceptor can take the form of a carbanion as in carboxylation of a Grignard reagent:



(22)

or an enolate:



(23)

The reaction mechanism which operates for PEP carboxykinase and PEP carboxytransphosphorylase probably follows reaction 23, with a reaction intermediate of the type portrayed by Utter and Kolenbrander (106) (Fig. 9). (The reaction mechanism for PEP carboxylase may be somewhat different because of evidence that the reactive species is HCO_3^- rather than CO_2, but this is still under investigation [106].)

We can thus look upon an enol structure as an "active substrate" for carboxylation, the reaction proceeding spontaneously without the need for an additional source of energy. The previous section has discussed the possibility that PEP may have arisen as an intermediate in primitive forms of glycolysis, particularly as it is an attractive compound with which to develop a further substrate-linked phosphorylation. Spontaneous carboxylation of PEP would yield a C_4 skeleton from which synthesis of several other familiar biochemical intermediates could follow, e.g., amino acids of the aspartate family. There would thus have been a strong evolutionary pressure towards development of a catalyst to speed up this spontaneous carboxylation, and, hence, the family of PEP-carboxylating enzymes may well be of ancient lineage.

The third spontaneous carboxylation reaction which is enzymically catalyzed is RuDP carboxylase. It has long been recognized (16) that the reactivity of RuDP towards carboxylation is due to the fact that it is perforce an acyclic sugar phosphate, since its carbonyl group at C_2 cannot participate in pyranose or furanose ring formation. Its structure remains open to enediol formation between C_2 and C_3, thus making carboxylation and hydrolysis a chemical feasibility (Fig. 10). Intensive research by several groups has confirmed the essential correctness of this reaction mechanism (48, 59, 62, 63, 97), although many detailed facets remain to be clarified, e.g., the quaternary structure of the enzyme, the nature of the activation of the enzyme by CO_2 and Mg^{2+}, the relation between, or the identity of, the activating CO_2 molecule and the substrate CO_2 molecule, the nature of the binding of RuDP to the enzyme, and the means by which proton

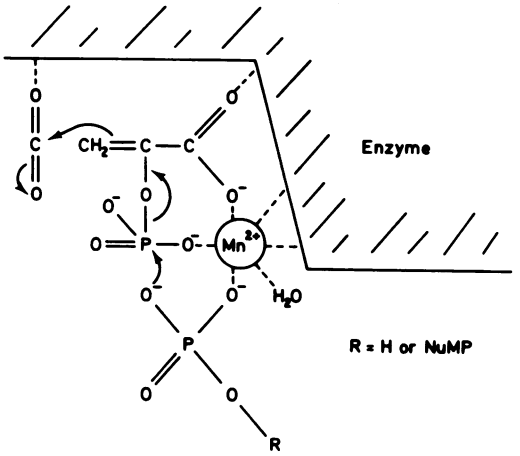


FIG. 9. *Mechanism for reaction catalyzed by PEP carboxykinase and PEP carboxytransphosphorylase.*

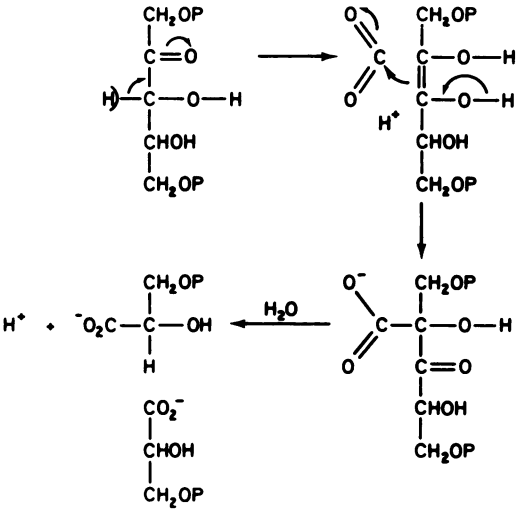


FIG. 10. *Mechanism for reaction catalyzed by RuDP carboxylase.*

release from C_3 is facilitated. For our purpose it is sufficient to point out that RuDP carboxylase catalyzes a carboxylation reaction chemically similar to those catalyzed by PEP carboxykinase and PEP carboxytransphosphorylase, all three reactions being carboxylations of an enolate. We may therefore speculate that these reactions are evolutionarily connected and examine what the implications of this might be.

Evolution of Ribulose Diphosphate Carboxylase

The essential structural feature of RuDP as a substrate for carboxylation is its acyclic form, which arises from preempting of ring formation by the phosphate group on C_5. The phosphate grouping on C_1 does not appear to play an essential chemical role, although presumably it might be involved in binding substrate to the enzyme. A primitive form of this enzyme might conceivably have developed initially with RuMP as substrate and catalyzed the formation of glycerate and phosphoglycerate.

If carboxylation of an enolate was the template for the development of PEP carboxylases and RuDP carboxylase, which kind of carboxylase evolved first (bearing in mind that RuDP carboxylase might have started out as an RuMP carboxylase)? We have suggested that pentose phosphates and PEP might have been intermediates in the core of primitive carbohydrate metabolism. Early selection pressures may have been greater on the development of carboxylation of PEP rather than RuMP or RuDP because it would lead to formation of a serviceable C_4 skeleton upon which could be developed syntheses of amino acids and porphyrins. Carboxylation of RuMP or RuDP would have led to formation of glycerate and/or phosphoglycerate. Breakdown of pentose by CO_2 to such products would appear to offer little as a potential catabolic pathway in comparison with glycolytic reactions because a potential site for substrate-linked phosphorylation, viz., triose phosphate dehydrogenation, would be sidestepped. Selection pressure in favor of RuDP carboxylation would instead come later on as cells developed the coupling of endergonic and exergonic reactions (see next section). It thus seems more likely to us that carboxylation of PEP would have antedated carboxylation of RuDP and a primitive PEP carboxylase might indeed have served as a template for the evolution of RuDP carboxylase as a fitter enzyme.

The fact that RuDP carboxylase and HuMP synthase both catalyze a condensation between C_1 and pentose phosphate and are key enzymes of similar-looking cycles can too easily lead to analogies being drawn which may be oversimplified, e.g., the suggestion that the term *autotrophy* be widened to include organisms capable of utilizing reduced C_1 compounds as the sole carbon source could lead to important biochemical differences being obscured rather than unified (111). From a mechanistic point of view, RuDP carboxylase catalyzes the carboxylation of an enolate while HuMP synthase catalyzes an aldol condensation; the difference between the two has probably kept them evolutionarily separate for a very long time indeed. We have suggested that RuDP carboxylase may have evolved from PEP carboxylases; the question as to whether electrophilic addition of CO_2 to an ene-diol and electrophilic addition of a carbonyl group to an activated methylene group ultimately arose from a common ancestral gene, as raised by McFadden (64), takes us back further still and remains open.

Evolution of the Ribulose Diphosphate Cycle

The RuDP cycle is a highly endergonic sequence of reactions (Tables 2 and 3), and biochemical development of this cycle would depend on a ready supply of reducing power and high-energy phosphate. The former would have been available from the reducing environment, but the latter may have taken a long period of biochemical evolution to appear. The advent of organic substrate-linked phosphorylation would not be sufficient in itself because there is no selective advantage for an organism to develop a metabolism based on autotrophy at the expense of heterotrophy when reduced carbon growth substrates are available. Pyrophosphate bond formation coupled to exergonic inorganic reactions and photosynthetic phosphorylation could have provided the thrust towards autotrophy. The use of the energy in the pyrophosphate bond, together with the use of reducing power, would enable endergonic reactions to be catalyzed and hence open the way to an increasing range of biosyntheses. Of central importance for our consideration is the reversal of triose phosphate dehydrogenase, i.e., reduction of a carboxyl group to an aldehyde group. When this could be catalyzed in a cell, the way would be open for feeding the core of primitive carbohydrate metabolism from a new direction instead of from formaldehyde.

The lack of selection pressure towards development of RuDP carboxylation, as compared to carboxylation of PEP, has already been mentioned; the ability to reduce phosphoglycerate to triose phosphate would transform this situation. It would enable the bridging of two proc-

esses: (i) the carboxylation of ribulose (di)phosphate to an intermediate highly susceptible to hydrolytic breakdown to two (phospho)glycerate molecules with (ii) a set of preexisting reactions which could interconvert triose phosphate and pentose phosphate. The bridging would open the way to a net synthesis of carbohydrate from the abundant carbon dioxide and hence be strongly selected for (Fig. 11). Templates for process (i) might already have existed in the PEP carboxylases; process (ii) would already have formed part of the core of primitive carbohydrate metabolism.

If RuMP instead of RuDP had been the substrate for a primitive carboxylase, it would have been necessary to phosphorylate a glycerate molecule in order to return both glycerate skeletons to the main network of carbohydrate metabolism. The energetics of such a cycle, which might be termed a RuMP cycle of CO_2 fixation, would be identical with that of a RuDP cycle, where the necessary phosphorylation takes place with pentose phosphate as substrate. Final selection of RuDP as the carboxylation substrate may have been governed by the advantages in cycle regulation, which phosphorylation of pentose phosphate might offer. Similarly, later adoption of the variant shown in Fig. 1 might also have been dictated by the advantages of having another irreversible enzyme in the cycle as a target for control. It is well-known that in the chloroplast, control of the cycle is effected by modulation of RuDP carboxylase, phosphoribulokinase, and fructose-1,6-diphosphatase (108).

We must suppose that the first autotrophs were anaerobes using energy from light or from inorganic redox reactions, as in present day photosynthetic bacteria or chemoautotrophs. Initially, the first photosynthetic bacteria may have used light energy only to generate high-energy phosphate by a system akin to cyclic photophosphorylation, the reducing power for biosynthesis coming from the many reducing agents present in the primitive environment. Olson has suggested (73) that the bacterial counterpart of noncyclic electron transport gradually moved towards the more negative part of the redox scale in a series of small mutational steps in response to a need for more powerful reductants in environments lacking H_2. The withdrawal of electrons for reducing purposes would have resulted in oxidation of the electron donor compounds such as sulfide and ammonia to sulfate and nitrate. The development of this line of adaptation might have led to one of the greatest single advances in biochemical evolution, viz., biological photolysis of water to oxygen. The

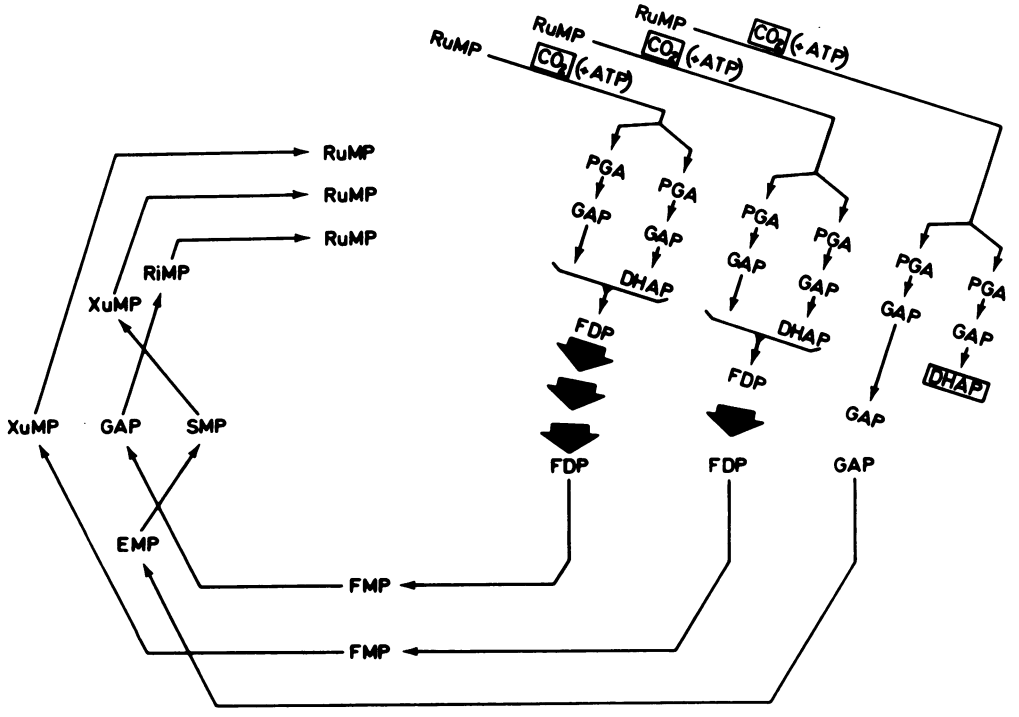


FIG. 11. *Possible evolution of RuDP cycle.*

impact of this on the development of aerobic metabolism lies outside the scope of this review.

Sulfate and nitrate are used extensively by contemporary microorganisms as electron acceptors in anaerobic respiration (dissimilatory reduction). Could such processes have featured in the evolution of primitive pre-photosynthetic organisms or did the bulk of the Earth's sulfate and nitrate arise from the activity of photosynthetic organisms? To the best of our knowledge, there is no direct evidence from the geological record as to whether sulfate and nitrate would have been available to pre-photosynthetic organisms at a period of 4 Gy or more ago. There is every thermodynamic reason to argue against their presence under a reducing atmosphere. It therefore seems reasonable to suppose that formation of sulfate and nitrate followed the evolution of photosynthetic organisms capable of oxidizing sulfide to sulfate (75), perhaps ammonia to nitrite (73), and, finally, water to oxygen. Broda has generalized such views (11, 12), but mention should nevertheless be made of the differing view of Egami (28) that formation and metabolism of nitrate could have antedated photosynthesis.

PRESENT-DAY RELATIONSHIPS BETWEEN METHYLOTROPHY AND AUTOTROPHY

In this article we have tried to present a logical sequence of chemical and biochemical evolution which might have led from primeval soup chemistry to the RuDP cycle by way of the RuMP cycle as a staging post. During this long sequence, extending over 3 Gy, many convergences and divergences must have occurred, which make the assignment of evolutionary relationships between present-day methylotrophs and autotrophs a complex and fascinating study. Much further experimental work needs to be done before some of the relationships are clarified or even become evident. Two examples of methylotrophy/autotrophy relationships between present-day organisms offering particular promise for further study may be mentioned.

The close morphological and biochemical relationships between the obligate methane utilizers and the nitrifying bacteria have frequently been stressed (81, 87, 99, 111). They both possess complex internal membrane assemblies responsible for oxidation of their very restricted range of substrates. They possess an incomplete tricarboxylic acid cycle, which further decreases their already limited metabolic versatility. They both oxidize their energy sources, CH_4 and NH_3, respectively, by mixed function oxidases. The methane mono-oxygenases (of at least some methane utilizers) are also capable of oxidizing ammonia to nitrite. Some of the methane utilizers use the RuMP cycle as a main carbon assimilation pathway, whereas the nitrifying bacteria use the RuDP cycle. Furthermore, a highly interesting finding that extracts of methane-grown *M. capsulatus* (Bath) contain low activities of RuDP carboxylase (9.4 nmol of CO_2 fixed per min per mg of protein) and phosphoribulokinase in addition to high activities of HuMP synthase (306 nmol/min per mg of protein) has been made recently by Taylor (103). The relative distribution of the RuDP carboxylase and the HuMP synthase between cytoplasm and particulate fraction makes it unlikely that the two activities are due to one enzyme. At the present time, the physiological role of RuDP carboxylase in this organism (and any other organisms found to resemble it) is not known, but this finding already opens up a new and important interrelationship between methylotrophy and autotrophy.

The second example concerns the autotrophic utilization of reduced C_1 compounds. Despite the relatively high cost in energy of assimilating reduced C_1 compounds by oxidizing them to carbon dioxide and fixing the latter autotrophically (Table 3), organisms are known which do this, e.g., *Alcaligenes* FOR1 (19), *Pseudomonas oxalaticus* (83, 84), and *Thiobacillus novellus* (20) during aerobic growth on formate; *Rhodopseudomonas palustris* during photosynthetic growth on formate (100); *Micrococcus denitrificans* during aerobic growth on methanol (24); and *Rhodopseudomonas acidophila* during photosynthetic growth on methanol (91). All but the first of these organisms are facultative autotrophs, and it may be that acquisition of the ability merely to oxidize C_1 compounds would, without the elaboration of further biosynthetic enzymes, confer upon such organisms the capability of growth on these compounds. The gain in nutritional versatility, at such little cost in synthesizing extra enzymes, probably outweighs the disadvantage of using an energetically unfavorable pathway. These organisms are probably autotrophs by design and methylotrophs by accident, and it may well be that a proper search will reveal that many more autotrophs can carry out this type of metabolism.

It should be emphasized again that in this article we have only been considering one net C_1 assimilation sequence, the RuMP cycle, as a possible template for evolution of autotrophy based on the RuDP cycle. We have not considered the position of the serine pathways, but if our thesis is accepted that there is a logical sequence from the formose reaction network to a primitive core of carbohydrate metabolism including the RuMP cycle, then it is unlikely

that the serine pathways would have led from thence to the RuDP cycle. Neither have we considered the position of the methanogenic anaerobes, some of which can grow on reduced C_1 compounds by fermenting them to methane and CO_2 and all of which can grow on H_2 and CO_2 by fermenting them to CH_4 (104, 112), because the nature of their assimilatory pathways is not yet known. When they are known, consideration of their evolutionary position will be most interesting, particularly as the methanogenic bacteria can obviously now all be classified as autotrophs.

CODA

Searching for "primitive" organisms may be even less realistic than searching remote swamps for specimens of *Tyrannosaurus rex,* the time span is so large and microorganisms are so adaptable (26). Nevertheless, bearing in mind the excitement caused by the finding of a possible microbial "monster" resembling the Precambrian microfossil *Kakabekia umbellata* lurking beneath the walls of Harlech Castle in Wales (96), it may still be worth asking if there is some microorganism which, if discovered, might be considered reminiscent of the primitive organisms portrayed in this article. A strict anaerobe performing a lactate fermentation of formaldehyde (reaction 14) would be one such case. Although its isolation would be technically difficult, involving growth on formaldehyde under strict carbon limitation in an anaerobic chemostat, some effort might nevertheless be worthwhile, because a successful outcome would show a metabolic pattern that might indeed be unmistakably primitive.

ACKNOWLEDGMENTS

We thank the many authors who let us have copies of manuscripts before publication. We are also very grateful for helpful advice from M. Calvin, C. D. Curtis, I. R. Kaplan, M. Schidlowski, D. A. Walker, and M. Whyte.

LITERATURE CITED

1. **Abelson, P. H.** 1966. Chemical events on the primitive earth. Proc. Natl. Acad. Sci. U.S.A. **55**:1365–1372.
2. **Abelson, P. H.** 1970. Prebiotic organic syntheses, p. 187–196. *In* L. Margulis (ed.), Origins of life. Gordon and Breach, New York.
3. **Anthony, C.** 1975. The biochemistry of methylotrophic micro-organisms. Sci. Progr. (London) **62**:167–206.
4. **Anthony, C.** 1978. The prediction of growth yields in methylotrophs. J. Gen. Microbiol. **104**:91–104.
5. **Babel, W., and K. Hofmann.** 1975. Korrelation zwischen assimilatorischen methylgruppenstoffwechsel und citrat zyklus bei obligat methylotrophen bakterien. Z. Allg. Mikrobiol. **15**:53–57.
6. **Babel, W., and D. Miethe.** 1974. Alternative zur Reaktionsfolge des allulose-6-phosphat-weges bei sinen methylotrophen Bakterium. Z. Allg. Mikrobiol. **14**:153–156.
7. **Bassham, J. A.** 1971. Photosynthetic carbon metabolism. Proc. Natl. Acad. Sci. U.S.A. **68**:2877–2882.
8. **Bassham, J. A.** 1971. The control of photosynthetic carbon metabolism. Science **172**: 526–534.
9. **Bellion, E., and J. Woodson.** 1975. Enzymes of C_1-metabolism in *Pseudomonas* MA-isofunctional isocitrate lyase, p. 81–90. *In* Microbial growth on C_1-compounds. Proceedings of the International Symposium on Microbial Growth on C_1 Compounds, Tokyo. Society of Fermentation Technology, Osaka, Japan.
10. **Beuscher, N., and G. Gottschalk.** 1972. Lack of citrate lyase—the key enzyme of the reductive carboxylic acid cycle—in *Chlorobium thiosulphatophilum* and *Rhodospirillum rubrum.* Z. Naturforsch. Teil B **27b**:967–973.
11. **Broda, E.** 1975. The beginning of photosynthesis. Origins Life **6**:247–251.
12. **Broda, E.** 1976. Evolution of the bioenergetic processes. Pergamon Press, Inc., Elmsford, N.Y.
13. **Buchanan, B. B., and R. Sirevåg.** 1976. Ribulose-1,5-diphosphate carboxylase and *Chlorobium thiosulphatophilum.* Arch. Microbiol. **109**:15–19.
14. **Butlerow, M. A.** 1861. Formation synthétique d'une substance sucrée. C. R. Acad. Sci. **53**:145–147.
15. **Cairns-Smith, A. G., and G. L. Walker.** 1974. Primitive metabolism. Curr. Mod. Biol. **5**:173–186.
16. **Calvin, M.** 1954. Chemical and photochemical reactions of thioctic acid and related disulfides. Fed. Proc., Fed. Am. Soc. Exp. Biol. **13**:697–711.
17. **Calvin, M.** 1969. Chemical evolution. Oxford University Press, Oxford.
18. **Calvin, M., and N. G. Pon.** 1959. Carboxylations and decarboxylations. J. Cell. Comp. Physiol. **54**:(Suppl. 1):51–74.
19. **Chandra, T. S., and Y. I. Shethna.** 1976. Isolation and characterization of some new formate utilizing bacteria. Curr. Sci. **45**:653–656.
20. **Chandra, T. S., and Y. I. Shethna.** 1977. Oxalate, formate, formamide, and methanol metabolism in *Thiobacillus novellus.* J. Bacteriol. **131**:389–398.
21. **Charalampous, F. C.** 1954. Mechanism of formation of erythrulose-1-phosphate by phosphoketotetrose aldolase of rat liver. J. Biol. Chem. **211**:249–262.
22. **Colby, J., and L. J. Zatman.** 1975. Tricarboxylic acid cycle and related enzymes in restricted facultative methylotrophs. Biochem. J. **148**:505–511.
23. **Colby, J., and Zatman, L. J.** 1975. Enzymological aspects of the pathways for trimethylamine oxidation and C_1 assimilation in obligate methylotrophs and restricted facultative methylo-

trophs. Biochem. J. 148:513–520.
24. **Cox, R. B., and J. R. Quayle.** 1975. The autotrophic growth of *Micrococcus denitrificans* on methanol. Biochem. J. **150**:569–571.
25. **Cox, R. B., and L. J. Zatman.** 1974. Hexose phosphate synthase in trimethylamine-grown *Bacterium* 4B6, a facultative methylotroph. Biochem. J. **141**:605–608.
26. **De Ley, J., and K. Kersters.** 1975. Biochemical evolution in bacteria, p. 1–77. *In* M. Florkin and E. H. Stotz (ed.), Comprehensive biochemistry 29B. Elsevier Publishing Co., Amsterdam.
27. **Dische, Z., and E. Landsberg.** 1960. A colorimetric procedure for the determination of triose phosphate and fructose-1,6-diphosphate in presence of other sugars. Biochim. Biophys. Acta **39**:144–147.
28. **Egami, F.** 1976. Comment on E. Broda's recent publications. J. Mol. Evol. **8**:387–388.
29. **Evans, M. C. W., B. B. Buchanan, and D. I. Arnon.** 1966. A new ferredoxin-dependent carbon reduction cycle in a photosynthetic bacterium. Proc. Natl. Acad. Sci. U.S.A. **55**:928–934.
30. **Ferenci, T., T. Strøm, and J. R. Quayle.** 1974. Purification and properties of 3-hexulose phosphate synthase and phospho-3-hexuloisomerase from *Methylococcus capsulatus*. Biochem. J. **144**:477–486.
31. **Ferenci, T., T. Strøm, and J. R. Quayle.** 1975. Oxidation of carbon monoxide and methane by *Pseudomonas methanica*. J. Gen. Microbiol. **91**:79–91.
32. **Fox, S. W., and K. Dose.** 1972. Molecular evolution and the origin of life. W. H. Freeman, San Francisco.
33. **Fujii, T., Y. Asada, and K. Tonomura.** 1974. Assimilative pathway of methanol in *Candida* sp. Incorporation of ^{14}C-methanol, ^{14}C-formaldehyde, ^{14}C-formate and ^{14}C-bicarbonate into cell constituents. Agric. Biol. Chem. **38**:1121–1127.
34. **Fujii, T., and K. Tonomura.** 1973. Hexosephosphate as an intermediate in the assimilation of methanol by *Candida* sp. Agric. Biol. Chem. **37**:447–449.
35. **Gabel, N. W., and C. Ponnamperuma.** 1967. A model for origin of monosaccharides. Nature (London) **216**:453–455.
36. **Garrison, W. M., D. H. Morrison, J. G. Hamilton, A. A. Benson and M. Calvin.** 1951. Reduction of carbon dioxide in aqueous solutions by ionising radiation. Science **114**:416–418.
37. **Getoff, N., G. Scholes, and J. Weiss.** 1960. Reduction of carbon dioxide in aqueous solutions under the influence of radiation. Tetrahedron Lett. **18**:17–23.
38. **Goldberg, I., J. S. Rock, A. Ben-Bassat, and R. I. Mateles.** 1976. Bacterial yields on methanol, methylamine, formaldehyde and formate. Biotechnol. Bioeng. **18**:1657–1668.
39. **Groth, W., and H. Suess.** 1938. Bemerkungen zur photochemie der Erdatmosphäre. Naturwissenschaften **26**:77.
40. **Haldane, J. B. S.** 1929. The origin of life. The Rationalist Annual, p. 3–10.
41. **Halmann, M., R. A. Sanchez, and L. E. Orgel.** 1969. Phosphorylation of D-ribose in aqueous solution. J. Org. Chem. **34**:3702–3.
42. **Hersh, L. B.** 1975. Growth of *Pseudomonas* MA on methylamine, p. 73–80. *In* Microbial growth on C_1-compounds. Proceedings of the International Symposium on Microbial Growth on C_1-Compounds, Tokyo. Society of Fermentation Technology, Osaka, Japan.
43. **Hofmann, K., J. Sawistowsky, and W. Babel.** 1975. Einfluss definierter substrat limitationen auf das enzym-und metabolitmuster des methylotrophen bakteriums *Pseudomonas* W6. Z. Allg. Mikrobiol. **15**:599–604.
44. **Horecker, B. L., O. Tsolas, and C. Y. Lai.** 1972. Aldolases, p. 213–258. *In* P. D. Boyer (ed.), The enzymes, 3rd ed., vol. 7. Academic Press Inc., New York.
45. **Horowitz, N. H.** 1945. On the evolution of biochemical syntheses. Proc. Natl. Acad. Sci. U.S.A. **31**:153–157.
46. **Horowitz, N. H., and S. L. Miller.** 1962. Current theories on the origin of life. Fortschr. Chem. Org. Naturst. **20**:423–459.
47. **Hoyle, F., and N. C. Wickramasinghe.** 1977. Polysaccharides and infrared spectra of galactic sources. Nature (London) **268**:610–612.
48. **Jensen, R. G., and J. T. Bahr.** 1977. Ribulose 1,5-bisphosphate carboxylase-oxygenase. Annu. Rev. Plant Physiol. **28**:379–400.
49. **Johnson P. A., and J. R. Quayle.** 1965. Microbial growth on C_1 compounds. Synthesis of cell constituents by methane- and methanol-grown *Pseudomonas methanica*. Biochem. J. **95**:859–867.
50. **Kato, N., H. Ohashi, T. Hori, Y. Tani, and K. Ogata.** 1977. Properties of 3-hexulose phosphate synthase and phospho-3-hexuloisomerase of a methanol-utilizing bacterium, 77a. Agric. Biol. Chem. **41**:1133–1140.
51. **Kato, N., H. Ohashi, Y. Tani, and K. Ogata.** 1978. 3-Hexulosephosphate synthase from *Methylomonas aminofaciens* 77a. Purification, properties and kinetics. Biochim. Biophys. Acta **523**:236–244.
52. **Kelly, D. P.** 1971. Autotrophy: concepts of lithotrophic bacteria and their organic metabolism. Annu. Rev. Microbiol. **25**:177–210.
53. **Kemp, M. B.** 1972. The hexose phosphate synthetase of *Methylococcus capsulatus*. Biochem. J. **127**:64P–65P.
54. **Kemp, M. B.** 1974. Hexose phosphate synthase from *Methylococcus capsulatus* makes D-*arabino*-3-hexulose phosphate. Biochem. J. **139**:129–134.
55. **Kemp, M. B., and J. R. Quayle.** 1966. Microbial growth on C_1 compounds. Incorporation of C_1 units into allulose phosphate by extracts of *Pseudomonas methanica*. Biochem. J. **99**:41–48.
56. **Kemp, M. B., and J. R. Quayle.** 1967. Microbial growth on C_1 compounds. Uptake of [^{14}C]-formaldehyde and [^{14}C]formate by methane-grown *Pseudomonas methanica* and determination of the hexose labelling pattern after

brief incubation with [^{14}C]methanol. Biochem. J. **102**:94–102.
57. **Kenyon, D. H., and G. Steinman.** 1969. Biochemical predestination. McGraw-Hill Book Co., New York.
58. **Krebs, H. A., and H. L. Kornberg.** 1957. A survey of the energy transformations in living matter. Ergeb. Physiol. **49**:212–298.
59. **Laing, W. A., and J. T. Christeller.** 1976. A model for the kinetics of activation and catalysis of ribulose 1,5-bisphosphate carboxylase. Biochem. J. **159**:563–570.
60. **Lawrence, A. J., M. B. Kemp, and J. R. Quayle.** 1970. Synthesis of cell constituents by methane-grown *Methylococcus capsulatus* and *Methanomonas methanooxidans*. Biochem. J. **116**:631–639.
61. **Löb, W.** 1908. Die einwirkung der stillen electrischen entladung auf feuchtes methan. Ber. Dsch. Chem. Ges. **41**:87–90.
62. **Lorimer, G. H., M. R. Badger, and T. J. Andrews.** 1976. The activation of ribulose-1,5-bisphosphate carboxylase by carbon dioxide and magnesium ions. Equilibria, kinetics, a suggested mechanism, and physiological implications. Biochemistry **15**:529–536.
63. **McFadden, B. A.** 1973. Autotrophic CO_2 assimilation and the evolution of ribulose diphosphate carboxylase. Bacteriol. Rev. **37**:289–319.
64. **McFadden, B. A.** 1976. Evolution of C_1-metabolism with a focus on CO_2 utilization, p. 267–280. *In* H. G. Schlegel, G. Gottschalk, and N. Pfennig (ed.), Symposium on microbial production and utilization of gases (H_2, CH_4, CO). Göttingen. Akademie der Wissenschaften, Göttingen.
65. **Meyerhof, O., K. Lohmann, and P. Schuster.** 1936. Über die Aldolase, ein Kohlenstoff-verkuiippendes Ferment. I. Mitteilung: Aldolkondensation von Dioxyacetonphosphorsäure mit Acetaldehyd. Biochem. Z. **286**:301–318.
66. **Miller, S. L.** 1953. A production of amino acids under possible primitive earth conditions. Science **117**:528–529.
67. **Miller, S. L.** 1955. Production of some organic compounds under possible primitive earth conditions. J. Am. Chem. Soc. **77**:2351–2361.
68. **Miller, S. L.** 1957. The mechanism of synthesis of amino acids by electric discharges. Biochim. Biophys. Acta **23**:480–489.
69. **Miller, S. L., and L. E. Orgel.** 1974. The origins of life on the earth. Prentice-Hall, Inc., Englewood Cliffs, N.J.
70. **Mizuno, T., and A. H. Weiss.** 1974. Synthesis and utilization of formose. Adv. Carbohydr. Chem. Biochem. **29**:173–227.
71. **Mueller, G. C., E. M. Quinn, and R. R. Rueckert.** 1955. Formation of 1-phosphoerythrulose-4-C^{14} by homogenates of Swiss chard leaves. Arch. Biochem. Biophys. **55**:408–414.
72. **Noltmann, E. A.** 1972. Aldose-ketose isomerases, p. 271–354. *In* P. D. Boyer (ed.), The enzymes, 3rd ed., vol. 6. Academic Press Inc., New York.
73. **Olson, J. M.** 1970. The evolution of photosynthesis. Science **168**:438–446.
74. **Orgel, L. E.** 1973. The origins of life: molecules and natural selection. Chapman and Hall, London.
75. **Peck, H. D.** 1974. The evolutionary significance of inorganic sulfur metabolism, p. 241–262. *In* M. J. Carlile and J. J. Skehel (ed.), Evolution in the microbial world. 24th Symposium of the Society for General Microbiology, Imperial College, London. Cambridge University Press, Cambridge.
76. **Ponnamperuma, C.** 1965. A biological synthesis of some nucleic acid constituents, p. 221–242. *In* S. W. Fox (ed.), The origins of prebiological systems and of their molecular matrices. Academic Press Inc., New York.
77. **Ponnamperuma, C.** 1972. The origins of life. Thames and Hudson, London.
78. **Ponnamperuma, C.** 1972. Exobiology. *In* A. Neuberger and E. L. Tatum (ed.), Frontiers of biology 23. North Holland Publishing Co., Amsterdam.
79. **Ponnamperuma, C., and N. W. Gabel.** 1974. The precellular evolution and organization of molecules, p. 393–413. *In* M. J. Carlile and J. J. Skehel (ed.), Evolution in the microbial world. 24th Symposium of the Society for General Microbiology, Imperial College, London. Cambridge University Press, Cambridge.
80. **Ponnamperuma, C., C. Sagan, and R. Mariner.** 1963. Synthesis of adenosine triphosphate under possible primitive earth conditions. Nature (London) **199**:222–226.
81. **Quayle, J. R.** 1972. The metabolism of one-carbon compounds by micro-organisms. Adv. Microb. Physiol. **7**:119–203.
82. **Quayle, J. R.** 1975. Unsolved problems in the microbial metabolism of methane and methanol, p. 59–65. *In* Microbial growth on C_1-compounds. Proceedings of the International Symposium on Microbial Growth on C_1-Compounds, Tokyo. Society of Fermentation Technology, Osaka, Japan.
83. **Quayle, J. R., and D. B. Keech.** 1959. Carbon assimilation by *Pseudomonas oxalaticus* (OX1). I. Formate and carbon dioxide utilization during growth on formate. Biochem. J. **72**:623–630.
84. **Quayle, J. R., and D. B. Keech.** 1959. Carbon assimilation by *Pseudomonas oxalaticus* (OX1). 2. Formate and carbon dioxide utilization by cell-free extracts of the organism grown on formate. Biochem. J. **72**:631–637.
85. **Racker, E.** 1961. Transketolase, p. 397–406. *In* P. D. Boyer, H. Lardy, and K. Myrbäch (ed.), The enzymes, 2nd ed., vol. 5. Academic Press Inc., New York.
86. **Reid, C., and L. E. Orgel.** 1967. Synthesis of sugars in potentially prebiotic conditions. Nature (London) **216**:455.
87. **Ribbons, D. W., J. E. Harrison, and A. M. Wadzinski.** 1970. Metabolism of single carbon compounds. Annu. Rev. Microbiol. **24**:135–158.
88. **Ribbons, D. W., and A. M. Wadzinski.** 1976. Oxidation of C_1 compounds by particulate frac-

tions from *Methylococcus capsulatus,* p. 359–369. *In* H. G. Schlegel, G. Gottschalk, and N. Pfennig (ed.), Symposium on microbial production and utilization of gases (H_2, CH_4, CO), Göttingen. Akademie der Wissenschaften, Göttingen.

89. **Rutten, M. G.** 1971. The origin of life by natural causes. Elsevier Publishing Co., Amsterdam.
90. **Sahm, H.** 1977. Metabolism of methanol by yeasts. Adv. Biochem. Eng. **6**:77–103.
91. **Sahm, H., R. B. Cox, and J. R. Quayle.** 1976. Metabolism of methanol by *Rhodopseudomonas acidophila.* J. Gen. Microbiol. **94**:313–322.
92. **Sahm, H., H. Schütte, and M-R. Kula.** 1976. Purification and properties of 3-hexulosephosphate synthase from *Methylomonas* M15. Eur. J. Biochem. **66**:591–596.
93. **Schidlowski, M.** 1976. Archaean atmosphere and evolution of the terrestrial oxygen budget, p. 525–535. *In* B. Windley (ed.), The early history of the earth. John Wiley & Sons, Inc., New York.
94. **Schopf, J. W.** 1972. Exobiology. *In* A. Neuberger and E. L. Tatum (ed.), Frontiers of biology 23. North Holland Publishing Co., Amsterdam.
95. **Schopf, J. W.** 1974. Paleobiology of the precambrian: the age of blue-green algae. Evol. Biol. **7**:1–43.
96. **Siegel, S. M., and C. Giumarro.** 1966. On the culture of a microorganism similar to the Precambrian microfossil *Kakabekia umbellata* (Barghoorn) in NH_3-rich atmospheres. Proc. Natl. Acad. Sci. U.S.A. **55**:349–353.
97. **Siegel, M. I., M. Wishnick, and M. D. Lane.** 1972. Ribulose-1,7-diphosphate carboxylase, p. 169–192. *In* P. D. Boyer (ed.), The enzymes, 3rd ed., vol. 6. Academic Press Inc., New York.
98. **Sirevåg, R., B. B. Buchanan, J. A. Berry, and J. H. Troughton.** 1977. Mechanisms of CO_2 fixation in bacterial photosynthesis studied by the carbon isotope fractionation technique. Arch. Microbiol. **112**:35–38.
99. **Smith, A. J., and D. S. Hoare.** 1977. Specialist phototrophs, lithotrophs, and methylotrophs: a unity among a diversity of procaryotes. Bacteriol. Rev. **41**:419–448.
100. **Stokes, J. E., and D. S. Hoare.** 1969. Reductive pentose cycle and formate assimilation in *Rhodopseudomonas palustris.* J. Bacteriol. **100**:890–894.
101. **Strøm, T., T. Ferenci, and J. R. Quayle.** 1974. The carbon assimilation pathways of *Methylococcus capsulatus, Pseudomonas methanica* and *Methylosinus trichosporium* (OB3B) during growth on methane. Biochem. J. **144**:465–476.
102. **Swenson, C. A., and R. Barker.** 1971. Proportion of keto and aldehyde forms in solutions of sugars and sugar phosphates. Biochemistry **10**:3151–3154.
103. **Taylor, S.** 1977. Evidence for the presence of ribulose-1,5-bisphosphate carboxylase and phosphoribulokinase in *Methylococcus capsulatus* (Bath). FEMS Lett. **2**:305–307.
104. **Thauer, R. K., K. Jungermann, and K. Decker.** 1977. Energy conservation in chemotrophic anaerobic bacteria. Bacteriol. Rev. **41**:100–180.
105. **Tonge, G. M., D. E. F. Harrison, and I. J. Higgins.** 1977. Purification and properties of the methane mono-oxygenase enzyme system from *Methylosinus trichosporium* OB3b. Biochem. J. **161**:333–344.
106. **Utter, M. F., and H. M. Kolenbrander.** 1972. Formation of oxaloacetate by carbon dioxide fixation on phosphoenolpyruvate, p. 117–168. *In* P. D. Boyer (ed.), The enzymes, 3rd ed., vol. 6. Academic Press Inc., New York.
107. **van Dijken, J. P., and W. Harder.** 1975. Growth yields of micro-organisms on methanol and methane. A theoretical study. Biotechnol. Bioeng. **17**:15–30.
108. **Walker, D. A.** 1977. Regulatory mechanisms in photosynthetic carbon metabolism. Curr. Top. Cell Regul. **11**:203–241.
109. **Walker, J. F.** 1964. Formaldehyde. Reinhold, New York.
110. **Wawszkiewicz, E. J., and H. A. Barker.** 1968. Erythritol metabolism by *Propionibacterium pentosaceum.* J. Biol. Chem. **243**:1948–1956.
111. **Whittenbury, R., and D. P. Kelly.** 1977. Autotrophy: a conceptual phoenix, p. 121–149. *In* B. A. Haddock and W. A. Hamilton (ed.), Microbial energetics. 27th Symposium of the Society for General Microbiology, Imperial College, London. Cambridge University Press, Cambridge.
112. **Zeikus, J. G.** 1977. The biology of methanogenic bacteria. Bacteriol. Rev. **41**:514–541.
113. **Zuckerman, B.** 1977. Interstellar molecules. Nature (London) **268**:491–495.