

The Fourth Symposium of
THE SOCIETY FOR
GENERAL MICROBIOLOGY

edited by

B. A. FRY & J. L. PEEL

**AUTOTROPHIC
MICRO-
ORGANISMS**

AUTOTROPHIC MICRO-ORGANISMS

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CONTRIBUTORS

- BAALSRUD, K., Biological Laboratory, University of Oslo.
- BISSET, K. A., Department of Bacteriology, University of Birmingham.
- BLINKS, L. R., Hopkins Marine Station of Stanford University,
Pacific Grove, California.
- BUTLIN, K. R., Microbiology Section, Chemical Research Laboratory,
Teddington, Middlesex.
- ELSDEN, S. R., Department of Microbiology, University of Sheffield.
- FOGG, G. E., Department of Botany, University College, London.
- GAFFRON, H., Institute of Radiobiology and Biophysics, University
of Chicago, Chicago, Illinois.
- GRACE, J. B., Department of Bacteriology, University of Birmingham.
- LARSEN, H., Department of Chemistry, Norges Tekniske Högskole,
Trondheim, Norway.
- LASCELLES, J., Microbiology Unit, Department of Biochemist
University of Oxford.
- LEES, H., Department of Biological Chemistry, University of Aberdeen.
- MEIKLEJOHN, J., Rothamsted Experimental Station, Harpenden,
Hertfordshire.
- POSTGATE, J. R., Microbiology Section, Chemical Research Labor-
atory, Teddington, Middlesex.
- SYRETT, P. J., Department of Botany, University College, London.
- WASSINK, E. C., Laboratory of Plant Physiological Research,
Agricultural University, Wageningen, Holland.
- WOLFE, M., Department of Botany, University College, London.
- WOODS, D. D., Microbiology Unit, Department of Biochemistry,
University of Oxford.
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EDITORS' PREFACE

This Symposium on 'Autotrophic Micro-organisms' has been organized along lines somewhat similar to the three previous symposia promoted and published by the Society for General Microbiology. Contributors were invited to write authoritative articles on specific subjects within the field of study of autotrophic micro-organisms. Their articles, which are collected together in this book, form the basis of short lectures by the contributors at the London meeting (April 1954). This volume is thus primarily intended to provide the background for the verbal communications and subsequent discussions, but the Editors believe that it will also prove useful as an account of the present position and trends in this expanding field of knowledge.

The period available for the preparation and editing of the individual contributions has been limited, and it has not been possible to allow authors to make major alterations or additions at the proof stage. The Editors apologize for any shortcomings or inaccuracies which may have arisen as a result.

Some contributors to the Symposium describe the nutritional type of a micro-organism in terms of a nomenclature, based on energy sources, which was put forward at a symposium held at Cold Spring Harbor in 1946. Since this terminology may be unfamiliar to many readers, it is reproduced on p. xi.

B. A. FRY
J. L. PEEL

*Department of Microbiology and Agricultural Research
Council Unit for Microbiology
University of Sheffield
9 November 1953*

NOMENCLATURE OF NUTRITIONAL TYPES OF MICRO-ORGANISMS BASED ON ENERGY SOURCES*

- A. PHOTOTROPHY—energy provided chiefly by photochemical reaction.
 - 1. *Photolithotrophy*—growth dependent on exogenous inorganic hydrogen-donors.
 - 2. *Photo-organotrophy*—growth dependent on exogenous organic hydrogen-donors.
- B. CHEMOTROPHY—energy provided entirely by dark chemical reaction.
 - 1. *Chemolithotrophy*—growth dependent on oxidation of exogenous inorganic substances.
 - 2. *Chemo-organotrophy*—growth dependent on oxidation or fermentation of exogenous organic substances.
- C. PARATROPHY—energy provided by host cell.

* (From *Heredity and Variation in Micro-organisms*, Cold Spr. Harb. Symp. quant. Biol. 11, 302 (1946).)

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THE NO MAN'S LAND BETWEEN THE AUTOTROPHIC AND HETEROTROPHIC WAYS OF LIFE

D. D. WOODS AND JUNE LASCELLES

*Microbiology Unit, Department of Biochemistry,
University of Oxford*

It is our task to introduce the subject of this Symposium simply because those with expert knowledge (and who are able to be with us) are naturally making their own contributions in the more specialized papers which follow. It was felt, however, that the Symposium should begin with some more general statement of the particular problems of the autotrophic way of life, and of its relationship to the way of life of the heterotrophic organisms with which the majority of microbiologists are more familiar. For it is certainly true, purely from the more statistical point of view, that autotrophic life among micro-organisms has received far less attention than its intrinsic interest merits; to balance this it has attracted some of the pioneers—Beijerinck, Winogradsky, Kluver, van Niel amongst others—in the development of general microbiology. The comparative lack of intensive research on this aspect of microbiology is not easy to understand. It was clear almost from the time of the discovery of the microbes concerned that their economic importance, especially in soil fertility and the cycle of nitrogen, sulphur and carbon in nature, was probably great. They were obviously organisms of the widest biochemical versatility likely to provide novel and interesting information. Their possible significance from the evolutionary point of view was often debated, yet such debate was handicapped by the rather scanty knowledge available concerning the basic life processes of many of the organisms. Happily, as the remainder of this Symposium will show, there is now developing an intense interest; the relatively few pioneers of the past may hope to find many more of their brilliant researches, and the fascinating organisms they discovered, subjected to detailed study at all the levels of investigation now available to the microbiologist.

It is presumably incumbent upon the first contributors to this discussion to attempt to explain or define at least the title of the Symposium. This is no easy matter; workers in this field have long been agreed that there is no sharp line of demarcation between, for example, autotrophic

bacteria and the rest of the bacterial kingdom. For reasons that will become clear later, we would ourselves prefer to speak of the autotrophic mode of life among micro-organisms rather than of autotrophic micro-organisms. The *ability* to live autotrophically is, as far as is known at present, more common than the *necessity* to live autotrophically. The word autotroph taken literally would seem to mean 'self-nourishing' or 'self-sustaining'; this is not very helpful without an implied qualification that the independence is in respect of more elaborate forms of carbon compounds. Only in recent years, with the discovery that certain photosynthetic bacteria are able to live with carbon dioxide as the only source of carbon and gaseous nitrogen as the only source of nitrogen, do we seem to have organisms with at least no visible means of support!

The autotrophic way of life could be defined as the ability to live and multiply on indefinite subculture in an environment containing carbon dioxide as the sole source of carbon; it necessarily follows that inorganic sources of nitrogen, sulphur and other elements are also sufficient. Such a definition implies a very high synthetic ability on the part of the organism since all new organic cell material must be fabricated ultimately from carbon dioxide and inorganic material. The definition may indeed be considered too exclusive. Is an organism to be said not to be living autotrophically simply because it cannot synthesize for itself from carbon dioxide minute traces of one or more substances of the vitamin type, which must therefore be supplied preformed? Such organisms do exist (e.g. certain strains of *Hydrogenomonas* and of *Athiorhodaceae* when living autotrophically); by all other criteria they are living a typically autotrophic existence, and it would seem reasonable to amend the phrase 'sole source of carbon' in the above definition to 'main or bulk source of carbon'. This is only one example of the sort of trouble that is met if any rigid definition is attempted; others will become obvious as the discussion proceeds. Werkman (1951) has indeed suggested that it is better to sense the differentiation between autotrophy and heterotrophy than to define it. Our task unfortunately is to convey this sense to others who, like ourselves, are not active workers in the field; in the absence of facilities for telepathic communication the only method would seem to be to put up a temporary definition of convenience as a general basis for discussion.

One matter should perhaps be made clear at this stage. Green plants, which photosynthesize higher carbon compounds from carbon dioxide, normally live a typically autotrophic life (at any rate as concerns bulk carbon nutrition). Photosynthesis is not, however, in any way tied to the autotrophic life; there are many bacteria which live heterotrophically

with the aid of photosynthetic processes and which certainly do not use carbon dioxide as the bulk ultimate source of carbon.

There are a number of excellent and brief accounts of the history, general physiology and biochemistry of autotrophic bacteria which it would be foolish to try to duplicate here (van Niel, 1943, 1944, 1949; Umbreit, 1947, 1951; Foster, 1951; Stephenson, 1949; Koffler & Wilson, 1951; Gest, 1951). The more general implications of these organisms in the biochemical approach to the problem of evolution amongst micro-organisms has been fascinatingly discussed by Lwoff (1944), Knight (1945) and van Niel (1949) among others. Lwoff (1944) has also attempted to classify and subdivide further the types of life process found in various micro-organisms, though he strongly emphasizes the absence of clear-cut boundaries. It seemed, therefore, that it would be best for us to point out, as simply as possible, what appear to be the main problems of the autotrophic life and how these may differ (if at all) from those of the heterotrophic life which most of us know much better. For a more detailed characterization of these problems we shall concern ourselves mainly with the properties of bacteria which have either the ability to live both heterotrophically and autotrophically, or which, in one way or another, seem to occupy the no man's land between the two ways of life. Some of these properties, now existing perhaps singly in isolated species, may, when combined in a single organism at some earlier phase of evolution, have dictated the way of life of the organism in a particular environment at that time.

The main problems to be solved seem to us to be biochemical in nature. For reasons of space we must use bacteria as main examples of the points we wish to make. In the course of a wide survey it is inevitable that we shall have to make general statements which may not be in accord with every fact gleaned from every bacterium. We can only hope to provide a very general picture which later contributors will expand and modify from their own special knowledge. In other words, we shall attempt mainly to provide a row of pegs on which later contributors may hang their hats or umbrellas. One difficulty we have is that pegs should be a rather permanent installation, whereas people may frequently purchase new hats and umbrellas, or even accidentally acquire those of other people! In order to keep the list of references within reasonable bounds we shall, whenever it is appropriate, cite one or other of the several excellent reviews as a source also of the original literature.

SOME PROBLEMS OF AUTOTROPHIC LIFE

The ability to use carbon dioxide as the main (if not the only) source of carbon for cell multiplication requires that the organism must have certain special biochemical abilities. This does not mean that all these abilities are peculiar to autotrophic organisms; some are certainly possessed by typical heterotrophs. Others whose nature is not yet known may well be possessed by other organisms. It is presumably only the combination of all such abilities in a single species which permits the autotrophic life, and it is for this reason that each such biochemical activity should be examined in detail wherever it may be found.

Firstly, the organism must have the enzyme systems necessary for bringing about an initial reaction resulting in the formation from carbon dioxide of a compound with two or more carbon atoms. This may need the actual enzymic activation of carbon dioxide itself, or only the formation or activation of some organic compound which then reacts spontaneously with carbon dioxide. At first sight it might seem that the bulk utilization of carbon dioxide requires the power to create carbon to carbon bonds between two C_1 residues. This may indeed be the case, but, on the other hand, it is necessary to remember that any culture must start from at least one organism which in turn contains many preformed higher carbon compounds. A cyclical process could therefore be envisaged (Lipmann, 1946; Calvin, 1949; Gaffron, Fager & Rosenberg, 1951; Buchanan *et al.* 1952) in which, for example, C_1 units are successively added to a pre-existing carbon chain and the original compound regenerated after splitting off the new carbon chains so built up. Similarly, even if there is *de novo* formation of C_2 units from C_1 units, such a cyclic mechanism might then proceed with the primary C_2 unit as acceptor. There is therefore no theoretical necessity for the bulk formation of carbon to carbon bonds between single carbon atoms, though it must be emphasized that this may happen.

Secondly, carbon dioxide presents the carbon atom to the cell in a highly oxidized form. On the average the carbon atoms of cell material are much less oxidized than in carbon dioxide. Their state in this respect is often taken to be represented by the empirical formula $[CH_2O]$ (cf. $[CHO_3]$ for the bicarbonate ion); van Niel (1936), however, has found evidence that in at least some organisms in the general group under discussion the average state of the carbon atom is even more reduced, and is more accurately represented by $[C_2H_3O]$. It is clear, therefore, that the overall utilization of carbon dioxide for growth requires linkage with active reducing systems, i.e. some oxidation process must occur

which is coupled to the overall reduction of carbon dioxide to new cell constituents. This does not mean of course that the primary reaction undergone by carbon dioxide is necessarily a reductive one, although this may seem probable. Detailed examples of such coupled oxidative reactions will be considered later in connexion with energy requirements.

The third special problem of autotrophic life concerns the overall energy requirement for growth. Presumably more energy will be required for each new cell produced than with an organism living heterotrophically. The required reduction of the carbon atoms of carbon dioxide is an endergonic process (except when molecular hydrogen is the reducing agent); furthermore, a far greater number of new carbon to carbon bonds must be created than in heterotrophic life where more complex carbon compounds must be provided preformed. Both heterotrophs and autotrophs will of course require energy-yielding reactions for coupling to the systems which synthesize other cell constituents from the C_2 , C_3 and C_4 compounds which are usually considered to be the key 'building stones'.

In biological systems the energy-yielding reactions are in the main oxidations. Oxidations of appropriate substrates could theoretically also serve as hydrogen donor systems for the reduction of carbon dioxide. Conversely, any substrate that the cell can use for the latter purpose must itself undergo oxidation and is therefore a potential source of energy to the cell. Whether or not it is an effective source of energy for autotrophic life presumably depends on the cell being able to bring about energy transfer from one system to the other, a process which, by analogy with similar events in yeast and animal tissues, would require a definite chemical link and possibly the intermediate formation of high-energy phosphate esters.

There are two main types of energy source for autotrophic life. In chemosynthetic autotrophy the oxidation of an inorganic substrate (usually rather specific for a given organism) provides both the necessary reducing system and the energy for growth on a medium containing carbon dioxide or bicarbonate as the sole source of carbon. Typical examples are given in Table 1; these organisms are usually known as the chemosynthetic- or chemo-autotrophs, but not all of them (see later) are confined to this way of life. The amount of substrate oxidized is much greater than would be required for the reduction of the amount of carbon dioxide actually incorporated into cell material. Some other bulk hydrogen acceptor is therefore required; in most cases this is molecular oxygen (that is, the organisms are strict aerobes when growing in this way), but there are anaerobic organisms known in which

nitrate (*Thiobacillus denitrificans*) and sulphate (*Desulphovibrio desulphuricans*) serve this purpose. Inorganic substances oxidized by various organisms include ammonia, nitrite, sulphur compounds (e.g. hydrogen sulphide, sulphur itself, sodium thiosulphate), hydrogen and ferrous iron. There has always been a little doubt about the autotrophic status of some of the iron-oxidizing microbes (Pringsheim, 1949), partly due to the difficulty of discriminating between biological oxidation of ferrous hydroxide and its spontaneous oxidation at normal pH values. A clear-cut case is, however, provided by the recent isolation (Temple & Colmer, 1951) of *Thiobacillus ferro-oxidans*, which is able to grow in acid media where spontaneous oxidation is very slow. There appears to be no case

Table 1. *Examples of bacteria able to grow chemo-autotrophically*

Organism	Substrates oxidized	Oxidant	End-product of oxidation	Reference
<i>Thiobacillus thio-oxidans</i>	Sulphur, thiosulphate and other inorganic S compounds	Oxygen	Sulphate	(1), (2)
<i>Thiobacillus denitrificans</i>	As above	Nitrate*	Sulphate	(1), (2)
<i>Nitrosomonas</i> spp.	Ammonia	Oxygen	Nitrite	(1)
<i>Nitrobacter</i> spp.	Nitrite	Oxygen	Nitrate	(1)
<i>Thiobacillus ferro-oxidans</i>	Ferrous salts or thiosulphate	Oxygen	Ferric salts or sulphate	(3)
<i>Hydrogenomonas</i> spp.	Hydrogen	Oxygen	Water	(1)
<i>Desulphovibrio desulphuricans</i>	Hydrogen	Sulphate and other inorganic S compounds	Sulphide	(4)

* Nitrate is reduced to molecular nitrogen during oxidation of sulphur compounds.

References: (1) Reviews of the literature in van Niel (1943), Stephenson (1949) and Foster (1951); (2) Bunker (1936); (3) Temple & Colmer (1951); (4) Butlin, Adams & Thomas (1949).

so far discovered of an organism using the oxidation of a higher carbon compound solely as reducing system and energy source for chemo-autotrophic growth, that is, with no liberation of carbon dioxide from substrate oxidation and no incorporation of carbon other than exogenous carbon dioxide.

A second group of micro-organisms is able to live autotrophically when supplied with radiant energy; all such organisms contain a chlorophyll pigment which is essential for the photosynthetic life. From the point of view of the present discussion we shall concern ourselves mainly with bacteria which can live photo-autotrophically. Such bacteria, although deriving their energy from light, still require the presence of an inorganic substrate (sulphur compounds or hydrogen) whose oxida-

tion provides the necessary reducing system for the overall carbon dioxide-fixation process. The organisms can live in this way anaerobically, and the amount of inorganic substrate oxidized is relatively small and in accord with the amount of carbon dioxide reduced and fixed by the cell. It might be thought that energy derived from this anaerobic oxidation might meet at least part of the total energy requirements, providing of course that the organism has the appropriate energy-transfer mechanisms. van Niel (1941) has pointed out, however, that such a contribution of energy, even if utilizable, is likely to be relatively small. The amount of substrate (e.g. hydrogen sulphide) oxidized could only account for fixation of one-eightieth of the total carbon dioxide actually fixed, assuming that the overall efficiency of the chemosynthesis were similar to that found with chemosynthetic organisms oxidizing the same substrate. With the photosynthetic autotroph *Chlorobium thiosulphatophilum* (which can utilize several substrates as reducing agent), Larsen, Yocum & van Niel (1953) have found that the amount of carbon dioxide fixed is largely independent of the nature of the oxidizable substrate, though the energy potentially available differs with the substrate. It is clear, therefore, that in photosynthetic autotrophy the bulk of the energy requirement for growth comes from radiant energy.

In photosynthesis by the higher plants, algae and other micro-organisms containing chlorophyll (e.g. diatoms) no extraneous oxidizable substrate is required to form the reducing system. The necessary overall reduction is made possible by the fact that the organisms are able to dispose of oxygen atoms through the evolution of molecular oxygen. The process is analogous in a way to the ability of a number of heterotrophic bacteria to split off molecular hydrogen and thus to carry out certain oxidations in the absence of any final hydrogen-acceptor. The evolution of oxygen is characteristic of plant photosynthesis, but does not occur with bacteria; hence the need for an oxidizable substance. Possible explanations for the differences between the photosynthetic process in bacteria and plants could not be made clear without detailed discussion at a biochemical level of current knowledge and thought concerning the mechanism of the primary photo-induced reaction; these matters will no doubt be dealt with by other contributors to the Symposium. There is, however, general agreement with van Niel's concept (1941) that the processes are fundamentally similar, and that photolysis of water provides the ultimate reducing system in plants and possibly the immediate reducing system in bacteria, though here an ultimate reducing system is required. Bacterial chlorophyll, except possibly that of green sulphur bacteria, differs slightly from plant

chlorophyll in chemical structure and in its absorption spectrum (see van Niel, 1944; Rabinowitch, 1951); in consequence photosynthesis is carried out by bacteria with light of a longer wavelength, and the absorption of each quantum would raise the energy level of the receptor molecule to a less extent than in plants. This may ultimately account for the observed differences in detail in the two processes.

In the succeeding sections we shall examine the ability of various bacteria which can only live heterotrophically to utilize metabolites (especially carbon dioxide) which are particularly implicated in autotrophic life. We shall then consider points which arise from the study of organisms which can live both autotrophically and heterotrophically, drawing our main examples from the photosynthetic bacteria and the so-called hydrogen bacteria.

HETEROTROPHIC METABOLISM OF SUBSTANCES USED IN AUTOTROPHIC LIFE

Utilization of carbon dioxide in heterotrophic life

It was mentioned earlier that the ability to utilize exogenous carbon dioxide, and even to incorporate its carbon atom into cell material, was not confined to organisms living autotrophically. Heterotrophic carbon dioxide fixation has been intensively studied and the results have been exhaustively and critically reviewed by Utter & Wood (1951) and by Ochoa (1951). It is proposed here to select points which seem germane to the present discussion.

Primary fixation reactions

There are now known a number of overall reactions by which carbon dioxide is thought to be incorporated into carbon compounds containing two or more carbon atoms by bacteria living heterotrophically; the principal types of reaction are shown in Table 2. The relative simplicity of the overall chemical changes and the fact that most of them have been demonstrated with cell-free enzyme preparations, if not purified enzymes, suggests that these may be primary carbon dioxide-fixation reactions, though there is certainly not complete agreement about this. Most of them have so far been shown to occur only by isotope exchange experiments; these demonstrate the potentiality of the cell to incorporate carbon dioxide by the reaction in question, but not of course that the intact cell does so to a significant extent.

Several points call for comment from the present standpoint. There is so far no definite enzymic reaction known in which two molecules of carbon dioxide (or indeed any two C_1 compounds) are condensed to

give a C_2 compound; all the known reactions require a preformed carbon chain of at least two atoms as acceptor.

In each of the reactions of Table 2 the carbon atom of carbon dioxide appears in the carboxyl (COOH) group of the product. The carbon atom has therefore undergone one stage of reduction, but is still considerably more oxidized than in the cell as a whole. This does not of course militate in any way against a carboxylation reaction as a primary fixation reaction in autotrophic life.

Table 2. *Some proposed overall reactions for heterotrophic assimilation of carbon dioxide by bacteria*

Type of reaction	Overall reaction	Experimental system	Reference
(1) $C_2 + C_1$	(a) $H_2 + CO_2 \rightleftharpoons \text{formate} + \text{acetate} \rightleftharpoons \text{pyruvate}$	Suspensions of <i>Bact. coli</i>	(1)
	(b) $H_2 + CO_2 + \text{acetate} \rightleftharpoons \text{pyruvate}$	Extracts of <i>Cl. butylicum</i>	(1)
(2) $C_3 + C_1$	(a) $\text{Pyruvate} + CO_2 \rightleftharpoons \text{oxaloacetate}$	Extracts of <i>M. lysodeikticus</i>	(1)
		Extracts of <i>Bact. coli</i> and <i>P. morganii</i>	(2)
	(b) $\text{Pyruvate} + CO_2 + \text{DPN}_{\text{red.}}^* \rightleftharpoons \text{malate} + \text{DPN}_{\text{ox.}}$	Extracts of <i>Lb. arabinosus</i>	(1)
	(c) $\text{Propionate} + CO_2 \rightleftharpoons \text{succinate}$	Suspensions of <i>Pr. pentosaceum</i>	(3)
(3) $C_4 + C_1$	$\text{Succinate} + CO_2 \rightleftharpoons \alpha\text{-ketoglutarate}$	Extracts of <i>Bact. coli</i>	(1)
(4) $C_5 + C_1$	$\alpha\text{-Ketoglutarate} + CO_2 + \text{TPN}_{\text{red.}}^* \rightleftharpoons \text{isocitrate} + \text{TPN}_{\text{ox.}}$	Extracts of <i>Esch. freundii</i>	(4)

* DPN and TPN=di- and tri-phosphopyridine nucleotide coenzymes respectively; ox.=oxidized form, red.=reduced form.

References: (1) Utter & Wood (1951); full references and a critical evaluation of the evidence will be found in this review. (2) Kaltenbach & Kalnitsky (1951*a, b*); *de novo* formation of oxaloacetate demonstrated in these systems. (3) Barban & Ajl (1951); for critical discussion of CO_2 fixation by *Propionibacterium* see also (1) and Leaver & Wood (1953). (4) Barban & Ajl (1953).

None of these reactions, as far as is known, results in the overall utilization by the microbes concerned of significant quantities of exogenous carbon dioxide as a source of carbon for new cell material. There is, however, no doubt that such reactions are of critical importance to the growth of the culture. Carbon dioxide (in traces) has long been known as an essential factor for the initiation of growth of certain bacteria living heterotrophically (Gladstone, Fildes & Richardson, 1935; see also review by Knight, 1945). There is evidence from nutritional experiments that the function of carbon dioxide may be in the initial synthesis of dicarboxylic acids such as glutamic acid, aspartic acid and their probable precursors, α -ketoglutaric and oxaloacetic acid (Lwoff &

Monod, 1947; Ajl & Werkman, 1948; Lyman, Moseley, Wood, Butler & Hale, 1947); this may well result from carboxylation reactions such as (2a), (2b) and (3) of Table 2. Similar experiments implicate carbon dioxide, perhaps less directly, at some stage in the synthesis by certain lactic acid bacteria of other amino-acids such as arginine, tyrosine and serine (Lyman *et al.* 1947; Lascelles, Cross & Woods, 1954; Cross, 1953), and in the synthesis of purine derivatives by *Streptococcus haemolyticus* (Pappenheimer & Hottle, 1940).

What evidence is there that the primary reaction undergone by carbon dioxide in autotrophic metabolism may be a carboxylation of the type listed in Table 2? With autotrophic bacteria there is almost no evidence on this point, but with algae, workers certainly seem in recent years to lean towards this idea. Later contributors will no doubt analyse the matter in detail, but briefly there is strong evidence for condensation of carbon dioxide with a C_2 compound as the primary reaction (Calvin, 1949; Gaffron *et al.* 1951). The carbon atom of the carbon dioxide appears primarily in the carboxylic group of phosphoglyceric acid, which is the product of the condensation. The nature of the C_2 acceptor is unknown, though it is not acetate or a derivative as in reaction (1) of Table 2. Cell suspensions of a photosynthetic bacterium (*Rhodospirillum rubrum*) have been found to incorporate isotope-labelled carbon dioxide into phosphoglyceric acid when incubated in hydrogen (Glover, Kamen & van Genderen, 1952), but the significance of the reaction in growth is not clear. Calvin & Massini (1952) also have evidence for the condensation of carbon dioxide with a C_3 compound to yield malate (possibly as in reaction (2)) as an early, and perhaps primary, reaction in photosynthesis by algae; they propose that such a C_3 - C_1 condensation follows the C_2 - C_1 condensation in the cycle of reactions by which the original C_2 compound is thought to be regenerated. Vishniac & Ochoa (1952a, b) have shown that illuminated chloroplast fragments can bring about the reduction of the phosphopyridine nucleotide coenzymes, and that this system can be coupled *in vitro* to a reductive carboxylation reaction (such as (2b) of Table 2) catalysed by enzymes from other sources; overall therefore, in this artificial system, the carbon of carbon dioxide is reduced and condensed with an acceptor molecule, the energy being ultimately obtained from light. A malic enzyme system from plant sources has also been found to be active in such a reconstruction (Tolmach, 1951; Arnon, 1951). It is not suggested that this is the primary carbon dioxide-fixation mechanism in plant photosynthesis (the reaction as so far demonstrated is slow), but only that it indicates interesting possibilities.