Book Reviews

The Carbon Reduction Cycle and Synthetic Reactions

The Photosynthesis of Carbon Compounds. Melvin Calvin and J. A. Bassham. Benjamin, New York, 1962. xii + 127 pp. Illus. Paper, $5.50.

Reviewing this book would be a simpler task if its title were "Organic Syntheses Associated With the Carbon Reduction Cycle in Unicellular Algae: An Enquiry Based Mainly on Experiences Gained at the Department of Chemistry, University of California, Berkeley." Such a title would circumscribe the contents rather precisely and would allow the reviewer to state that the book provides more than the title promises. On several occasions the authors touch on themes that go beyond their own immediate practical experience, and they put forward some interesting speculation on new pathways of intermediary metabolism, which, if correct, will be important for the interpretation of that process in all organisms known to contain a pentose carboxylation mechanism. By contrast, the present all-embracing title is likely to give the expert a feeling of disappointment with the contents of the book and the lay-scientist or the uninitiated bio-chemistry student an unwarranted belief that the book covers most of what he ought to know about the subject.

I

Let us first look at the book from the narrower viewpoint of the title suggested above. A quarter of a century ago, after the discovery of enzymatic fixation and reduction of carbon dioxide in nonphotosynthetic bacteria, it became clear that the study of photosynthesis ought to be divided into two classes of problems—the photochemistry of pigments and the reduction of carboxyl groups. And the latter might not be, strictly speaking, a photochemical process at all. The discovery and elucidation of the fantastically complex carbon fixation mechanism, now known as the Calvin-Benson cycle, confirmed the view of photosynthesis as a combination between two quite dissimilar components. Now we have learned that among several loosely bound partial systems, which together constitute the mechanism of photosynthesis, the fixation and reduction of carbon dioxide may be the one most remote from the photochemistry proper. The authors rightly point out that "Biosynthetic reactions in plants cannot be classified as photosynthetic or nonphotosynthetic on the basis of direct photochemical action, because all reactions in the photosynthetic pathways are probably 'dark' reactions." Since experiments with extracts have shown that the fixation of carbon dioxide will proceed when TPNH and ATP (triphosphopyridine nucleotide and adenosine triphosphate) are added to the proper enzyme system in the dark, they propose to call photosynthetic all reactions that occur in the chloroplasts whenever "the conversion of light energy results in the formation of ATP and TPNH and perhaps other unknown factors. When these cofactors are formed by the light reaction and are used to bring about the synthesis of carbon compounds, we may consider the reactions to be photosynthetic."

The book is divided into a dozen short chapters: "Carbon reduction cycle of photosynthesis"; "Evidence for the carbon reduction cycle"; "The carboxylation reactions"; "Balance among synthetic pathways"; "Photosynthesis vs. other forms of biosynthesis"; "Amino acid synthesis"; "Carboxylic acids"; "Carbohydrates"; "Fats"; "Pigments"; "Aromatic nuclei"; "Other biosynthetic products"; and "References." Some chapters are no longer than a page, thus containing no more information than necessary to make a point. They are preceded by a prologue and introduction intended to bring the discussion that follows into proper focus within the large field of photosynthesis. There is a quick retelling of such familiar stories as the biochemical formation of light-absorbing pigments, in particular of porphyrins; the point is made that succinate and glycine, the starting materials for porphyrin synthesis, originate in the carbon cycle.

In addition, the book contains 40 pages on which are reprinted three of the authors' original publications that they consider fundamental. Unfortunately, special references are not inserted into the main text to call the reader's attention to specific problems that are discussed in these reprints. (Appending reprints of, or selected pages from, the original announcements of important discoveries to a survey type monograph should be emulated, if the book is intended for students. Next to work in the laboratory, reprints of original work convey the best impression of what science in the making is like.)

These chapters are the frame and background for the main theme: namely the question of whether the intermediates of the carbon reduction cycle can be used for specific synthetic side reactions in an extracurricular manner (as it were) the moment they become available. Tracer carbon experiments have repeatedly shown that many simple compounds appear very quickly, or simultaneously, with the sugar phosphates in illuminated cells. The most interesting pages of the book contain speculations on the existence of new, more direct, and energetically more economical pathways for the synthesis of amino acids (serine, alanine, and aspartic acid), carboxylic acids, and fats under conditions in which reducing compounds, energy-rich phosphate derivatives, and intermediates of the reduction cycle are all available in high concentration—that is, in the light. Glycolic acid is formed preferentially at low carbon dioxide pressures, and in tracer experiments both carbons appear equally labeled. This is discussed rather thoroughly, and a scheme is shown which describes the pathways from the carbon reduction cycle to glycolic acid and acetylphosphate. (A misprint in figure 7 may be more troublesome to the student than the one in equation 4, on page 6. The latter is obvious, while in figure 7 it is not immediately clear whether acetyl or glycolyl phosphate is intended to be on the designated spot.)

The present discussion on the kinetics and the mechanism of the appearance of malic acid, equally-labeled glycolic acid, and the amino acids mentioned above is reminiscent of the early days when it became necessary to account for the quick labeling of the entire molecule of phosphoglyceric acid (PGA), which obviously required a short metabolic cycle of some kind. Not until 1953, after several years of debate about the merits of one rather two carboxylations in the cycle, were the attempts given up to solve the problem on the basis of C_4 dicarboxylic acids.

This older history is here condensed into one sentence: "The carbon reduction cycle in essentially the form shown in Figure 2 was mapped during the period between 1946 and 1953." For the carboxylation reaction with ribulose diphosphate, there was no analogy, and it had therefore not been anticipated. The same was true for the particular form of carbohydrate rearrangements which came to light with Benson's discovery of C_5 and C_7 sugars among the earliest products of carbon incorporation. It is quite possible that the continuation of the new investigations described here will bring more such surprises.

All the foregoing can be summarized by saying the book will serve the young biochemist as an adequate introduction to a complex of problems centered around the famous discoveries made in the authors' laboratory and also as a guide to promising new enquiries on the course of photobiological syntheses.

II

In view of the book's title, however, and its far-reaching propositions about the synthetic capacities of chloroplasts, several topics must be mentioned, which the book might easily have considered, but which it does not. This lack is surprising, because references to additional very pertinent information are found in several of the annual review volumes. And it would seem important to have the authors' opinion on these matters.

The number of scientific publications has been increasing in an exasperating way. It is understandable that, within the frame of a short original paper, it is virtually impossible to cover the literature in the manner considered proper a generation ago. Thus, scientists are now willing to accept such an excuse as the one offered in the reprint on page 79: "We shall not here even try to outline all of the various forms of evidence which have been adduced in support of such a scheme, but only to point out additional bits which have been added in recent years, and particularly those which stem from our own work." The more so, this should not be the motto governing the writing of even a small monograph. The student who cannot afford one of the larger handbooks hopes to find a balanced selection of references when he buys a book of 125 pages, which deals with one particular subject of general importance.

A main point the authors want to make is "that chloroplasts synthesize a complete spectrum of biochemical products, all of which might reasonably be considered to be photosynthetic products." The key to these wide-ranging syntheses of carbon compounds in the light is the carbon reduction cycle, its detailed mechanism, and its exclusive location in the chloroplasts.

A few years ago Kandler and Gibbs discovered that sugar phosphates emerging from the cycle are not symmetrically labeled. Richter found no aldolase in photosynthesizing blue-green algae. These blue-green algae have no chloroplasts, merely an inner envelope of widely spaced lamellae, but they are obligately photoautotrophic, while most of the chloroplast-containing plants are not. Other interesting observations on the appearance of organic substances in assimilating algae are concerned with the role of nitrogen supply as a switch from sugar and protein to fat production, that of carbon dioxide supply on the appearance of decarboxylations following a short period of intense illumination, and that of phosphorylation in directing the flow of light energy into alternate pathways when sugars are given to assimilating algae. None of this is discussed or referred to.

But more important even is the occurrence of the carbon reduction cycle in nonphotosynthesizing but autotrophic organisms like *Thiobacillus denitrificans*, another topic which is not mentioned. Obviously the key mechanism is not the exclusive property of pigmented structures. Any source of ATP and reduced coenzymes can set it running.

We face not merely a semantic quibble but a true problem of intermediary metabolism when we compare syntheses made in the dark with those made in the light. The authors do not mention the fact that algae like *Chlorella* can grow beautifully in the dark, pigments, chloroplasts and all, using one single source of carbon-glucose or acetate. The only difference, apart from rates, between growth that takes place in the dark and growth that takes place in the light is the ultimate source of carbon, which is in one case sugar and in the other carbon dioxide. Similarly Spoehr showed many years ago that higher plants can grow to maturity and can reproduce, if given sugars. Thus, the old definition—"the primary function of photosynthesis is to form carbohydrate only"—was a very good operational one. In a mature leaf, the photosynthetic apparatus acts very nearly like a sugar factory, but on the other hand, it has been obvious and has often been pointed out that a microorganism which multiplies when the light is on must, during this time, synthesize all other compounds as well. And whenever the rate of growth approaches the rate of photosynthesis (a function of light intensity) the tracer carbon must appear rapidly in a host of other organic compounds.

III

Is this a question of the chloroplast saying to the mitochondrium "Anything you can do I can do better?" Isolated chloroplasts or their fractions must be prodded into organic photosynthetic activities by an additional supply of assorted cofactors and enzymes, so easily do they lose such vital components. Shall we believe that an entire set of enzymes which belong exclusively to the chloroplast becomes useless when night falls, while the cell as a whole grows and divides rather preferentially during this time? In unicellular algae, the lamellae or the chloroplast occupy a very large portion of the cell. Perhaps one should turn the question around and ask: Which among the synthetic pathways so preponderant during periods of illumination are still being used, though at a diminished rate, when, in absence of light, the respiring mitochondria have to provide ATP and reduced coenzymes?

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