COMPARATIVE BIOCHEMISTRY OF PHOTOSYNTHESIS

A Report of the Seminar Held in Gwatt, Switzerland, 21–26 July 1969

Helmut METZNER

Institut für Pflanzenphysiologie der Universität, Auf der Morgenstelle, 14 Tübingen, Germany

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The Institutes of Organic Chemistry and Plant Physiology of the University of Bern and the Institute of Chemical Plant Physiology of the University of Tübingen jointly organized this small seminar. There were 30 participants from Belgium, Bulgaria, Germany, Great Britain, and Switzerland. Like the seminar at Freudenstadt (August 1968) this latest one was intended to encourage a group of young scientists and graduate students to study some selected publications and to discuss their own research problems in the informal atmosphere of a small resort. The organizers had mixed some outstanding papers with contributions containing inconsistent data, contradictions, weak arguments, and false conclusions. The ensuing discussions demonstrated that the participants really appreciated this unusual procedure and they quite soon understood how to find the weak points of our present-day hypotheses.

Since it would be impolite to quote the sources of the good and bad information discussed, the following paragraphs only summarize the essentials of the debates and the most important suggestions for further studies. They will not refer to the many methodological questions which were raised nor to yet unconfirmed observations which some speakers reported.

To concentrate the debates only two topics of photosynthesis research were chosen, the very first and the very last steps on the pathway to carbohydrates. The primary event is the quantum absorption by the pigment molecules of the green plant cell, the final step the synthesis of sugar molecules.

The first day was devoted to the key chromatophore pigment chlorophyll *a*. We know that all organisms which perform a complete photosynthesis starting from water protolysis possess this porphyrin pigment, the synthesis of which from δ-aminolevulinic acid is rather well understood. Nevertheless it is still controversial whether we have to assume more than one form of chlorophyll *a*. Spectroscopic measurements have repeatedly shown that the *in vivo* spectrum of green cells or isolated chloroplasts is much more complicated that the absorption spectrum of a molecular solution. It still has to be decided whether this observation reflects the existence of several pigment aggregates of different sizes or the existence of more than one form of chlorophyll *a*.

Several methods are available for extracting chlorophyll-protein complexes from cell material. This can be done either by purely physical disruption of the cells or by their treatment with surface-active agents like sodium dodecyl sulphate or Triton X-100. Studies on the photochemical behaviour of such complexes demonstrate that the isolated particles only give an electron transfer from suitable electron donors to ferredoxin but no water-splitting reaction. The system catalyzing the light-induced evolution of molecular oxygen seems to be bound to structural elements which cannot be isolated in the form of defined particles. In spite of the many different methods which have been used to localize the chlorophyll *a* in the membranous structures of the chloroplasts we have very little reliable information on the arrangement of the plastid pigments inside the plastids. Electronmicrographs show several subunits or "knobs" of different diameters, but the question of what chemical properties are connected with these morphological elements is still open.

The discussions on the role of chlorophyll were supplemented by the description of the unusual photochemical properties of chlorophyll films in contact with semiconductor surfaces. As has been shown during the last months, illuminated chlorophyll-coated AgCl crystals split water and transfer the electrons and protons released to suitable acceptor molecules.

The next day was reserved for the second group of chromatophore pigments the carotenoids. After an introduction by E.C.Grob (Bern) about our present-day knowledge of their chemistry and biosynthesis, several contributors dealt with various aspects of the reaction sequence which leads from the colorless tetraterpenes to the coloured carotenoids. In this connection genetic experiments have proved valuable and efforts to use suitable micro-organisms – perhaps even isolated chloroplasts – for the study of these transformations promise success. The search for the role of the carotenoids has been made especially in assimilating cells. A light-dependent cycle connects the zeaxanthin with the epoxy-compound violoxanthin. It can be demonstrated that the incorporated oxygen comes from the water, not from the atmosphere, so there should be a connection between the early process of water oxidation and the transformation of the carotenoids. These pigments are known to be antioxidants and other roles have been ascribed to them. In experiments with carotenoid-less mutant strains it was found that the cells suffer severely from bright illumination. Bleaching of the chlorophyll can only be prevented in very weak light. The ability of some chlorophylls to transfer absorbed light energy to chlorophyll *a* is quite well documented. How the connection between these two pigment systems might be made is doubtful; we do not even know how the carotenoids are incorporated into the structure of the chloroplasts.

The final stage of photosynthesis is represented by those processes which lead to the synthesis of carbohydrates and nitrogen-containing metabolites. We are, however, convinced that these reactions are not part of photosynthesis in the strict sense. The real photosynthetic process ends with the production of the so-called "assimilatory power", i.e. a mixture of reduced pyridine nucleotides and ATP. During the seminar all aspects of ATP production were excluded. Here the alternative hypotheses of chemical and chemi-osmotic coupling between electron transport and phosphorylation are still controversial; these questions will be treated at a separate meeting.

The introduction of radioactive carbon isotopes greatly promoted the study of light-dependent CO_2 incorporation into plant cell constituents. The admirable success of Calvin's group led to the assumption that the pathway demonstrated for *Chlorella* and some other green algae might be the only one for CO_2 uptake and reduction. The third day was devoted to the thorough discussion of the mechanism of CO_2 fixation. The first step is generally assumed to be the fixation of CO_2 to a keto compound. Despite great efforts to understand the primary events in the reaction between CO_2 and carboxydismutase we do not even know whether the enzyme reacts with CO_2 or with HCO_3^-. Research in recent years has shown that ribulose diphosphate is not the only CO_2 acceptor. Tropical grasses like sugar cane use phosphoenolpyruvate to fix CO_2 and there may also be other acceptors.

An ever increasing number of studies concentrates on the regulation of the Calvin cycle. It can be demonstrated that the enzymes involved have quite different sensitivities towards various inhibitors and light also influences carbon incorporation. As several authors have found with algal cells, the spectral composition of the light has a strong influence. Plant cells illuminated with red light preferentially synthesize carbohydrates; cells under blue light, amino acids. The extensive discussions of published material showed that in no case has a direct influence of light on an enzyme been proven. Instead we have to assume that light alters the relative amounts of those regulators which – like ATP or NADPH – indirectly influence the enzyme activities. Some additional data, presented by Dr. Erismann (Bern), demonstrated also that the nature of the nitrogen source – NO_3^- or NH_4^+ – has a decisive influence on the pathway of carbon incorporation.

These considerations point to the interesting field of "photorespiration". Experiments with different plants have shown that many green cells respire more strongly if illuminated. The substrate of this photorespiration is obviously different from that of the regular dark respiration; until now nobody has proved the identity of this metabolite. Photorespiration is enhanced by blue light whereas light of longer wavelengths has a much weaker effect.

Big gaps in our present knowledge were demonstrated in a special evening session in which members of the Plant Physiology Institute of the University of Bern reported on their results on sulphur incorporation into illuminated leaves. The details of this pathway are still poorly understood, but the experiments promise interesting results.

The last day was concerned with the comparative aspects of photosynthetic CO_2 fixation and was introduced by R.C.Fuller (Oak Ridge). Studies on CO_2 incorporation have accumulated many data which require a critical evaluation from a taxonomic point of view. A group of "specialists" are the photosynthetic bacteria which reduce CO_2 without the concurrent evolution of oxygen. These organisms demonstrate many interesting pathways which have been lost by algae and higher plants. The first cells which perform a "regular" photosynthesis including water splitting are the blue-green algae. Some forms, however, take up and consume organic molecules even in the light; in many cases it is an open question whether the cells can exist without some preformed organic compounds in the medium. Even in the groups of higher organized algae the pathways may be different. It should be emphasized that the reserve carbohydrates of the green algae – and the mosses, ferns, and flowering plants – are composed of glucose units, whereas the red algae preferably use galactose units as building blocks. The reserve carbohydrates of the brown algae, in which mannitol is one of the early fixation products, are composed of mannose moieties. These data demonstrate the existence of a phylogenetic evolution of CO_2 assimilation beginning with the incomplete cycles of some bacteria and ending with the sequence observed in flowering plants.

The discussions were moderated and summarized by Erismann (Bern), Fuller (Oak Ridge), Grob (Bern), Metzner (Tübingern) and Sironval (Gorsem). They were supplemented by new – mostly unpublished – data presented by the participants. All who visited this seminar left the beautiful Lake of Thun with the impression that they had learned a great deal. Small conferences of this kind encourage the free exchange of ideas and arguments much more that do formal symposia with long lists of invited speakers. The discussion cannot, perhaps, be prepared as carefully as the sessions of a summer school, but assistants and students appreciate the freedom to dwell on arguments and conclusions originating in informal roundtable discussions.

The two meetings in Freudenstadt and Gwatt encouraged the organizers to plan a third conference of the same type which Dr. Sironval has promised to invite to Belgium next year.