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ALGAE AND PROGRESS IN PHOTOSYNTHESIS RESEARCH

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Abstract:

As we all know photosynthesis is the most fundamental life process on earth. Really it is the reaction of life, since all forms of life require energy for maintenance and propagation. The solar energy is captured by green plants and then transformed into stored chemical energy in the form of a wide variety of reduced carbon compounds. In addition photosynthesis stored this solar energy for millions of years in the form of organic fossils, which are now used as fuels. Really we are not going to follow the history of photosynthesis, but to follow, as far as possible, the role of algae in progress of photosynthesis research.

Although photosynthesis has been intensively studied by scientists for hundreds of years, the mechanism of this process remained till to the middle of the twentieth century unknown. It should also be admitted that even now, we know very little about certain aspects of photosynthesis. Attempts are still going on. Using algae which could be easily and efficiently cultivated under controlled conditions, these aspects might be fully resolved.

Our work will be presented in three successive parts (Eras) hoping to succeed in scanning, at least, the most leading experiments appreciating the historical role of algae in photosynthesis.

I- OXYGEN EVOLUTION AND CARBON FIXATION: SOME LEADING HISTORICAL EXPERIMENTS.

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Introduction:

In our meetings on algae, we are always accustomed to exhibit algal, biodiversity in different habitats as well as their economical benefits. In this lecture we will touch another scientific benefit of algae, i.e. the role of algae in progress of photosynthesis research.

Although photosynthesis has been touched by scientists for hundreds of years, yet the mechanism of this process remained till to the middle of the twentieth century unknown. It should also be admitted that even now, we know very little about certain aspects of photosynthesis. Attempts are still going on.

Really we are not going to follow the history of photosynthesis but to follow, as far as possible, some of the leading experiments using algae appreciating the role of algae in progress of photosynthesis research.

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Algae and photosynthesis:

Investigation of photosynthesis under controlled conditions represents an extremely useful tool for clarifying the mechanism and dependence of photosynthesis on the surrounding factors. This could be realized using algae, which could be cultivated under controlled conditions. These algal cultures should have remarkable similarities among all cells of the same species, so that the effects of individual variations as well as the variations in illumination and nutrition are eliminated. This could not be realised in detached leaves of higher plants, especially when subjected to long exposure to factors, since their metabolism could be altered from that of the intact plant. In addition the green algae, like higher plants, are organisms, whose pigments include chlorophylls a, and b and various carotenoids and xanthophylls. Also they store starch and contain cellulose in their cell walls. Therefore algae are more suitable for quantitative research for the reasons:

(1)- have short life cycle, (2)- can be grown in all laboratories under fairly standard conditions, (3)- algal life cycle could be completed in few days, using certain light-dark periods leading to synchronous culture, in which all the cells of algal population are of the same age, (Myers and Clark, 1944), (4)- algal culture when subjected to some specific nutrional and environmental conditions under repeated dilution, it could grow continuously giving balancedly growing population for months and (5)- algae of different colours enable the researchers to follow the role of different pigments in photosynthesis. These abovely mentioned advantages could not be realized in higher plant leaves.

Versus to the above advantages, many trials were made to use chlorplast suspensions of higher plants, to follow the photosynthetic activity under variable conditions. It was found that the carbon fixation rates were less than one tenth of those observed in leaves (Arnon, 1960). However the intermediate products of photosynthesis were nearly the same as those observed by Calvin group in algal cultures (Hall and Roa, 1981). Recently there are many trials to cultivate the higher plants as cell-suspension culture using tissue culture technique; to be easily studied imitating the algal cultures. However these methods are very sofisticating compared to algal cultures.

Therefore the use of algae in photosynthesis research remained the most appropiate methods and of remarkable advantages. *Chlorellas* have been widely used under three species names; *Chlorella vulgaris Chlorella pyrenoidosa* (fuska), and *Chlorella ellipsoidea*. Also *Scenedesmus* species were used; *S. obliquus*, *S. quadricauda*, *S. basiliensis*. The species, *Ankistrodesmus*, *Raphidium*, *Chlamydomonas*, *Euglena*, *Anabaena*, *Anacystis* and *Nostoc*, were used in special experiments.

Algal chloroplasts:

In Eukaryotic algae, chloroplasts show an enormous variation in size, shape and number per cell. This photosynthetic apparatus in green algae is nearly similar to that of higher plants (Prebble, 1981). It is surrounded by an outer normal plasma membrane. The inner membrane, bearing pigments, is organized into flattened sacs (thylakoids), which are stacked to-gather to form the grana (Fig. 1). Nearly all Algal chloroplasts contain long thylakoids; most of which extend from one end of the plastid to the other. Chloroplasts with the most complicated type of internal structure are found amongst *Chlorophyta*, which are the most similar to those of higher plants. They have clearly recognizable grana and with some thylakoids extending through the stroma from one granum to another.

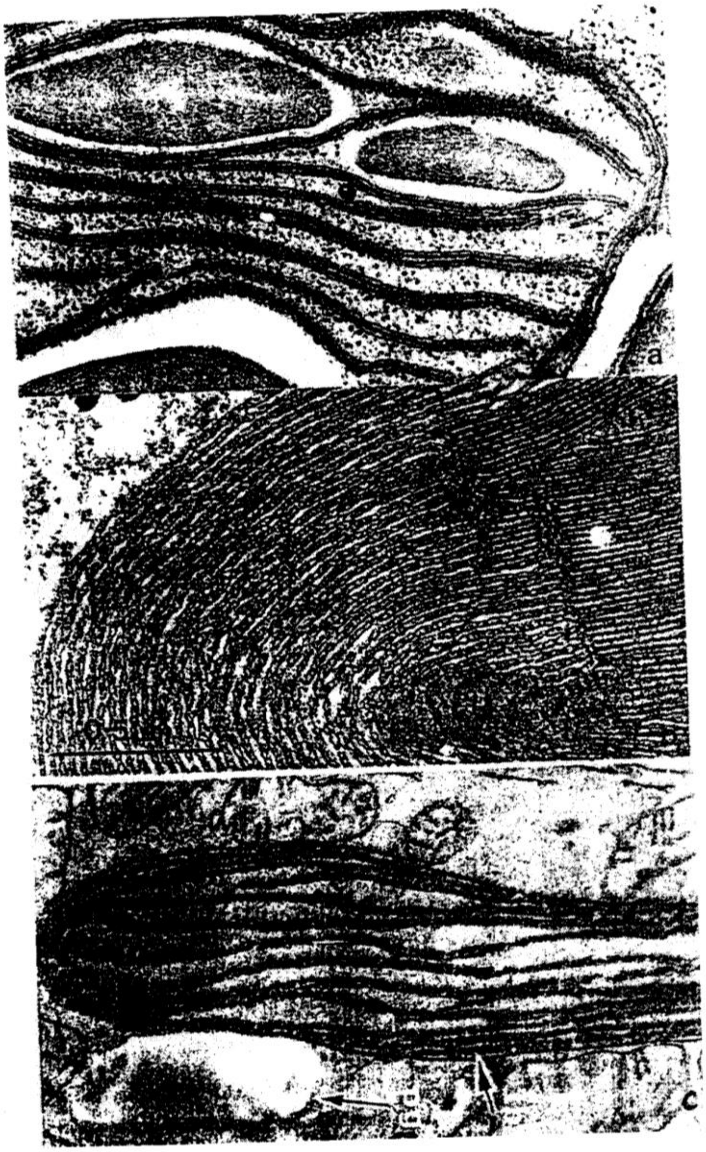

Fig. 1: Chloroplasts of the green algae, *Chlorella pyrenoidasa, Caulerpa cactoides* and *Euglena gracilis*

Algae and History of Photosynthesis:

Really we are not going to follow the history of photosynthesis, but to follow, as far as possible, the role of algae in progress of photosynthesis research. It is quite known that though plants of divergent aspectes were used in photosynthesis studies, most of the leading experiments were carried out with unicellular green algae; *Chlorella, Scenedesmus* and the unicellular flagellate *Euglena* (Hall and Rao, 1981). In this lecture we will pick up some of these leading experiments.

As far as we know the earliest and frequently quoted experiment of photosynthesis using algae was conducted by Engelmann (1882). In this experiment Engelmann could detect the direct connection between oxygen evolution by green cells and the role of light, and even more, the role of light spectrum in photosynthesis. Engelmann placed a filament of the green alga *Spirogyra* with its specially arranged chloroplasts on a microscope slide together with a suspension of oxygen-requiring motile Bacteria. After the alga being illuminated, it was found that the bacteria moved towards the green bands of the algal filament.

In another experiment, Engelmann (1884) illuminated different parts of the chloroplast with different colours of the light using a prism. He found that the largest number of bacteria surrounded those parts of the algal filament that were in red and blue regions of the spectrum (Fig.2). Engelmann concluded that red and blue light are most effective for producing oxygen during photosynthesis. This is a leading experiment, which had early touched the role of light, light spectra, and of chlorophylls in oxygen evolution during photosynthesis. These results opened the way to works done later indicating that chlorophyll plays the main role in energy capturing.

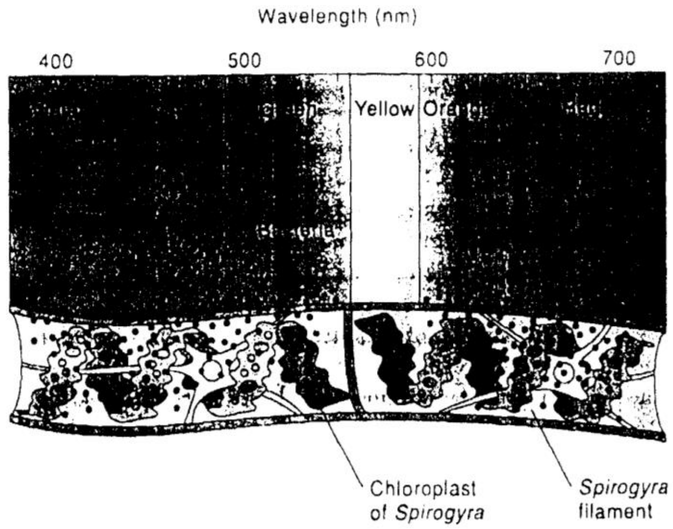

Fig. 2: Schematic representation of Engelmann's experiment.

In 1919 Warburg introduced to the study of photosynthesis a new method (manometric technique) and a new experimental material, the green alga *Chlorella*. *Chlorella* has proved to be an excellent experimental organism. Warburg (1920) observed that photosynthesis under continuous light was less than that of cells exposed to intermittent light. In another experiment Warburg and Negelein (1923) gave another indication that there is a dark reaction (Blackman, 1905) in photosynthesis. Warburg (1924) studied the effect of light intensity and some other factors on O_2-evolution by *Chlorella* cultures. Consequently the basics of the theory of the limiting factors were laid. In the course of time other algae with special pigments or metabolic or nutritional characteristics have been used to attack certain portions of the photosynthesis problem.

Emerson and Arnold (1932a, 1932b) illuminated suspensions of *Chlorella* cells with light flashes (lasting about 10^{-5} seconds each) and dark periods (lasting about 0.06 second each) for completion of photosynthesis. They could give an additional evidence for the two reactions (light reaction and dark reaction) of photosynthesis, which were first investigated by Blackman (1905). Emerson and Arnold suggested in addition, that under the conditions of intermittent light, the dark reaction might have proceeded to completion during the dark period. This was the first attempt to measure the rate of the dark phase in photosynthesis as an event apart from the whole process. In addition Emerson and Arnold mentioned for the first time that for the reduction of one molecule of CO_2 a photosynthetic unit containing about 2500 chlorophyll molecules is involved.

Ruben and Kamen (1940) ascertained the production of Oxygen from water. They exposed a culture of *Chlorella* to water containing heavy oxygen (^{18}O). The oxygen evolved was ^{18}O indicating that O_2 in photosynthesis originates from water.

Fan *et al.* (1943) reported that by feeding *Chlorella* with certain reducible substances in the absence of CO_2, *Chlorella* gave off appreciable quantities of O_2, which comes from water. These ideas supported that CO_2 uptake and O_2 production occur in separate reactions. Therefore the works came after were concerned with these separate reactions of photosynthesis.

The path of carbon in photosynthesis:

The path of carbon in photosynthesis, the major work of Calvin and his group (1946 $\rightarrow$ on) has been carried out using suspensions of unicellular green algae (mostly *Chlorella* or *Scenedesmus*). These works represent some of the most important discoveries in modern Biology. For his work in elucidating the path of carbon in photosynthesis, Calvin received the Nobel Prize for chemistry in 1961.

As it has been mentioned above, algae could be obtained in a reproducible biological form relatively easily and in any amount. They could be grown in the laboratory in a continuous culture, which could be maintained over periods extending beyond several months (Myers and Clark, 1944).

Radioactive carbon (^{14}C) was first used by Ruben and Kamen (1940) in studying the pathway of carbon in photosynthesis. It should be mentioned that photosynthesizing organisms do not discriminate between $^{12}CO_2$ and $^{14}CO_2$ and the cells incorporate ^{14}C as normal (Bassham and Calvin, 1957).

Using *Chlorella* culture Calvin and his co-workers followed the pathway of carbon in photosynthesis using radioactive carbon; (Benson and Calvin, 1947; Calvin and Benson, 1948). They exposed a dense culture of *Chlorella*, after being introduced in a lollipop- like-chamber (Fig.3), to radioactively labeled $^{14}CO_2$. After preliminary experiments, they reduced the time of exposure of algae to labeled carbon (^{14}C) to only one second. They used a special system (Fig. 4). In this apperatus, the time of exposure of algae to radioactive carbon was determined by the length of tubing between the point of injection and the methanol killing solution.

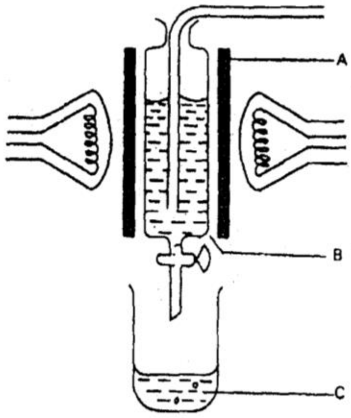

Fig. 3: Schematic representation of the apparatus for the fixation of $^{14}CO_2$ by unicellular algae A) heat filter B) lollipop C) boiling ethanol

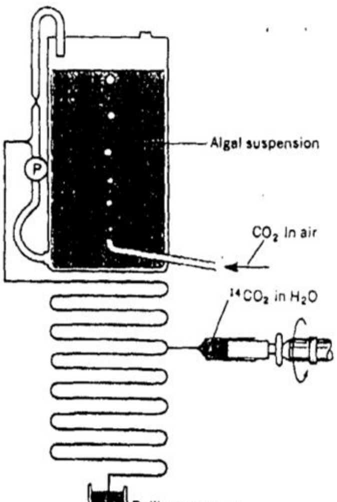

Fig. 4: Flowsystem for short exposures of algae to $^{14}CO_2$.

Using chromatography and autoradiography (Fig. 5), they could follow the path of carbon to carbohydrates. Most of the radioactivity in short-time experiments appeared in 3-phosphoglyceric acid (3 PGA). Working further they could reconstruct the sequence of events from time of entry of carbon atom into the plant, as carbon dioxide until it appears in the variously, more or less, finished products of photosynthesis. In addition to short time experiments (1-5 seconds) they used low temperature (2 °C), to slow down all the reactions, which enable to see the earliest products of carbon fixation. They needed to work for 10 years to succeed in following the pathway of carbon in photosynthesis (Fig.6).

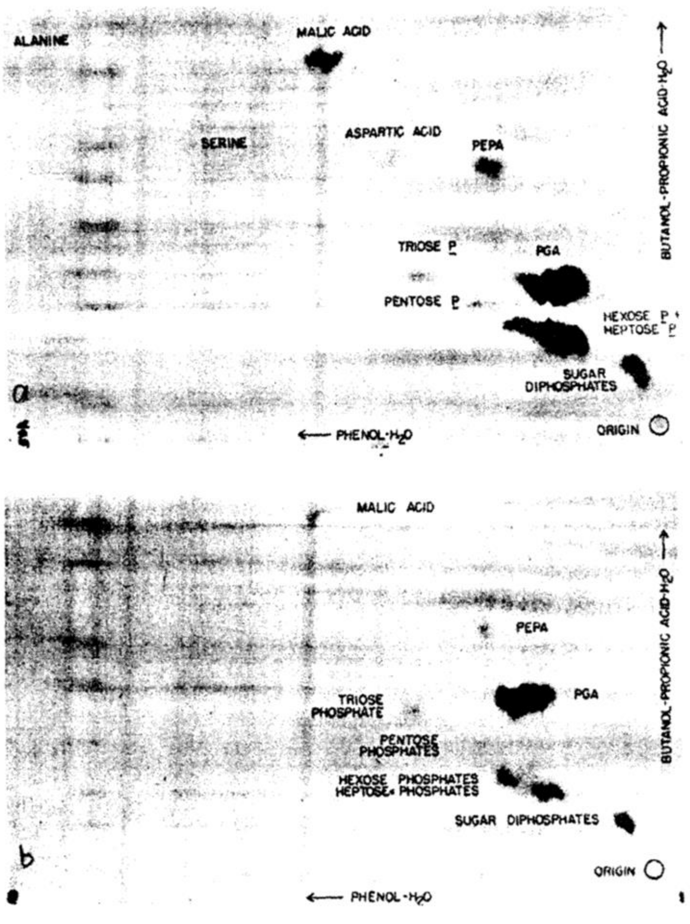

Fig. 5: Radioautographs of two-dimentional paper chromatograms of prooducts formed by *Chlorolla pyrenoidosa* during photosynthesis with $^{14}CO_2$ for a) 7 seconds and b) 2 second.

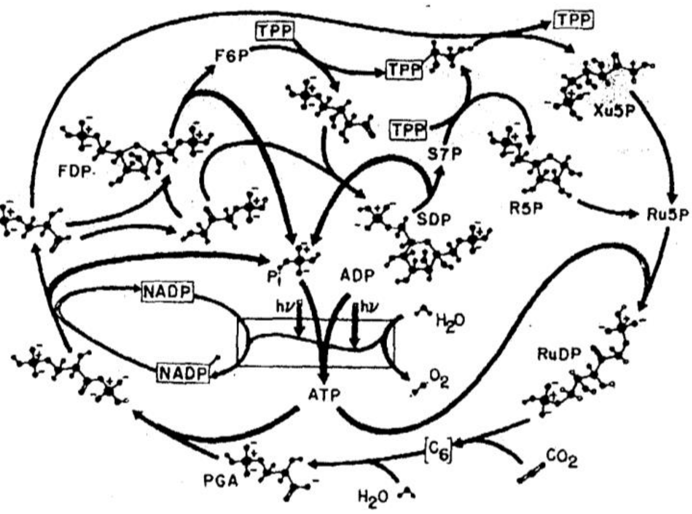

Fig. 6: Schematic representation of the Calvin cycle.

After this pathway has been ascertained (Bassham and Calvin, 1957; Calvin and Massini, 1952; Calvin, 1962; Metzner, 1969) many research workers used these techniques to study the effects of some factors on the degree of labeling of the various photosynthetic intermediates. In this respect Tolbert (1958, 1963) using Chlorella cullures, studied the effects of variable CO_2 or O_2 concentrations on the labeling of some of the intermediate compounds. He found that glycolic acid was highly labeled under low CO_2 and high O_2 concentrations. These results lead later to formulate the process of photorespiration (Hess and Tolbert, 1967).

Some other experiments were also conducted to follow the degree of labeling of the intermediate products of variously aged cells (Ahmed, 1968; Ahmed and Ries, 1969) using *Synchronized Chlorella* cultures (Lorenzen, 1957; Tamiya, 1957).

The dark reaction could be regarded as being now understood, as it has been exhibited in this part of the lecture. However the steps of the light reaction, which need unimaginable efforts from the scientists, are still under investigation. I will leave this part in relation to algae to my colleagues, who will continue this lecture.

Summary

The role of algae in the history and progress in photosynthesis research (first Era) could be evidenced by the following leading experiments, which ,without algae could not. at that time, be realised.

1 - Engelmann using *Spirogyra* (1882-1884) could detect for the first time:
   a) **oxygen evolution** in photosynthesis.
   b) **role of light and different light spectra in oxygen evolution**, and
   c) **role of chlorophylls in oxygen evolution.**

2 - Warburg and his group (1919-1924) introduced a new manometric method and a new excellent experimental organism (*Chlorella pyrenoidosa*) in studies of photosynthesis. Using continuous and intermittent light, they ascertained the presence of light and dark reactions in photosynthesis. In addition they formulated the theory of the limiting factors.

3 - Emerson and Arnold (1932) illuminated *Chlorella* suspension with light flashes (10^{-5} second each) and dark periods (0.06 second each). They could obtain an additional evidence for the dark reaction in photosynthesis. In addition they introduced, for the first time, the role of a photosynthetic unit (2500 chlorophyll molecules) in the reduction of CO_2.

4 - Ruben and Kamen (1940) exposed *Chlorella* suspension to water containing **heavy** oxygen (^{18}O). They found that the oxygen evolved was heavy (^{18}O), which gave a good evidence that oxygen is evolved from water.

5 - Fan *et al* (1943) using *Chlorella* gave an additional evidence that CO_2 - uptale and O_2 – production occur in separate reactions (now dark and light reactions).

6 - Calvin and his group (1946 →) constructed their experiments using
a) continuous cultures of *Chlorella* or *Scenedesmus*
b) radioactive carbon (^{14}C) and
c) newly developed chromatography and autoradiography.

They needed to work for 10 years to follow the pathway of carbon in photosynthesis, which was then summerised in Calvin Cycle (1957).

7 - Tolbert using chlorella (1958-1967) could formulate the glycolate pathway giving hints to photorespiration. In addition some other related experiments (Ahmed 1968, Ahmed and Ries, 1969) were conducted using synchronous cultures of *Chlorella pyrenoidosa* to compare the pathway of carbon in variously aged cells.

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