Let the sun shine in

New light is shed on photosynthesis studies as researchers celebrate a 200-year-old field

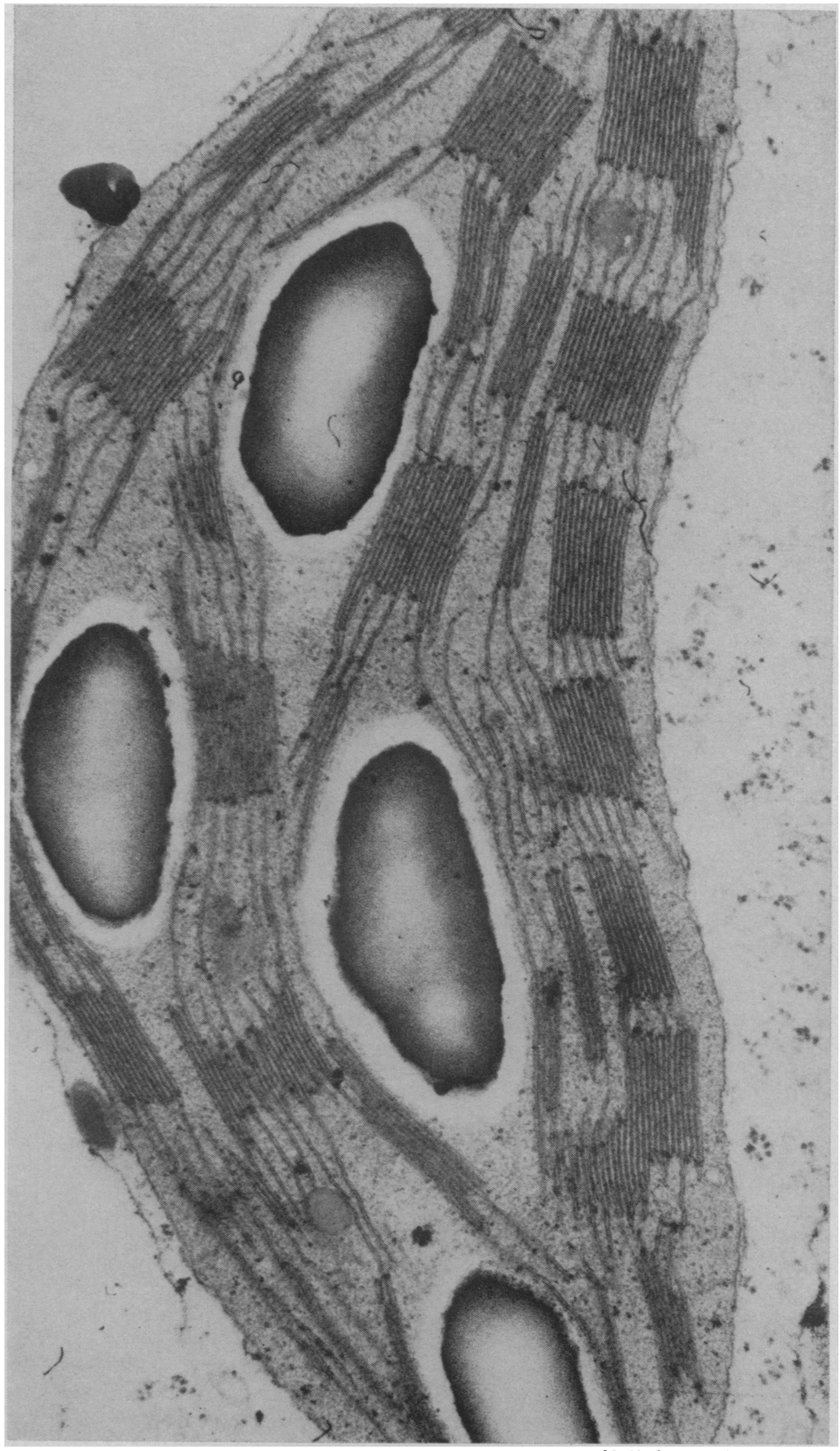
by Robert J. Trotter

In 1770 Joseph Priestley, a radical English clergyman and occasional chemist, was minister of Mill Hill Chapel in Leeds. Living near the local brewery he became interested in the "fixed air" or carbon dioxide produced by fermentation. This scientific interest led to experimentation and, in turn, to his discoveries of what we now call oxygen, ammonia, hydrochloric acid, hydrogen sulfide, nitrous oxide, nitric oxide, sulfur dioxide and silicon fluoride.

Another important discovery resulting from Priestley's experiments with carbon dioxide was "the improvement of air by plants." On Aug. 17, 1771, he placed a sprig of mint into a glass jar standing inverted in a vessel of water and "found that the air would neither extinguish a candle, nor was it at all inconvenient to a mouse." This discovery, 200 years ago, that oxygen is produced by green leaves was the beginning of studies in the field of photosynthesis.

To celebrate this discovery, the National Academy of Sciences opened its annual meeting last month in Washington with a bicentennial symposium on photosynthesis. The chairman was Dr. Kenneth V. Thimann of the University of California at Santa Cruz.

Two years after Priestley's discovery a Dutchman named Jan Ingenhousz heard of it and decided to conduct his own experiments on the effects of plants on air. He observed not only the improvement of air by plants, but that the improvement was due to a chemical action that sunlight caused to occur in green leaves. Three years later a Swiss churchman named Jean Senebier added carbon dioxide to the growing amount of information on photosynthesis when he declared that "air liberated by plants exposed to the sun is the product of the transformation of fixed air by means of light." Now, only one more reagent directly involved in photosynthesis remained to be discovered and added to the equation. This



M. K. Corbett, Univ. of Md.

Tobacco chloroplast magnified 40,000 times: The work of photosynthesis takes place within the parallel membranes and produces kernels of organic material.

was done in 1804 by a French scholar named Theodore de Saussure who published a treatise in which he concluded that water was a necessary ingredient in photosynthesis.

With the addition of this last reagent, the photosynthesis equation could be written but it could not be balanced. This was done in 1845 by a German, Dr. Julius Robert Meyer, who wrote that "green organisms absorb the light of the sun and use this power to produce a continuously accumulating chemical difference." He had completed the equation by balancing the energy involved and fulfilling the law of the conservation of energy. The equation for photosynthesis is:

$$CO_2 + H_2O + \text{light} \xrightarrow{\text{green plant}} O_2 + \text{organic matter} + \text{chemical energy}$$

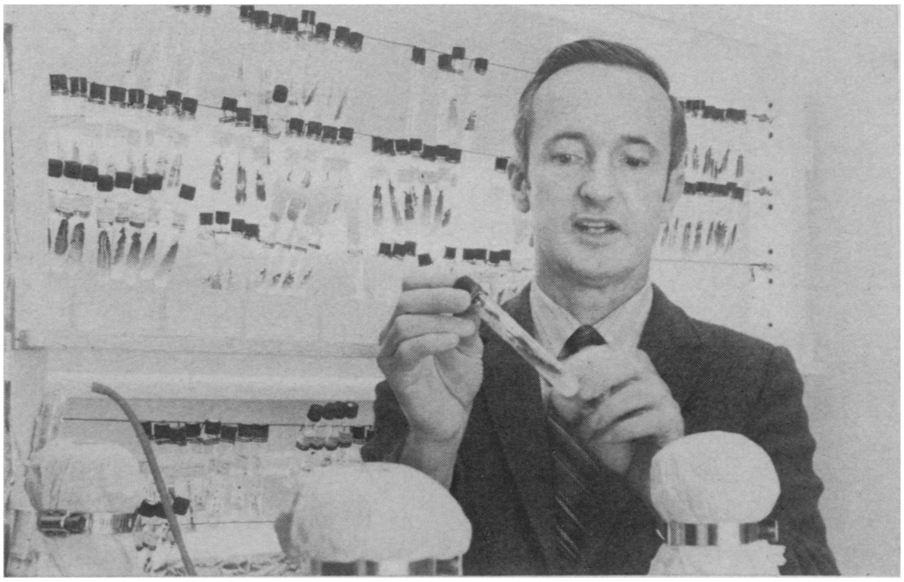
Thus, life on earth obtains its substance and energy through the process of photosynthesis. Green plants use the sun's energy to synthesize carbohydrates and other organic compounds. These compounds are transformed to provide cellular constituents and food for the living organisms on the planet, from the simplest bacteria to the most highly developed animals, including man. The oxygen produced goes into a gigantic ventilation system that continually replaces oxygen used up in respiration and combustion.

Most school children learn this much, but a complete equation does not mean a complete answer. Since Meyer's time scientists have been and still are attempting to answer basic questions about the process of photosynthesis.

Most of the important work is taking place now or has been completed during the past 50 years. The physics, photochemistry, biochemistry, plant physiology and ecology of photosynthesis have each grown beyond the capacity of single individuals to comprehend. Millions of dollars have been spent and the yield of information has been high. But the effect has been curious. As Dr. C. Stacey French of the Carnegie Institution of Washington's Department of Plant Biology in Stanford, Calif., puts it: "The number of questions remaining to be solved has increased rather than decreased because far more significant questions can now be asked."

It is now known that light energy, water, carbon dioxide, nitrates and sulphates are combined in the choroplasts (subcellular food factories) of leaves to produce organic compounds. The young leaves primarily synthesize amino acids (the building blocks of proteins) for cell growth and division. The more mature leaves concentrate on the production of carbohydrates needed for nourishing other organs of the plant.

By 1953, with the use of radioactive carbon, researchers were able to follow the path of carbon in this process and to map out the basic reactions in the production of organic compounds. Since then they have been attempting to discover the exact processes that enable cells to produce carbohydrates and proteins in varying amounts at different times.



LRL

Bassham: Producing proteins to feed the undernourished may soon be possible.

A noted specialist in this area of photosynthesis is Dr. James A. Bassham, associate director of the Lawrence Radiation Laboratory's Chemical Biodynamics Laboratory in Berkeley. Leaves, he explains, are able to change over from synthesis of carbonhydrates to synthesis of proteins and back again with the help of certain enzymes. These are in turn affected by ions (electrically charged atoms or molecules) that help the enzymes to influence various speeds and types of synthesis. Using radioisotopes to tag suspected chemicals and follow their progress through living cells, Dr. Bassham and his associates have found that the enzyme pyruvate kinase regulates the reactions in which most amino acids are synthesized. The enzyme sucrose phosphate synthetase acts as a control to regulate the flow of carbon in the synthesis of sucrose (a carbohydrate).

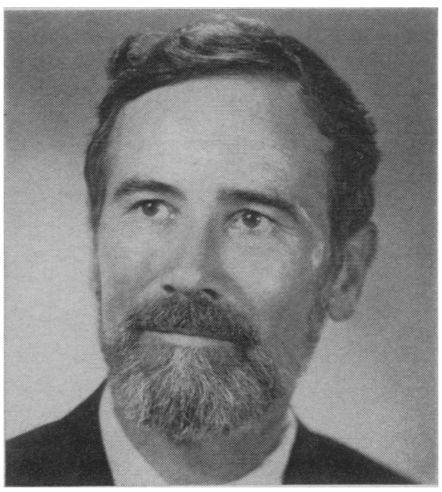
Researchers can activate these controls artificially by adding ammonium ions. This results in increased protein synthesis. "It is perhaps not too much to hope," reports Dr. Bassham, "that in the future, application of a suitable chemical spray to mature leaves which are producing mostly sucrose may be able to switch their metabolism for a time back to protein production. Since the direct utilization of green leaves by humans may prove to be an efficient agricultural usage in a protein-hungry world, such an induced switch might prove to be a valuable aid to reducing protein deficiencies in some areas of the world."

Thus, studies in carbon metabolism may eventually lead to the understanding and control of photosynthesis. But scientists must first understand the physical and chemical mechanisms of the system.

The green chlorophyll and other pigment molecules act as light harvesting antennas to absorb the energy of the sun and deliver it to the reaction centers of the plant cells where oxygen and organic matter are produced. To study these processes, says Dr. Roderick K. Clayton, professor of biology and biophysics at Cornell University, it is necessary to ask three questions:

- How does the antenna deliver energy to the reaction centers?
- How is photochemistry brought about at the reaction centers?
- What are the primary products of the photochemical act, and how are they used efficiently and safely?

Partial answers have been found by



Cornell Univ.

Clayton: How is energy delivered?

separating the light harvesting antennas of photosynthetic bacteria from the reaction centers, and studying them separately and together. One tool used in this study is the measurement of fluorescence given off by chlorophyll. Any light energy not used in photosynthesis is returned in the form of fluorescence. Its intensity gives clues to the path and use of energy. For example, if the reaction centers are unable to function, the fluorescence becomes more intense in the reaction centers and spreads back into the antennas.

These and other studies have shown that when a particle of light strikes the antenna it puts a molecule (usually chlorophyll) into an excited state. This excitation is passed from one molecule to another throughout the antenna until it reaches the reaction center. It takes about a billionth of a second.

In the reaction center a specialized chlorophyll molecule transfers one electron to a nearby electron-acceptor molecule. Some energy is given off in the form of heat, and a relatively stable pair of molecules is produced: oxidized chlorophyll and a reduced acceptor. The early stages of this process are complete in about seven-trillionths of a second. "We do not know what the electron acceptor molecule is," Dr. Clayton says, "but we are beginning to learn about its physical and chemical properties."

The speed of this reaction and the orderly sequence of electron donor and acceptor molecules in the system keep the primary products from reacting wastefully with each other or harmfully with other substances in their surroundings. As long as this system is allowed to follow its natural path it leads to oxygen evolution and the formation of reducing agents that help in the fixation of carbon dioxide and eventually the production of organic material.

These answers, however, relate only to the physical and chemical aspects of Priestley's discovery. There are actually two systems in photosynthesis: first, the "photo" or light phase in which light energy is caught and converted into chemical energy and, second, the "synthesis" or chemical phase in which energy from the first phase is used to convert carbon dioxide into organic material.

The chemical energy necessary for biosynthesis in all living cells (plants, animals or bacteria) is supplied by two substances. One is adenosine triphosphate (ATP) and the other is the reduced form of nicotinamide adenine dinucleotide phosphate ($NADPH_2$). ATP provides the energy for the reactions and $NADPH_2$ provides the hydrogen atoms necessary to reduce carbon dioxide to the level of carbohydrates.

In the "photo" phase the energy of the sun is used to generate these two products. The process by which plants use light energy to generate ATP was discovered in the laboratory of Dr. Daniel I. Arnon of the department of cell physiology at the University of California at Berkeley. It was named photosynthetic phosphorylation (photophosphorylation). This type of ATP formation is peculiar to chlorophyll pigments and is not the same as ATP formation in other forms of living cells.

Dr. Arnon and his associates further discovered that there are two forms of photophosphorylation, cyclic and noncyclic. In the first only ATP is produced. In the second, ATP is formed, oxygen is given off and ferredoxin (an iron-sulfur protein found in chloroplasts) is reduced. The reduced ferredoxin in turn reduces NADP and supplies the $NADPH_2$ necessary for carbon dioxide reduction.

The latest experiments have shown that photosynthesis in green plants includes two photosystems: Photosystem I, which proceeds best in "long" wavelength light and is used in cyclic photophosphorylation; and Photosystem II, which proceeds best in "short" wavelength light and is used in noncyclic photophosphorylation.

The most recent discovery by Dr. Arnon and his associates has shown that the short wavelength light of Photosystem II triggers two reactions. The researchers worked with fragmented chloroplasts of spinach at the temperature of liquid nitrogen (more than 300 degree below zero F.). At this temperature all molecular activities stop except those involving light energy and electrons. They found that the green pigment of chlorophyll traps light energy and uses it to whirl electrons out of the chlorophyll molecules. The excited electrons are accepted by an unknown substance called Component 550 (its peak in the absorption spectrum is at the 550 millimicron point). These electrons then pass through reactions that yield ATP. They then receive another boost of light energy and proceed to the molecules of ferrodoxin and on the production of $NADPH_2$. The ATP produced in Photosystem II is not adequate for complete photosynthesis, so Photosystem I must proceed simultaneously and supply the extra ATP.

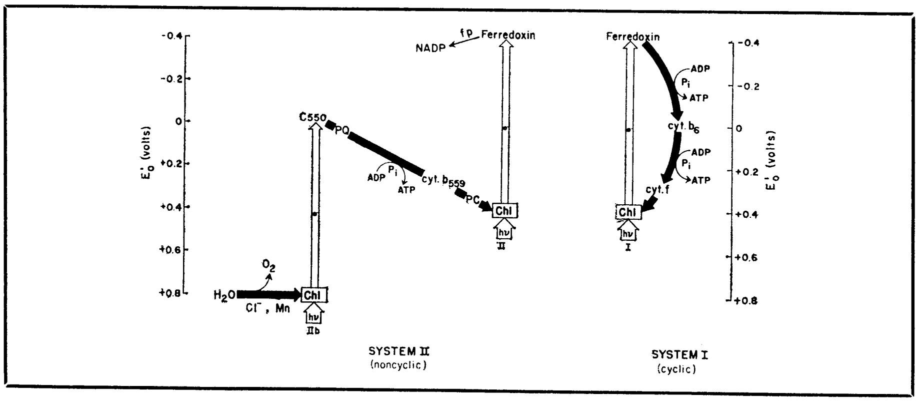
Another object of continuing research is chlorophyll. Dr. French calls it the most striking substance involved in photosynthesis.

This green material had been extracted from leaves, studied and even synthesized. But, extracted chlorophyll is unfortunately only part of the complex existing in living cells. In its functional state, chlorophyll is combined with insoluble proteins, carbohydrates and carotenoids. "The main reason for our ignorance about the chemistry of this natural green coloring matter," says Dr. French, "is that the material itself is not soluble." It thus cannot easily be prepared in pure form for chemical analysis.

For this reason Dr. French has concentrated on studying the absorption and fluorescence spectroscopy of chlorophyll. There are, however, drawbacks to this method. Using even the smallest particles of distinguished chloroplasts there is still some scattering of light to distort the results.

Future programs, using various methods of spectrophotometry and computer analysis of spectra should yield more reliable results. Dr. French hopes this work will lead to understanding the particular forms of chlorophyll that participate in the two known photochemical reactions of photosynthesis, and at what wavelengths they operate.

As programs such as these continue, the gaps in photosynthesis knowledge are being bridged. Scientists hope that before the 300th anniversary of Joseph Priestley's discovery, there will be answers to all of the still-unanswered questions. When that happens the equation of photosynthesis will be completely solved. □



Arnon