The program was concluded by a study of the "Oxidation of Neoprene," as reported by E. K. Gladding (Du Pont), who stated that oxidation of poly-2-chlorobutadiene (neoprene) and poly-2,3-dichlorobutadiene is accompanied by the formation of peroxidic materials, carbonyl compounds, acids, and acid chlorides. The acid chloride function is a characteristic oxidation product of these chlorine-substituted polymers, and experiments with model compounds show that it is formed from a configuration equivalent to two adjacent monomer units of the polymer. The colored products that form during neoprene oxidation are believed to consist of structures that can arise through polymerization (followed by elimination of HCl), of β-chloro-α, β-unsaturated carbonyl, or carboxylic acid intermediates formed by oxidation.

A Discussion of Photosynthesis

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ASPECTS OF PHOTOSYNTHESIS from the initial absorption of light to the ultimate syntheses were scrutinized by some 80 research workers of many scientific disciplines at Gatlinburg, Tennessee, October 29–November 1, 1952.[1] The free discussion that was encouraged at this meeting yielded many clues for further study of the photosynthetic process.

Light utilized in photosynthesis is absorbed by the several chlorophylls and bacteriochlorophylls, and by open chain tetraphyrolles and carotenoids of various organisms. The absorbed energy is transferred between molecules of these pigments, as determined by the molecular structures and permitted by the organization of the pigments in the grana. The energy is used to effect the chemical transfer of hydrogen from some molecule, such as water, to unidentified primary acceptors. The acceptors then transfer hydrogen to compounds formed during carbon dioxide fixation, probably both by known and still unknown enzymatic systems.

Energy level systems for porphins and tetrahydroporphins were considered as a basis for discussion of the spectroscopic properties of related, but less symmetrical, chlorophylls that are effective in photosynthesis (J. R. Platt, University of Chicago). Such molecules have a triplet level of energy lower than the first excited singlet level that gives rise to fluorescence emission. This triplet level, having a long life of the order of 10^{-4} seconds, is responsible for photochemical reactions. The separation between those two levels determines how much of the energy of the absorbed light is degraded and, therefore, is not available for chemical activation in photosynthesis. Unfortunately, no direct calculation of this separation is available. Estimates, based on the measured values for the levels of several other compounds, however, indicate that the quantity might be as small as 5–10 kcal. for chlorophyll (M. Kasha, Florida State University; Platt).

Another singlet level may lie very close to the fluorescent level, but transitions between the former and the ground level are not observed, since they would be of the forbidden N-II type. If such a level lies between the fluorescent singlet level and the lowest triplet level, radiationless transitions between the two would be facilitated and fluorescence weakened.

Attention next turned to some reversible photochemical changes of chlorophyll, which are probably related to the primary acts of photochemical reactions sensitized by chlorophyll *in vitro* and possibly of photosynthesis. It is known that the red absorption of oxygen-free solutions of chlorophyll in methanol is reduced when such solutions are illuminated with intense, visible light. When the light is extinguished, the solution very quickly returns to its original color.

Experiments were described in which chlorophyll was dissolved in a rigid solvent, at liquid-nitrogen temperatures (H. Linschitz, Syracuse University). Under these circumstances the color change was much more marked, but the time required for the dark reversal of the photochemical process was too short to measure. If certain quinones were added, the photoproduct was stable so long as the solvent remained rigid. If the solution was warmed, the original color of the chlorophyll returned instantly when the solvent glass melted. The absorption of the labile photoproduct of chlorophyll was much less than that of normal chlorophyll in the red and blue but had a marked maximum in the green. This photochemical reaction is possibly an oxidation of the chlorophyll

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[1] The conference was supported by the National Science Foundation, the Office of Naval Research, and the Atomic Energy Commission. This report combines summaries for individual sessions, which were prepared by R. S. Livingston (University of Minnesota), E. Rabinowitch (University of Illinois), C. S. French (Carnegie Institution of Washington), F. Daniels (University of Wisconsin), B. Kok (Wageningen University), and M. Kamen (Washington University), who served as chairmen. Discussions were introduced by J. R. Platt (University of Chicago), E. C. Wassink (Wageningen University), R. Hill (University of Cambridge), M. Calvin (University of California, Berkeley), F. S. Brackett (National Institutes of Health), and H. Gaffron (University of Chicago).

resulting in formation of a semiquinone and of a corresponding oxidized chlorophyll radical.

Results of some recent experiments, in which solutions of chlorophyll in methanol were illuminated at room temperature with an intense light flash of short duration, were described (R. S. Livingston, University of Minnesota). The course of the back reaction that takes place in darkness was followed by the use of a photomultiplier and an oscilloscope. Analysis of data obtained by this method indicated that at least two different photolabile products are formed at comparable concentrations by the flash. One of these disappears by a first order reaction, or by a self-quenching process, with a half-life of almost 3×10^{-4} seconds, and reforms the normal molecule. About 20 per cent of the primary product forms a second labile product which has a spectrum similar to that observed in the rigid solvent at low temperatures. This product is probably a radical formed by a reaction of the primary product with a molecule of the solvent, since it reforms chlorophyll by a second order reaction.

The observation made by Krasnovskii that chlorophyll, when dissolved in liquid pyridine, is photoreduced by ascorbic acid has been verified (A. S. Holt, University of Illinois). Chlorophyll can be reformed in darkness from this reduction product by the addition of suitable oxidizing agents. The absorption spectrum of the reduced chlorophyll is similar to that of the reversible oxidation product of chlorophyll by quinones. Both resemble the absorption spectrum of "brown phase" of the Molisch phase test of chlorophyll.

The action spectra of fluorescence reveal the transfer of excitation energy between the several photosynthetic pigments ending in the pigment with the lowest excitation level (E. C. Wassink, Wageningen University). Measurements of fluorescence intensity of chlorophyll in plants and of bacteriochlorophyll in purple bacteria were considered as means of tracing the excitation energy, after this energy has been concentrated in the pigment with the lowest excitation level. This concept, however, was vigorously challenged. The fate in terms of "energy transfer" to an unknown chemical system was considered as one in which the electronic excitation energy is converted into chemical energy—e.g., of an oxidation-reduction system.

Recent experiments give new information about energy transfer between pigments. The action spectra of fluorescence in algae and purple bacteria demonstrate complete transfer of energy from chlorophyll *b* to chlorophyll *a*, and from the phycobilins in red and blue-green algae to chlorophyll *a*, and from two forms of bacteriochlorophyll (absorption maxima at 8000 A and 8500 A) to a third form—bacteriochlorophyll (8900 A) (T. M. N. Duysens, University of Utrecht). The energy transfer was found to be 40 per cent effective between carotenoids and bacteriochlorophyll in purple bacteria; complete between fucaxanthol and chlorophyll in brown algae; and only about 20 per cent effective between carotenoids and chlorophyll in red algae. These results closely parallel those obtained in the study of the action spectra of photosynthesis, thus proving that the latter is brought about exclusively by the excitation energy of chlorophyll *a* or of bacteriochlorophyll (8900 A) (C. S. French, Carnegie Institution; Duysens). The most interesting result is that this rule remains true even in those red or blue-green algae where a large part of chlorophyll *a* seems to be ineffective in producing either fluorescence or photosynthesis. In algae of this type, the energy absorbed by the red and blue pigments seems to be transferred to the small part of chlorophyll *a* that is present in fluorescent and photosynthetic form, but the larger part of chlorophyll *a* is useless because of contamination with a small amount of an unknown pigment—probably so-called chlorophyll *d*, which drains the excitation energy away and dissipates it through its own fluorescence. For some unknown reason, the contaminated chlorophyll *a* is unable to supply energy to the photosynthetic apparatus.

Reactivation of "inactive" chlorophyll *a* in red algae takes place with prolonged illumination by red or blue light, and renewed loss of activity follows illumination by green light (C. Yokum, Harvard). Extremely weak chemiluminescence that follows photosynthesis has a spectrum similar to that of chlorophyll *a* fluorescence (W. A. Arnold, Oak Ridge National Laboratory). The fluorescence is emitted whether photosynthesis is brought about by light absorbed by chlorophyll *a* itself, or by light absorbed by phycoerythrin, again confirming that the former pigment is the only one closely associated with the photochemical process in photosynthesis.

Absorption spectra of chlorophyll, bacteriochlorophyll, and their derivatives in microcrystalline form and in the form of monolayers on water give insight into the mechanism of energy transfer between identical molecules (E. E. Jacobs, University of Illinois). The results were interpreted as indicating that resonance interaction between closely packed pigment molecules produces shifts of the red absorption bands by 1500–2000cm^{-1} toward longer waves. The "red shift" in monolayers was about 90 per cent of that in three-dimensional microcrystals, indicating preferential resonance energy transfer in one plane. In microcrystals, the shift was found to be a function of crystal dimensions, becoming "saturated" in crystals containing 10^6 molecules. Theoretical discussions (H. Kallman, New York University, and others) showed that doubt remains as to the reliability of estimates of the extent of long-range transfer of energy between unlike molecules.

Although unknown steps remain in the energy transfer, the photochemical reacting system of the plastids can be followed *in vitro*, as was first accomplished by R. Hill (University of Cambridge) in 1938. Hill reported that the reaction rate is increased by a protein fraction of the cytoplasm for which a partial concentrating has been accomplished. Rates of oxygen evolution have now been obtained that approach the rate of photosynthesis *in vivo*. A cellular fraction that promotes a high initial rate of reaction, even at low light intensities, was described (R. W. Lumry, University of Utah). The removal of this fraction enhanced the stability of the plastid preparation.

The lack of an induction period for the "Hill reaction" was shown by a methemoglobin reduction method that is free from the lags inherent in gas measurements (Hill). The properties of cytochrome *f*, present in plastids, and the possible participation of cytochrome *f* as an intermediate of photosynthesis were discussed. The hope is raised that the substance or chain of intermediates directly acted upon by energy transferred from chlorophyll *a* can be found.

The rate of oxygen evolution in the "Hill reaction" was found (Lumry and J. D. Spikes, University of Utah) to vary with light intensity in the form of a rectangular hyperbola. Extrapolation to zero light intensity indicated that between 6.7 and 8.2 quanta are required for production of one oxygen molecule. These values are markedly lower than those recently reported with quinone by O. Warburg.

That free radicals, such as HO_2 and OH, may take part in the early photoreactions was discussed (N. Uri, University of Chicago). These might be detected during photosynthesis by their catalytic effects on some polymerizations.

Interpretation of oxidation-reduction potential measurements under nonequilibrium conditions was discussed (D. Goddard, University of Pennsylvania; Rabinowitch, University of Illinois; and Uri). The way in which these measurements are widely used in biological work was challenged (Rabinowitch), and the confusion was traced to a failure to distinguish between the free energy change in a reaction, which determines the equilibrium, and a free energy of activation of an activated complex, which determines the rate.

The organization of the plastids was illustrated by electron micrographs (S. Granick, Rockefeller Institute for Medical Research; and A. E. Vatter, University of Illinois). The course of the formation of grana in higher plants, their platelike shape, and organization in lamellae were very clear, as was the presence of a membrane. Permeability of the membrane to various organic compounds can be followed by osmotic swelling (L. R. Blinks, Hopkins Marine Station; and J. H. McClendon, University of Minnesota).

The controversial subject of the minimum quantum requirement of photosynthesis was considered first from an experimental approach not dependent upon manometric measurements. A value of 6.5–8.0 quanta/O_2 was shown to be a minimum over short time periods above light compensation for respiration in a uniformly illuminated suspension of unstirred *Chlorella pyrenoidosa*. High initial values were not observed; instead, even under aerobic conditions, a brief induction period is noted during which the rate rises. In these experiments (F. S. Brackett and R. Olsen, National Institutes of Health), oxygen was determined by a polarographic method that permitted measurements at intervals of 10 seconds.

In measurement of quantum requirement a correction has to be made for respiration, which itself might be influenced by light. A mass spectrographic method (A. H. Brown, University of Minnesota) making use of O^{18} indicated that respiration was not stimulated in the light. This mass-spectrographic method had a time resolution of about one minute, the order of that for manometric methods (Riley, National Institutes of Health).

Polarographic measurements indicate systematic departures from monotonous changes in oxygen concentration during photosynthesis, similar to those of slower types found manometrically. These were eliminated when glucose was present. Similar changes were evident when pH was followed with a time resolution of seconds in photosynthetic algae suspensions (Gaffron, University of Chicago).

Measurements of minimum quantum requirements per oxygen molecule evolved by Warburg manometric methods still lead some investigators to results of 4 quanta or less (Burk, National Institutes of Health) and other investigators to 8 or more quanta (Emerson, University of Illinois). Thin algal suspensions and geometrically limited illumination are often, but not always, used by both groups of workers. Causes for differences in results apparently cannot be accounted for by intermittency of illumination or peculiarities of the algae. The report of marked effects upon algae of growth conditions, such as light intensity, however, might prove important (H. Tamiya, Tokugawa Institute).

Evidence for a lower quantum requirement below compensation for respiration by light was discussed (B. Kok, Wageningen University). While no marked changes were found in respiration at compensation by mass spectrographic methods (Brown), the fluctuations observed polarographically are of an order to explain the effect (Brackett, Olsen).

An initial photoreaction having a unit quantum requirement per molecule of oxygen was reported for *Chlamydomonas moeweisii* in carbonate buffer (pH 8.8), as well as for chlorella in acid medium (Burk).

The over-all energy requirement for reduction of a mol of CO_2 to sugars by the photosynthetic system must be at least 184 kcal. (Franck, University of Chicago). Since the energy available from the triplet level of chlorophyll *a* is probably less than 36 kcal., at least 5 quanta/O_2 would be required. Fewer quanta might conceivably be required below compensation (or for short periods of time) if internal cycles existed for giving nonphoto-energy back into the CO_2 reduction (Franck; Kok; Strehler, Oak Ridge National Laboratory). The "Hill reaction," interestingly enough, might require only about 3.5 quanta/O⁻ in the limit, since entropy changes involved in atomic rearrangements are minor (Franck).

The course of carbon in photosynthesis is now understood in its major outline (Calvin, Benson, Bassham, *et al.*, University of California). Current experimental interest is moving toward solutions of the kinetics for accumulation of various compounds. The general technique is to add $C^{14}O_2$ at zero time to a photosynthesizing system and to determine the course of appearance with time of radioactivity in each carbon atom of metabolic intermediates. A method was given for solving the linear differential equations involved as carbon appears in more and more complex compounds, as the carbon course branches into several products, and as parts of molecules recycle toward the initial reactions.

Carbon first appears in the carboxyl group of phosphoglyceric acid PGA (Calvin *et al.*; Fager, University of Chicago). Since no probable two-carbon precursor of PGA has been found, attention is now turning to the possibility that CO_2 enters by addition to a more complex molecule. Time-activity curves for C^{14} incorporation indicate that the carboxyl carbon of PGA appears in the #5C of sedoheptulose and the #3C of the pentose. These compounds appear to be recycled back for the PGA initially formed upon fixation of CO_2.

The distribution of carbon in intermediate metabolic stages is influenced by inhibitors such as phenylurethane and dinitrophenol (Burris, University of Wisconsin). These inhibitors lead to a striking decrease in the relative C^{14} content of PGA to that of alanine after 30 seconds of photosynthesis. Sequence of light and dark periods and other changes also influence the distribution of C^{14} from $C^{14}O_2$ in the metabolic pools (S. Aronoff, Iowa State College).

Various approaches to the central problem of the mechanisms intervening between the light-absorption act and the appearance of reduced products of photosynthesis were considered (Gaffron). There are two broad assumptions: One holds that light absorption leads to the formation of a specific reductant unique to the photochemical system, with a unique reaction chain eventually coupling with the respiratory metabolism. The other holds that a nonspecific process gives rise to a multiplicity of reductants not unique to the photic metabolism but coupled directly to typical respiratory reaction chains.

Data presented may indicate that, during back reactions induced by photochemical oxygen, coupling with phosphorylation to form adenosine triphosphate and other forms of "high-energy" bonds occurs (Strehler). It is suggested that a number of quanta could cooperate in this fashion to produce an aggregate of such compounds, which by dismutation could raise one or more substances to a potential capable of CO_2 reduction. A number of experimental studies showing light-stimulated esterification of phosphate were presented (Wassink), as also were data on the existence of a phosphatase in extracts from green leaf material that is somewhat inhibited by light (Tamiya).

Experiments suggesting the existence of a new TPN-linked triose phosphate dehydrogenase were reported (D. Arnon, University of California). A partially purified preparation has been found to affect the apparent reversible oxidation of 3-phosphoglyceraldehyde without addition of either phosphate or arsenate. A number of alternative explanations, not involving the existence of a new enzyme, were developed in discussions of this report (M. Gibbs, Brookhaven National Laboratory; R. S. Bandurski, California Institute of Technology; and B. Vennesland, University of Chicago). Further purification of the system will probably lead to clarification of the phenomenon involved.

Data and experiments showing the versatility of the chloroplast suspensions in coupling reductively to a large variety of DPN- and TPN-linked systems while actively carrying out a "Hill reaction" were reviewed (W. Vishniac, Yale). The question of the significance of these observations on the nature of the natural hydrogen transfer system was considered (H. Gest, Western Reserve; S. Granick; E. Rabinowitch; and J. Rosenberg, University of Chicago). In this connection, the experiments attempting to isolate a protein fraction from the supernatant of centrifuged chloroplast suspensions which catalyzes the fixation of CO_2 in phosphoglycerate have been continued. As yet only small, albeit reproducible, fixations primarily in phosphoglycerate and phosphopyruvate have been observed with the partially purified preparations when added to chloroplast suspensions otherwise inactive in fixation (E. Fager, University of Chicago). The fixations that are observed, at present, appear to be inhibited by TPN, DPN, and ATP.

It was also reported that extracts of *R. rubrum* exhibit what is apparently the anaerobic analog to the "Hill reaction" of green plant extracts (Vernon, Kamen, Washington University). A light-sensitized oxidation of reduced cytochrome or indophenol accords with the inference that the other end of the reaction involves liberation of H_2 or its equivalent. The extracts fail to show any reduction capacity at present, but further efforts are being made to search out the reducing substances expected to be formed.

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