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THE ENERGY CONVERSION APPARATUS IN PHOTOSYNTHESIS

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Kenneth Sauer

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ABSTRACT

An analysis of outstanding problems still presenting difficulty with respect to understanding the quantum-conversion process in photosynthesis is presented. Considerations of how some of these difficulties may be overcome are included. The dynamic process of energy conversion is considered in terms of photon absorption, electronic energy transfer, trapping in long-lived excited states, primary oxidants and reductants, and the electron transport chain leading to products representing stored chemical potential. The physical structure of the apparatus accomplishing this energy conversion is sought in the framework of the concept of the photosynthetic unit. The nature of this unit - its size, composition, arrangement and orientation of components, internal electrical and polarizability properties, and assembly and aggregation in the chloroplast - and the problems related to its determination are essential considerations in the overall approach to the understanding of the mechanism of energy conversion.

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** U.S. Public Health Service Fellow, 1960-63.

INTRODUCTION

Without doubt the greatest gap in our present knowledge about photosynthesis concerns the nature of the mechanism whereby radiant energy from the sun is converted into high-energy, stable chemical intermediates useful for the biosynthesis of organic molecules by the plant. This is not to say that our knowledge of the so-called "dark reactions" whereby plant cellular materials are synthesized is complete or even approaching completion; however, the major efforts of the past 15-20 years in the laboratories of Melvin Calvin, A.A. Benson, J.A. Bassham, Daniel I. Arnon and many others have produced an outline of the most important steps in the biosynthetic pathway (Calvin, 1962), and we have every reason to believe that this outline will prove to be essentially correct in the light of future experiments.

The magnitude of the efforts to find out the nature of the primary light-to-chemical energy conversion process is no less impressive; yet, a wholly satisfactory and widely accepted explanation of the mechanism has not resulted. Certainly one of the principal reasons is the fact that we are attempting to chart an area of nature which appears to be unique and for which we have no simple, readily understandable analogy.

I would like to begin by breaking down what we know about the energy conversion process into a sequence of events described in general terms, in spite of the obvious danger of thereby introducing specifications which may, in the long run, turn out to be misleading or incorrect.

(1) We can consider the process of photosynthetic quantum conversion to originate with the absorption of visible or near-visible radiation by the various pigment molecules associated with photosynthetic organisms. This absorption process, which by the Franck-Condon Principle must occur in about 10^{-15} sec following the capture of a photon, leads directly to the production of an excited electronic state of the pigment molecule involved. This process is reasonably well understood and need not concern us in principle in its particular manifestation in photosynthesis.

(2) The initially produced electronic excitation energy can, in general, be readily and rapidly (i.e. in 10^{-9} to 10^{-11} sec) transferred from one pigment molecule to another--either of like or of different kind - with the necessary restriction that in all such processes energy conservation must be maintained. In essence this means that all such transfers must occur to a state which contains the same or less electronic energy.

(3) The termination of the pathway of initial electronic excitation occurs when the energy becomes trapped in a state either which is relatively long-lived and/or from which it can be converted to some other energy form.

(4) At this or some closely subsequent point it is generally assumed that the primary oxidizing and reducing species are produced.

(5) The primary oxidant and reductant then proceed to drive what is commonly called the electron transport system; whereby

(6) The energy originally present in the photon is converted with relatively high efficiency to thermodynamically stable chemical intermediates. In this process in higher plants and algae, water is split, oxygen is liberated, TPN is reduced and ADP is phosphorylated to produce ATP. The dark, enzymé-catalyzed biosynthetic reactions then follow.

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It is necessary to point out that even this generalized statement of the process, which is consistent with much of the recent research on the energy conversion pathway, would by no means receive approval by all the reputable workers in the field. It probably does, however, represent a majority view.

For our purposes it will be desirable to discuss the energy conversion apparatus of photosynthesis under two major headings. Under the first of these I shall raise questions concerning the detailed mechanism whereby the dynamic processes described above are carried out. Under the second I shall propose methods of investigation into the physical nature of the structure that is able to perform the energy conversion so rapidly and so well.

The Dynamic Processes of Energy Conversion

Excited electronic states. Letting pass for the present the process of photon absorption itself, let us first consider the nature of the excited state produced and the manner in which energy may

be transferred from one excited state to another. A rather large variety of pigments can occur in photosynthetic systems: the several kinds of chlorophylls and carotenoids plus the phycobilins generally make up the principal complement. With some justice the question may be raised as to whether any of these besides chlorophyll a in green plants and marine algae, bacteriochlorophyll in photosynthetic bacteria, etc., are really essential to normal reasonably efficient photosynthesis. Although distinct functions, including that of light-gathering, can be demonstrated for the so-called "accessory pigments", it has not been demonstrated unequivocally that they play a necessary role in photosynthesis (Haxo, 1960). In nearly all cases studied so far, it appears that energy absorbed by accessory pigments (and in the short wavelength absorption bands of chlorophyll a) is transferred to a low-lying excited electronic state of chlorophyll a as a necessary prerequisite to its use in the photosynthetic pathway (Duysens, 1952). Recent studies on the various proposed roles for carotenoids, for example, as hydrogen or oxygen carriers or as oxidation inhibitors either have provided evidence to the contrary or have raised serious questions about the validity of the propositions (Yamamoto, Chichester and Nakayama, 1962 a,b; Sauer and Calvin, 1962b). These points still await a final answer, but for our present purposes we can consider the participating excited electronic states of chlorophyll a or bacteriochlorophyll alone, and can limit our discussion of energy transfer to that involving these states.

The electronic transitions responsible for all the readily observed visible and near infrared absorption bands of the chlorophylls are of the $\pi-\pi^*$ singlet class. The corresponding transition dipoles all lie in the plane of the porphyrin ring of the chlorophyll molecule. Several discussions of the particular molecular orbitals associated with the various components have appeared in the literature. (Platt, 1956; Seely, 1957; Gouterman, 1961). The lowest lying of these states in chlorophyll a has a natural lifetime in vivo of only 1.5×10^{-8} sec, as measured from fluorescence yields and decay times (Brody, 1957; Brody and Rabinowitch, 1957).

Long-lived excited states. Many workers feel that a longer-lived, electronically excited state is required for the energy to be localized for a sufficient time so that its conversion into high-energy chemical intermediates can be effected. Two such states have been proposed: one is the lowest triplet state of chlorophyll and the other is that resulting from an $n-\pi$ transition - possible owing to the participation of a carbonyl group in the ring conjugation of chlorophyll a. Both these excited electronic states have been observed in vitro; however, no direct evidence has been presented for their participation in the photosynthetic energy conversion process in vivo. On the contrary, experiments of Witt and coworkers (1960) show that photo-induced absorption changes attributed to triplet chlorophyll can be observed only when absorption changes associated with the functioning photosynthetic apparatus are quenched. Further

search for the participation of such long-lived states *in vivo* is called for. In the case of the triplet state, it would be reasonable to look for associated electron spin resonance signals. The photo-induced esr signals at g = 2.00, which are easily produced and readily observed in nearly all functioning photosynthetic organisms, do not result from a triplet state. Triple resonances at g = 2.00 give rise to very broad absorption spectra, owing to the zero-field splitting of the triplet energy levels and to the random orientation of the chlorophyll molecules in the bulk material (Ingram, 1958). Illumination of the sample with polarized light, so as to excite preferentially those molecules of a given orientation, will probably not give much improvement owing to the rapid, efficient energy transfer which occurs among chlorophyll molecules *in vivo*. It may be, however, that a search with high-sensitivity apparatus at g = 4, where the broadening effects do not operate, will produce evidence bearing on the participation of the triplet state. Studies with pure crystals of chlorophyll *a* would constitute a valuable preliminary to the search in biological materials.

An alternative method for the possible observation of long-lived excited state participation presents itself in the recently rediscovered magnetic optical rotation effect, first noted by Faraday. Experimentation along these lines is currently under way in our laboratories in Berkeley. Faraday's observation, stated in its simplest form, was that transparent substances placed in a suitably oriented magnetic field may induce a rotation of the plane of

polarization of incident plane-polarized light, even if the material does not contain asymmetric sites. It is known that the Faraday rotation exhibits dispersion characteristics in the vicinity of absorption bands of atoms or molecules (Waring and Custer, 1960; Hameka, 1962). Furthermore the selection rules governing these Faraday rotation anomalies are not the same as those governing normal electronic transitions observed spectrally in the absence of a magnetic field. Thus, it is possible that electronic transitions which are forbidden on the basis of their electric dipole transition probabilities will nevertheless exhibit dispersion effects in their Faraday rotation spectra. In this way it may be possible to locate the proposed long-lived excited states of chlorophyll in vivo and, by studying the effect of suitable exciting light, to determine whether they play a role in the photosynthetic energy conversion process.

Electron-hole conduction mechanism. A suggestion by Katz (1949) prompted Sogo, Pon and Calvin (1957) to propose that the heart of the energy conversion process consists of the production of electrons and holes in conduction bands of the type observed in molecular crystals. These electrons and holes, resulting from the trapping of excitation energy at suitable sites in the chloroplast, are thought to be the primary reductant and oxidant species responsible for originating the subsequent reactions of the electron transport chain, for the oxidation of water, and for the ultimate production of molecular oxygen and reduction of TPN. One very nice feature of this proposal is that the rapid, independent migration of holes and electrons in a suitable matrix provides a convenient explanation for the absence of a significant back-reaction of the primary oxidized and reduced species once they are produced. The

absence of appreciable back-reaction is essential for photosynthesis of high efficiency. Although experiments on suitable model systems have demonstrated that the photo-production of such charge carriers is of common occurrence (Kearns, in press), only circumstantial evidence is thus far available to support their participation in photosynthetic energy conversion. This evidence is chiefly the observation of photo-induced absorption changes, electron paramagnetic resonance absorption, and dielectric and conductivity changes, all of which can occur rapidly and sometimes reversibly even at the temperatures of liquid nitrogen, and, in one study, of liquid helium. Although most of the techniques used to date have not been capable of measuring time constants shorter than about 1 sec, the studies by Witt and coworkers (1960) of absorption changes produced by light from a flash lamp have produced effects with time constants of 10^{-5} sec or shorter. The accurate measurement of the rise and decay times, especially of the phenomena which appear to be temperature independent, is a challenge to experimental ingenuity. Such information would be invaluable for the assignment of these effects, which apparently do not involve the migration of individual atoms or molecules.

Integration of low-level signals. A paramount experimental problem which must be overcome arises from the fact that, for the phenomena listed above, the observed experimental signals ride on a noise level which is scarcely less than the quantity sought. Simply improving the time response of the instrument will not, in general, provide the necessary retention of discrimination. A solution to this problem, and to many related measurements of time transients where

low signal-to-noise ratios are a problem, may come in the application of one of a number of recently-developed integrating techniques. Particularly in situations where the transient phenomenon is readily reversible and can be repeated again and again, the coherent information in the photo-induced signal can be added by a suitable "integrating" device faster than will be the random or incoherent noise. A somewhat crude, but more familiar, analogy is present in the measurement of radioactivity. For all but the smallest number of counts, the precision with which the radioactivity can be determined increases with the square root of the total number of counts in the sample. The accuracy improves as well, since long counting times and extended background determinations improve the systematic corrections which need to be applied. In principle, any specified level of precision can be obtained if the experimenter has the patience to wait long enough.

Improvement of signal-to-noise can be achieved for many relatively time-independent phenomena simply by lengthening the time constant of the measuring device and waiting sufficiently for the indicating meter to achieve a good approximation of the final rest point. Galvanometer and balance damping and electronic filtering or narrow-banding are familiar techniques of this kind. They can generally be extended in a simple fashion to improve the sensitivity and precision of measurements such as absorption and fluorescence spectra, electron paramagnetic resonance spectra, or quantum requirement measurements.

The patient researcher who is willing to trade time for information stands to gain considerable improvement over many conventional procedures current in laboratory practice. The

simple integration method of extending measuring time constants is not a panacea, however. There are phenomena such as the study of rapid transients, irreversible processes, unstable systems, and potential saturation effects (as in nuclear magnetic resonance) where other methods are demanded.

By way of illustration, consider the problem of measuring low level photo-induced absorption spectral changes in photosynthesis. A major source of noise in such measurements arises simply from the statistical emission of photons by the measuring light source. This noise is unavoidable; it is also "white" noise, in the sense that it contains all frequency components up to a very high level. For fairly monochromatic light from a 500 watt tungsten light suitably incident on a sample, the inherent noise in the measuring beam alone in the frequency range 0 to 1 megacycle may correspond to a signal-to-noise of 1000:1. Thus, an absorption change amounting to a few tenths percent with a microsecond time constant would be barely observable under the best of conditions; accurate kinetic measurements would be out of the question. Increased incident light intensity, more efficient optics, electronic narrow-banding techniques, etc., can be used to give some improvement, but the best solution to the problem lies in another direction.

Through the proper application of electronic or other time-gating techniques it is possible to pick out two representative points in the duration of the transient phenomenon and to study at some length the resulting signal from these two points only. This is done by repeated induction of the transient and by proper phasing of the gate positions, as is illustrated for a representative transient signal in Fig. 1. In case A, the difference between the signal

amplitudes at gate positions 1 and 2 is nearly zero, whereas in case B the corresponding amplitude difference is substantially larger. In the case of a real transient signal riding on a very high noise level, the difference in the amplitudes at the two gate positions can be "integrated" by repetition for as long as necessary to achieve a specified signal-to-noise. The examples of such measurements shown in Fig. 2 were obtained through the use of a "boxcar" integrator of the type described by Blume (1961). The device operates essentially on the principle of increasing the charge on a capacitor in proportion to the magnitude of the amplitude difference between the two gates and in proportion to the number of repetitions of the transient observed. It is possible to adjust the RC time-constants of the "boxcar" circuit so that it corresponds to different numbers of transient repetitions. In Fig. 2 are shown three situations corresponding to 10^2, 10^4 and 10^6 transients, respectively, in the duration of the RC time constant of the circuit. In each case the first half of the trace would result from the gate arrangement sketched in Fig. 1A, and the second part as in Fig. 1B. The integrated traces are recorded on an XY-pen recorder with a writing speed of 3 inches/minute. By moving the position of Gate 2 through the complete transient, as indicated in Fig. 1C, the entire profile may be precisely recorded over a time which may be a million or more times longer than the duration of a single transient pulse. A corresponding improvement of one thousand fold or more in signal-to-noise may be expected. An illustration

of a ten-fold overall improvement in signal-to-noise resulting from the use of the scanning "boxcar" technique is shown, from top to bottom, in the three curves at the right in Fig. 3. The curves at the left were obtained by reducing, from top to bottom, the high frequency cutoff of an AC coupled amplifier. The resulting improvement in signal-to-noise in this case is accompanied by serious distortion of the shape of the transient due to the loss of the high-frequency components necessary for its accurate representation. No such distortion need occur if integrating techniques are properly applied. An elegant example of the sampling method has been reported for the measurement of fluorescence rise and decay curves with time constants of the order of 10^{-9} sec (Bennett, 1960).

The integration process can be carried out more efficiently if more than two gates are applied to each transient. Multi-channel pulse-height analyzers are commercially available which readily handle 256, 400, 512 and up to 8000 and more channels. A representation of this gating pattern is shown in Fig. 1D. The improvement in discrimination of low level signals using techniques of this kind can be astounding. Many measurements associated with photosynthesis are susceptible to substantial improvements through proper coupling with modern electronic devices. No less numerous are applications to other areas of cell biology; e.g., in the study of nerve impulses, membrane potential transients, visual pigment responses, mechanical changes in cell conformations and the kinetics of reactions involving very small amounts of substrates or very few enzyme molecules as catalysts.

Electron spin resonance. Electron spin resonance signals, due presumably to molecules containing single unpaired electrons,

have been observed in most photosynthetic organisms. As shown in Fig. 4, the signals are of two types. One has a narrow (~10 gauss) band-width, g = 2.002, is strongly light dependent and normally absent in the dark, and exhibits relatively fast, largely temperature-independent, kinetics (< 1 sec, limited by the instrument). This signal has been shown to be associated with the pigmented structures of chloroplasts and bacteria (i.e., the quantasomes and chromatophores, respectively) and seems to be due to a species with a reversible oxidation potential of +0.45 volts (chlorophyll positive ion?). The second signal is broader (~20 gauss) with g = 2.005, is somewhat light dependent but remains strong in the dark, and exhibits slow (minutes), temperature-dependent rise and decay times. This signal appears to be associated with some component which is either soluble in the stroma of the chloroplast or is at least readily extractable from the chloroplast in aqueous media (Androes, Singleton and Calvin, 1962). Fine structure has occasionally been observed for this esr signal. The true assignment of the molecular species responsible for either of these signals has not yet been achieved, nor has it been conclusively demonstrated that the associated species are involved in the direct pathway of photosynthetic energy conversion, although the evidence to support such a view is mounting. Further information about these resonances may be expected from measurements of the rapid time constants - perhaps through the use of integrating techniques as described above, from the precise determination of quantum yields and action spectra, by carrying the measurements down to 1°K, and by double resonance ENDOR techniques to show with which nuclei the electrons interact most strongly.

Electron transport reactions. In general, the identification of the carriers of the electron transport chain is far from complete. A number of possible carriers - such as cytochromes b_6 and f in green plants and RHP and cytochrome c_2 in bacteria, plastoquinones (three types), vitamin K, coenzyme Q_7 and ferredoxin (PPNR) - have been identified and partially characterized. The nature of the participation of these substances in electron transport and, in particular, the coupling to substances adjacent in the electron transport chain and to phosphorylation, are all little understood. Of particular interest would be the characterization of the primary chemical donors and acceptors associated with the site at which quantum conversion occurs. Other problems relating to two-wavelength enhancement and to two non-overlapping pigment systems, to the mechanism of oxygen formation, etc., are still mystifying. In most cases classical experimental techniques have much to offer to the kineticist interested in unravelling the complex reactions associated with electron transport.

Quantum efficiency of photosynthesis. A major outstanding issue which is still unresolved today after more than three decades of investigation is the minimum quantum requirement of carbon fixation in normal photosynthesis. Despite the large number of measurements which suggest that about eight quanta are required per CO_2 fixed, there remains an important and perhaps now even a growing school which feels that the quantum requirement may be as low as three quanta per CO_2. For a number of mechanisms which have been proposed to account for energy conversion in green plant photosynthesis it makes a big difference whether the quantum requirement is at least eight or whether it is significantly less than this value. One would think

that the distinction between a quantum requirement of three and a requirement of eight or more would be readily settled experimentally, in spite of the admitted complications of the overall process. Still, an unambiguous answer eludes us today. It may be that we cannot formulate a meaningful answer to this question until we know more about the detailed mechanism, including the many feed-back channels, which is operative in the overall photosynthetic process. With this view, the most profitable approach at the present stage in our knowledge may be to attempt reliable measurements on the various partial reactions of photosynthesis, including those which are exhibited as physical changes or transients, the Hill reaction, photophosphorylation and TPNH formation. Experiments along these lines are under way, but much more needs to be done.

Quantasome and Chromatophore Structure

The second major aspect of the problem of photosynthetic energy conversion on which I would like to make some comment is the question of the physical nature or structure of the apparatus involved. The suggestion of Emerson and Arnold (1932), based on their studies using flashing light, that there exist photosynthetic units within the chloroplast has received elegant confirmation within the past few years. Studies using electron microscopy (Park and Pon, 1961; Healy and Park, private communication) coupled with the demonstration of certain aspects of photosynthetic activity in chloroplast subunits produced by sonic rupture, strongly support this concept of a viable photosynthetic unit. Whether this unit contains 100 or 400 chlorophyll molecules will undoubtedly be answered by research presently under way. We cannot underemphasize the importance of the finding that efficient quantum conversion related to the photosynthetic pathway can be accomplished by particles -

called quantasomes - which are 100-200 Å in diameter, less than one hundredt the diameter of the intact chloroplast. A similar situation obtains for photosynthetic bacteria, where the chromatophore or its subunit constitute an analogous unit of activity. The problem of determining the structural characteristics of the site of quantum conversion, difficult as it is, is immeasureably simplified for the photosynthetic units in comparison with what it would be if the entire chloroplast or bacterium were involved.

Chemical composition. Preliminary analytical data have been obtained for spinach quantasomes (Park and Pon, 1963). The principal components are protein (50% of the dry weight) and colorless lipids, probably phospholipids, (30%). Chlorophyll makes up nearly 10% of the total dry weight. The remainder consists of a variety, perhaps a large variety, of components, including carotenes and xanthophylls (4%), at least three plastoquinones (total 1%), cytochrome b_6 and vitamin K. A number of additional ones will undoubtedly be found. Measurements on the monomeric quantasome suggest a molecular weight of 1.6×10^6, based on an experimentally determined density of 1.18, which is intermediate between those of lipid and of protein. The number of chlorophylls per unit can be estimated in various ways: the range runs from 115 to 180. One manganese, five iron and five copper atoms are present in some form in each quantasome monomer. An extension of such measurements is an essential prerequisite to the conceptual reconstruction of the quantasome.

With particles of the intermediate complexity of quantasomes and chromatophores, analytical techniques based on physical properties of the components can be brought to bear. Both infrared and nuclear

magnetic resonance spectra of the materials would be expected to give complex but possibly very useful information. Coupled with extraction and separations techniques it may be possible to learn the nature of the components present (e.g., the colorless phospholipids) and perhaps about their intermolecular association. The low inherent sensitivity for biological materials of nuclear (especially proton) magnetic resonance techniques can be greatly enhanced by coupling the spectrometer with a multi-channel analyzer, as described in other terms above. This application has recently been explored by Klein and Barton (Lawrence Radiation Laboratory, Livermore) and by a group at Varian Associates (Palo Alto, California).

Internal structure and aggregation. Beyond the straightforward quantitative determination of the quantasome or chromatophore components it is essential to learn the manner in which they are assembled. After ascertaining the macromolecular properties of monomer quantasomes, one will want to learn whether major subunits exist, as is the case for the elementary particles of mitochondria. The new developments in high-resolution electron microscopy by Dr. Fernandez-Moran will constitute an invaluable tool with which to study this problem. The possibility of utilizing the natural contrast of unstained material will open wide areas of application presently only dreamed of. The quantasomes would seem to offer particular advantages for the studies using natural contrast electron microscopy, since each particle contains naturally atoms of iron, manganese and copper, as well as the considerable amount of magnesium-containing chlorophyll.

Presumably the principal structural materials of the quantasomes are

the protein and the phospholipids. On the basis of recent electron microscopic studies, carried on by R.B. Park at Berkeley, on lipid-extracted vs unextracted quantasome aggregates there is good reason to believe that the lipid and protein do not form a relatively homogeneous lipoprotein network. One model would suggest a two-phase separation between a hydrophilic protein region and a lipophilic phospholipid region. The site of energy conversion may be closely associated with the boundary between these two regions. Studies of electric dichroism of quantasome aggregates suggest that an essential element of the energy conversion site - the quantatrope - is a region containing a small fraction of the chlorophyll a as specially oriented molecules./Low temperature transient studies of various kinds, (Sauer and Calvin, 1962a) as listed previously, suggest further that oriented or fixed donor and acceptor molecules may be incorporated in this site. Further studies of the nature of the site and of the associated molecules are under way; these are based largely on polarization (dichroism) measurements on oriented materials. In addition, one may hope that the gigantic strides made in recent years in the decoding of complex molecular structure from X-ray diffraction patterns will soon carry forward to cover a still greater level of complexity represented by the various sub-cellular particles of mitochondria and chloroplasts. Thus far, only a few interplanar spacings have been obtained through diffraction techniques applied to chloroplast grana (Kreutz and Menke, 1960); the precise and complete determination of the internal molecular structure of quantasomes and chromatophores presents a challenge of unparalleled complexity and significance to the investigators of macromolecular conformations.

Profile of dielectric properties. The dielectric and other electromagnetic properties of quantasomes, and especially the manner in which these properties vary within the quantasome, may be of primary significance to the energy conversion process. A number of the reactants and products of intermediary electron transport are ionic or other water soluble species. These can be separated or confined by bounding lipid regions, which in turn will harbor other potential cofactors in the process. This separation in space may be essential to an orderly and efficient energy conversion process. The transport of metabolites such as ATP and TPNH, and even O_2 from the active sites and ADP, phosphate, TPN^+ and water to the sites is closely related to the manner in which the quantasomes are put together in the lamellae and grana of the chloroplast.

Measurement of the internal distribution of the various electrical properties of small particles is not an easy task. It may be, however, that through the use of sensitive selective extraction methods it will be possible to piece the picture together from a series of bulk measurements. However, I think this is unlikely. We may well consider how experiments might be designed to map out the internal dielectric and polarization properties of quantasomes and chromatophores. Whether we have a protein nucleus covered with a lipid coating, whether it is a two- or three- or a multilayered sandwich, or whether some more complex basic pattern is involved is essential to our understanding of photosynthetic transport properties.

Summary and Review.

All in all, the outstanding problems associated with the

quantum conversion process in photosynthesis constitute an imposing challenge to present-day ingenuity and experimental techniques. The questions I have raised are going to be answered - some of the answers are being formulated even now. There is no reason why studies along any of the lines I have suggested cannot profitably be started tomorrow. Still, these are intended to be merely suggestive directions to catalyze response among those who would contribute to the solution of one of nature's cleverest puzzles.

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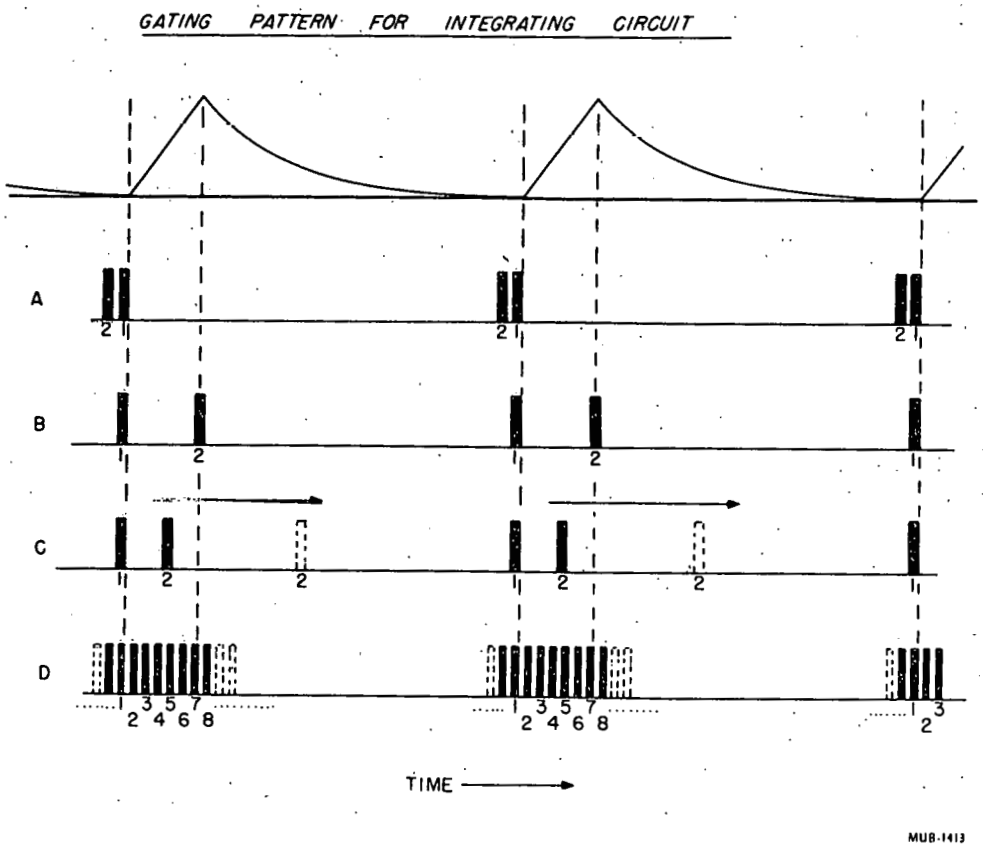

Fig. 1. Gating patterns for various modes of integrating signals from a hypothetical transient repeatedly presented. Gate 1 represents a reference position; gates 2, 3, 4, etc. represent sample positions. For further details, see text.

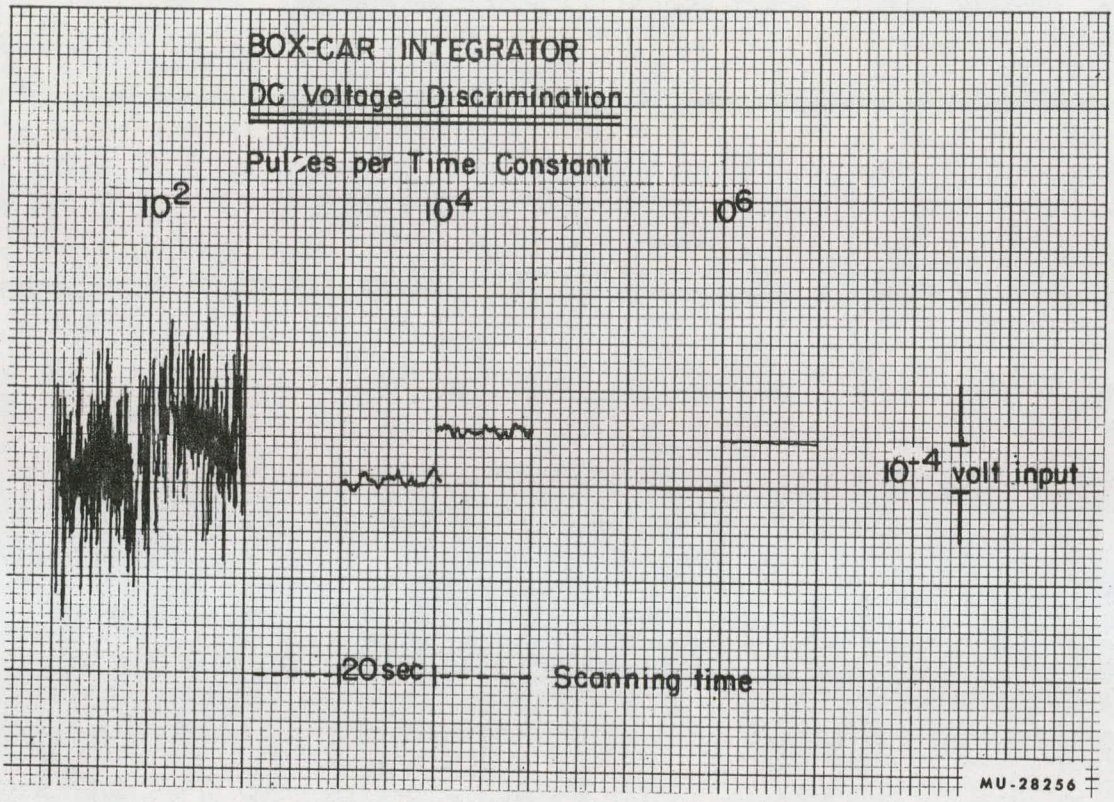

Fig. 2. Integration of noisy dc signals using a "boxcar" circuit. For the three cases shown, the signal-to-noise ratios are in the proportion 1:10:100. Traces provided by I. Kuntz.

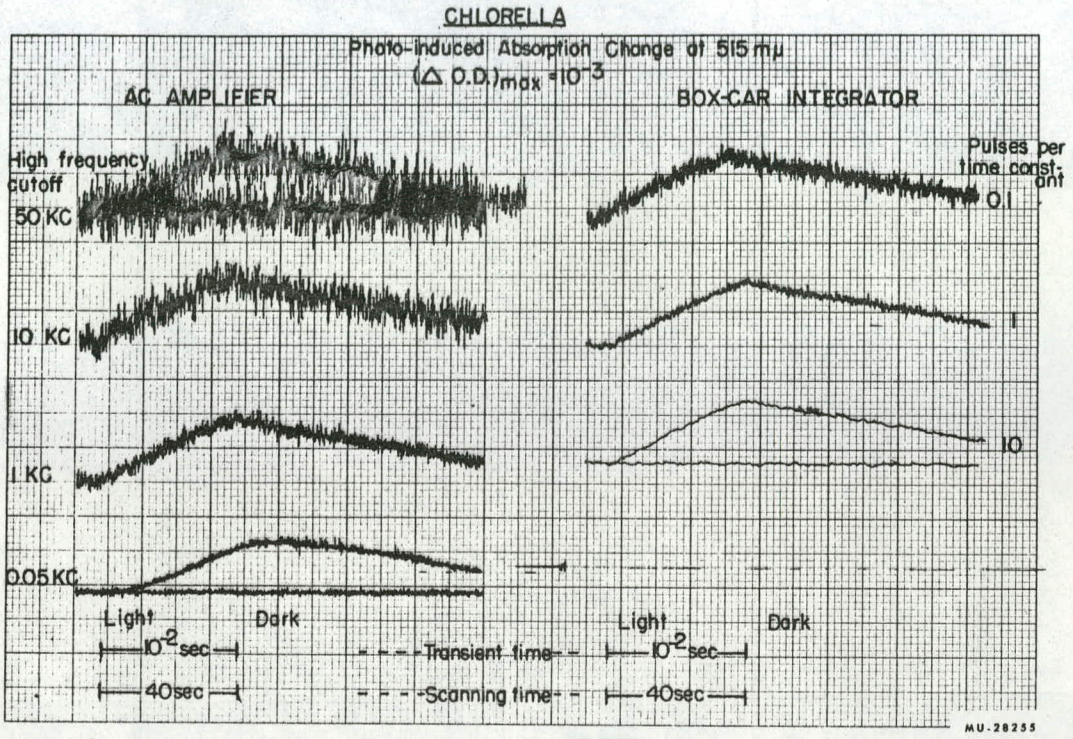

Fig. 3. Integration of transient photo-induced absorption changes. Traces at left were obtained using several different high-frequency limits for an ac amplifier. Traces at right were obtained using the "boxcar" integrator. Measurements provided by I. Kuntz.

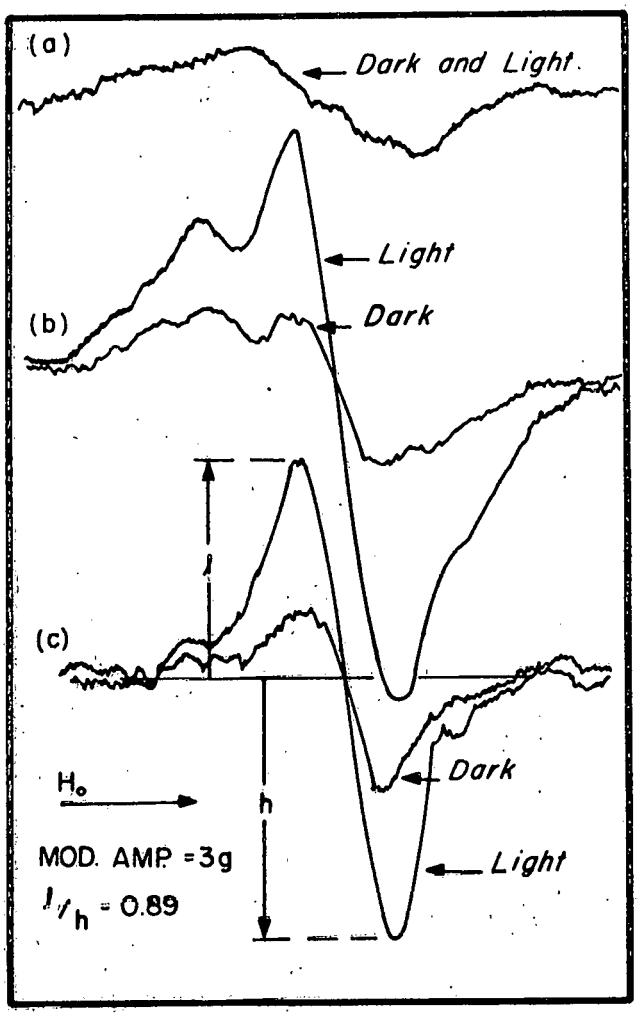

Fig. 4. Separation of overlapping electron spin resonances in spinach chloroplasts. Signals from (a) colorless soluble stroma materials leached from chloroplasts, (b) whole spinach chloroplasts, (c) washed chloroplast fragments (quantasomes). Androes and Calvin (in press).