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BIO-ORGANIC CHEMISTRY ANNUAL REPORT

December 1963 through December 1964

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Contents

FOREWORD

Most of the work of the Bio-Organic Chemistry Group stems from two primary concerns: First, the development of radioactive isotopes and their application principally to chemical and biological systems, and, second, the interaction of biological and chemical systems with radiation. In this annual report we have selected a few of our activities for description in order to indicate the scope of the Group's productivity. Many of the particular projects involve the interaction of radiation with both chemical and biological systems as well as the use of the tracer method to define the nature of those interactions.

Our studies in photosynthesis and radiation chemistry have continued on an expanding frontier which includes both the metabolic behavior, the physical-chemical structure, and the energy conversion process, a few aspects of which are described in this report. It is perhaps worth mentioning specifically that the radiation chemistry and the fundamental problems of information transfer in biology have led us into the problems of chemical evolution as well as into research on brain mechanisms and learning.

In succeeding years we hope to select for specific presentation other aspects of our activity which are only peripherally mentioned in this report, and thus give proper balance and coverage over the years.

Melvin Calvin, Director
Bio-Organic Chemistry Group
Lawrence Radiation Laboratory

Bio-Organic Chemistry Annual Report

December 1963 through December 1964

M. Calvin, Director

Lawrence Radiation Laboratory and Department of Chemistry
University of California, Berkeley, California

February 9, 1965

1. PHOTOSYNTHESIS OF CARBON COMPOUNDS

J. A. Bassham

The mapping of the photosynthetic pathways from carbon dioxide to end products and the elucidation of the mechanisms of individual reactions along these pathways have been of continuing interest to this Group for some years. Earlier work resulted in the mapping of the basic pathway for the fixation and initial reduction of carbon dioxide. This pathway is the carbon reduction cycle, or Calvin cycle.[1]

Electrons and chemically energetic cofactors which drive the carbon reduction cycle are formed by the "light reactions" of photosynthesis which convert absorbed electromagnetic energy to chemical energy. The reduced and energetic cofactors not only drive the basic cycle, but also reduce nitrate and sulfate, and convert intermediate compounds of the cycle to end products, including fats, proteins, carbohydrates, nucleic acids, pigments, etc.

Present research projects include studies of (a) the mechanism of transfer of electrons and "high-energy phosphate" to the reactions of the carbon reduction cycle, and mechanisms by which reactions of the cycle are mediated, (b) location of "branch points" along the cycle at which intermediate compounds are "drained off" from the cycle for use in biosynthetic pathways leading to end products, and the regulatory mechanisms at these points, and (c) details of biosynthetic paths leading to end products. The latter paths are considered photosynthetic if they utilize cofactors formed by the light reactions.

During 1964, studies of the photosynthesis of amino acids and proteins were reported.[2,3] Among the conclusions reached were the following:

1. Carbon reduction during photosynthesis produces amino acids directly in actively-turning-over pools in the chloroplasts.
2. Amino acids from these pools are used directly in the formation of protein, probably in the chloroplasts.
3. The principal incorporation of ammonium ion into amino groups is via reductive amination to give glutamic acid.
4. The photosynthetic pool of glycine is very small under the conditions used, but this small pool (compared to total glycine pool is utilized for photosynthetic protein synthesis.

Since most of our results were obtained with unicellular algae, it was desirable to check our findings with other plants. During the year we constructed and used an apparatus that permitted kinetic tracer experiments with Lemna, as well as other higher plants.

We are currently investigating the effects of different light colors on the distribution of photosynthetic products in Chlorella and in Nostoc. Previous studies of the effects of colored light on products of carbon fixation in whole plants have mostly been conducted prior to knowledge of the action spectra of so-called "two-light" effects. If current theories about the operation of two pigment centers in photosynthesis are correct, one might expect that light used only by the "long-wavelength" center might produce relatively more "high-energy phosphate" (such as ATP) in comparison with reduced cofactors (such as NADPH) than light utilized by both pigment centers. Since the cofactor requirements differ for the biosynthesis of various end products, one might expect to cause variation in

the distribution of end products by varying the illumination wavelength in an appropriate way. On sudden change from one light wavelength to another, transient changes in the concentration of intermediate compounds of the cycle might also be expected. Preliminary results suggest that intact cells of green plants such as Chlorella may be less sensitive to wavelength than isolated and broken chloroplasts from leaves. However, we are hopeful that blue-green algae, Nostoc, may exhibit more pronounced effects, because of the greater wavelength separation of the absorption spectra of the two pigment systems.

We are studying the formation of glycolic acid during photosynthesis using ^{14}C and T (^{3}H). Previous work on this problem has been somewhat inconclusive owing to (a) glycolic acid volatility, and (b) difficulty in counting small amounts of T, in the presence of ^{14}C. These problems have been overcome, and early results indicate that the glycolic acid is not formed by a reductive pathway from CO_2 (as has been sometimes suggested),[4] but is probably formed oxidatively from sugar phosphates of the carbon reduction cycle, as we suggested several years ago.[5]

Our major effort during the past year has been directed to the mechanisms by which the reactions of the carbon reduction cycle, including those using cofactors from the light reactions, are mediated. In a recent review[6] we outlined reasons for postulating that the cycle in vivo may be mediated by an organized system of enzymes, possibly including multifunctional enzymes, in close (but easily disrupted) association with the lamellar structure of the chloroplasts. If this hypothesis is correct, it may be that some of the electrons and "high-energy phosphate" are transferred from the photoelectron transport system, known to be located in the lamellae, directly to the enzymes of the carbon reduction cycle. Conceivably, such an arrangement could provide catalysis for an in vivo reductive carboxylation, in contrast to the known non-reductive carboxylation mediated by the isolated enzyme, carboxydismutase.

In an attempt to obtain additional kinetic evidence from the in vivo system which might bear on these proposals, we have undertaken a series of studies to investigate the transient effects of the addition of various chemicals to the algae photosynthesizing with $^{14}CO_2$ under steady-state conditions. With the substances which penetrate the cell rapidly and produce immediate effects, rapid transient changes in the levels of intermediates of the carbon reduction cycle can be seen. This type of chemically induced change in the metabolite concentration provides a more direct kind of information about the mode of action of the chemical than does the pre-incubation of the whole cells with inhibitor followed by the application of tracer to the already damaged system. Observation of the immediate effects of a chemical on a steady-state system seems more likely to provide information about the primary site of action.

We have administered to whole chlorella cells chemical compounds known to produce large effects on the photoelectron transport system or photophosphorylation system in isolated, or broken, chloroplasts. Other substances tried were suspected of having an inhibitory action on one or more steps of the cycle. Not all such substances produced effects with whole cells, perhaps owing to their failure to penetrate the cell wall. However, we have been able to find several substances which cause complete inhibition of photosynthesis within 2 minutes after their addition to the algaw which were photosynthesizing under steady-state conditions. We found that methyl lipoic and lipoic acids produce rapid reversible inhibition of photosynthesis accompanied by very rapid transient changes in the levels of intermediate compounds of the photosynthetic carbon reduction cycle. Therefore we tested several other lipid-soluble compounds. Of these substances, hexylresorcinol caused rapid inhibition. We have studied the transient changes in the levels of radioactive intermediate compounds of the photosynthetic carbon reduction cycle and metabolically related substances which result from addition of each of these various inhibitors.

For the purposes of summarizing the results of these studies with inhibitors, the reactions of the carbon reduction cycle may be simplified to the following:

I. The phosphorylation of ribulose-5-phosphate (Ru5P with adenosine-triphosphate (ATP) to give ribulose-1, 5-diphosphate (RuDP).

II. The carboxylation of RuDP to give an unstable 6-carbon compound which is hydrolytically split to give two molecules of 3-phosphoglyceric acid (PGA).

III. The phosphorylation of PGA to give phosphoryl-PGA, followed by reduction with reduced nicotine adenine dinucleotide ($NADPH_2$) to give 3-phosphoglyceraldehyde (PGAl).

IV. Isomerization and condensation of PGAl, with itself, and with erythrose-4-phosphate, to give fructose-1, 6-diphosphate (FDP) and sedoheptulose-1, 7-diphosphate (SDP).

V. Conversion of FDP and SDP to their respective monophosphates, F6P and S7P,

by removal of the phosphate group at the number one carbon position.

IV. Transketolase and other reactions to make eventually three molecules of Ru5P from five molecules of PGAl, thus completing the cycle.

When the light is turned off, the supply of ATP and $NADPH_2$ stops, and Reactions I and III stop. This causes the concentration of RuDP to drop rapidly to zero (due to dark carboxylation) and the concentration of PGA to rise (no longer reduced to PGAl) and then to fall (conversion to alanine and other products outside the cycle) after carboxylation has stopped owing to RuDP's being used up. These effects are reproduced almost exactly by the addition of dichlorophenyl methyl urea (DCMU) and by carbonyl cyanide chlorophenylhydrazone (CCCP). Presumably CCCP uncouples photophosphorylation, cutting off the supply of ATP. DCMU halts photoelectron flow from water, resulting in cutting off the supply of both ATP and $NADPH_2$.

Addition of Vitamin K_5--known to stimulate cyclic photophosphorylation in isolated, illuminated chloroplasts--apparently stimulates ATP production at the expense of NADP reduction in vivo as well. The level of PGA increases, indicating that its reduction to PGAl is blocked by the absence of $NADPH_2$. That NADP is now in its oxidized form is also indicated by an abnormal appearance of 6-phosphogluconic acid, the presumed result of oxidation of glucose-6-phosphate. Most of the sugar phosphates are greatly depleted, since PGA reduction stops whereas ATP production favors the continued phosphorylation of Ru5P to give RuDP, which is then carboxylated. However, it is interesting to note that though there is an initial dip in levels of FDP and SDP, because of slowing down of PGA reduction, there is a subsequent rise in these diphosphates, indicating inhibition of the removal of the number one phosphate (Reaction V). This phenomenon may indicate a relation between removal of the number one phosphate of FDP and SDP and photophosphorylation. After several minutes' addition of Vitamin K_5, photophosphorylation may be presumed to be blocked because of conversion of all ADP to ATP.

In addition of hexylresorcinol, like DCMU and CCCP, slows Reaction III (the conversion of PGA to PGAl), but it also blocks the conversion of FDP and SDP to the monophosphates--again an inhibition of the phosphate removal. Since there is no indication of oxidation of NADP (as in the case with Vitamin K_5) it could be that the slowing of the reduction of PGA is again due to blocking of photophosphorylation.

Finally, addition of fatty acids ranging in chain length from C_6 to at least C_{12} (where solubility becomes very limiting) and of lipoic acid causes reversible inhibition of photosynthesis which is accompanied by apparent blocking of Reaction II, the carboxylation (as indicated by a very rapid drop in PGA level and a momentary rise in RuDP level), followed in a few seconds by blocking of both Reaction I (RuDP level drops again) and Reaction V, the removal of number one phosphate groups from FDP and SDP. The latter effect is quite pronounced, with FDP and SDP levels rising threefold or more, and the corresponding monophosphates dropping by a similar factor, in some cases. The simultaneous blocking of Reactions I and V once again suggests a relation between removal of number one phosphate groups and photophosphorylation.

Several laboratories have reported that chloroplast suspensions show light-induced reversible light-scattering and absorption changes.[7-9] Work at the C. F. Kettering Research Laboratory[10] suggests that these changes are produced by high-energy intermediates of photophosphorylation. Recent unpublished work in our Laboratory indicates that similar changes can be produced in whole Chlorella cells and are greatly increased by the presence of fatty acids, but abolished by the photophosphorylation uncoupler, CCCP. Thus it appears that these physical effects, photophosphorylation, and the removal of FDP and SDP phosphates may all be related. It is hoped that further studies of both metabolic and physical effects of inhibitors will yield clues about the nature of the energy transfer process in photosynthesis.

References

1. J. A. Bassham, A. A. Benson, Lorel D. Kay, Anne Z. Harris, A. T. Wilson, and M. Calvin, J. Am. Chem. Soc. 76, 1760 (1954).

2. J. A. Bassham and Martha Kirk, Biochim. Biophys. Acta 90, 553 (1964).

3. J. A. Bassham, Bronislawa Morawiecka, and Martha Kirk, Biochim. Biophys. Acta 90, 542 (1964).

4. M. Stiller, Ann. Rev. Plant Physiol. 13, 151 (1962).

5. M. Calvin and J. A. Bassham, The Photosynthesis of Carbon Compounds (W. A. Benjamin, Inc., New York, 1962).

6. J. A. Bassham, Ann. Rev. Plant Physiol. 15, 101 (1964).

7. L. Packer, Biochim. Biophys. Acta 75, 12 (1963).

8. M. Itoh, S. Izawa, and K. Shibita, Biochim. Biophys. Acta 66, 319 (1963).

9. A. T. Jagendorf and J. Hind, in Photosynthetic Mechanisms of Green Plants (Natl. Acad. Sci. -Natl. Res. Council, Washington, D. C., 1963), p. 599.

10. R. A. Dilley and Leo P. Vernon, Biochem. Biophys. Res. Commun. 15, 473 (1964).

2. BIOSYNTHESIS OF OPIUM AND TOBACCO ALKALOIDS

H. Rapoport

Opium Alkaloids

Earlier work from these laboratories established the rapid de novo synthesis of thebaine from $^{14}CO_2$ and its primary in the morphine-codeine-thebaine group of opium alkaloids. The next problem to which we turned our efforts was to ascertain the precursor of thebaine. Feeding experiments in other laboratories indicated that the benzyltetrahydroisoquinoline compound, reticuline, might be the precursor. We have obtained evidence, derived from short-term exposures to $^{14}CO_2$ of both seedlings and budding seedlings and budding plants of Papaver sommiferum, which now establishes reticuline as the true biosynthetic precursor of thebaine and, via thebaine, of codeine and morphine.

First the natural occurrence of reticuline in very low concentrations in 120-day-old poppies and 5-day-old seedlings was established by gas-liquid chromatography. Then several hundred 5-day-old seedlings were exposed to $^{14}CO_2$ for 2.5 hr and the alkaloids were isolated. From the relative amounts and specific activities of reticuline and thebaine, reticuline's essential role as precursor was demonstrated. Similar experiments and similar results were obtained with older plants.[1]

Tobacco Alkaloids

Plants of Nicotiana glutinosa were grown in an atmosphere containing $^{14}CO_2$ for periods varying from 2 hr, the shortest time at which incorporation of radioactivity into nicotine, isolated separately from the root and aerial portions, was degraded, and the activity in the pyridine ring, the N-methyl group, and carbon-2' of the pyrrolidine ring was determined. These data were correlated in terms of (a) the rate of incorporation of CO_2 into nicotine, (b) the site of nicotine syntheses, (c) the relative rate of N-methyl syntheses, and (d) the relative rates of syntheses of the pyridine and pyrrolidine rings. The conclusions thus reached have been compared with those in the literature derived from grafting experiments and from feeding precursors other than CO_2. Evidence also indicated independent nicotine synthesis in both root and aerial portions.

Radioactive nicotine, isolated from the root and aerial portions after a 6-hr exposure to $^{14}CO_2$, was degraded and the activity determined in the pyridine ring, the N-methyl group, and C-2' and C-5' of the pyrrolidine ring. Equal activity was found at C-2' and C-5', consistent with the glutamate-symmetrical intermediate hypothesis. However, the labeling pattern required is not derivable from any of the known glutamate biosyntheses. An attempt was made to reconcile the various data by suggesting a new glutamate biosynthesis.[2,3]

References

1. R. O. Martin, M. E. Warren, Jr., and H. Rapoport, J. Am. Chem. Soc. 86, 4726 (1964).

2. W. L. Alworth, R. C. DeSelms, and H. Rapoport, J. Am. Chem. Soc. 86, 1608 (1964).

3. W. L. Alworth, A. A. Liebman, and H. Rapoport, J. Am. Chem. Soc. 86, 3375 (1964).

3. THE STRUCTURE AND CHEMISTRY OF CHLOROPLASTS

Roderic B. Park

Previous work in this and other laboratories has shown that the photosynthetic process is distributed between two morphologically distinguishable portions of the chloroplast. The quantum conversion reactions and associated electron transport reactions, including phosphorylation, were shown to take place in the internal membrane system of the chloroplast. The CO_2 fixation reactions were shown to be associated with the matrix material surrounding the membranes, the stroma. During the past year we have continued our studies of the ultrastructure, chemical composition, and interaction of these chloroplast substances.

The Internal Membranes of Chloroplasts

The internal membranes of the chloroplast are easily separated from the stroma material of the chloroplast by washing the chloroplasts in dilute buffer. When such membranes are shadow cast and viewed in the electron microscope the membrane is seen to consist of particulate units which we initially described as 200-Å oblate spheroids. We termed these structures quantasomes, with the thought that this particle might represent the smallest membrane unit capable of quantum conversion. Experiments described during the past year[1] have reinforced this hypothesis. We noted that chloroplast membrane structure, as observed in commercially grown spinach, appeared to vary with the season of the year. The most highly structured membranes appeared in winter-grown spinach. Short days maintain the vegetative condition in spinach, long days being required demonstrated quantasome structure, but also that the quantasomes often existed in very highly ordered or paracrystalline arrays. An example of such an array is shown in Fig. 1. The existence of extended arrays such as that in Fig. 1 have allowed a more accurate determination of quantasome dimensions than was previously possible. The quantasomes in Fig. 1 average 185 by 155 Å, with a thickness of 100 Å. We have measured the quantasome buoyant density in the ultracentrifuge as 1.17. This value, combined with the quantasome volume determined from electron microscope measurements, may be used to calculate the quantasome molecular weight. The molecular weight of a single quantasome from the paracrystalline array shown in Fig. 1 is 2.0×10^6. The distribution of compounds in a membrane unit of this size may be determined from studies on the chloroplast membrane composition. The distribution of lamellar substances based on a quantasome molecular weight of 2×10^6.

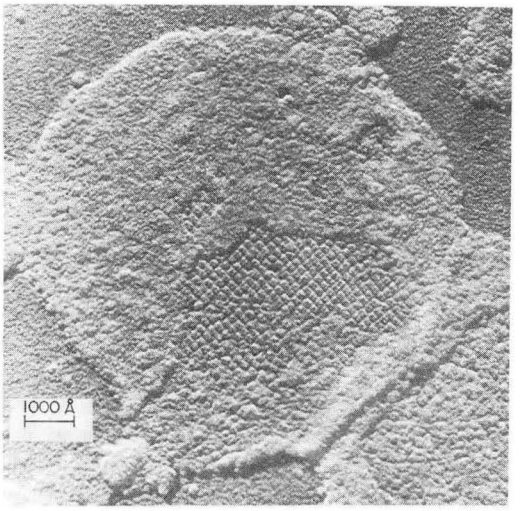

Fig. 3-1. Electron micrograph of shadow cast preparation of spinach chloroplast membranes. These membranes contain 11% chlorophyll by weight and perform the quantum conversion reactions and associated electron transport reactions of photosynthesis.

Lipid (composition in moles per mole quantasome)		
230 chlorophylls		206,400
160 chlorophyll a	143,000	
70 chlorophyll b	63,400	
48 carotenoids		27,400
14 β-carotene	7,600	
22 lutein	12,600	
6 violaxanthin	3,600	
46 quinone compounds		31,800
16 plastoquinone A	12,000	
8 plastoquinone B	9,000	
4 plastoquinone C	3,000	
8-10 α-tocopherol	3,800	
4 α-tocopherylquinone	2,000	
4 vitamin K_1	2,000	
116 phospholipids (phosphatidylglycerols)		90,800
144 digalactosyldiglyceride		134,000
346 monogalactosyldiglyceride		268,000
48 sulfolipid		41,000
? sterols		15,000
unidentified lipids		175,600
	Total	990,000
Protein		
9,380 nitrogen atoms as protein		928,000
2 manganese		110
12 iron including two cytochrome		672
6 copper		218
	Total	930,000
Total lipid + protein		1,920,000

The original data for this compilation are given in reference 1. The 12 iron atoms in the protein include one cytochrome f and one cytochrome b_6. Thus the quantasome contains at least one of all the membrane-bound components known to be involved in electron transport. This, combined with the fact that it contains 230 chlorophylls, retains the possibility that the quantasome is a physiological photosynthetic unit. This hypothesis can be fully verified or disproved only with the isolation of the quantasome.

Figure 1 also shows that the quantasome is composed of about four subunits. These subunits exist on about 90-Å centers, and may be related to the 90-Å periodicity which is seen in cross sections of chloroplasts and many other membranes (mitochondrial and retinal rod membranes).

During the past year we have endeavored to find out how the lipid and protein components of the quantasome are associated with one another. Each component accounts for about 50% of the total quantasome mass. In earlier studies we had attempted to extract the lipids from aqueous suspensions of lamellae followed by viewing the shadow cast extracted lamellae in the electron microscope. These initial efforts were largely unsuccessful because of the water insolubility of the exposed lamellar proteins, which were very prone to aggregation and precipitation. To overcome this difficulty we developed a technique for extracting lamellae which were already adhered to carbon films. This involved sequentially extracting the carbon films with their adhering membranes for several minutes each in 100% acetone, acetone-petroleum ether, and petroleum ether. We know from large-scale extraction of lyophilized lamellae that more than 90% of the lamellar lipids are removed by such an extraction procedure. Direct evidence that the extraction procedure actually removed the photosynthetic pigments was obtained by spectrophotometric observations of the carbon-covered grids before and after extraction. Electron microscope observations of extracted shadow cast preparations showed that the quantasome arrays were still evident in the residual protein and, in fact, were in much greater relief than the intact membrane. This result suggests two conclusions: First, that it is the protein framework of the membrane which determines the membrane surface morphology, and second, that the lipid is wrapped around the proton framework, yielding the increased relief when it is extracted. This result supports the unicellular theories of membrane structure.

The Stroma of Chloroplasts

Carboxydismutase preparations made by Rabin and Trown[2] were observed by negatively staining with neutral phosphotungstic acid. Carboxydismutase as observed by this technique has outer dimensions varying between 80 and slightly more than 100 Å. Carboxydismutase contains a prominent central core and seems to consist of a cylinder of six or more subunits.

Studies on the interaction of carboxydismutase and the chloroplast internal membranes are presently being carried out, with the freeze-etching technique of Mühlethaler and Frey-Wyssling. We hope to find out why isolated chloroplasts show only about 5% of the photosynthetic rate of the *in vivo* system. The possibility that the stroma proteins become uncoupled from the membrane during the isolation procedure can be tested by the freeze-etch method.

References

1. R. B. Park and J. Biggins, Science 144, 1009 (1964).

2. P. W. Trown and B. R. Rabin, Proc. Natl. Acad. Sci. U. S. 52, 88 (1964).

4. PROBLEMS IN BIOLOGICAL ORGANIZATION AND REGULATION

V. Moses

An active research program has recently been developed in the Laboratory to study control mechanisms in biological systems. This program was started at the beginning of 1963, and for the first year or so involved only two people who investigated in a preliminary manner a range of topics which looked promising for further exploration. The number of people concerned grew rapidly during 1964, and now totals four graduate students, four postdoctoral fellows, and a technician, under the overall supervision of a senior member of the permanent scientific staff of the Laboratory. With two exceptions to be noted below, all the current studies are being performed with bacteria, since in the present state of our scientific capability these organisms are for technical reasons the most suitable to use, and the manner of their biological responses probably has general validity among living organisms.

It has long been known that bacteria and other microorganisms would in many instances synthesize new specific enzymes when presented in their environment with certain substrates whose metabolism depended on the presence of these particular enzymes. Although the cells were obviously capable of the formation of these catalysts under the appropriate circumstances, they did not in fact make them unless their corresponding substrates were introduced into the environment. These substrates were thus in some way initiating or triggering in the cells the formation of the enzymes necessary for their metabolism. It gradually came to be recognized that mechanistically the failure to make enzyme when the appropriate substance was absent from the medium was due to an unknown factor which repressed the capability within the cell. In the presence of the specific substrate (or inducer) this repression was relieved and the enzyme was made. Removal of the inducer restored the repression. By certain genetic modifications of the cells it was possible to breed bacteria in which the repression was permanently relieved and which therefore no longer required the presence of inducer in order to make enzyme.

A model to account for these and other observations was proposed some years ago, mainly by J. Monod and F. Jacob in Paris. They suggested that the genetic information necessary to make enzymes was present in the DNA of the cell (structural genes). They envisaged that the use the cell made of this information was controlled by other sections of the DNA, termed regulator genes. It was imagined that the regulator genes contained information for the synthesis of specific products, the nature of which is not known, and whose function is in some way to block specifically the expression of the genetic information contained in the structural genes. However, these substances, called "repressors," are believed to interact very specifically and loosely with inducers to form altered repressors which no longer possess repressive properties. With repression relieved, it is believed that the genetic information contained in the structural genes of DNA is transcribed into a complementary form of RNA, called "messenger RNA." The latter is then believed to travel to the site of protein synthesis on the ribosomes, where the appropriate amino acids, each carried by a specific form of another type of RNA--"transfer" or "soluble" RNA--are lined up successively in the correct sequence to make the polypeptide chain according to information borne by the messenger RNA. Eventually a complete polypeptide chain is produced which folds to form the finished protein. Messenger RNA is believed to be an unstable molecule, and only a few protein molecules are made with each messenger RNA ceases, and the latter, in turn, is governed by the presence or absence of inducer. Removal of an inducer results in the restoration of the original properties of the corresponding repressor, which would bring enzyme production to a rapid halt by blocking the further production of messenger RNA. This model for enzyme induction has widespread implications throughout biology, although it must be noted that it is not universally accepted. It is supposed, for example, the cellular differentiation--the process by which all the multiplicity of cell types are developed within an organism from a single cell, the fertilized egg--might be effected by the appropriate switching on and off of the syntheses of various enzymes and other proteins at different stages of development. In another connection it has been suggested that learning and the storage of memory may be closely tied up with the synthesis of certain specific proteins as a consequence of incoming mental stimuli. Another theory would relate the development of cancer, in many ways a process of de-differentiation, to an interference in some specific way with these control mechanisms of cellular activity. And lastly, the immune response--the ability of higher animals to

produce specific antibodies to invading antigens--has many features in common with enzyme induction in bacteria and other lowly creatures. Enzyme induction can in some cases be directly demonstrated in animals, but technically this is difficult, and the range of investigative procedures which may be employed with animals or animal tissues does not permit the great flexibility possible with bacteria.

Even in bacteria, other control mechanisms exist in addition to enzyme induction (or its converse, enzyme repression: the synthesis of the specific enzyme only in the absence of a specific compound). Some of these concerned with intermediary metabolism are well understood: the inhibition of an enzyme by the product of the reaction it catalyzes, or retroinhibition, the specific inhibition of the enzyme catalyzing the first reaction of a sequence by the final product of that sequence. These types of controls tend to operate as negative feedback mechanisms, correcting any disproportionality of function which may arise in the metabolism of the cell. Others are less well characterized. It has often been observed that the inducible synthesis of certain enzymes concerned especially with carbohydrate metabolism is inhibited ("repressed") by the presence of other carbohydrates. The earliest repressing type of carbohydrate to be recognized was glucose, and the effect was firt called the "glucose effect"; more recently it has been realized that glucose is not unique in this respect and that many rapidly metabolized sugars can show the same sort of effect. The phenomenon is now termed "catabolite repression." It is known to exert its action in a manner different from the regulator gene described above, but in general the effect is poorly understood.

In our preliminary explorations of the field, an observation was made which suggested to us that although the messenger RNA for inducible enzymes might decay rapidly, messenger RNA for enzymes not under inducible control might be much more stable and persist for a long time in a functional form. We were led to this conclusion in part by observing the synthesis of inducible and constitutive (not under the control of external inducers) proteins under various physiological conditions. When bacterial cells are growing rapidly, the introduction of an inducer results in the first appearance of inducible enzyme activity about 3 minutes later. If cells were allowed to outgrow their carbohydrate food supply, and to remain in a state of starvation for about an hour, it was found that the restoration of carbohydrate resulted in the immediate resumption of growth. However, if an inducer was added together with the carbohydrate, then the delay in the first appearance of enzyme was increased from 3 minutes to about 15 minutes. We were unable to reduce this delay period by adding the inducer before restoring the carbohydrate supply. This was true even if the inducer had been present before the previous phase of growth had come to a halt, and had been present ever since. Thus, cells which make enzyme inducibly while they are growing stop doing so when they enter a state of starvation. Not only do they stop making enzyme then, but they also lose the capacity to make it again immediately after carbohydrate is added again to the culture, and have even a reduced ability to acquire this capacity within the standard time of 3 minutes. Growth of the cells, however, which involves the synthesis of many other proteins not under external inducible control, shows no lag when starvation is suddenly terminated, indicating that for these proteins the synthetic capacity does not decay during the period of starvation. Further investigations of this phenomenon led to the suggestion that the factor in the synthesis of inducible enzymes which decayed was messenger RNA (known for these enzymes to be unstable), while the retention of the capacity for other proteins resulting in growth indicated that these were not dependent on such an unstable messenger molecule. These findings have important implications both for the evolution of control by enzyme induction and for cellular differentiation. If many enzymes and proteins which are not controlled inducibly are made with stable messenger RNA, then it might be difficult for the cell to exert much control over their rate of synthesis. However, it may not be important for the cell to be able to do this, as differentiation may require control over a relatively small proportion only of the total cellular protein complement. As yet we have no information to explain chemically or biologically such a range of different stabilities for different messenger RNA molecules.

Studies by us of catabolite repression have been concentrated mainly on a strain of bacterium in which glucose exerts a transitory inhibition only. The addition of glucose to cells synthesizing an inducible enzyme halts inducible enzyme formation within a few minutes, but this resumes about 10 minutes later even though the glucose has not been exhausted. A number of other carbohydrates and related substances have been tested in this system. Ribose, galactose, and glucose are active in this respect, while deoxyribose and deoxyglucose are inactive. Among other compounds tested it was found that the four ribonucleosides are active, while the deoxyribonucleosides, purine and pyrimidine bases, are inactive. Studies with labeled substances have shown that explanations for this effect based on the idea that entry of one of the

active carbohydrates into the cell in some way expels the inducer are not adequate. We currently incline to the view that catabolite repression in the system under study arises from the ability of the cells to produce excessive amounts of high-energy-containing compounds from the high rates of oxidation of the active carbohydrates. The high-energy-containing compound, perhaps a nucleotide polyphosphate, then either directly or indirectly represses the formation of the inducible enzyme. At present both the site of action of such a compound and its identity are unclear. Such an explanation has the advantage of being sufficiently general to account for the wide range of active compounds, the only criterion for activity in a compound being its ability to produce excessive amounts of high-energy-containing intermediates beyond the cell's immediate requirements. In these circumstances it would be economic for the cell to repress the formation of enzymes, which would result in the metabolism of yet more substances to produce further and more excessive amounts of the postulated high-energy-containing compound. Partial inhibition of protein synthesis by one of a number of drugs would also decrease the requirement for energy in the cell, and this too would then be demonstrated as catabolite repression. Such relationships between inhibition of protein synthesis and the onset of catabolite repression are well known. Experiments are currently in progress to test these hypotheses.

A number of drugs have been described in the literature as being specifically inhibitory for the replication of DNA; mitomycin C and phenethyl alcohol are two well-known examples. We have been studying the effects of these compounds in bacterial systems in order to be able to investigate more fully the interrelations between DNA synthesis and the formation of other macromolecules in the cell. Much information has already been obtained about the mode and consequences of action of both of these inhibitors. It appears that phenethyl alcohol must act additionally at sites other than DNA synthesis, and may also be effective in inhibiting protein synthesis at a point other than the genetic level. DNA, RNA, and protein synthesis seem all to be inhibited in concert by suitable concentrations of phenethyl alcohol, though some recovery may take place and not all three types of synthesis may recover equally. With mitomycin C, total DNA synthesis ceases immediately, though incorporation of precursors into DNA does not. RNA synthesis and the synthesis of an inducible enzyme slow down and stop about an hour after the addition of the inhibitor, but protein synthesis continues for a further hour. Following this, however, some breakdown of protein occurs, and it appears possible that this final protein to be made may be an abnormal species which is metabolically unstable. Further investigation is expected to yield more information on these data.

One of the most important facets of the theory of enzyme induction due to Monod and Jacob, and for which there is perhaps less direct evidence than for any other aspect, is the repressor, the hypothetical substance which acts to prevent the transcription of messenger RNA unless it is able to combine with inducer. Without accepting in full this theory, and thus not necessarily acknowledging that the receptor for the inducer must act in this way, we can have no doubt that the inducer interacts with a cellular molecular species, the chemical nature of which at present may only be surmised. One part of our program is to attempt to isolate and characterize the receptor for the inducer, whatever its function may be. Three properties of this receptor are known beyond doubt: it exists; there must be at least one molecule of it in every cell; its specificity requirements for the inducer with which it will interact have in at least one case, that of β-galactosidase, been described in detail. Making only these assumptions, we are attempting to isolate this compound. The isolation procedure requires that the compound's presence be detected and its concentration measured, and for this it is necessary to obtain a cell-free system capable of synthesizing the enzyme under inducible control in which the repressing action of the proposed repressor may be tested.

On cell-free preparation has been reported in the literature which has the necessary properties, and we are attempting to make this preparation function in our hands. Such a preparation obtained from cells constitutive for the enzyme under study (β-galactosidase) should synthesize the enzyme without addition of inducer. Isolates from the corresponding inducible cells, depending on their content of repressor, should serve to inhibit the synthesis of the enzyme in the preparation from constitutive cells, and should enable us to recognize the presence of the repressor. We will thus be able to use this assay in a purification procedure, and also investigate the antagonistic action of the inducer against the repressive action of the repressor. The quantities of purified repressor likely to be obtained on the most optimistic basis are minute, and it may be that the only way in which it will be possible to investigate the repressor of chemical techniques will be to start with a highly radioactive repressor obtained from generally labelled cells.

This in turn raises radiation problems. Since the proportion of the total cell material which is likely to be represented by the repressor may be extremely small, perhaps one part in a million or so, it will be necessary to label the cells to a million times the extent needed to label the repressor, since there is no way of labeling the latter specifically. Care must be taken both to avoid radiation damage, and to select as a source of radioactive foodstuff for the cells a substance as inexpensive as possible. The most promising source of labeled food for the bacteria is hydrolyzed radioactive Chlorella grown on labeled bicarbonate, the cheapest and most highly specifically radioactive of all carbon compounds obtainable. Preliminary experiments have shown that it is indeed possible to obtain radioactive bacteria in this way.

A further project which is just starting is to investigate the whole phenomenon of enzyme induction and synthesis under conditions of deuteration of the cells and the medium. It is currently believed that processes such as the transcription of DNA into RNA and the translation of RNA into protein are highly dependent on hydrogen bonding. The chemical characteristics of this bonding would be expected to change when the naturally predominant isotope of hydrogen, protium, is replaced by the heavy isotope, deuterium. Plans are being made to study a number of aspects of transitions from a protonated to a deuterated system. A deuterated environment is in many ways a particularly interesting one to study. In the evolutionary history of a bacterium, the organisms may have been exposed to a very wide variety of chemical substances to which in time they have adapted by a process of mutation and selection. A great deal of the memory of these events is recorded in the current genetic information of the cells. But one environment which the organism is most unlikely to have encountered in its history is a highly deuterated one, and it is therefore hardly possible that there has been any development of a specific adaptive mechanism which is recorded genetically. In other words, when we expose a cell to a highly deuterated medium we are exposing it to a condition for the first time in its evolutionary history, and moreover to a situation which is likely to have profound effects at the levels of nucleic acid and protein synthesis. A detailed analysis of cellular response to these conditions is thus of great interest in a study of the interaction of biological material with its surroundings.

Two areas of research activity are not concerned with bacteria. One of these is a study of the mechanism of action of a plant growth-promoting substance, N, N'-dinitroethylenediamine. This compound has a remarkable effect on the growth of some higher plants. A single application of a weak solution to the growing point of a pea plant results in an almost twofold increase in the weight of the final plant under appropriate growth conditions. With no further application, successive generations of the plants are also abnormally large and the effect has to date been followed into the fifth generation. Since detailed biochemical studies on higher plants are relatively difficult, we have been studying the effects of this compound in microorganisms, in some of which it also has a growth-promoting effect. Generally the compound has no effect on cells which are growing in luxury conditions of nutrition, but in some organisms will stimulate cells which are developing certain conditions of nutritional deficiency. These effects are being studied in the green alga Euglena, which has shown sensitivity to the compound. Some difficulties have been encountered in obtaining reproducible results, but it is hoped shortly to have a system which will permit systematic study. It seems possible that the compound is acting in a nonspecific way on inducibly controlled syntheses of growth-limiting proteins, permitting to some extent the relaxation of repressive control, thus overcoming particular growth limitations. The fact of the persistence of the effects in higher plants over several generations suggests very strongly that the compound is active at a genetic level, but the observation of the generality of its action in partially overcoming many sorts of growth restriction suggest that it does not indeed possess one specific site of action.

Another possible triggering mechanism of great theoretical and practical importance is the production of cancerous tissue in animals and humans by the action of certain chemical compounds. For example, a range of malignant transformations may be produced in certain tissues by treatment with one of a number of polycyclic hydrocarbons. These hydrocarbons are bound by DNA, and a correlation has been shown to exist between the degree of binding to DNA and the carcinogenic potency of the chemical agent. However, what is not clear is whether the binding of the carcinogen to DNA is the primary cause of tumor formation, or whether it is a coincidental and irrelevant effect. One would want to know whether cancer results from a direct alteration of the DNA by interaction with the carcinogen, or whether a specific receptor, sensitive to the presence of the carcinogen, is modified in some way by the latter to trigger off a series of events culminating in malignancy. This is being tested in the following way. It is known that within one living animal different types of carcinogenic agents stimulate the development

of tumors in different tissues, yet it is believed that all the cells of the organism contain a full complement of DNA. This might mean that the DNA content of the cell is not immediately relevant to the chemical production of a malignant transformation, though this information can also be interpreted to mean that the carcinogens succeed in gaining access to some areas of the animal but not to others. A more critical type of experiment would be to investigate the response of tissue culture cells derived from different organs of one animal to a range of chemical carcinogens. A study may then be made of the specific chemical structure required of the carcinogen to produce a malignant transformation. Since all the tissues from one animal contain the same DNA, a range of specificities for the carcinogen receptor will indicate that this receptor is not DNA. Such an investigation is currently being carried out, together with a detailed study of the metabolic fate of one particular carcinogen, ethyl carbamate (urethane) using tracer techniques.

Finally, studies in biological organization which were started some years ago are being continued with bacteria. Recent evidence from a variety of sources has indicated that a high degree of intracellular organization of intermediary metabolism exists, and that parallel reaction sequences, following the same chemical pathways, may coexist within the cell in different environmental circumstances, and result in different end products. An experimental design for the overall study of such metabolic behavior was evolved and has already been tested in a preliminary manner with Ehrlich ascites tumor cells. The results of this test suggested that there was in those cells indeed a high degree of behavioristic individually between different parallel pathways, a phenomenon described as metabolic compartmentalization. This study is currently being extended to bacterial systems, largely for technical convenience, but also because the results will be useful in the interpretation of other parts of the program. Essentially the method involves tracer-type investigations of a selected area of metabolic activity with a view to determining whether or not the metabolic fate of a particular compound is dependent on its route of formation or mode of entry into the cell. A metabolic fate not so dependent would suggest that no matter how a compound arises within the cell or enters metabolic sequences its ultimate metabolism is the same, and thus there would be no need to invoke the parallelism referred to above. A fate dependent on origin, however, would indicate that functional compartmentalization exists within the cell, and that this must ultimately be an expression of structural compartments. The latter unfortunately are difficult to delineate, particularly if no information is available regarding their individual metabolic roles. It is therefore hoped that a behavioristic study of metabolic organization will eventually permit a full structural analysis, and this in turn will greatly aid our understanding of overall cellular cohesion and of biological control and variation mechanisms at a molecular level.

5. PHYSICAL CHEMISTRY OF BIOLOGICAL SYSTEMS

Kenneth Sauer and Melvin P. Klein

The common aim of much of the research undertaken in the Laboratory is to relate molecular and anatomical structure to biological function. There is little difficulty in describing the structures or possible gross variations when the structural elements are of sufficient size to permit observation in either the light or electron microscope. It is necessary to resort to other means of observation, however, when the structural elements are smaller than the resolving power of the microscopes. The most general and versatile methods rely on spectroscopic techniques. Virtually any molecule or aggregate of molecules could be uniquely characterized if all of its spectroscopic properties could be measured. If it were possible to carry out such an ambitious program, the data in themselves would be of relatively little use if a firm theoretical basis for their origin were absent. Therefore it is important that we collect, for a wide variety of molecules subject to a range of conditions, as many spectral data as possible, in order to establish the potential use of the different techniques in reflecting structural properties as well as changes in these properties.

Electron spin resonance (ESR) is one such spectroscopic technique which has contributed useful information about free radical intermediates in enzyme-substrate interactions and about the role of transition metal ions. A number of iron-containing proteins exhibit ESR signals which occur at a g value of 4.3. In general, the resonances are structureless and their interpretation is based on a model proposed several years ago. During the past year we have investigated extensively the ESR spectrum of the iron-containing cyclic hexapeptide known as ferrichrome A, which is isolated from the smut fungus, Ustillago sphaerogena. The low-temperature ESR spectra of ferrichrome A exhibit several components, and to account for all of these components theoretically we have proposed a new model which has wide applicability for the interpretation of the ESR spectra of iron compounds, especially iron-organic materials.

For several years many laboratories have studied the ESR signals which result when photosynthesizing materials are illuminated while in the ESR spectrometer. Significant progress has been made in understanding the origin of these signals. It is observed that if the samples are kept in the dark there is little or no ESR signal and that when the light is admitted the signals increase to an equilibrium value. When the light is removed the signals decay, with both fast and very slow time constants (seconds and 1/2 hour), eventually reaching a very small intensity. With the advent of new signal-processing techniques it has been possible to measure the rise and decay kinetics in a much shorter time than was possible previously. In samples of chromatophores prepared from Rhodospirillum rubrum, a red photosynthetic bacterium, we have been able to correlate the kinetic responses of the photoinduced ESR signal exactly with the transient optical density changes which occur at 433 mμ.[1] The spectral region at 433 mμ is not related to any of the optical features associated with the chlorophylls; it is therefore unlikely that the ESR signals reflect any direct involvement of chlorophyll. It is probable that the signals reveal a free radical intermediate in the biochemical reduction and a quinone is a likely species. To separate the biochemical steps from the photophysical steps, we have been extending the ESR experiments to ever lower temperatures. At the temperature of liquid helium very little motion of atoms or molecules can occur, thus effectively suppressing most chemical reactions. At these low temperatures, however, electronic and molecular excitations still occur. Observations on Rhodospirillum rubrum thus far indicate that in chromatophore fragments as the temperature is lowered the transients become more rapid while the total signal intensity remains about constant. There is a noticeably different behavior between the chromatophores and whole cells. In whole cells there is no large light-induced component at low temperature and, although a small light-induced signal is observed, its kinetics are somewhat slower than at room temperature. While this difference is not surprising, since damage must inevitably occur when the cells are broken, it will be instructive to ascertain how alterations in the cell are responsible for the difference in signal behavior and to attempt to correlate these observations with others made in this Laboratory.

Of the many possible modes by which the photo excitation of the plant pigments is stabilized, the triplet state has been discussed frequently. The triplet state, by definition, has unpaired electrons. These unpaired electrons are the basis for ESR measurements. In recent years there have been many reports of ESR investigations on photoinduced triplet

states. A limited search for such triplet signals in the photosynthetic materials has not been successful, but will receive greater attention in the immediate future. The low-temperature experiments are also expected to reveal if some of the transition metal ions, which are ubiquitous constituents of photosynthetic materials, are directly involved in the electron transport pathway.

The study of model systems is useful on its own as well as in providing interpretable guides to the properties of an intact biological system. The close relation between the porphyrins and many biological pigments makes them especially attractive models. ESR and optical spectroscopy of a variety of metal-free porphyrins and porphyrins incorporating transition metals are continuing. ESR has been observed in Mn^{24}, Co^{2+}, V^{2+}, Fe^{3+}, Cu^{2+}, and Cr^{3+} phthalocyanines in pyridine solutions at 77°K. The spectra are interpretable in terms of ligand field theory, and reveal subtle details of bonding between the metal and the host. Facilities are now available for studying the optical absorption spectra at liquid helium temperatures. With the rapid progress in the theoretical predictions of such spectra there is good expectation that the optical properties will be largely interpretable.

Nuclear magnetic resonance (NMR) has become, in recent years, an important adjunct in the arsenal of analytical techniques. Proton NMR when applied to organic molecules of relatively low molecular weight has been particularly informative. Again, by virtue of the new signal-processing techniques it has been possible to extend NMR techniques to very small quantities of material with a molecular weight as great as the chlorophylls. It has been possible to obtain the chlorophyll a spectrum in solution.

The technique of recoil-free γ-ray spectroscopy or the Mössbauer effect has been applied with success to the study of the iron in ferrichrome A, the mammalian iron storage protein ferritin, and to a number of iron chelates. The results with ferrichrome A have been very rewarding; the spectra exhibited, for the first time, the existence of a magnetic hyperfine interaction in a paramagnetic iron compound. The temperature dependence of this hyperfine interaction established that the hyperfine field had its origin in the unpaired 3d electrons of the iron ion. A theory has been developed which described adequately the shape of the spectrum, the magnitude of the hyperfine splitting, and the strong temperature dependence of the electronic relaxation times of the Fe^{3+}. The interpretation of the Mössbauer results utilized results from the ESR work described above, and the two spectral investigations were shown to be complementary.

The nature of the iron in ferritin has been the subject of discussion for a number of years. The magnetic susceptibility of ferritin, as measured near room temperature, is consistent with an electron spin of 3/2. The Mössbauer spectra show that all the iron is in the Fe^{3+} valence state, that the iron ions reside in positions which have relatively low symmetry, and that at helium temperatures the iron becomes magnetically ordered. At the lowest temperatures, the spectra are virtually indistinguishable from $\alpha \cdot Fe_2O_3$. While this latter material is antiferromagnetic at room temperature, the ferritin becomes magnetically ordered at much lower temperatures. This is a reflection of the small number of iron ions in any single micelle, about 400, and this observation sets a new lower limit on the number of magnetic ions required to exhibit collective behavior.

Techniques, developed in the Laboratory, for measuring very small optical density changes as well as the time dependence of such changes are being applied to the study of the mechanisms of photosynthesis.[2]

The optical properties of phosynthetic organisms exhibit reversible changes upon illumination; the magnitudes, signs, wavelengths, and kinetics of change depend on the wavelength and intensity of the actinic light. When the optical density increases upon illumination with actinic light it is assumed that some new compound or chromophore is created and, conversely, a decrease in optical density is though to reflect a decrease in the concentration of a component. Detailed examination of these changes has yielded a large quantity of data. Current efforts are being applied toward interpreting the origins of these spectral properties and fitting the kinetic behavior into a pattern which can be reconciled with a consistent theoretical flow diagram. These experiments are tantalizing, indeed, for they offer the possibility of learning the nature and kinetics of some of the detailed chemical steps involved in the transformation of light into chemical energy. It will be interesting to measure the temperature dependence of these transient optical properties, for this may further help to distinguish the photophysical from the biochemical.

In pursuit of the correlation between structure and function in photosynthetic systems, detailed studies have been undertaken to seek correlations between the internal organization of chloroplast subunits and their

photophysical or photochemical efficiences. It has long been known that agents such as organic solvents and detergents are effective in altering the environment of pigment molecules such as chlorophyll and carotenoids. In a recent study, the effects of several organic compounds and surface-active agents on the absorption spectra, chlorophyll fluorescence, and Hill-reaction activity of quantasome subunits from spinach chloroplasts were observed to fall into two general classes.[3] Added acetone, methanol, urea, or sodium dodecyl sulfate induces a loss in Hill activity at appreciably lower concentrations than are required to give a pronounced fluorescence increase or blue shift in the chlorophyll absorption maximum at 678 mμ. On the other hand, quantasomes treated with the nonionic surface-active agent Triton X-100 retain Hill activity at higher concentrations than are sufficient to produce pronounced absorption and fluorescence changes. The principal conclusions reached from this study are:

(a) the position of the chlorophyll absorption maximum in the red is not a sensitive measure of the ability of photosynthetic materials to carry out the Hill reaction,

(b) energy diverted from normal photochemical pathways does not in general immediately appear as enhanced fluorescence, and

(c) detergent concentrations comparable to the chlorophyll concentrations of quantasomes are capable of profound effects on physical properties.

One of the best measures of the efficiency of a photochemical reaction is the quantum requirement or its reciprocal, the quantum yield. When such measurements are made with use of monochromatic light and at a number of wavelengths, action spectra can be constructed to show the effectiveness of various regions of the absorption spectrum in producing photochemistry. Action spectra have been measured for the photosynthetic Hill reaction of chloroplasts.[4] When potassium ferrycyanide is used as the oxidant for the photo-induced oxidation of water to molecular oxygen mediated by chloroplasts, the action spectrum shows a marked decrease in efficiency at wavelengths longer than 680 mμ. Pigments absorbing at these long wavelengths apparently do not participate in reactions leading to oxygen evolution. When catalytic amounts of the cofactor dichlorophenolindophenol (DCPIP) are added to the system, a virtually identical action spectrum is obtained. This evidence suggests that the two substances, ferricyanide and DCPIP, act in about the same place with respect to the light reactions of photosynthesis. This is contrary to earlier results based on their differing abilities to couple to photosynthetic phosphorylation. Recent work from other laboratories tends to support this near identity of the sites of action.

Although the overall absorption spectra of molecules are directly related to their electronic structures, there are other molecular properties which are not specifically revealed by absorption spectroscopy. Such structural properties are exemplified by helical content of proteins or nucleic acids and the conformation and conformational changes of proteins. These parameters are potentially accessible from measurements of the optical rotatory dispersion (ORD) or its Kramers-Krönig conjugate, the circular dichroism (CD). Each of the effects may have a magnetically induced counterpart, known respectively as magnetic optical rotatory dispersion (MORD) and magnetic circular dichroism (MCD). Progress in these areas has been hampered by lack of adequate experimental data because suitable equipment was not available. Furthermore, the theoretical or even empirical developments required for interpretation are missing as a result of the paucity of experimental data. To satisfy this requirement we have constructed a spectrometer for measurement of either CD or MCD capable of recording changes as small as 10^{-5} optical density unit over the spectral range from 220 mμ to 850 mμ. In addition, a recording spectropolarimeter has been modified to permit measurements of MORD with a sensitivity of about 10^{-3} degree from 200 mμ to 700 mμ.

Synthetic polynucleotides undergo various structural changes upon changes in temperature and solution environment--e.g., pH and salt concentration--as evidenced by hydrodynamic and hypochromism measurements. These polymers exhibit large conformation-dependent MCD. The natural optical activity has yielded some structural information, but the solution structures of these polymers are largely unknown. The biological activity of polymers appears to be strongly dependent on short-range order. The MCD of polymers of known structure will be useful for testing the general theories of magnetically induced optical activity, with the ultimate aim of using the theory to elucidate new information on the short-range solution structure of polyribonucleotides.

These same goals will be furthered by testing the spectra of small molecules as well as by studying impurities in inorganic crystals; for these latter systems, the spectra and wave functions are very well known. This knowledge may render their MCD spectra tractable to theoretical treatment.

Cerruloplasmin, a mammalian globular

protein implicated in Wilson's disease, is known to bind eight copper ions. From ESR, NMR, and optical measurements, it has been known that roughly half the copper ions are reversibly reducible but that some of the ions are more accessible than the rest. It is reasonable to assume that a conformational change in the protein is responsible for the variation in accessibility. Large structural changes are observed by means of ORD and MORD as the copper is oxidized, reduced, or removed.

The problem of the preparation of polynucleotides by nonbiological methods is far from being resolved. Studies aimed at finding satisfactory ways of preparing these important substances are under way in many laboratories.

The product of the reaction of ethyl ether and phosphorus pentoxide, usually called "ethyl metaphosphate," has recently become interesting for the synthesis of polynucleotides as well as for the synthesis of polysaccharides and polypeptides. However, several recent attempts to synthesize these biologically important substances gave inconsistent results. To clarify these problems, the real nature of the structure of "ethyl metaphosphate" and the kinetics of its reactions with simple nucleophilic substances--e. g., water and ethanol--should be known, but were, unfortunately, lacking. Further progress in the synthesis of polynucleotides by means of this potentially useful starting material depends, therefore, on the solution of the problems described above. Phosphorus NMR[5] and IR measurements were thought to be useful for such studies. In this way the kinetics of the reaction of phosphorus pentoxide with diethyl ether was investigated and the structure of "ethyl metaphosphate" resolved.[6] It is usually a mixture of cyclotetrapolyphosphate ester, isocyclotetrapolyphosphate ester, and linear tetrapolyphosphate ester, and contains hydroxyl and ethyl groups in a probably random distribution. Instead of a linear tetrapolyphosphate ester, bicyclotetrapolyphosphate ester can be present in the mixture. The composition of an actual preparation depends on the amount of ether used and on the time of heating. The effectiveness of "ethyl metaphosphate" for the dehydrating polymerization depends, therefore, on the condition of its preparation.

The kinetics of the hydrolysis and ethanolysis of "ethyl metaphosphate" was also studied. By the addition of specific amounts of water and ethanol, substances could be prepared which consisted mainly of linear tetrapolyphosphate, tripolyphosphate, and pyrophosphate esters. Similar degradations of the original "ethyl metaphosphate" can be caused by various organic solvents normally employed in dehydrating polymerization processes.

The data obtained show that variable results, which actually have been reported, are to be expected if the identical conditions for the preparation of "ethyl metaphosphate" as well as for the preparation of the polynucleotides by means of this substance are not maintained. Further progress in the synthesis of polynucleotides, polypeptides, and polysaccharides should be possible by means of the many polyphosphate esters now available through the studies described.

References

1. R. H. Ruby, I. D. Kuntz, Jr., and M. Calvin, Proc. Natl. Acad. Sci. U. S. 51, 515 (1964).

2. I. D. Kuntz, Jr., P. A. Loach, and M. Calvin, Biophys. J. 4, 227 (1964).

3. K. Sauer and R. B. Park, Biophys. Acta 79, 476 (1964).

4. J. Biggins and K. Sauer, Biochim. Biophys. Acta 88, 655 (1964).

5. G. Weill, M. Klein, and M. Calvin, Nature 200, 1005 (1963).

6. G. Burkhardt, M. P. Klein, and M. Calvin, J. Am. Chem. Soc. 87, 591 (1965).

6. OPTICAL PROPERTIES OF BIOPOLYMERS

I. Tinoco, Jr.

The main emphasis in 1964 was on the absorption and rotation of light by oligonucleotides. From the measured optical properties of oligomers of known base sequence, one can predict the properties of other oligomers. This can lead to the identification and characterization of oligonucleotides obtained from biologically important ribonucleic acids.

The oligomers studied were obtained by pancreatic ribonuclease hydrolysis of yeast RNA. The hydrolysate was treated with phosphomonesterase to remove terminal phosphates and chromatographed to separate polymers according to chain length. Then the dimer and trimer fractions were further chromatographed to obtain the individual species. The dimer fraction contains adenylyl-(3'-5') cytidine (ApU), adenylyl-(3'-5') uridine (ApU, GpC, and GpU). In addition ApA and ApG were obtained from other sources. The trimer fraction was resolved into ApApC, ApApU, ApGpC + GpApC, ApGpU, GpApU, GpGpC, and GpGpU. Each species was studied in solutions of three different pH values over a wavelength range of 200 to 350 mμ. Some of the dimers were also studied as a function of temperature. The general conclusions are that the optical rotation of the oligomers is much more sensitive to conformation than the absorption, and that the rotation of the trimers can be calculated from that of the dimers. Therefore, the rotation is sensitive to sequence, but can be predicted from the dimer rotation.

Another problem studied by optical rotation measurements was the folding of DNA inside the head of bacteriophage. There is a linear relation between the amount of DNA inside the phage head and the specific rotation per g/ml of DNA. One interpretation of this result is that high DNA content corresponds to a tightly folded DNA, which has a different rotation from that of free DNA in solution.

This work was done in collaboration with Mr. Charles R. Cantor, Mr. Myron M. Warshaw, and Dr. Marcos F. Maestre.

7. THE 10-kV ION ACCELERATOR ("ISOTOPE SEPARATOR")

Helmut Pohlit, Wallace Erwin, and Richard M. Lemmon

For about six years, members of this Group have been working on, and doing research with, an ion accelerator designed to direct a beam of carbon ions onto organic targets. The most recent general description of this instrument, together with the results of some irradiations, were reported in R. T. Mullen's Ph. D. thesis (The Chemical Interaction of Accelerated Carbon-14 Ions with Benzene, UCRL-9603, March 1961). Since that time much development work has been expended on the instrument. There is no doubt that the accelerator has the potential of contributing greatly to our understanding of "hot" and "epithermal" atom processes. However, for this potential to be realized, the following factors must be both known and controllable:

(a) the total number of accelerated ions hitting the target;

(b) the energy of the ions (and their atomic or ionic state); and

(c) the density of the ions at the target--at least in comparison with the density of target molecules.

During 1964 studies and improvements were made on the accelerator as listed below.

Studies on the Ion Source

For a long time we have been troubled by instabilities in our ion source, which is of the "capillary-arc" type. We first investigated the possible remedial influence of the addition of a noble gas (argon) to our source gas (CO_2), but found this to be of little help. Eventually, we found that one precondition for consistency in striking the arc was the condition of the surface inside the arc chamber. Deposits of cathode material and combustion products of the feed gas apparently produce an insulating layer; this layer must be periodically removed (metal polish) for satisfactory operation of the arc.

In order that we might have better control of the feed gas pressure in the arc chamber, a vacuum thermocouple gauge was connected with the low-pressure gas input line close to the entrance to the chamber. This new gauge enabled us to determine that the lowest pressure at which an arc could be maintained (with CO_2) was about 15 microns, and that the best normal operating pressure was about 30 μ. This control of the input gas pressure not only enables us to keep the arc operating better, but also enables us to use a minimum amount of feed gas (important with $^{14}CO_2$) to achieve a desired ion beam.

Many mechanical changes were made in the anode and cathode of the ion source in the hopes that control of the anode-cathode distance would result in a more stable arc. However, so long as the distance between the electrodes, exceeded a minimum of about 0.5 cm, the positions of cathode and anode were found to have no effect upon striking the arc, or upon the normal operating conditions for CO_2, CO, or N_2.

In order to establish an arc through the capillary, one has first to strike an arc between the rhenium filament and the arc chamber wall. This initial arc, at rather high pressures (about 1 mm), is drawn through the capillary to the anode. We made extensive measurements of the anode and wall voltages and their respective currents to determine how they influence--under different gas pressures and filament electron emissions--the arc-striking and maintaining processes. These findings have led to a considerable increase in our ability to maintain a steady arc.

We have always observed that the extracting (or ion-accelerating) field causes the arc to be more sensitive. It is conceivable that the part of the extracting field that penetrates through the extraction hole into the arc plasma alters the electrical and thermodynamical properties of the plasma. Discharges lead to the extinction of the arc. Other discharges are observed to occur where no appreciable electrical potential is present. These, clearly, are traveling waves that are generated and amplified in the arc plasma. One cause has been primary discharges from insulating pieces located in the accelerating field. They are charged by the impinging ions; these pieces discharge occasionally, thus creating sources for electrons which, in turn, effect the "macro" discharge. Removing or refining the potential of insulating pieces has removed part, but not all, of the difficulty.

Studies on the Peformance of the Ion Beam

We have found that our carbon ion beam was spread over an area of about 1.5×3.0 cm at the target. That this is not due to charge repulsion is evident from comparisons with other accelerators, and from considerations of space-charge neutralization by ionization

of the residual gas.[1] We checked the possibilities of inadequate focusing voltage or too-high residual gas pressure, or both, but found that neither was responsible for the spread in our ion beam. Finally, through the use of fluorescent screens (ZnS) and Faraday cages, we found that incorrect alignment of the component parts of our apparatus (ion source, accelerating section, magnetic field, and target section) was the cause. After realignment of the ion accelerator, a single-mass beam at the target had a width of about 0.6 mm and a height of about 3 cm. It was this high because the magnetic field can focus only in a plane perpendicular to its field lines.

Deceleration of the Ion Beam

Since a major goal of this work is to perform ion irradiations at low energies (less than 100 eV), attempts were made to learn about beam parameters at the target when the ions are decelerated close to the target.

The decelerating field itself was established between two parallel plates, perpendicular to the direction of the beam, with circular orifices (diameter = 6.3 cm, spacing = 3 cm). The first plate was on ground potential, the second on the decelerating potential. Connected with the second plate was the entire target section together with a surrounding metal case. The beam intensity was measured by isolating the target from the case, connecting it through a 1-megohm resistor, and measuring the voltage drop across the resistor. Upon decelerating down to energies of about 800 eV no effects, other than those also observed at high energies, could be found. Benzene was condensed continuously on the target cold finger with various rates but the beam retained its shape and the measurement did not fail. Thus, in case of irradiations with energies between 800 V and 10 kV the energy is sufficiently accurately determined by the difference of accelerating and decelerating voltage. The total number of impinging ions (total current) can be measured as described above and by electrical integration over the time of irradiation.

The ion density at the target can be measured with a very thin rod which can be moved by known amounts in front of the target. This rod is also biased and the irradiating ion current is measured in the same way as above. The ratio of the number of beam particles to target particles over the beam cross section is still unknown. But as long as this number is small enough so that no ion-track overlap can occur it is of little interest.

Measurements of the efficiency of retrapping the condensed benzene in the present trapping arrangements show that virtually all the benzene introduced is recovered. However, there are no measurements, as yet, of the extent of escape of lighter irradiation products such as methane during and after an irradiation. With the exception of this last point, all parameters mentioned at the beginning of this report are thus known or measurable in the energy range from 800 V to 10 kV. Below this energy, different difficulties arise. They are under investigation and will be discussed in a subsequent report.

Reference

1. L. P. Smith, W. E. Perkins, and A. T. Forrester, Phys. Rev. 72, 989 (1947).

8. FATE OF THE RADICAL INITIATOR IN THE RADIOLYSIS OF CRYSTALLINE CHOLINE CHLORIDE

Margaret A. Smith and Richard M. Lemmon

Crystalline choline chloride, $(CH_3)_3NCH_2CH_2OH^+Cl^-$, is of considerable interest to radiation chemists because of its extraordinarily high decomposition G value. Under certain conditions of irradiation it decomposes by a chain reaction that gives $G_{(-M)}$ values as high as 55,000.[1] The anomalous radiation sensitivity is shown only by the crystalline form; in solution, the compound exhibits normal radiation stability.[2] The main radiolysis products are trimethylamine and acetaldehyde.[3,4]

Electron spin resonance studies of the irradiated crystals have shown the appearance of a free radical with a postulated structure approximating $\cdot CH_2CH_2OH \leftrightarrow CH_2\dot{C}H_2OH$. This structure appears to be stabilized by hydrogen bonding and by crystal cage effects. Kinetic studies indicate that the ESR-observed radical acts to initiate, rather than to propagate, the chain reaction.[1]

During 1964 a study was made of the fate of the observed free radical among the possible dimerization products, 1, 4-butanediol and 2, 3-butanediol, and the disproportionation product, ethanol. The results of this work indicate that a previously postulated[1] radical termination mechanism, reaction of the chain-propagating radical ($CH_3\dot{C}HOH$) with a different radical (presumably $(CH_3)_3\overset{+}{N}\cdot$), is essentially correct. Although a few percent of the chain-propagating radicals disproportionate to ethanol and acetaldehyde, most of them terminate by reaction with the $(CH_3)_3\overset{+}{N}\cdot$.

The details of this work have been submitted for publication in the Journal of the American Chemical Society.

References

1. R. O. Lindblom, R. M. Lemmon, and M. Calvin, J. Am. Chem. Soc. 83, 2484 (1961).

2. R. M. Lemmon, P. K. Gordon, M. A. Parsons, and F. Mazzetti, ibid. 80, 2730 (1958).

3. B. M. Tolbert, Adams, Bennett, Hughes, Kirk, Lemmon, Noller, Ostwald, and Calvin, ibid. 75, 1867 (1953).

4. R. M. Lemmon and M. A. Smith, ibid. 85, 1395 (1963).

9. HYDROCARBONS OF BIOLOGICAL ORIGIN FROM A ONE-BILLION-YEAR-OLD SEDIMENT

Geoffrey Eglinton, P. M. Scott, Ted Belsky, A. L. Burlingame, and Melvin Calvin

Two experimental methods are now being used to study the origin of terrestrial life and the time of its first appearance. The "primitive atmosphere" experiments[1] demonstrate that a wide variety of small molecules of biological significance can be formed in the laboratory from mixtures of extremely simple substances like methane, ammonia, and water. Geologists and geochemists examine ancient sediments for fossil organisms and determine the chemical nature of the imprisoned organic matter.[2-4] Well-defined morphological remains from earlier than about 600 million years ago are scanty, and generally are difficult to relate conclusively to specific living things.[5] A firm correlation between the morphological evidence and the organic matter present in the same rock would permit a systematic search for chemical evidence of early life in the ancient sediments. Certain classes of organic compounds--the alkanes,[6] the long-chain fatty acids,[7] and the porphyrin pigments[8]--show promise as biological markers, since they are evidently stable for long periods of time under geologic conditions. These compounds are valid as biological markers only insofar as they cannot be synthesized in significant proportions by abiogenic means. For this reason "primitive atmosphere" experiments play an important role. The range of compounds based on the isoprenoid subunit is particularly useful, for here we have a high degree of structural specificity coupled with a widespread distribution in nature. Thus pristane (2, 6, 10, 14-tetramethylpentadecane) and other isoprenoid hydrocarbons have been isolated from crude petroleums of moderate ages (Mesozoic and Paleozoic) in concentrations vastly greater than those anticipated for individual branched alkanes in a thermally derived mixture.[9] Pristane is a known constituent of living things--zooplankton,[10] fish and whale oils,[11] wool wax,[12] and marine sponges[13]--but the original source of the mineralized material may be the phytol portion of chlorophyll degraded either biogenically or abiogenically.[9, 14] There is every prospect that the isoprenoid hydrocarbons, and the related alcohols and acids, will be useful biological markers.

During 1964 we were able to identify phytane (2, 6, 10, 14-tetramethylhexadecane) and pristane in the oil that seeps in small quantities from the Precambrian Nonesuch shale in Michigan. This rock[15] is of the order of 1 billion (10^9) years old. The identification of these hydrocarbons augurs well for the extension of such analyses to even older Precambrian formations.

The details of this work were published in Science 145, 263 (1964).

References

1. N. H. Horowitz and S. L. Miller, Fortschr. Chem. Org. Naturstoffe 20, 423 (1962).

2. P. H. Abelson, ibid. 17, 379 (1959); P. E. Cloud, Jr., and P. H. Abelson, Proc. Natl. Acad. Sci. U. S. 47, 1705 (1961).

3. I. A. Breger, Ed., Organic Geochemistry (Macmillan, New York, 1963).

4. U. Colombo and G. D. Hobson, Eds., Advances in Organic Geochemistry (Macmillan, New York, 1964).

5. E. S. Barghoorn and S. A. Tyler, Ann. N. Y. Acad. Sci. 103, 451 (1963); J. S. Harrington and P. D. Toens, Nature 200, 947 (1963); C. G. A. Marshall, J. W. May, and C. J. Perret, Science 144, 290 (1964); A. G. Vologdin, The Oldest Algae in the USSR (Academy of Sciences of the U. S. S. R., Moscow, 1962).

6. W. G. Meinschein, Space Sci. Rev. 2, 653 (1963).

7. P. H. Abelson, T. C. Hoering, and P. L. Parker, in Advances in Organic Geochemistry (see Ref. 4), p. 169; J. E. Cooper and E. E. Bray, Geochim. Cosmochim. Acta 27, 1113 (1963).

8. H. N. Dunning, in Organic Geochemistry (see Ref. 3), p. 367.

9. J. G. Bendoraitis, B. L. Brown, and L. S. Hepner, Anal. Chem. 34, 49 (1962); for other papers, J. J. Cummins and W. E. Robinson, J. Chem. Eng. Data 9, 304 (1964).

10. M. Blumer, M. M. Mullin, and D. W. Thomas, Science 140, 974 (1963).

11. B. Hallgren and S. Larsson, Acta Chem. Scand. 17, 543 (1963); G. Lambertsen and R. T. Holman, ibid., p. 281, and references cited therein.

12. J. D. Mold, R. K. Stevens, R. E. Means, and J. M. Ruth, Nature 199, 283 (1963).

13. W. Bergmann, in Organic Geochemistry (see Ref. 3), p. 534.

14. E. G. Curphey, Petroleum, London 15, 297 (1952).

15. W. S. White and J. C. Wright, Econ. Geol. 49, 675 (1954); W. S. White, ibid. 55, 402 (1960).

10. CYANAMIDE: A POSSIBLE KEY COMPOUND IN CHEMICAL EVOLUTION

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During 1964 we pursued a number of studies designed to elucidate a possible role for cyanamide (H_2H-CN), and its dimer, in chemical evolution. The results reported from many laboratories over the past decade[1-4] make it clear that all of the necessary biological monomers could have been formed on the primitive earth. By "biological monomers" we mean compounds such as the amino acids (on the way to proteins), the simple sugars (on the way to carbohydrates and nucleic acids), and the purines (on the way to nucleic acids). There is no doubt that such compounds would have been formed on the earth under its assumed early atmosphere (composed mostly of CH_4, NH_3, N_2, H_2O, and H_2).

There now looms the problem of finding reasonable mechanisms by which the biological monomers may have condensed to form the polymers under primitive earth conditions. The condensations under consideration (amino acids to proteins, sugars to carbohydrates, sugars to sugar phosphates, purines to nucleosides, and nucleosides to nucleotides) are all dehydration reactions, and a number of suggestions have been made as to how they took place. One of them is that a dehydrating condition could have arisen in evaporating ponds.[5] A second is that the reaction may have taken place upon contact with suitable mineral surfaces.[6] However, it seems to us that a more likely approach to the problem of biological polymer appearances would be to find conditions that would result in dehydrations in dilute aqueous media.

In considering compounds that can effect dehydration polymerizations, our attention was drawn to HCN plus uv light, and to the carbodiimides.[7] The substituted carbodiimides are widely used to effect dehydration condensation, for example, of amino acids to peptides,[8,9] and of alcohols (including sugars) with phosphoric acid to form phosphate esters;[10] in both these examples, water may be present in the reaction mixture. However, there is little reason to suppose that any significant quantity of the dialkyl carbodiimides was present on the primitive earth. A more likely candidate is cyanamide itself, a compound which hydrolyzes relatively slowly, that was probably present on the primitive earth, and that is a tautomer of carbodiimide (H_2N-C≡N⇌HN=C=NH).

We have tested the effect of cyanamide, or its dimer, in promoting the following condensations in dilute aqueous solutions: (a) glucose plus orthophosphoric acid to glucose-6-phosphate; (b) adenosine plus orthophosphoric acid to adenosine-5'-phosphate; (c) orthophosphoric acid to pyrophosphoric acid; (d) glucose to disaccharides; and (e) amino acids to dipeptides. In all these systems with the exception of (d) we have found that the cyanamide does indeed produce the condensation. In general, the experiments were carried out at concentrations (of both cyanamide and the other reactants) of 1 to 10 millimolar, at room temperature. The solutions were allowed to stand for about 1 day, and then were analyzed by paper chromatography. The yields of the condensed products were usually about 1 to 2%. The results of this work have been reported in Proc. Natl. Acad. Sci. 52, 27 (1964), and in a forthcoming paper, Science 147 (in press).

We have also found that the cyanamide dimer, H_2N-C(=NH)-N=C=NH, is formed upon ultraviolet irradiation of dilute HCN and NH_4CN solutions, and by the electron irradiation of a mixture of methane, ammonia, and water. These results lend further support to the idea that cyanamide may have played a key role in chemical evolution. These results were reported in Science 147, 149 (1965).

References

1. S. L. Miller, J. Am. Chem. Soc. 77, 2351 (1955).

2. J. Orό and A. P. Kimball, Arch. Biochem. Biophys. 94, 217 (1961).

3. C. Ponnamperuma, R. Mariner, and C. Sagan, Nature 198, 1199 (1963).

4. C. Ponnamperuma, R. M. Lemmon, R. Mariner, and M. Calvin, Proc. Natl. Acad. Sci. U. S. 49, 737 (1963).

5. S. W. Fox and K. Harada, J. Am. Chem. Soc. 82, 3745 (1960).

6. J. D. Bernal, "The Origin of Life on Earth," in Proceedings of the First International Symposium, Moscow, USSR, 1957 (Pergamon Press, London, 1959).

7. M. Calvin, AIBS Bull. 12, No. 5, 29 (1962).

8. J. C. Sheehan and G. P. Hess, J. Am. Chem. Soc. 77, 1067 (1955).

9. J. H. Bradbury and D. C. Shaw, Australian J. Chem. 12, 300 (1959).

10. H. G. Khorana, Some Recent Developments in the Chemistry of Phosphate Esters of Biological Interest (John Wiley and Sons, New York, 1961), p. 134.

11. SOME ASPECTS OF THE PHOTOCHEMISTRY AND RADIATION CHEMISTRY OF THYMINE

Joan Friedman

The reaction of thymine, one of the pyrimidine bases of DNA, to light and ionizing radiation is important in the responses of whole cells to these stresses. These cell responses include mutation, radiation damage repair, and cell reproductive death. Three problems in the photochemistry and radiation chemistry of thymine have been studied in this Laboratory: the mechanism of thymine dimerization by uv light; the possible reversal of dimerization by use of visible light and the dye, acridine orange (AO); and some of the ionizing radiation chemistry of thymine.

Mechanism of Thymine Dimerization

Thymine is known to form cyclobutane dimers upon irradiation in dilute frozen aqueous solution. Dimers are also formed in vivo in the DNA of microorganisms when irradiated by uv light. Dimer formation in solution and in crystals deposited in thin layers is very much less efficient than in ice. Two aspects of the mechanism of dimerization were studied here. One was the role of ice in the dimerization; the second was the investigation of the nature of the excited species and intermediates. Thymine-2-C^{14} was irradiated both in thin films and suspended in hexane to test the role of water, in slow and quick frozen solutions to test the function of the ice, and in the presence and absence of oxygen to determine the nature of the excited state. The results obtained indicate that water is necessary for the dimerization--i.e., no dimers were found upon irradiation of thin films or the hexane suspension. Although others have obtained dimers upon irradiation of thin films, S. Y. Wang has determined that dimer formation in these films depends to a great extent on the humidity of the surrounding atmosphere. It was found that a higher yield of dimers was obtained in the quick-frozen solutions. This is interpreted as being a surface effect. More smaller microcrystalline regions of thymine would be expected to be formed in the quick-frozen solutions and thus present more surface area to the incident light. No oxygen effect was noted in thymine dimerization in ice. This might be interpreted as suggesting that long-lived triplet diradicals are not important in the dimerization in ice, but it is most likely that the oxygen is not sufficiently free to diffuse through the compact ice structure to get close enough to the radicals, if any, to quench them. Many details of the thymine dimerization mechanism have yet to be worked out.

A Possible Model for the Photoreactivating Enzyme: Reversal of Dimerization by Visible Light

Reversal of thymine dimerization has been implicated in the phenomenon of photoreactivation in certain microorganisms. Since the thymine dimer starts absorbing light appreciably only at wavelengths shorter than about 2400 Å, and photoreactivation occurs only upon irradiation with light at longer wavelengths than 3200 Å, there must be a chromophore in the system different from the thymine dimer which in some way transfers its energy to the dimer to cause it to split. We used the dye acridine orange (AO) as a model chromophore because it binds to DNA and has an absorption maximum in the visible region. To find out if AO plus visible light could split thymine dimer to yield the monomer, we looked for interaction between the dye and both thymine and thymine dimer. We used uv-difference spectroscopy after irradiation of the samples with visible light, and fluorescence of AO as affected by thymine and the dimer. A search was made for products of visible-light irradiation of thymine and AO by using C^{14}-thymine and C^{14}-thymine dimer, in both the presence and absence of air. We also measured the bleaching of AO alone in the presence of air.

An enhancement of fluorescence from AO was always observed in the presence of thymine dimer, although the magnitudes varied widely from day to day. The wavelength of maximum emission was not changed. The fluorescence enhancement might be evidence for a specific interaction between dye and pyrimidine which would be a prerequisite for energy transfer. When illuminated in frozen, deaerated solution, samples of AO and a mixture of thymine and the dimer gave in one case a small decrease in percent dimer over the control irradiated without AO, and in another case (at higher concentrations of AO), negative results. It may be that the initial results were fortuitous or that the higher concentrations of AO used lead to self-quenching rather than energy transfer. Samples were illuminated in the absence of oxygen to eliminate the photooxidation of AO which occurs rapidly when AO is irradiated in air.

Radiation Chemistry of Thymine

Two separate problems in the radiation chemistry of thymine are described here. In the first we were concerned with the products formed upon irradiation of thymine and, in particular, with whether ionizing radiation could produce, even in small yield, the same biologically significant product as uv irradiation (the dimer). To this end, we irradiated thymine in frozen aqueous solution and in the solid state. As in most of the uv irradiations, no attempt was made to deaerate the frozen solution.

The second problem is concerned with the radiation-induced reaction of thymine with ethanol. This reaction was noticed in the course of a search for a sensitizer to ionizing radiation (analogous to uv sensitizers). Benzophenone, dissolved in a small amount of ethanol, was added to a water solution of thymine, degassed, and irradiated in a Co^{60} source. The thymine appeared to be efficiently converted to two major products, but upon further study it was shown that the ethanol, rather than the benzophenone, was responsible for the reaction. For comparison, the effect of ethanol on the radiation decomposition of uracil was also studied.

Results of this investigation show that the sensitivity of thymine to ionizing radiation depends strongly on the physical state of the pyrimidine. In frozen solution, at a radiation dose twice that needed to decompose more than 50% of the thymine in a dilute solution (about 2 megarads), thymine is essentially not decomposed at all. In the dry solid, thymine survives a radiation dose of 95 megarads essentially intact. The resistance of thymine to decomposition in the solid is most likely due to the difficulty of inducing radical formation in this resonance-stabilized molecule. In solution, the solvent molecules are the primary target of the ionizing radiation, and reactions of the radicals thus formed with thymine are responsible for its decomposition. The radiation resistance in ice is probably due to the decreased mobility of these solvent radicals. In addition, excited water molecules lose energy to the ice lattice instead of ejecting electrons to form radicals. The same thymine-water configuration that favors formation of thymine dimers upon uv irradiation does not favor their formation when hit by ionizing radiation; no dimers were observed in these irradiations. The photochemical excitation is concentrated wholly in α, β-unsaturated carbonyl π-system of the pyrimidine, whereas radical formation by ionizing radiation is nonspecific. The probability of a particular radical's being produced by the action of ionizing radiation depends primarily on the concentration of sites and next on the stability of the radical being formed. Thus, the probability that the 5, 6-double bond of thymine would be excited by the direct action of rays is very small. Apparently, the solvent radicals do not attack thymine in such a way as to produce dimers.

In the presence of ethanol, thymine is more sensitive to the action ionizing radiation than in its absence in aqueous deaerated solutions. At a radiation dose of 2.35 megarads, the G value of thymine increases from 1.8 in the absence of ethanol to 2.7 when ethanol is present as about 1% of the solution. A study of the irradiations, using ethanol-C^{14}, shows that the major irradiation products are ethanol adducts. The structures of these products are not known, although one of the products, characterized by its intense uv absorption and high R_f in the paper chromatographic solvent systems used, probably contains 4 moles of ethanol to one of thymine (determined by double-labeling the products with thymine-methyl-H^3 and C^{14}-ethanol). This conclusion, of course, would not be valid if one or more of the methyl hydrogens were replaced in the course of the reaction, since this would change the specific activity of the tritium on the thymine moiety.

Uracil is less sensitive to radiation decomposition in the absence of oxygen than thymine. Its G value in deaerated aqueous solution is 1.2. In the presence of a 4.5% ethanol solution, the G value was estimated to be roughly 1.0. Since the major product of irradiation, amounting to about one-third of the starting material, was the same in both the presence and absence of ethanol, it is concluded that the presence of ethanol affects the radiation decomposition of uracil very little (if at all), and if ethanol does increase the G value of uracil, it does not do so by reacting with it, as in the case of thymine.

Elucidation of the mechanism of the thymine-ethanol reaction rests heavily on identification of products. Although only a few major products are formed, there is always a large number of other products present, which makes the isolation problem very difficult. The greatest success in separation of products here was achieved with unidimensional paper chromatography on thick Whatman 3MM paper; however, it was impossible to completely free any one product from the soluble material on the paper that was eluted off with the products. Two possible products that might have been expected to form (O,O'-diethylthymine and N, N'-diethylthymine) were synthesized, but were found not to correspond to any of the radiation products.

12. BIOCHEMICAL AND ANATOMICAL CHANGES IN THE BRAIN AS A CONSEQUENCE OF EXPERIENCE

Edward L. Bennett, Marie Hebert, and Hiromi Morimoto

(in collaboration with Professor Mark R. Rosenzweig, Professor David Krech, Barbara Olton, and Jo Ann Keller, Department of Psychology, and Dr. Marian C. Diamond, Department of Anatomy, University of California)

A search for responses of the brain to experience, in the context of a larger project dealing with relations of intelligent behavior in animals, has been in progress in our Laboratories for about 7 years. It has long been speculated that mental activity leads to changes in the size of the brain, in the interconnections of its cells, and in its chemical composition. However, brain physiologists and anatomists have been singularly unsuccessful in finding any solid evidence to support theories which have demanded such changes. In our experiments, we have varied the experience given to different groups of animals (generally littermate rats) and subsequently have examined the brain for anatomical and biochemical effects of the differential experience. In a typical experiment, littermate rats (to reduce random variation among subjects) are assigned at weaning to the desired experimental conditions. One such condition, termed Environmental Complexity and Training (ECT), has generally been designed to enhance the experience of the animals. Animals in this condition are housed in groups of 10 to 12 in large cages provided with "toys." Every day they are placed for 30 minutes in a square field 90 cm on a side where the pattern of barriers is changed daily. After some weeks they are also given one or two trials a day in various standardized mazes for sugar pellet rewards. Another condition, termed Isolated Control (IC), has been designed to reduce the experience of the animals. This is achieved by housing animals individually in cages in a dimly lit and quiet room where they cannot see or touch another animal (although they can hear and smell them). A third, intermediate condition frequently used is termed Social Control (SC); in this condition animals are maintained under what might be called normal colony conditions, three rats to a cage, and exposed to the ongoing activities of the room but with no special or unusual treatment. Typically, animals are kept under these experimental conditions for 80 days and then they are killed for analysis of the brain. The brain is generally divided by gross dissection into four samples from the cortex and one from the subcortex. In some experiments the brain is studied by suitable histological techniques.

Inasmuch as our methods and results have been recently comprehensively reviewed, [1] in this brief report we summarize in outline form only our principal conclusions, which are as follows:

1. Male rats given enriched experience develop, in comparison to restricted littermates, the following changes:

(a) Greater weight and thickness of cerebral cortical tissue.

(b) An increase in total acetylcholinesterase (AChE) activity in the cortex, the gain in enzymatic activity being less than the gain in tissue weight.

(c) An increase in total cholinesterase (ChE) activity in the cortex, this gain being greater than the gain in tissue weight.

(d) An increase in total DNA, indicating an increase in the total number of cortical cells (a provisional result subject to additional replicate experiments).

(e) No change in protein, hexokinase activity, or serotin per unit weight of brain tissue, indicating that the added cortical tissue is normal in its chemical endowment in these respects.

(f) An increase in the diameter of capillaries in the cortex. This indicates that the enriched-experience animals have greater cortical blood supply than do their impoverished littermates.

(g) An increase in the number of glial cells in the cortex but no change in the number of neurons, as counted by newly devised anatomical procedures. An increase in cell number (point d above), and the greater increase in ChE (point c) than in AChE (point b), are all consistent with an increase in glia, since glia contain chiefly ChE, while neurons contain chiefly A hE. Thus three lines of evidence, two biochemical and one anatomical, converge nicely.

2. Changes with experience develop as readily in adult as in young rats. To date we have demonstrated greater weight and an increase in AChE and ChE in adult rats.

3. The greater the difference in richness of experience between groups of animals, the greater the differences that develop in their brains--i. e., the difference in cortex weights is greater between the ECT and IC animals than between the SC and IC animals.

4. Control experiments have demonstrated

that these cerebral effects cannot be attributed in any large measure to differential handling, to differential locomotor activity, to the social isolation stress of restricted animals, or to differences in body weight.

5. The main effects, as far as we have tested, occur in all the strains of rats that we have used--six strains--and in the two inbred strains of mice that we have used.

6. Changes produced in the brain by a specified environment tend to last while environmental conditions remain constant, but they can be modified or reversed by altering the animals' experience.

7. The changes produced by differential experience are characteristically different in magnitude from one functional cortical region to another.

8. Studies involving blinding or raising animals in the dark demonstrate that we can produce effects of experience selectively in one or another functional region of the brain. Furthermore, decreased development of one area of the cortex produced by lack of stimulation in one sensory channel appears to be accompanied by compensatory development of other areas, but only if overall enrichment of the environment is maintained.

9. In experiments involving genetic selection for brain chemistry, we have been able to produce considerably larger differences than in experiments in which we have manipulated the environment. It is clear that both hereditary and environmental factors must be taken into account in the determination of brain chemistry.

As pointed out in the Science article, "we wish to make clear that finding these changes in the brain consequent upon experience does not prove that they have anything to do with storage of memory. The demonstration of such changes merely helps us to establish the fact that the brain is responsive to environmental pressure--a fact demanded by physiological theories of learning and memory." [1]

Reference

1. E. L. Bennett, M. C. Diamond, D. Krech, and M. R. Rosenzweig, Science 146, 610 (1964).

13. HISTOLOGICAL STUDIES ON THE MECHANISM OF D_2O-INDUCED STERILITY IN MICE

Ann M. Hughes and Laurel E. Glass*

In studies in which normal females were mated to D_2O-treated male mice, it has been shown that D_2O produces sterility in the males;[1, 2] however, the site of the damage has not been demonstrated. Since embryonic death after implantation did not increase in such females, cleavage-stage embryos were examined 18 to 72 hours after mating.[3] Most of the embryos present in the oviducts were either one-called or, though "multicelled", were abnormal and degenerating; few normal embryos were found. These data did not indicate whether developmental failure was due to failure of the sperm to fertilize the egg or to a failure of the fertilizer egg to develop. By use of standard histological techniques, eggs obtained from females 12 to 16 hours after mating have been examined in an attempt to resolve this question.

Whole oviducts from mated females were fixed in Bouin's, embedded in paraffin, serially sectioned at 10 μ, stained with haematoxylin-eosin, and examined with a light microscope at magnifications up to 640X.

By two weeks on D_2O, the average number of fertilized eggs had dropped from 5.8 per female to 3.8 per female; after the males had been on D_2O four weeks, no fertilized eggs were obtained from mated females. The examination of "live" eggs by phase-contrast microscopy has confirmed the above results.

Under the conditions of this experiment, sperm penetration usually occurred in 6 to 8 hr after the females were removed from the males. Thus, by collecting eggs 12 to 16 hr after copulation, one could be sure that sperm entry would have been effected, yet most eggs should not be old enough to start degenerating. The results presented here clearly indicate that the failure of the D_2O-treated males to produce viable offspring, as previously reported, was due to a failure of the sperm to fertilize the egg and not to a failure of the subsequent development of the fertilized egg.

Autoradiographic studies of eggs from females mated to D_2O-treated males (in which the sperm are labeled isotopically) and electron microscopy of sperm from D_2O-treated males are also in progress in further attempts to pinpoint the site of action of D_2O.

References

1. A. M. Hughes and M. Calvin, Science 127, 1445 (1958).

2. A. M. Hughes, E. L. Bennett, and M. Calvin, Proc. Natl. Acad. Sci. U.S. 45, 581 (1959).

3. A. M. Hughes, E. L. Bennett, and M. Calvin, Ann. N. Y. Acad. Sci. 84, 763 (1960).

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PUBLICATION LIST, 1964

1. Inhibition of Carboxydismutase by Iodoacetamide. B. R. Rabin and P. W. Trown. Proc. Natl. Acad. Sci. U. S. 51, 497 (1964).

2. Transient EPR and Absorbance Changes in Photosynthetic Bacteria. R. H. Ruby, I. D. Kuntz, Jr., and Melvin Calvin. Proc. Natl. Acad. Sci. U. S. 51, 515 (1964).

3. A Contribution to the Understanding of Primary Quantum Conversion. R. H. Ruby, I. D. Kuntz, Jr., and Melvin Calvin. Bull. Soc. Chim. Biol. 46, 75 (1964); in English and French.

4. Cerebral Effects of Environmental Complexity and Training Among Adult Rats. M. R. Rosenzweig, E. L. Bennett, and David Krech. J. Comp. Physiol. 57, 438 (1964).

5. Reliability and Regional Specificity of Cerebral Effects of Environmental Complexity and Training. E. L. Bennett, David Krech, and M. R. Rosenzweig. J. Comp. Physiol. Psychol. 57, 440 (1964).

6. Aggregation of Chlorophyll a. A. F. H. Anderson and Melvin Calvin. Arch. Biochem. Biophys. 107, 251 (1964).

7. Oxidation States of Manganese Methyl Pheophorbide a in Aqueous Solution. P. A. Loach and Melvin Calvin. Nature 202, 343 (1964).

8. Mechanism of the Action of Carboxydismutase. B. R. Rabin and P. W. Trown. Nature 202, 1290 (1964).

9. The Biosynthesis of Nicotine in Nicotiana glutinosa from Carbon-14 Dioxide. W. L. Alworth, R. C. DeSelms, and H. Rapoport. J. Am. Chem. Soc. 86, 1608 (1964).

10. The Quantasome: Size and Composition. R. B. Park and J. Biggins. Science 144, 1009 (1964).

11. Two-Dimensional High Voltage, Low Temperature Paper Electrophoresis of ^{14}C-Labeled Products of Photosynthesis with $^{14}CO_2$. W. Rohr and J. A. Bassham. Anal. Biochem. 9, 343 (1964).

12. The Chemical and Photochemical Reduction of Flavin Nucleotides and Analogs. G. K. Radda and Melvin Calvin. Biochemistry 3, 384 (1964).

13. Exoenzyme Kinetics with Applications to the Determination of Nucleotide Sequences. C. R. Cantor, I. Tinoco, Jr., and L. Peller. Biopolymers 2, 51 (1964).

14. Quinone and Pigment Composition of Chloroplasts and Quantasome Aggregates from Spinacia oleracea. H. K. Lichtenthaler and M. Calvin. Biochim. Biophys. Acta 70, 30 (1964).

15. A Rapid Method for the Identification of Small Quantities of Lipid-Soluble Vitamins and Quinones in Biological Material. H. K. Lichtenthaler. J. Chromatog. 13, 166 (1964).

16. Atom to Adam. Melvin Calvin and G. J. Calvin. Proc. Am. Phil. Soc. 108, 73 (1964).

17. Cyanamide: A Possible Key Compound in Chemical Evolution. G. Steinman, R. M. Lemmon, and M. Calvin. Proc. Natl. Acad. Sci. U. S. 52, 27 (1964).

18. Mechanism of Action of Carboxydismutase. P. W. Trown and B. R. Rabin. Proc. Natl. Acad. Sci. U. S. 52, 88 (1964).

19. Hydrocarbons of Biological Origin from a One-Billion-Year-Old Sediment. G. Eglinton, P. M. Scott, T. Belsky, A. L. Burlingame, and Melvin Calvin. Science 145, 262 (1964).

20. Genetic Effects of Deuterium Oxide in Drosophila melanogaster. Ann. M. Hughes, P. E. Hildreth, and G. W. Becker. Genetics 49, 715 (1964).

21. Absorption Changes in Bacterial Chromatophores. I. D. Kuntz, Jr., P. A. Loach, and Melvin Calvin. Biophys. J. 4, 227 (1964).

22. The Biosynthesis of Nicotine in Nicotiana glutinosa from Carbon-14 Dioxide. Formation of the Pyrrolidine Ring. W. L. Alworth, A. A. Liebman, and H. Rapoport. J. Am. Chem. Soc. 86, 3375 (1964).

23. Atom to Adam. Melvin Calvin and G. J. Calvin. Am. Scientist 52, 163 (1964).

24. The Interaction between Mn(IV) Hematoporphyrin IX and Water. Paul A. Loach and Melvin Calvin. Biochim. Biophys. Acta 79, 379 (1964).

25. The Nucleic Acid Content of Spinacia oleracea Chloroplasts Isolated by Nonaqueous Technique. John Biggins and R. B. Park. Nature 203, 425 (1964).

26. Chemistry and Photochemistry of Iron and Manganese Porphyrins. Melvin Calvin, P. A. Loach, and A. Yamato. In Theory of Complex Structures, Ed. by B. Jezouska-Trzebistowska (Pergamon Press, London, 1964), pp. 13-34.

27. Primary Quantum Conversion: EPR Evidence. R. H. Ruby and Melvin Calvin. In Bacterial Photosynthesis Ed. by A. San Pietro (Antioch College Press, Yellow Springs, Ohio, 1964), pp. 335-341.

28. Studies on the Tautomerism of Amides. L. Skulski, G. C. Palmer, and M. Calvin. Ann. Soc. Chim. Polon. (Roczniki Chemii) 38, 789 (1964).

29. Molecular Orientation in Quantasomes. II. Absorption Spectra, Hill Activity, and Fluorescence Yields. K. Sauer and R. B. Park. Biochim. Biophys. Acta 79, 476 (1964).

30. Action Spectrum of the Hill Reaction with Ferrycyanide and Ferricyanide/Indophenol by Isolated Chloroplasts. J. Biggins and Kenneth Sauer. Biochim. Biophys. Acta 88, 655 (1964).

31. Protein Synthesis during Photosynthesis. J. A. Bassham, B. Morawiecka, and M. R. Kirk. Biochim. Biophys. Acta 90, 542 (1964).

32. Photosynthesis of Amino Acids. J. A. Bassham and M. R. Kirk. Biochim. Acta 90, 553 (1964).

33. Reticuline as the Benzyltetrahydroisoquinoline Precursor of Thebaine in Biosynthesis with Carbon-14 Dioxide. R. O. Martin, M. E. Warren, Jr., and H. Rapoport. J. Am. Chem. Soc. 86, 4726 (1964).

34. Chemical and Anatomical Plasticity of Brain. E. L. Bennett, M. C. Diamond, David Krech, and M. R. Rosenzweig. Science 146, 610 (1964).

35. Rotating Stopcock and Aspirator Type Samplers. K. K. Lonberg-Holm. In Rapid Mixing and Sampling Techniques in Biochemistry (Academic Press Inc., New York, 1964), pp. 275-287.

36. Multichannel Syringe-Type Sampler for Large-Scale Experiments. V. Moses and K. K. Lonberg-Holm. In Rapid Mixing and Sampling Techniques in Biochemistry (Academic Press Inc., New York, 1964), pp. 311-317.

37. Techniques for Following Rapid Inhibitor-Induced Changes in the Operation of the Photosynthetic Carbon Reduction Cycle. J. A. Bassham and M. R. Kirk. In Rapid Mixing and Sampling Techniques in Biochemistry (Academic Press Inc., New York, 1964), pp. 319-330.

38. Failure to Train Planarians Reliably. E. L. Bennett and Melvin Calvin. Neural Research Program Bull. 2 (4), 3 (1964).

39. A Technique for the Investigation of Intermediary Metabolism of Cells Grown in Tissue Culture. V. Moses and K. K. Lonberg-Holm. Acta Unio Intern. Contra Cancrum 20, 1066 (1964).

The following Ph. D. theses were completed during 1964:

1. Gordon A. Pryor, Brain Serotonin and Behavior in Selected Strains of Rats, UCRL-11179, Jan. 1964. (Dept. of Psychology.)

2. David F. Ilten, The Electrical, Magnetic and Optical Properties of Some Complex Organic Systems, UCRL-11374, July 1964. (Dept. of Chemistry.)

3. John Biggins, Studies on the Structure and Function of the Chloroplast Lamellae, UCRL-11863, Dec. 1964. (Dept. of Plant Physiology.)

Abstracts and Papers were presented at meetings as follows:

1. Strain differences in cerebral response to environmental complexity and training. M. R. Rosenzweig, D. Krech, and E. L. Bennett. Fed. Proc. 23, 255 (1964).

2. Effects of Post-Weaning Blinding and Light Deprivation on Retinas and Brain

of Rat. E. L. Bennett, M. R. Rosenzweig, and D. Krech. Fed. Proc. 23, 384 (1964).

3. Genetic Selection of Rats for Cerebral Acetylcholine Concentration. E. L. Bennett. Proc. VIth Intern. Biochem. Congress, New York, 1964, III-4.

4. Attempt to Study Metabolic Compartmentalization. V. Moses, Julia J. Chang, and K. K. Lonberg-Holm. Proc. VIth Intern. Biochem. Congress, New York, 1964, IX-59.

5. Lipoic Acid Induced Transient Changes in Concentrations of Intermediate Compounds of the Photosynthetic Carbon Reduction Cycle. J. A. Bassham and M. Calvin. Proc. VIth Intern. Biochem. Congress, New York, 1964, X-5.

6. Some Morphological and Physical Properties of Chloroplast Lamellar Proteins. R. B. Park and J. Biggins. IVth Intern. Photobiology Congress, Oxford, England, 1964, p. 82.

7. Action Spectra of Electron Transport Reactions by Chloroplasts and Quantasomes. J. Biggins and K. Sauer. IVth Intern. Photobiology Congress, Oxford, England, 1964, p. 116.

8. Photosynthesis and Amino Acid Biosynthesis. J. A. Bassham. Xth Intern. Botanical Congress, Edinburgh, Scotland, 1964, Abstracts, p. 162.

In addition, the following scientific lectures were also presented by members of the Bio-Organic Chemistry Group during 1964:

1. R. B. Park

   Quantasome of the Chloroplast. American Society of Cell Biology, Cleveland, Ohio, Nov. 1964.

2. M. P. Klein

   ESR in Ferritin, Ferrichrome, and Ferredoxin. International Conference on Magnetic Resonance in Biological Systems, Boston, Mass., July 1964.

3. R. H. Ruby

   Transient ESR Signals in Photosynthetic Systems. International Conference on Magnetic Resonance in Biological Systems, Boston, Mass., July 1964.

4. V. Moses

   Molecular Regulation and Its Possible Evolutionary Significance. Symposium on "Evolving Genes and Proteins," Rutgers University, New Brunswick, N. J. Sept. 1964.

5. Melvin Calvin

   a. Chemical Evolution. National Science Foundation, Washington, March 1964.

   b. Primary Quantum Conversion in Photosynthesis. Rockefeller Institute, May 1964.

   c. Coordination Chemistry of Manganese and Porphyrins. F. P. Dwyer Memorial Lecture, University of New South Wales, Sydney, N. S. W., Australia, June 1964.

   d. Point In Time. Russell Grimwade Lecture, University of Melbourne, Melbourne, Australia, July 1964.

   e. Energy Transfer in Photosynthesis. IVth International Photobiology Congress, Oxford, England, July 1964.

   f. Chemical Evolution. General Congress Lecture, Xth International Botanical Congress, Edinburgh, Scotland, August 1964.