UCRL -8273

UNIVERSITY OF CALIFORNIA

Radiation Laboratory

BERKELEY, CALIFORNIA

UCRL-8[illegible]

UNIVERSITY OF CALIFORNIA
Radiation Laboratory
Berkeley, California

Contract No. W-7405-eng-48

THE NURTURE OF CREATIVE SCIENCE AND THE MEN WHO MAKE IT

Melvin Calvin

May 1, 1958

Printed for the U. S. Atomic Energy Commission

THE NURTURE OF CREATIVE SCIENCE AND THE MEN WHO MAKE IT

Melvin Calvin

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

May 1, 1958

ABSTRACT

This report describes the development of work that began as an investigation of photosynthesis and that continues in this direction, but which has as a new product some entirely strange results. Photosynthesis, the process upon which all life on earth today is ultimately dependent, achieves the conversion of electromagnetic energy from the sun into chemical energy in the form of plant material by the reduction of CO_2 from the atmosphere with the liberation of O_2 to the atmosphere. It has been possible to describe in some detail the way in which the plant accomplishes the reduction of carbon dioxide, using radioactive carbon as a tracer. The status of our present knowledge, and how we attained it, together with some prospectus of the future and what we can look forward to, is the principal theme of this discussion.

One of the avenues that we were pursuing for the photosynthetic work was the possible exploration of the path of hydrogen from the water molecule to the sugar molecule. To do this we felt it necessary to grow our plants in heavy water (D_2O). This has led to some entirely unexpected and new observations on the effect of deuterium on cellular development which may open an avenue for the investigation of growth and reproduction that is completely new to us. Another unanticipated development in the course of our investigation of photosynthesis has been the evolution of concepts of the electronic behavior of macro-molecules which could lead, on the one hand, to the creation of organic solar converters and, on the other, to new thoughts on the relationship between micromolecular structure and visible macrostructure and function in living organisms.

This work, which has been the product of the activities of many people from many existing divisions of science--physics, chemistry and biology--has led to a new synthesis, not only in the original area of thought but perhaps in others undreamed of at its inception. The background for this type of inter-disciplinary research activity is described, and the possible future of such research establishments in the United States is discussed.

THE NURTURE OF CREATIVE SCIENCE AND THE MEN WHO MAKE IT*

Melvin Calvin

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

May 1, 1958

INTRODUCTORY REMARKS

Mr. Carmichael, Mr. Taylor, Mr. Rittenberg, members of the New York Section, ladies and gentleman. It is a bit difficult to find the precise and correct words to express to you my feelings at the moment. To accept this medal is an honor indeed, and I hope the years to come will justify Mr. Carmichael and his colleagues in their selection. As for Mr. Rittenberg, I can only say that the thought may have occurred at the time he suggests and in the way he suggests.

I do have one bit of information which Mr. Rittenberg overlooked. In fact, I am not at all sure that any researcher could have discovered this bit of information. I am not trying to describe to you the time at which the decision to go into this work was made -- that Mr. Rittenberg has already done. I would like to describe to you the moment (and, curiously enough, it was a moment) when the recognition of one of the basic facets in the photosynthetic carbon dioxide cycle occurred. It so happened that we didn't have a freezer in our house and I had to go down, very likely, to a place where they have some deep-freeze equipment and where my wife kept certain items that we needed to keep for a long period of time, such as an occasional steak. One day we were down there collecting either the evening's dinner or the next morning's breakfast, and I was waiting in the car. I had had for some months some basic information from the laboratory which was incompatible with everything that, up until then, I knew. I was sitting at the wheel, waiting while my wife went in to get what she went after, and I think I was parked in the red zone (I'm not sure) when the recognition of the missing compound occurred. It occurred just like that --quite suddenly--and suddenly, also, in a matter of seconds the cyclic character of the path of carbon became apparent to me, not in the detail that you will see later, of course, but the original recognition of phosphoglyceric acid, and how it got there, and how the acceptor might be regenerated all occurred in a matter of 30 seconds. So there is such a thing as inspiration, I suppose, but you have to be ready for it. I don't know what made me ready for it at that moment, except I didn't have anything else to do. I had to sit and wait, and perhaps that in itself has some moral. It is this kind of thing that I would like to tell you a little more about.

Your chairman and your awards-committee chairman both emphasized the fact that Mr. Nichols, in establishing the medal, wanted to recognize basic and original research in chemistry. Mr. Nichols was an industrialist and yet he recognized the need for basic research.

We have heard much in the past months of the existing or impending shortage of scientists and engineers in our country and the need for accelerating or

---

*Transcription of Nichols Medal Address, New York Section, American Chemical Society, March 14, 1958. The preparation of this paper was sponsored by the U. S. Atomic Energy Commission.

transforming our educational program to produce them. I am quite confident that most of you here tonight will recognize, with me, that for today and for the immediate future at least, we do not lack scientific and engineering manpower of the type usually envisaged in these cries for "more", although we must, of course, continue to expand our ability to produce them for the longer future. Recruiting for industry in chemistry add physics has been considerably more selective this year than in the past few years. In fact, some of may engineering colleagues tell me that entire engineering organizations are ready and waiting to undertake jobs when they are given the word.

It is my feeling that what we lack today and are not providing for the future is not the numbers but the few men capable of generating the entirely new and original notions that could put these many scientists and engineers and organizations to work. In order to find these few people, there must be a climate for the development of many. An essential feature of this climate is an atmosphere of curiosity about the nature of the world around us and the freedom to satisfy that curiosity. My very presence here tonight to receive the honor that you have bestowed upon me is evidence of your regard for the product of such an atmosphere.

DISCUSSION

I would like to tell you the story of the development of the work that started out as an investigation of photosynthesis and that continues in this direction but which has many new ramifications and some entirely strange results. Photosynthesis, the process upon which all life on earth today is ultimately dependent, achieves the conversion of electromagnetic energy from the sun into chemical energy in the form of plant material, by the reduction of carbon dioxide from the atmosphere with the liberation of oxygen to the atmosphere (and you will see a little more of that in a moment). It has been possible to describe in some detail the way in which the plant accomplishes the reduction of CO_2, using radioactive carbon as a tracer. The status of our present knowledge, and how we attained it, together with some prospectus of the future and what we can look forward to, is the principal theme of this discussion.

Figure 1 will give you some idea of the problem as we faced it. The two molecules that are involved in the photosynthetic process in the beginning are the two at the bottom--water on the left, and carbon dioxide on the right. Light energy somehow breaks the water molecule, producing something that eventually becomes oxygen, with a sequence labeled A, B, and C, and an active hydrogen (H) of some sort, which acts upon carbon dioxide or a derivative of carbon dioxide producing compounds along the sequence X, Y, and Z, ultimately to form carbohydrate, that is, reduced carbon.

When the war was over in 1945, radioactive carbon (carbon-14) became available to us in quantity. It could be followed through a series of transformations such as were unknown and which are labeled X, Y, and Z in Fig. 1. We undertook to follow that path of tracer carbon by feeding labeled carbon to the plant, as shown in Fig. 2. Labeled carbon enters the leaf through a series of compounds, eventually coming out as sugar. It is easy to see that if we were to kill the leaf (or stop the process of photosynthesis), we would find those compounds that were on the route from carbon dioxide to sugar containing the radioactivity (indicated by the blips on the detectors down below). Those compounds that were not along the path between carbon dioxide and sugar, such as Q in Fig. 2, would not be radioactive, and thus it would be possible to map the route from carbon dioxide on the one hand to sugar on the other hand.

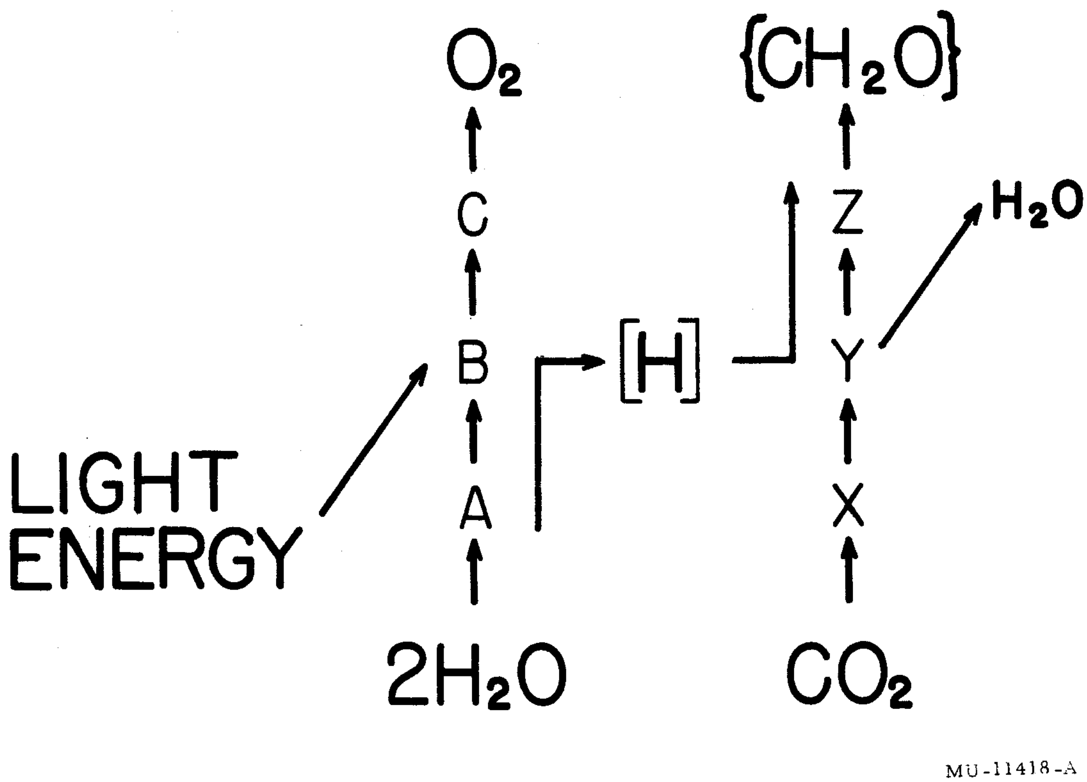


Fig. 1. Elementary photosynthesis scheme

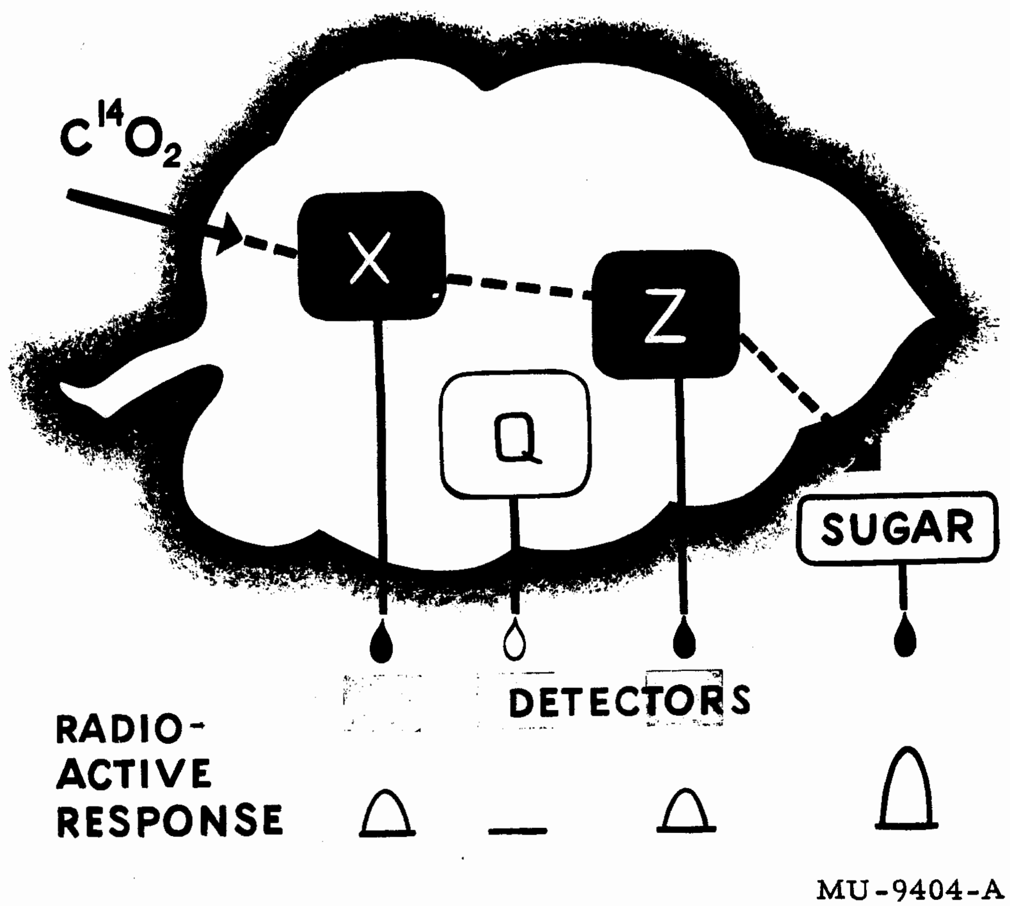


Fig. 2. Schematic representation of C^{14} labeling in the leaf.

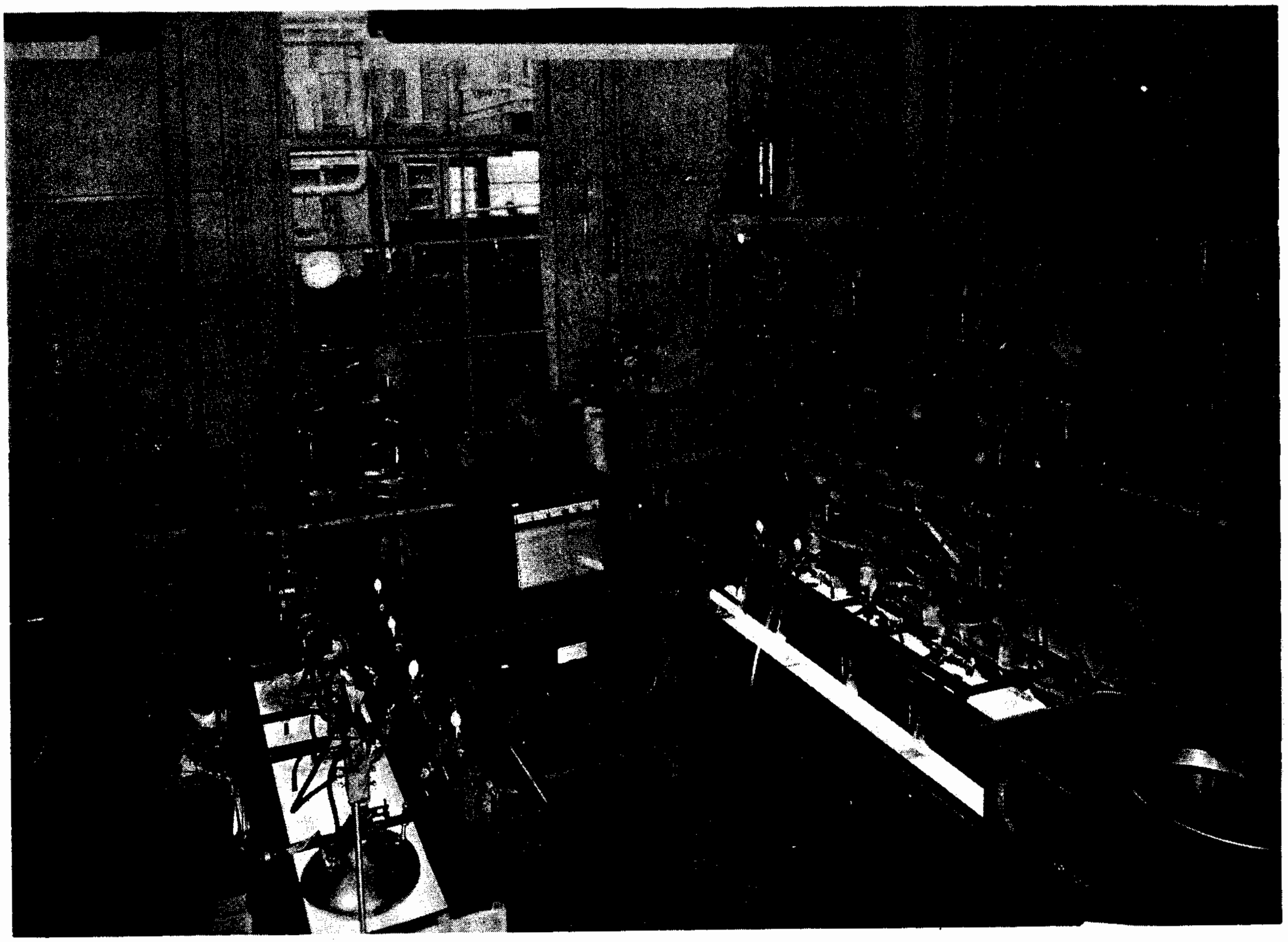
In order to do this kind of experiment we had to have a plant material which was extremely reproducible in its behavior. We had to become "farmers", but a special kind of farmer. We had to grow our plants in a reproducible way so that they would do the same thing on Tuesday as they did on Monday, but that is not simple with living organisms unless they are specially cared for. So we had to devise our own "farm" on which we could grow the organisms we would use for this study in such a way that we could depend upon their identical behavior from day to day.

Initially, after the selection of pure cultures and their maintenance in 150-cc flasks, we grew the algae in 1-liter shake flasks, as illustrated in Fig. 3; this is the type of algae-culture equipment that we used for many years, up until about 3 years ago as a matter of fact. Our present farm consists of a continuous-tube culture (Fig. 4) of unicellular green algae whose density is controlled by a photocell. The continuous control automatically feeds medium into the culture tube when the algae gets dense enough. When we need the algae cells, we can harvest them in a sterile manner. The biological material that we ordinarily use is the unicellular green algae <u>Chlorella</u>, whose culture can be controlled to produce a very reproducible organism. Figure 5 shows a photomicrograph of <u>Chlorella</u>, and this will give you some idea of the size; the scale, as you can see is in microns -- the cells are very small. The cup-shaped chloroplasts which contain the photochemical apparatus (chlorophyll) show near the center.

Having devised the "farm" and grown the algae, we then expose the algae, in the "lollipop" (Fig. 6) to radiocarbon for various periods of time. Following the exposure, the algae are killed in a variety of ways, and an extract of the algae which will be used for analysis is prepared by vacuum concentration. The method of analysis that we use is paper chromatography and, in fact, our entire operation is dependent upon this analytical procedure. A small bit of the extract is placed on a corner of a piece of filter paper which is hung in a trough (Fig. 7) and suitable solvent added to the trough are allowed to run over the paper, thus separating the mixture of compounds into their various constituents. This is done successively in both directions of the paper. We then find which constituents are radioactive by exposing the paper to an X-ray film. Wherever there is a radioactive compound on the paper, the film becomes black, and we thus know where the radioactive compounds are on the paper. Then we proceed to determine what they are. From their position on the paper, we have a clue to what they are but not a complete identification.

As a result of this operation of paper chromatography followed by radioautography we see in Fig. 8 a chromatogram showing what happens after 30 seconds exposure to radiocarbon, and you will see that there are about a dozen compounds that contain radioactivity. Thirty seconds, then, is much too long an exposure, and so we shortened the time; Fig. 9 shows a chromatogram of a 10-second exposure, and you can see one compound predominating. I might add that it has taken about 10 years to identify the compounds whose spots appear on Fig. 8, and there are still spots on the film whose identity is unknown to us.

The result of all this, is an analysis of the path of carbon in photosynthesis, which is shown in Fig. 10. I wanted to show you how detailed the path of carbon in photosynthesis really was. We now know every one of those compounds. To give you a better (and simpler) idea of what we have done, I have schematized this entire cycle into a leaf (Fig. 11) which is the one shown in Fig. 2, in which we [illegible] compounds labeled [illegible] and [illegible] to show you what the cycle amounts to in



ZN-1960

Fig. 3. Shake-flask apparatus for algae culture.

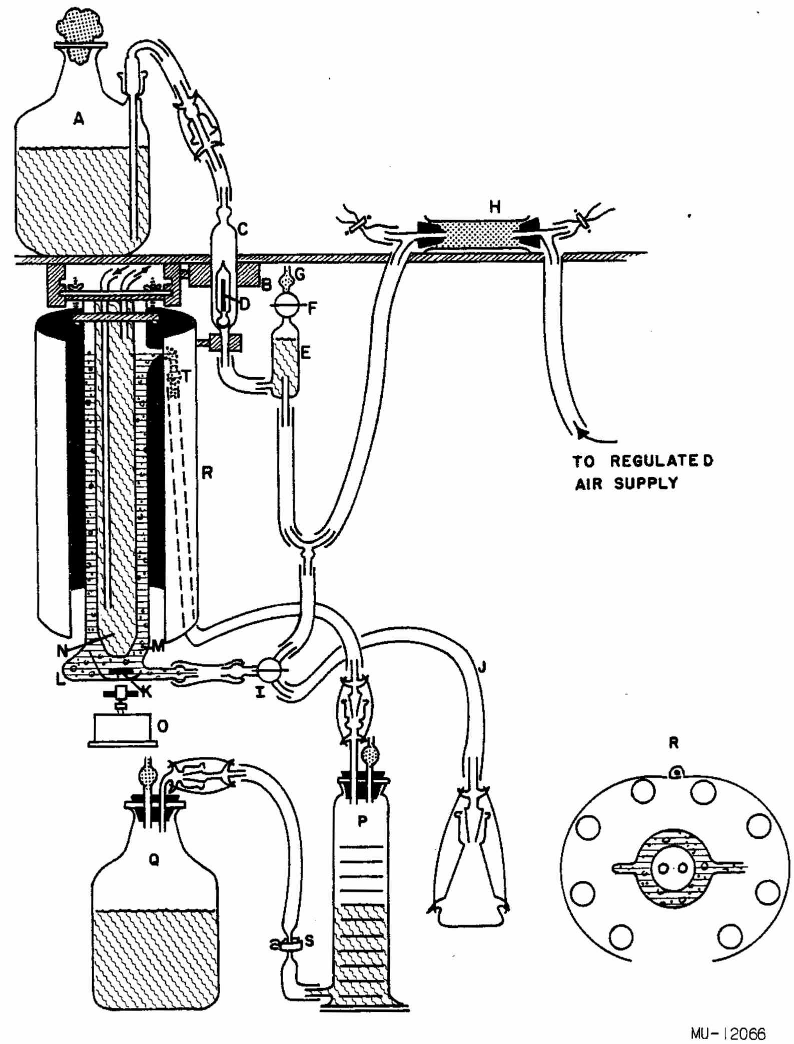
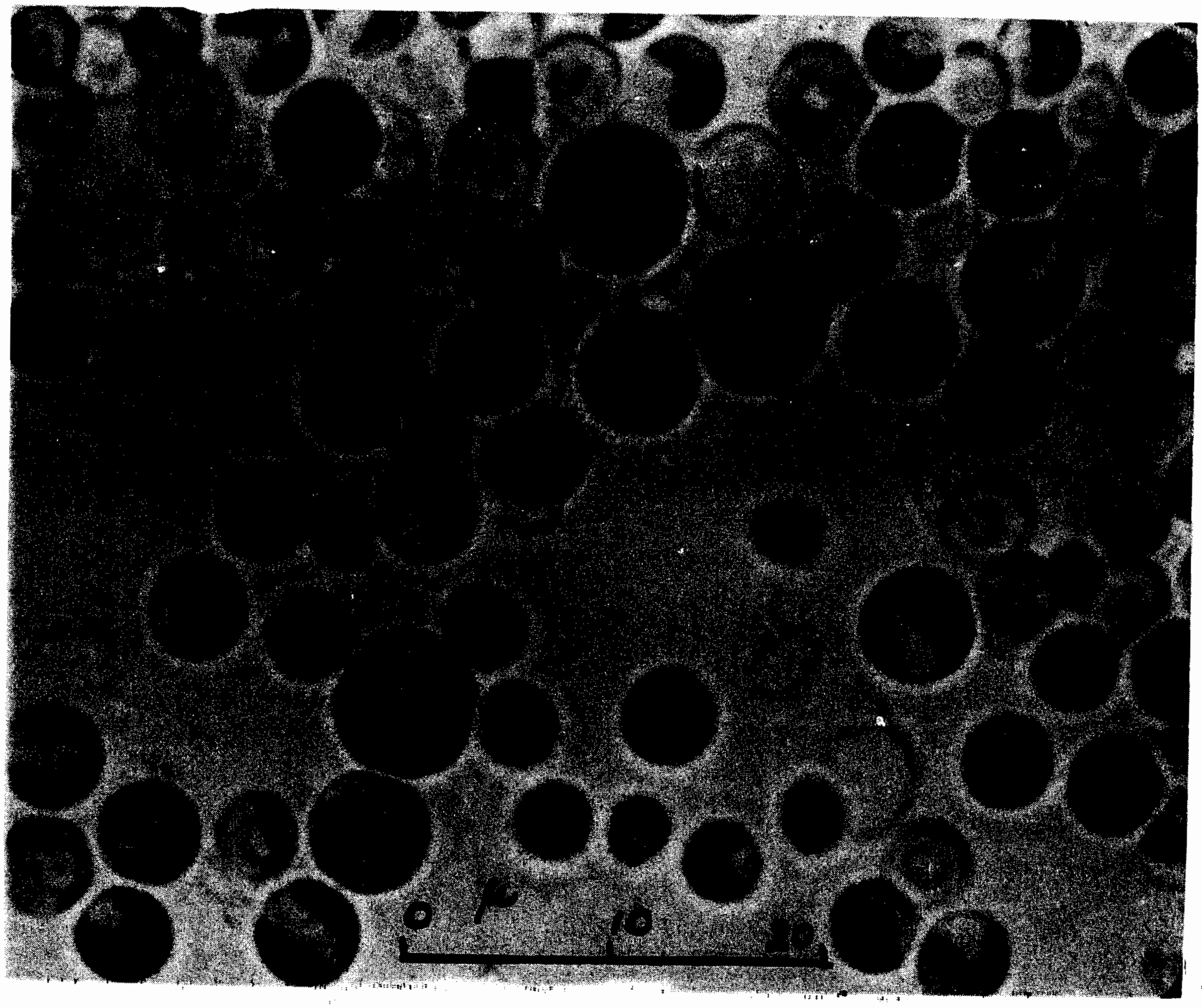
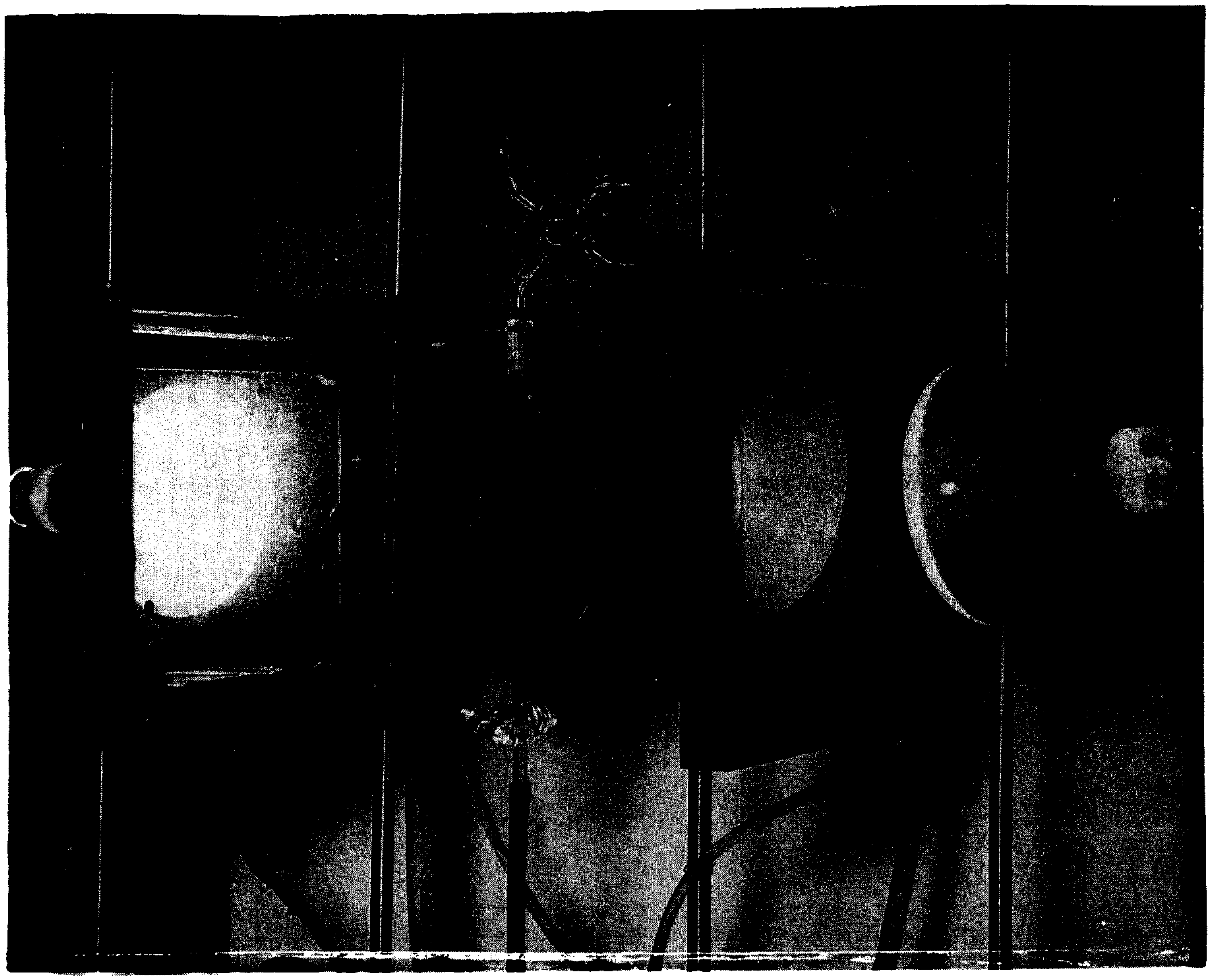


Fig. 4. Constant-density algae-culture apparatus: A, 16-liter carboy of medium; B, solenoid; C, glass tube with D, magnet; E, bubble trap; F, stopcock; G, cotton-packed air outlet; H, cotton-packed air filter; I, three-way stopcock; J, draining and inoculating tube; K, magnet; L, fin; M, encased algae culture; N, water bath; O, magnetic stirrer; P, collecting graduate; Q, reservoir; R, jacket with eight fluorescent lights; S, pinch clamp; T, overflow outlet. Inset is cross section showing jacket with eight fluorescent lights.



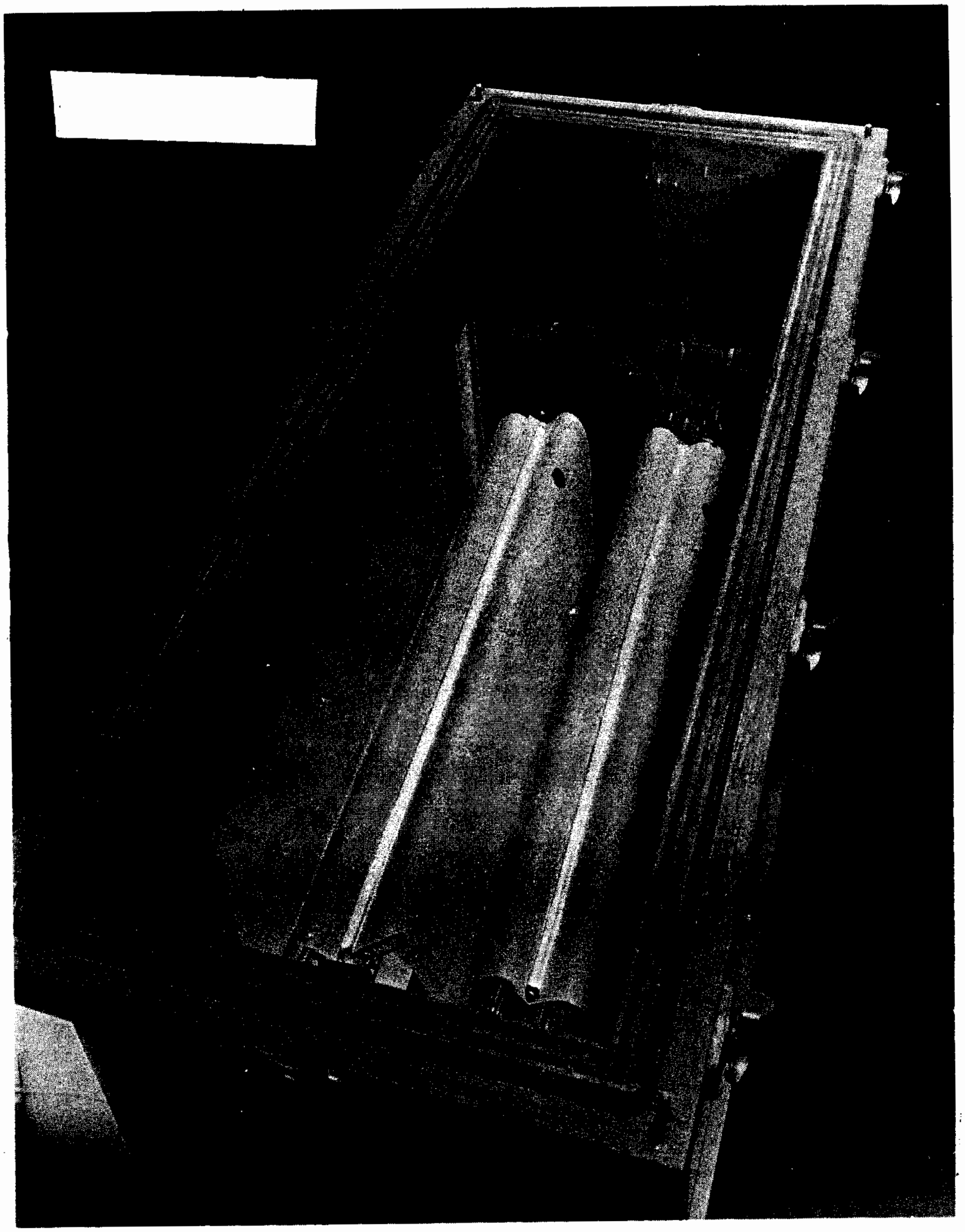
ZN-1750

Fig. 5. Photomicrograph of <u>Chlorella</u>.



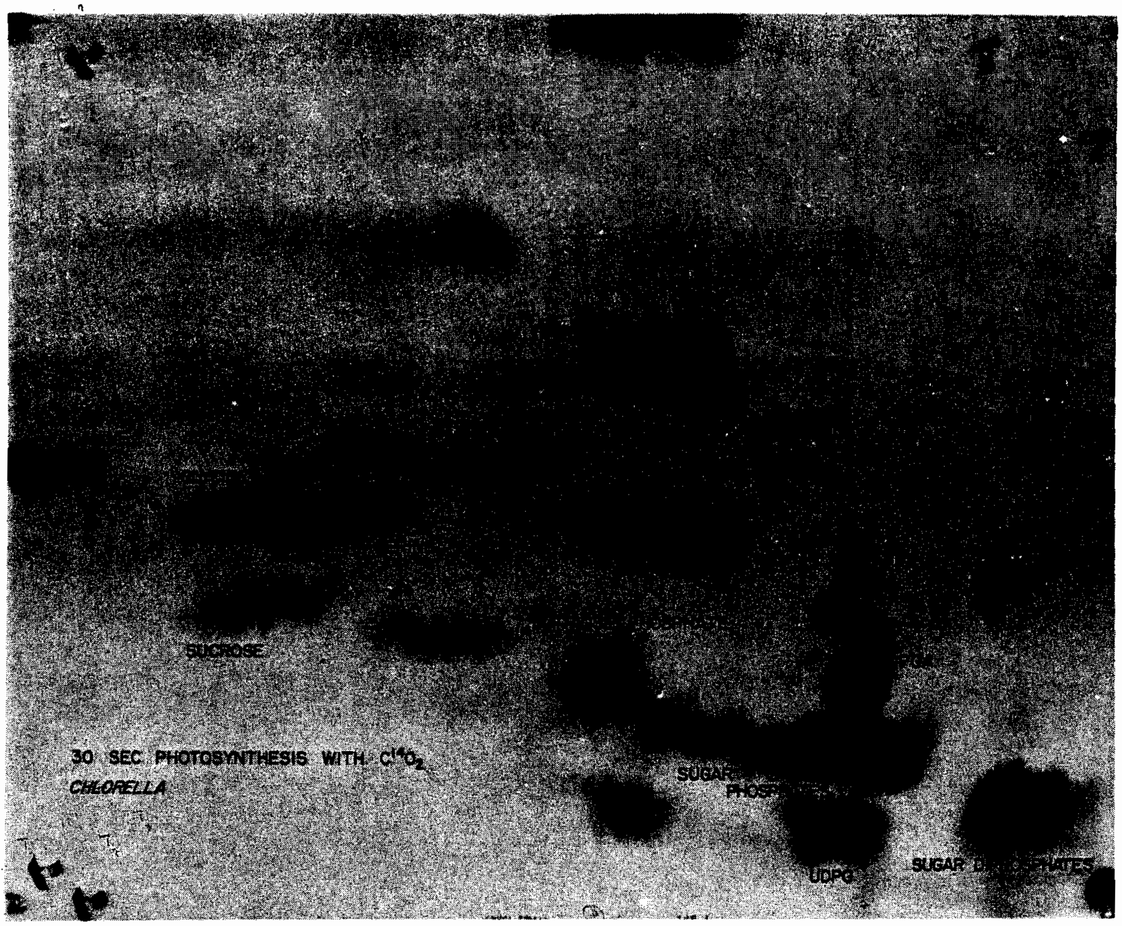
ZN-952

Fig. 6. "Lollipop".



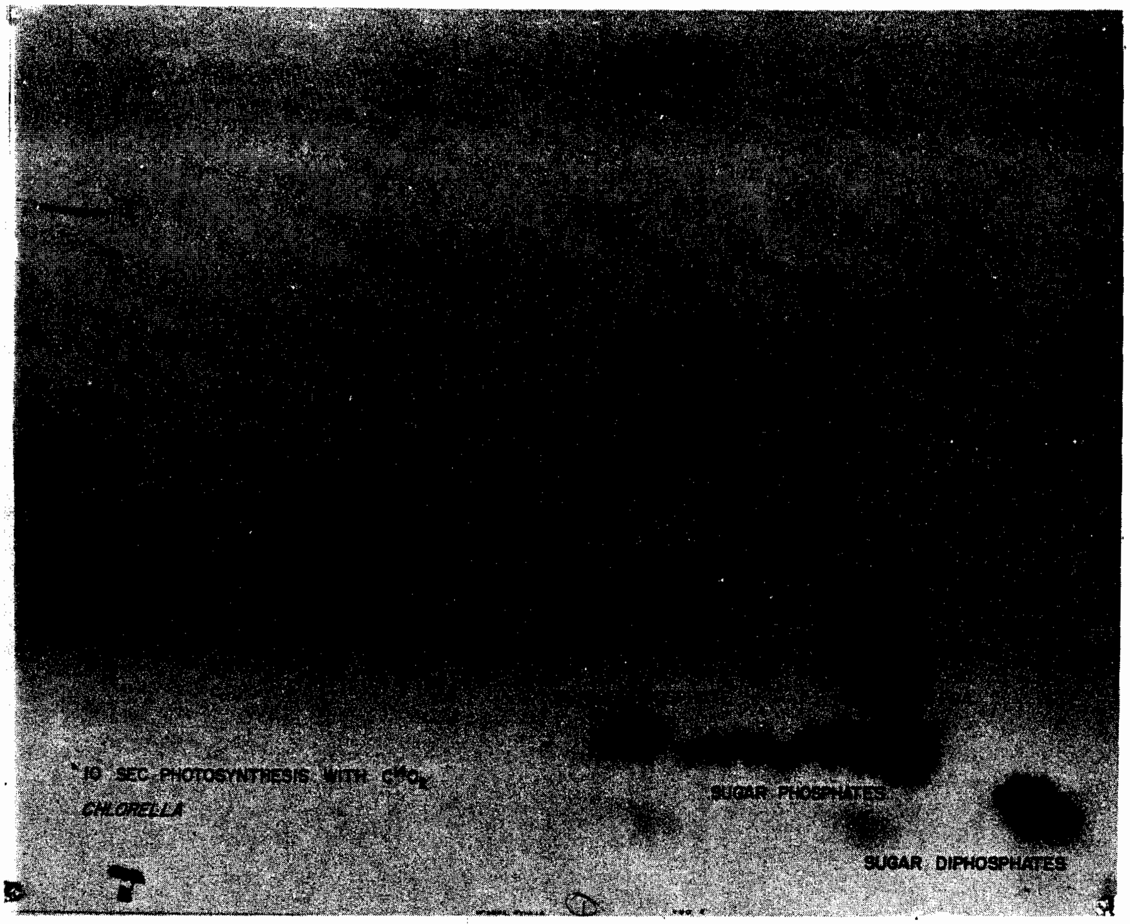
ZN-1957

Fig. 7. Top view of chromatograms in chromatographic trough.



ZN-1959

Fig. 8. Chromatogram of extract from algae indicating uptake of radio-carbon during 30 seconds of photosynthesis.



ZN-1958

Fig. 9. Chromatogram of extract from algae indicating uptake of radio-carbon during 10 seconds of photosynthesis.

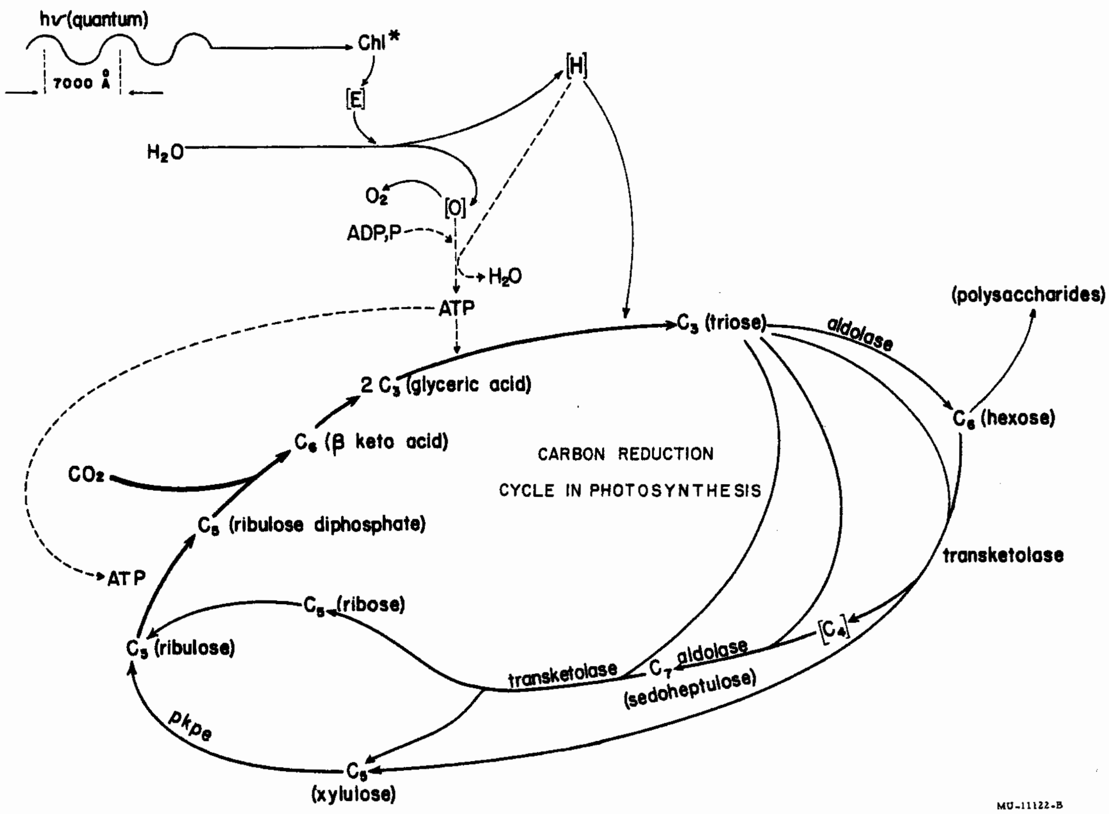


Fig. 10. The path of carbon in photosynthesis.

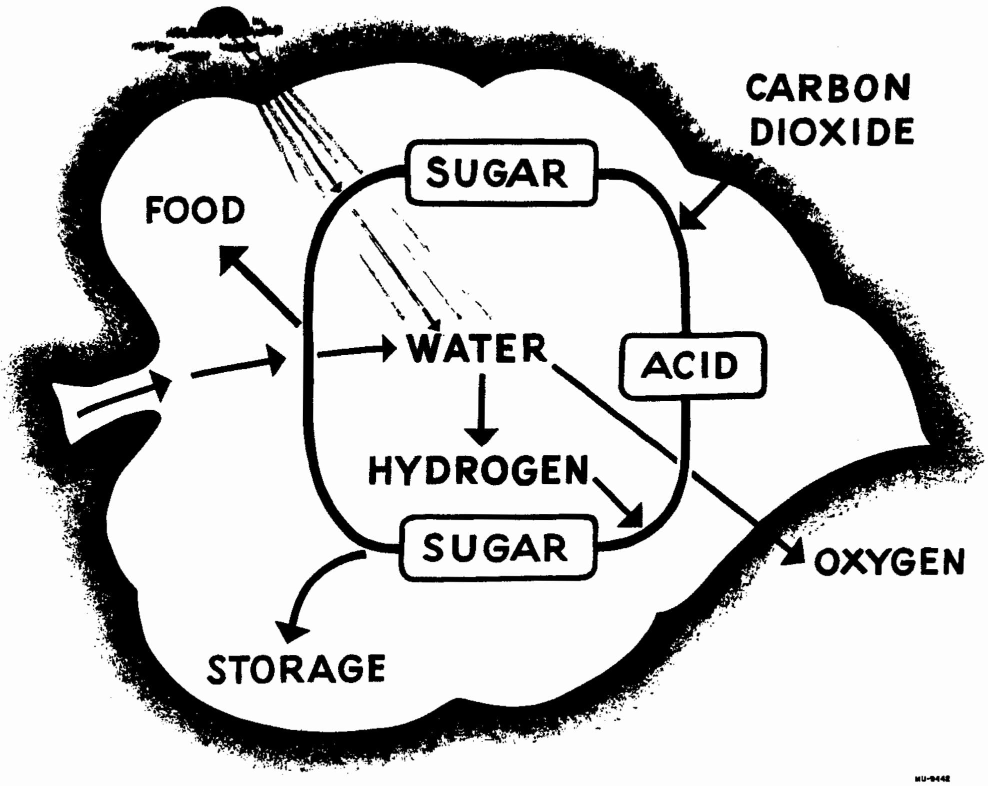


Fig. 11. Schematic diagram of photosynthesis in leaf.

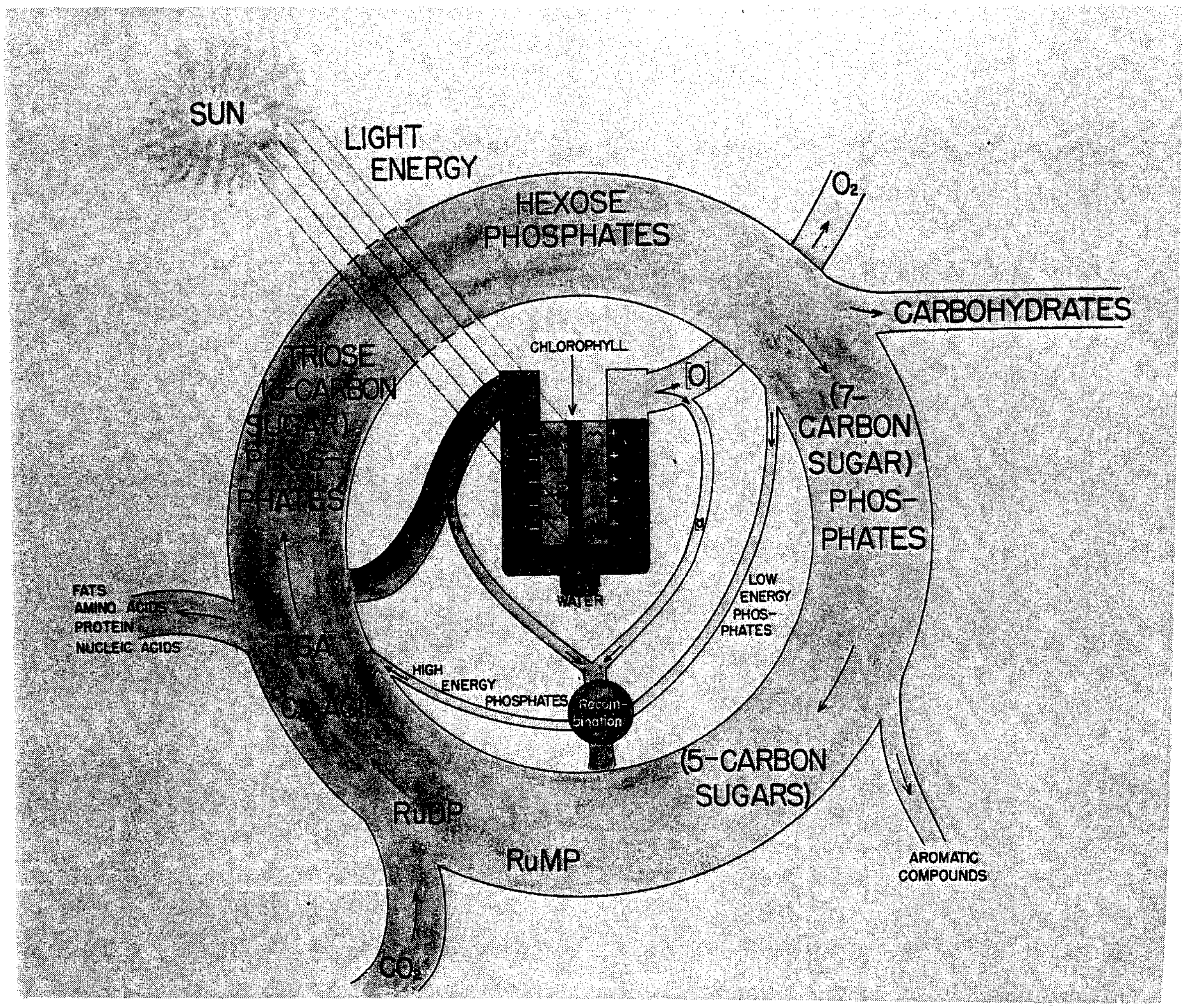
actual fact. The carbon dioxide enters the leaf at the upper right, and is combined with the sugar. The sugar, in combination with the CO_2 forms an acid. That acid is then reduced to sugar again, by the hydrogen which the sun makes from the water. The sunlight is shown shining on the water in Fig. 11 but it is, of course, actually absorbed by chlorophyll as shown in Fig. 12. Following absorption, an "active" hydrogen is made and that active hydrogen, following the arrow on the lower right, joins the acid and sugar, thus converting the acid to a sugar. The sugar undergoes a series of rearrangement and comes back again to the original sugar itself, and thus the cycle is completed. You see we have mapped the path of carbon in photosynthesis in great detail. More recently we set out to map the path of hydrogen, which is really an unknown quantity. All we know is that it starts from water and ends on the sugar.

An early approach was to use deuterated cells to follow the path of hydrogen, but this was abandoned when it was found that such cells showed distinct pathological characteristics and that the quantities required for the detection apparatus (in this case nuclear magnetic resonance) would involve the use of large amounts of cell material and would necessitate the isolation of intermediates on a large scale. For example, the unicellular green algae, <u>Chlorella</u>, when placed in water containing as much as 30% deuterium, while it continues to grow, ceases to divide, and giant cells result.

There remained the possibility of using the radioactive isotope of hydrogen, tritium, to follow the uptake of hydrogen from radioactive water by cells carrying out photosynthesis. Many difficulties had to be overcome, not the least of which was the very weak energy of the beta-particles emitted by tritium and the large amount of dilution of the tracer by the enormous amounts of water which are inevitably present in any biological system. The experiments with tritium were performed essentially in the same way as the carbon experiments described earlier. This work is still in the preliminary stages, but the results so far have shown that tritium is incorporated into a number of compounds in the course of three minutes, and these substances appear to be the same ones as those containing C^{14} after the cells are exposed to $C^{14}O_2$, though the relative distribution of activities is quite different with the two tracer substances.

Our experiments with heavy water on <u>Chlorella</u>, which indicated an interference with cell division but not directly with growth, suggested work on cancer cells whose characteristics are oriented toward rapid division or multiplication. We studied the effect of D_2O on the survival of mice with ascites tumor. Mice inoculated with ascites tumor and maintained on 25% and 30% D_2O drinking water showed an improved survival time of a few days, whereas 40% D_2O drinking water (a toxic concentration) had no effect on survival time. It has been postulated that the increased survival time of the mice maintained on the 25% to 30% D_2O is due to a decreased rate of cell division. This inhibitory effect has theoretical interest but no therapeutic value at the moment.

Another area in which the property of inhibiting cell division might show is in fertility, and accordingly, the control (normal) animals in the cancer study were mated and observed. This led to perhaps the most obviously interesting result of our experiments with D_2O which is the fact that male mice who receive drinking water containing in it up to 30% deuterium are incapable of fertilization. This effect is reversible, for when these mice are returned to normal water they recover their ability to produce offspring in



ZN-1748

Fig. 12. Photosynthesis of food from carbon dioxide.

about 65 days; this is 5 days to clear out the heavy water, 40 days for spermatogenesis and 20 days for gestation. The importance of this last observation with respect to the nature of growth and cell division and its possible application to a fundamental study of genic material, such as nucleic acid, has only begun to be explored.

The mechanism by which we believe this action occurs has to do with the fact that most of the giant molecules in the living organism retain the particular shape they have because of bonds within them formed by the hydrogen atoms of their structure. When these hydrogen atoms are replaced with deuterium atoms, we may expect these structures to change markedly. This should prove to be an important tool in the investigation of these most basic substances in biology.

Another unanticipated development from our investigation of photosynthesis has been the evolution of concepts of the electronic behavior of macromolecules which could lead, on the one hand, to the creation of organic solar converters and, on the other, to new thoughts on the relationship between micromolecular structure and visible macrostructure and function in the living organism.

CONCLUDING REMARKS

You can see how this work, which has been the product of the activities of many people from all existing divisions of science--physics, chemistry, and biology--has led to a new synthesis, not only in the original area of thought but perhaps in others undreamed of at its inception.

In order to foster this sort of thing, it seems to me our methods of educating scientists must be carefully examined. This education must be such as to enable the young scientist to explore deeply and well some particular area of natural phenomena. There is no substitute for this sort of concentrated activity and concentration of thought. However, it must be accompanied by the conviction that the student is free to follow, and, in fact, has the duty to follow, the exploration of any natural phenomena into whatever area the light may lead him. In this way will the creation of new horizons overlapping existing divisions of science be encouraged. Without it, we will be limited to the classifications and subdivisions of science developed during the 19th and early 20th centuries, and our thoughts, conceptions, and even practical developments will be circumscribed by the very words and modes of expression which each scientific subdivision of today tends to use.

In order to avoid academic prejudice, I felt it encumbent on me to try and find an external expression of what I wanted to say, and I found it in the words of Harold Gershinowitz, the president of the Shell Development Company (also of California):

> "In the large sense there can be no such thing as undirected research in industry. It may start out thus, but essentially it is directed into types of activity that the managers of that research laboratory have confidence will at some time have application. Therefore, I think it is very important that there should be somewhere a place in the research organization of the nation where really undirected investigation can take place."

And I add, on a large scale.

I would suggest that the creation of several dozen laboratories or institutes devoted to unrestricted basic research (such as I have described to you) throughout the country, located, as they must be, at the universities, would be a partial answer, at least, to our problem of producing the creative thinking in all areas of science that we so sorely need. The funds of the creation of these laboratories may come from a variety of sources. It would be highly desirable if they could come from the foundations and private contributions, but it is difficult to see that amount of money necessary for the creation of several dozen of these laboratories to be forthcoming from such sources.

One might expect that the managers of our industrial complex would appreciate the value of such uncomitted research establishments outside their own research and development laboratories, and help to find ways and means of providing the facilities and continued support that they would need. However, it appears that the only single source capable of achieving the creation of these laboratories, and on the scale we need them, will be the people as a whole, probably through the function of one or more of their Federal agencies, such as the National Academy of Sciences or the National Sciences Foundation.

These institutes must be part and parcel of the fabric and development of our universities. Their staffs could be about one-third academic and permanent supporting personnel, one-third visiting postdoctoral people, and one-third graduate students. The absolute numbers would depend upon the individuals concerned and the areas of their work. One might expect them to vary from less than a dozen to maybe 10 or 20 times that many.

We would thus not only provide the climate for the generation of new ideas but also for the discovery and development of the young men capable of producing them. Without these, we as a nation will not survive in the class in which we now stand. Unless we contribute in this large way to the development of the human race, we will have to sink back into a subsidiary position in the intellectual activities of the world.

Information Division
br