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# PHOTOSYNTHESIS

## (The Path of Carbon in Photosynthesis and the Primary Quantum Conversion Act of Photosynthesis)

Melvin Calvin

November 22, 1952

Berkeley, California

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## ABSTRACT

This constitutes a review of the path of carbon in photosynthesis as it has been elaborated through the summer of 1952, with particular attention focused on those aspects of carbon metabolism and its variation which have led to some direct information regarding the primary quantum conversion act. An introduction to the arguments which have been adduced in support of the idea that chlorophyll is a physical sensitizer handing its excitation on to thioctic acid, a compound containing a strained 1, 2-dithiolcyclopentane ring, is given.

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* Transcription of the Harrison Howe Lecture, delivered at Rochester, New York, November 22, 1952.

** The work described in this paper was sponsored by the U. S. Atomic Energy Commission.

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## (The Path of Carbon in Photosynthesis and the Primary Quantum Conversion Act of Photosynthesis)

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Most of you are undoubtedly familiar with what the term "photosynthesis" means. It is at least used commonly to describe that process by which green plants convert the energy of sunlight (electromagnetic energy) into the potential energy of reduced carbon compounds, simultaneously evolving molecular oxygen. In 1946 the long-lived radioisotope of carbon, carbon-14, became available to us and we set out to pursue the work on the path of carbon itself from carbon dioxide to the reduced carbon compounds, using labeled carbon as the means of tracing that path.

The process of photosynthesis can be divided, both theoretically and actually physically, into two rather distinct parts and Figure 1 shows diagrammatically that sort of division. The three elements that we are primarily concerned with - carbon, hydrogen and oxygen - start out into the plant as $CO_2$ and water, and through the agency of light oxygen is evolved and reduced carbon is generated. The action of the light can be separated fairly distinctly, both physically and theoretically, from the reduction of $CO_2$. The right hand side, in a sense, is the "photo" part of photosynthesis and the left is the "synthetical" part of photosynthesis. Now, with the radioactive carbon we are able to trace the synthetic part from $CO_2$ through a sequence of intermediates into the reduced carbon that the plant generated. The "photo" part, however, does not seem to involve carbon or $CO_2$, or the direct reduction of $CO_2$, and so for a number of years we confirmed the theoretical separation. Toward the end of the last five years we more or less comforted ourselves with the notion that all we had learned from our studies with radiocarbon was something that we should call "phytosynthesis" rather than photosynthesis. (I have

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tried to use this term a number of times without success, the reason being that people take it for a typographical error and always change it; so I don't try any more.) In any case, I will give you briefly the results of these studies on the path of carbon - the synthetical part - and then show you that particular point in which these studies, which for a long time we thought were completely divorced from the "photo" part of the reaction, have lead us to make a suggestion, at least, of what the primary quantum conversion act of photosynthesis is. This is what I am going to try to bring you today.

Let us briefly review the path of carbon in photosynthesis - how we have studied it and what kind of information has arisen from this study. The method of study in such a system as this is pretty straightforward. One simply arranges a green plant of some sort in a steady state of photosynthesis. This might be represented diagrammatically in Figure 2. The "plant" is placed in a situation corresponding to the box and a steady state of photosynthesis is set up; the plant absorbs $CO_2$, light and water, and the $CO_2$ is being converted through a series of intermediates into reduced compounds (carbohydrates) of some sort or other, evolving oxygen in a steady state condition. At a time $t = 0$, we will inject the labeled carbon into the entering stream of $CO_2$ and trace its path along inside the leaf, or the green plant. It is quite clear that if we wait long enough labeled carbon will find its way into carbohydrates and other reduced materials of the plant. The experiment is to shorten the time during which the carbon has been traveling along this road until we find the earliest compounds into which that carbon is incorporated. Thus we are able to trace the path along which it travels and the branches and cycles it might go through.

In order to do this steady state experiment, it is necessary to have more or less reproducible organisms, and Figure 3 shows our algae "farm". We use green algae for this continuous culture system (although blue-green algae and purple bacteria are sometimes used); these are harvested every day and a new culture medium drawn in. Figure 4 will show the exposure apparatus. The algae suspension is placed in this flat circular vessel which we call the "lollipop" (for obvious reasons) and the light source is on either side passing through the infrared filters; the $CO_2$ is entering in continuous flow through a bubbler tube. At a time $t = 0$ we allow radiocarbon to enter with the $CO_2$, and at suitable intervals of time we open the stopcock, draw out the algae suspension into alcohol and thus stop the biological reactions that are going on in as

mild a manner as possible so as not to destroy or convert too badly the compounds into which the radiocarbon has been incorporated. We then proceed to make analyses of the extract from the algae to try and determine which compounds in the extract have radiocarbon in them and determine the order of appearance of radioactivity in those compounds.

Now, the method that was first attempted for this analytical purpose was the usual method of isolation; that is, we wanted to try to extract from the solution the various organic materials that were present and determine which one of them had in it radioactive carbon. This was a very laborious, slow procedure, and after about a year of it we had identified only a single radioactive compound which was very much of the total radioactivity that had been fixed. It was clear that by this method we would not achieve our goal very soon (perhaps not in our lifetime) and some other method of analysis, much more rapid and general, had to be found. This was actually presented to us by the method of paper chromatography, invented by Consden, Martin and Synge in England, which was adapted for our purposes very well.

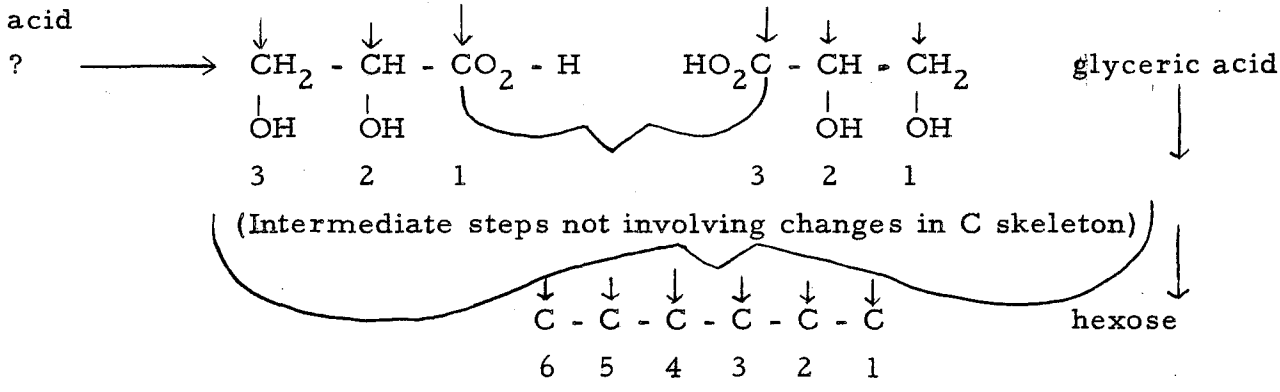
The method of paper chromatography hardly needs any general description - most of you are undoubtedly familiar with it. It depends upon a difference in the relative solubilities of organic substances in two or more solvents. One puts a bit of the extract containing the mixture of compounds on a corner of a piece of filter paper and allows the mixture of solvents to run over that spot. The compounds which are most soluble in the moving solvent run the most rapidly; the compounds which are least soluble move less rapidly, and thus you get the compounds separated along the movement path of the solvent. The compounds which do not separate in that particular set of solvents may be extracted from the paper and run in other solvents, or the paper turned around, and another set of solvents used on it. Actually, we have done most of our work with two-dimensional chromatograms and one can then use the coordinates of the compound with respect to the origin and the series of solvents used as a means of identification of that compound, provided you can determine the coordinates.

In order to determine the coordinates on the paper with respect to the origin, one must be able to find the compound on the paper. The amount of compound we use is so small that even if it were colored you wouldn't be able to see it. The method that was originally developed by Consden, Martin and Synge was for amino acids, for which there was a fairly general spray test

which will develop a color on a paper wherever there is an amino acid. However, we did not know what we were looking for chemically, all we knew was that we were looking for a compound containing radioactive carbon. This general fact provided us with the universal detection method which we required, namely, the radioactivity itself. One could work slowly over the paper, using a Geiger counter, and find out where the radioactivity was. This method has indeed been used and we are using it today - when we have a great deal of time or when we are in a very great hurry. The reason for this is that when we are in a hurry we usually know approximately where to look on the paper and for what we are looking, so we can put the counter down on the spot where we think the compound should be and see if it is there. When you don't know what you are looking for and don't know where it might be located you would have to use a very small Geiger counter and explore the entire paper with the counter. This isn't practical yet, so we had to turn to other methods. That other method was provided for us by the fact that the beta-particles from carbon-14 are relatively soft and do not go very far and do expose photographic film in the way that light does. Thus, we had the ideal method: place the X-ray film in contact with the paper and wherever there is a radioactive spot on the paper, that is, a compound containing radioactive carbon, the X-ray film is exposed. When we develop the X-ray film, a black spot appears, its darkness being relatively proportional to the amount of radioactivity in the compound. We could thus locate radioactive compounds readily and easily and Figure 5 shows such a chromatogram made from ten minute photosynthesis in $C^{14}O_2$ by Scenedesmus. This is a photograph of the X-ray film after it has been developed. However, we don't get the names developed on the X-ray film; they must be placed by hand and the proper placing of those names constituted the major activity of the laboratory for about five years. However, you will notice that there are still spots which do not have any names. We hope that we will be able to give names to those, one of these days. You can see that ten minutes was too long for determining the early compounds of photosynthesis. Sucrose, as well as a variety of other compounds, we know to be late in the photosynthetic scheme. So, we shortened the time to one minute (Figure 6). You will see that there are still far too many compounds labeled to allow us to draw any conclusions as to what is first, but at least we have eliminated a large number and are practically limited to the phosphates as early products of one minute photosynthesis. Sucrose, alanine, serine, etc. which were in the ten minute picture have not yet appeared. Practically everything that shows any intensity

is a phosphate of one sort or another. However, one minute was still too long, and as is shown in Figure 7 we have gone down to fractions of a minute and find practically nothing but phosphoglyceric acid. When you go to still shorter times there is still less of the other compounds and one could quantitate the whole thing by simply making a series of such papers of different times (one second, two, four, eight, etc.), determining the amount of radioactivity in each one of these spots and making graphs such as those shown in Figure 8. I might say that this figure should not be used for true steady state conditions beyond three minutes.

It is quite clear that the very first thing to show was phosphoglyceric acid. This was the first important definitely confirmed observation and the next problem that arises is the one in which we must determine not only which compound contains the radioactivity, but since these compounds contain more than one carbon atom, to determine which carbon atom became labeled first. This involved the problem of degradation which is the next thing we developed. The phosphoglyceric acid was degraded and the sugar that appeared on the film from the same experiment was purified and also degraded and we found the distribution of radiocarbon in the phosphoglyceric acid and hexose to be that shown in Figure 9. This happens to be 15 second photosynthesis by barley, and the distribution is 50 percent in the carboxyl-carbon with the remainder split equally between the alpha- and beta-carbons of the phosphoglyceric acid; 50 percent in carbons 3 and 4 of the hexose with carbon atoms 2 and 5 and 1 and 6 of the hexose dividing the remainder in about the same way as the alpha- and beta-carbon atoms of phosphoglyceric acid. I might say that we made a whole series of degradations of this sort, using shorter and shorter times. The first carbon atom, then, to be labeled is the carboxyl-group of the phosphoglyceric acid




The length of the arrows above each C atom is proportional to the amount of label in that atom.

so the $CO_2$ presumably enters that position first. The other two come in about equally, the hexose being labeled equally in the center two carbon atoms

(3 and 4) and then in the 1, 2 and 5, 6 corresponding to this.

Immediately the suggestion arose that the hexose was made probably by a reversal of the well-known glycolytic sequence; that is, the phosphoglyceric acid is reduced by some agent, probably from the light, to phosphoglyceraldehyde; and some of the phosphoglyceraldehyde isomerized to give dihydroxyacetone, and then the $C_1$ carbon atoms of each of these compounds combining in what looks like an aldol condensation giving fructose diphosphate. This will give us hexose labeled in the two center carbon atoms. The label coming into α and β of glyceric acid would produce label in 1, 2 and 5, 6 of the hexose. This sort of thing was borne out by all our further tests - there is a variety of other tests which can be and have been made - and it seems clear now that the hexose (sucrose) is made by a combination of two triose molecules to give centrally labeled hexose and then peripherally labeled hexose. The hexose, in turn, goes, through a suitable coenzyme (uridine diphosphoglucose), on to fructose 1-phosphate to give sucrose phosphate with the phosphorus probably on the No. 1 carbon atom of the fructose moiety. But this is really part of the intermediary (carbohydrate) metabolism of plants and is not very close to photosynthesis itself, so I am not going to spend any more time on that aspect of it.

The next problem, now that we have made the hexose and know approximately how it is put together, is to determine what the $CO_2$ acceptor is and where it comes from. Apparently we add $CO_2$ to something and that something is either a two-carbon fragment itself or very quickly splits off its equivalent after carboxylation. This carboxylation giving phosphoglyceric acid might not actually be on a two-carbon piece but on something which simultaneously with the carboxylation splits to give the three-carbon fragment. In any case, we have to generate the equivalent to this two-carbon piece in such a way that it comes out equally labeled in the two-carbon atom.

The question remains - where and how that two-carbon piece arise? In the search for other materials that might be a source for it, we have found besides the hexose (exclusive of malic acid - there is evidence that malic acid is not on the direct line) the five-carbon keto sugar, ribulose, and the seven-carbon keto sugar, sedoheptulose. Kinetic studies indicate that the heptose and fructose seem to be acquiring label at the same rate at least initially, i. e., they seem to be formed in parallel reactions from the same precursor. Furthermore the label shows up in the center carbon atoms of the seven-carbon chair earlier than it does near the ends. Both ribulose and

sedoheptulose seem to be universally present in all plants and their active pools are generally quite small and very rapidly saturated with labeled carbon from carbon dioxide. These and other such data have led us to propose tentatively a cycle for the regeneration of the two-carbon precursor, involving both the seven- and five-carbon sugars, and which is shown schematically in Figure 10. It resembles very much the tricarboxylic acid cycle in that we have a three-carbon compound circulating continuously in the inner ring and upon it the four-carbon compound is split into two. Actually, the details of this cycle as well as corrections and changes will be forthcoming as the years go by with the accumulation of more and better data of the foregoing type together with studies of more or less isolated enzyme systems which might be involved. I would like to turn now to a discussion of the "photo" part of photosynthesis, describing how we were led by these isotope studies to the possibility of making a suggestion as to the nature of the primary quantum conversion act. By the primary quantum conversion act, we mean that particular set of motions of nuclei and electrons which results in the conversion of electromagnetic excitation in chlorophyll into some form of chemical bond energy. Unfortunately, what I have to say is not the sort of thing in which I can point to a certain set of observations and then deduce unequivocally a result from those observations - a rather simple set of observations. All I am able to do tonight is try to indicate the direction in which all the observations point and see what, if anything, lies along that road. Whether what we see actually is the primary quantum conversion act or not, I am afraid, remains yet to be proven. However, there are, as you will see, strong indications of a single act which might be involved.

During the course of the study of the path of carbon, one of the observations that was made very early in the work, some four or five years ago, was that while the light is on, carbon which is freshly incorporated from carbon dioxide into the plant is prevented from entering into the well-known tricarboxylic acid cycle; and that immediately the light is turned off, such carbon can find its way directly into the tricarboxylic acid cycle. It is worth while to recall here what is meant by the TCA (Krebs) cycle and to indicate the reaction by which carbon atoms are introduced into it. The reaction of oxalacetic acid with a two-carbon piece which we now know to be the thiol ester of acetate on Coenzyme A may be taken as the point of entry (Figure 11). Such a condensation leads to citric acid, which then goes through a dehydration, a rehydration, and oxidation, going to oxalsuccinic acid. The oxalsuccinic acid can lose first of all a β-carboxyl and then be oxidatively

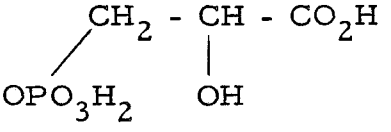
α-decarboxylated giving succinic acid. The succinic acid is dehydrogenated to fumaric acid; the fumaric acid is hydrated to malic acid, and the malic acid finally oxidized to oxalacetic, thus completing the cycle. In effect, in passing through this tricarboxylic acid cycle, we have combusted the two carbon atoms to carbon dioxide, not necessarily the two entering originally, but two carbon atoms have eventually emerged as carbon dioxide from the cycle, having gone in as an acetyl group. This reaction, the condensation of acetyl-CoA with oxalacetic acid, is the only one known at present to feed carbon into the tricarboxylic acid cycle.

Now, the experimental observation which I mentioned a while back is that while the light is on freshly assimilated carbon dioxide finds its way into the compounds of this cycle extremely slowly. The moment the light is turned off, freshly assimilated carbon finds its way directly into the compounds of this cycle. It seems worth while to show you just what kind of experimental data that is, and Figure 12 shows some of it. You will note here that while the light is on, with the plants getting labeled carbon dioxide, the sucrose is increasing very rapidly, the malic acid increases rapidly and saturates. On the other hand, the glutamic acid and the citric acid, which are taken as representative compounds of the tricarboxylic acid cycle, are increasing very slowly while the light is on. Notice what happens immediately the light is turned off. The citric acid immediately starts to rise, and the glutamic acid rises very rapidly. When the light is turned on, it stops rising. The next figure (Figure 13) shows an even more spectacular representation of the same result in an independent experiment. This shows the absolute amount (the numbers are proportional to the absolute amount) of radioactivity to be found in each of the compounds, glutamic acid, citric acid, alanine and sucrose, under the specific sets of conditions shown alongside the bar - 60 seconds light, 60 second light-60 seconds dark, and 120 seconds light - which are the same for each of the four compounds. You will notice that for the compounds belonging to the tricarboxylic acid cycle, namely, the citric acid and glutamic acid, it is during the dark period that the rate of incorporation of newly assimilated carbon - this is the carbon that was assimilated in the first 60 seconds - is enormously greater than it is in the corresponding light periods; whereas in compounds which are not directly associated and dependent upon the operation of the tricarboxylic acid cycle, the rate of incorporation is pretty much dependent upon the light and is not inhibited by the light. This phenomenon is the basic on which I want to expand upon.

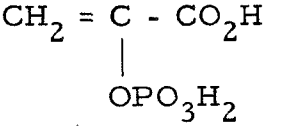
The interpretation can be made of this phenomenon in terms described by Figure 14, in which we write a photosynthetic cycle, driven by the light, with water and carbon dioxide entering, oxygen removed, and the carbon compounds running around in it in a suitable fashion. This photo-synthetic cycle, which has been discussed earlier and which contains such compounds as phosphoglyceric acid, triose phosphates, hexose, heptose and pentose phosphates, is the source of various precursors to the storage and structural products, carohydrates, fats and proteins. While the light is on, that is what is going on. But immediately we turn the light off, there is some direct connection between the compounds in the photosynthetic cycle and the tricarboxylic acid cycle, which was discussed in detail earlier. In the dark a direct connection is established, as well as the one through the normal storage products in the plant.

Furthermore, we know something about the nature of that direct connection. First of all, it is inhibited by the light, and secondly, the only way in which we know that carbon can be fed into that tricarboxylic acid cycle is through the condensation reaction of oxalacetic acid with acetyl Coenzyme A. Now, some years ago when we first encountered this phenomenon, we suggested that the way in which the light prevented the new carbon from getting into the tricarboxylic acid cycle was that in some way light reduced the concentration of some pool leading to it. At that time, we didn't know about acetyl-CoA; we simply wrote it as acetate - as the two-carbon carbon dioxide acceptor, and we suggested that the light kept it in a reduced form, more reduced than acetate, so that it could not participate in the tricarboxylic acid cycle. What we would like to do now, as a result of further information, is to suggest precisely how this condensation is prevented in the light.

When you look at the compounds which we now believe participate in the photosynthetic cycle, you will note that the first one to be formed, or at least the first one that we can isolate, is a three-carbon compound, phosphoglyceric acid, and a close relative of it, phosphoenolpyruvic acid.

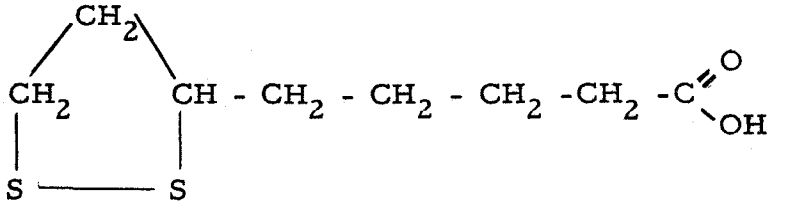


phosphoglyceric acid



phosphopyruvic acid

Two-carbon compounds do not appear very early. Furthermore, the mechanism by which we can get from these three-carbon compounds to acetyl-CoA, which is what we need to have entry into the tricarboxylic acid cycle, is now beginning to be a little clearer, as a result of the work of a group at Illinois (Gunsalus) and Texas (Reed), on what is called the pyruvic acid oxidase factor, and as a result of the work also of a group at Lederle Laboratories (Jukes, Stokstad, Brockman) on the chemistry of a similar or identical substance recognized as a growth factor for certain protozoa by Kidder (Amherst). The reaction seems now fairly clearly defined. Pyruvic acid interacts with the pyruvic acid oxidase factor to give "active acetyl" plus carbon dioxide. And this "active acetyl" may be degraded to acetic acid by hydrolysis or it may be transferred to the -SH group of Coenzyme A to make acetyl-CoA. Thus a way is provided by which the freshly incorporated carbon of the photosynthetic cycle gets into the tricarboxylic acid cycle. Now the question which must be answered in detail is how is it that the light prevents this from happening? In order to do that, we have to have a closer look at what that pyruvic acid oxidase factor is. It has been described in the past few years, having gone under several different names. The Lederle group has called it protogen, following Kidder, because it was a growth factor for protozoa; the group at Illinois (Gunsalus and his associates) have called it pyruvic acid oxidase factor, because they found it to catalyze this particular reaction, and the identity of the two factors was recognized several years ago. The actual chemistry of it has only appeared in the last few months, and it now seems quite certain that the central prosthetic group, or co-factor, if you like, is a molecule having the following structure:



(Synthetic) 6,8-thioctic acid

It is an octanoic acid. The Lederle group has synthesized a number of them having different size rings and it seems quite clear that this is the one which is the most active one.

Now how does this actually function in oxidizing pyruvic acid and forming the "active acetyl". It seems that the most likely form of function is as follows: (Figure 15). The acetyl thiol ester first formed exchanges with the sulfhydryl of Coenzyme A, giving the acetyl-CoA and the reduced sulfur compound. This reduced sulfur compound then is reoxidized by suitable oxidizing agents in the cell, ultimately leading to molecular oxygen, and can then repeat its oxidative decarboxylation of pyruvic acid. Now note what has happened here. The oxidized, disulfied form of this thioctic acid is required to carry out this oxidative decarboxylation of pyruvic acid to produce acetyl-CoA, which we need to run the tricarboxylic acid cycle. We suggested some months ago that the light in some way kept more of the thioctic acid in the reduced, dithiol form, while in the dark the steady-state ratio was shifted toward the disulfide form. In this dithiol form it, of course, would not be capable of carrying out an oxidative decarboxylation of pyruvic acid to produce acetyl-CoA. At that time, we did not suggest how the light produced this reduced thioctic acid. It just seemed that this explanation fit all the observations so well that it was a reasonable one. Somehow the light produced a reductant which reduced the disulfide to the disulfhydryl. What I would like to do tonight is go one step further and suggest that it is the direct transfer of the electronic excitation energy from chlorophyll to the disulfide which results in its reduction. It might be of interest to describe for you the chain of events and observations leading to this notion.

Through the kindness of Dr. Jukes of Lederle Laboratories, we were able to obtain a few milligrams of two of the synthetic thioctic acids which they had prepared, namely, the 5, 8-thioctic acid and 6, 8-thioctic acid. Upon receiving them, we noted that the 5, 8-acid was colorless while the 6, 8-acid was yellow. Looking at the structures of these compounds, it was a little surprising to me to believe that so much color difference could be due simply to the change from a six- to a five-membered ring. Furthmore, no known saturated disulfides showed light absorption extending into the visible range. We measured the absorption spectrum of the two acids in alcoholic solution and found the maximum for the 6, 8-acid at 3320 Å and that of the 5, 8-acid at 2860 Å. Clearly either the 6, 8-acid was not of that structure or else the structure itself had peculiar properties. The possibility also existed that some impurity was responsible for the yellow color. After some discussion, we decided to try and make a compound which could have relatively little ambiguity about its structure; i.e., one with only three carbon

atoms in it besides the disulfide link. This was done by allowing $Na_2S_2$ to react with trimethylene dibromide and extracting a bright yellow product from the reaction mixture with petroleum ether. We took the absorption spectrum of that product and Figure 16 shows that absorption spectrum and that of the two thioctic acids which we had, as well as of an ordinary open chain disulfide, N-propyl disulfide. You will note that as you increase the strain in the disulfide bond, from an open-chain to a six- and a five-membered ring, you do indeed shift this absorption spectrum toward the visible, and the correspondence between this spectrum of the trimethylene disulfide and that of the substance in the bottle that was labeled 6, 8-thioctic acid is so good that there seems to be hardly any question but what it is indeed 6, 8-thioctic acid. This immediately led to the next notion that this ring strain was a crucial matter. Perhaps it was possible that the light was directly producing this reduced thiotic acid, or some conjugate of it. How could this come about?

An examination of the kinetic facts of photosynthesis, together with the structural consideration of the plant itself, leads to a relatively simple conception of this. First of all, let me say a word or two about the structure of the chloroplast or its smaller organized constituent, the granum. There appears to be a relatively highly concentrated package of chlorophyll, together with a number of other things, particularly carotenoids. One could visualize its structure diagrammatically as determined principally by the flat prophyrin ring, arranged in an ordered array of some sort, in these single packages. For approximately each thousand chlorophyll molecules contained in the granum, there is one of these disulfides in some conjugate coenzyme form situated around on its surface. The light may be absorbed anywhere in this package. There are many experimental models now for energy transfer within this sort of array. In effect, a quantum absorbed by any one of these molecules is absorbed by them all, or it is available to them all. Because of the intrinsically stable nature of this electronic excitation (lifetime probably longer than one hundred-thousandth of a second) it has time to wander around in this condensed phase until it finds itself adjacent to an absorbed disulfide. There is certainly a chemical interaction here which immediately leads to the dropping of that quantum into the energy of two sulfur free radicals. This is presumed possible primarily because of the strain in the ring. Now, we require some knowledge of the energy needed to split a disulfide link. We have available only somewhere around between 30-40 kcal. This roughly only - the excited state of chlorophyll in the condensed phase is in this range. I won't try to discuss the evidence for the

interaction between chlorophyll molecules - there is such interaction - the absorption spectrum of chlorophyll in the living plant corresponds approximately to that of a close-packed array of porphyrin molecules, and such things have actually been reproduced by Holt and Jacobs at Illinois showing the interaction between molecules in synthetically constructed micelles or microcrystals. We have roughly somewhere between 30 and, at the maximum, 40 kcal. to dissociate this disulfide link. Now what evidence I could find about the disulfide link all gave values higher than this, but not much higher. They gave values ranging from 55-70 kcal. depending upon where you looked. These were all open-chain disulfides - things like $H_2S_2$ and $R_2S_2$. I might say that for $H_2S_2$ a value of between 60 and 70 was usual, and for the $R_2S_2$ it was approximately 10 kcals. lower, between 50 and 60, depending on the size of the R group; but in all cases, they are open disulfide, no closed rings.

In view of the spectral relationships which you saw a moment ago, it seems certain that the energy required to open the disulfide in this strained five-membered ring is less than that required to open the disulfide in an open chain compound. If one assumes that to a first approximation the spectral differences shown in Figure 16 are due primarily to differences in the energy of the ground state, with the excited state in each case corresponding to a separation of the two sulfur atoms, one arrives at a decrease in the dissociation energy in passing from the open chain (no strain) to the five-membered ring of approximately 25-30 kcals. This would bring the dissociation energy down to around 30 kcals., which is in the right range. This, as you can well imagine, is a relatively critical quantity for this whole interpretation and must be accurately known.

After the initial conversion of the quantum into the potential energy of the dithiyl radical, this free radical can pick hydrogen off suitable hydrogen donors - and I cannot at this moment specify what those hydrogen donors are, except to say that it is not likely that the hydrogen donor is water itself because the energy available seems hardly enough to do it. In any case, it does pick up hydrogen directly from some hydrogen donor, forming the disulfhydryl compound. This disulfhydryl compound can go on and reduce other secondary reducing agents in the cell, and thus lead to the reduction of carbon dioxide. The compound which donated these hydrogens will lead ultimately to molecular oxygen.

While a survey of the distribution of protogen using bioassay methods has revealed its presence in a wide variety of biological materials, it seemed to indicate the highest concentrations in green plant tissues such as dried alfalfa

and algae. However, it remains for us to demonstrate that one can isolate these cyclic disulfides from the plant and that the light action is very closely associated - very directly concerned - with the opening of the disulfide ring. We have done a great number of model experiments in the laboratory to demonstrate that photochemically we could open that disulfide ring in model systems, and I shall briefly recite them for you. For example, by putting a little zinc porphyrin in with the disulfide in a non-aqueous medium (anaerobically), the porphyrin absorbing in the visible range, one could show the photochemically induced disappearance of the disulfide. The photochemical (as well as thermal) fading of the picrahydrazyl, induced by the disulfide, has been demonstrated. The photochemically induced dehydrogenation of tetralin, induced via the disulfide, was also demonstrated. So that there is a wide variety of model experiments, all demonstrating the possibility of this disulfide fission, both directly, and indirectly, by electromagnetic energy absorbed by some other molecule. I am sure that you will agree when we say that it is not sufficient to demonstrate this thing in a model. There is already some more direct evidence. We have been able to demonstrate an acceleration of the rate of oxygen evolution by illuminated Scenedesmus using quinone as the oxidizing agent (Hill reaction) by treating the algae with 6,8-thioctic acid. The 5,8-isomer is ineffective. However, I hasten to add that we are now suggesting not merely some possible mode of action. I think we are going one step further and suggesting precisely that act in which the quantum is converted from electromagnetic into chemical energy.

So, our next problem, assuming now that the primary recipient of the quantum is a disulfide and that in receiving that quantum it is opened into a dithiyl radical, is to try to specify the precise species from which these two sulfur free radicals snatch the hydrogen and how the residual fragment goes on to molecular oxygen.

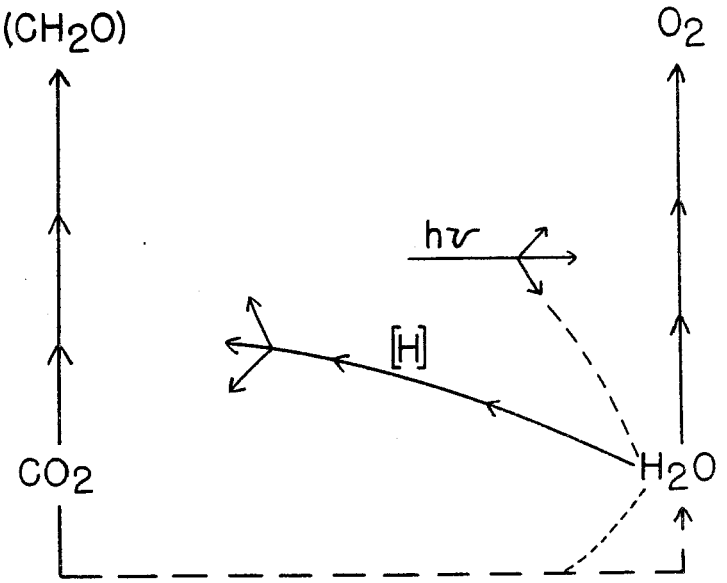



Fig. 1

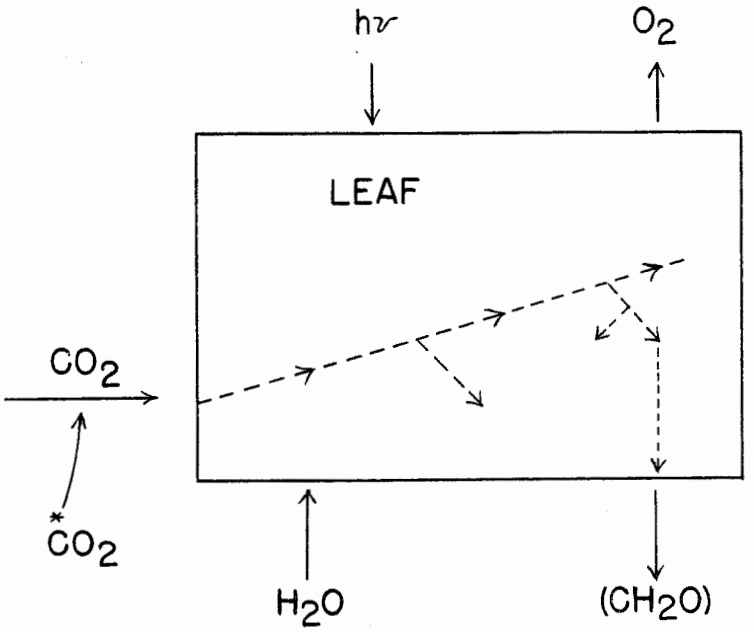



Fig. 2

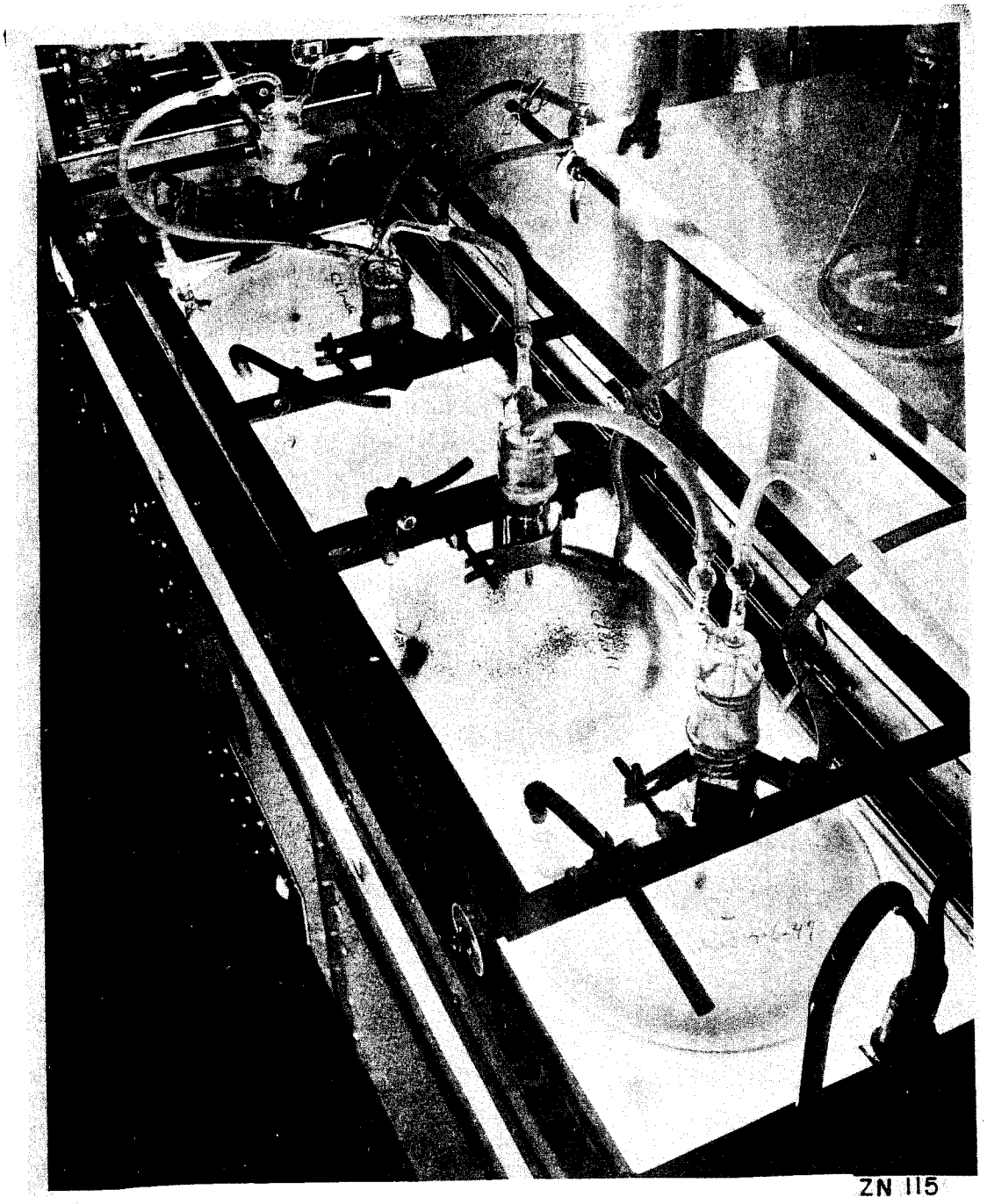
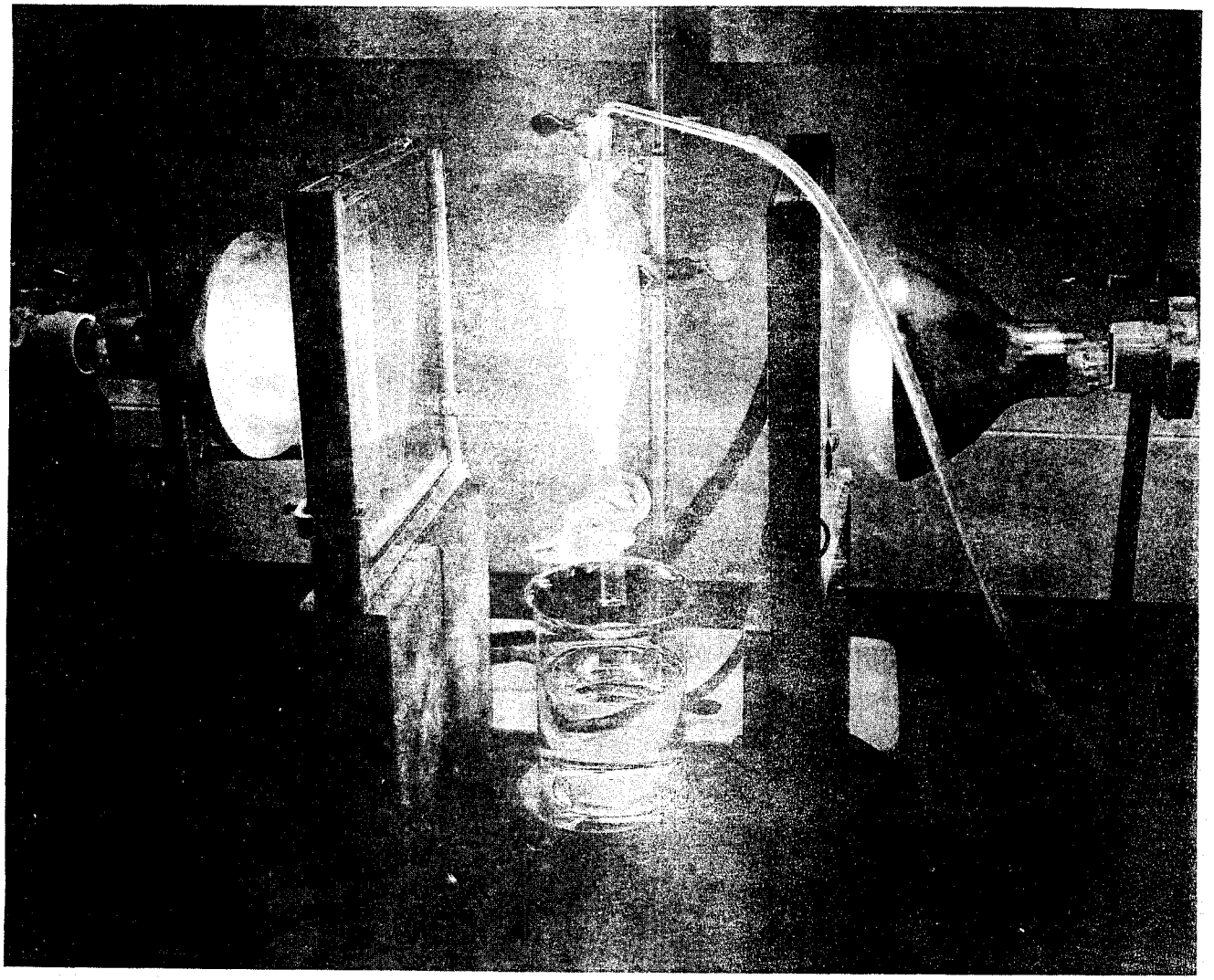



Fig. 3



ZN-481

Fig. 4

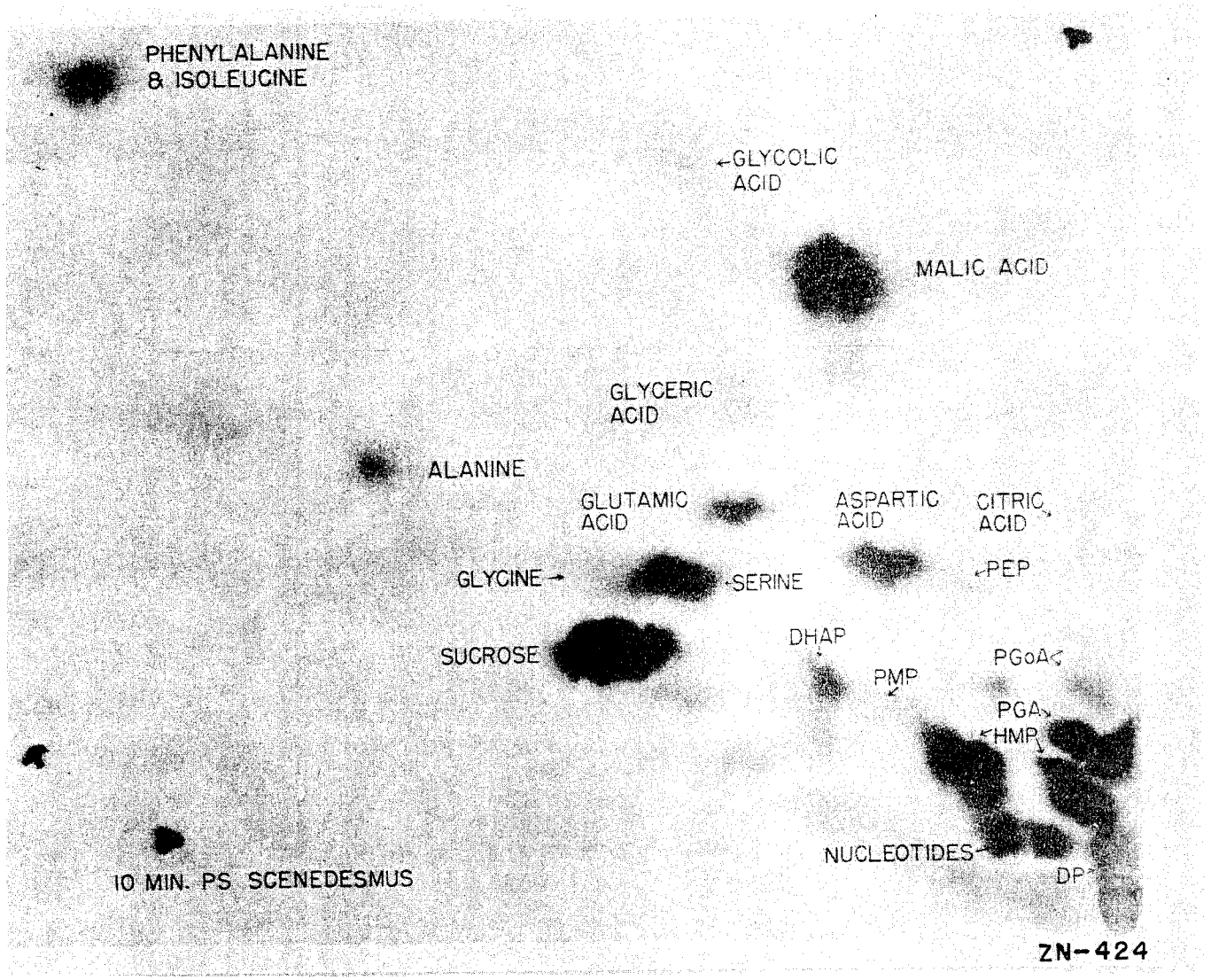

PHENYLALANINE
& ISOLEUCINE
GLYCOLIC
ACID
MALIC ACID
GLYCERIC
ACID
ALANINE
GLUTAMIC
ACID
ASPARTIC
ACID
CITRIC
ACID
GLYCINE
SERINE
PEP
SUCROSE
DHAP
PMP
PGoA
PGA
HMP
NUCLEOTIDES
DP
10 MIN. PS SCENEDESMUS
ZN-424

Fig. 5

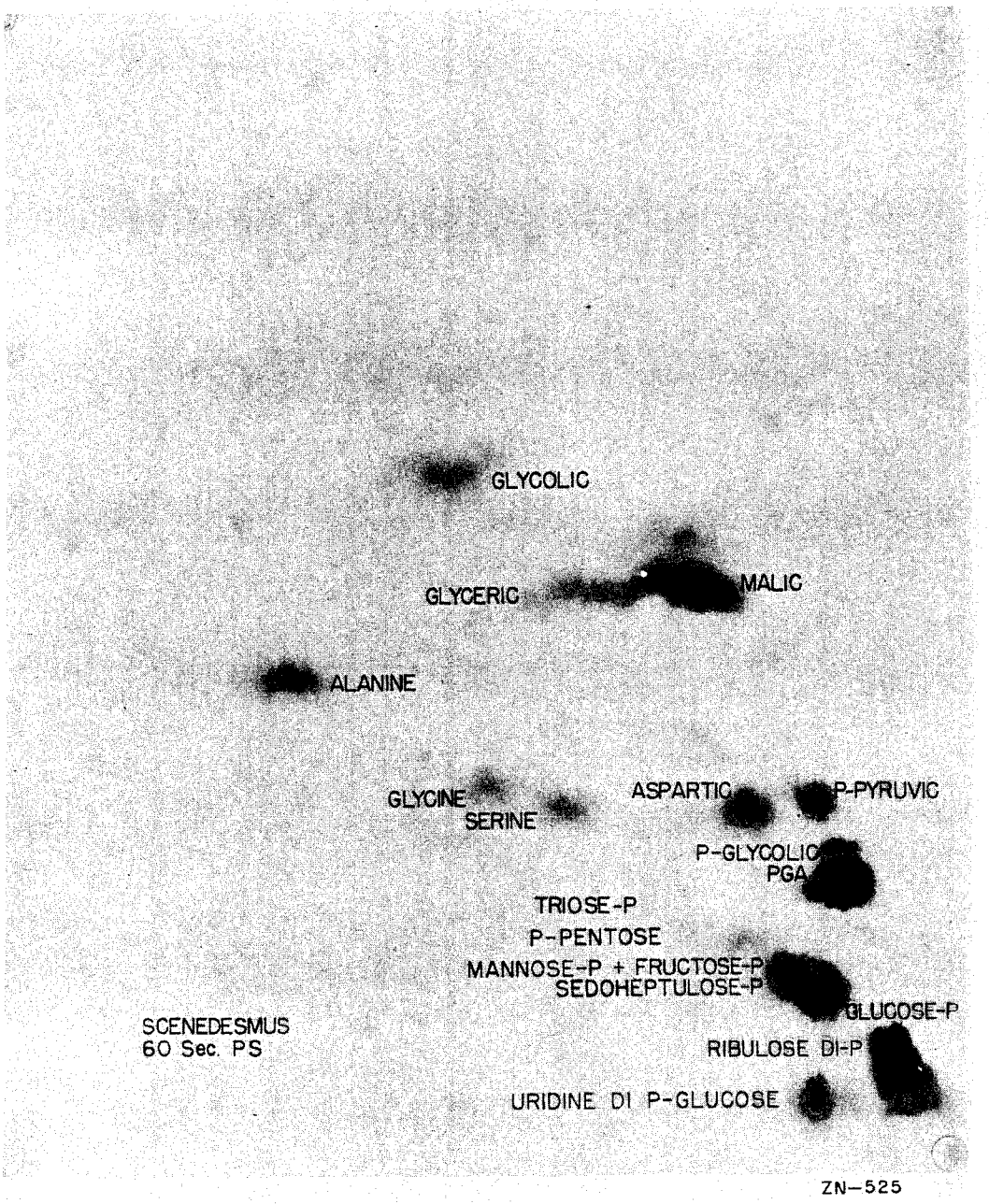

GLYCOLIC
GLYCERIC
MALIC
ALANINE
GLYCINE
SERINE
ASPARTIC
P-PYRUVIC
P-GLYCOLIC
PGA
TRIOSE-P
P-PENTOSE
MANNOSE-P + FRUCTOSE-P
SEDOHEPTULOSE-P
GLUCOSE-P
SCENEDESMUS
60 Sec. PS
RIBULOSE DI-P
URIDINE DI P-GLUCOSE
ZN-525

Fig. 6

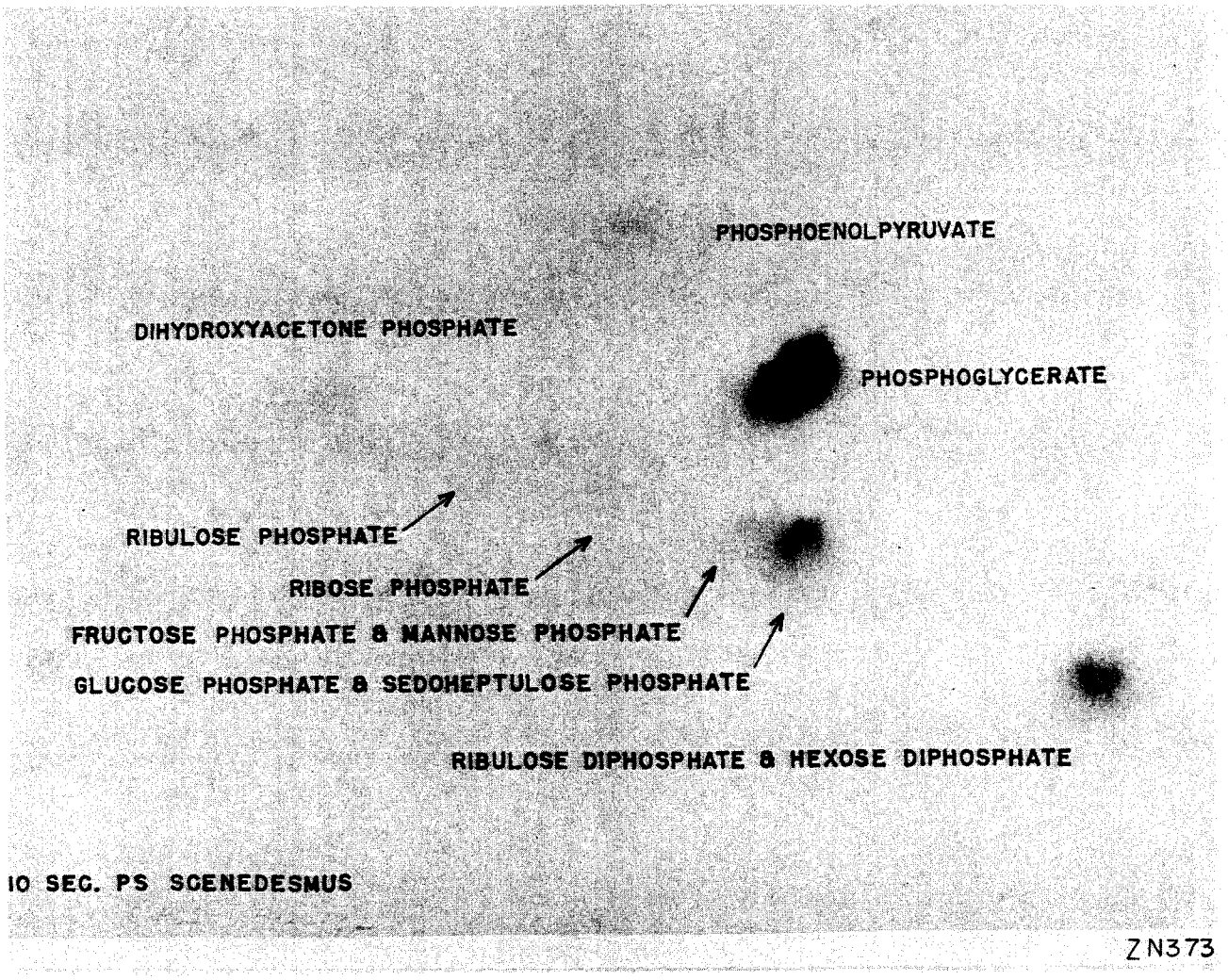

PHOSPHOENOLPYRUVATE
DIHYDROXYACETONE PHOSPHATE
PHOSPHOGLYCERATE
RIBULOSE PHOSPHATE
RIBOSE PHOSPHATE
FRUCTOSE PHOSPHATE & MANNOSE PHOSPHATE
GLUCOSE PHOSPHATE & SEDOHEPTULOSE PHOSPHATE
RIBULOSE DIPHOSPHATE & HEXOSE DIPHOSPHATE
10 SEC. PS SCENEDESMUS
ZN373

Fig. 7

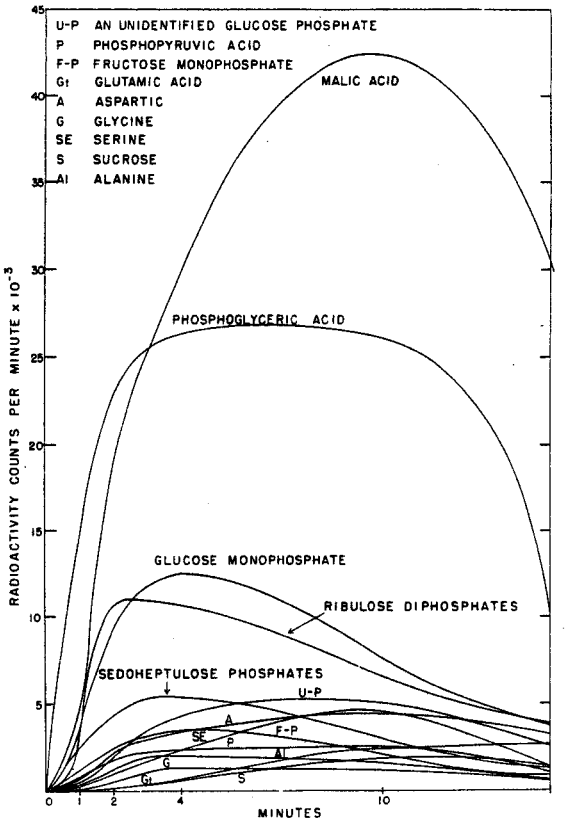
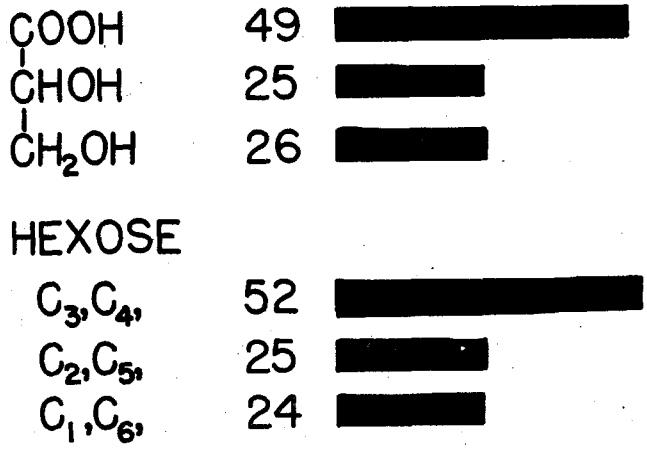
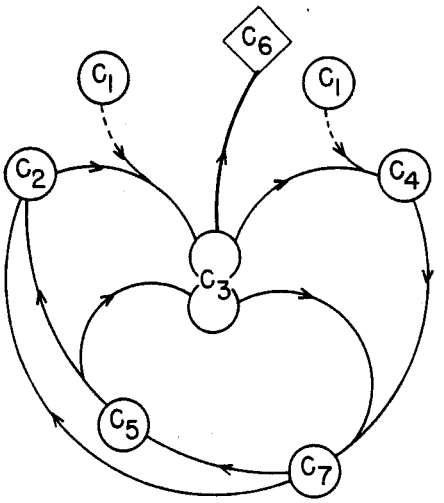

U-P AN UNIDENTIFIED GLUCOSE PHOSPHATE
P PHOSPHOPYRUVIC ACID
F-P FRUCTOSE MONOPHOSPHATE
Gt GLUTAMIC ACID
A ASPARTIC
G GLYCINE
SE SERINE
S SUCROSE
Al ALANINE
MALIC ACID
PHOSPHOGLYCERIC ACID
GLUCOSE MONOPHOSPHATE
RIBULOSE DIPHOSPHATES
SEDOHEPTULOSE PHOSPHATES
U-P
A
SE
P
F-P
Al
G
Gt
S
RADIOACTIVITY COUNTS PER MINUTE x 10⁻³
MINUTES
0
1
2
4
10
5
15
20
25
30
35
40
45

**Fig. 8**



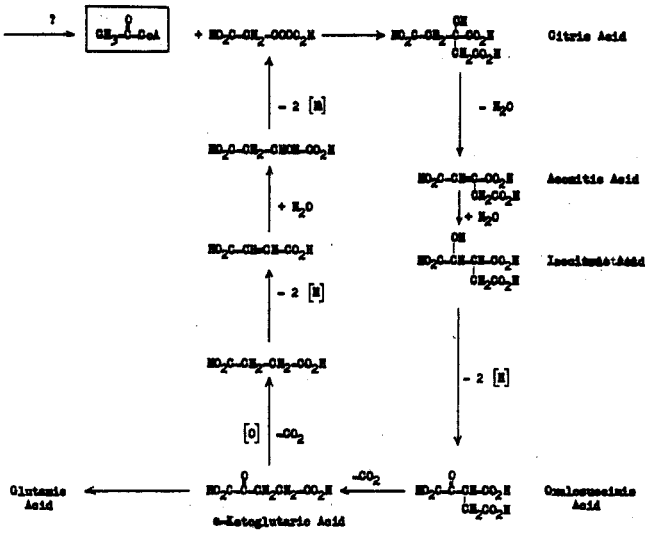

15 SEC. P.S. BARLEY

Fig. 9




PROPOSED CARBON CYCLE FOR REGENERATION OF TWO-CARBON $CO_2$ ACCEPTOR

Fig. 10




TRICARBOXYLIC ACID CYCLE

Fig. 11

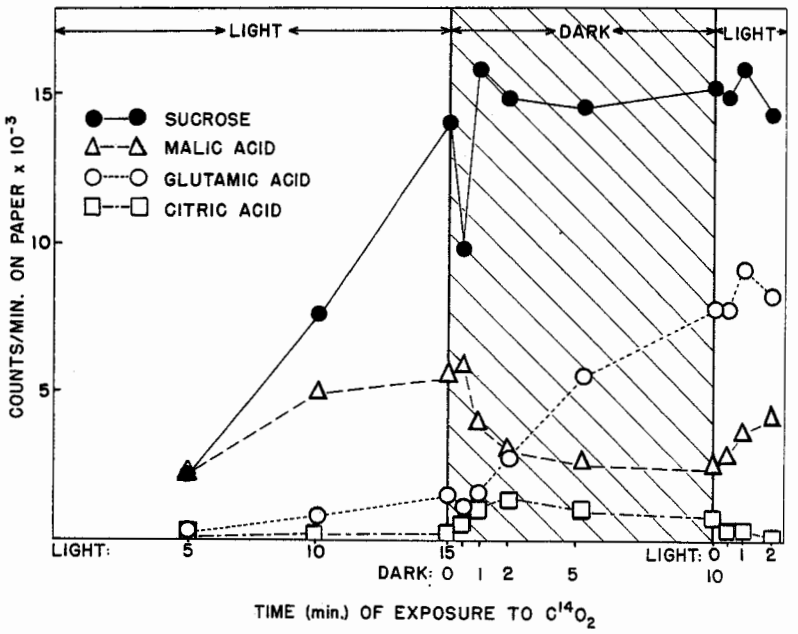

LIGHT
DARK
LIGHT
SUCROSE
MALIC ACID
GLUTAMIC ACID
CITRIC ACID
COUNTS/MIN. ON PAPER x 10⁻³
15
10
5
LIGHT: 5 10 15
DARK: 0 1 2 5
LIGHT: 0 1 2 10
TIME (min.) OF EXPOSURE TO C¹⁴O₂

Fig. 12

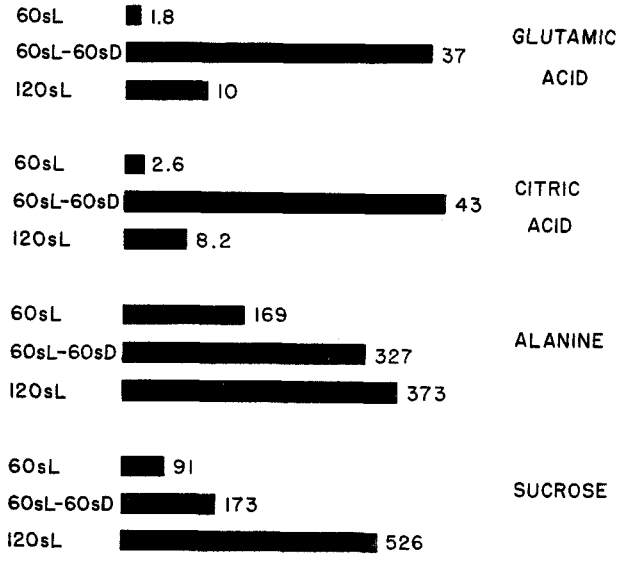

60sL
1.8
60sL-60sD
37
120sL
10
GLUTAMIC ACID
60sL
2.6
60sL-60sD
43
120sL
8.2
CITRIC ACID
60sL
169
60sL-60sD
327
120sL
373
ALANINE
60sL
91
60sL-60sD
173
120sL
526
SUCROSE

Fig. 13

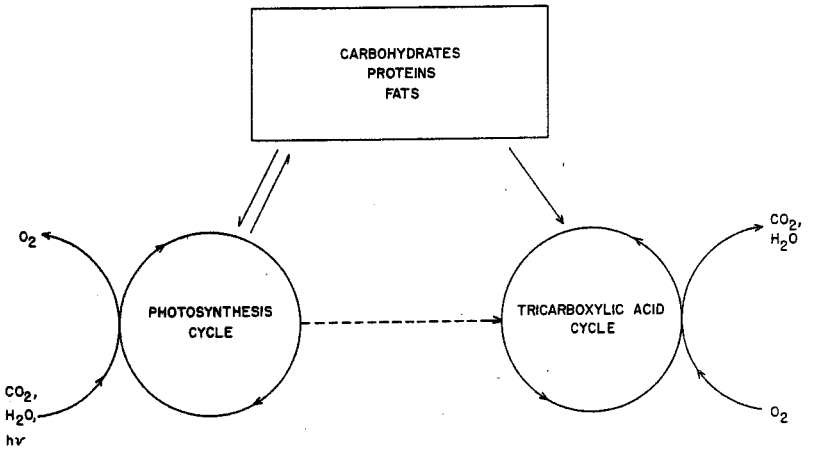

CARBOHYDRATES
PROTEINS
FATS
$O_2$
PHOTOSYNTHESIS CYCLE
$CO_2$, $H_2O$, $h\nu$
TRICARBOXYLIC ACID CYCLE
$CO_2$, $H_2O$
$O_2$

Fig. 14

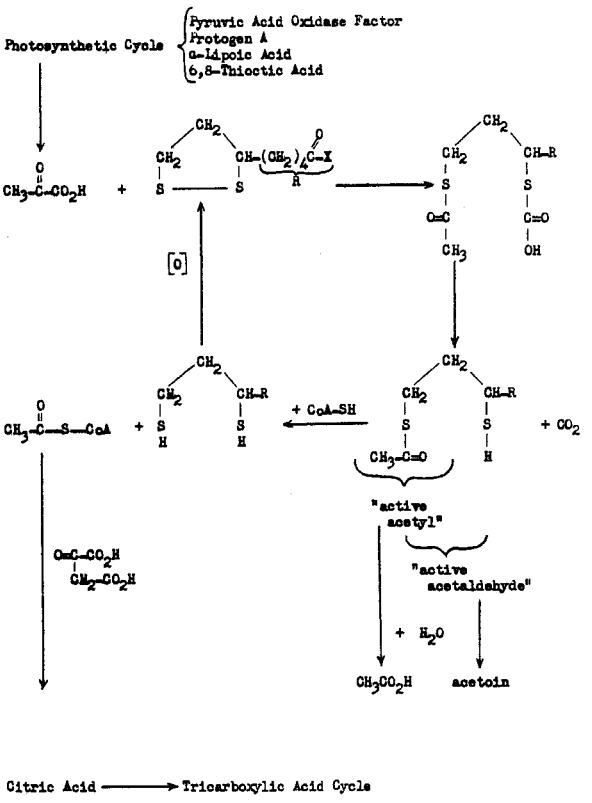

Photosynthetic Cycle
Pyruvic Acid Oxidase Factor
Protogen A
α-Lipoic Acid
6,8-Thioctic Acid
[O]
+ CoA-SH
+ CO2
"active acetyl"
"active acetaldehyde"
+ H2O
CH3CO2H
acetoin
Citric Acid
Tricarboxylic Acid Cycle

Fig. 15

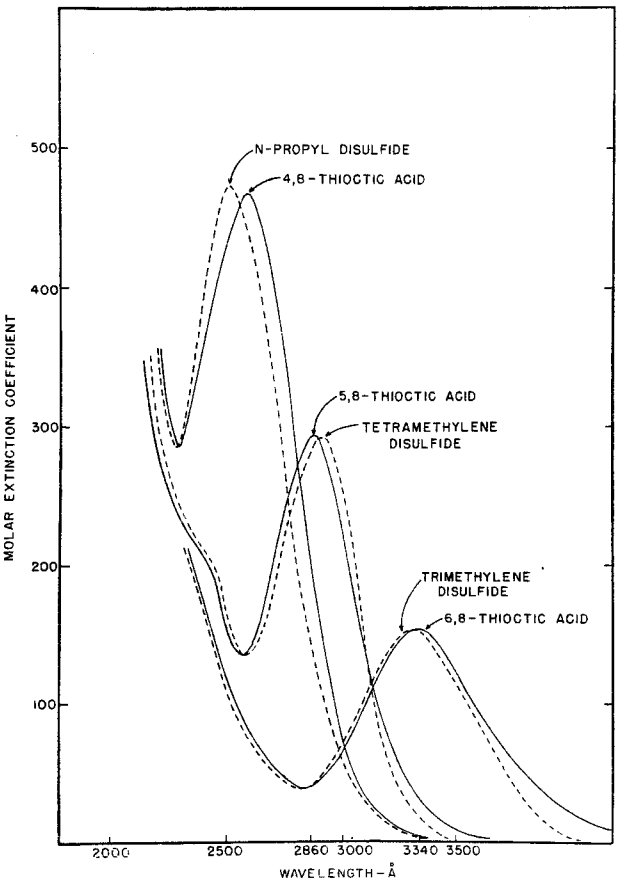

N-PROPYL DISULFIDE
4,8-THIOCTIC ACID
5,8-THIOCTIC ACID
TETRAMETHYLENE DISULFIDE
TRIMETHYLENE DISULFIDE
6,8-THIOCTIC ACID
MOLAR EXTINCTION COEFFICIENT
500
400
300
200
100
2000
2500
2860
3000
3340
3500
WAVELENGTH-Å

Fig. 16