*The Photosynthetic Carbon Cycle.**

THE CENTENARY LECTURE, DELIVERED BEFORE THE CHEMICAL SOCIETY, IN THE ROOMS OF THE INSTITUTION OF CIVIL ENGINEERS, LONDON, ON OCTOBER 20TH, 1955, AND AT THE UNIVERSITY, GLASGOW, ON OCTOBER 28TH, 1955.

By MELVIN CALVIN.

SOME of you may have heard parts of this Lecture three or four years ago but it has materially changed since then in two ways. One is that we now know what the photosynthetic carbon cycle is, and it has been carried out, I can tell you now, in its entirety in the absence of living materials or formed parts of living cells. It has been carried out by a separate collection of soluble chemicals so that we are quite sure what it is, and now we can talk about it with a high degree of confidence.

When the work first began, we called it " the path of carbon in photosynthesis," which is a more or less general description of how carbon passes through the plant from the condition of carbon dioxide to the condition of carbohydrate. At the time that the title was invented we really did not know what kind of sequence of chemical transformations would be involved. One of the reasons that the title has changed from " the path of carbon " to " the photosynthetic cycle " is that now we know that it is a cyclic operation and that we can define exactly the compounds involved.

The discussion will be roughly divided into two fairly distinct parts : the first part involves the description of the set of chemical transformations which the carbon atom of carbon dioxide undergoes on its way from carbon dioxide to carbohydrate; and the second part describes the state of our knowledge of how the electromagnetic energy which is captured by the chlorophyll in the green plant is used to accomplish that series of transformations, since the series of transformations—carbon dioxide plus water to give carbohydrate and oxygen—is an upgrade one, that is, one in which energy is stored. That energy, ultimately, as we know, must come from the electromagnetic radiation absorbed by the chlorophyll.

Now, actually, the latter part of the discussion is on a different plane with respect to the degree of certainty than is the way in which the energy is used in transforming the carbon skeleton. I appraise you of this fact at the beginning so that you may be conscious of the transition some time toward the middle of this discussion.

This question of the biosynthesis of carbohydrate from carbon dioxide was originally the exclusive province of organic chemists. As a matter of fact, the founders of organic chemistry are rather closely associated with the early attempt to describe the biosynthetic mechanism of carbohydrate synthesis. Liebig, Emil Fischer, and, more recently, Willstätter and Stoll are very closely associated with the early attempts to describe the sequence of transformations that lie between carbon dioxide and carbohydrates. In general, these speculations were based upon structural analogies—upon the structural features of carbohydrates—and also upon certain kinds of deductions with respect to the nature of the reactions which the chemist could carry out and is familiar with in the laboratory. As it turns out, you will see that none of the speculations of Liebig, Fischer, or Willstätter has been substantiated, and in place of these we have discovered that the natural route by which carbon is converted from carbon dioxide into sugar is, in some senses, much simpler than the routes proposed by these earlier chemists and, in another sense, much more complex. Thus, the principles of the transformations are simpler, but the actual details of the transformations are more complex. I hope, perhaps, to be able to point out some of these things.

Willstätter and his predecessors were confined more to structural analogy in their attempts to devise a biosynthetic route. We were much more fortunate—roughly 40 years later—because we have in our hands a method of examining the detailed stages of the transformation which is independent of structural analogy. This method depends upon the availability of tracer carbon, that is, a long-lived radioactive carbon. This one happens to be ^{14}C which has a half-life, as you know, of 5000 years so that there is ample time, although I must admit that at the beginning of this work it looked as though we might not have plenty of time. The method, in principle, was very simple. We knew that the green plant absorbs carbon dioxide and ultimately, through the agency of the absorbed light, causes it to react with water, or the elements of water, and finally to produce carbohydrate. We know this much : but we didn't know what lay between

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

the two ends, and in principle it became a straightforward matter to feed the plant with labelled carbon dioxide and take a series of time snapshots as the carbon passes from carbon dioxide into the plant through all the chemicals that it has to go through and arrives in carbohydrate. It is clear that there must be a sequence of intermediates—the early ones should become labelled first and the later ones later, and eventually, of course, the label will appear in carbohydrate. So the principle of what I will call " appearance-rate experiments " is perfectly straightforward. These were the first types of experiments which we performed.

We set up a plant in what we may call a " steady state " of photosynthesis: that is, the plant was absorbing carbon dioxide, light, and water, and ejecting, or, if you like, throwing off oxygen and storing away carbohydrate; this is a " steady state." Carbon is coming in at one end and the carbohydrate is being deposited at the other, and in between—or somewhere along the line—oxygen is evolved. Now, at a given instant of time we inject into the incoming stream of carbon, labelled carbon atoms in the form of labelled carbon dioxide. Then we take a series of photographs in time of the chemical distribution of the radiocarbon as it passes into the plant. We will then have performed, in essence, the basic experiment of an appearance-rate curve. The problem, of course, is, first, to select the proper biological material, and, secondly, to devise a method which will give us the proper photograph of the distribution of the radiocarbon in the plant. The first of these was practically determined for us. There was little choice remaining because of the highly developed state of knowledge of algal growth. Two requirements were determining. One was the desire we had to handle the biological material as nearly as possible in the same way as chemicals. One cannot do that with an entire plant but one can with a bottle of algæ; one cannot treat it quite like a bottle of chemicals off the shelf, but this was our predisposition and for this reason we used algæ primarily for our early experiments. The second factor was that the algæ which we used had been used previously in a wide variety of photosynthetic experiments, so that the general technique of growing them in a small way had been already developed. We had to develop it much further because our method of examining the algæ was much more precise and more detailed than had previously been used. Therefore, algæ that were grown in batches each day were not good enough for our purposes. We soon found out that they were not chemicals—that they varied from day to day if good care was not taken of them—and it became necessary for us to provide our own algæ farm in which we grew the algæ under extremely closely controlled conditions so that the suspension of the algæ that we used each day would do the same thing each day. This was, you might say, an elementary requirement of scientific investigation—that the bottle which you believed to be the same shall actually be the same. As I said, it required the development of our highly controlled algæ farm and we have been improving it ever since as our methods of investigating the product became more delicate and more precise.

Of course, we begin to detect variations even in the algæ of today and we have to continue to improve their culture. Another sort of thing which we have done, I might say, is to use a wide variety of plants, and the conclusions which we have drawn with algæ we have been able to demonstrate throughout the plant kingdom—in fact, from the blue-green algæ which are very closely related to bacteria through plants such as the mosses and grasses, to the leaves of the higher plants.

The plant material having been selected for our major work, namely, the algæ, it was easy enough to set them up in a steady state of photosynthesis and then to take little samples of them at various intervals of time after the radiocarbon had been introduced and drop them into boiling alcohol which, in effect, stops all the biological reactions, destroying the activity of the enzymes involved in the reactions. Then it became necessary for us to devise a method of analyzing the extract that was so produced.

These extracts were first mixture-extracts—made by extracting first with 80% alcohol, then with 20% alcohol. Some of the materials were soluble in the 80% alcohol which was the original solution. Then it turned out that a good fraction of what we wanted was not as soluble in 80% alcohol as would be desirable, and the second extraction of the material was made with 20% alcohol. The two extracts were put together and the analysis made of the mixture. In the beginning we used classical organic analytical methods. We attempted to extract each compound that was present in macroscopic amounts—to purify it, crystallize it, and to measure its radioactivity. This was a very laborious procedure, and after a couple of years we identified one compound and it had only a small fraction of the total radioactivity that was in this plant after a minute or two. It was quite clear that we had to devise something more rapid—a general method of analysis—and this led to a combination of the elegant method of paper chromatography which had been developed for the analysis of protein hydrolysates in

FIG. 1.

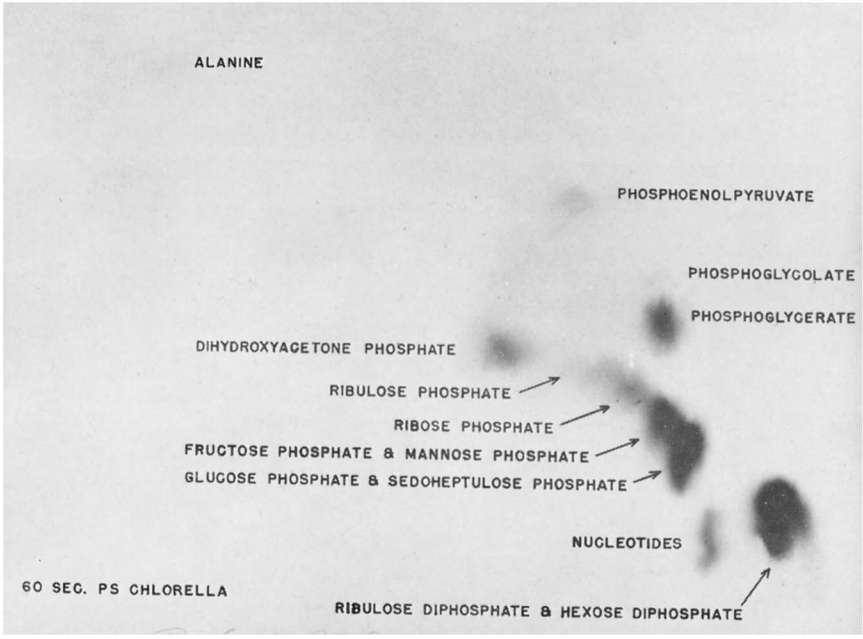
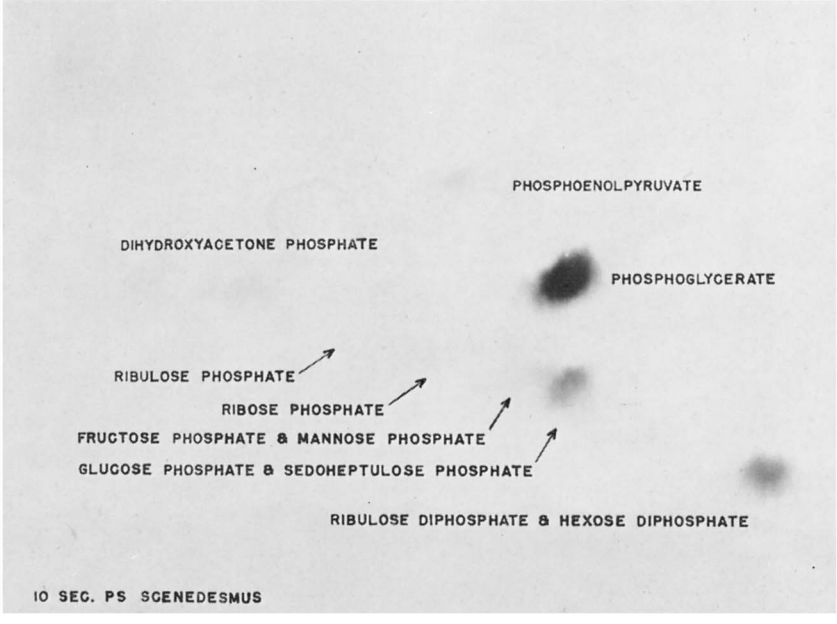


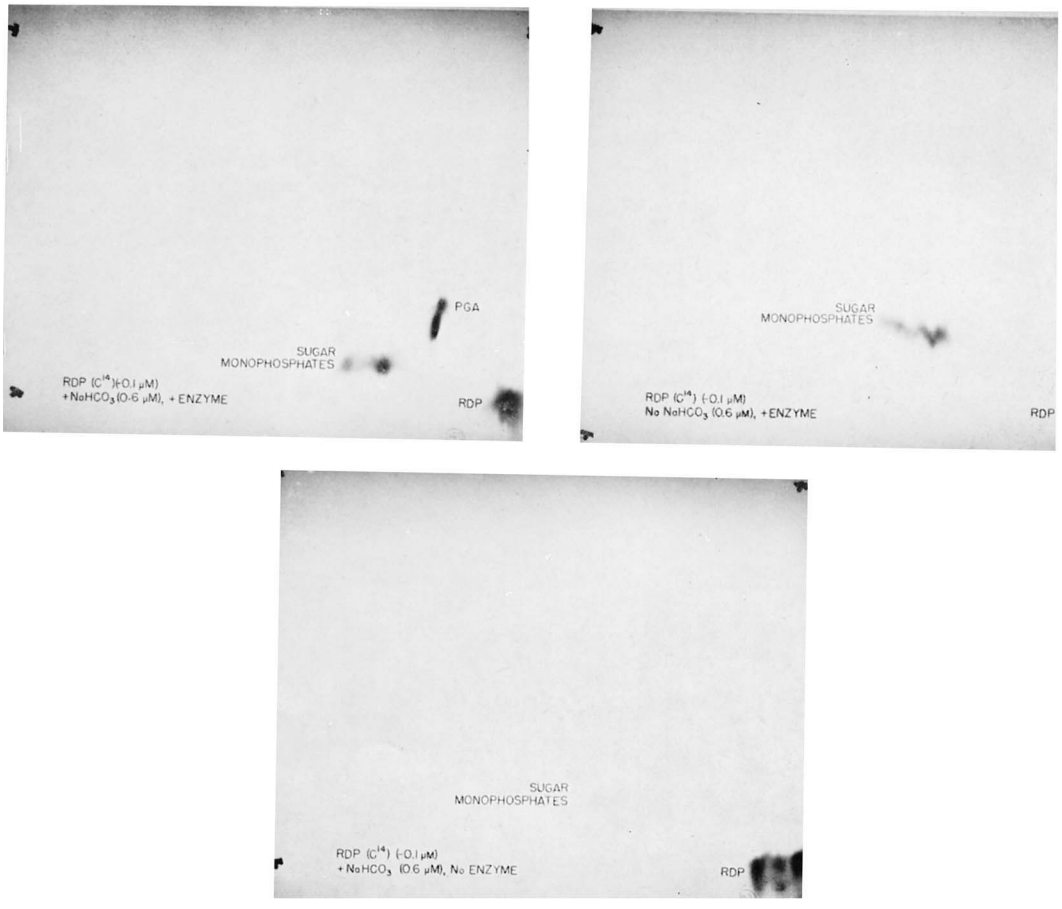
FIG. 2.



FIGS. 1 *and* 2. *Chromatogram of extract from algæ indicating uptake of radiocarbon during photosynthesis by* (*Fig.* 1) Chlorella (60 *seconds*) and (*Fig.* 2) Scenedesmus (10 *seconds*).

[*To face p.* 1896.

FIG. 14. *Chromotograms showing effect of enzyme action on ribulose phosphate.*[8]



England together with our method of examining the extracts for radioactivity. The two things together provided us with an extremely rapid, general, and useful tool for the analysis of the extract. The principles of two-dimensional paper chromatography have been amply described elsewhere and will not be repeated here.

We are not interested in all of the compounds in the extract. We are interested only in those which have radiocarbon in them. I might say the amount of material we put on the paper is so small that, in general, when the chromatograms are made the dried paper looks just as it did at the beginning, except for small pencil marks where the original spot was. The chlorophyll actually is often visible on the paper, but most of the paper is quite colourless. We do not know what compounds we are looking for, not even their chemical nature, so we could not examine the paper by ordinary chemical means—that is, by colour tests—the only thing that characterizes them with certainty is the radioactive carbon content, and those are the ones in which we are interested. This, of course, makes it very simple—we need no chemical spray to find the compounds we want on the paper. All we have to do is find where the radioactive areas are, on the paper, which will then locate the compounds in which we are interested.

There are several ways in which this can be done. One is to put a small boy to work with a Geiger counter and have him explore the paper inch by inch, and we do that occasionally when we have a great deal of time or when we know approximately where to look. But the best way has turned out to be to place an *X*-ray film in contact with the paper, of the same size as the paper and wherever there is a radioactive spot the *X*-ray film will be exposed. Before the exposure, we place a radioactive mark at two corners of the paper so that the relation of the film to the paper can be reproduced. Then we develop the film after a suitable length of exposure and get a series of dark spots on the film for the particular compounds in which we are interested.

The next problem is the identification of the chemical character of each of these areas. Here we begin to use our information as to the relation between the chemical structure and how the compounds migrate—that is our first approximation. Then we use the more general technique of cutting out the radioactive spots of the paper, eluting them with a suitable solvent, performing a chemical operation on the resulting 0·2—0·3 c.c. of solution, then putting it back on paper and again running the paper in two-dimensional chromatography. From the behaviour before and after the chemical treatment and the location of the material in each case, we eventually can narrow down the chemical character of the material, and the final identification of the material is achieved by mixing with the radioactive material the supposed chemical which it is, in an amount sufficient to give a colour test. By that time there will already be enough information about the chemical character to have devised a suitable colour test for it on the paper and then to show the absolute coincidence of the radioactivity and the colour test. The amount of chemical which is extracted from the plant and is radioactive is negligible compared with the amount we add for the chromatography, so that the colour test is given only by the material which we add. Radioactivity is given only by the material which was extracted from the plant and the two things coincide exactly; not " nearly " in the centre of gravity of the spots but in the shape—the spots are never simple, they have very irregular edges. The two things have to correspond exactly in every respect before we consider the identity of compounds established.

This, then, is the general analytical technique. The results of the first experiments are shown in the first two Plates. Fig. 1 shows the result of a 60-sec. exposure; this happens to be for one type of alga; the black spots that we get are clearly separated. In 60 sec. there are perhaps a dozen or more compounds formed with radiocarbon in them and some of them contain large amounts of radiocarbon; so from this alone we recognize that 60 sec. is much too long.

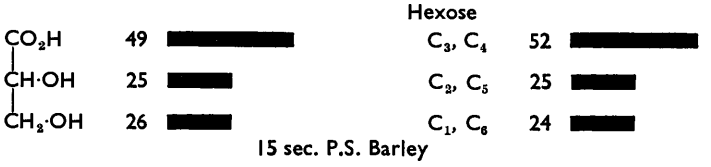
Now Fig. 2 shows a much shorter exposure time of 10 sec. with different but very closely related algæ. To a chemist they are very much alike, but it turns out that for other purposes—we will see later in the discussion—the difference between these two organisms has become of significance. For the most part the earlier compounds have disappeared and one compound has become predominant, namely phosphoglyceric acid. When this was done on as quantitatively kinetic a basis as we could later do it, and a plot was made in terms of percentage of the total radioactivity which occurs in each compound, and this percentage then extrapolated back to zero time, this compound (phosphoglyceric acid) does indeed extrapolate to very nearly 100%.

Now phosphoglyceric acid (PGA) is a compound which contains three carbon atoms, and the next question that arises is which of the three carbon atoms contains the greatest amount of radioactivity. This was established by breaking the acid apart by established chemical

methods, so that we knew precisely which carbon atom was which, in a separated form. This was done, with the result shown in Fig. 3.

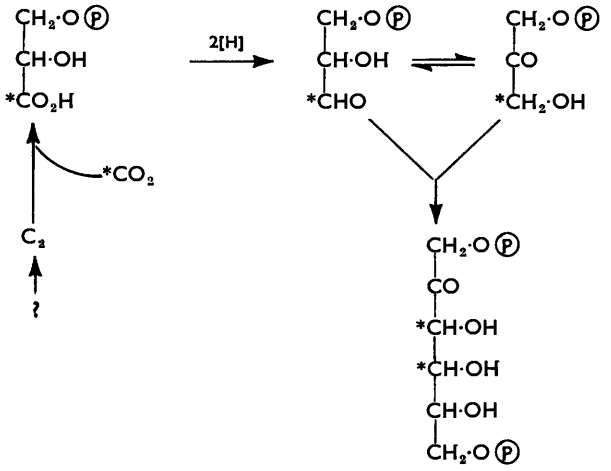
This shows the distribution of radioactivity in the three carbon atoms; it is a 15-second photosynthetic experiment in barley. Actually, it looks as though I have done this with a purpose : there are three different plants here; that is, *Chlorella* on the first figure, *Scenedesmus*

FIG. 3. *Distribution of labelled carbon in photosynthesis experiments.*



on the second, and barley on the third. I assure you this was quite accidental; I didn't pick them out to have three different organisms. But it does illustrate the basic point I tried to make earlier, namely, that the fundamental conclusions which were drawn have actually been checked with about twenty different plant materials and they differ only quantitatively—qualitatively they are the same. Notice that the distribution of the carbon in glyceric acid from 15-seconds photosynthesis in barley shows most of the radioactivity in the carboxyl group and the rest of it equally distributed between $C_{(\alpha)}$ and $C_{(\beta)}$. From the same experiment we extracted some hexose and degraded it, and here you notice that the distribution of radioactivity is such that it appears that the middle two carbon atoms of the hexose are formed from the carboxyl group of the glyceric acid and the outer four correspond to the α- and β-carbon atoms of the glyceric acid. This very early finding suggested immediately that the hexose was made by a reversal of the well-known glycolytic fission of the six-carbon piece into two three's, in such a manner that the middle carbon atom arises from the carboxyl group of the phospho-

FIG. 4. *Path of carbon from* CO_2 *to hexose during photosynthesis.*



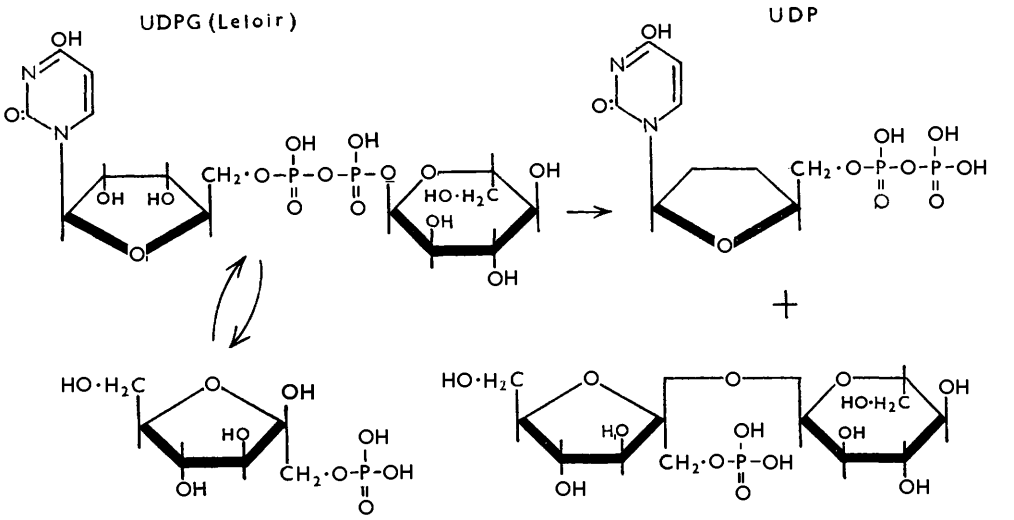
([H] denotes some available reducing agent.)

glyceric acid which is just reduced to an aldehyde group. I might say that this has since been confirmed in a variety of ways and the detail of this relation is shown in Fig. 4, in which the phosphoglyceric acid is shown as first reduced with two equivalents of reducing agent to form labelled glyceraldehyde. There is one point that must now be emphasized, namely, that as the time is shortened the radioactivity appears more and more in the carboxyl group of the glyceric acid. So in the very shortest times which we have been able to use it is practically all in the carboxyl group and none in the α- or β-carbon atom.

Fig. 4 shows the relation of the glyceraldehyde to the corresponding keto-triose which then come together head-to-head, to form the hexose with the two erstwhile carboxyl carbon atoms in the middle. It is the well-known aldolase fission of fructose diphosphate in the reverse direction, which is well established in yeast, and now we have it going forward in the green

plant—at least from the phosphoglyceric acid to fructose diphosphate. The fate of the fructose diphosphate has been elaborated further since most of the carbon eventually shows up in some form of free carbohydrate. As a matter of fact, in most of our plants it shows up first as sucrose itself. It became of interest to us to see if we couldn't find out how sucrose was made from fructose diphosphate. This turned out to be not too difficult after some of our additional spots had been identified. One of them, namely, the uridine-diphosphoglucose (UDPG), turned out to be of great importance. When we recognized it, we knew only that it was one of the very early labelled compounds and labelled only in the glucose moiety. The questions how it got there and what its fate was immediately arose and we suggested an answer which has since been confirmed by individual enzymic experiments by Leloir. The chemical and how it is used are shown in Fig. 5.

FIG. 5. *Uridine-diphosphoglucose reaction with fructose diphosphate.*



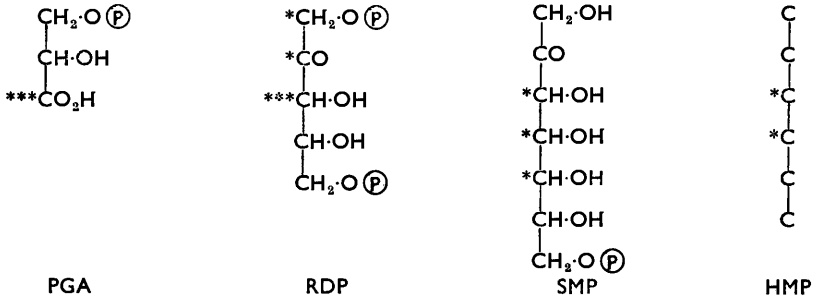
The first unphosphorylated carbohydrate compound that we could identify was sucrose and, therefore, it became necessary for us to devise a way of going directly from the phosphorylated hexoses to sucrose. Now one of the early intermediates which we identified was uridine-diphosphoglucose, and the label was all on the glucose. This is a very labile compound; it is extremely easily hydrolyzed to free glucose. It is one of the few glucose compounds that is more easily hydrolyzed than glucose 1-phosphate. We suggested. since we saw no free labelled hexose, that is, no unphosphorylated hexose, that the sucrose was synthesized by an exchange reaction between the glucose of uridine-diphosphoglucose and the proton of the 2-hydroxyl group of fructose 1-phosphate to form urdine diphosphate and sucrose phosphate. Now the phosphorus is at position 1 of the fructose moiety and then this loses its phosphate to yield sucrose.

As I said earlier, this basic notion has since been confirmed by Leloir who has prepared enzyme extracts which will carry out such reactions. The first which he found used, not fructose phosphate, but fructose itself and uridine-diphosphoglucose to make sucrose directly, and more recently he has found an enzyme system which will catalyze the reaction of uridine-diphosphoglucose with fructose phosphate to make a sucrose phosphate. I might say that we had also found sucrose phosphate in the plant, although in extremely minute amounts. Thus, we have completed the sequence from carbon dioxide all the way to the major product, sucrose, beginning with phosphoglyceric acid as the first identifiable compound isolated from the plant.

Now a glance at Fig. 4 implied that the reaction was such as to indicate that something must combine with the carbon dioxide to make directly carboxyl-labelled phosphoglyceric acid. The arithmetic suggests that this something should be a two-carbon compound or one which yields a two-carbon compound extremely easily. In the search for that precursor to phosphoglyceric acid—that acceptor of carbon dioxide which leads to phosphoglyceric acid—we identified

everything but a two-carbon compound. Instead of finding it, we found a five-carbon sugar, ribulose diphosphate (RDP), and a number of related five-carbon sugars, ribose 5-phosphate and ribulose 5-phosphate (Fig. 6). We found, still more surprisingly, the seven-carbon sugar, sedoheptulose monophosphate (SMP). There is also sedoheptulose diphosphate present, but ribulose diphosphate and sedoheptulose monophosphate are the compounds which are present in largest amounts, although the diphosphate of the C_7-sugar and the monophosphate of the C_5-sugar are also present. Of course, we found, in addition to fructose diphosphate, fructose monophosphate, glucose monophosphate, and mannose monophosphate: these are all to be understood in Fig. 6 in the group of hexose monophosphates (HMP). But no two-carbon pieces appeared that might conceivably be progenitors of the α- and β-carbon atoms of phosphoglyceric acid. Obviously, it became necessary for us, not only to identify these compounds and to get their appearance curves as whole compounds, but also to go much further than that: actually to degrade all of these compounds, one carbon atom at a time, and draw appearance curves of the individual carbon atoms in these compounds.

FIG. 6. *Distribution of radioactive carbon in certain sugars.*



Since our experiments to determine the first isolable compound were so successful, one might ask why we did not take the phosphoglyceric acid (and triose) out of the calculation and simply plot the percentage of the radioactivity in the remaining compounds as a function of time and see which next approached 100% after the phosphoglyceric acid and triose had been subtracted. We tried that, but we were shocked and puzzled to discover that no one compound extrapolated back to 100% at zero time but three of them extrapolated back to finite values—none of them 100%. They all added up to 100%, but none predominated over the others. These three were the pentose, the heptose, and the hexose, and we could not distinguish one as dominant over the others. You will note later why that situation arose. The simple appearance-curve analysis broke down and it failed us on the very next step of the analysis. Even when we proceeded to the individual carbon atoms, it failed us. We could notice as we analyzed the distribution of carbon in these four compounds, including phosphoglyceric acid (see Fig. 6), which seem to be the basic ones of the whole group, that there were very peculiar relations between them. The stars represent the approximate degree of relative radioactivity among the carbon atoms in each of these compounds. I shall not go through the very complex and rather involved detailed arguments which permitted us to determine the relations between these compounds. You will note that there is no simple relation between them; *e.g.*, the five-carbon fragment does not appear with this distribution anywhere in the seven- or the six-carbon materials; the six-carbon fragment does not occur with its own distribution anywhere in the seven-carbon material; in fact, there is no simple relation but we were able to devise an unequivocal relation between them by careful analysis of the kind that led to the establishment of the relation between the triose and the hexose. We were able to establish the connection between the hexose, the pentose, and the heptose. Figs. 7 and 8 show the relations we were able to deduce.

Fig. 7 shows the route of formation of the pentoses. The right-hand symbol is the distribution of the label that we find in the pentose, and once we realized that the pentose (ribulose diphosphate was what we were analyzing) might possibly arise from two different sources it became possible for us to devise a unique scheme which would give it. This, then, provides the source of the pentose—the triose to which the top two carbon atoms of the heptose have been added to give two pentoses, *viz.*, ribulose 5-phosphate, which comes from the top two carbon atoms of heptose plus the triose, and the ribose monophosphate which comes from the

bottom five carbon atoms remaining from the heptose. The two together then are both converted into ribulose diphosphate and the analysis of the ribulose diphosphate, which is what we performed, gives the distribution which we found. While this is fine—it gives us the relation between the pentose, the triose, and the heptose—we still have to devise a way of creating the heptose. Now notice that we cannot create that heptose from pentose because we are using it to make pentose. So we have to devise another way of making the heptose. There is only a limited number of ways in which this can be done. It could be made, presumably, from hexose plus one carbon atom, but there is no way in which to make it from one carbon atom and hexose with the type of label which we have. If that were the case—if that one carbon atom were to come from carbon dioxide—then eventually, in the shortest possible time, in the heptose there should appear at least one carbon atom which is " hotter " than all the others, and this was never the

FIG. 7. *Formation of five-carbon sugars from sedoheptulose phosphate* (SMP).

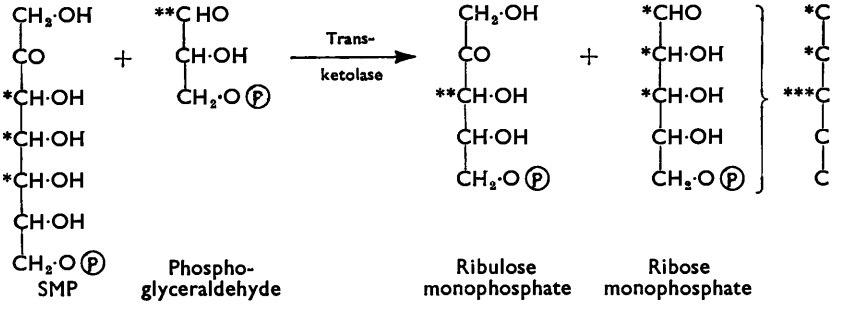
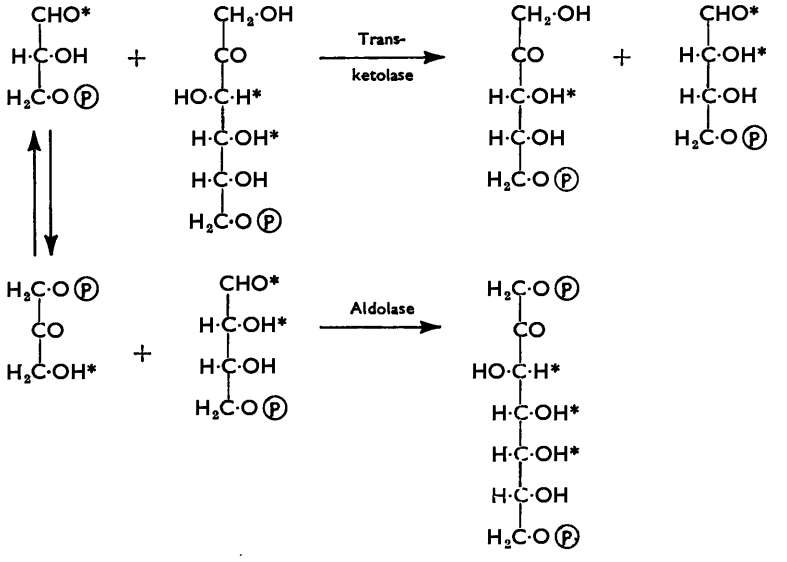


FIG. 8. *Formation of a heptose from triose and hexose.*



case; even in a fraction of a tenth of a second the heptose had the distribution shown. If anything, $C_{(3)}$ and $C_{(5)}$ were always equally labelled (or very nearly always equally) and $C_{(4)}$ less so at the shortest times (a fraction of a tenth of a second). So we had to devise another way of making this heptose and there is only one way left. If one may not use either the hexose and a one-carbon fragment, or the pentose and a two-carbon fragment, one must make it from a tetrose. If one makes it from the tetrose, the other part must be a triose—and we have plenty of triose. We now have the problem of making a tetrose with the proper labelling. Fig. 8 shows how this can be done.

We take the same reaction which we used to make the pentose from the heptose : the top two carbon atoms of the keto-hexose added to triose make another pentose and a tetrose with the required type of labelling. This tetrose, then, combines with the triose to make (with the

same enzyme which makes the hexose from two trioses, that is, one ketose and one aldose) the heptulose diphosphate with the distribution we need. This, then, is the source of the heptose. Again it comes from hexose and triose *via* the reactions of Fig. 8. I might say that as this work progressed, as we devised these schemes and came to the conclusion that this must be the way in which these compounds are formed, the enzymologists shortly after our discovery of the universal appearance of the seven-carbon sugar in all plants, found it also in animal tissue and then they proceeded to search for the enzyme that would do this reaction—and, indeed, they found it. All of these reactions have now been carried out as separate reactions by suitable enzymes. Thus, we now have found the relation between the triose, pentose, heptose, and hexose, but none of this had led us to the answer we were seeking, namely, the precursor of the phosphoglyceric acid.

This required a different type of experiment, in which the radioactivity was used to determine the total pool sizes in the plant. In other words, by following the appearance curves long enough we have found, as shown in Fig. 9, that certain of the compounds " saturated " and then did not become more highly radioactive. Certain others continued to increase in radioactivity as the photosynthesis proceeds. Now such a one is sucrose which is a storage product (an end product); its radioactivity rises as long as radiocarbon is put into the plant. But the activity

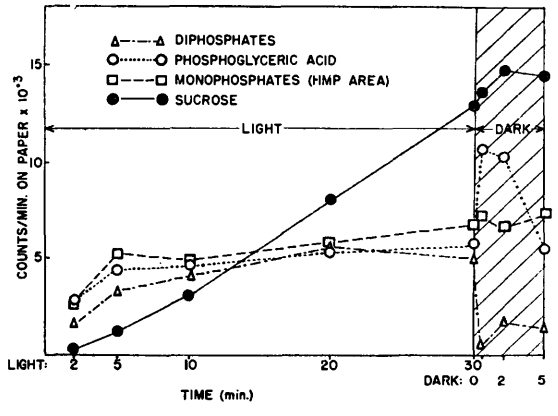


FIG. 9. *Effect of light and dark on activities of phosphates and sucrose.*

of others rises and these become " saturated " within five minutes. Much more carbon has gone into the plant than exists in these three radioactively saturated compounds but most of it is stored in sucrose. This immediately suggests that these three compounds are involved in some sort of a cyclic operation and become " saturated " very quickly, as the amount of carbon going through to sucrose exceeds in amount the total amount of these three compounds. They will, of course, become " saturated " with respect to radioactivity which will not change further as carbon passes through these compounds and is laid up in sucrose.

This was more evidence, then, for the cyclic system but it still did not explain what the carbon dioxide acceptor was. This explanation came only when we turned off the light in such a steady-state experiment to see what happens to the actual amounts of some of the compounds. Now these that " saturate " are the three we are most interested in; the triangular points are mostly ribulose diphosphate, the circles are phosphoglyceric acid, and the squares consist mostly of the hexose monophosphate with some heptose monophosphate. Now notice what happens on suddenly switching off the light, which provides the reducing agents necessary to reduce the carbon to sugar: activity of the ribulose diphosphate suddenly falls and that of the phosphoglyceric acid suddenly rises. Now it is already clear that the triose has its origin in the phosphoglyceric acid by reduction, and as soon as the light is removed reduction of glyceric acid to glyceraldehyde ceases. So the activity of the acid starts rising, but the corresponding fall in the ribulose diphosphate focused our attention on this as the possible source of the two carbon atoms, *i.e.*, of the precursor of the phosphoglyceric acid. The fact that this diphosphate activity fell by approximately the same amount as that of phosphoglyceric acid rose suggested that the two were closely related, and that in the way that we needed to make ribulose diphosphate the carbon dioxide acceptor and its five carbons plus one carbon dioxide molecule make two phosphoglyceric acid molecules. Now this completes a cyclic sequence which will be shown diagrammatically below.

Fig. 9 is included more or less for historical purposes because it is the first run of this sort

which focused our attention on ribulose diphosphate as a carbon dioxide acceptor. Since that time we have done the experiment again and Fig. 10 is a nice illustration of what happens when you know what you have. It shows the same experiment done two years later : here the ribulose diphosphate activity falls and the phosphoglyceric acid activity rises, beautifully confirming the original experiment in which there were only one or two points but from which we made the proper guess.

FIG. 10. *Light–dark transients in phosphoglyceric acid and ribulose diphosphate activities.*

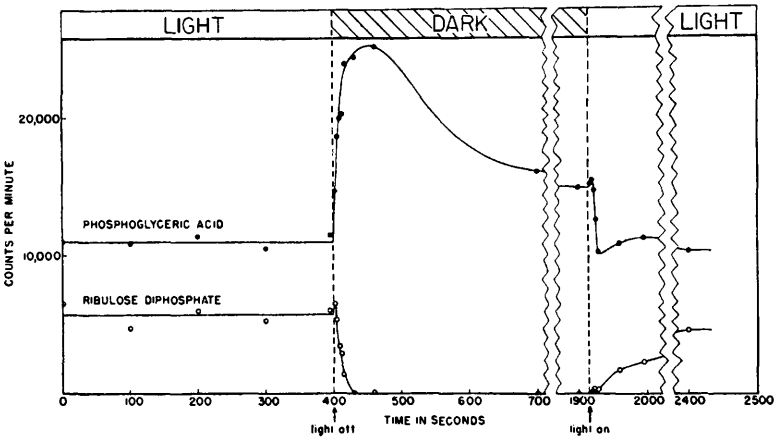
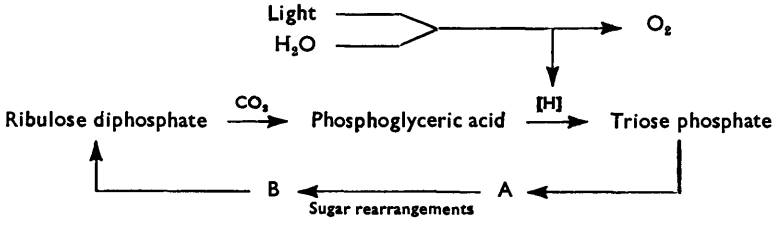


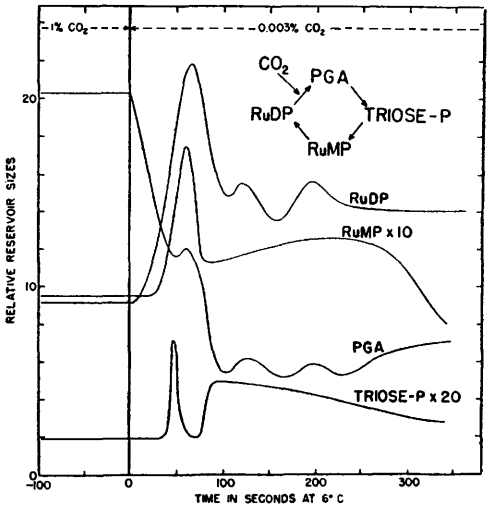
Fig. 11 shows diagrammatically the cycle which we had been leading up to. Here is our phosphoglyceric acid which is reduced to triose phosphate by a reducing agent. The reducing agent has its origin in the light. The light in some way photolyzes water to produce a reducing agent and an oxidizing agent which ultimately becomes molecular oxygen. The reducing agent is used to reduce phosphoglyceric acid to triose. The triose then undergoes—this is already at the sugar level now—a series of transformations such as those we have already seen in the relation between pentose, hexose, and heptose—that is what A, B, etc., are, and we will come back to that in a moment—eventually leading to ribulose diphosphate. Everything builds up to ribulose diphosphate which then carboxylates to give phosphoglyceric acid. The light experiment (Fig. 11) is the one in which we turned off the source of [H], in effect, by turning

FIG. 11. *Suggested cyclic scheme for relations in photosynthesis.*



off the light. We block that point of the cycle and what has happened is that phosphoglyceric acid has built up and ribulose diphosphate has fallen, because if reduction of phosphoglyceric acid is blocked there is no source for the ribulose diphosphate but the carbon dioxide is still there to carboxylate it to give phosphoglyceric acid. This immediately suggests another type of experiment to check this simple diagrammatic cycle. There are two points at which we get into this cycle as far as this diagram is concerned. We can get into it, or effect it, by changing the level of reducing agent and that is what we did by changing the light—we can get into it by changing the level of carbon dioxide, keeping the illumination constant. This is the other possible way in which we can induce transients in these pools. We did that experiment : it is much more difficult to do but it has been done. The predicted result is that ribulose diphosphate activity should build up and that of phosphoglyceric acid decrease in the initial phases of that transient. We cannot see easily what happens afterwards, as will be discussed below.

Fig. 12 shows the results of such an experiment, showing the effect of dropping the carbon dioxide pressure from 1% to 0·003%. Now it takes a few seconds actually to get the carbon dioxide out of the system—to change it (it is not like light which can be turned out). So the Figure shows that our prediction is fully borne out. The first thing that happened after the carbon dioxide dropped was that the phosphoglyceric acid curve fell. On the other hand, the ribulose diphosphate curve rose, as predicted. This Figure actually contained a lot more information than that. If these things are related in a cyclic system as we had proposed and we suddenly throw into this cyclic system a change of one of the external conditions, which is involved in maintaining a steady state, we should see a transient travel around the cycle in both directions. We should see the transient going forward—clockwise—the phosphoglyceric acid curve is falling, then the triose curve should fall, then the ribulose monophosphate curve should fall, then the ribulose diphosphate curve should fall. That is the transient going around the forward way. On the other hand, in the transient going around the other way, the ribulose diphosphate activity should rise, then the ribulose monophosphate activity should rise and the triose activity should rise and the phosphoglyceric acid activity should rise. Now this pair



TRANSIENTS IN THE REGENERATIVE CYCLE

FIG. 12. *Transients in the regenerative cycle.*

of transients travelling around the cycle will eventually meet and there is then an oscillation. After the first one it is pretty hard to predict by simple inspection, although I suppose it would be possible, if one were absolutely right and knew all the rate constants, to put this thing into a calculator and await the result. But, qualitatively, it is good enough for the present purpose.

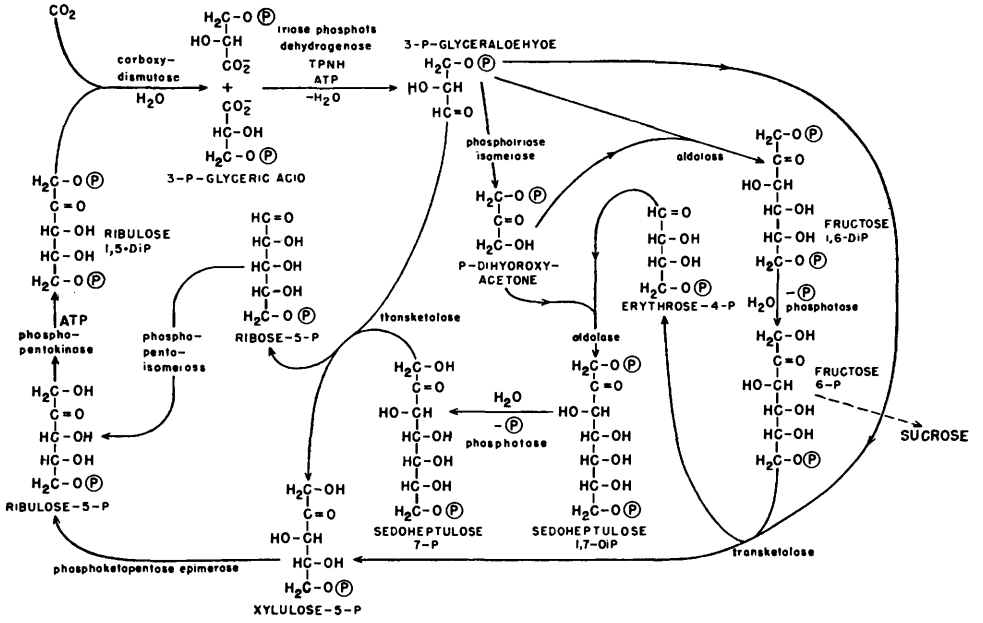
Now the first curve to rise—in the rising transients in the counterclockwise direction—is that of ribulose diphosphate, the second thing, that of ribulose monophosphate, and the third that of the triose. The first curve to fall on the forward cycle is that of phosphoglyceric acid, the second that of the triose, then that of ribulose monophosphate and, finally, that of ribulose diphosphate. This we consider a very good confirmation of the cyclic operation which we have proposed. The rest of the fluctuations are much more complex because they are interrelated, now, with the take-offs from this cycle, and these things are for further investigation. The method, I think, is established and is a very powerful one in examining the interrelations of a complex system of this sort.

The details of the sugar transformations leading to the carboxylation of ribulose diphosphate we have already seen, so that we can now set up the entire cycle (Fig. 13). It contains all of the elements which we have discussed one at a time up to now. It contains the formation of the phosphoglyceric acid from ribulose diphosphate and carbon dioxide, also the reduction of the phosphoglyceric acid to phosphoglyceraldehyde; then the phosphoglyceraldehyde undergoes four different reactions in parallel. That is why we never see (as mentioned earlier) a second compound as the successor to phosphoglyceric acid (and triose). The steady-state level of triose was so small that we could seldom use it. We had to use things like the hexose, the pentose, and the heptose, and these all come in later. The triose and the hexose make the

tetrose and the pentose; the tetrose and the triose then get together to make the heptose almost certainly as the diphosphate, which then loses one phosphorus atom to make the heptose monophosphate, which then undergoes the same transketolase reaction as the first one—the top two carbon atoms of the heptose combining with triose to make the two pentoses. Now these reactions in all cases lead to ribulose 5-phosphate which finally requires another phosphorus atom at position 1 to make the 1 : 5-diphosphate. Thus, we are back again to a system capable of carboxylation.

This, then, is the entire cycle. One starts with one each of these molecules and then by going around the cycle six times ends with one extra hexose molecule which we can now take off to form sucrose, according to the reaction developed earlier. As a matter of fact, carbon can be taken off at various other points of this cycle to make other important biological materials, but this is the major route from carbon dioxide to sucrose *via* this cycle.

FIG. 13. *The photosynthetic carbon cycle.*



There is one other thing to mention in connection with this cycle—it completes the story. As stated above, the aldolase reaction (and isomerase reaction) had been known ten to fifteen years ago, in connection with the reverse process, the breakdown of sugar, so that they were well known as individual steps. As the work went along and the new substrates (ribulose and sedoheptulose) were discovered and the relations between them defined, the enzymes responsible for the required reactions were partially purified by the enzymologists. About the time when we finally decided what the new carboxylation was, which had not yet been described enzymically, it seemed wise for us to become enzymologists at least for a short time and try to demonstrate this reaction in a cell-free system. And we did that. We made extracts from both algæ and spinach and we got our ribulose diphosphate out of the plant by paper chromatography and cutting out the proper areas of the paper. This was the quickest way to get it rather than to try to synthesize it; it has not been synthesized yet. We tested the extracts for this reaction. It was all done by our technique of chromatography with radioautography. I might say, first, that if we used unlabelled ribulose diphosphate and labelled carbon dioxide in our reaction mixture and put the enzyme in, then all we got was labelled phosphoglyceric acid. But this could have happened in a variety of ways. The phosphoglyceric acid so obtained was carboxyl-labelled. Now we wanted to do it the other way. We wanted to take labelled ribulose diphosphate, so that we would know exactly what happened to all those five carbon atoms, and then unlabelled carbon dioxide and the enzyme and see what happened. That should make phosphoglyceric acid, of which one molecule was labelled equally in all three (starting with uniformly labelled rubulose) carbon atoms and it should make one phospho-

glyceric acid which was labelled only in the bottom two and not in the carboxyl group, so that the average of the two would be that the carboxyl was slightly less radioactive than the other two, instead of the opposite as in the first experiment. Fig. 14 (Plate facing p. 1897) shows the kind of data obtained; we see what happens with labelled ribulose diphosphate, unlabelled sodium hydrogen carbonate, and enzyme; phosphoglyceric acid is formed—some of the ribulose diphosphate is not used. It is a short time—I think it is only about a two-minute experiment—two minutes' exposure to (unlabelled) sodium hydrogen carbonate in these three chromatograms. There is apparently some enzyme in this preparation which converts some of the ribulose diphosphate into ribulose monophosphate, but most of it becomes phosphoglyceric acid. If the hydrogen carbonate is omitted, no phosphoglyceric acid is formed. This is the important thing. It is the hydrogen carbonate which does it; and if the enzyme is omitted nothing happens—one has just the ribulose diphosphate (with some ribulose monophosphate which is present in the sugar preparation). So we have demonstrated this reaction—ribulose diphosphate plus carbon dioxide to give nothing but phosphoglyceric acid. There is a small amount of side-reaction because the enzyme preparation which we have called carboxydismutase is not yet clean in this experiment and we have not yet seen the intermediate six-carbon compound.

FIG. 15. *Carboxydismutase reaction.*[9]

Substrate, enzyme added	Origin	RuDP-^{14}C residue	PGA
$H^{14}CO_3^-$ + carboxydismutase	0	0	0
RuDP + $H^{14}CO_3^-$ + carboxydismutase	6000	0	6000
RuDP-^{14}C + HCO_3^- + carboxydismutase	5600	639	4218
RuDP-^{14}C + HCO_3^- + boiled carboxydismutase	5600	5142	58

(RuDP = ribulose disphosphate; PGA = phosphoglyceric acid.)

Fig. 15 shows the result of direct counting of chromatograms such as Fig. 14, and it confirms what has just been said. When there is no hydrogen carbonate with the enzyme nothing happens: the same in the absence of ribulose diphosphate. With unlabelled ribulose diphosphate and labelled hydrogen carbonate and the enzyme, all the activity is in phosphoglyceric acid and in the carboxyl group. With the labelled ribulose and the unlabelled hydrogen carbonate and the enzyme, most of the activity is in phosphoglyceric acid. Finally, with labelled ribulose and the unlabelled hydrogen carbonate and the boiled enzyme preparation nothing much has happened; the ribulose diphosphate has remained unchanged.

FIG. 16. *Mechanism of the carboxylation reaction.*

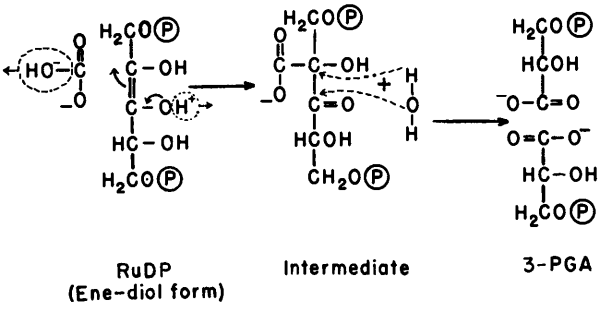
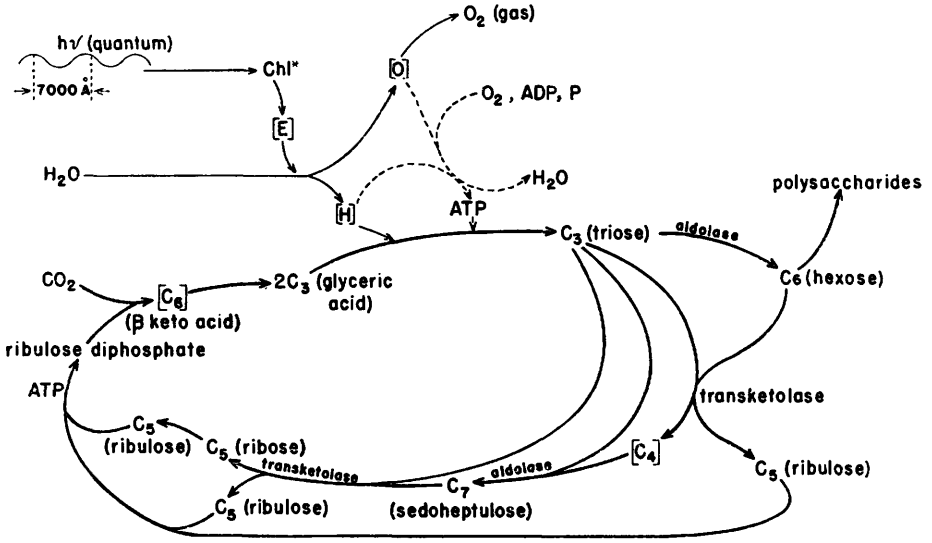


Fig. 16 gives a suggested mechanism for this carboxylation. The ribulose diphosphate in the enediol form can pick up bicarbonate ion with the elimination of the elements of water. This water molecule then can attack the resulting α-hydroxy-β-keto-acid in a standard fashion to hydrolyze it between the α- and the β-carbon atom and give two molecules of phosphoglyceric acid. We have never seen this intermediate and I think you can see why we would never see it under our isolation conditions. It would either be decarboxylated back to ribulose diphosphate, being an α-hydroxy-β-keto-acid, or it would be spontaneously hydrolyzed non-enzymically to give phosphoglyceric acid. This would be all we would see on our chromatograms. It is probably worthwhile hunting for this C_6 acid as an intermediate. Maybe one day somebody will do it.

We know now every step in the carbon cycle and I might add, parenthetically, that the entire cycle has been carried out in a soluble system. It has been done in a flask, so to speak, with a mixture of soluble enzymes and reagents. One feeds in carbon dioxide and takes out glucose, and this can be done now without the presence of any of the organized structures of the cell,

such as plastids, mitochondria, etc. Only soluble substances have been used, such as protein molecules and reagents—no organized particulate structures. This is not a light reaction because the reducing agent is supplied in the form of reduced pyridine nucleotide and the phosphorus is supplied in the form of adenosine triphosphate (ATP). Fig. 17 shows the photosynthetic cycle in a schematic way but emphasizes more the reagent requirements. It will be noted that all that is required to make it go are some reducing agents and some adenosine triphosphate (ATP). We need this triphosphate to make 1 : 3-diphosphoglyceric acid and to make ribulose diphosphate, and we need reducing power to reduce the glyceric acid (diphosphate). Those are the only two chemical reagents that are required to convert carbon dioxide into sugar. Now as stated above, this has actually been done by supplying the reducing agent in the form of reduced nucleotide and adenosine triphosphate as such, as indicated by Racker.[6]

FIG. 17. *Proposed cycle for carbon reduction in photosynthesis.*



Normally in the living green organism these two reagents must, of course, find their ultimate origin in the light reaction itself. This also is shown diagrammatically in Fig. 17. The quantum is absorbed by the chlorophyll, giving excited chlorophyll. The excited chlorophyll molecule then goes through some sort of an energy transformation, as yet unknown, to a situation (labelled [E] in Fig. 17), which ultimately uses that energy to split the water molecule into a reducing agent, [H], and some sort of an oxidizing agent [O]. The oxidizing agent eventually can rearrange itself and come out as oxygen gas. The reducing agent can be used to reduce the glyceric acid. In addition to that, we must supply the adenosine triphosphate. Now the way—one way at least—in which we know that large amounts of adenosine triphosphate can be made is by the combination of a reducing agent such as the pyridine nucleotide either with molecular oxygen or with some intermediate on the way to oxygen. We can now define the precise quantitative requirements for the reduction of *one carbon dioxide molecule.* We can say it requires four of these reducing hydrogen atoms and we can show that what is required are three molecules—at least three—of adenosine triphosphate. If these are the reagent requirements to run this cycle and these reagents must have their ultimate origin in a photochemical reaction, the question arises how can these two things be produced by the photochemical reaction. In general, the high-energy phosphate, as stated above, can be produced—and we know of several such methods of producing it—by some oxidation reaction. Now the oxidation can use pyridine nucleotide as substrate, can use molecular oxygen as the oxidizing agent or, presumbly, something else in between. This is a back-reaction, then, of the primarily produced or secondarily formed reducing agent with (*a*) a primarily produced oxidizing agent before the latter yields oxygen, or (*b*) with oxygen itself.

Now since we know that adenosine triphosphate can be made by the absorption of oxygen, oxidizing reduced pyridine nucleotide, we could suppose that, if the rate of reduction of carbon were sufficiently slow so that there would be time for some of this intermediate reductant which

is formed to be reoxidized by oxygen to make adenosine triphosphate, or if some of the stored sugar might be reoxidized by oxygen to make adenosine triphosphate, we should then have a quantum requirement which would be much lower than if all of the adenosine triphosphate had to be made directly by means of the immediately absorbed light. Now the reducing agent and the adenosine triphosphate are both used together; that is, one needs four equivalents of hydrogen and three molecules of adenosine triphosphate to reduce one molecule of carbon dioxide. The rate of reduction of carbon at the higher rates of photosynthesis may be ten or twenty times (sometimes more) the maximum rate of oxygen absorption and respiration. Now if carbon dioxide is taken in and reduced at a very high rate, clearly the adenosine triphosphate

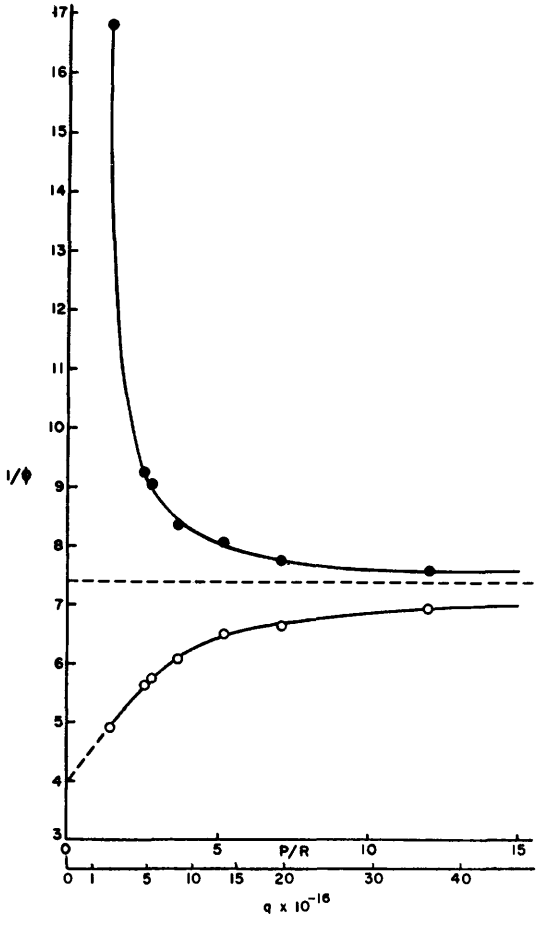
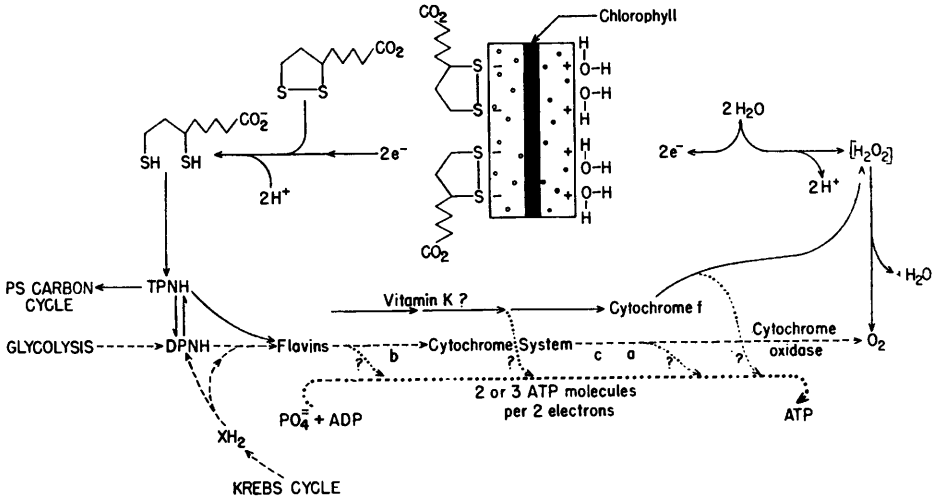


FIG. 18. *Quantum requirement of photosynthesis by* Chlorella.[4]
● Uncorrected.
○ Corrected.

that would be required to reduce carbon at that rate could not possibly all be made by a respiratory mechanism, that is, by a mechanism which involves the reabsorption of oxygen and the creation of the high-energy phosphate by an ordinary combustion process. It is partly for this reason that Fig. 17 shows the broken line from the intermediate oxidant so that at very high rates of reduction the adenosine triphosphate would be made nearer to the primary photochemical reaction than at low rates of reduction. At very low rates of carbon reduction the adenosine triphosphate required to reduce it could be made by the oxidation of sugar at some other place and then brought to where it is needed for the reduction of glyceric acid. Now we can make the single likely assumption that a quantum of light can excite only one electron, not more, in the basic physical sense, and that it takes one quantum to excite one electron. Thus, there is a 1 : 1 relation between quanta and electrons and the excited electrons are the reducing agents—the [H] of Fig. 17. With this assumption it should be possible to find conditions under which the adenosine triphosphate is provided by a respiratory mechanism and the electrons are provided by the light. In that case, one should find conditions in which the

quantum requirement may be as low as four because only four electrons are needed to reduce the carbon dioxide. However, at very high rates of photosynthesis we need not only four electrons but also three adenosine triphosphate molecules, and we know that these cannot be produced by a respiratory mechanism; they must be produced also by the reoxidation of some of these electrons that are made by the primary photochemical reaction, as shown above. Then the quantum requirement must be higher. Now we can accept the quantitative relations indicated in the studies of oxidative phosphorylation in which it is known that the passage of a *single electron* from the reducing level of reduced pyridine nucleotide right up to molecular oxygen can generate about 1·5 adenosine triphosphate molecules; in other words, two electrons can generate about 3, or sometimes as many as 4 adenosine triphosphate molecules (it has not yet been completely established). So at very high reduction rates, since three adenosine triphosphate molecules are needed for every four hydrogen atoms used as reducing agents and since it takes two electrons to make at least three adenosine triphosphate molecules by reoxidation, the minimum quantum requirement under those conditions should be around six, that is, four electrons for reducing and two more to make the required three adenosine triphosphate

FIG. 19. *Suggested nature of the photochemical apparatus and its relation to other functions.*



- - - - Oxidative, or respiratory pathways. —— Reductive, or photosynthetic, pathways.

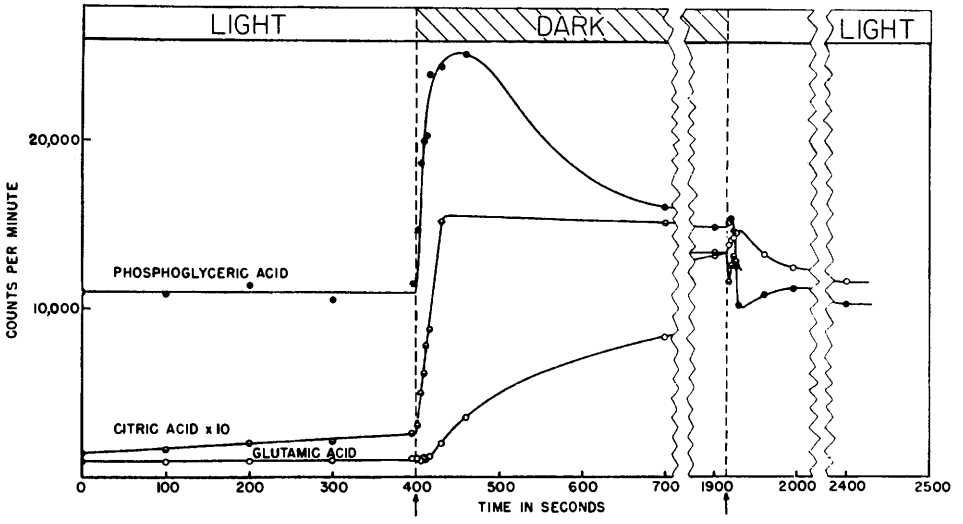
molecules. And this we have been able to demonstrate (Fig. 18).[4] We have been able to find conditions for which at high rates of photosynthesis the quantum requirement is a little more than six (6·5); and as the photosynthetic-to-respiratory rate ratio (P/R) is reduced the quantum requirement falls: we have extrapolated it to zero P/R. It is not a bad extrapolation, but it is actually from five down to four. There is no reason, I suppose, why it should not go down to 3·5. But for theoretical reasons we would prefer it to be nearer 4, although this is not essential.

Thus we have shown that at low rates of photosynthesis we can use some of the respiratory energy to make the adenosine triphosphate and thus use the quanta solely for reducing power and get a quantum requirement as low as four. At the high levels, however, we must not only make the reducing agent but we must also make the adenosine triphosphate by the recombination of the primarily formed oxidizing and reducing agents and thus produce a quantum requirement as high as six or seven. Now the question arises what do we know about the production of the reducing agent (and the oxidizing agent) and what do we know about the way in which the intermediate reductant and the intermediate oxidants can recombine to make adenosine triphosphate. And here, we enter, partly at least, the realm of conjecture; partly there is good reason for what we say.

Fig. 19 shows a proposed scheme in which this accomplished:[2] it includes the reduced pyridine nucleotide which helps run the photosynthetic carbon cycle; this is the ultimate reducing agent. In addition to the reducing agent, we must have adenosine triphosphate. This scheme provides a tentative proposal for the recombination of the reducing agent of pyridine nucleotide and the intermediate oxidant which I have here bracketed as hydrogen peroxide

although its nature is really unknown. We may combine these two through some sequence very much like the respiratory sequence, which involves flavins, cytochromes, cytochrome oxidase, and molecular oxygen, but here we by-pass to cytochrome f which is an iron–hæm compound such as exists in animal tissue, but very characteristic of green materials. We propose that through some sequence very similar to the respiratory system, which is called the oxidative phosphorylation system, we will produce adenosine triphosphate by recombining the electrons which have been raised to a reduction level such as that of reduced triphosphopyridine nucleotide by light, with the intermediate oxidant. We thus make two or three adenosine triphosphate molecules for every two electrons that pass through this system. The broken line is what happens to the respiratory system alone. The solid line is presumably what happens through some unknown sequence here to cytochrome f in the photosynthetic system. Now one may ask, where did all this come from, and this is a proper question. In the upper left-hand part of Fig. 19 we designate the reaction of the photoexcited electrons taking place at this point with the sulphur compound and in the upper right-hand part the reaction of the remaining positive charge or " hole " taking place at this point with the water molecules. Here we have already made a great jump. First of all, we have indicated some sort of structural feature for the primary photochemical apparatus. There is very ample justification for this. I might say

FIG. 20. *Light–dark transients in phosphoglyceric, citric, and glutamic acid activities.*

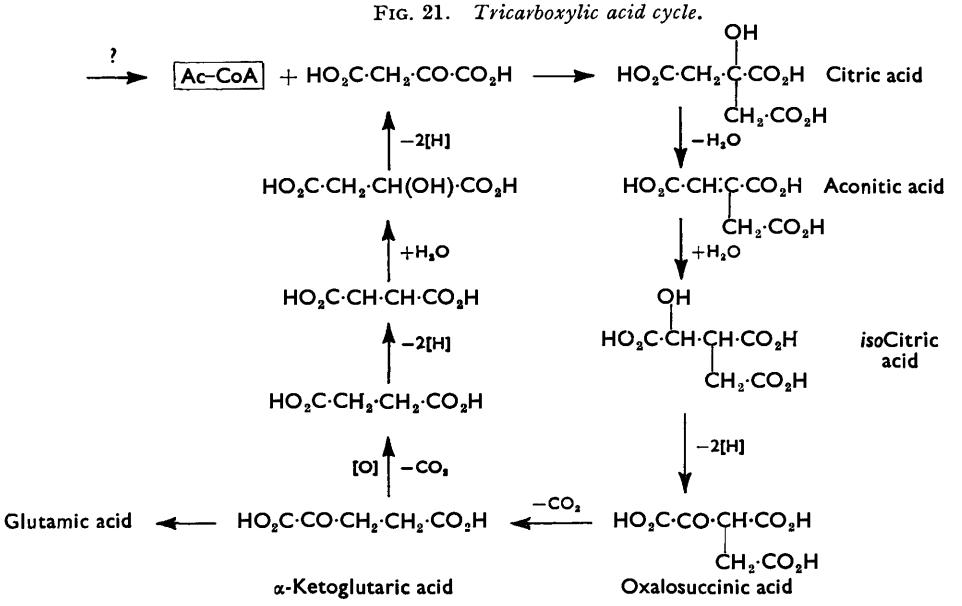


that electron-microscope pictures, particularly the most recent ones of Sjostrand [7] in Stockholm, have shown the layered structure of the small green units called the chloroplast fragments, or grana, within the individual cells. They are layered, 60 Å per layer, and the chlorophyll alternates with something else in these layers. It is suggested that this layered structure is a kind of photobattery, in which the energy is absorbed by the chlorophyll layer and lifts the electron into conduction bands where it then migrates to one side of the layer; the " hole," that is the positive charge, in an ordered lattice migrates the other way. The " holes " are immediately trapped by the donation of electrons to them, *i.e.*, by neutralization, from the water molecules. This reaction (upper right, Fig. 19) takes place at the edge of the layer, producing some intermediate oxidant which here is given as peroxide but whose identity is yet to be determined, with suitably labelled oxygen atoms. Since the " holes " are immediately trapped on one side the electrons on the other side have no place to go to and can remain for quite a long time, waiting for something to pick them up. We have proposed that the substance that picks up the electrons is a sulphur compound, a five-membered disulphide ring, which for very special reasons is capable of doing this, giving a dithiol which then can reduce the pyridine nucleotide and carry the reduction further. We prefer sulphur to the pyridine nucleotide for a number of reasons. The sulphur compound has a reduction potential slightly more reducing than reduced pyridine nucleotide (a couple of hundredths of a volt) and, therefore, it would reduce pyridine nucleotide, but the reverse would not take place readily. In addition, there was another reason for focusing our attention on this sulphur compound, which came from our studies of the

carbon cycle. This involves another one of these steady-state experiments in which we see what happens to the level of certain compounds when light is turned off. Earlier this focused our attention on ribulose diphosphate as the primary carbon dioxide acceptor. Now I am going to show you how our attention was focused on this disulphide compound as the electron acceptor in the primary photochemical reaction. Fig. 20 shows the kind of data to which I am referring.

Here, again, a steady state is established, but now instead of looking at ribulose and the sugars we are looking at something else. In addition to phosphoglyceric acid, whose activity rises, as already shown, when illumination ceases, another very interesting phenomenon occurs. While the light is on no radioactivity moves from the phosphoglyceric acid into two characteristic compounds, citric acid and glutamic acid. But immediately illumination ceases the radioactivity appears in citric and glutamic acid. The citric acid scale has been magnified ten times; there is very little citric acid, but there is an enormous change in the amount when the light is turned off. The other compound, the glutamic acid, is shown on the same scale as the phosphoglyceric acid; the changes in the two correspond (approximately quantitatively); that is, the disappearance of phosphoglyceric acid in the first few minutes in the dark is accounted for by the appearance of the radioactivity in glutamic acid. The actual amount of citric acid,

FIG. 21. *Tricarboxylic acid cycle.*



however, stops rising very quickly. This demonstrates that the carbon is passing from phosphoglyceric acid through citric acid into glutamic acid. An argument similar to that used earlier can now be made, demonstrating that this is a cyclic system and glutamic acid is the storage product. Citric acid immediately calls to mind the Krebs cycle and this has been very well established as a route for the combustion of sugars through pyruvic acid; Fig. 21 shows this cycle.

Now the only way to bring carbon from glyceric acid into this cycle is through pyruvic acid and pyruvic acid oxidase. Pyruvic acid oxidase is the system which converts pyruvic acid into carbon dioxide and acetyl coenzyme A. Without going into the details of the structure of coenzyme A, it suffices to remember that it contains thiol as an active group and it is the transfer of the acetyl group of pyruvic acid to the thiol group which constitutes the pyruvic acid oxidase reaction. Let us emphasize, once again, that the only way from phosphoglyceric acid into the Krebs cycle is througy acetyl coenzyme A *via* pyruvic acid oxidase. Fig. 22 shows this light and dark relationship diagrammatically.

Here is shown the photosynthetic cycle building up sucrose and phosphoglyceric acid and we know that in the light the carbon dioxide and water go in, with oxygen coming out, and we generate reduced carbon which goes into carbohydrates, fats, proteins, etc., in the plant. Then, gradually, these things will leak over into the respiratory system and the tricarboxylic acid cycle shown in Fig. 21. In light they pass over rather slowly from the photosynthetic cycle to the

Krebs cycle. They have to go through all the storage pools of the plant on the way. But when illumination ceases, in 30—60 seconds, a very large proportion of the material in the photosynthetic cycle, particularly the phosphoglyceric acid, passes directly into the tricarboxylic acid cycle. The possibility exists that this change may be due to a diversion by diffusion of

FIG. 22. *Schematic relations between the photosynthesis cycle, the tricarboxylic acid cycle, and storage products in the plant.*

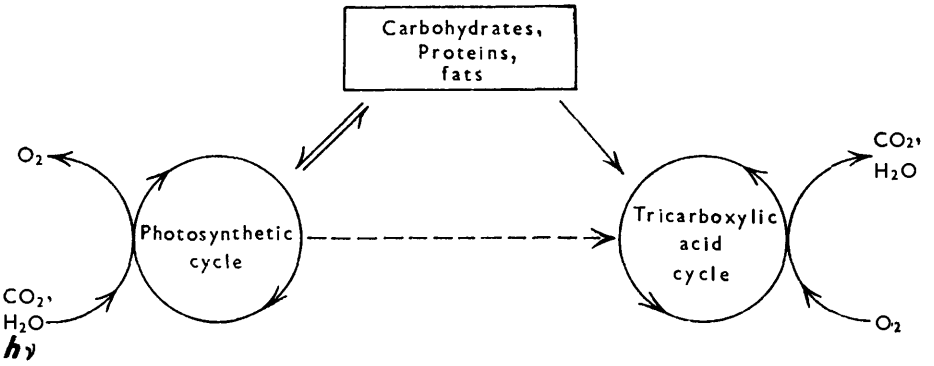
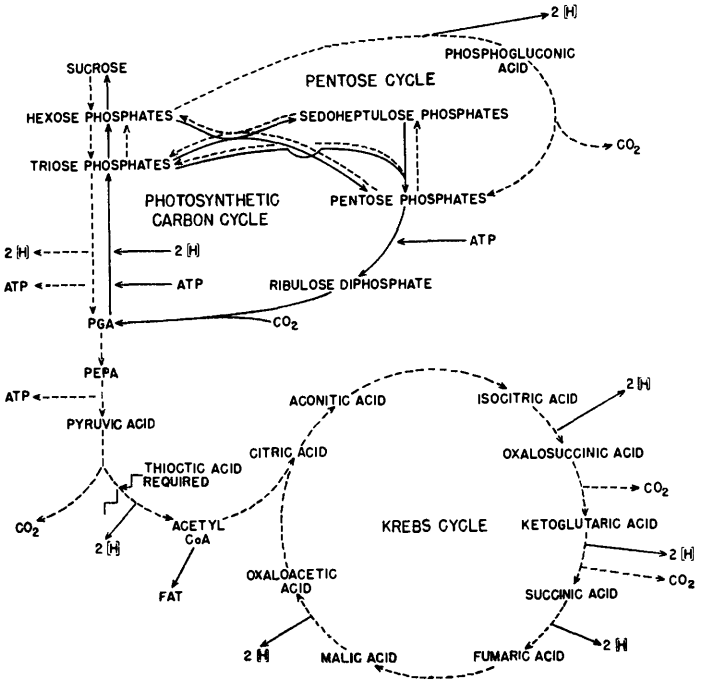


FIG. 23. *Some relations between the photosynthetic cycle and the respiratory cycles*; (1) *Krebs cycle and* (2) *pentose cycle.*



– – – – Oxidative pathways. —— Reductive, photosynthetic pathways.

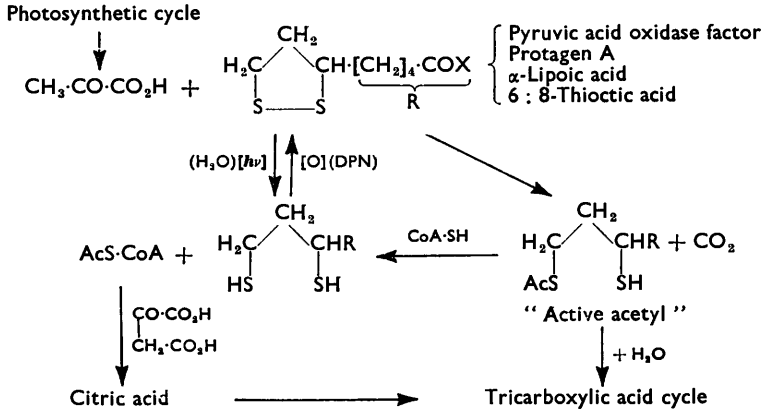
the phosphoglyceric acid of the photosynthetic pool into the normal Krebs cycle pool of the cytoplasm since it is no longer reduced in the photosynthetic cycle on cessation of illumination. We have considered this possibility but have rejected it as unlikely. The change in the rate of appearance of this carbon in (for example) citric acid upon going from light to darkness is at least 20 times the maximum change in concentration of photosynthetic (labelled) phosphoglyceric acid and hence *more* than 20 times the change in concentration difference between the

photosynthetic pool and the cytoplasmic Krebs cycle pool. A correspondingly large change in concentration gradient would be required to change the rate of diffusion from one pool to the other.*

There is then some kind of valve system between the two cycles, which in the light is closed but in the dark is opened. This is a basic notion which we must realise. Now remember that the only way for the intermediates to get into the photosynthetic cycle—the direct way—is *via* phosphoglyceric acid through phosphopyruvic acid, pyruvic acid, pyruvic acid oxidase, to acetyl-coenzyme A, and then into the cycle as illustrated above. The light in some way controls that reaction. About two years ago when the nature of pyruvic acid oxidase was discovered it turned out to be this five-membered cyclic acid—thioctic or lipoic acid—and this cyclic disulphide has some very curious properties.

The point at which the valve works is shown in Fig. 23. Here is shown the photosynthetic cycle in all the details, as well as the route from phosphoglyceric acid to phosphopyruvic acid, pyruvic acid, and right through pyruvic acid oxidase to make acetyl-coenzyme A and into the citric acid cycle. This shows in detail the two cycles that were indicated diagrammatically in Fig. 22, but it shows precisely where this valving (disulphide) compound is functioning in bringing the pyruvic acid to the acetyl-coenzyme A. Fig. 24 shows how this valving actually works.

FIG. 24. *Mechanism of photochemical control of the relations between the photosynthesis cycle and the tricarboxylic acid cycle.*



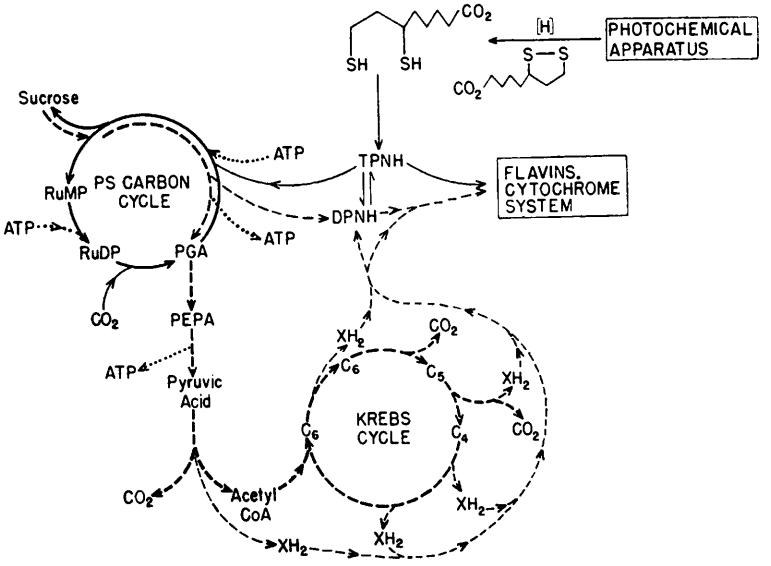
The coenzyme for pyruvic acid oxidase was demonstrated to be this five-membered ring with some carbon atoms on the side and the " X " we are now in the process of determining. It looks as though it might be a glyceride of some kind, but that has not been established and is not important for this particular argument. The important thing is to notice that in order for the two carbon atoms (α and β) of the pyruvic acid to get into the acetyl-coenzyme A we must have available the disulphide form of the thioctic (lipoic) acid. It is an oxidizing agent which oxidizes pyruvic acid to make acetyl-lipoic acid and carbon dioxide. Then the acetyl-lipoic acid exchanges with the hydrogen atom of the SH of coenzyme A (CoA) to make the dithiol and acetyl-coenzyme A which then reacts with the oxaloacetic acid to form citric acid, and thus passes into the tricarboxylic acid cycle. The dithiol, then, has to be reoxidized by something, usually pyridine nucleotide, to give the disulphide back so that it can function again. The light prevents this leakage from the photosynthesis cycle direct to the Krebs cycle. I suggest that the light does it by reducing the disulphide; at this point we do not say how the light reduces the disulphide. It shifts the equilibrium from the disulphide to the dithiol and if the compound is in the dithiol form it cannot carry out the oxidase function. Thus, we shut off the connection between pyruvic acid and the tricarboxylic acid cycle.

The final suggestion is that the light does this directly—that there is nothing between the electron which is activated by the light in the chlorophyll layers and the disulphide—that it is this which picks up the electrons off the layer of chlorophyll. The proof of this is not going to be easy. This is in a class with most of these colloidal phenomena in biological systems, in

* A question by Prof. G. E. Briggs (Cambridge) has reminded us of the apparent lack of a definitive discussion of this point.

which there is a very complex structural element involved, and the efficiency of the process depends on the existence of that complex structural element. We have been able to demonstrate, for example, that the addition of external lipoic acid to a suitable system will, indeed, increase the efficiency with which oxygen is evolved.[5] We have also shown that the addition of lipoic acid will decrease the lifetime of the excited states that are waiting for a converter. Rembember that I pointed out that the " hole " is trapped so that the electron remains and we have been able to show by flashing-light experiments that when lipoic acid is added the length of time that those electrons remain is decreased. But the direct demonstration of the suggestion that lipoic acid, or a very similar sulphur compound, is the direct receiver of the photoexcited electrons is going to be extremely difficult. However, as just mentioned,

FIG. 25. *Suggested nature of the photochemical apparatus and its relation to other functions.*



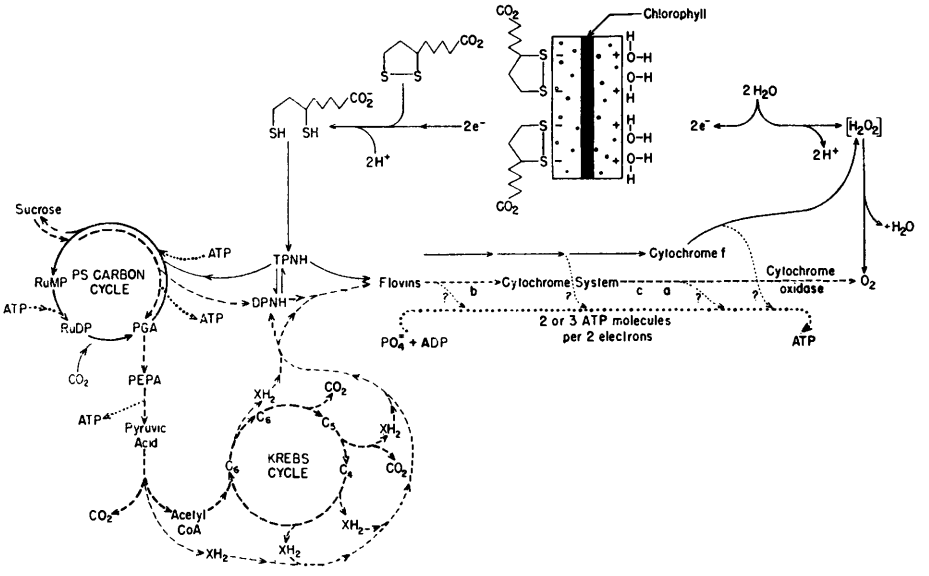
– – – · Oxidative, respiratory pathways. —— Reductive, photosynthetic pathways.

there are various indications which tend to confirm this notion. It may ultimately turn out that it is not lipoic acid itself but some derivative of it; or it may turn out that there is one compound lying between the photoexcited electrons and the sulphur, but it cannot be very much that lies between them because the valving is so swift and so complete. This constitutes one of the major problems that remain. The other is the nature of the intermediate oxidant that was mentioned earlier.

Fig. 25 is the system I have been describing, here shown in some detail, with different aspects emphasized. Here the photochemical apparatus is only indicated, producing reduced disulphide which then reduces the pyridine nucleotide which then runs the photosynthetic cycle. Some of this reduced pyridine goes back through the flavin–cytochrome system to produce the adenosine triphosphate which is necessary to run the photosynthetic cycle. The rest of it is used for the reduction. The relation between the photosynthetic cycle and the tricarboxylic acid, or Krebs, cycle is more clearly emphasized. Here is also shown the way in which adenosine triphosphate can be made in the dark, or at very low light intensities, by using up some of the stored sugar in the Krebs cycle. Coming in at the upper left-hand part of the scheme, the sugar can produce pyruvic acid which can then run through the Krebs cycle to produce reduced diphosphonucleotide, which can then run through the cytochrome system to oxygen to produce adenosine triphosphate. This would permit a quantum requirement of four. Some adenosine triphosphate may come this way, so that most of the reducing power can be used for carbon reduction and then we get a quantum yield of four. When the adenosine triphosphate requirement is so great that we have to burn some of the reduced pyridine as well, then we get a quantum requirement of six. Finally, Fig. 26 shows the whole system together—the photochemical apparatus, the photosynthetic carbon cycle, and the Krebs cycle. Also shown is the oxidative cycle which produces the adenosine triphosphate and about which least is

known. The photosynthetic cycle is well established. The nature of the primary electron is certainly not well established. The nature of the intermediate oxidant is a major problem. This last problem is one of those which we are now actively engaged with, by the use of suitable tracers. We cannot use ^{18}O because one must then know what to look for. The intermediate should be isolated and put into the mass spectrograph, and if, this intermediate is unknown, it is a little hard to get it out. So we are trying to devise other ways of tracing this intermediate. The way in which we are focusing our attention now is ^{17}O and we will use nuclear reasonance as the means of tracing. It is not going to be easy because the sensitivity is not great, but I think we will be able to identify the nature of these intermediate oxidants all included in the $[H_2O_2]$ of Fig. 26.

FIG. 26. *The complete photosynthetic cycle.*



That pretty well defines what we know and what remains to be known, at least today. How big this oxygen problem is going to be I do not know, but judging from what happened to the " Path of Carbon " I expect that before this is finished the " Path of Oxygen " is going to be as big and as complicated.

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