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THE PATH OF CARBON IN PHOTOSYNTHESIS. XVII.

PHOSPHORUS COMPOUNDS AS INTERMEDIATES

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Studies of carbon dioxide fixation in green plants using the  $C^{14}$  isotope have shown that in very short times phosphoglyceric acid contains most of the radioactivity.<sup>1,2,3</sup> The tracer is present almost entirely in the carboxyl group.<sup>4</sup> The importance of organic phosphates in the subsequent metabolism of phosphoglyceric acid can be seen from the accompanying photographs.

Figures (1) and (2) show radioautographs of a photosynthetic experiment with Scenedesmus. Figure (1) indicates the compounds containing  $P^{32}$  after exposure to radioactive phosphate for twenty hours, whereas Figure (2) is a photograph of the same chromatogram showing the carbon-labeled compounds

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after five minutes in radioactive  $\text{CO}_2$ . Many of the phosphates also contain a carbon label.

Even after sixty seconds photosynthesis by Chlorella, by far the major part of the radioactivity is in the form of phosphates (Figure (3)).

Since the discovery of radioactive phosphoglyceric acid as a primary carboxylation product, our attention has been directed towards the path by which the radiocarbon becomes converted into the various cell constituents.

The effect of numerous external factors on the total fixation and on the fixation pattern, as shown by radioautographs of the two-dimensional paper chromatograms, has been investigated. Before these results could be satisfactorily interpreted, it was essential that each radioactive spot on the chromatograms could be identified, and much of our work has been directed to this end.

The identification of small quantities of radioactive compounds has required the use of new techniques, the most important of which is the paper chromatography of the unknown compound together with non-radioactive carrier. Complete coincidence of the radioautograph and the spot produced by a color reaction of the carrier is strong evidence for the identity of the two. The effect of different chemical treatments or enzymes on the  $R_f$  values of unknown compound and carrier provides further confirmation. Ion exchange chromatography, solvent distribution, co-crystallization of derivatives, determination of radioactivity distribution among individual carbon atoms in fully labeled compounds and the value of  $\text{C}^{14}/\text{P}^{32}$  ratios in doubly labeled compounds have all been used to characterize and identify unknown radioactive areas.

The separation and identification of the organic phosphates which are among the early products of photosynthesis will now be described.

(1) Phosphoglyceric Acid - Carrier-free radioactive phosphoglyceric acid has been isolated from Scenedesmus which had photosynthesized for five seconds in  $C^{14}O_2$ .<sup>2</sup> The yield of crystalline barium salt contained one-third of the total radioactivity fixed by the algae. It was directly compared with authentic barium-3-phosphoglycerate and the two shown to be identical in phosphorus analysis, elution characteristics from Dowex A-1 resin, and the molybdate-enhancement of molecular rotation. Hydrolysis gives radioactive glyceric acid identical with an authentic specimen.

(2) Hexose Monophosphates - During the first few seconds of photosynthesis, a characteristic radioactive area appears on the chromatogram below the phosphoglyceric acid. Treatment of the compounds in this area with phosphatase yields several other compounds with the properties of sugars. These sugars have been identified by co-chromatography as glucose, fructose and mannose. The glucose and fructose phosphates are stable to mild acid hydrolysis and, when run in *t*-butanol/picric acid/water as solvent, co-chromatograph with glucose-6-phosphate and fructose-6-phosphate, respectively. The mannose phosphate is likewise stable to mild acid hydrolysis and is probably identical with the mannose phosphate isolated from the hexose monophosphates of yeast, i.e., the 6-phosphate.<sup>5</sup>

On many of the two-dimensional chromatograms, the hexose phosphate area is divided into two distinct halves. The left-hand spot, on treatment with phosphatase, gives fructose, mannose and a trace of glucose. The right-hand spot, on similar treatment, gives glucose and a heptose sugar, sedoheptulose, which is discussed in the following section.

The quantity of radioactive mannose phosphate is considerably higher in the algae, Chlorella and Scenedesmus, than in the leaves of the higher plants e.g., soy bean, sugar beet and barley.<sup>6</sup>

(3) Sedoheptulose Phosphate - The "hexose monophosphate" area was found to give, on treatment with phosphatase, a sugar which was not identical with any of the common hexoses. This sugar has been shown to be sedoheptulose (D-altroheptulose).<sup>7,8</sup> The evidence for its structure can be summarized:

(a) The radioactive spot co-chromatographs with authentic sedoheptulose.

(b) Treatment with 1 N hydrochloric acid at 100° for five minutes gives a radioactive compound which co-chromatographs with authentic sedoheptulosan. The equilibrium value of the ratio of the two radioactive areas after acid treatment is about 4:1, the value obtained for sedoheptulose and sedoheptulosan.

(c) Periodate oxidation of the uniformly labeled sugar gives the theoretical quantities of radioactive formic acid and formaldehyde. Similar treatment of the acid transformation product gives the theoretical amount of formic acid and no formaldehyde.

(d) The hydrogenation product co-chromatographs with sedoheptitol and on periodate oxidation yields the theoretical amount of formaldehyde and formic acid.

From the chromatographic coordinates of the phosphate, it is probably a monophosphate. The location of the phosphoryl group has not been established, but in view of its relative stability towards acid, it is probably not on the C<sub>1</sub> or C<sub>2</sub> hydroxyls.

Robison, Macfarlane and Tazelaar<sup>9</sup> isolated a heptulose phosphate from a yeast juice fermentation. We have been able, through the courtesy of Drs. M. G. Macfarlane and J. Baddiley, to examine concentrates of this ester.

It behaves chromatographically in the same way as the radioactive sedoheptulose phosphate, and gives a heptulose on treatment with phosphatase which is readily converted to an anhydride by acid. The  $R_f$  values of these products are identical with those of authentic sedoheptulose and sedoheptulosan.

(4) Pentose Monophosphate and Diphosphate - When the slowest-moving radioactive spot (in both solvents) is treated with phosphatase, it is converted mainly into a sugar which has the chromatographic properties of a pentose. The sugar has been identified as ribulose.<sup>8,10</sup> It co-chromatographs with ribulose, and on epimerization in pyridine gives ribose and arabinose. The 2,4-dinitrophenylosazone crystallizes with that of D-arabinose. Catalytic reduction yields a compound which co-chromatographs with ribitol. When the original phosphate is allowed to oxidize in the air, phosphoglyceric and phosphoglycolic acids are formed. A determination of the  $C^{14}/P^{32}$  ratio in the compound saturated with  $P^{32}$  and  $C^{14}$  indicates that the compound is a diphosphate. It is concluded on this evidence that the unknown compound is ribulose-1,5-diphosphate.

When the diphosphate area is treated with phosphatase, varying small amounts of glucose, fructose and mannose are produced.

Pentoses are also liberated when a radioactive area to the left of the hexose monophosphates is hydrolyzed by phosphatase.<sup>10</sup> We have recently been able to separate this area into two spots, one of which gives ribose and the other ribulose on hydrolysis (Figures (4) and (5)). In all probability, these are monophosphates.

(5) Triose Phosphates - To the left of the pentose monophosphate area there is a radioactive spot which yields dihydroxyacetone on phosphatase treatment. Co-chromatography of the hydrolysis product with

dihydroxyacetone, detected by the yellow color it gives with the aniline trichloroacetate spray, shows complete coincidence of color and radioactivity (Figure (6)). Experiments to demonstrate the presence of 3-phosphoglyceraldehyde have been inconclusive, due probably to the difficulty of chromatographing this compound or its hydrolysis product.<sup>2</sup> The triose phosphate equilibrium is, in any case, strongly in favor of phosphodihydroxyacetone.

(6) Phosphoglycolic Acid - A characteristic radioactive spot appears above that due to phosphoglyceric acid on chromatograms of C<sup>14</sup>- and P<sup>32</sup>-containing metabolites. It is hydrolyzed by phosphatase to give glycolic acid, identified by co-chromatography with an authentic sample.

(7) Phosphopyruvic Acid - Authentic phosphopyruvic acid has the same R<sub>F</sub> values as a radioactive spot in the standard solvents. Acid hydrolysis of the radioactive spot gives pyruvic acid, identified by its distribution coefficients between ether and water, its volatility and the formation of a 2,4-dinitrophenylhydrazone which co-crystallized with that from pyruvic acid.<sup>2</sup> The same spot appears on chromatograms of metabolites labeled with P<sup>32</sup>. The action of phosphatase on the carbon-labeled compound causes volatilization of the radioactivity.

(8) Sucrose Phosphate - When a hexose monophosphate area from sugar beet was hydrolyzed with an invertase-free phosphatase preparation, a compound was formed which had the chromatographic coordinates of sucrose. It was shown to be sucrose by co-chromatography, and by enzymatic hydrolysis to glucose and fructose, identified by co-chromatography.

The hexose monophosphate area from another chromatogram of the same photosynthetic experiment was rechromatographed in t-butanol/picric acid/water and gave, besides other radioactive sugar phosphates, a slow-moving radioactive spot. This was freed from picric acid and free sugars by one-

dimensional chromatography in phenol and treated with phosphatase. Chromatography in two dimensions gave a radioactive spot in the same position as sucrose. When the other spots from the picric acid chromatogram were treated in the same way, no sucrose was produced.

Although the occurrence of a phosphate of sucrose has been postulated by several workers, we believe that this is the first direct evidence of its presence in plants. Of other plant sources so far examined, algae appear to contain no detectable sucrose phosphate under our conditions, and other leaves contain much less than sugar beet.

From the position of the compound on paper chromatograms, it is probably a monophosphate, but we have, as yet, no evidence as to the location of the phosphate group in the molecule.

(9) Nucleotides<sup>11</sup> - On all chromatograms from plants which have been allowed to photosynthesize in  $C^{14}O_2$  for periods of longer than thirty seconds there is a spot which appears below the hexose monophosphate area. Early work had shown that it contains glucose, easily liberated by acid hydrolysis. In our earlier publications it has been named "Unknown Glucose Phosphate". It has now been shown that when phosphatase acts on the spot, glucose, mannose and galactose are formed, identified by co-chromatography. These three sugars possess the major part of the carbon radioactivity, the glucose being the largest single repository for the radioactivity.

When working with plants which had photosynthesized for some time in  $C^{14}O_2$  (five-ten minutes) it was found that phosphatase-treatment of the unknown spot gave, in addition, radioactive uridine and radioactive inosine. The uridine was identified by co-chromatography, the inosine by its  $R_f$  value in three solvents and by its acid hydrolysis to hypoxanthine and ribose. The ribose was identified by co-chromatography and the hypoxanthine by identity of  $R_f$  values in three solvents with an authentic sample.



Acid hydrolysis of the original spot gave adenine, identified by the u.v. absorption spectrum and  $R_f$  value; adenosine-5'-phosphate, identified by the u.v. absorption spectrum and  $R_f$  value in t-butanol/boric acid/water; uridine-5'-phosphate, identified by  $R_f$  values, u.v. spectrum and reactivity towards sodium periodate. The inosine in the phosphatase-treated product arises by enzymatic deamination. A sample of adenosine was deaminated, when subjected to the same conditions, to give inosine.

When the original compound was extracted from a two-dimensional chromatogram and subjected to rechromatography, at least eight spots appeared on the chromatogram. Of these, five have been identified. They are: (i) Glucose; (ii) galactose; (iii) mannose; (iv) adenosine-5'-phosphate; (v) uridine-5'-phosphate.

Of the remainder there are, besides the original compound, two other radioactive spots. On treatment with phosphatase, the latter each give glucose and galactose. One spot moves in the hexose monophosphate area, and the other moves nearly as far as the free sugars in both phenol and butanol/propionic acid. There is evidence that the fast-moving spot is a mixture of cyclic phosphates of glucose and galactose. When it is treated with a phosphatase preparation for a short length of time, glucose, galactose and a spot in the hexose monophosphate area are produced, while some of the original remains unhydrolyzed.

When the original compound produced during photosynthesis is allowed to decompose slowly on a dried chromatogram, the fast-moving phosphate is the first decomposition product to be formed. We believe, therefore, that the two unknown decomposition products are (a) the 1,2-cyclic phosphates of glucose and galactose and (b) the 2-phosphates of glucose and galactose.<sup>12,13</sup>

When the original unknown compound is chromatographed in ethanol/ammonium acetate at pH 7.5,<sup>13</sup> the main radioactive spot moves at the same  $R_f$  as uridine diphosphate glucose,<sup>\*\*\*</sup> and gives uridine, glucose and galactose, as well as traces of other sugars, on phosphatase treatment. Another spot on the ethanol/ammonium acetate chromatogram absorbs strongly in the u.v., has the same  $R_f$  as adenosine triphosphate, and gives adenosine on treatment with "Polidase."

For these reasons, we believe that the original spot contains at least four different compounds: (i) uridine diphosphate glucose; (ii) uridine diphosphate galactose; (iii) adenosine triphosphate; and (iv) a compound giving mannose on hydrolysis.

One significant observation with regard to the mannose is that when the original spot decomposes, mannose does not appear in either the "cyclic phosphate" or the "2-phosphate" regions of the chromatogram. If the mannose is present originally in a nucleotide structure of the UDPG type, and if the configuration at the glycosidic center is the same as in the other compounds of this group, then this behavior can be explained. Mannose differs from glucose and galactose in the configuration of the hydroxyl group at  $C_2$ , and cannot, therefore, form an alpha 1,2-cyclic phosphate. A mannose-nucleotide structure would decompose to give free mannose. The observation of Leloir and Cabib<sup>14</sup> that yeast contains a compound which yields mannose, guanine and phosphate on hydrolysis is of great interest in this connection.

In comparative experiments, it has been found that soy bean and sugar beet leaves contain little or no radioactive mannose, while the algae,

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Scenedesmus and Chlorella, give rise to mannose derivatives in both the hexose monophosphate and nucleotide areas of the chromatogram.

### KINETICS

The second part of the main problem has been to find out the actual mechanisms by which carbon dioxide becomes reduced. We know what are the main radioactive compounds appearing during short periods of photosynthesis in the presence of  $C^{14}O_2$ . It remains to find how they are interrelated. When algae, undergoing photosynthesis in a steady state, are given a sample of  $C^{14}O_2$ , it is clear that the labeled carbon will pass through a sequence of intermediates between  $CO_2$  and the ultimate products of plant synthesis. It will, of course, take time for the carbon to reach and saturate each of these intermediates. Furthermore, since practically all of these compounds contain more than one carbon atom, the overall rate of appearance of radioactivity in all of the carbon atoms must be the sum of the rates of appearance in each one. Thus, the experiment to be performed consists in stopping the photosynthesis after suitable periods of contact with  $C^{14}O_2$  and analyzing the products with respect to the distribution of radioactivity (1) among the compounds and (2) among the carbon atoms in each compound.<sup>6</sup> A wide variety of steady states depending on the effect of external variables, e.g., light intensity,  $CO_2$  pressure, temperature and pH, and the effect of inhibitors on (1) and (2) are examined.

Isotopes other than those of carbon can be used in these studies. Experiments have been initiated to study the rapidity with which labeled phosphate is incorporated into algae. In sixty seconds there is appreciable incorporation, both in the light and in the dark, and the compounds in which the label is found are mainly those which the carbon experiments have shown to be early products of photosynthesis. (See Figure (7)).

In the work to find out the first stable reduction product of carbon dioxide, algae were allowed to photosynthesize for very short periods in  $C^{14}O_2$ . Phosphoglyceric acid was found to contain most of the radioactivity. This compound was hydrolyzed to glyceric acid, and the label in each position determined by degradation with periodate.<sup>4</sup> It can be seen from Table 1 that the carboxyl group is the first to become labeled, followed by the  $\alpha$ - and  $\beta$ -carbons, which are equally labeled.<sup>15</sup>

Table 1

Distribution of Radiocarbon in Phosphoglyceric Acid  
and Hexose Labeled During Photosynthesis

Compound	Conditions			
	Preillum 2 Min. Dark	4 Sec. PS Photosynthesis	15 Sec. PS	60 Sec. PS
COOH	96.0	87.0	49	44
$\alpha$ CH(OH)	2.6	6.5	25	30
$\beta$ CH <sub>2</sub> OH	1.7	6.8	26	25
Hexose (from su- crose)	C <sub>3,4</sub>		52	
	C <sub>2,5</sub>		25	
	C <sub>1,6</sub>		24	

When the sucrose is isolated from short-term photosynthetic experiments, and the radioactive hexose obtained from it are degraded, carbon atoms 3,4 are found to be most active (Table 1). The other carbon atoms are equally labeled. This, together with the fact that in the formation of sucrose and fructose moiety becomes labeled before the glucose, indicates that sucrose

is formed by a reversal of the normal glycolytic pathway. Since sucrose is the first free sugar to become radioactive it appears that neither free glucose nor free fructose is directly involved in sucrose synthesis. The actual step by which sucrose is formed is something of a mystery. The sucrose-forming enzyme from Pseudomonas saccharophila catalyzes the reaction:<sup>18,19</sup>



So far it has not been possible to isolate a similar enzyme from green plants.<sup>19</sup>

It is of interest to speculate as to the role of the UDPGlucose and UDPGalactose which have now been found in green plants. UDPGalactose has hitherto been found only in yeasts which have been galactose-adapted. It appears that in green plants it figures in galactose formation from glucose. Leloir is of the opinion that UDPG has some other function than the inter-conversion of galactose and glucose.<sup>14</sup> We believe that these compounds may well be involved in the synthesis of polysaccharides in the plant, functioning in a manner similar to that of glucose-1-phosphate in the numerous phosphorylase reactions. In particular, we would like to suggest that it may be involved in sucrose synthesis, by the following series of reactions (Figure (8)). In Figure (9), the labeling of the unknown glucose phosphate (mainly UDPGlucose and UDPGalactose) is more rapid than that of sucrose, and this would agree with the mechanism just proposed. Recent work by Putman and Hassid<sup>16</sup> gives further support for the idea that only phosphorylated derivatives of glucose and fructose are involved in sucrose synthesis in higher plants. It has been found that in sucrose synthesis from labeled glucose in leaf punches, no free fructose is formed, although the sucrose becomes equally labeled in both the glucose and fructose portions. Conversely, when labeled fructose is used, no free labeled glucose appears, while the sucrose is uniformly labeled.

It is possible that compounds of the UDPG type could be concerned in the transformation of sugars and their subsequent incorporation into polysaccharides. Uridine diphosphate would thus serve as a carbon carrier in the same way that the pyridine nucleotides and flavin nucleotides are involved in hydrogen transport, the adenylic acid system in phosphate transfer and coenzyme A in the transfer of acyl groups. There is already some evidence for the existence of other members of the uridine diphosphate group.<sup>13,17</sup>

The early kinetic and degradative experiments indicated that the path of carbon in photosynthesis involved a primary carboxylation to give phosphoglyceric acid whose conversion to sucrose was essentially a reversal of glycolysis. More refined kinetic data have fully supported this work.<sup>6</sup> In the ideal experiment described above, if the rate of appearance of a radioactive compound is plotted against time, only those compounds which have no appreciable stable reservoirs lying between them and the  $C^{14}O_2$  should show a finite slope at zero time. In other words, if the percentage distribution of  $C^{14}$  amongst a group of compounds is plotted against time, the earliest-labeled compounds will show a negative slope, and the reservoir of a precursor will become saturated before that of its product.

Phosphoglyceric acid (Figures (10) (11)) has a finite slope at zero time and is also the first product to become saturated. Phosphoenol pyruvic acid, on the other hand, although it apparently has a finite slope at zero time, does not become saturated until after phosphoglyceric acid (Figure (11)). It is possible that phosphoenol pyruvic acid has an initial zero slope, followed by rapid equilibration with phosphoglyceric acid.

The rapid labeling of malic acid (Figure (10)) might indicate that it, too, is a primary carboxylation product. This can, however, be accounted

for by considering that malic acid is in rapid equilibrium with a compound arising by carboxylation of a  $C_3$  compound derived from phosphoglyceric acid. Degradation experiments on the radioactive malic acid produced during short-term photosynthesis are in progress. The malonate-inhibition experiments of Bassham et.al.<sup>4</sup> make it unlikely that malic acid is directly concerned in photosynthetic carbon dioxide reduction.

From the foregoing work, it seems that  $CO_2$  reacts with a  $C_2$  compound to give phosphoglyceric acid. The nature and the origin of this compound are the next questions which must be answered. The new kinetic data lead to the conclusion that sedoheptulose phosphate and ribulose diphosphate are concerned in the early stages of  $CO_2$  fixation. The early labeling, and the fact that neither sugar is stereochemically related to glucose while being related to each other, suggests that these compounds may be concerned in the regeneration of the  $C_2$  acceptor for carbon dioxide.

There is a growing body of evidence that ribose phosphate and ribulose phosphate can undergo scission to give a  $C_2$  and a  $C_3$  fragment.<sup>20</sup> Lactobacillus pentoaceticus will ferment arabinose-1- $C^{14}$  to give lactic acid together with acetic acid labeled exclusively in the methyl group.<sup>21</sup> Similar results have been reported for Lactobacillus pentosus.<sup>22</sup> There is evidence, too, that ribulose phosphate and sedoheptulose phosphate are enzymically interconvertible.<sup>23,24</sup>

It appears, then, that sedoheptulose phosphate may be converted successively to ribulose diphosphate and to triose phosphate, with the liberation of  $C_2$  fragments which are themselves, or may be converted to, the  $CO_2$  acceptor. The sedoheptulose phosphate itself could arise by an aldolase reaction between triose phosphate and a tetrose, presumably erythrose, produced by reduction of a secondary carboxylation product. The operation of this cycle may be as illustrated in Figure (12).

The  $C_4$  secondary carboxylation product would be in rapid equilibrium with malic acid. In confirmation of this scheme it has been found that when the sedoheptulose phosphate from short term experiments is degraded (by periodate oxidation of the sedoheptulosan derived from it), the  $C_4$  atom is more radioactive than for a uniformly labeled compound (Table 2).

The data in Table 3 are a striking indication of the rapidity with which the sedoheptulose phosphate becomes labeled.

Other schemes involving a  $C_7 \rightarrow C_5 \rightarrow C_3 \rightarrow C_7$  cycle are, of course, possible, as well as one involving the  $C_5$  or  $C_7$  compounds as reservoirs of reactive  $C_2$  fragments. The necessity of a second carboxylation reaction for the production of a  $C_4$  compound can be obviated by a  $C_6 \rightarrow C_4 + C_2$  reaction, the  $C_4$  then entering into the  $C_7$  cycle as in Figure (12). (cf. Gaffron et al.<sup>3</sup>).

The exact nature of the  $C_2$  fragment is, as yet, unknown.

For an understanding of the results of kinetic experiments, it is necessary to know the steady state concentrations of several key metabolites, and the way in which these are affected by the variables just mentioned. In preliminary experiments using Scenedesmus grown in radioactive phosphate buffer, it appears that variations in light intensity and  $CO_2$  pressure have a profound effect on the relative quantities of organic phosphates in the cell. These results are now being directed towards the design of kinetic experiments whose conditions will conform more exactly to the study of the path of carbon during steady state photosynthesis.



Table 2  
 Degradation of Sedoheptulosan  
 Percentage of Radioactivity in C<sub>4</sub>

Sample	% Activity	No. of Expts.
Calc. for Equal Labeling	14.3	--
5 Min. Soy Bean	14	2
20 Sec. Soy Bean	21	1
10 Sec. Soy Bean	28	3

Table 3  
 10 Second Photosynthesis Soy Bean.  
 Percentage Radioactivities (C<sup>14</sup>)

Compound	Percent
Phosphoglyceric acid	32
Sedoheptulose phosphate	24
Fructose phosphate	19
Triose phosphate	8
Glucose phosphate	6
Pentose phosphate	5
Diphosphates	4
Phosphoenol pyruvate	3

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CAPTIONS TO FIGURES

- Figure 1 - Radiogram of extract of Scenedesmus grown for 20 hours in radioactive phosphate and allowed to photosynthesize for 5 minutes in  $C^{14}O_2$ . A sheet of exposed film was used to filter out the  $C^{14}$  radiation.
- Figure 2 - Radiogram of same extract as in Figure 1. The exposure was made after the phosphorus radiation had decayed.
- Figure 3 - Radiogram of cold alcohol extract of Chlorella which had photosynthesized for 60 seconds in  $C^{14}O_2$ .
- Figure 4 - Identification of ribose monophosphate.
- Figure 5 - Identification of ribulose monophosphate.
- Figure 6 - Phosphodihydroxyacetone identification.
- Figure 7 - Radiograms of Scenedesmus exposed to radioactive phosphate for one minute. Light and dark.
- Figure 8 - Scheme for sucrose synthesis from phosphorylated intermediates.
- Figure 9 -  $C^{14}O_2$  fixation in the sugar phosphates and sucrose during photosynthesis by Scenedesmus at  $15^\circ C.$ <sup>6</sup>
- Figure 10 -  $C^{14}O_2$  fixation in the major soluble compounds during photosynthesis by Scenedesmus at  $15^\circ C.$ <sup>6</sup>
- Figure 11 -  $C^{14}O_2$  fixation in  $C_2$ - and  $C_3$ -compounds during photosynthesis by Scenedesmus at  $15^\circ C.$ <sup>6</sup>
- Figure 12 - Proposed carbon cycle for regeneration of two-carbon  $CO_2$  acceptor.

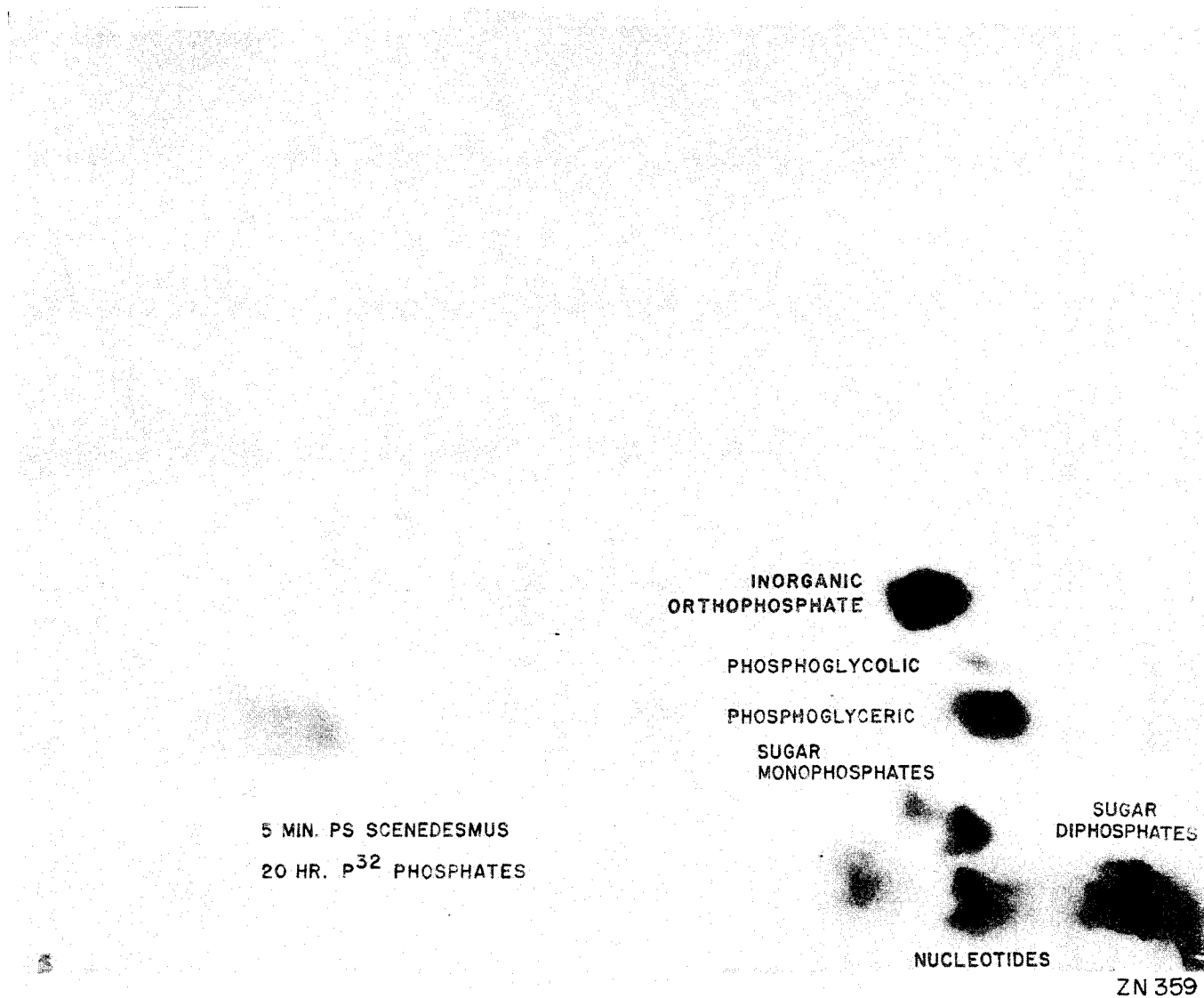


Fig. 1

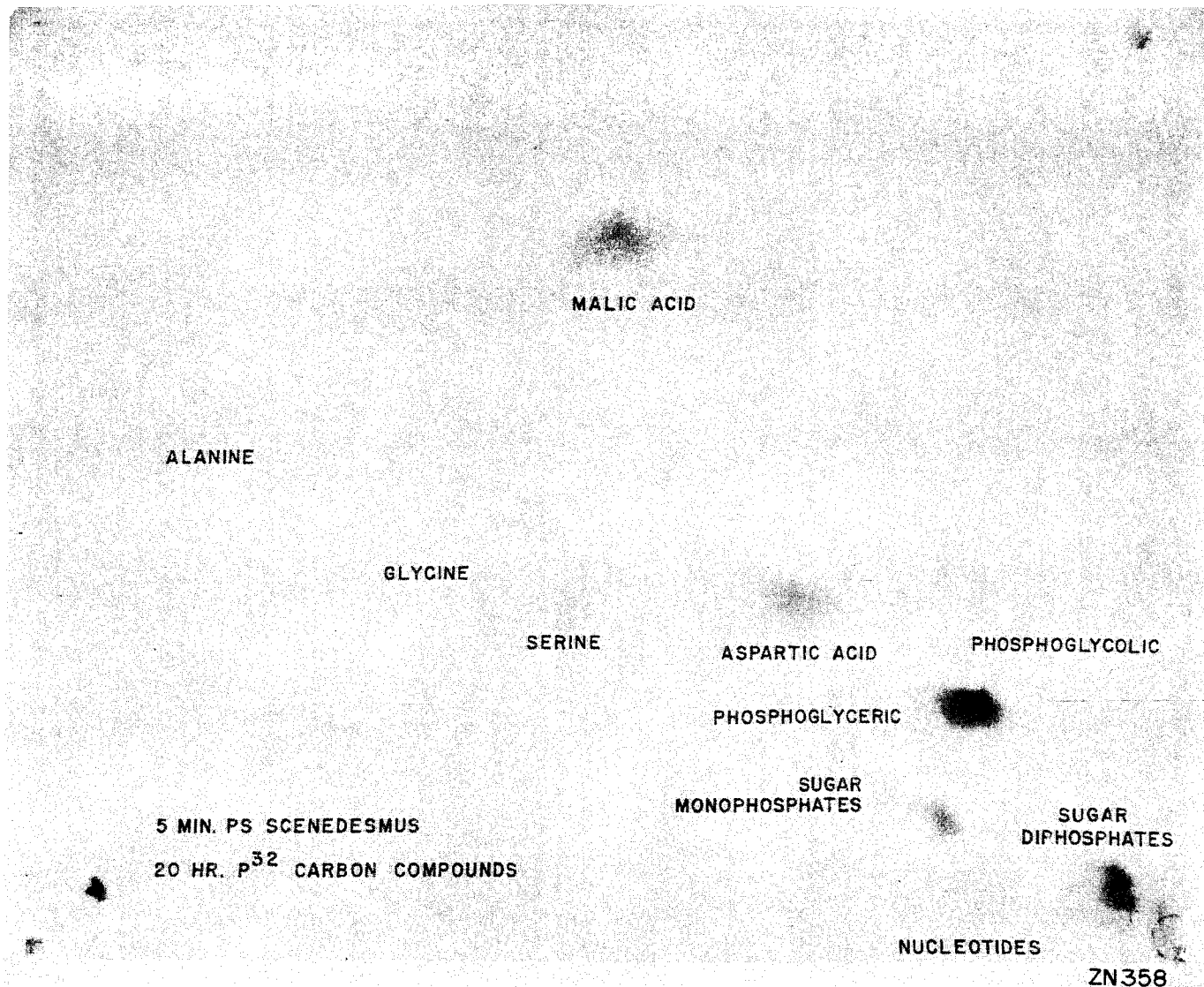


Fig. 2

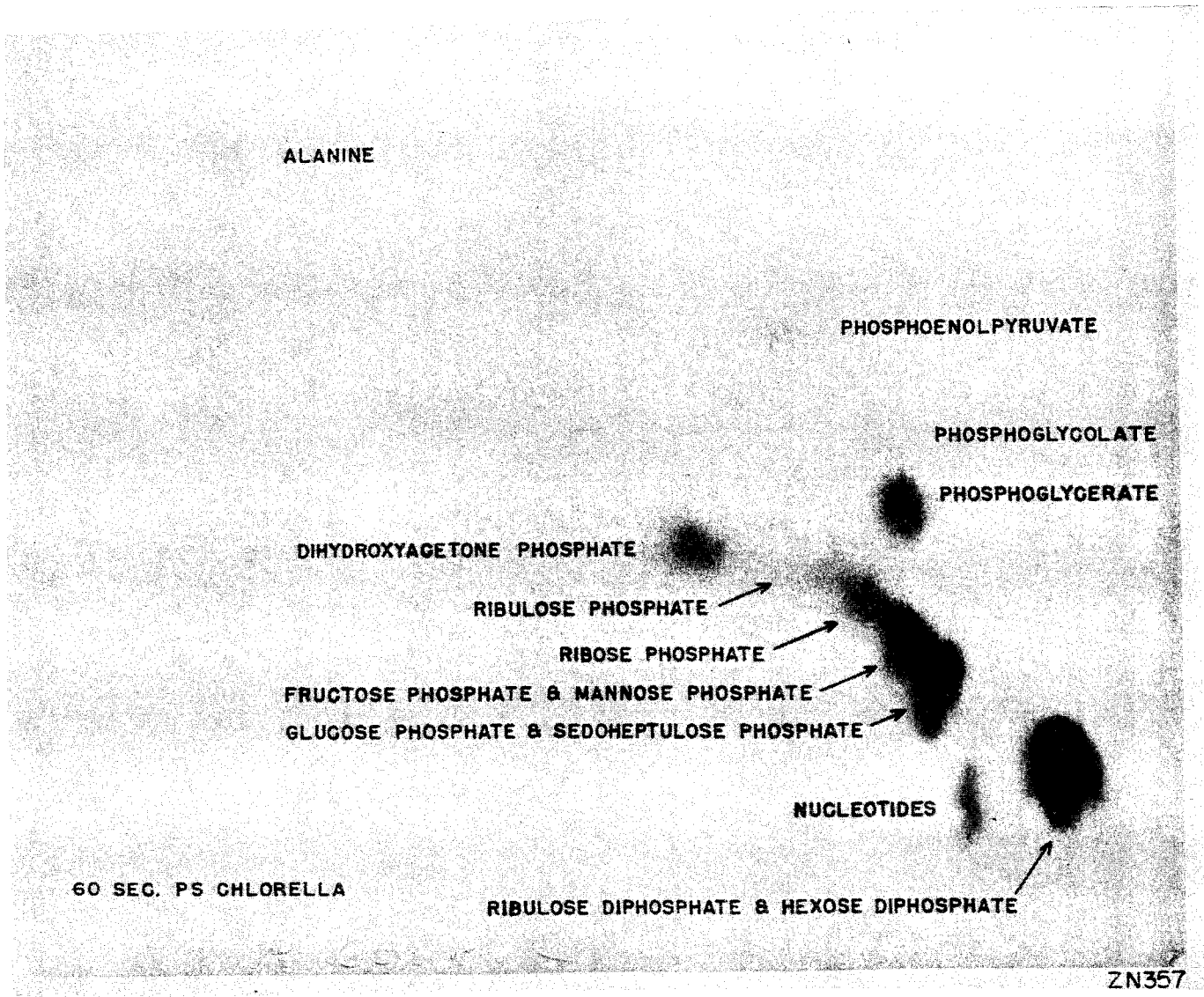


Fig. 3

CO-CHROMATOGRAPHY OF CARRIER SUGARS WITH PHOSPHATASED  
LABELED SUGAR PHOSPHATES FROM 60 SEC. PS CHLORELLA

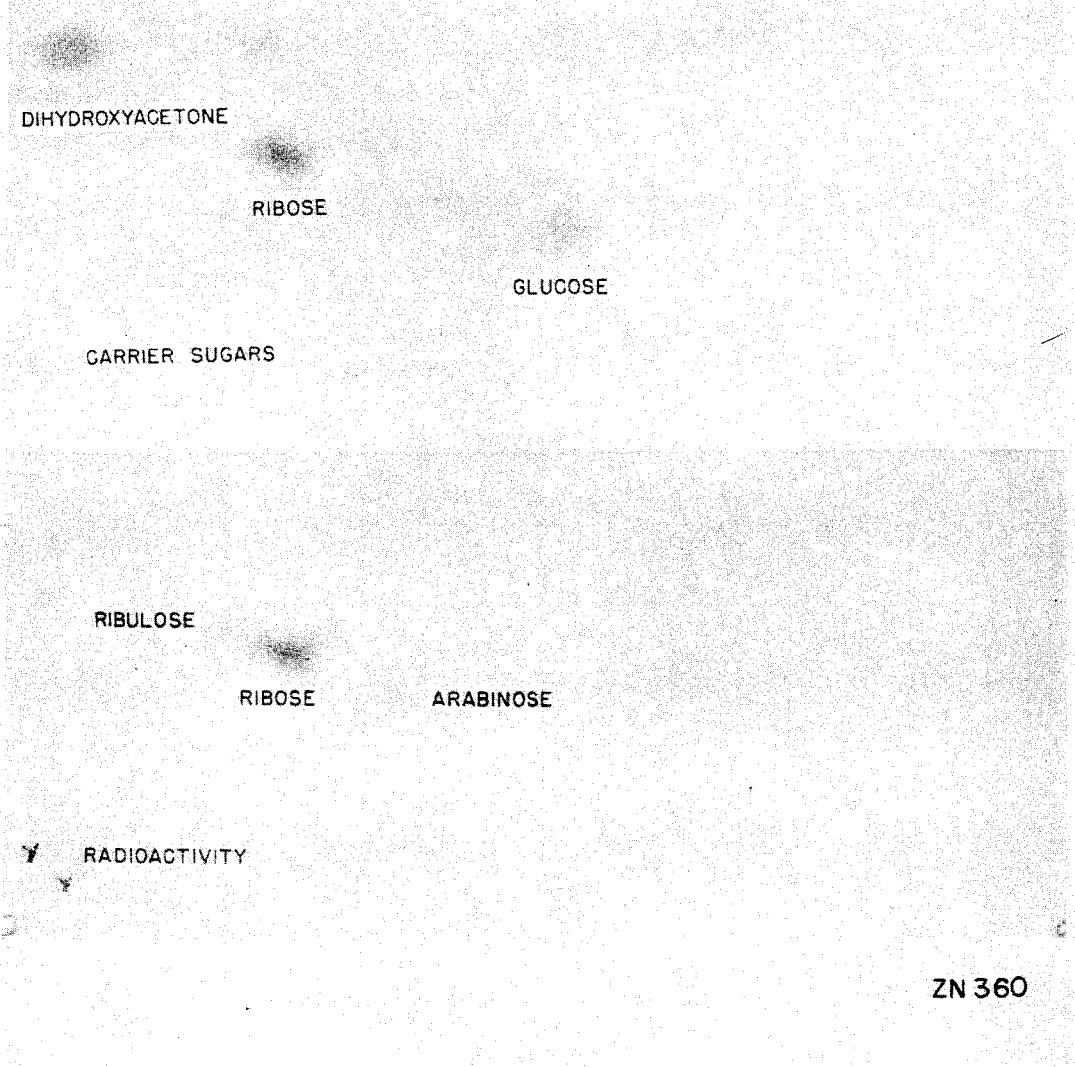


Fig. 4.



CO-CHROMATOGRAPHY OF CARRIER SUGARS WITH PHOSPHATASED  
LABELED SUGAR PHOSPHATES FROM 60 SEC. PS CHLORELLA

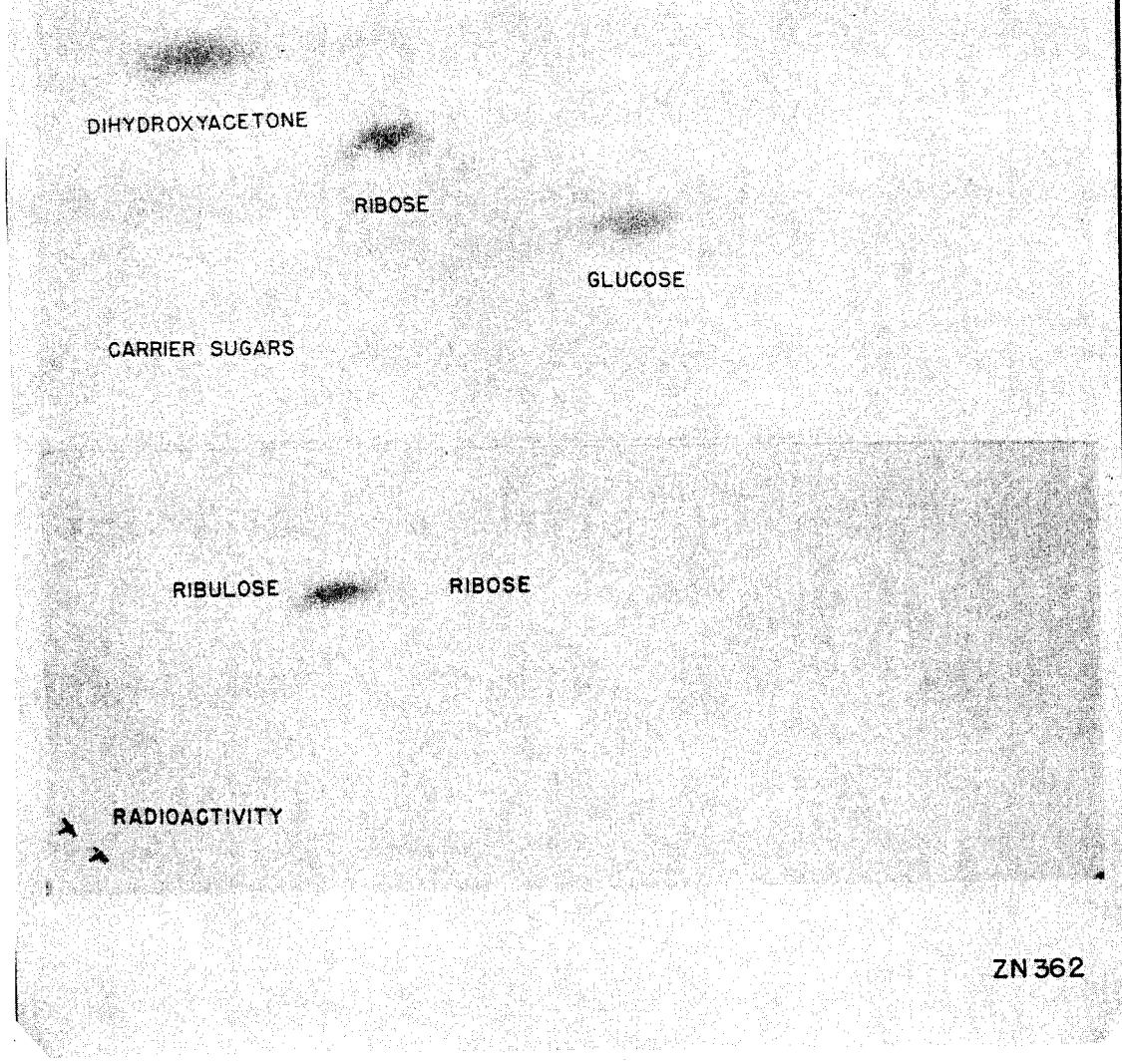


Fig. 5

CO-CHROMATOGRAPHY OF CARRIER SUGARS WITH PHOSPHATASED  
LABELED SUGAR PHOSPHATES FROM 60 SEC. PS CHLORELLA

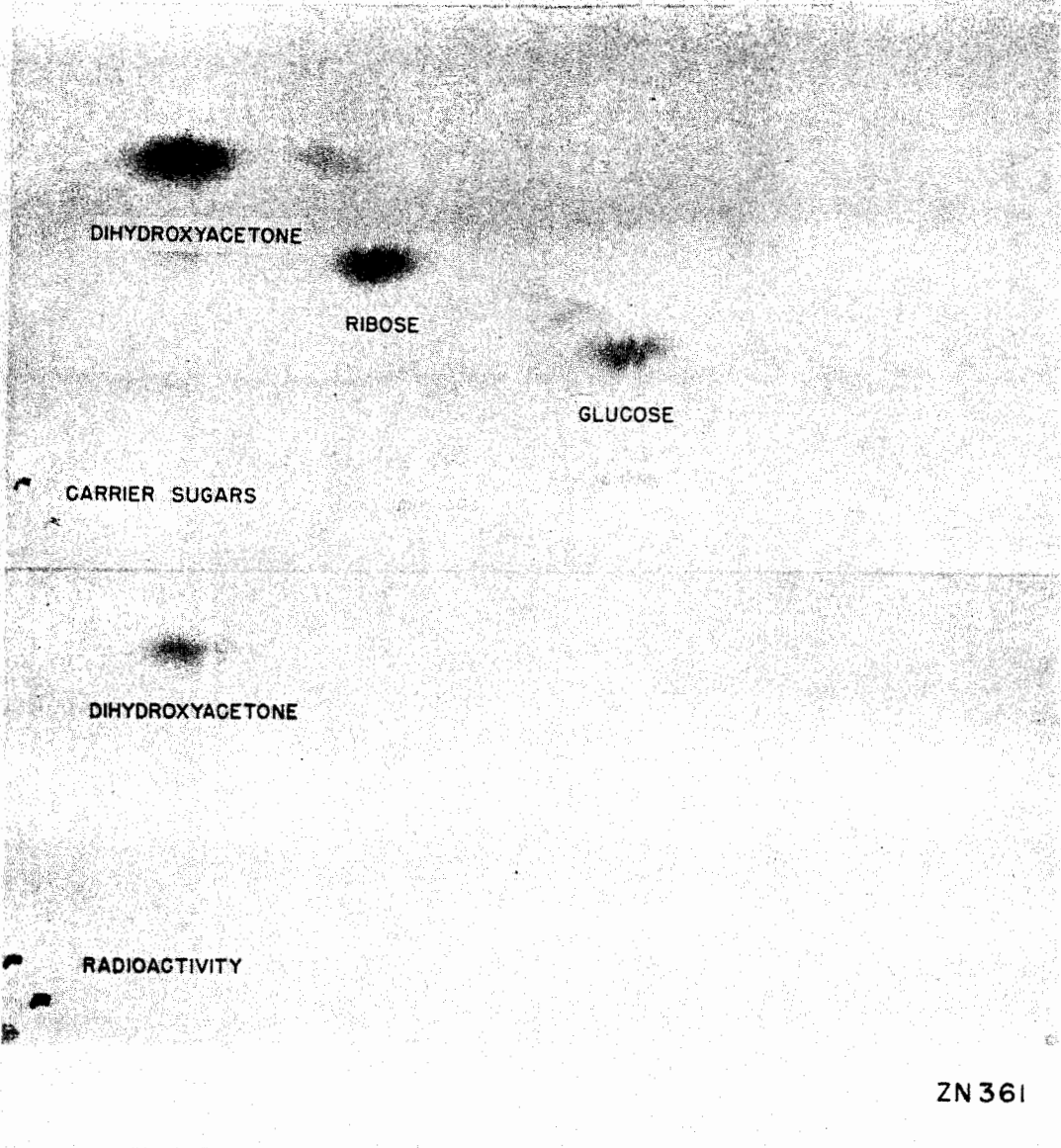
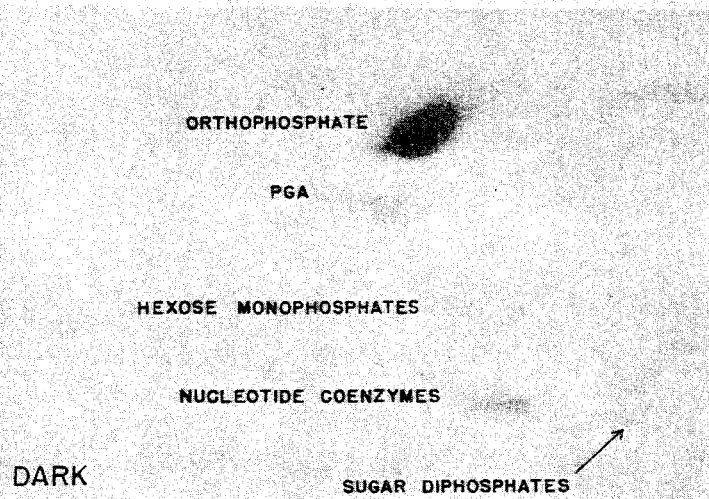
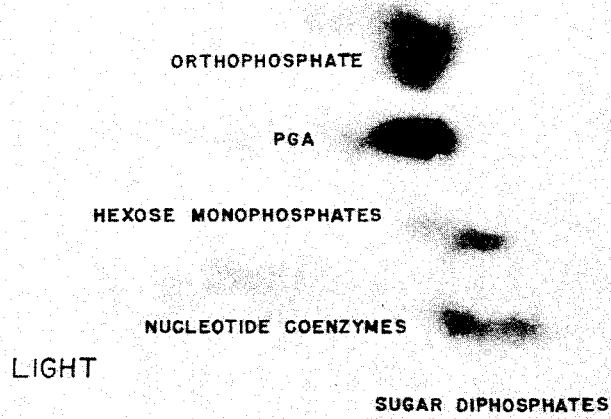


Fig. 6

FIXATION OF P<sup>32</sup> BY SCENEDESMUS  
IN ONE MINUTE



ZN363

Fig. 7



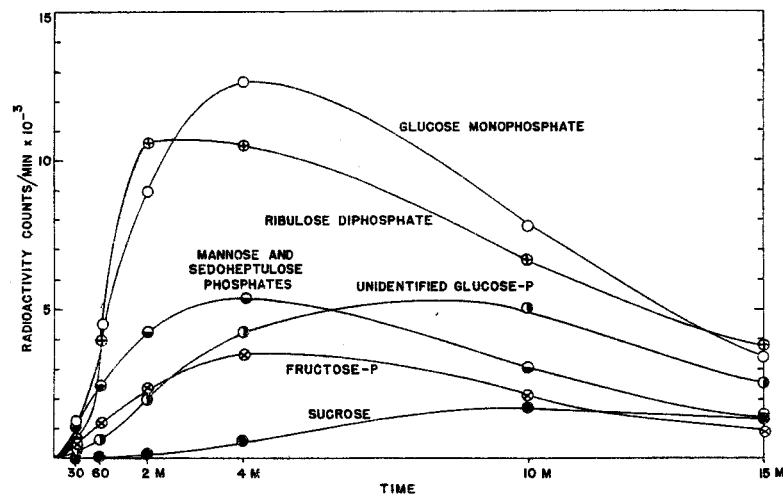


Fig. 9

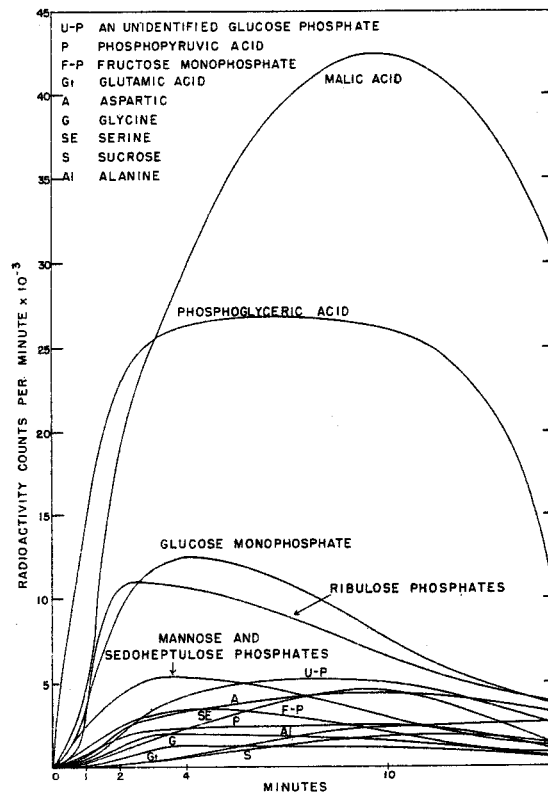


Fig. 10

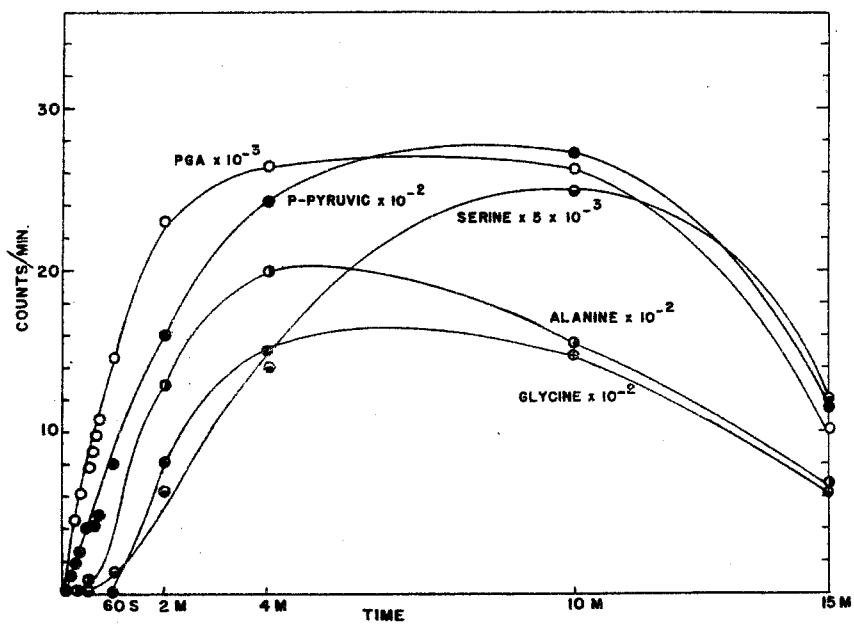
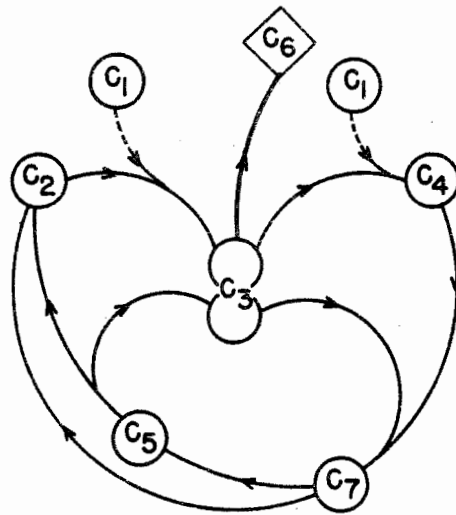


Fig. 11



PROPOSED CARBON CYCLE FOR REGENERATION  
OF TWO-CARBON CO<sub>2</sub> ACCEPTOR

Fig. 12