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THE PATH OF CARBON IN PHOTOSYNTHESIS. VII

RESPIRATION AND PHOTOSYNTHESIS

by

A. A. Benson and M. Calvin

Berkeley, California

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THE PATH OF CARBON IN PHOTOSYNTHESIS. VII RESPIRATION AND PHOTOSYNTHESIS*

by

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ABSTRACT

The relationship of respiration to photosynthesis in barley seedling leaves and the algae, Chlorella and Scenedesmus, has been investigated using radioactive carbon dioxide and the techniques of paper chromatography and radioautography.

The plants are allowed to photosynthesize normally for thirty seconds in $C^{14}O_2$ after which they are allowed to respire in air or helium in the light or dark.

Respiration of photosynthetic intermediates as evidenced by the appearance of labeled glutamic, isocitric, fumaric and succinic acids is slower in the light than in the dark.

Labeled glycolic acid is observed in barley and algae. It disappears rapidly in the dark and is maintained and increased in quantity in the light in CO_2-free air.

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* This work was sponsored by the United States Atomic Energy Commission.

ABSTRACT, Cont. - -

Radiograms of algal and barley extracts in which the C^{14} was reduced in the dark by preilluminated plants show the same compounds as produced in normal photosynthesis. This includes a considerable amount of sucrose.

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RESPIRATION AND PHOTOSYNTHESIS

A. A. Benson and M. Calvin

July 21, 1949

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

Previous work (1,2) has shown that illumination of an algal suspension in the absence of carbon dioxide greatly enhances its ability to fix carbon dioxide in an immediately following dark period. The kinetics of the generation and decay of this ability have been determined, and it was shown that less than one minute of illumination was sufficient to bring this fixing ability almost to its saturation value. Other experiments (3) which determined the dependence of dark fixation rate on carbon dioxide pressure showing it to be very similar if not identical with the dependence of steady state photosynthesis upon carbon dioxide pressure were used as a partial argument in support of the suggestion that the enhanced dark fixation immediately

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* This work was sponsored by the United States Atomic Energy Commission.

(1) M. Calvin and A.A. Benson, "The Path of Carbon in Photosynthesis I", Science, 107, 476, (1948).

(2) A.A. Benson, M. Calvin et al. Chapter 19, "Photosynthesis in Plants", Iowa State College Press, (1949).

(3) A.A. Benson and M. Calvin, "Cold Spring Harbor Symposia on Quantitative Biology", 13, 6, (1948).

following illumination in the absence of carbon dioxide was indeed the process of carbon dioxide reduction taking place in photosynthesis.

Nevertheless, kinetic arguments involving unknown chemical reactions being what they are, it was still conceiveably possible that this enhanced dark fixation was due only to the reversibility of the respiratory and fermentative decarboxylations. The photosynthesis occurring during pre-illumination presumably reduced the carbon dioxide partial pressure by reactions as yet unknown thus shifting the fermentative and respiratory reaction equilibria in the direction of decarboxylation. Upon the introduction of carbon dioxide in the dark, the equilibria are shifted in the opposite direction thus giving the enhanced fixation but by processes presumed to be quite different from those taking place in the light. (Figure 1.)

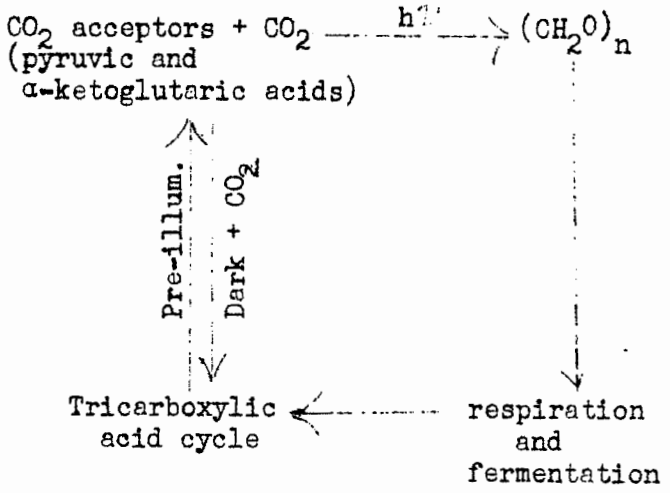

Figure 1.

If this be the case then the products formed in dark fixation of carbon dioxide should be the same following preillumination in the absence of

carbon dioxide as they are following a dark saturation with carbon dioxide, and quite different from those formed in the light. If the preilluminated dark fixation were the same process that takes place in ordinary photosynthesis, the products formed should be the same as those found in an equivalent period in the light and different from those formed in the dark without preillumination. If some of the compounds or closely related substances which appear in respiration and fermentation are also intermediates in photosynthesis, they should be formed in all three cases.

Figure 2 is a set of radiograms showing the products formed in each of the three cases for algae and for barley leaves. (See 30s. PS.C., 2m.D.PIC., 45m.D.C., 30s.PS.B., 2m.D.PIB., 50m.D.B. in Table I.) It is apparent that the products formed in dark fixation following preillumination in the absence of carbon dioxide correspond very closely to those formed in direct photosynthesis and not to all of those formed in dark fixation following dark saturation with carbon dioxide. These latter are indeed the substances expected from the simple reversibility of respiratory and fermentative reactions. We can thus confirm the suggestion, at first made on the basis of kinetic studies (3), that all of the reactions lying between carbon dioxide and sucrose are dark reactions. The reducing energy required to achieve this transformation is supplied by the photochemical reaction involving the photolysis of water. Furthermore the reducing power so provided is in the form of a definite chemical species rather than in the form of some excited electronic state of a molecule. This conclusion has been reached previously as a result of

TABLE I

PERCENTAGE DISTRIBUTION OF RADIOACTIVITY AMONG AQUEOUS ALCOHOL-SOLUBLE NON LIPID PRODUCTS

	Alanine	Aspartic	Glutamic	Glutamine	Glycine	Serine	Succinic	Fumaric	Glycolic	Isocitric (citric?)	Malic	Sucrose	Phosphate Esters
30s.PS.C.[a]	6.1	0.8				1.3			0.5		0.9	0.5	89[l]
2m.D.PI.C.[a]	39	5.5	0.4			7.3	0.4			0.2	2.4	6.7	38
45m.D.C.[a]	11	8.7	23	1.5		4.0	17	7.5		1.9	21	1.2	1.2
30s.PS.B.[b]	1.1	0.1			12	4.9			8.3		0.5	15	59[m]
2m.D.PI.B.[a]	33	3.4	1.2			2.2					4.9	1.9	53[n]
50m.D.B.[a]	2.2	28[z]	17	8.2		4.7				6	31		2.9
30s.PS.-150s.L.[c](He)C	23	17	0.4			3.8					8.4	27	21[p]
30s.PS.-150s.D.(He)C[d]	27	3.6	3.1			4.5		0.9		0.5	3.4	7.2	50[q]
30s.PS.-150s.L.C.[e]	8.0	6.1	0.5		4.1	8.3			6.7	0.6	6.7	31	25[r]
30s.PS.-150s.L.Sc.[f]	1.2	39	1.5		0.9	3.7			3.7	2.2	25	1.9	56[s]
5m.PS.C.[g]	1.9	3.0	0.4			2.0	0.2			0.1	1.9	23	65[t]
30s.PS.-2m.L.B.[h]	2.2	1.4			7.4	6.7			23		2.3	45	11[u]
30s.PS.-2m.D.B.[b]	4.6	0.8			0.7	17			0.2	0.5	0.7	34	42[v]
30s.PS.-40m.D.B.[i]	6.4	1.6	2.3	1.4		7.3				0.8	2.1	62	16[w]
60s.PS.B.[j]	7.7	2.6			3.3	3.7			2.0		3.3	27	50[x]
5m.PS.B.[k]	8.1	0.7	0.8		4.5	4.3			6.4	0.2	2.6	65	6[y]

TABLE I Cont. - -

Percentages were determined by direct counting of areas on the paper chromatogram defined by the radiogram.

S.-seconds, m.-minutes, PS.-photosynthesis with $C^{14}O_2$, C.-Chlorella pyrenoidosa, Sc.-Scenedesmus D-3, L.-light, D.-dark, B.-barley seedling leaves, PI.-5-10 min. preillumination in helium, He.-helium.

All treatments subsequent to photosynthesis were done in CO_2-free air unless specified as in He.

[a] In these experiments less than 5% of the total radioactivity fixed was insoluble in alcohol-water. [b] 2.5% insol. [c] 25% insol. [d] 6% insol. [e] 30% insol. [f] 30% insol. [g] 45% insol. [h] 10% insol. [i] 6% insol. [j] 3.2% insol. [k] 4.5% insol. [l] includes - Phosphoglyceric acid 24 (PGA), Hexose monophosphates 60 (HMP), Hexose diphosphates 3.9 (HDP), Triose-phosphates 0.5 (TrP). [m] includes free glyceric acid 10. [n] includes free glyceric acid 2. [p] includes PGA 1.3, HMP 14, HDP 4, P-Pyruvic 1.8. [q] includes PGA 6, HMP 35, HDP 7, P-Pyruvic 1.8. [r] includes PGA 3.2, HMP 13, HDP 7. [s] includes PGA 25, HMP 11, HDP 18, P-Pyruvic 2.2. [t] includes PGA 26, HMP and HDP 39, P-Pyruvic 0.6. [u] includes free glyceric acid 0.7. [v] includes free glyceric acid 15. [w] includes free glyceric acid 9. [x] includes free glyceric acid 6. [y] includes free glyceric acid 0.6. [z] includes asparagine 2.

studies of fluorescence (4) and of the comparative biochemistry of certain bacteria. (5) The continued carbon dioxide absorption in the dark immediately following a strong illumination (6,7) constitutes the direct observation of the effect. It is of interest to note that the successful separation of the photochemical apparatus for the splitting of water and the evolution of oxygen (8) from the carbon dioxide reducing system has been accomplished.

Figure 2 shows that a number of radioactive compounds are common to all three of the radiograms. These are particularly alanine, serine, aspartic acid and malic acid. They may be taken as indicators for the presence of the corresponding keto-acids, pyruvic and oxaloacetic acids under all three circumstances. It is thus apparent that some compounds are involved as intermediates in both the photosynthetic and the respiratory cycles. Whether the common reservoirs of the two cycles are identical or are physically separate in the organism remains to be determined.

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(4) E. Katz and E.C. Wassink, Enzymologia, 6, 152 (1939).

(5) Van Niel, C.B., Adv. Enz. 1, 263 (1941)

(6) E.D. McAllister and J. Myers, Smithsonian Instn. Publ. (Misc. Coll.), 99, No. 6 (1940).

(7) R. Emerson and C.M. Lewis, Am. J. Botany, 28, 789, (1941).

(8) R. Hill, Nature, 139, 881 (1937).
R. Hill and R. Scarisbrick, Nature, 146, 61, (1940).

A number of experiments have been done in an attempt to determine something about the relationship between the photosynthetic and respiratory paths. A simple design for such an experiment would be to introduce the label into some of the photosynthetic intermediates by a short period of photosynthesis in radioactive carbon dioxide (9) and follow this by a suitable period of respiration in either dark or light. The result of such an experiment on algae is shown in Figure 2. One effect is immediately apparent. In those cases in which the algae have not been given a dark period either during or after exposure to C^*O_2 there is little or no labeled glutamic and citric (iso) acid formed, (see 30s.PS.C., 30s.PS.-150s.L.(He)C. and 5m.PS.C., Table I.) If, however, they are given as little as 150 sec. of dark time following 30 sec. of photosynthesis in C^*O_2, relatively large amounts of labeled glutamic and citric (iso) acids appear. (See 30s.PS.-150s.D.(He)C. Table I). If the dark period is aerobic the amounts of these compounds which appear are somewhat larger than under anaerobic conditions. It is thus clear that the light not only initiates a series of reactions constituting the photosynthetic reduction of carbon dioxide but also in some way inhibits certain other reactions. Although the nature of the inhibition is as yet unknown, it is clear that light

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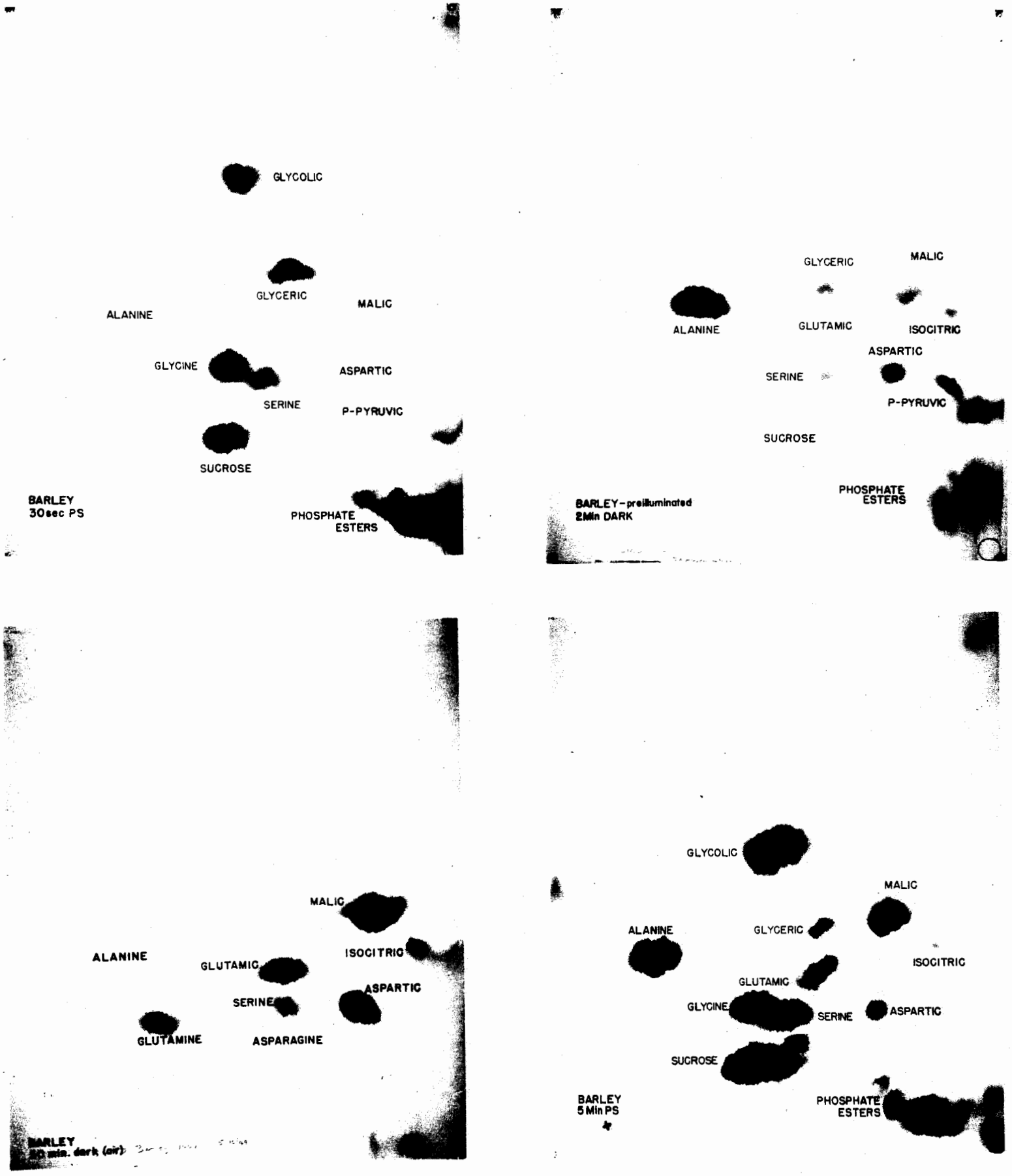
(9) The experimental methods have been described previously. M. Calvin and A.A. Benson, Science, 109, 140, (1949); "The Path of Carbon in Photosynthesis V. Paper Chromatography and Radioautography of the Products." A.A. Benson et. al. J.Am. Chem. Soc. in press.

prevents some of the newly formed photosynthetic intermediates from participating in the tricarboxylic acid cycle as represented by glutamic and (iso) citric acids. In the barley leaves the formation of labeled glutamic acid is so slight even after relatively long dark periods that we cannot yet say whether the same phenomenon occurs or not. It is indicated by a comparison of the iso-citric contents of 30s.PS.B., 5m.PS.B., 30s.PS.-2m.L.B. and 30s.PS.-2m.D.B. in which the last contains more of this acid than any of the first three. In the barley experiments another effect can be seen, which involves the conditions under which labeled glycine and glycolic acid are found. Glycine and glycolic acid, formed in the light from radiocarbon dioxide, disappear in as little as two minutes in the dark. They appear in detectable amounts in as short a period as five seconds of photosynthesis in radiocarbon dioxide and are maintained in the light in the absence of carbon dioxide with the glycolic acid increasing. (see 30s.PS.B., 30s.PS.-2m.D.B., and 30s.-2m.L.B.) The same effect has been observed with algae. Additional experiments with algae showed that the presence of oxygen during the subsequent illumination enhanced the formation of labeled glycine and glycolic acid. In the case of glycine for which the total amount present may be estimated from the amount of the ninhydrin color produced on the paper, it appears that the short dark periods reduce not only the amount of labeled glycine but the total glycine as well. This would seem to indicate that the size of the reservoirs of free glycine and presumably of glycolic acid vary considerably with illumination and may possibly be related to the photosynthetic path of carbon. Degradation studies now under way should lead to a detailed exposition of the sequence of compounds involved.

C^{14} Radiograms of Extracts of Plants Exposed to $C^{14}O_2$ Under Selected Conditions.

Figure 2.



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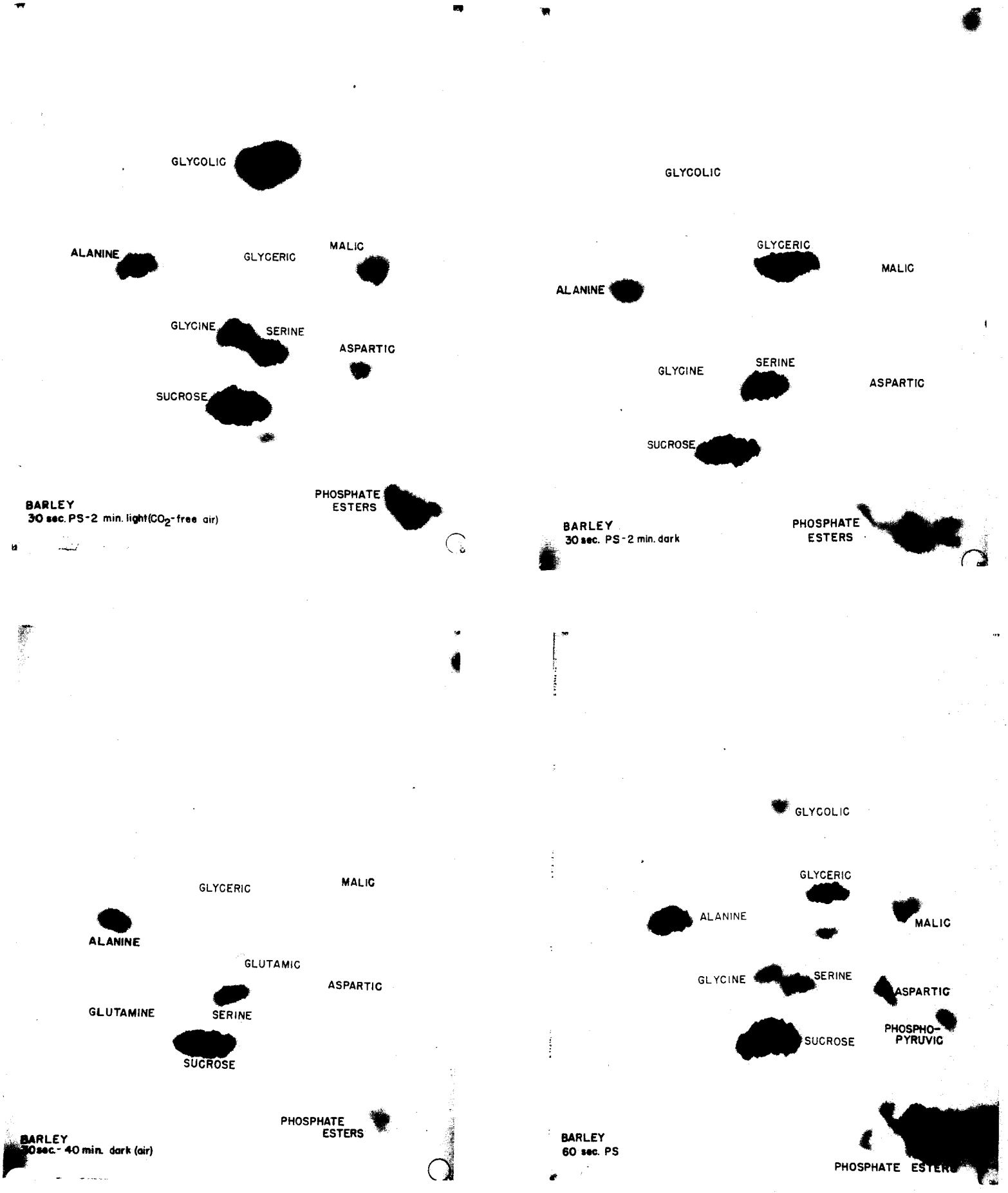

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