Localization of Carboxydismutase & Triosephosphate Dehyrogenases in Chloroplasts [1, 2]

U. Heber, N. G. Pon, & M. Heber

Institute of Agricultural Botany, University of Bonn, Germany, & Lawrence Radiation Laboratory, University of California, Berkeley, California

Isolated chloroplasts form labeled sugar phosphates in the light in the presence of $C^{14}O_2$ and suitable cofactors (1, 4, 5, 15, 30). They contain, therefore, all the enzymes needed for the uptake and reduction of CO_2 and the transformation of the resultant compounds (22). It is not known, however, whether the photosynthetic formation of sugars (or sugar phosphates) in the leaf cell is confined to the chloroplast or proceeds in chloroplast and cytoplasm alike. In the latter case a specific function of the chloroplast would consist in the light dependent formation of ATP and TPNH[3]. Enzymes of photosynthetic dark reactions would then be distributed throughout the plasm. In the former case at least some of these enzymes could be expected to be located exclusively in the chloroplasts.

The following article deals with the localization of three enzymes of carbohydrate metabolism, namely carboxydismutase[4] and the TPN and DPN-dependent triosephosphate dehydrogenases. In the experiments a nonaqueous method of chloroplast isolation has been employed (6, 7, 18, 31) since chloroplasts isolated by one of the common aqueous methods are exposed to leaching of enzymes (19) and other substances (32). In contrast to these latter methods, a diffusion of hydrophilic compounds such as enzymes, sugars, amino acids etc. from their original location within the cell does not take place in the frozen dried tissue preparations used in the nonaqueous procedure (Heber, unpublished results). Thus this procedure is well suited for localization studies. It has been employed previously to determine the distribution of enzymes (19, 31), sugars (18), amino acids (20), and inorganic ions (32) between chloroplasts and the remainder of the leaf cell.

Material

Mature leaves of *Tetragonia expansa* Murr, *Nicotiana Tabacum* L., *Secale cereale* L., *Trifolium repens* L., *Vicia faba* L., ssp. minor, and *Spinacia oleracea* L. were obtained directly from the field. Spinach sometimes was purchased at a local market.

Methods

Chloroplast Isolation. The procedure is described in detail elsewhere (18) and consists briefly of the following steps:

I. Freeze-drying of leaves, frozen quickly in liquid air or dry ice and petroleum ether, at −20 to −25° in vacuo.

II. Grinding of the dry leaves in petroleum ether/CCl_4 (20/80 v/v) in a cooled Waring blendor for 5 minutes.

III. Centrifugation of the broken material at different densities of the organic solvent (different ratios petroleum ether/CCl_4) and collection of the chloroplast-containing fractions.

It was important to apply further purification steps in addition to the normal procedure to obtain pure preparations (21). The chloroplast isolation was performed mostly at 0° and only in few cases at room temperature. In the former case, very little chlorophyll was extracted (or destroyed) by the organic solvent (0–5 %). In the latter case, more chlorophyll was lost (up to 25 %). The results of chlorophyll determinations of the fractions were corrected for the loss of chlorophyll due to extraction as follows: The total chlorophyll content of all the fractions was compared with that of the starting material. From this the percent loss of chlorophyll due to extraction into petroleum ether/carbon tetrachloride and due to destruction could be calculated. Values were then corrected assuming proportionate losses in all fractions.

Fractions Used for Enzyme Determinations. Enzymes were determined in the chloroplast fraction, in unfractionated tissue and in chloroplast-depleted tissue, which is the material remaining after the removal of the chloroplast fraction. Since yields of chloroplasts were in the order of 15 to 50 % in our isolation procedure, chloroplast-depleted tissue contained between 50 and 85 % of the chloroplasts of intact tissue.

---

[1] Received Sept. 17, 1962.

[2] This work was sponsored in part by the United States Atomic Energy Commission and in part by the Deutsche Forschungsgemeinschaft.

[3] Abbreviations: TPN and TPNH, oxidized and reduced triphosphopyridine nucleotide, respectively; PGA, phosphoglyceric acid.

[4] Carboxydismutase is synonymous with ribulose-1,5-diphosphate carboxylase.

Comparable conditions were maintained by treating all fractions as much as possible in a similar manner. Time of exposure to organic solvents, temperature environments, and drying conditions were the same for all three fractions of a given leaf preparation. Chlorophyll was determined according to Arnon (2) and protein according to Lowry et al. (23), after appropriate checks with the Kjeldahl nitrogen procedure.

Enzyme Determinations. Carboxydismutase (25). Small test tubes contained a solution of the following in μmoles: tris, 15.0; $MgCl_2$ 2.0; cysteine 0.5; ribulose-1,5-diphosphate, ca. 0.4; and $NaHC^{14}O_3$, 0.5 (8 μc). The pH was 8.0. The reaction was started by adding 100 μl of solution containing ca. 30 μg of protein, and terminated by adding 50 μliters of 50 % CCl_3COOH after 10 minutes at room temperature. C^{14}-PGA was counted after spreading an aliquot on an aluminum planchet (11). All assays were performed in duplicate or in triplicate. Results are expressed in counts per minute of labeled PGA per mg protein in the reaction time. The reproducibility was better than ±5 % in most cases.

TPN-Dependent Triosephosphate Dehydrogenase. An optical assay method was used (8, 34). The reaction mixture contained in a final volume of 2.5 ml in μmoles: tris, 100; $MgSO_4$, 20; cysteine, 13; glutathione, 2.6; PGA, 22.5; ATP 2.75; and 0.2 mg protein. The pH was 7.5. The reaction was started by adding 0.4 μmole of TPNH. The same mixture was used as control except that PGA was omitted. Readings were made every 30 seconds at 340 mμ. The decrease of the optical density in the first minute of the reaction was used to calculate the reaction rate. Since the addition of phosphoglycerate kinase did not stimulate the reaction but was slightly inhibitory, it was assumed that endogenous phosphoglycerate kinase was available in excess. No phosphoglycerate kinase was added, therefore, in the routine determinations. The TPNH consumption of the controls did not exceed 1.5 μmoles/mg of protein/hour. Results are expressed in Bücher units per mg of protein. One Bücher unit equals a turnover rate of 0.03 μmole coenzyme/100 seconds or 1.08 μmoles/hour at 25°. The reproducibility of the results was better than ±3 %.

DPN-Dependent Triosephosphate Dehydrogenase. The same procedure as described above was used except that TPNH was substituted by DPNH. Pyruvate kinase was determined according to Bücher and Pfleiderer (10).

Calculations. The chloroplast protein/total protein ratio of leaf cells and the yield of chloroplasts from the dried leaves are easily calculated from the available chlorophyll and protein data. Since the chlorophyll is located entirely in the chloroplasts the fraction of the chloroplast protein present in the total protein of a cell is given by the equation $x/y = z$, where x is the average chlorophyll content of the total protein and y the chlorophyll content of the protein of the isolated chloroplasts. Similarly, the percent yield of the isolated chloroplasts (w) is calculated from the equation $100 \cdot u/v = w$, when u is the total amount of chlorophyll in the isolated chloroplasts and v is that in the tissue prior to the isolation procedure.

The specific activity of the cytoplasm is determined in two ways: A, by comparing the specific activity of chloroplast protein with that of intact tissue, and B, by comparing the specific activity of chloroplast protein with that of chloroplast-depleted tissue. A. In the equation $z \cdot a + (1 - z)\, b = c$, where a is the specific activity of chloroplasts, c is that of intact tissue, and z is the ratio of chloroplast protein to total protein, only the specific activity of the cytoplasm b is unknown. B. The equation $(z - z \cdot w/100)\, a + (1 - z)\, b = d(1 - z \cdot w/100)$ is a modification of equation A and allows comparison of the activity of chloroplasts with that of chloroplast-depleted tissue obtained from the same isolation procedure. The specific activity of chloroplast-depleted tissue is designated by d.

If the specific activities of the proteins in cytoplasm and chloroplast are known, the distribution of an enzyme within the cell can be calculated. The value $100\, z \cdot a / [z \cdot a + (1 - z)b]$ is the percentage of the total enzyme content of the cell present in the chloroplast fraction.

However, the chloroplast fraction was not pure. Therefore, the obtained values were corrected where necessary, for a cytoplasmic contamination of the chloroplast fraction. This was done as follows: The percentage of the total pyruvate kinase of the cell present in the chloroplast fraction was determined as shown above. This percentage was considered a direct measure of the cytoplasmic contamination of the chloroplast fraction (19). Evidence will be presented in a later publication that pyruvate kinase is not present in chloroplasts in vivo. Then z (chloroplast protein/total protein) was corrected for the cytoplasmic contamination of the chloroplast fraction yielding a lower value.

Using the original value of z and the experimentally obtained values of a, c, and d, the specific activity of the cytoplasmic protein was determined for the enzyme whose distribution was under investigation. The (calculated) specific activity of cytoplasmic protein is not affected by a cytoplasmic contamination of the chloroplast fraction. We then inserted the corrected value of z in the equations A and B. Using the specific activities of cytoplasm (as calculated), intact tissue and chloroplast depleted tissue (as determined experimentally), we obtained by recalculating corrected values of the specific activity of chloroplasts a. The corrected specific activity of the chloroplasts and the corrected value of z were then used to calculate the percentage of the total enzyme content of the cell present in the pure chloroplasts.

The above equations are derived without regard for the localization of activators and inhibitors within specific parts of the cell. For aldolase the specific occurrence of an inhibitor in non-chloroplastic parts of the cell has been reported (19, 31).

Results

Carboxydismutase. The activity of carboxydismutase in different cell fractions is shown in table I. From these values and additional data on the protein distribution within the cell and the yield of chloroplasts, the localization of carboxydismutase

Table I

Activity of Carboxydismutase in Different Cell Fractions
One unit carboxydismutase = 10^5 counts/minute labeled PGA/mg protein per 10 minutes incubation.

Experiment no.	Material	Chloroplasts	Intact tissue	Chloroplast depleted tissue
1	Nicotiana	20.3	10.5	...
2	Nicotiana	0.93	0.6	...
3	Tetragonia	10	8	...
4	Tetragonia	23.2	14.1	11.7
5	Spinacia	9.0	5.7	...
6	Spinacia	20.2	14.7	11.8
7	Spinacia	11.2	7.5	6.7
8	Spinacia	8.4	8.5	5.8
9	Spinacia	20.2	19.2	14.6

was calculated using methods A and B (table II). Results of method A differ considerably, but in experiments 1, 2, and 7 practically all of the carboxydismutase was detected in the chloroplasts while in all cases the major part of carboxydismutase was found there. Sometimes, however, the calculated specific activity of the cytoplasm (experiments 3, 6 & 8, method A) suggests that in fact not all of the carboxydismutase is located in the chloroplasts and that a relatively low level is maintained also in the cytoplasm.

Another explanation is possible, however. During freeze-drying and subsequent treatment with organic solvent, carboxydismutase loses part of its activity. The chloroplasts were exposed to grinding, centrifugation, and frequent changes of solvent. These conditions were different from those experienced by the intact tissue. Hence, in some cases, the chloroplasts lose more activity than the intact tissue and, therefore, an apparently high content of carboxydismutase in the cytoplasm results.

Table II

The Localization of Protein & of Carboxydismutase Activity in the Chloroplasts As Compared With the Whole Leaf Cell

Experiment no.	Material	% chloroplast protein in total cell protein	% of the total carboxydismutase activity present in chloroplasts A*	B**
1	Nicotiana	56	110	...
2	Nicotiana	61	95	...
3	Tetragonia	61	77	...
4	Tetragonia	54	89	100
5	Spinacia	57	90	...
6	Spinacia	58	80	93
7	Spinacia	64	96	98
8	Spinacia	73	72	96
9	Spinacia	67	...	88

* Calculated according to method A.
** Calculated according to method B.

While intact tissue is not derived from the same preparation as the chloroplast fraction, chloroplast-depleted tissue is obtained in the same procedure. Therefore, we compared the activity of chloroplasts with that of chloroplast-depleted tissue and calculated the specific activity of the cytoplasm according to method B (table II, B). The results indicate higher percentages of carboxydismutase in the chloroplasts than those calculated by method A for the same experiments. It amounts in most cases to practically 100 %. It can, therefore, be concluded that carboxydismutase is located mainly in the chloroplasts. A very low level of carboxydismutase in the nonchloroplastic part of the plasm cannot, however, be ex-

Table III

Activity & Distribution of TPN-dependent Triosephosphate Dehydrogenase in Different Cell Fractions

Experiment no.		1 Trifolium	2 Secale	3 Vicia	4 Spinacia	5 Spinacia
% Chloroplast protein in total protein		80	68	76	70	61
Specific activity*						
in chloroplasts		44	50.5	26.8	40	36
in intact tissue		38.5	31.6	22.1	26.5	28.5
in chloroplast-depleted tissue		33.6	28.2	16.6	26.1	21.5
in cytoplasm	A**	15.5	0.0	7.1	0.0	17
	B***	3.5	0.0	0.0	5.3	9.7
Yield of isolated chloroplasts in % of total chloroplast content of tissue		30.2	29	50	36	47
% of the total dehydrogenase activity present in chloroplasts	A**	92	109	94	106	77
	B***	98	105	102	95	85

* Specific activity in Bücher units/mg protein.
** Calculation performed according to method A.
*** Calculation performed according to method B.

cluded, especially since some plant species contain in the nonchloroplastic part of the cell inhibitors of CO_2 fixation which may or may not mask the action of carboxydismutase (21).

TPN-Dependent Triosephosphate Dehydrogenase. Experiments showing the activity of TPN-dependent triosephosphate dehydrogenase in different cell fractions and the results of calculations are listed in table III. The calculation shows as in the case of carboxydismutase that the TPN-dependent triosephosphate dehydrogenase is located in the chloroplasts. The amount of TPN-triosephosphate dehydrogenase calculated for the cytoplasm lies within the limits of experimental error. A possible exception is experiment 5, where spinach was purchased from a local market. This material might have been damaged.

DPN-Dependent Triosephosphate Dehydrogenase. Table IV shows the results of determinations of DPN-dependent triosephosphate dehydrogenase in different cell fractions. Contrary to the results obtained in the case of the two other enzymes (tables I & III), the specific activity is lowest in the chloroplasts and highest in the chloroplast-depleted tissue. The calculations reveal that approximately 50 % of the enzyme is located in the chloroplast fraction. However, the chloroplast fraction was not pure and the contamination with cytoplasm had to be taken into account. The amount of pyruvate kinase present in the chloroplast fraction served as indicator of a cytoplasmic contamination (19, see also Methods). Considering this contamination, between 20 and 40 % of the total amount of DPN-dependent triosephosphate dehydrogenase was found in the chloroplasts (table IV).

Discussion

The method described above gives direct qualitative and, under certain circumstances, quantitative evidence for the localization of substances in chloroplasts or cytoplasm. When the specific activity of an enzyme in the tissue increases after removal of chloroplasts, this particular enzyme may be located preferentially in the cytoplasm. When the specific activity decreases, the enzyme may be located mainly in the chloroplasts. No change in the specific activity may indicate an equal distribution within the cell plasm.

A contamination of the isolated chloroplasts by cytoplasm does not affect the results of quantitative localization studies when the enzyme is located entirely in the chloroplasts. Since the chloroplasts are compared with the remainder of the cell, even contaminated chloroplasts contain all the activity. When the enzyme occurs in chloroplasts as well as in the nonchloroplastic part of the cell plasm or in the latter only, then contamination of the chloroplasts leads to inaccurate results and the purity of the chloroplast preparation becomes of particular importance. The purity of isolated nonaqueous chloroplasts has been considered in detail previously (19–21, 31–33). A rough criterion for the purity of a chloroplast preparation of leaves is a chloroplast protein to total protein ratio from 0.55 to 0.65 in mature leaves while higher ratios may indicate contamination with cytoplasmic protein. A better criterion is given by enzymic measurements: an enzyme not present in vivo in the chloroplasts and found in the preparation is a direct measure of a contamination (19).

The question of whether or not the chloroplasts were pure is of limited interest in the case of carboxydismutase and TPN-dependent triosephosphate dehydrogenase, since practically all the enzyme was found to be located in the chloroplasts and a contamination does not influence the final result. In the case of DPN-dependent triosephosphate dehydrogenase the influence of a contamination shall be considered more closely. The equations for calculating the enzyme distribution define the cytoplasm here as a nonchloroplastic part of the cell, while the

Table IV

Activity & Distribution of DPN-Dependent Triosephosphate Dehydrogenase in Different Cell Fractions

Experiment no.		3 Vicia	4	6	7 Spinacia	8
% chloroplast protein in total protein		76	70	60	62	67
% chloroplast protein in total protein, corrected		69	60	50	57	59
Specific activity*						
chloroplast fraction		12.8	14.1	15.8	17.4	10.0
chloroplasts (corrected)		6.9	10.8	14.0	15.3	6.3
intact tissue		15.4	19.2	18.3	19.9	18.1
chloroplast-depleted tissue		16.6	21.5	20.0	25.0	19.5
cytoplasm	A**	23.6	31.2	22.0	26.6	34.0
	B***	21.5	32.4	22.8	32.4	30.2
Yield of isolated chloroplasts in % of total chloroplast content of tissue		50	36	55	40	45
% of the total dehydrogenase activity of the cell present in chloroplasts	A**	36	36	40	43	22
	B***	30	33	37	39	22

* Specific activity in Bücher units/mg protein.
** Calculation performed according to method A.
*** Calculation performed according to method B.

chloroplast fraction covers chloroplasts plus a certain amount of cytoplasmic contamination. Therefore the specific activity of the chloroplast fraction does not necessarily reflect the specific activity of chloroplasts in their morphological sense, while the calculated specific activity of the cytoplasm is not influenced by a contamination of the chloroplasts with cytoplasm, but only by the normal limits of error of the applied methods. This served as the basis for elimination of the influence which cytoplasmic contamination of the chloroplast fraction would exert on the final result. We first determined the specific activity of the cytoplasmic proteins using data uncorrected for cytoplasmic contamination of the chloroplast fraction. By recalculating with corrected values, we then obtained the percentage of enzyme present in pure chloroplasts. Thus between 20 and 40 % of the total amount of DPN-dependent triosephosphate dehydrogenase of the leaf cell was found to be located in the chloroplasts.

Many workers have studied the localization of enzymes inside the cell. Only in few cases, however, was the quantitative distribution determined, since most experiments used subcellular particles isolated in an aqueous medium. Thus only a minor part of the total carboxydismutase content of the leaf was recovered in the chloroplasts after their isolation from buffered solutions (25, 26, 28). Using a non-aqueous procedure (31), however, Smillie and Fuller (28) found that the activity of carboxydismutase parallels the chlorophyll content of preparations obtained in the fractionation of frozen dried leaves.

Nothing is known so far on the quantitative localization of triosephosphate dehydrogenases, although the TPN-linked enzyme was found only in green plant parts (14, 16) or autotrophic organisms (13). Arnon (3) considered at first that the enzyme was located in the cytoplasm, but Rosenberg and Arnon (27) later found part of the enzyme in the chloroplasts. Brawerman and Konigsberg (9) showed parallel formation of chlorophyll, chloroplasts, and TPN-linked triosephosphate dehydrogenase in Euglena cells and discussed a possible induction of the synthesis of this enzyme by TPNH. Their results indicated the localization of a considerable fraction of the TPN enzyme in the cytoplasm.

From our experiments we conclude, on the other hand, that TPN-dependent triosephosphate dehydrogenase as well as carboxydismutase are located entirely or nearly entirely in the chloroplasts of mature leaf cells. Carboxydismutase catalyzes the uptake of CO_2 into ribulose diphosphate to form PGA. TPN-dependent triosephosphate dehydrogenase can subsequently reduce PGA after its phosphorylation to the triose level. The localization of both enzymes in the chloroplasts is further evidence for their importance in photosynthesis. At the same time the results suggest that at least part of the photosynthetic dark reactions occur exclusively in the chloroplasts.

Losada, Trebst, and Arnon (22) found about equal amounts of DPN- and TPN-dependent triosephosphate dehydrogenases in isolated chloroplasts, although only in minor amounts. From their data approximately 0.2 Bücher units of these enzymes per mg protein can be calculated for the chloroplasts as compared with values ranging from 10 to 50 Bücher units per mg chloroplast protein in our experiments. Only the TPN enzyme was effective in bringing about the reductive fixation of $C^{14}O_2$ in the light. On the other hand, Smillie and Fuller (29) found predominantly the DPN-dependent enzyme in photosynthetic bacteria. They concluded that this enzyme plays a major role in bacterial photosynthesis. Our calculations reveal for leaves of Vicia and Spinacia the presence of about 30 % of the total DPN enzyme of the cell in the chloroplasts.

The reaction rates of the examined enzymes in frozen dried tissue preparations can be calculated from the data in tables I, III, and IV. The highest activities of carboxydismutase observed in chloroplasts of Nicotiana, Tetragonia, and Spinacia leaves were about $2 \cdot 10^6$ counts/min per mg of protein in 10 minutes or ca. 2 μmoles CO_2 uptake per mg of protein in an hour, corresponding to 30 μmoles/mg of chlorophyll per hour. The loss of activity due to freeze-drying and treatment with organic solvents was not determined for carboxydismutase, but amounts in the case of other enzymes to ca. 30 to 50 %. If this applies also for carboxydismutase about 60 μmoles CO_2 uptake/mg of chlorophyll per hour are catalyzed by carboxydismutase under the reaction conditions. This value is to be compared with a maximal photosynthetic CO_2 uptake of 100 to 200 μmoles/mg of chlorophyll per hour by the intact leaf (17, 35). Other workers, however, have found higher values for carboxydismutase (24).

Activities of TPN-dependent triosephosphate dehydrogenase observed in chloroplasts of Trifolium, Secale, Vicia, and Spinacia leaves were in the range of 30 to 50 Bücher units/mg chloroplast protein. This is equivalent to ca. 450 to 750 μmoles of PGA reduced/mg chlorophyll per hour without regard for enzyme destruction due to freeze-drying and organic solvent. Apparently TPN-linked triosephosphate dehydrogenase is available in large excess as compared with the maximal efficiency of photosynthesis in the intact leaf.

Reaction rates of DPN-dependent triosephosphate dehydrogenase are considerably less than half that of the TPN enzyme in chloroplasts, but amount in the cytoplasm up to more than 30 μmoles of PGA reduced/mg of protein per hour.

Summary

A method has been developed to determine quantitatively the distribution of enzymes between the chloroplasts and the remainder of the cell. It is shown that carboxydismutase and TPN-dependent triosephosphate dehydrogenase are located entirely or nearly entirely in the chloroplasts. DPN-dependent triosephosphate dehydrogenase occurs in chloroplasts as well as in the cytoplasm, but in the latter place at a higher concentration. Only about 30 % of the total amount of the DPN enzyme of the cell were found in the chloroplasts.

Acknowledgment

Two of us (U. H. & M. H.) wish to thank Professor Melvin Calvin for providing the laboratory facilities with which part of this work was completed.

Literature Cited

1. Allen, M. B., D. I. Arnon, J. B. Capindale, F. R. Whatley, & L. J. Durham. 1955. Photosynthesis by isolated chloroplasts. III. Evidence for complete photosynthesis. J. Am. Chem. Soc. 77: 4149–55.
2. Arnon, D. I. 1949. Copper enzymes in isolated chloroplasts. Polyphenoloxidase in *Beta vulgaris*. Plant Physiol. 24: 1–15.
3. Arnon, D. I. 1952. Glyceraldehyde phosphate dehydrogenase of green plants. Science 116: 635–37.
4. Arnon, D. I., M. B. Allen, & F. R. Whatley. 1954. Photosynthesis by isolated chloroplasts. Nature 174: 394–96.
5. Arnon, D. I. 1960. The chloroplast as a functional unit in photosynthesis. In: Encyclopedia of Plant Physiology, Ruhland, W., ed. Vol V, I: 773–829. Berlin.
6. Behrens, M. 1938. Zell- und Gewebetrennung. In: Handbuch biol. Arbeits-Methoden, Abderhalden, ed. 1363–96. Berlin.
7. Behrens, M. & R. Thalacker. 1957. Gewinnung von Chloroplasten in nicht-wässrigem Milieu. Naturwiss. 44: 621.
8. Beisenherz, G., H. J. Boltze, Th. Bücher, R. Czok, K. H. Garbade, E. Meyer-Arendt, & G. Pfleiderer. 1953. Diphosphofruktose-Aldolase, Phosphoglyzeraldehyd-Dehydrogenase, Milchsäure-Dehydrogenase, Glyzerophosphat-Dehydrogenase, und Pyruvat-Kinase aus Kaninchenmuskulatur in einem Arbeitsgang. Z. Naturforschg. 8 b: 555–77.
9. Brawerman, G. & N. Konigsberg. 1960. On the formation of the TPN requiring glyceraldehyde-3-phosphate dehydrogenase during the production of chloroplasts in *Euglena gracilis*. Biochim. Biophys. Acta 43: 374–81.
10. Bücher, Th. & G. Pfleiderer. 1955. Pyruvate kinase from muscle. In: Methods in Enzymology, S. P. Colowick & N. O. Kaplan, eds. New York. vol I, 435–40.
11. Calvin, M., C. Heidelberger, J. C. Reid, B. M. Tolbert, & P. E. Yankwich. 1949. Isotopic Carbon. New York.
12. Crane, F. L. 1961. Structure & Function of mitochondria. Ann. Rev. Plant Physiol. 12: 13–34.
13. Fuller, R. C. & M. Gibbs. 1950. Intracellular & phylogenetic distribution of ribulose-1,5-diphosphate carboxylase & d-glyceraldehyde-3-phosphate dehydrogenases. Plant Physiol. 34: 324–29.
14. Gibbs, M. 1952. Triosephosphate dehydrogenase & glucose-6-phosphate dehydrogenase in the pea plant. Nature 170: 164–65.
15. Gibbs, M. & M. A. Cynkin. 1958. The conversion of carbon-14 dioxide to starch glucose during photosynthesis by spinach chloroplasts. Nature 183: 1241–42.
16. Hageman, R. H. & D. I. Arnon. 1955. Changes in glyceraldehyde phosphate dehydrogenase during the life cycle of a green plant. Arch. Biochem. Biophys. 57: 421–36.
17. Hamdorf, G. 1959. Experimentelle Untersuchungen zur Erfassung der maximalen photosynthetischen Leistung bei Landpflanzen. Flora 147: 521–52.
18. Heber, U. 1957. Zur Frage der Lokalisation von löslichen Zuckern in der Pflanzenzelle. Ber. Dt. Bot. Ges. 70: 371–82.
19. Heber, U. 1960. Vergleichende Untersuchungen an Chloroplasten, die durch Isolierungsoperationen in nicht-wässrigem und in wässrigem Milieu erhalten wurden. I. Ermittlung des Verhältnisses von Chloroplastenprotein zum Gesamtprotein der Zelle. II. Kritik der Reinheit und Fermentlokalisationen in Chloroplasten. Z. Naturforschg. 15 b: 95–109.
20. Heber, U. 1962. Untersuchungen zur Physiologie und Biochemie der Chloroplasten im Zusammenhang mit der Proteinsynthese. Habilitationsschrift Bonn.
21. Heber, U. & E. Tyszkiewicz. 1962. The rate of photosynthesis in isolated chloroplasts. J. Exptl. Bot. 13: 185–200.
22. Losada, M., A. V. Trebst, & D. I. Arnon. 1960. Photosynthesis by isolated chloroplasts. XI. CO_2 assimilation in a reconstituted chloroplast system. J. Biol. Chem. 235: 832–39.
23. Lowry, O. H., N. J. Rosebrough, A. L. Farr, & R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193: 265–75.
24. Peterkofsky, A. & E. Racker. 1961. The reductive pentose phosphate cycle. III. Enzyme activities in cell-free extract of photosynthetic organisms. Plant Physiol. 36: 409–14.
25. Pon, N. G. 1960. Studies on the carboxydismutase system & related materials. Thesis, University of California UCRL-9373.
26. Racker, E. 1957. The reductive pentose phosphate cycle. I. Phosphoribulokinase & ribulose diphosphate carboxylase. Arch. Biochem. Biophys. 69: 300–10.
27. Rosenberg, L. L. & D. I. Arnon. 1955. The preparation & properties of a new glyceraldehyde-3-phosphate dehydrogenase from photosynthetic tissues. J. Biol. Chem. 217: 361–71.
28. Smillie, R. M. & R. C. Fuller. 1959. Ribulose-1,5-diphosphate carboxylase activity in relation to photosynthesis by intact leaves & isolated chloroplasts. Plant Physiol. 34: 651–56.
29. Smillie, R. M. & R. C. Fuller. 1960. Glyceraldehyde-3-phosphate dehydrogenases in plants & photosynthetic bacteria. Biochem. Biophys. Res. Commun. 3: 368–72.
30. Smillie, R. M. & G. Krotkov. 1959. Enzyme activities of subcellular particles from leaves. VI. Photosynthetic phosphorylation & photosynthesis by isolated chloroplasts from pea leaves. Canad. J. Bot. 37: 1217–25.
31. Stocking, C. R. 1959. Chloroplast isolation in nonaqueous media. Plant Physiol. 34: 56–61.
32. Stocking, C. R. & A. Ongun. 1962. The intracellular distribution of some metallic elements in leaves. Am. J. Bot. 49: 284–89.
33. Thalacker, R. & M. Behrens. 1959. Uber den Reinheitsgrad der in einem nichtwässrigen spezifischen Gewichtsgradienten gewonnenen Chloroplasten. Z. Naturforschg. 14 b: 443–46.
34. Warburg, O. & W. Christian. 1939. Isolierung und Kristallisation des Proteins des oxydierenden Gärungsfermentes. Biochem. Z. 303: 40–68.
35. Willstätter, R. & A. Stoll. 1918. Untersuchungen über die Assimilation der Kohlensäure. Berlin.