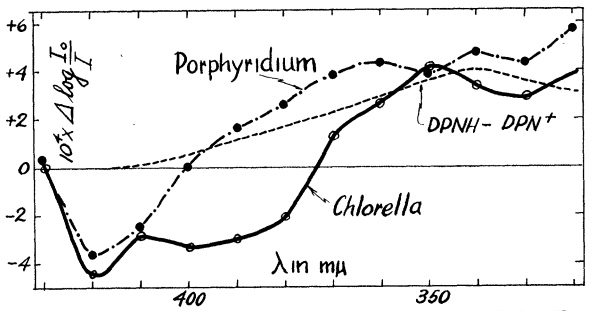

Fig. 2. Difference spectra of *Porphyridium* and *Chlorella*, light minus darkness, and difference spectrum of DPN. The optical density of the *Porphyridium* suspension corrected for scattering was 0.47 at 630 mμ, that of *Chlorella* 0.60 at 680 mμ.

The difference spectrum of *Chlorella* as well as that of *Porphyridium*, were measured for suspensions freshly prepared from growing cultures. Both spectra show an increase in absorption around 350 mμ (Fig. 2). The absorption of pyridine nucleotide increases upon reduction in the same region. Unfortunately the difference spectrum does not have several sharp peaks like the difference spectrum of the cytochrome pigments. Thus we can say only that the difference spectrums of the algae in the ultraviolet support the working hypothesis that upon illumination of photosynthesizing cells a pyridine nucleotide becomes reduced to be reoxidized within a few seconds in the dark.

The broadening of the peak at 420 mμ in the *Porphyridium* spectrum of Fig. 2, as compared with the corresponding peak in the spectrum of Fig. 1, may be caused by the different growth conditions or subsequent treatment of the culture. The decrease in absorption in the *Chlorella* spectrum around 390 mμ may be the result of the activity of the pigment or pigments that are responsible for the maximums at 480 and 520 mμ.

The optical density of the *Chlorella* suspension at 680 mμ corrected for scattering was 0.60. The measured optical density of chlorophyll and the change in optical density of pyridine nucleotide indicate that one molecule of nucleotide is reduced in light per about 100 chlorophyll molecules. When DPN^+ (diphosphopyridine nucleotide) was added to a final concentration of $10^{-4}M$ to a chloroplast suspension, in our apparatus a partial reduction of added DPN^+ could be observed. The ratio of reduced to oxidized form was about 1/2000. This explains why the reduction of DPN^+ by illuminated chloroplasts could not be detected before by conventional spectroscopy. This experiment shows, in a direct way, that DPN^+ can be reduced by illuminated chloroplasts, and confirms a conclusion previously reached in an indirect way (*12*).

The results of these experiments, combined with those of others, make plausible the following hypothesis, which can be tested by further experiments. Chlorophyll *a*, directly or indirectly excited by light, reacts with an oxidized pyridine nucleotide, PN^+, and an unknown oxidant, ZH, to give reduced pyridine nucleotide, PNH, and Z a strongly oxidizing compound. This reaction is followed by various dark reactions. Z is used for the greater part to oxidize water. Part of the Z oxidizes a cytochrome, presumably cytochrome *f*. The PNH also participates in two reactions. The main part is used for reduction of CO_2; a smaller part of the PNH is used for the reduction of the cytochrome (via intermediate enzymes). The oxidation of PNH by Z, mediated by the cytochrome, generates ATP to assist in the reduction of CO_2.

References and Notes

* Present address, Biophysical Research Group, Bijlhouwerstraat 6, Utrecht, Netherlands.

1. L. N. M. Duysens, "Transfer of excitation energy in photosynthesis," thesis, Utrecht (1952).
2. ———, *Carnegie Institution of Washington Year Book* **52**, 157 (1953).
3. ———, *Nature* **173**, 692 (1954).
4. ———, *Science* **120**, 353 (1954).
5. R. Hill and R. Scarisbrick, *New Phytologist* **50**, 98 (1951).
6. H. Lundegårdh, *Physiol. Plantarum* **7**, 375 (1954).
7. I take pleasure in thanking E. I. Rabinowitch, R. Emerson, and the University of Illinois for the courtesies and privileges extended to me. I am indebted to J. Coleman and J. Hoogeweg, whose assistance made the completion of this investigation possible in the short time available. This work has been carried out with the assistance of the U.S. Office of Naval Research.
8. H. E. Davenport and R. Hill, *Proc. Roy. Soc. London* **B139**, 327 (1952).
9. A. L. Lehninger and S. W. Smith, *J. Biol. Chem.* **181**, 415 (1949).
10. J. A. Bassham *et al.*, *J. Am. Chem. Soc.* **76**, 1760 (1954).
11. D. I. Arnon, M. B. Allen, and F. R. Whatley, communication to 8th International Botanical Meeting, Paris (1954).
12. W. Vishniac and S. Ochoa, *Nature* **167**, 768 (1951); L. J. Tolmach, *ibid.* **167**, 946 (1951); D. I. Arnon, *ibid.* **167**, 1008 (1951).

7 September 1954.

Carbon Dioxide Fixation by Roots

G. E. Graf and S. Aronoff

Department of Genetics and the Institute for Atomic Research and Department of Botany and Plant Pathology, Iowa State College, Ames

In the course of recent work we have had occasion to analyze the pattern of carbon dioxide fixation by roots, primarily the onion and soybean (*1*). Although this subject has been investigated (*2*) with barley roots, some differences in the distribution of radioactivity make it appear worth while to report our results.

Six to eight excised roots approximately 1 cm long were sealed in a vial containing 1 ml of distilled water and $NaHC^{14}O_3$ (0.125*M*). In a control experiment to test the possible effects of excision, onion roots about 10 cm long were passed through a glass tube bent at a 90° angle. The bend was sealed with mercury, permitting exposure of the roots alone to radiocarbon dioxide without removal of the roots from the bulb. The pattern of fixation of excised and attached roots proved to be identical. After 1 hr of exposure to the radioactive solution, the roots were dropped into boil-

ing 80-percent ethanol within the outer member of a homogenizer, ground with the inner member, and refluxed gently for 10 min. In some cases the roots were frozen and ground in liquid nitrogen prior to extraction, resulting in identical chromatograms. Following concentration in small glass cones, the extracts were transferred to Whatman No. 1 sheets for chromatography. Two-dimensional, ascending chromatograms were made, first using 80-percent phenol and then butyric acid, butanol, and water (2 : 2 : 1) as solvents.

In general, the *complete* coincidence of a spot eluted from the radioactive chromatogram upon two-dimensional chromatography with the pure compound was considered sufficient identification (*3*). However, additional proof was obtained for glyoxylic and glycolic acid by derivation. Carrier glyoxylate was added to the radioactive compound and the phenylhydrazone was prepared. The specific activities remained unchanged through three recrystallizations. Similarly, the specific activity of the *p*-nitrobenzoylate of glycolic acid remained unchanged.

A typical radiogram is shown in Fig. 1. As with barley, by far the most prominent spot was malic acid. Activity was also found in alanine, glutamine, glutamic, aspartic, citric, succinic, isocitric, glyoxylic, and glycolic acids. The presence of the last two compounds is somewhat unusual and generally not included in fixation or exchange reactions accompanying the tricarboxylic acid cycle. In contrast with Poel's results, no spot corresponding to tyrosine was found in any experiments with the onion or soybean, or in a single experiment with barley. There seems, indeed, to be no valid reason, at our present state of knowledge, to expect this compound to possess any appreciable activity. As was reported previously (*1*), the pattern of fixation was not altered by a number of drugs, even when respiration was strongly inhibited. Glyoxylic acid has been found in cell-free bacterial preparations (*4*), and a major role in metabolism has been postulated for this compound. The possibility that glycolic and glyoxylic acids are degradation products of our extraction and/or chromatographic techniques cannot be ignored, but they would have to result from compounds other than those found on our chromatograms.

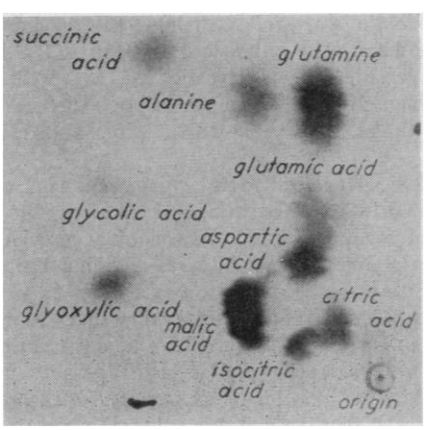

Fig. 1. A typical radiogram.

Poel's finding of the diminution of radioactivity in nutrient solution, as compared with distilled and tap water, is difficult to interpret. We have found that, on immersing soybean roots in increasing concentrations of KCl (0, $2.00 \times 10^{-4}M$, $2.00 \times 10^{-3}M$, and $2.00 \times 10^{-2}M$), there was an increase in radioactivity (1730, 2050, 3010, and 4610, respectively) (*5*). The stimulation of root respiration by increasing salt concentration is a well-known phenomenon. This increased rate of production of (nonradioactive) carbon dioxide is, of course, accompanied by an increased rate of production of organic acids partly capable of exchange and fixation. These effects will oppose each other in terms of the final radioactivity found—that is, whether increased respiration results in increased radioactivity will depend in large measure on the rate of enzymatic exchange and fixation as compared with the dilution of the specific activity of external carbon dioxide by the additional carbon dioxide produced within the roots. If the latter far exceeds the former, then it is conceivable that increased respiration will result in diminished activity. Other factors, however, such as the absolute increase in external carbon dioxide tension (which is reflected, then, in the ratio of root to solution volume), will have to be considered.

References and Notes

1. S. Aronoff and G. E. Graf, *Nature* **172**, 1043 (1953).
2. L. W. Poel, *Nature* **169**, 501 (1952); *J. Exptl. Botany* **4**, 157 (1953).
3. We are indebted to C. A. Hargreaves for the gift of isocitric lactone.
4. J. J. R. Campbell, R. A. Smith, and B. A. Eagles, *Biochim. et Biophys. Acta* **11**, 594 (1953); R. A. Smith and I. C. Gunsalus, *J. Am. Chem. Soc.* **76**, 5002 (1954).
5. We acknowledge with gratitude the assistance of A. Kuksis in this series of experiments.

22 October 1954.

Rapid Method for Determining Mean Values and Areas Graphically

S. I. Askovitz

Ophthalmology Research Laboratory,
Albert Einstein Medical Center,
Philadelphia, Pennsylvania

The observed data in many fields of scientific investigation are recorded on continuous graph paper, charted either manually or by various motor-driven devices. It is frequently necessary to determine the mean value of the recorded quantity from such a chart, or to estimate areas bounded by such graphs. A simple method (*1*) was devised for accomplishing either of these ends. This method requires only a pencil and ruler (or straightedge) and can be completed by means of a single broken line without lifting the pencil from the paper. The technique was originally