The fact that materials possessing the biological activity of α-lipoic acid can be recovered from diverse biological sources, coupled with the fact that α-lipoic acid has extremely high biological activity and possesses a catalytic role (in the oxidative decarboxylation of pyruvate), suggests that it is a new member of the family of B vitamins. Further research is in progress on its composition, structure, and biological activity.

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The Green Pigment and Physiology of Guard Cells

R. O. Freeland

Department of Botany,
Northwestern University, Evanston, Illinois

There is widespread uncertainty in the minds of botanists as to the composition and physiology of the green pigment located in plastids in the guard cells of most leafy plants. Sayre (*1*) concluded from his extensive study of the physiology of the stomates of *Rumex patientia* that the plastids in the guard cells are different structurally, physiologically, and genetically from the chloroplasts of the mesophyll cells. Using microchemical methods, he was unable to obtain a positive test for chlorophyll in the plastids of guard cells but stated that there is no conclusive proof that it is not chlorophyll, because of the extreme difficulty of making the test upon such small bodies.

The present paper deals with two problems related to guard cells, the determination of the absorption spectrum of the green pigments, and a test for photosynthesis in them.

After examining many plants, the leaves of *Hymenocallis littoralis,* Salisb., were found to be most suitable for investigation. In this species, large pieces of epidermis can be stripped from the leaves, and the presence of a heavy cuticle renders it relatively easy to smooth and straighten them for cleaning and inspection. The latter is important because freshly stripped pieces of epidermis were always found to have some fragments of mesophyll adhering to them. Each piece of epidermis, after being mounted on a glass plate, was scraped on the inner surface with a safety razor blade and then scrubbed with a soft bristle brush and water until it was free of all adhering cells and plastids from the mesophyll. Each strip of tissue was inspected under a microscope and if satisfactory was placed in a darkened test tube of cold acetone to which a pinch of $CaCO_3$ had been added. This procedure was continued at intervals over a period of a month or more until a 30-ml test tube full of loosely packed epidermal tissue was accumulated.

The plant material, plus a little quartz sand, was ground in the acetone with a mortar and pestle. After filtering, the acetone solution was light-green in color. The acetone extract was added to 50 ml of a petroleum ether-acetone mixture (10:1) in a separatory funnel. Gentle rotation and the addition of a small quantity of water brought about separation of the two solvent phases. The water-acetone layer was discarded, and the remaining petroleum ether was washed repeatedly with water. Then enough benzene was added to the petroleum ether to give a solution composed of 9 parts petroleum ether to 1 part benzene. This solution, containing the pigments, was passed, by gravity flow, through an adsorption column 1.5 cm in diameter, consisting from top to bottom of 20 cm powdered sugar, 5 cm $CaCO_3$, and 5 cm alumina. Finally, the column was treated with 10% benzene in petroleum ether in an attempt to resolve any possible components of the adsorbed pigment in a well-defined chromatogram. Only one pigment layer, blue-green in color, was visible in the column. After drying the column by suction with air, this layer was removed. The pigment was eluted from the sugar with a 1:1 solution of methanol and ethyl ether. The extract was freed of methanol by repeated water washing in a separatory funnel. The resulting ethyl ether containing the green pigment was studied immediately in a Coleman Junior spectrophotometer. The data on light absorption in terms of density are presented in Fig. 1. The entire experiment, from the collection of epidermal tissue to pigment analysis, has been repeated with the same qualitative results. For comparative purposes the pigments were extracted from the mesophyll tissues, separated, and studied in the same way. The chromatogram for the pigments from the mesophyll showed the usual distribution from top to bottom of the adsorption column of xanthophyll,

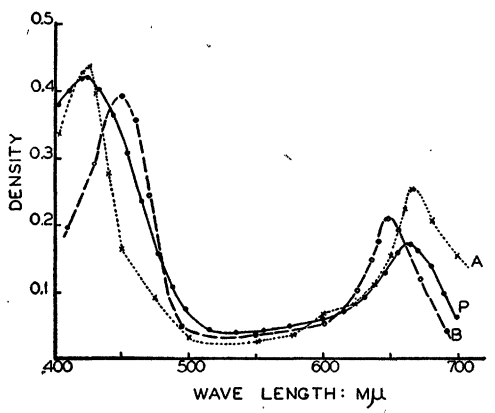

FIG. 1. Absorption data for ethyl ether solutions of the green pigments extracted from the leaves of *Hymenocallis. P,* pigment from the guard cells: *A* and *B*, chlorophylls *a* and *b*, respectively, from the mesophyll. (Density = − log Transmittance.)

chlorophyll *b*, chlorophyll *a*, and carotin. Absorption data for chlorophylls *a* and *b* are presented in Fig. 1.

Absorption spectra of chloroplast pigments in various physical states and in different solvents are well known. The principal absorption maxima for chlorophylls *a* and *b* in ethyl ether are 430 and 660 mμ, and 455 and 642 mμ, respectively (*2*). The chlorophylls from the mesophyll of *Hymenocallis* were found to have such absorption maxima (Fig. 1) and were unquestionably chlorophylls *a* and *b*. The green pigment isolated from the guard cells of the epidermal tissue has two absorption maxima, at 420–430 mμ and 660–670 mμ, indicating that it is predominantly chlorophyll *a*. The breadth of the absorption band below 475 mμ for the guard cell pigment, and the lack of complete conformity in shape between the density curves for the guard cell pigment and chlorophyll *a*, suggest the possibility that traces of either carotinoids or chlorophyll *b* or both were associated with the green pigment extracted from the guard cells. Although the developed chromatogram of the guard cell pigments showed only one visible band, it should be noted that the quantity of pigment being handled was quite small.

Numerous and varied experiments have been performed using luminous bacteria, *Photobacterium fischeri*, which glow in the presence of oxygen, to test for photosynthesis in guard cells. Each experiment consisted of three groups of culture vessels, test tubes, or van Tieghem cells, all of which contained active bacteria. One set of cultures contained in addition several strips of epidermal tissue; one set contained a small piece of *Elodea, Anacharis canadensis;* and one set was left without any chlorophyllous tissue. The circumambient solution consisted either of the liquid culture in which the bacteria were growing or of a solution of 0.1% $KHCO_3$, to which a high concentration of the bacteria had been added. After the culture vessels were sealed to exclude further access to air, they were placed in a darkroom, and the fluid in them was deoxygenated either by passing a slow stream of nitrogen gas through each or by the organisms themselves. After deoxygenation was completed, as indicated by the cessation of light from the cultures, the test material was exposed to light for various periods of time and then observed as quickly as possible in the dark. Exposures to several intensities of both natural and artificial (Mazda) light were tried. In every experiment the check cultures, containing *Elodea,* were luminous after exposure to light and the cultures without green tissues were not. Evolution of oxygen in the test cultures containing strips of epidermal tissue, as indicated by the bacteria becoming luminous, was not observed. In brief, all tests for photosynthesis in guard cells by this method were negative.

According to Curtis and Clark (*3*), Alvim through the use of the starch test has obtained evidence that photosynthesis occurs in the guard cells of bean plants. The presence of chlorophyll in the guard cells of *Hymenocallis* suggests but does not prove that photosynthesis takes place in them, since chlorophyllous plants are known which do not carry on photosynthesis (*4*). Failure to demonstrate photosynthesis in guard cells through the use of oxygen-sensitive, luminous bacteria does not prove conclusively that the process does not occur in them, for several reasons. In the cultures containing epidermal tissue there was, relatively, a very small number of green cells in comparison with the total number of nongreen cells. Use of oxygen by the latter may have made it impossible to detect any luminosity of the bacteria in the vicinity of the guard cells even with a microscope. Furthermore, submergence of the epidermal tissue plus the rather drastic treatment to which it was subjected in preparation for the tests may have inhibited photosynthesis.

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Growth of Human Leukemic Leucocytes *in Vitro* and *in Vivo* as Measured by Uptake of P^{32} in Desoxyribose Nucleic Acid[1,2]

Edwin E. Osgood, Jonah G. Li,[3] Harold Tivey, Marie L. Duerst, and Arthur J. Seaman

Department of Medicine,
Division of Experimental Medicine,
University of Oregon Medical School, Portland

By determination of uptake rates of radioactive phosphorus (P^{32}) into desoxyribose nucleic acid (DNA), it is possible to obtain a quantitative measure of the rate of formation of human leucocytes in culture (*1*), and by parallel determinations in the cell-donor patient with leukemia, treated with P^{32}, to compare the rate *in vitro* with that of the same population of leukemic cells in the patient. This information is otherwise unobtainable, since leucocyte death is occurring concurrently with cell division, both in the culture and in the patient. The rate of cell formation supplements the information on the rate of cell differentiation, obtainable from total and differential cell counts, and the rate of mitosis obtainable with colchicine. It is the purpose of this paper to present evidence for the initial statement, and to outline briefly the techniques used and the preliminary results.

[1] A preliminary report.

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[3] Present address: Division of Preventive Medicine, University of California School of Medicine, San Francisco.