Reports

Photochemical Activity of Digitonin Extracts of Chloroplasts

Digitonin, a nonionic detergent, has been used extensively to extract the photosensitive pigment-protein complex rhodopsin from the retinal rods of the eye. A light-dependent reaction, which is analogous to that occurring in the intact retina, can be measured spectrophotometrically with the extracted rhodopsin. This photochemical reaction "bleaches" rhodopsin and results in a shift of the absorption peak (from about 500 mμ to about 365 mμ). In the dark, rhodopsin is reformed, and this reaction is accompanied by a shift to the original absorption peak. Similarly, a photosynthetic pigment-protein complex, referred to as "chloroplastin" (*1, 2*), can be extracted by digitonin from plant chloroplasts, which appears to be homogeneous in the analytical ultracentrifuge and on electrophoresis. When chloroplastin is bleached by light, no shift in spectrum similar to that which occurs in the case of rhodopsin is observed. Chloroplastin bleaches at a rate proportional to the total amount of photo- and thermal energy absorbed, with a resultant steady decrease in optical density and with the disappearance of the maximum chlorophyll absorption peak at 675 mμ; it is bleached to unknown products (*3*).

Experimental attempts were made to promote with chloroplastin some photochemical reactions analogous to those of photosynthesis (*4*). The chloroplasts were isolated from *Euglena*, as has been previously described (*1*), and extracted with recrystallized, freshly prepared 2-percent aqueous digitonin (*5*) buffered at a *p*H of 8.4. Three different assay methods were used: assay by (i) the rate of photoreduction of the dye 2,6-dichlorobenzenoneindophenol, (ii) the evolution of oxyen, (iii) the conversion of inorganic phosphate.

The rate of photoreduction of the dye 2,6-dichlorobenzenoneindophenol ($3 \times 10^{-5}M$) was measured at 600 mμ (*6*) [after illumination for 1 to 4 minutes with 300 ft-ca (7)], 30°C, and a final *p*H of 7.1 ± 0.1 ($0.1M$ phosphate buffer). In 20 percent of the preparations photoreduction of dye was complete within 2 minutes, with no accompanying reduction in darkness.

In those extracts of chloroplastin which exhibit active dye reduction, an absorption peak at 488 mμ of one of the *Euglena* carotenoids increases in optical density in the light and then decreases in the dark to its original density. This reaction can be repeated several times by placing the reactants alternately in light and darkness, without further addition of dye; this indicates that there is a reoxidation in the dark. Also this seems to indicate that there is an increase in the amount of carotenoid absorbing at 488 mμ simultaneously with the dye reduction. A cytochrome *c* photooxidase has been demonstrated in similar digitonin extracts by Nieman and Vennesland (*8*). In their experiments, cyanide was used to inhibit enzymatic dark oxidations; in ours, cyanide was not used.

Extracts yielding active photoreduction of dye were tested for their ability to cause photolysis or evolution of oxygen. Photolysis was measured manometrically in completely anaerobic Warburg vessels with KOH in the center well, and the dye was tipped from a side arm at zero time. The reaction conditions were the same as for dye reduction except that the system for photolysis measurements was made oxygen-free to permit a qualitative identification of oxygen evolved by bacterial bioluminescence. In some preparations of chloroplastin, photolysis occurred and yielded 20 to 30 μl of oxygen in 2 minutes, and there was a distinct luminescent glow, which persisted for almost a minute after an anaerobic suspension of *Photobacterium phosphoreum* in 3-percent NaCl-phosphate buffer was injected, in darkness, through a rubber serum-bottle stopper (*9*). Controls in the absence of light and others without dye did not evolve gas.

In the experiments in which photolysis occurred, a light-catalyzed conversion of inorganic phosphate into labile phosphate was observed over a 1-hour period in an anaerobic system similar to that described above, containing six cofactors and adenosine monophosphate. The reaction vessels contained 2 ml of chloroplastin (having a chlorophyll concentration of about $10^{-5}M$), 20 μmole of Mg^{++}, 30 μmole of α-ketoglutarate (*10*), 0.3 μmole of riboflavin-5-phosphate (*10*), 0.6 μmole of menadione (*10*), 2 μmole of ascorbate (*11*), about 5 μg of cytochrome *c* (*11*), 55 μmole of adenosine monophosphate (*10*), and 4 μg of inorganic phosphate, and the preparation was held at the same temperature, *p*H, and light intensity as in the procedures described above.

Inorganic and labile phosphate were measured by Fiske and Subbarow (*12*) and Crane and Lipmann (*13*) techniques. In one case, 700 μg of inorganic phosphate disappeared, but only 180 μg of labile phosphate could be found; in another, 550 μg of inorganic phosphate disappeared and 200 μg of labile phosphate was found. These experiments were immediately repeated with the addition of glucose and hexokinase (*10*) and the glucose-6-phosphate formed was determined by triphosphopyridine nucleotide (*10*) reduction at 340 mμ in the presence of glucose-6-phosphate dehydrogenase (*11*). In this way, 80 to 90 percent of the inorganic phosphate that disappeared was accounted for as labile phosphate. The phosphate conversion that occurred in the dark control was only 3 to 4 percent of that found in the light. Whether all of the cofactors play a role in this reaction is as yet not known.

We have made extensive efforts to prepare more uniform chloroplast extracts with digitonin and other detergents from isolated *Euglena* and spinach chloroplasts, but we have been unable to account, as yet, for the variability in activity from preparation to preparation. These results, though, indicate that some typical photosynthetic reactions can be observed with the digitonin extractable complex (the chloroplastin) from chloroplasts (*14, 15*).

RUSSELL A. EVERSOLE*
JEROME J. WOLKEN
Biophysical Research Laboratory,
Eye and Ear Hospital, and
University of Pittsburgh Medical School,
Pittsburgh, Pennsylvania

References and Notes

1. J. J. Wolken, *J. Cellular Comp. Physiol.* 48, No. 3, 349 (1956).
2. ——— and F. A. Schwertz, *Nature* 177, 136 (1956); chloroplastin is a digitonin extract of chloroplasts containing chlorophyll and carotenoids combined with proteins, lipids, and

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All technical papers are published in this section. Manuscripts should be typed double-spaced and be submitted in duplicate. In length, they should be limited to the equivalent of 1200 words; this includes the space occupied by illustrative or tabular material, references and notes, and the author(s)' name(s) and affiliation(s). Illustrative material should be limited to one table *or* one figure. All explanatory notes, including acknowledgments and authorization for publication, and literature references are to be numbered consecutively, keyed into the text proper, and placed at the end of the article under the heading "References and Notes." For fuller details see "Suggestions to Contributors" in *Science* 125, 16 (4 Jan. 1957).

lipoproteins. *Euglena* chloroplastin appears to be similar to Smith and Youngs' chlorophyll holochrome.

3. J. J. Wolken and A. D. Mellon, *Biochim. et Biophys. Acta* 25, 267 (1957).
4. D. I. Arnon *et al., Proc. Intern. Congr. Biochem. 3rd Congr. Brussels, 1955* (1956), pp. 227–232.
5. The digitonin $C_{55}H_{90}O_{29}$–D-58 used in this study was procured from Fisher Scientific Co., Pittsburgh, Pa.
6. H. W. Milner *et al., Arch. Biochem. Biophys.* 28, 193 (1955).
7. General Electric 20-watt daylight fluorescent tubes were used.
8. R. H. Nieman and B. Vennesland, *Science* 125, 353 (1957).
9. M. B. Allen *et al., J. Am. Chem. Soc.* 77, 4149 (1955).
10. The α-ketoglutarate, riboflavin-5-phosphate, menadione, adenosine monophosphate, hexokinase, and triphosphopyridine nucleotide used in this study were procured from Nutritional Biochemicals, Inc., Cleveland, Ohio.
11. The ascorbate, cytochrome *c*, and glucose-6-phosphate dehydrogenase used in this study were procured from General Biochemicals, Inc., Chagrin Falls, Ohio.
12. C. H. Fiske and Y. Subbarow, *J. Biol. Chem.* 66, 375 (1925).
13. R. K. Crane and F. Lipmann, *ibid.* 201, 235 (1953).
14. We thank Dr. Frank H. Johnson of Princeton University for providing the bioluminescent bacteria.
15. This study was aided in part by grants from the American Cancer Society (CP-69B), the U.S. Public Health Service [B-397(C3)], and the Stoner Mudge Foundation.

* Present address: Vitamins, Inc., Chicago, Ill.

31 December 1957

Gases in Glaciers

Gas trapped in the bubbles of glacier ice may originate from two different sources, depending on the manner in which the ice was formed. If the temperature is always below freezing in the area of ice formation, as in parts of Greenland and Antarctica, there is no melting, and the ice is formed simply by compaction of snow crystals. Air is trapped between the crystals, and the composition of the gas in such ice should be uniform and the same as that of the atmosphere (Table 1) at the time the ice was formed. If there are no losses or additions, it seems probable that the air will be preserved in its original state for the life of the ice.

If melting is involved in the formation of the ice, as in the case of the more temperate glaciers, the gas will have a different composition. Water equilibrated with air at 0°C contains 2.9 percent (by volume) of dissolved gases (Table 1). Since gas is insoluble in ice (*1*), the gas will separate from the water, on freezing, and will contribute to the gas content of the ice. Since meltwater is not evenly distributed in the firn, there will be variations in the composition of the gas enclosed in the ice thus formed.

Because the atmospheric gases dissolve in water in proportions that differ from their proportions in the air, it is possible to estimate the amount of water involved in formation of the ice (*2*). For this purpose the ratio of the inert gases—that is, the ratio of argon to nitrogen—is considered to be constant. Such an estimate gives, therefore, evidence about the climate at the time the ice was formed and, in theory, should also yield information about the composition of the atmosphere at that time.

The gas enclosed in a temperate glacier in Norway has been systematically examined (*3*). When the ice was formed, it contained a gas which was mostly air, with a small admixture of gas that had been separated from water by freezing. This was evident from the variations in composition from bubble to bubble and from elevated carbon dioxide values. However, during their life in the glacier, the oxygen, argon, and carbon dioxide components of the gas had been systematically removed. This was attributed to a leaching process which takes place during the warm summers of the temperate climate, when the temperature of the ice approaches 0°C and slight films of water containing dissolved gas may migrate through intercrystal boundaries.

In glaciers or icecaps where the temperature of the ice remains below zero, it would appear that the ice is an excellent preservative of the enclosed gas. Glacier ice is pure and comparable in salt content to distilled water (*4*), and there is not enough contaminating matter in the ice to produce a chemical reduction of the oxygen. Also, gas diffusion through ice is extremely slow—less than that of helium through glass (*5*)—and because of the enormous distances within a glacier it seems unlikely that the composition of the gas could change appreciably by diffusion through the ice, even over long periods of time.

It is well established that there have been climatic cycles since the last ice age, with temperatures warmer and colder than at present (*6*). The composition of the atmosphere has been known, through analysis, for only about 100 years, during which time the carbon dioxide content seemingly has not increased measurably (*7*). The theory has has been postulated, though, that a high carbon dioxide content would result in a warm climate, through a "greenhouse" effect (*8*); this theory lends special interest to the study of the carbon dioxide content of the air.

Table 1. Composition of dry gas.

Component	Atmospheric air (% by volume)*	Water equilibrated with air at 0°C, 760 mm-Hg† (% by volume)
Nitrogen	78.09	61.54
Oxygen	20.94	34.90
Argon	0.94†	1.86
Carbon dioxide	0.03	1.75

* Data from R. J. Hock *et al., J. Meteorol.* 9, 441 (1952).
† Data from *Handbook of Chemistry and Physics* (ed. 36, 1954–55).

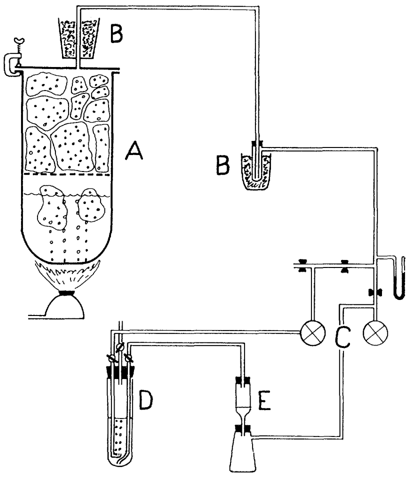

Fig. 1. Schematic diagram of equipment for extraction of carbon dioxide: *A,* 130-lit vacuum-tight ice-melting pot; *B,* vapor traps containing salt-snow mixture; *C,* high-vacuum pumps for taking vacuum and transporting the gas; *D,* barium hydroxide absorption tube; *E,* filtration unit for collecting and washing barium carbonate.

What was the climate and what was the composition of the atmosphere in ages past? Greenland and Antarctica are covered by large ice sheets, in which some of the ice may be 20,000 years old or more. It seems likely that analysis of the gas contained in this ancient ice may provide some answers to these questions.

Samples of glacier ice from three locations near Thule, northwest Greenland, have been collected and analyzed for enclosed gas (*2*). The amounts of oxygen, argon, and carbon dioxide in these samples were all equivalent to, or higher than, those of these gases in air and varied within each piece of ice. This indicated that the ice was formed in a period when the summers, at least, were warm in these latitudes. Using the ratio Ar/N_2, we calculated values for oxygen and carbon dioxide and compared these calculated values with the observed values. The oxygen content of the gas was close to that expected, but the values for carbon dioxide were scattered around a value twice as high as that for present-day air. One explanation might be that the composition of air was different with respect to carbon dioxide when this ice was formed.

Information thus obtained about ancient atmosphere and climate is of little value unless the age of the ice is known. When it was found that the carbon dioxide content of the Greenland ice sam-