5% methanol. The methanol was then removed from the clear, green extracts by contact with water. In a few minutes, the absorption maxima of the solutions shifted to longer wavelengths, chlorophyll *a* from 663 to about 710 mμ, chlorophyll *b* from 643 to 690 mμ. Chlorophyll *a′* behaved like chlorophyll *a,* chlorophyll *b′* like chlorophyll *b.*

For reproducibility of these spectral shifts, low-boiling petroleum ether should be employed instead of higher-boiling fractions (50°–60°). The chromatogram should be developed extensively in order to remove colorless, fatty substances. Only the more concentrated regions of the chlorophyll zones in the chromatographic columns should be utilized, thereby reducing the amount of colorless contaminants. With extracts of some plants, readsorption of the chlorophylls was necessary. The removal of the methanol and residual propanol from the petroleum ether solutions must be complete. The entire preparation, including extraction, separation, and recovery, should be carried out in a short time (about 1 hr).

Chlorophylls in the alcohol-free petroleum ether were present in insoluble, colloidal, or microcrystalline form. These suspensions were turbid and were very weakly fluorescent. When the suspensions were centrifuged or were filtered through paper or shallow layers of powdered sugar, most of the pigments were removed, leaving very light green solutions with absorption maxima at shorter wavelengths. Chlorophylls *b* and *b′* yielded such dilute solutions that a depth of 10 cm was required in order to reveal the spectral absorption maximum at 645 mμ. Resuspension of the centrifuged pigments in fresh petroleum ether provided turbid mixtures with reappearance of the spectral absorption maxima at the longer wavelengths. Agitation of these centrifuged chlorophyll preparations with water in a stream of nitrogen also provided suspensions with absorption maxima at the longer wavelengths. The addition of traces of fats, sterols, higher aliphatic alcohols, and paraffin to the petroleum ether plus methanol solutions, followed by removal of the methanol with water, yielded solutions or suspensions with spectral absorption maxima ranging from those of the true solutions of the chlorophylls to those of the suspensions of the purified pigments.

The purified chlorophylls precipitated from petroleum ether were not altered chemically, and they were not isomerized. These preparations redissolved rapidly in petroleum ether in the presence of a little methanol or acetone. The resultant solutions contained the original unaltered chlorophyll, as shown by spectral absorption properties and by the formation of a single zone in the chromatographic column. They also formed a single zone when adsorbed with some of the unprecipitated chlorophyll. The precipitation, dissolution, and adsorption were repeated several times with a single chlorophyll preparation without evidence of alteration.

In summary, the absorption maxima of the chlorophylls purified by chromatographic adsorption are shifted to much longer wavelengths when these pigments are precipitated from solution in petroleum ether. These spectral shifts, which are analogous to those observed by cooling solutions of the pigments in hydrocarbon solvents, are due to a change in the physical state of the pigments, not to isomerization. The differences between the spectral properties of chlorophyll *a* in plants and in various pigment preparations support the view that this photosynthetic substance occurs naturally in a unique combination or association with other constituents of the chloroplasts.

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Manuscript received February 21, 1952.

Equilibria between Species of Chlorophylls in Solution[1]

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In order to clarify the interpretation in our previous communication (*Science,* 114, 275 [1951]), to which H. H. Strain refers in the preceding article, we shall discuss some aspects of our work in more detail and present more recent results in confirmation of our point of view. At the same time, we shall show that the behavior of the colloidal solutions of chlorophyll studied by Dr. Strain cannot account for the changes we have found in the spectra of chlorophyll in solution.

The existence of isomers was not inferred from shifts in wavelength of any of the spectral peaks with temperature, since all the peaks of each species moved toward longer wavelengths as the temperature was lowered. Rather, the presence of two isomers in each of the chlorophylls in the ether-hydrocarbon solvent was implied by the coexistence of two similar systems of peaks, the relative intensities of which varied reciprocally upon change of temperature. Actually, the corresponding peaks were clearly resolved in the blue region of the spectra of chlorophylls *a*, *b*, and *b′*, and on the short wavelength side of the red peak of chlorophyll *a*. However, the main red peak in each of the three chlorophylls appeared single. It was surmised that the main red peaks of the pairs of isomers were superimposed on each other and that they shifted with temperature to about the same degree. Indeed, there were indications that the superpositions were not exact.

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[1] Research carried out at the Brookhaven National Laboratory, under the auspices of the U. S. Atomic Energy Commission.

We have since found that in another solvent the isomers exhibit a pair of peaks in the red, which are resolved almost as well as those in the blue. In Fig. 1 is shown the effect of temperature on the main red peak of chlorophyll b' dissolved in a solvent consisting of 10% diisopropyl amine in 1 : 1 propane and propene. At 230° K, only one band, at 6460 A, is present; at 170° K and at 75° K two components are clearly evident at 6480 A and 6610 A. The one at the longer wavelength is more intense at lower temperatures. The relative intensities of the two components of this band vary reciprocally, and reversibly with temperature; and equilibrium is quickly established. Along with these changes of the red band are similar reciprocal relations among the components of the blue band, such as were described in our previous paper. Altogether similar changes were observed in the red band of chlorophyll b in this solvent.

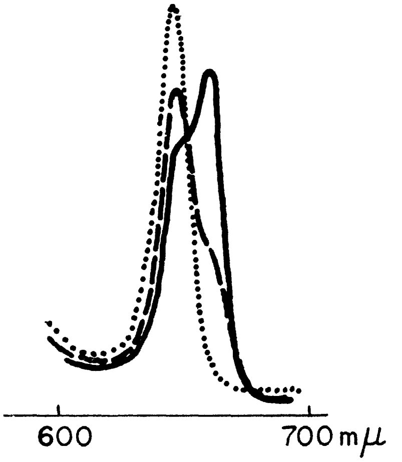

FIG. 1. Absorption spectrum of the intense red band of chlorophyll b' dissolved in a solvent consisting of 10% diisopropyl amine in 1 : 1 propane and propene : .... 230° K, -------- 170° K, ———— 75° K.

The following considerations rule out, we believe, the possibility of an explanation of these phenomena in terms of the existence of chlorophyll in the colloidal state. All the solutions remained clear, and over the entire range of temperatures at which the reciprocal transformations of the spectral systems took place, the transparency remained practically unaltered as far into the ultraviolet region as we examined—3000 A—where scattering from colloidal particles would have seriously affected the transparency. At the lowest temperature, about 150° K, at which both species were clearly observable, the equilibrium between them was rapid. At such low temperatures a decided time lag would have been required if coagulation processes were involved, since they would doubtless have been diffusion-controlled.

Manuscript received June 12, 1952.

Comments and Communications

Two Long Climatic Records[1]

FOSSIL pollen profiles from sediments formed outside the limits of continental glaciation are of interest because they give a record not interrupted by the presence of ice in sedimentary basins. They also yield information as to the nature and extent of climatic trends correlative with ice movement.

The left-hand profile in Fig. 1 is based upon a reconnaissance drilling by H. T. Stearns in Valle Grande, a caldera and former lake bed shown in the Jemez Springs and Santa Clara quadrangles, northern New Mexico, and here reported by permission of the Atomic Energy Commission. The material consisted of chips washed out in drilling, bagged to give a representation of conditions at 5′ intervals. Thus the profile provides only a rough approximation except near the top, where the results of our own close-interval sampling to a depth of 14 m are included.

The right-hand profile presents a composite of three cores within the limits of Mexico City. Material for analysis was obtained by precision coring and generously furnished by L. Zeevaert at the suggestion of A. R. V. Arellano, of the Instituto de Geología.

All pollen and spores were counted, but only those of upland forest trees are here considered. From their totals at each level, the percentages of those clearly indicative of moist conditions were calculated. These percentages were then plotted against depth in meters. Essentially, the values represented are:

Mexico	New Mexico
Oak–alder–fir	Spruce–fir
Oak–alder–fir–pine	Spruce–fir–pine–oak

The separation of various species of pine was not feasible at this juncture. These vary somewhat in their requirements, although generally indicating dryness. The same may be said of oak in New Mexico. The net effect of these limitations, however, has been, not to invalidate our results, but to make them more conservative than they otherwise would have been, especially in the emphasis of moist peaks.

Groups of values to the left of the shadowed band (Fig. 1) indicate dry intervals, those to the right indicate moist, and levels that we consider correlative are connected. Data given are based upon a previous report (Sears, P. B. *Bull. Geol. Soc. Am.*, **63**, 241 [1952]). Moist maxima in New Mexico may safely be

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[1] The work on which this report is based was supported in part by a grant from the Penrose Fund of the Geological Society of America.