

and victorin strongly indicate that both may have a similar mode of action. Susceptible tissues which have previously been exposed to victorin fail to respond to the addition of DNP. If victorin has an effect similar to that of DNP, it is probable that the rate-limiting phosphate acceptor systems are by-passed.

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9. I wish to express appreciation to H. E. Wheeler, under whose direction this research was conducted, and to thank J. Baker and W. K. Porter for making facilities available, as well as for their helpful suggestions and criticisms.
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## Crystallization of Chlorophylls

It has been pointed out that the successful crystallization of the chlorophyll pigments depends on high purity and the presence of water (1). We wish to report (2) the observation that the precipitation of the chlorophylls from highly impure extracts in organic solvents by washing with water constitutes a coprecipitation of crystalline chlorophyll *a* with amorphous chlorophyll *b*. This observation was made during a systematic spectrophotometric survey of each fraction obtained during the preparation of microcrystalline chlorophylls according to the method of Jacobs *et al.* (1). A sample of the petroleum ether extract of the pigments (see below) showed a pronounced absorption band with a peak at 745 mμ. According to Jacobs and Holt (3), this absorption band (corrected for scattering) is associated with a microcrystalline suspension of chlorophyll *a*.

The following example provides a generalized description of our procedure. Four pounds of fresh spinach was blended with acetone and filtered through a pad of Hyflo Super Cel on Whatman No. 1 filter paper in a large Büchner funnel. About 600 ml of solution passed through the filter before chlorophyll appeared. This solution, containing some of the yellow pigments and acetone soluble lipids, was discarded. After further acetone extraction of the

gross content of the chlorophylls, the pigments were transferred in a separatory funnel to a single 500-ml portion of Skelly solvent F by successive treatment of 1-liter portions of the acetone extract with 2.5-liter portions of distilled water. Crystallization of chlorophyll *a* was already apparent in the petroleum ether after the second transfer from acetone (dashed curve of part A, Fig. 1). The solid curve of part A, Fig. 1, was obtained after all the pigments had been transferred to the petroleum ether. The dashed curve represents the spectrum of a 1:7.5 dilution with petroleum ether, while the solid curve represents the spectrum of a 1:30 dilution. The pigments were precipitated in the centrifuge and washed several times with fresh petroleum ether.

The separation of chlorophylls *a* and *b* was achieved at this point by the chromatographic procedure utilized by Jacobs *et al.* (1). The crystallization of the individual chlorophylls was accomplished in a manner somewhat similar to that used by these authors. The isopropyl alcohol-pentane solution of chlorophyll obtained as the effluent from the chromatographic column was thoroughly washed with water. During this procedure, microcrystals of chlorophyll *a* appeared as shown by the absorption spectrum (part B, Fig. 1). Chlorophyll *b* was removed from the sucrose adsorbent with acetone. This was followed by transfer of the chlorophyll *b* to petroleum ether by addition of water. Thorough water washing of the petroleum ether layer was continued until microcrystalline chlorophyll *b* appeared as shown by the absorption spectrum (part B, Fig. 1). Collection of the crystals was considerably simplified by the use of a model L Spinco ultracentrifuge (20,000 *g* for up to 30 minutes).

It should be remarked that all operations were carried out in a cold room at 4°C. Chromatography of the pigments at room temperature resulted in obvious color changes while the pigments were still on the sucrose adsorbent.

In addition to the spectrophotometric studies presented here, other physical studies of these crystals have been carried out (4). G. Donney of the Geophysical Laboratory of the Carnegie Institution of Washington took several x-ray powder diagrams of our crystalline preparations (5). It was found that the powder diagrams of the mixture of chlorophylls *a* and *b* precipitated from petroleum ether were identical with the powder diagrams of the pure chlorophyll *a* crystals. Such an observation has also been made with artificial mixtures of chlorophylls *a* and *b* (6). Since the spectrum of the redissolved precipitate shows the presence of chlorophyll *b*, this indicates that chlorophyll *b* in an amor-

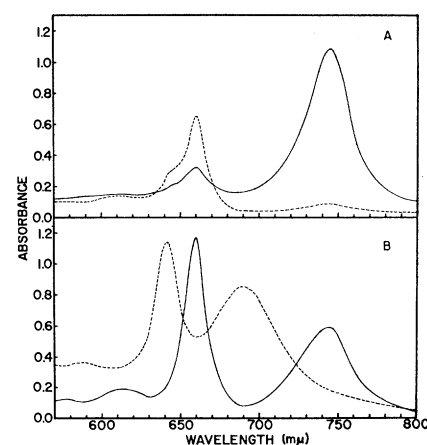


Fig. 1. Absorption spectra of precipitated chlorophylls in petroleum ether. *A*, Spectra of mixed chlorophylls at two stages of crystallization (see text). *B*, Spectra of pure chlorophyll *a* (solid curve) and chlorophyll *b* (dashed curve) showing absorption bands for both dissolved and microcrystalline chlorophylls. The spectra were obtained with a Cary model 14 spectrophotometer with a 1-cm cell; they have not been corrected for scattering.

phous form coprecipitates with the microcrystalline chlorophyll *a*. The less likely possibility that a mixed crystal with practically unchanged parameters is formed is not excluded by these considerations.

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#### References and Notes

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2. This work was supported by research grant No. C-3370 from the U.S. Public Health Service. We gratefully acknowledge this assistance.
3. E. E. Jacobs, A. S. Holt, *J. Chem. Phys.* 22, 142 (1954).
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5. We wish to extend our thanks to Dr. Donney for making the x-ray powder diagrams. Dr. Donney will carry out an independent detailed analysis of the crystallographic structure of our chlorophyll *a* crystals. We also wish to thank Dr. I. Fankuchen of Brooklyn Polytechnic Institute for making a low-angle x-ray powder diagram of the chlorophyll *a* microcrystals.
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## Inhibition of Adrenal Steroid 11-Oxygenation in the Dog

Inhibition of adrenal cortical secretion by a direct action on steroid biosynthesis has been described following administration of amphenone B [3,3-di(*p*-aminophenyl)butanone-2 dihydrochloride], but limitations imposed by the toxic effects of this substance have led to the search for other inhibitory agents. Recently, the