turation (*9*) when assessments of the same roentgenograms are compared with Greulich-Pyle (*10*) standards. This is the greatest retardation in skeletal maturation reported for a number of preadult Peruvian series (*11*). Furthermore, the Vicos Indian boys show a mean deceleration of growth from about 10 to 15 years of age compared with other series from the Peruvian Sierra. After 15 years of age, the Vicos boys show a belated growth spurt that may relate to puberty. The slow and retarded growth, maturation, and phalangeal mineralization of the Vicos boys and the ultimately small body size of the adult men (*12*) probably reflect body economy in utilizing the meager amount of available calcium, as well as an inadequate total nutritional environment.

Clearly, the photodensitometric analysis of bone mineralization fits in closely with the growth and maturational data and provides revealing information on the relationship of food to physique. After several years on a diet raised to adequate levels, the Vicos Indian boys will be restudied with respect to any alterations in their developmental patterns (*13*).

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14 March 1958

Increased Ascorbic Oxidase Activity Induced by the Fungal Toxin, Victorin

The toxin theory of disease has been an intriguing concept ever since the discovery of diphtheria toxin in 1888, by Emile Roux and Alexandre Yersin. In the field of animal pathology, the theory has led to brilliant advances in the understanding and control of disease. The application of this concept to the field of plant pathology, although admittedly less fruitful, has in the last decade yielded important results.

The studies of Braun (*1*), Dimond and Waggoner (*2*), Gäumann (*3*), and Gottlieb (*4*), to mention a few, clearly demonstrate that plant pathogens are capable of producing metabolites in culture which, when they are applied to the host, produce injurious effects in some cases similar to those encountered in natural infection. However, proof that plant pathogens produce toxins which are directly and solely responsible for the symptoms found in infected plants is still lacking.

Victorin, the toxin produced by the fungus *Helminthosporium victoriae* M. and M., first described by Meehan and Murphy (*5*), offers a unique tool for investigation of the toxin theory in relation to plant disease. These workers, as well as Litzenburger (*6*) and Luke and Wheeler (*7*), observed that, unlike other phytotoxins which generally lack the host specificity exhibited by the pathogen involved, victorin affected only those varieties of oats that were susceptible to the fungus (hybrids derived from victoria). Luke and Wheeler (*7*) also demonstrated that high yields of toxin could be obtained from highly pathogenic isolates of the fungus, whereas nonpathogenic strains failed to produce toxin.

Studies by Romanko (*8*) disclosed that victorin caused three- to five-fold increases in respiration of oat tissues of varieties susceptible to *H. victoriae* but that it failed to produce any appreciable effect on the respiration of Camellia, a resistant variety. This was apparently the first report of a toxin's producing such an effect only in plants susceptible to the pathogen. Further studies carried out by me (*9*) have shown that this increase in respiration is directly proportional to the concentration of toxin applied (original culture filtrate, containing 1000 units per milliliter as measured by the method of Luke and Wheeler, diluted 2.5×10^{-2}) (Fig. 1).

When cuttings were placed in toxin for 4 hours and exposed to light, and the enzymatic activity was measured 12 hours later, ascorbic oxidase, the major terminal oxidase system found to be in operation in susceptible oats, was found to be four times as high as that in comparable tissues (Fig. 2). The possible reasons for this increase in enzyme activity are at present not known, but in view of Newcomb's (*10*) findings that auxin (indoleacetic acid) greatly increases ascorbic oxidase activity in tobacco pith cells grown in culture, the results obtained appear to be significant. Tests with oats of resistant varieties showed that treatment with the same concentration of victorin failed to produce any detectable effect on ascorbic oxidase activity.

Romanko's studies (*8*) suggested that the increase in respiration of susceptible tissues caused by victorin may be due to uncoupling of phosphorylation from oxidation. Studies with 2,4-dinitrophenol (DNP), a proven respiratory uncoupler,

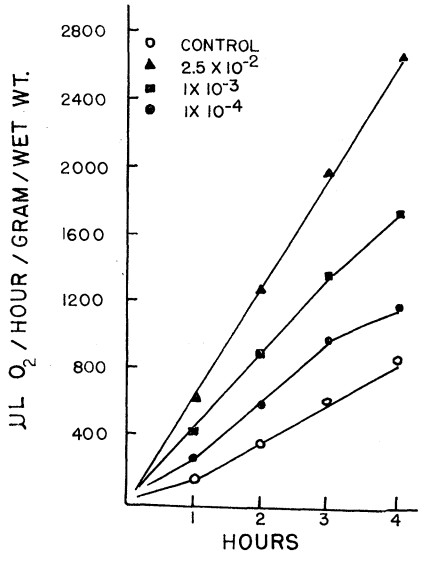

Fig. 1. Increase in respiration of susceptible oat tissue as a function of toxin concentration.

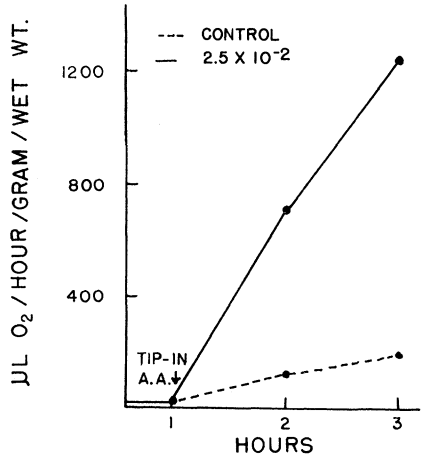

Fig. 2. Increase in ascorbic acid oxidase activity of homogenates from susceptible oat plants treated with victoria.

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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31 March 1958

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 amorphous 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.

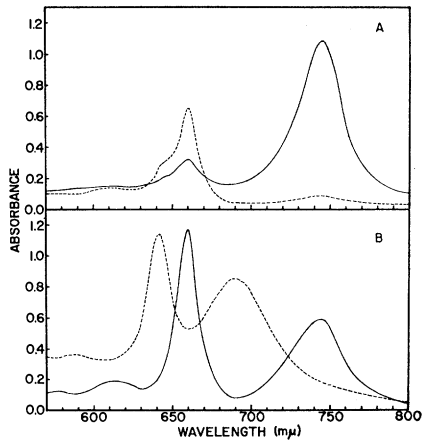

Fig. 1. Absorption spectra of precipitated chlorophylls in petroleum ether. *A*, Spectra of mixed chlorophylls at two stages of crystallization (see text). *B*, Spectra of pure chlorophyll *a* (solid curve) and chlorophyll *b* (dashed curve) showing absorption bands for both dissolved and microcrystalline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

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15 April 1958

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