

In the Chicago area such an organization has been in operation for some time. At the suggestion of the down state committee a joint meeting was arranged. This met in the Laboratory of Physics of the University of Illinois on Saturday, April 23, 1938.

A program of ten papers was presented, dealing with the present-day problems of the physics teacher, by representatives from the University of Chicago; Western State Teachers College, Macomb; Eastern State Teachers College, Charleston; the University of Illinois; Woodrow Wilson Junior College, Chicago; and Bradley Institute.

Active interest was expressed throughout, and discussions followed each paper. Among the important recommendations discussed and passed were: (1) Full

cooperation with the state committee in its endeavor to raise the teaching standard of physics in the state. (2) The joint session favors that a minimum of 16 hours of college physics be required of prospective high-school teachers of physics; however, that this recommendation should not be immediately applied to teachers already in service.

The attendance was thirty-two. Twenty-one of the leading educational institutions of the state were represented at the meeting. The down state club continued the general committee—Bocksthaler, Palmer, Paton—with instructions to arrange for the next meeting.

CHAS. T. KNIPP,
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SPECIAL ARTICLES

SOLUTIONS OF CHLOROPHYLL-PROTEIN COMPOUNDS (PHYLLOCHLORINS) EXTRACTED FROM SPINACH

THE differences in solubility, fluorescence and absorption spectrum between the green pigments in the leaf and the chlorophylls extracted in solvents such as alcohol have been ascribed either to dispersion of the green pigments in the leaf, or to adsorption or combination of the chlorophyll with lipid or protein.¹ We have prepared aqueous solutions of the green pigments which show characteristic protein properties and which resemble the pigments in the leaf. To distinguish them from the chlorophylls we have adopted the name *phyllochlorin* for these chromoproteins, as suggested by Mestrel.

Our extracts have been prepared using dilute aqueous digitalin, a solvent currently used for the photosensitive retinal pigments.² Ordinary leaf press juice or distilled water extracts show the green pigments not in true solution,³ but in a fine suspension whose particles are visible under the microscope and can be retained on a fine filter.

About 100 gm of fresh spinach is thoroughly ground with fine sand, water is added to make 100 ml, and the suspension filtered through a coarse fluted filter. The moist cake is reground and again extracted. To the combined extracts is added 5 gm of Filter-Cel.⁴ per 100 cc, and the whole is filtered through a thin layer

of Filter-Cel. on a Buchner funnel. The deep yellow-brown filtrate is discarded. The cake is washed in distilled water several times until the filtrate shows no trace of yellow color. It is then extracted with 25 ml of 1 or 2 per cent. aqueous digitalin;⁵ the result is a dark green solution which shows no trace of suspended material under an oil immersion lens. Similar preparations can be made with 4 per cent. purified bile salts. More dilute extracts are obtained in concentrated (40–50 per cent.) urea solutions. Digitalin solutions of the phyllochlorin kept for some weeks in the cold room (5° C.) show a little precipitated pigment which does not redissolve.

The absorption bands of the phyllochlorin (Fig. 1),

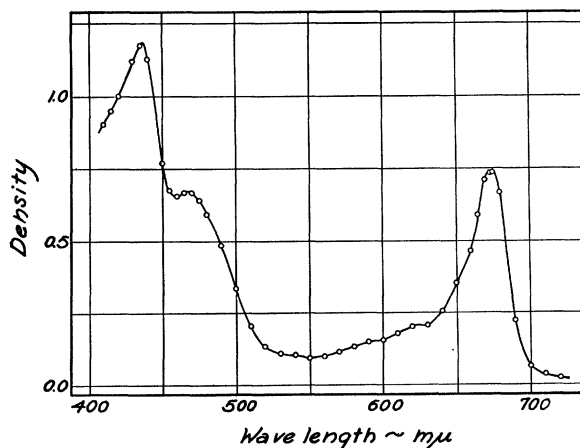


FIG. 1. The absorption spectrum of a phyllochlorin solution prepared with 2 per cent. digitalin and diluted 1 to 10 with distilled water. The density values are for a 5 mm depth of solution.

measured with Shlaer's spectrophotometer,⁶ are like those of the leaf and are shifted towards the longer

⁵ Crystalline digitalin, Eimer and Amend, New York.

⁶ S. Shlaer, *Jour. Opt. Soc. America*, 28: 18, 1938.

¹ Reviewed by H. Mestrel in "The Investigation of the Pigments of the Living Photosynthetic Cell," in *Contributions to Marine Biology*, Stanford University Press, 1930.

² e.g., K. Tansley, *Jour. Physiol.*, 71: 442, 1931; A. M. Chase and C. Haig, *Jour. Gen. Physiol.*, 21: 111, 1938; G. Wald, *Nature*, 140: 545, 1937.

³ T. B. Osborne and A. J. Wakeman, *Jour. Biol. Chem.*, 42: 1, 1920. Such suspensions have also been studied by V. Lubimenko, *Rev. Gen. de Bot.*, 39: 547, 1927; and B. Hubert, *Rec. trav. bot. néerl.*, 32: 324, 1935.

⁴ Filter-Cel., Johns-Manville, New York.

wave-lengths as compared with the natural mixtures of chlorophyll *a* and *b*.⁷ The three bands are at 437, 470 and 675 m μ , while those for chlorophyll are at 420, 465 and 660 m μ . The 420 and 660 m μ maxima of chlorophyll have about the same height, while for phyllochlorin solutions the 437m μ maximum is always 60 per cent. higher than the 674 band. This suggests the presence of carotenoids associated with phyllochlorin, such as French⁸ found for the chromoprotein solutions from photosynthetic purple bacteria.

Boiling a neutral digitalin extract shifts the red absorption band towards the shorter wave-lengths. When a solution is made strongly acid or weakly acid and boiled, the solutions turn yellow, corresponding to the formation of phaeophytins. A digitalin extract saturated with solid ammonium sulfate precipitates the phyllochlorin only after several days, but when boiled forms a bright green viscous mess. No pigment is lost on prolonged dialysis (about two weeks) of a digitalin extract, and only a part of the pigment precipitates. This precipitate, separated by centrifuging, does not readily redissolve in digitalin solution. The pigment which remains in solution is now readily precipitated with high concentrations of ammonium sulfate. Such precipitates are easily redissolved in digitalin solution but not in water. It is likely that the solvent action of the digitalin and the bile salts is due to the formation of coordination compounds which are not broken up even on prolonged dialysis. Phyllochlorin is precipitated and the chlorophyll extracted by strong alcohol, methyl alcohol or acetone but not by petroleum ether in agreement with the effects of these solvents on the leaf. Phyllochlorin solutions show a positive Biuret reaction.

In agreement with observations of the green leaf, phyllochlorin solutions show little or no red fluorescence when irradiated with blue light (436 m μ). This is in contrast with the strong red fluorescence of alcoholic chlorophyll solutions. Phyllochlorin solutions are quite stable to visible light.

The behavior of phyllochlorin solutions in strong centrifugal fields is being investigated in collaboration with Dr. E. G. Pickels⁹ using an air-driven ultracentrifuge.¹⁰ Preliminary studies show that the phyllochlorin when subjected to a force of 160,000 gravity can be sedimented completely through a 10 mm column of the liquid medium within three hours, leaving no color in the supernatant fluid. Our best preparation

showed two sedimentation boundaries which correspond to particles of high molecular weight, *i.e.*, above 70,000. The two boundaries retained their identity with respect to their sedimentation rates when studied by the light absorption method in the red and blue regions corresponding to the absorption maxima of phyllochlorin in the visible, and in the ultra-violet region characteristically absorbed by proteins. One boundary sedimented almost twice as fast as the other; these more rapidly moving and presumably heavier particles showed a greater total absorption in each of the two regions of the visible spectrum than did the smaller particles.

It is tempting to assume that these two proteins correspond with phyllochlorins *a* and *b*. The similarity of sedimentation properties throughout the spectrum indicates that the additional blue absorption is characteristic of the phyllochlorins and not of some other component.

It now appears that the classical organic chemical studies of the chlorophylls and carotenoids were concerned with the prosthetic groups of extremely complex specific catalysts, perhaps analogous to the hemoglobins and enzymes such as cytochrome, catalase and the yellow respiratory enzyme. Presumably there are many additional components concerned in photosynthesis, since phyllochlorin does not carry on photosynthesis *in vitro*.

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OVINE AND BOVINE LISTERELLOSIS IN ILLINOIS

THE pathogenesis of organisms of the genus *Listerella* and their possible etiologic significance in rodent septicemia have been reported in England¹ and South Africa,² while in New Zealand³ sheep suffering from an encephalitic syndrome, designated "circling disease," has been associated with or attributed to *Listerella* infection. In the United States, *Listerella* has been isolated from cattle,⁴ sheep⁵ and man,^{6,7} displaying symptoms of encephalitis, and from chickens⁸ that disclosed lesions of necrotic myocarditis. So far as

¹ E. G. D. Murray, R. A. Webb and M. B. R. Swann, *Jour. Path. and Bact.*, 29: 407, 1926.

² J. H. H. Pirie, *So. African Inst. Med. Res.*, 3: 163, 1927. Cited by Gill.

³ Dudley A. Gill, *Vet. Jour.*, 87: 60, 1931 and 89: 258, 1933; *Australian Vet. Jour.*, 13: 46, 1937.

⁴ F. S. Jones and R. B. Little, *Arch. of Path.*, 18: 580, 1934.

⁵ Erwin Jungherr, *Jour. A. V. M. A.*, 91: 73, 1937.

⁶ Caspar G. Burn, *Jour. Bact.*, 30: 573, 1935; *Am. Jour. Path.*, 12: 341, 1936.

⁷ E. W. Schultz, M. C. Terry, A. T. Brice and L. P. Gebhardt, *Proc. Soc. Exp. Biol. and Med.*, 31: 1021, 1934. Cited by Burn.

⁸ C. V. Seastone, *Jour. Exp. Med.*, 62: 203, 1935.

⁷ *c.f.* E. Rabinowitch and J. Weiss, *Proc. Roy. Soc. London, A*, 162: 251, 1937.

⁸ C. S. French, Abstract in the Proceedings of the American Society of Biological Chemists, Baltimore meeting, March 30-April 2, 1938.

⁹ Of the Laboratories of the International Health Division, The Rockefeller Foundation, New York.

¹⁰ J. H. Bauer and E. G. Pickels, *Jour. Exp. Med.*, 65: 565, 1937.