SCIENTIFIC SECTION

NOTES ON THE SPECTRUM AND PHARMACOLOGY OF CHLOROPHYLL.*

BY FREDERICK GRILL.

The chlorophyll granules have long been known to botanists and the part that these granules play in photosynthetic assimilative processes in plants have received the attention of plant physiologists. The chloroplast or the plasmic base of the chlorophyll granule has been discussed in the botanical literature and there has been much speculation as to the genesis of the chloroplast and as to the similarities of the chloroplast of the plant and the erythrocyte of the animal. Dr. Albert Schneider in his lecture course in botany suggests that, "chlorophyll granules are to the plants what erythrocytes are to the animals."

The chemical study of chlorophyll dates from the year 1819, when this name was first applied to the green pigment found in plants, by two investigators, Pelletier and Caventou. However, it was not until 1912, that the chemical composition of chlorophyll was definitely worked out. Only recently has any attention been given to the pharmacology of the pigment, and the experimental evidence indicates that chlorophyll may prove of great value in internal medicine (7).

The chemistry of chlorophyll is complex. Chlorophyll is a nitrogenous compound of organic nature. Several authorities assign to it the empirical formula ($C_{55}H_{72}O_6N_4Mg$). In the year 1912, Willstätter and Isler (13) definitely demonstrated that chlorophyll is a mixture of two pigments to which they assigned the following formulas:

Chlorophyll *a* $C_{55}H_{72}O_5N_4Mg$
Chlorophyll *b* $C_{55}H_{70}O_6N_4Mg$.

The structural formulas indicate that these two pigments are both esters of methyl and phytyl alcohol. Magnesium (11) is apparently a very important constituent of chlorophyll, whereas iron is, perhaps, the most characteristic mineral in hemoglobin (blood).

Among the substances associated with chlorophyll are three pigments of a yellow or reddish brown color, named, carotin, xanthophyll and fucoxanthin. Collectively these pigments are known as the carotinoids. The solubilities of the pigments are very similar to that of chlorophyll, and it is due to this that their removal from chlorophyll is so difficult.

The more recent investigations of the chemical constitution of chlorophyll showed an interesting relationship between it and the hemoglobin of blood. Haematinic acidimide obtained from chlorophyll has also been obtained from the hemoglobin of blood. Hemoglobin is hydrolyzed by dilute acids or alkalis forming haematin, which contains iron. The iron may be removed from the haematin by treating with hydrogen bromide in acetic acid solution. The iron-free compound thus formed is known as haematoporphyrin. Both the haematin and the

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haematoporphyrin on oxidation yield haematinic acidimide which is also derived from the chlorophyll. Willstätter and Asahina (12), by the reduction of chlorophyll, have obtained three pyrrole derivatives, namely, phyllopyrrole, haemopyrrole, and iso-haemopyrrole. Haemopyrrole has been obtained from the reduction of haematoporphyrin. These several characteristics show a chemical relationship between the chlorophyll of plants and the hemoglobin of blood.[1]

Among the interesting experiments which indicate a biological relationship between hemoglobin and chlorophyll are those by Manoiloff (6). He has arrived at a method whereby it is possible to differentiate between the chlorophyll of male and of female plants of the dioecious groups, as hemp. Manoiloff also demonstrated a differential test, using blood, to determine the sex of animals. Even the sex of the foetus *in utero* may be ascertained by this method using a small sample of the blood of the mother. Manoiloff claims to get about 75 per cent positive results and thus yields about the same percentage of positive results as the famous Wassermann reaction for syphilis. Lack of time did not permit repeating or checking the Manoiloff experiments although they are extremely simple. The differentiating reactions are based on the appearance of color when the reagents are applied seriatim according to directions.

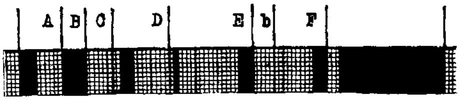


Fig. 1.

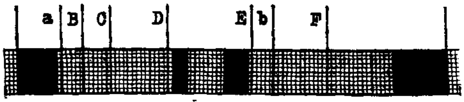


Fig. 2.—Spectrum of oxy-hemoglobin in dilute blood.

The spectrum of chlorophyll is characteristic. According to Seeker (9), a freshly prepared alcoholic extract of chlorophyll in the proper concentration shows five absorption bands. There is a broad dark band at Fraunhofer line C; three light bands, one between C and D, one close to D and one near E; and the fifth band beginning about F and taking in all of the blue and the violet. The appearance of the spectrum changes with the age of the solution. Hemoglobin (oxy-hemoglobin) in diluted blood shows absorption spectra similar to those of chlorophyll. There are four bands in the spectrum of oxy-hemoglobin. One at the extreme left near Fraunhofer line a, one near D, one near E and the fourth one beginning about G and cutting out the violet.

The absorption bands of chlorophyll in different solvents varies somewhat in position. There is also a marked difference due to the concentration of the solutions used, and the thickness of the layer through which light must pass. The figures show some of the spectra obtained with different solutions. In the stronger solutions, a third dark band appears at the extreme left of the spectrum, and a dark band at F does not seem to be split. The essential characteristics of the spectrum of chlorophyll are the presence of at least one very dark band in the red, bands in the yellow and green and absorption of the blue beginning at F.

The spectrum of the chlorophyll of the living leaf differs somewhat from the spectrum of the chlorophyll extracts, in the position of the bands.

[1] Hass, Paul and Hill, T. G. "An Introduction to the Chemistry of Plant Products," Vol. I, 223–242, Longmans, Green & Co., London (1921).

Heat seems to have no appreciable effect on the spectrum of chlorophyll. Age effects the spectrum of an alcoholic extract of chlorophyll. There is a darkening and widening of the bands; the whole spectrum appears to be reduced in intensity; and the solution loses its intense green color, developing a brownish tinge. On the other hand, the yellow to red color by reflected light does not change with age. The spectrum offers a simple and rapid means of identifying chlorophyll.

The extracts of chlorophyll used in these experiments were obtained from fresh actively growing immature oat plants, *Avena sativa.* The solvents used were 95% alcohol, ether, chloroform and acetone. Alcohol and acetone extracts were most commonly employed. The usual method of extraction was to use 10 Gm. of the fresh leaf, washed and blotted, and then cut up into small pieces. The cut leaf was crushed in a mortar, with a portion of the solvent and the material filtered. The mortar and the filter were washed with repeated portions of the solvent until the volume measured up to 100 cubic centimeters. This made approximately a 10 per cent solution by weight of the chlorophyll-bearing leaf.

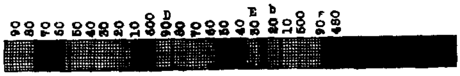


Fig. 3.—Spectrum of chlorophyll in the living leaf.

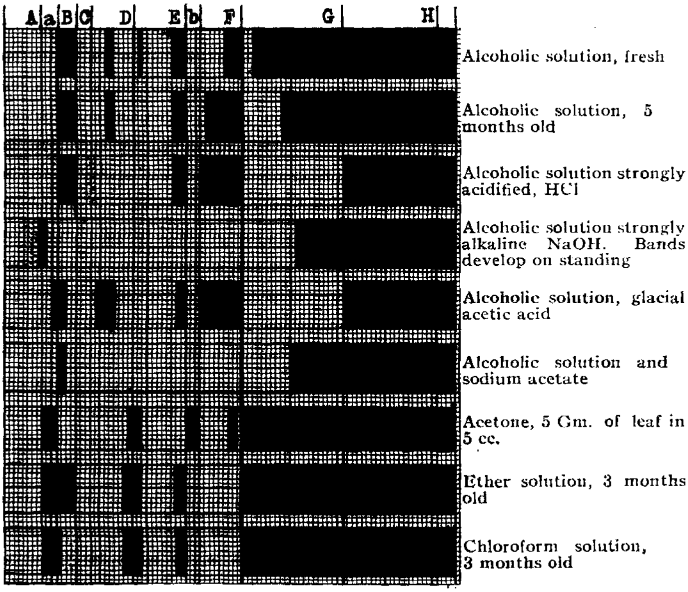


Fig. 4.—Spectrum of chlorophyll in different solvents.

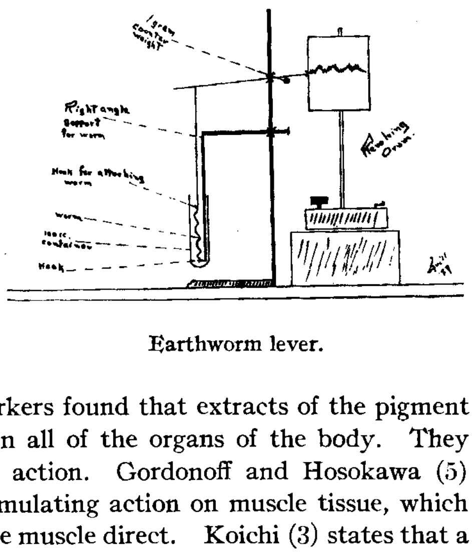
The solubilities of chlorophyll in various solvents are as follows: Alcohol 95 per cent, very soluble; acetone 95 per cent, soluble and of a brilliant green color; benzol, only slightly soluble; chloroform, soluble; ether, very soluble; water, insoluble, merely forming a suspension of the chlorophyll granules.

The majority of the extracts prepared with organic solvents exhibited a marked difference in color by reflected and transmitted light. Transmitted light shows a brilliant green color, the intensity of which is proportional to the strength of the solution and to the volume of light. Reflected light shows a brownish green to wine color the intensity of which is proportional to the concentration of the solution and the intensity of light.

The extracts were examined by means of a simple direct vision hand spectroscope. The heart and muscle tests were made by means of the usual lever attachment for the heart muscle of the frog; and the earthworm tests were made by means of a lever as suggested by Trendelenburg (10), and Schneider (8).

Trendelenburg, in his work on anthelmintics, suggests the use of muscle segments of the worm, thereby eliminating the disturbing action of the nerve supply. According to Gordonoff the action of chlorophyll extracts is on the muscle tissue direct. Even though the nervous organization of the earthworm is of a low or primitive type, the physical activities of this animal are greatly modified by the ganglia located in the anterior segments of the sexually mature animals. Sexual maturity was adjudged by the fully developed clitellum. General stimulation of the organisms is much under the influence of the nerve supply as a whole, and it was thought that for the purpose of the experiments whole worms would yield the more striking results. Perhaps for purposes of making bio-assays of chlorophyll preparations, which are beginning to appear upon the market, earthworm segments could be used.

The reason why the worm was selected for these experiments was that since it is a non-chlorophyll eating animal, it might, therefore, show unusual susceptibility to the action of chlorophyll. The results of the tests proved this to be the case. A normal tracing of the worm suspended in frog saline was taken, noting the quantity and the quality of the spasmodic contractions. After a normal tracing was obtained, the desired volume of chlorophyll extract was added to the normal saline solution; and the changes in the tracings noted and recorded in the usual manner. A series of six to ten tracings were obtained from one worm.



Earthworm lever.

The pharmacologic tests with the extracts of chlorophyll have revealed some interesting results. Gordonoff and co-workers found that extracts of the pigment produce a general stimulating action upon all of the organs of the body. They also demonstrated a blood regenerating action. Gordonoff and Hosokawa (5) proved that chlorophyll has a marked stimulating action on muscle tissue, which action is not *via* the end plates, but on the muscle direct. Koichi (3) states that a solution of chlorophyll in olive oil produces a slight bradycardiac action on the frog. Experiments by Bürgi (1) also show that there is a cardiac stimulation (tonus) due to chlorophyll. There is increase in the force of the heart contractions in cardiac insufficiency, and the action of the isolated frog heart is continued for periods of time far in advance of the heart not exposed to the action of chlorophyll.

Other investigators obtained similar results with the extract of chlorophyll or with some of its commercial preparations. Koenigsfeld (2) and Gordonoff and Amakawa (4) demonstrated a diuretic action due to chlorophyll. Koenigsfeld used a commercial preparation *chlorosan*. It is interesting to note that these men have obtained measurable results from very high dilutions of chlorophyll.

A guinea-pig of approximately 300 Gm. received a large dose (2.5 cubic centimeters) of contused green leaf in water, injected hypodermically. This material contained the cellular dedritus of the leaf consisting of chlorophyll granules, parenchymatous tissue, ducts, silica, bast fiber, etc. No appreciable effects followed within forty hours after the injection was made. Later an infection developed at the site of inoculation, no doubt due to the use of the non-sterile material. The animal soon recovered from this and appeared considerably stimulated as evidenced by increased appetite and physical activity. This test would seem to indicate that chlorophyll granules, not the extract, in large doses is non-toxic. Another observation of interest was that solids (cellulose, silica, etc.) injected hypodermically produced no marked effects. Possibly the animal's ability to overcome the secondary infection was due to the action of chlorophyll and that the later stimulating effect was due to the chlorophyll extractives formed in the tissue cells.

Some experiments on frogs were done as follows. A solution of chlorophyll (3 Gm. of chlorophyll bearing leaf macerated in 30 cubic centimeters of 50 per cent glycerin) was prepared. One cubic centimeter of this solution was injected into the anterior lymph space of the frog. There was an immediate increase in the respiratory rate followed by a decrease. Fifteen minutes later a second dose of 1 cubic centimeter of this extract was injected. Respiration almost ceased and there was a tendency to convulsive muscular contractions and then a lapse, into a paralytic state. After a lapse of several hours or longer the frogs returned to normal and exhibited evidences of marked stimulation. Several experiments of this kind were made using extracts of chlorophyll in alcohol in place of the glycerin extract. The results in every case were closely similar. These reactions would indicate a marked respiratory and general systemic stimulating effect due to chlorophyll.

Skin reactions on the forearm showed no results. For the skin reaction tests a concentrated ether extract was used.

In the earthworm (*Lumbricus terrestris*) tests, acetone and alcoholic solutions of chlorophyll were used. Check tests and control tests were made to allow for or to eliminate the action of the solvents. The following is a summary of the observations and tests made.

1. According to the observations of recent investigators (Willstätter and others) there is a chemical affinity between chlorophyll and hemoglobin.

2. According to Manoiloff, and others, there is a biological or physiological relationship between chlorophyll granules and erythrocytes. By means of certain reagents it is possible to determine the sex of animals, even the sex of the young *in utero*, employing the blood as the test substance; and the sex of plants (dioecious group) using chlorophyll extracts and the same reagents as those employed to determine the sex in animals.

3. Chlorophyll extracts, but not the chlorophyll granules themselves, have a general stimulating action on all the tissues and organs of animals, this stimulating action may be elicited by very minute doses of the extract. The eating of even large quantities of vegetables rich in chlorophyll does not yield the same degree of stimulation as does the administration of very minute amounts of the extract of chlorophyll granules.

4. The earthworm appears to be very susceptible to the action of chlorophyll extract and this easily obtainable and easily controllable animal may prove of great

value in making bio-assays of the chlorophyll preparations which are beginning to appear on the market.

5. Chlorophyll extracts apparently slow the action of the heart, at the same time increasing the force of the contraction, suggesting a digitalis action. The heart action (in the frog) appears to assume a dicrotic character with large doses.

6. The spectrum of chlorophyll resembles that of blood.

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THE OCCURRENCE AND ALKALOIDAL CONTENT OF VARIOUS EPHEDRA SPECIES II.

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In This Journal, Vol. XVI, No. 4, April 1927, we reported on the occurrence and alkaloidal content of several Ephedra species. We are now in a position to report on two species which were briefly mentioned in that article, and which we have since obtained and analyzed for alkaloidal content.

EPHEDRA TRIFURCA, TORREY.

The specimens of this plant were gathered for us by Frank A. Thackery of the United States Department of Agriculture, Bureau of Plant Industry, Sells, Arizona. The species was identified by Dean J. J. Thornber of the University of Arizona. The plants were received in October and were in full bloom. Figure 1