Papers Presented to Local Branches

CHLOROPHYLL[1]

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The Dutch scientist, Ingenhouss, was probably the first one to discover that carbon dioxide is assimilated by the plant. This was in 1779. His observations were confirmed by Theodore de Saussure, of Geneva, in 1804. Their announcements caused a sensation and were thoroughly ridiculed and really not accepted until Liebig's time. The name Chlorophyll was given to the green coloring matter of the plant by Pelletier and Caventou in 1817. This term "chlorophyll" applies strictly to the coloring matter and not to the so-called chlorophyll granules of the plant cell.

Chlorophyll does not occur alone, but always associated with two yellow coloring matters, namely, Carotin and Xanthophyll. These substances are embedded in granules of albumenoid composition. The shape of these granules is, in most plants, that of a lentil. In some algae we find them, however, in the shape of bands, or plates or stars. In a few cases, the chlorophyll is evenly distributed over the entire plasma. In the higher forms of plants, we find chlorophyll bodies in all *green* plant parts, namely, in the leaves, and here again in or below the palisade cells which line the upper side of the leaf, which side is exposed to the light. The chlorophyll bodies, called chloroplasts, consist of one or probably two albumenoid substances of a spongy texture, in the meshes of which the coloring matters, together with other substances (oil), are embedded. The body is surrounded by a very fine plasma membrane. Stoma as well as membrane are soft and plastic; the granules can therefore change their shape readily. A cell densely filled with granules—sometimes we find fifty to sixty granules in one cell—will show them in triangular, quadrangular or polyangular form. The membrane, however, prevents the granules from forming one mass. Upon the death of the cell, the membrane disintegrates and granule-body and contents form one shapeless mass. At the same time, the bright green color is changed to a brown, due to the presence of acid cell juices which can and do now attack the coloring matter. For this reason, our dried drugs, especially those which have not been dried carefully, present a brownish-green appearance.

Besides the coloring matters, we find certain colorless bodies and protein masses in the chloroplast. The chlorophyll is looked upon as the substance which assimilates the CO_2; the colorless bodies probably assist in the storing of the starch formed and in changing it into soluble diastase. Under the influence of light,

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CO_2 and H_2O are changed into starch and this is stored. When the starch production rests (at night) the starch is changed into diastase. The function of these chloroplasts is of greatest importance for the life of the plant as well as for our own. They convert the energy of the sun into vital energy. CO_2 and H_2O are formed into starch and other substances which serve as food for plant and animal.

In plant parts not exposed to light, so-called chromoplasts or bodies containing a yellow coloring matter take the place of chloroplasts. These chromoplasts, however, occur also in plant parts exposed to light, such as certain flowers, and are often formed from the chloroplasts. We have evidence of this in the change of color when apples or oranges ripen.

Starch is the first *visible* product of the chromoplast's photosynthesis, although simpler intermediate compounds are doubtless first formed. This starch is converted into soluble form at night and carried, in solution, into plant parts requiring nourishment.

Several conditions are absolutely necessary for the formation and function of chlorophyll. First of all and most important is light. Most plants fail to produce chlorophyll in darkness, although some do (conifers and maple). Light, especially the red rays, acts as stimulus. The work of chlorophyll cannot be done in darkness, nor can protoplasm produce chlorophyll without it. Plants grown in the dark or at low temperature are called "etiolated." They are of a yellowish color, and turn green on exposure to light. The coloring matter "etiolin" is most likely an intermediary substance in the formation of chlorophyll and is changed into this substance upon absorption of red light rays.

Iron is not a constituent of chlorophyll, but it also seems to act as a stimulus upon the living protoplasm to produce chlorophyll. On the other hand, neither light nor iron alone can bring about the production of chlorophyll. The cell must contain certain specific chromoplasts. The cells of animals, fungi and certain phanerogamic parasites do not contain these chromoplasts and do, therefore, not form chlorophyll.

As the presence of certain granules is necessary to form chlorophyll, so will chlorophyll do its work only when in the granule and again then only when contained in the living protoplasmic cell. Isolated chloroplasts continue for a time to absorb CO_2 and give off oxygen. If anaesthetized by ether, they will only absorb light rays, but no longer take up CO_2 nor give off oxygen.

The conditions and factors necessary for the change taking place in the cell are: CO_2, H_2O, warmth, light of definite wave length, chlorophyll and protoplasm. We might compare the cell to a factory. Light is the stimulus which sets the machinery going. Water and CO_2 are the raw materials, chlorophyll is the machinery itself. Starch is the food product turned out.

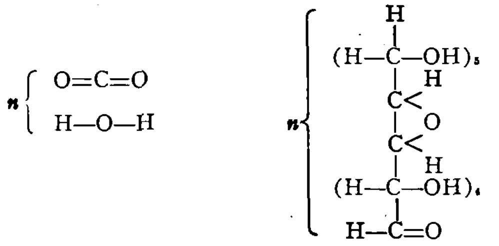
How is this wonderful work accomplished? Only very little is known about it.

As I stated before, starch is the first *visible* product of this photosynthetic process, but it is surely not the first and only product. The starch molecule is very complex, carbon dioxide and water very simple in chemical construction. We must assume, therefore, that a number of simpler compounds are first formed and these again are changed into starch.

We can represent the change as follows:

$$n(6CO_2+5H_2O)=n(C_6H_{10}O_5+6O_2), \text{ or}$$

graphic:



In one plant (Tropaeolum) it has been found that the formation of sugar precedes that of starch.

Van Baeyer proposed the following series of changes, as probable:

$$CO_2+H_2O=HCOOH+O=HCOH+O_2$$
$$6n(HCOH)=n(C_6H_{12}O_6)=n(C_6H_{10}O_5)+H_2O.$$

Objections to this theory are that HCOH and HCOOH are poisonous substances which would kill the cell, unless their conversion into harmless substances is instantaneous. Another series of reactions must, therefore, take place simultaneously.

Emil Fischer believes that the formation of a compound of CO_2 with HCOH precedes that of sugar and starch.

The amount of CO_2 taken up by plants is enormous. 10,000 L air contain 4-5 L of CO_2, which weigh 8-10 gm. Of this 3/11 is carbon. So in 10,000 L of air we have about 2 gm. C. A tree weighing 5000 kg. contains about 2,500,000 gm. of C. To get this amount, the tree must absorb and assimulate the CO_2 of 12,000,000 cc. of air. This figure is astonishingly large. However, we must not forget that the air contains about 3000 billion kg. of CO_2. This alone is sufficient to sustain plant life. Furthermore, the supply is constantly replenished by decaying matter, animal breathing, burning of wood and coal and by volcanoes.

Properties: Chlorophyll is an unstable substance. It is destroyed by strong sunlight, most readily by the red rays. This destruction seems to be an oxidation process, as it takes place only in the presence of oxygen. Chlorophyll is readily soluble in ether, alcohol, fatty and ethereal oils, petrolatum, petroleum, carbon disulfide. Alcoholic solutions are emerald green in transmitted light and show a blood-red fluorescence. The spectrum shows seven absorption bands and is absolutely characteristic. Benzin dissolves the chlorophyll and carotin from an alcoholic solution and leaves the xanthophyll in the alcohol.

Two general methods can be used for the separation of chlorophyll from its yellow companions.

Fremy's process: Shake one volume of alcoholic solution of chlorophyll with a mixture of two volumes of ether and one volume of concentrated HCl. Upon separation, the upper layer contains the golden yellow xanthophyll, the lower acid layer is colored bluish-green and contains a decomposition product, phyllocyanin.

Kraus' method: A 65% alcoholic solution of chlorophyll is shaken with twice its volume of benzin (.714). The upper layer is green, due to chlorophyll; the lower one is yellow, due to xanthophyll.

Very little indeed was known of the chemistry of these coloring matters until Willstatter and his assistants took up the work. Today we know many interesting facts regarding their chemistry, and thanks to this genial research chemist, a large amount of work has been and is now being done on chlorophyll. Judging by his past most successful work, we can confidently expect that the chemistry of these interesting substances will be completely cleared up within reasonable time.

I will give a summary of his work of the last six or seven years, and in order not to tire you, I will omit all purely chemical discussions and theories and present facts and how they were found.

Hoppe-Seyler, von Tschirch, Schenck, Marchkowski and many others had experimented with chlorophyll and came to divergent conclusions in many cases.

To obtain chlorophyll in comparatively pure form, Kraus' method was used and improved considerably by the substitution of wood alcohol for ethyl alcohol. Grass, spinach, and many other chlorophylls were used. It was observed that chlorophyll forms a colloidal solution with water. This colloidal solution was used for purposes of further purification. Ether does not extract chlorophyll from it, but its impurities, especially the carotin. The colloidal solution is pale green, turbid, not fluorescent. The chlorophyll can be recovered from it either by salting out and extracting with ether, or by adding an acid, when the chlorophyll is changed to its insoluble form, deeply colored, when it can be extracted with ether. The chlorophyll, thus purified, was subjected to acid and alkali of different strength and under different conditions and a number of reaction products obtained.

Chlorophyll
- Acids: Phytines—Phaeophytine { Phytochlorine; Phytorhodine }
- Alkalis: Phyllines—Glaucophylline—Rhodophylline { Pyrrophylline; Phyllophylline }

We will now take up the action of acids. Nitric acid destroys chlorophyll with separation of a colorless oil, which comes from the alcohol rest of the chlorophyll ester.

Reaction with other acids is marked by a color change and a splitting out of magnesium. The presence of Mg in the chlorophyll molecule was proven beyond doubt in all experiments; 2½ to 3½% of MgO is found in the ash. It is very readily split off by acids. HCl in 11% solution changes chlorophyll into a compound, which is soluble in ether with olive-green color. Warmed with alcohol, it assumes a bright red color.

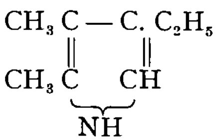
Compounds obtained by action of acids are called "Phytines," those obtained by action of alkalis "Phyllines." Acid action also produces an ester without basic or acid properties—consequently chlorophyll itself is an ester. Phosphorus was not found at any time, consequently an older theory that chlorophyll was similar to lecithin was proven incorrect. On treating an alcoholic solution of chlorophyll with an alcoholic oxalic acid, a product not readily soluble in alcohol was formed.

It was extracted with chloroform, to which it imparts a brown color. Isolated, this substance was called Phaeophytine. It is waxy, almost black; in solution, olive-brown with red fluorescence. With metals, zinc, copper of iron, it becomes chlorophyll-like. When this phaeophytine is saponified with alkali, it splits off an alcohol, $C_{20}H_{40}O$, which was called Phytol, and which was subsequently found in all chlorophylls.

In contradistinction to action of acids, alkalis do not split off the magnesium; the principal products of alkaline hydrolysis are deep green alkali salts which contain the Mg in complex form, most likely attached to the nitrogen.

On treating chlorophyll with alcoholic KOH on the water bath, it takes on a beautiful fluorescence. At 140° C., a crystallizable compound is formed. At 200°, it changes into another compound, which is red and which is termed Rhodophyllin. All of these still contain Mg. They show marked properties as to crystallization and solubility. To obtain rhodophyllin in pure state, it was necessary to use bomb tubes. In the ash of the compound thus prepared, Willstatter found to his surprise zinc oxide. Upon investigation, this was found to come from the glass. It had replaced the Mg in the molecule. This shows how easily the Mg can be replaced. Further experiments were carried out in a silver cup enclosed in an autoclave. Addition of water precipitated the rhodophyllin, which is purified by NH_4OH extraction of ethereal solution. Upon analysis it was found to be $C_{33}H_{34}N_4O_4Mg$. The present accepted formula for Haemin is $C_{34}H_{34}N_4O_4FeCl$. The difference of the one C atom in such a large molecule may be overlooked and Willstatter believes that the Rhodophyllin is very closely related to Haemin, and surely possesses the same nucleus.

Another chlorophylline, which was called Phyllopophyrine, showed the formula $C_{32}H_{36}O_2N_4$, which corresponds to Haematoporphyrin, a derivative of our blood coloring matter, $C_{32}H_{36}O_6N_4$. They differ only by the O_4. Both substances must have the same nucleus, for on reduction each one forms Di-methyl-ethyl pyrrol

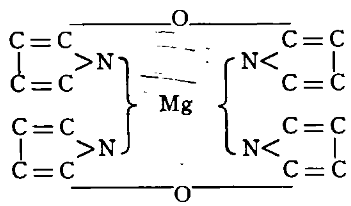


These facts point to a genetic relationship of the two substances, haemoglobin and chlorophyll, although their physiologic functions are entirely different. Chlorophyll contains magnesium, haematin contains Fe; these metals undoubtedly exercise a catalytic action and the assimilation of CO_2 is a function of the basic metal Mg. Plant life is mainly synthetic; carbohydrates, fats and albumens are built up of the simplest inorganic substances. Animal life needs iron to carry on its analytic functions by oxidation and Fe acts as the oxygen carrier.

The question as to whether the chlorophylls of all plants are alike was answered in the affirmative after an examination of several hundred different plant extracts. Discrepancies in the results were found to be due to an enzyme action. When a green plant is extracted with alcohol, an enzyme which accompanies the chlorophyll, becomes active and alcoholizes it; the phytol rest is replaced quantitatively by C_2H_5. The solvent will show a brownish color. This can be avoided by a rapid extraction, or by addition of a small amount of alkali. Some plants are richer in chlorophyllase than others. Water enhances the action of this enzyme, wood alcohol retards it. Chlorophyll and chlorophyllase appear to be specific for one another. Other esters, such as spermaceti, were not affected by chlorophyll, nor did other enzymes, such as pancreatin, split chlorophyll. The enzyme not only splits chlorophyll, but also forms it. Upon addition of phytol to phytol-free chlorophyll esterification took place; this enzyme, no doubt, plays an important part in the formation of chlorophyll in the plant.

The chlorophyll of all plants showed the presence of Mg and of 1-3 of its molecule of phytol. The final reaction products of alkalis on phaeophytine were two substances which are the components of chlorophyll: one bluish-green, photochlorine; the other yellow-green, phytorhodine, which were isolated and analyzed. A structural formula was found for phytol.

Absolutely pure chlorophyll was obtained and found to be crystallizable, forming a blue-black powder with metallic lustre, readily soluble in alcohol and ether, but insoluble in benzin. The solutions are blue-green. It contains 5% MgO in its ash, its composition corresponds to $C_{55}H_{72}O_6N_4Mg$ and most likely possesses the following nucleus:



The yellow coloring matters have also been examined. Berzelins found a yellow substance and called it Xanthophyll. Arnaud found another substance the formula for carotin to be $C_{40}H_{56}$, a hydrocarbon. (Benzin extraction). and named it Carotin. Willstatter isolated them and examined them. He found Xanthophyll is obtained by precipitation from alcoholic solution with petroleum benzin. It crystallizes readily, forms red plates. Its formula is $C_{40}H_{56}O_2$. We have here a simple relation to carotin. Xanthophyll is its oxide. Both are unsaturated and absorb oxygen readily, even by mere exposure to air. They probably are the vegetable oxygen carriers.

SOME ACTIVITIES OF THE AMERICAN MEDICAL ASSOCIATION AND THEIR VALUE TO PHARMACISTS[1]

L. E. WARREN, PH. C., B. S., CHICAGO.

As the name implies, the American Medical Association is an association of physicians. In a general way it fulfills the functions for medicine that the American Bar Association does for law, the American Pharmaceutical Association does for pharmacy, and the American Dental Association does for dentistry. The American Medical Association has a membership of about 38,000.

The American Medical Association is ruled by a body of representatives called the House of Delegates. These are elected by the several state associations, by

[1]Read before the Chicago Branch A. Ph. A., Nov. 26, 1912.