permits us therefore to understand such a confused phenomenon as death, but we hope that this investigation will permit us to understand many phenomena of life if we try to obtain the principal substances composing living protoplasm, compounds of proteids and lipoids, artificially.

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OUR SEARCH FOR CHLOROPHYLL AND FOR THE VITAMINS

INTRODUCTION

DURING the past decade there is probably no subject that has been given more attention, by scientific groups as well as by the public in general, than that of vitamins. On the other hand, chlorophyll has been given little if any attention even by those who should be studying it most. Vitamins are as ubiquitous in our present-day literature as chlorophyll is in nature. Volumes have been written about both subjects, but still we are in the dark ages regarding the rôle of chlorophyll and the origin of the vitamins. This paper is written to review briefly the struggle of man to know something regarding these two subjects. My purpose is to raise questions in the minds of scientifically-minded men rather than to answer any question regarding chlorophyll or vitamins. The future only can satisfactorily answer these questions.

A—CHLOROPHYLL

History of the Chemical Nature of Chlorophyll

About ninety years ago Berzelius attempted to isolate the green pigment from leaves by the use of strongly reactive reagents and succeeded in obtaining only products of radical decomposition. Thirteen years later Verdeil pointed out a possible relation between chlorophyll and blood. Both pigments were believed to contain iron. The chief result of the earlier period of investigation, in which strong chemical reagents were used, was that chlorophyll was found to be related to hemin.

Nearly fifty years ago the relation between blood and chlorophyll was further strengthened by the work of Hoppe-Seyler. Methods of handling the pigments became more careful, for chemical investigation became more and more dependent upon spectral analytical methods. Willstätter says that the method was far overrated, for it did not prevent serious errors, since many important changes of chlorophyll and its derivatives exert no influence upon the absorption spectrum, while certain insignificant changes of constitution produce disproportionately great changes in the spectrum. Workers of this day found phosphorus and potash present in their preparations of chlorophyll; chlorophyll was considered a lecithin. From the chlorophyllan which was obtained, phylloporphyrin was prepared and this definitely established the blood-chlorophyll relation.

After Hoppe-Seyler, chemical workers made no attempt to isolate or analyze chlorophyll. They believed that its isolation was impossible on account of its alterability, chemical indifference and extreme solubility. Some thirty years ago Schunck and Marchlewski analyzed the acid and alkaline decomposition-products of chlorophyll, but learned nothing regarding its chemical characteristics. The chemists of the day did not seriously consider observations made by physicists or botanists. The optical treatises of Stokes gave important hints regarding the existence of two components of chlorophyll, while Borodin made fascinating microscopical observations.

Twenty-two years ago Willstätter published his first paper on chlorophyll. Since then he and his collaborators have deduced the characteristics of its constitution from a consideration of the derivatives that were formed upon reaction with acids and alkalies. In a few years they obtained pure chlorophyll (1911) but learned practically nothing new regarding its chemical or physical properties. Chemically, chlorophyll was found to be composed of carbon, hydrogen, oxygen, nitrogen and magnesium, and formulae have been given for chlorophyll *a* and chlorophyll *b*. These formulae are only tentative and much more work is needed to ascertain the exact chemical formula for each of the two components. There seems to be no doubt that chlorophyll and hemin each contains four pyrrol nuclei, yet how these nuclei are linked together in the molecule is a matter for much further consideration.

Willstätter's Search for Chlorophyll

The story of the search for chlorophyll is indeed a very fascinating one. It also is the record of a man who attempted and accomplished what chemical workers of an earlier day said was impossible.

Much of the work was very laborious and the yields must often have been most disheartening. This was especially true of his first attempts to obtain chlorophyll by methods of fractionation. His work can not be fully appreciated unless something is known regarding the amount of materials used and the number of men assisting him. In all more than eighteen highly trained investigators assisted him in the work on chlorophyll. For more than seven years several men working at the same time were actively engaged in solving the chemical nature of chlorophyll. It was in 1911 that the tremendous amount of labor which he and his coworkers had put forth was crowned with success. It was then that pure chlorophyll was first isolated. To-day, chlorophyll can be isolated from living or dried leaves, and the methods have been developed so that we may obtain the pure green pigment in amounts sufficient for any purpose.

It probably will be a revelation to even those who have been most interested in chlorophyll to know what vast quantities of material were necessary in the study of chlorophyll and its derivatives. In working on the derivatives, phytochlorin and phytorhodin, lots of ten and twenty-three kilograms of ground nettle-leaves were used in a single extraction. In a further study of the composition of chlorophyll in which magnesium was found to be present instead of phosphorus, iron or potash fifty kilograms of leaf-meal were used at one time. In the preparation of pheophytin, lots of one hundred and four hundred kilograms of leaf-meal were extracted. In all eight hundred and sixty grams of phytol were obtained from three kilograms of pheophytin, which was obtained from more than one thousand kilograms of dried leaves. Even then the chemistry of phytol was not successfully accomplished and at a later time another worker again undertook more work on the chemistry of phytol. To-day, even more can be learned about phytol. To work out the chemistry of rhodophyllin, 40- to 70-kilogram-lots of leaves were used. One hundred-kilogram-lots were used in learning what we know about the phyllins. I have said nothing yet about the number of one-, two-, ten- and twenty-kilogram-lots of leaves used in obtaining information regarding: ethyl chlorophyllide from thirty-five different plants, the fractional separations carried out in the isolation of the two components of chlorophyll, the preparation of chlorophyll, pheophytin and phytol from seventy species of various classes of plants, the quick and slow extraction of the chlorophyll of more than two hundred species of plants of many classes in order to compare the amount of phytol present in the chlorophyll obtained or of many of the other phases of the investigations on chlorophyll. As yet I have not even mentioned the amount of materials that must have gone down the sink.

It would be very difficult indeed to give even a rough estimate of the vast amount of solvents used in the study of chlorophyll. Petroleum ether, methyl and ethyl alcohol, acetone and ether were used by the barrels and possibly by the carloads. Let us take one example:

In obtaining pure chlorophyll from one kilogram of dried leaves about six liters of acetone, seven of petroleum ether, one of ether and four of methyl alcohol are necessary. To fractionate eight grams of chlorophyll into chlorophyll *a* and chlorophyll *b* about ten liters of ether, forty-five liters of methyl alcohol and fifteen liters of petroleum ether are required. When you get through with the fractionation you may or you may not have chlorophyll *a* and chrolophyll *b*, all depending upon how well you know and use your knowledge of the nature of chlorophyll.

I have purposely given the separation of chlorophyll *a* from chlorophyll *b* as an example, for here is where we are going to have to begin to learn more about chlorophyll, by studying its two components each in the pure state. Most of the work on chlorophyll is simple and easy when compared with the fractionation of the two components, but even yet the amount of organic solvents necessary is quite large.

At first, the preparation of pure chlorophyll was as difficult an undertaking as is now the separation of the pigment into its two components, for it was a fractionation method. The discovery, that chlorophyll of a certain degree of purity is not soluble in petroleum ether when free from alcohol, led to the easy preparation of pure chlorophyll and there is no reason to believe that some day chlorophyll *a* and chlorophyll *b* may not be easily separated or if not easily separated they will be easily determined quantitatively, which should greatly aid us in the solution of problems concerning chlorophyll.

The Absorption Spectra of Chlorophyll and its Derivatives

Willstätter has given us many measurements of the absorption spectra of chlorophyll and its derivatives. Two methods were used to obtain these results—the photographic and visual. Willstätter says that a comparison of the photographs and diagrams convinces us that photographic reception and representation of the spectra are not, as is generally assumed, a method possessing greater objectivity than observations with the eye and graphical reproduction of the measurements that are thus obtained. The photographic method is also characterized by considerable subjectivity which is conditioned by the sensitiveness of the plate, the illumination, the procedure and by the reproduction. Even though photograms reproduce all the observed absorption bands correctly according to their position, nevertheless their boundaries and relative intensities are somewhat less exactly shown than in the case of direct observation with the eye, the sensitiveness of which exceeds that of the photographic plate.

Since the days when the work on chlorophyll was completed, tremendous advances have been made by the bureau of standards in measuring the absorption spectra of solids and of liquids. The optical transmissive and absorptive properties of solutions of dyes are of primary and fundamental importance in practical analysis and identification as well as in theoretical studies of chemical constitution. Much work has already been done on this subject, but the results are largely qualitative or only crudely quantitative. Observations made of the edges of the absorption bands at different concentrations or thicknesses are only roughly quantitative. It is only in very recent years that any work of an exacting nature has been done and practically all of this has been done at the Bureau of Standards.

At the present time four methods of measuring the spectral transmissive properties of dyes have been developed. These methods cover the range from 240 to 1,360 millimicrons. The work done by Willstätter covers only the visible spectrum, four hundred to seven hundred millimicrons. The photographic method with the Hilger sector photometer covers the range 240 to 500; the photoelectric null method covers 380 to 600; the visual, using the König-Martens spectrophotometer, 436–710 and the thermoelectric method covers 600–1,360. Consequently, methods are now available for studying very accurately the absorptive properties of chlorophyll in the ultra-violet and infra-red regions of the spectrum as well as in the visible.

Plant physiologists have done practically nothing in the way of an exact study of factors affecting even the visible portion of the chlorophyll spectrum. That portion of the spectrum beyond the visible region has hardly been given the slightest serious consideration. The plant physiologist needs to know everything that can possibly be known regarding the absorption curve for chlorophyll. Our knowledge by all means should extend into the regions beyond the visible, for who knows what may be discovered. After the spectral transmissive properties of chlorophyll from 240–1,360 mμ are very accurately known, then and then only can we begin to study the exact effect of any given wave-length of light upon the chlorophyll molecule. The effect of each wave-length should be studied before we can say that we know very much regarding the effect of light upon chlorophyll formation or decomposition. Practically all we now know about light and chlorophyll is concerned with the mass-effect of visible light upon a mass of chlorophyll, which has not even been quantitatively determined. There is a tremendous opportunity for study in this field. Of course many obstacles will have to be overcome, but they are evidently not so unconquerable as was the preparation of chlorophyll for so many years. The instruments are available and chlorophyll of sufficient purity can be prepared, so there is no real reason why work on this important problem should be longer delayed. It is a problem of pure science and one that is fundamental in plant physiology and bids fair to offer its share of intellectual and spiritual contributions to the welfare of mankind.

The problem becomes all the more interesting when we consider that chlorophyll is being continually formed and broken down in plants. By such a consideration very much more chlorophyll is involved in the growing season than we have been generally led to believe. This fact alone would tremendously increase the importance of chlorophyll and would lead us to suspect that chlorophyll is perhaps something more than we had formerly considered it to be. When we consider that about one per cent. of the dry weight of green leaves is chlorophyll, then and then only do we begin to realize the enormous amount of chlorophyll which is produced annually by all green plants. The amount produced then must be several per cent. of the weight of the dry organic matter produced per year by green plants.

B—Vitamins

History of the Chemical Nature of Vitamins

Ninety-four years after Pelletier and Caventou (1818) proposed to call the green pigment present in plants chlorophyll, Funk proposed the name vitamin for substances which were necessary in a complete diet. Seventeen years ago, the year (1911) that Willstätter first prepared pure chlorophyll, Funk claimed to have isolated vitamin B from rice-polishings. The evidence indicated that it was a crystalline nitrogenous compound belonging to the pyrimidine group and that it was possibly a constituent of nucleic acid. One year later oryzanine was obtained and was found to contain carbon, hydrogen, oxygen and nitrogen. Attempts have been made to isolate vitamin A from cod-liver oil and analysis of the products obtained gave only carbon, hydrogen and oxygen; nitrogen was not found. Funk, however, feels that in the interest of future investigation the question regarding the presence of nitrogen in vitamin A should be left open. Attempts at preparing pure vitamin B have been made by several workers, but as yet the pure product has not been obtained. Analysis of the products seems to show that it contains carbon, hydrogen, oxygen and nitrogen; workers seem to agree that the compound is of a cyclic nature.

At the present time about all that can be said is that no one as yet has been able to isolate a vitamin in pure form. In regard to the chemistry of vitamin, little of real significance has been accomplished. The chief advances, however, have been made in regard to their physical properties. We do not even know what they look like, whether they are crystalline or amorphous substances, whether they have color or taste. Some one has said that the vitamins may even be a form of energy and not a substance at all. Our knowledge about vitamins consists chiefly of their effects upon animals. It is known that they are present in practically all plant-products though often in only very minute quantities. The greatest progress has been made in regard to our knowledge of their presence in food-products. They seem to play a very different rôle in nutrition from the other food-constituents. They regulate and control certain vital processes in the animal organism. Fractions having high-vitamin concentrations have been obtained by various investigators from cod-liver oil, from yeast and from rice-hulls. Fractions ten thousand times the original concentration in cod-liver oil have been obtained. In spite of this high degree of concentration we are far removed from the isolation of the various vitamins and the determination of their chemical composition. A host of investigators, however, have established that these chemically-unknown substances are of vital importance.

Since there are no known chemical tests for the vitamins, progress in their isolation depends largely upon the success of the physiological tests available for guiding the fractionation. Improvement in the accuracy and rapidity of these tests is undoubtedly a matter of great importance.

During the past few years there has been a revival of the interest in the chemistry of vitamins. Attempts have been made at isolating A, B and D in crystalline form. Many years have been required to obtain these elusive compounds even in the state of purity now reported. Seidell looks forward to the synthesis of the vitamins and to their extensive application to the nutritional needs of man.

The Search for Vitamins

Only a part of the story of the search for vitamins can be written, for these substances have not yet been isolated in pure form. The work has always been very laborious and the yields have been anything but encouraging.

In 1911, Funk reported the results of his attempts in isolating 0.4 grams of a colorless crystalline substance from fifty kilograms of rice-polishings obtained from Malay. Later from 380 kilograms of the polishings he obtained 2.5 grams of the crude product which contained vitamin B. His analysis showed that nitrogen was present in the product obtained. From seventy-five kilograms of dried yeast he obtained 0.45 grams of colorless needles and from one hundred kilograms of yeast 2.5 grams of crude product containing vitamin B was obtained. This product produced 1.6 grams of crystals.

After repeated trials crystals of oryzanine picrate have been obtained from three hundred grams of fat-free rice-bran. Vitamin B has been sought from several other sources. Seidell has modified the method of obtaining vitamin B several times, but as yet only a crude product has been produced. Synthetic experiments have been resorted to by Williams and Seidell, but to date nothing definite has been found regarding vitamin B.

From 25-kilogram-lots of cod-liver oil, attempts have been made to obtain vitamin A, but as yet only crude fractions have been obtained. Cod-liver oil is probably the only substance from which attempts have been made to isolate vitamin A. Some even are led to suspect that the vitamin is present only as impurities in the fractions which have been obtained. It is known that codfish do not synthesize vitamin A. The vitamin has been found to come from the food of the codfish, which is small fish. The small fish feed on plankton, which consists of copepods, and the copepods live on diatoms, which have been shown to be able to synthesize vitamin A. At the present time many observations point to the conclusion that only plants which contain chlorophyll are able to produce vitamin A. Vitamin A may be present in many food-products, but its ultimate origin seems to be chlorophyll-containing plants.

No exhaustive attempt as yet has been made to isolate vitamin C. Fractions containing vitamin D have been obtained from malt-house combings and from yeast. Other vitamins have been reported, but nothing at all definite is known regarding their chemical nature, for as yet no attempt has been made to isolate them.

There are several reasons why it is so difficult to obtain the vitamins in a pure state. The starting materials, such as cod-liver oil, yeast and rice-hulls, are very complex materials from which it is possible to isolate many organic compounds. The vitamins are present in only very minute quantities in any raw material. Cod-liver oil can be fractionated so that there is ten thousand times as much in a given sample as there was in the original material. If we consider that the concentrated fraction containing vitamin is pure vitamin, then the vitamin present in the original cod-liver oil must have been less than one hundredth of one per cent. Since we are quite positive that the fraction was not pure vitamin, then we can be just as positive that the amount of vitamin present in the original material must be very small indeed. Of course the laboratory reagents and manipulations destroy or lose much of the original vitamin. At the present time no color or chemical tests are known which will aid in the various steps of purification. Feeding experiments, which require much time, are used and these rarely give uniform results.

The most urgent problem at the present time seems to be concerned with the chemical nature of the vitamins, for only when reasonably pure preparations of the active substances are available can we expect to gain a clearer knowledge of their physiological action upon the human body. The medical practice to-day is sorely in need of just such knowledge as only the study of vitamins in a pure state will give.

Value of Vitamin Studies

Since practically every person is somewhat familiar with vitamins little need be said about the value of studying them. Vitamins are believed to eliminate certain specific diseases such as beriberi, scurvy, rickets and many others. Vitamins are generally believed to reduce the prevalence of many other diseases and greatly increase the resistance of the human body to all kinds of infection. Even sterility is believed to be produced by a lack of one of the vitamins. As to their exact value in bodily metabolism, medical science is not yet prepared to tell us. Vitamins are not only of interest to men of science and of medicine, but to those engaged in political economy. The recent world war, which greatly interfered with the normal movement of foodstuffs and consequently caused the wretchedness of stricken Europe, is only one striking example.

Just how valuable the vitamins are in animal economy we yet have very little knowledge. In regard to the value of the vitamins in plant-economy we are completely in the dark. Funk and many others would like to know the rôle of vitamins in the vegetable kingdom, which means that we must know their rôle in plant physiology. When we have isolated and are able to use pure vitamins in our scientific investigation on plants and animals then we will be able to discuss very ably the value of vitamins and their rôle in nature. There seems to be every reason to believe that vitamins are very intimately concerned in the rôle of all organic life. A most fundamental study of their chemistry and rôle in nature then should be thoroughly in harmony with progress in pure science, and any avenue suggested leading to their possible isolation should be seriously considered.

CONCLUSION

Any one interested in light, in chlorophyll or in the vitamins should not fail to learn all they can regarding the interrelation of these subjects. The chemist, the physicist and the plant-physiologist should each be interested from the bearing these subjects have upon one another and also upon his own field. To say that no relation exists only reveals our ignorance and we as scientifically-minded men can say that no relation exists between these subjects only after we know much more about them. At present we all must confess that our knowledge regarding these subjects is very elemental. Funk believes that most of the problems discussed by those interested in vitamins have only been scratched on the surface, due to the fact that such slow progress has been made in the chemistry of the vitamins. The exact nature of light, of chlorophyll and of the vitamins is as yet foreign to our knowledge.

Reports on the isolation of pure chlorophyll up to the day of Willstätter have been only reports of failures, and to date all attempts to isolate a vitamin have met with the same fate. Many of the problems which Willstätter undertook and solved would have thoroughly disheartened many of the most seasoned investigators. Attempt after attempt to ascertain the real nature of the vitamins have only met discouragement. But since wise men know that failures are the greatest stepping-stones to success in any endeavor we now find ourselves in a position to strive harder than ever to unravel the real nature of that which we would know.

I have attempted to review in a very brief way our knowledge of the chemistry of the vitamins and of chlorophyll, and at the same time show the great amount of work necessary to find out what little we now know regarding these subjects. The isolation of chlorophyll from plant-tissue undoubtedly will be an easy matter when compared with the isolation of vitamins from raw materials. Chlorophyll is highly colored and relatively abundant in plant-tissue while vitamins are present in only very small amounts and are probably colorless or only slightly colored. Color changes in the isolation of chlorophyll tell us instantaneously when we have altered the molecule, but we have no such guide in the isolation of a vitamin. No investigator can say when we will be able to isolate a pure vitamin from the raw materials in which it is found. Any study of the chemistry of vitamins knows that their isolation is confronted with many obstacles which as yet have not been overcome.

I have presented an hypothesis which undoubtedly many will say is misleading. To them I can say that often in science some of the most misleading hypotheses have led to favorable solutions of many scientific questions. At any rate, we all can say that if the subjects discussed herein remain quiescent, progress will never be made. We solve only the problems which we most actively think about, and certainly these subjects are worthy of our concentrated thought.

In the world of inorganic chemistry the atom is as complicated as the solar system and has its own microscopic astronomy of electrons. In the world of organic chemistry, chlorophyll is about as complicated a substance as can be found. In ordinary chemical processes the whole atom gets heated when electrons shift. But the right radiation or an electron stream of the right velocity will loosen the electrons without wasting energy in heating up the mass of the atom. Now the green leaves of plants are marvels of efficiency in using directly the light of the sun to form organic matter. Somehow, nobody knows just how, sunlight causes electronic shifts which tie the atoms to form all sorts of organic substances. The plant grows, develops and so we have more organic matter of all kinds. It is the most fundamental mysterious reaction in nature and one on which our food and our lives depend. Chemists have not yet duplicated such a silent efficient process. The atom is the key to many chemical problems of to-day and chlorophyll has a very good chance of becoming the key to much which concerns all living processes. Who among us is prophet enough to say what the chemistry of to-morrow will be?

We now know what master chemists can do with the atom, for from it they get power-radio, television, the vitaphone and thousands of other wonders. We know what a master chemist can do in the way of analyzing chlorophyll and breaking it up into the components, but what we want to know is how the Great Master Chemist of the Universe very quickly and so quietly produces such large amounts of organic matter, nitrogen-compounds in particular which we as investigators are now considering as the basis of all life as we know it.

Men everywhere are spending huge sums of money to make practical applications of discoveries which have been made in the field of pure research. Our advance, however, will be measured not so much by the practical applications we make, for practical applications are comparatively easy, as by the progress we make seeking knowledge regarding fundamental things, of which we are now in almost complete ignorance. If chlorophyll is in any way related to the vitamins and if the vitamins play as large a rôle in maintaining health as they are assumed to play, then certainly a knowledge of chlorophyll will do something to reduce the $15,000,000,000 annual loss of this nation due to sickness alone. The problem of pure research is not so much a problem of getting or giving of money as it is of getting workers who passionately give of themselves in the pursuit of truth for truth's sake. The most essential quality of an investigator is the spirit within and this quality is not purchasable with money.

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SCIENTIFIC EVENTS

THE AMERICAN STANDARDS ASSOCIATION

RECONSTRUCTION of the American Engineering Standards Committee to keep pace with the growth of the industrial standardization movement in the United States is now under way, according to an announcement by the committee. The principal features of the reconstruction are the definite federation of national organizations, under the name American Standards Association, in such a way that trade associations interested in standardization may more readily join in the direction of the movement; placing the technical work of approving standards in a standards council, and concentrating administrative and financial responsibility in a board of directors composed of twelve industrial executives.

The reorganization has been unanimously approved by the main committee of the A. E. S. C., and is now being voted upon by the membership. The action of the committee results from more than a year's intensive consideration of the subject by the main committee and rules committee. The latter was enlarged for the purpose to include a representative of each of the 19 member-bodies desiring representation.

Among the conditions which led to the reorganization are the growth of the trade association movement together with the predominating position which the trade association is coming to have in the field of industrial standardization, and the increasingly important direct part which the plant executive is playing in the standardization activities within his plant and in the movement as a whole. Recognition of this latter condition is reflected in the make-up of the board of directors, which will control the general administrative and financial affairs of the association. The industrial executives composing this board will be elected on nomination of member-bodies and will serve for three years.

Approval of standards and matters of procedure will be in the hands of a standards council. The council will be composed of not more than three representatives of each of the member-bodies, the councilors also serving for a period of three years.

The objects of the association, as stated in the new constitution, will be: To provide systematic means by which organizations engaged in industrial standardization work may cooperate in establishing American standards in those fields in which engineering methods apply, thus avoiding duplication of work and the promulgation of conflicting standards; to serve as a clearing house for information on standardization work in the United States and foreign countries; to further the industrial standardization movement as a