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THE CHEMICAL AND PHYSICAL COMPOSITION OF PROTOPLASM[1]

IT is a very great pleasure for me to take part in such a joyful event as the dedication of a new institution devoted to the botanical study and researches. Botany is only a part of biology, and I should like to speak, in my address, on one of the most important problems not only of botany but of biology in general, on the chemical and physical composition of protoplasm.

It is known that life is concentrated in the living contents of cells. The most important part of these contents is protoplasm. It represents the medium in which all other organs of the cell perform their functions. If protoplasm dies no phenomena of life can occur, and the organism becomes a sacrifice of death. It is therefore necessary for all biologists and physicians to know the properties of living protoplasm, its chemical and physical composition. This composition plays a very important part in life and death.

But how can we investigate the chemical composition of protoplasm if at the first touch of our chemical reagents living matter dies? How can we define this composition if we can investigate only the products of destruction of protoplasm?

In order to answer this question we must remember the procedure employed by chemists in their investigations of the chemical composition of new substances. They endeavor first of all to decompose the unknown substance and to determine the chemical composition of products of its destruction. From this chemical composition they arrive at the conclusion concerning the chemical structure of the unknown substance. Then they try to obtain this substance synthetically.

Therefore, in order to determine the chemical composition of living protoplasm, we must first of all investigate the chemical composition of the products of its destruction. Then we must endeavor to form a scheme of the chemical structure of the principal compounds composing living protoplasm. Finally, we must try to obtain these principal compounds artificially.

It is evident that the substances of dead protoplasm we can investigate chemically in our laboratories arise from the substances of living protoplasm. They

[1] Address delivered at the dedication of the new botany building of Wellesley College, Wellesley, Mass., November, 1927.

must be regarded as the products of decomposition of the substances forming living protoplasm. The chemical investigation of dead protoplasm was made only in some cases, namely, it was made on protoplasm of plasmodium of Myxomycetes, on leucocytes and on red blood cells. All kinds of protoplasm proved to contain water, protein substances, fats, sterines and phosphotides; besides, they contained carbohydrates, products of the decomposition of proteins and a small quantity of salts. The dry protoplasm of plasmodium contains, for instance, as principal substances, nucleoproteids (about 50 per cent.) fats, sterines and phosphotides (about 10 per cent.). For the sake of abbreviation we shall call fats, sterines and phosphotides simply "lipoids." The dry protoplasm of leucocytes contains about 80 per cent. nucleoproteids and 16 per cent. lipoids. Finally, the dry protoplasm of red blood cells consists of about 95 per cent. red proteid, haemoglobin, and about 5 per cent. lipoids.

We must, therefore, conclude that the principal component part of protoplasm consists of proteids and lipoids. The protein substances and lipoids can be discovered, by microchemical analysis, also in other cells of plants and animals. At the same time, carbohydrates and products of the decomposition of protein substances represent, evidently, only an admixture because they can be absent, as, for instance, in red blood cells, while salts are always present in a small amount.

It is evident that protein substances and lipoids can represent products which arise from the substances of living protoplasm and form there some compounds which are destroyed by the reagents used in our chemical analysis. But they can be present in living protoplasm in free state. In order to get an idea about the chemical structure of these compounds we can not content ourselves with a chemical analysis and we shall endeavor to apply some physical methods, too.

As is known, living protoplasm possesses the so-called selective permeability. It is very permeable to water and narcotic substances which are soluble in lipoids, as, for instance, alcohol, ether and so on, but it lets salts, sugar and other substances, soluble in water, through with difficulty. The selective permeability of protoplasm disappears after its death. Dead protoplasm lets all substances dissolved in water through very easily. According to the well-known German botanist, Pfeffer, we supposed twenty years ago that the surface of protoplasm is covered by a membrane, by the so-called "plasmamembrane," which only possesses the selective permeability, while the inside of protoplasm is as permeable to all substances as gelatine jelly. According to recently published investigations, Pfeffer's theory proved to be wrong; it was based on an incorrect interpretation of experiments. Protoplasm has no "plasmamembrane," and all its parts possess the selective permeability. We must, therefore, conclude that death is accompanied by changes in protoplasm which bring about the disappearance of the selective permeability of its whole mass. What changes can occur there?

As is known, death can be produced by the agents which alter protein substances chemically, as, for instance, by corrosive sublimate, strong acids, high temperature and strong light. At the same time these agents do not alter lipoids. It is therefore very probable that death is accompanied by some changes in the proteinic part of the compounds forming living protoplasm. On the other hand, it is impossible that protein substances cause its selective permeability; indeed, these substances can not absorb liquid narcotic substances, but they absorb sugar and salts. If living protoplasm had consisted of free protein substances, if it had contained lipoids as an admixture, it would absorb salts and sugar very easily, but it would not let narcotic substances pass through. We arrive therefore at the conclusion that the compounds forming living protoplasm contain not only a protein group, but also substances which absorb narcotics and do not absorb salts and sugar. We can scarcely doubt that these substances are lipoids. Such conclusion is confirmed by the fact that reagents reacting chemically with lipoids (as, for instance, saponin) are poisonous and produce death. On the other hand, all chemical changes of protein substances brought about by different agents annihilate the selective permeability of protoplasm, that is, cause the protoplasm to get rid of its lipoids. Therefore, the lipoids are maintained, in living protoplasm, by proteids; they probably form some chemical compounds with proteids.

Our conclusion is confirmed by the fact that the concentration of narcotics, which is sufficient to bring about the coagulation of protein substances in living protoplasm, is smaller than the concentration of the same narcotic producing the coagulation of protein substances of our laboratories, as for instance, albumen of eggs. Moreover, the greater the solubility of a certain narcotic in lipoids is, the smaller is the concentration of this narcotic which produces the coagulation of proteins of protoplasm in comparison with the concentration of the same narcotic which produces the coagulation of albumen. The close connection between proteids and lipoids in living protoplasm is proved once more by the well-known phenomenon that living protoplasm can not be stained by anilin dyes, while dead protoplasm absorbs these

dyes very easily. Indeed, all protein substances absorb anilin dyes very easily, even if they are dissolved in water; therefore, they can not be in a free state in living protoplasm. Some lipoids, as, for instance, lecithin, absorb anilin dyes, too, and are freed in protoplasm only by death. Finally proteolytic enzymes which dissolve free proteins do not act on living protoplasm at all.

There is, therefore, good reason to believe that the principal substances of living protoplasm represent chemical compounds of proteids and lipoids, but these compounds are so unstable as to be compared with explosive substances; they are destroyed, like the latter, not only by chemical agents and high temperature but also by purely mechanical effects, as, for instance, by a blow. The destruction of these compounds leads immediately to death. But it is quite equitable to ask: Why must the destruction of the compounds of proteids and lipoids in protoplasm bring about death?

In order to answer this question we consider the physical structure of living and dead protoplasm. The living protoplasm has a colloidal structure.

As is known, the colloidal structure, the colloidal state, is the state of a very great dispersion of substances. Substances are dispersed into very minute particles in a mostly liquid medium, which is called the dispersion medium. If we dissolve, for instance, albumen of eggs in water we obtain a colloidal solution in which albumen forms the dispersed phase, water forms the dispersion medium. If we dissolve two or more colloidal substances in water we have two or more dispersed phases in the same solution. The formation of a colloidal solution depends certainly upon the chemical and physical properties of both the dispersion medium and the dispersed phases. If, for instance, water of an albumen solution is replaced by alcohol the colloidal solution can no more exist; and in general if the dispersing medium of a colloidal solution is altered or destroyed the dispersion can not be maintained and the dispersed phases become precipitated. On the other hand, if the dispersed phases themselves are changed chemically, they usually can not remain in the solution and coagulate.

Living protoplasm represents a colloidal solution because it shows all properties of colloidal solutions and, namely, the properties of hydrophilic colloidal solutions. Botanists know very well that the aggregate state of protoplasm of plant cells is liquid. Recently it could be shown that not only plants but also animals possess liquid protoplasm. Its liquid aggregate state is proved by the following well-known facts: living protoplasm which had got rid of the cell walls takes the form of a globe in water; only liquids take this form if they are in another liquid; moreover, in living protoplasm are very often observed movements of particles or the protoplasm flows like a liquid; finally all liquid bodies, as, for instance, water or oil, take in living protoplasm the form of globules.

Although the principal mass of living protoplasm is liquid, it can contain gelatinous granules or fibrils. Moreover, the aggregate state of protoplasm is changeable: the liquid protoplasm can become rigid, partly or in its whole mass. Changes of the aggregate state of protoplasm are often observed on the surface of amoeba and plasmodium. In the axial parts of pseudopodia of foraminifera this change can also be produced by changes of water content of protoplasm. Still oftener strong changes of internal friction of protoplasm are observed. All these changes of the aggregate state and viscosity of protoplasm sometimes proceed very quickly and can be explained only by its colloidal structure. Only hydrophilic colloidal solutions can change their aggregate state and viscosity so rapidly without any change of temperature, as, for instance, it is observed on solutions of gum Arabic or albumen which become gelatinous by evaporation and melt again if they are mixed with water. The often observed disappearance and new appearance of granules in protoplasm are so like the same phenomena in colloidal hydrophilic solutions that one can not now doubt that protoplasm of plants and animals represents a colloidal (hydrophilic) solution.

We have concluded that the principal chemical compounds composing living protoplasm consist of proteids and lipoids. We may now ask: Do these compounds form the dispersion medium or dispersed phases of protoplasm? The above-mentioned behavior of living protoplasm in the solution of anilin dyes and its selective permeability show us that these compounds form the dispersion medium of protoplasm. Indeed, its principal mass remains colorless in relatively strong solutions of anilin dyes, while the most granules absorb them. On the other hand, the osmotic properties of protoplasm are due to the dispersion medium.

It is, therefore, very comprehensible that the destruction of the chemical compounds of proteids and lipoids must produce death. If the dispersion medium of a colloidal system is destroyed the whole system is destroyed, because the colloidal dispersed phases of protoplasm can not remain unchanged too: they must coagulate or dissolve in the surrounding liquid and are therefore lost for life. Death must occur because all phenomena of life depend on the colloidal structure of protoplasm.

The investigation of chemical and physical composition of protoplasm, although it is not yet finished,

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