If variation was markedly greater in the early periods of the existence of living matter, it is clear that it would have been possible for evolutionary change to have been effected much more rapidly than at present-especially when we remember that the world was then comparatively unoccupied by organisms, and that with the change of conditions consequent on the cooling and differentiation of the earth's surface, new places suitable for organic life were continually being formed. It will be observed that the conclusion we have now reached, viz, that variation was much greater near the dawn of life than it is now, and heredity a correspondingly less important phenomenon, is a deduction from the selection theory. It becomes, therefore, of some interest to inquire whether a suggestion obtained by a perfectly legitimate mode of reasoning receives any independent confirmation from other sources. The first source of facts to which we turn for such confirmation must obviously be paleon-But paleontology unfortunately tology. affords us no help. The facts of this science are too meagre to be of any use. Indeed, they are wanting altogether for the period which most immediately concerns us-namely, the period when the existing forms of life were established. This took place in the prefossiliferous period, for in the earliest fossiliferous rocks examples of almost all existing groups of animals are met with.

But although paleontology affords us no assistance, there is one class of facts which, when closely scrutinized, do lend some countenance to the view that when evolutionary change was at its greatest activity, *i. e.*, when the existing forms of life were being established, variation was considerably greater than it is at the present day.

But as this address has already exceeded all reasonable limits, and as the question which we are now approaching is one of very great complexity and difficulty, I am reluctantly compelled to defer the full consideration and treatment of it to another occasion. I can only hope that the farreaching importance of my subject and the interest of it may to some extent atone for the great length which this address has attained.

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## THE PROTEIDS OF LIVING MATTER.\*

OF all the phenomena of nature vital phenomena have always appeared to the human mind the most complicated and intricate, so much so that even many scientific men have ascribed them to an inexplorable cause—the so-called vital force. This 'vitalism' is adhered to by many even to-In scrutinizing the various vital dav. phenomena we observe, however, a great difference in the degree of complexity. There are on the one hand actions of an admittedly purely chemical, physical, and mechanical nature; and on the other those of organization, genetic differentiation, and of irritability on which differences of opinion still exist. The former appear of relatively simple character compared with the latter, which seem to offer difficulties of explanation insurmountable for science in its present state of development.

Protoplasm even of the simplest cells represents a highly complicated machinery. The organization corresponds to the construction of a machine, while its motive power consists in various forms of energy. Hence, two principal questions arise : (1) How is the machinery constructed? (2) What is the nature of the primary energy moving the machinery? The latter question is of a simpler kind than the former. We

<sup>\*</sup>A paper read before the joint meeting of the Biological and Chemical Societies of Washington, May 5, 1900.

know that cells produce heat by the process of respiration (some of the lowest forms of fungi also by fermentative action) and that this heat energy is necessary for carrying on the various functions of life, for which purpose it can be transformed into chemical, electrical and mechanical energy in the cells. But how are the cells enabled to bring on the active oxidation phenomena that characterize the respiration process, and by what special contrivance can the heat energy thereby produced be converted into other forms of energy?

The conception of the nature of living protoplasm has changed with the progress of time. Formerly and by some authors even at the present time, it was defined as a changing mixture of different substances and all compounds found in the protoplasm were considered indispensable and intrinsic parts of it. Compare for instance, the publications of Reinke on the protoplasm of *Æthalium septicum*. Recently also Verworn has returned to this old conception.

But such a view cannot be logically entertained when we see that a certain protoplasm does continuously the same kind of work like a mechanism of a fixed structure. This mechanism consists here in a specific structure built up of easily changeable proteins requiring a certain amount of water and mineral salts. The amount of imbedded material, however, may continuously change. This material consists either of thermogens, as fat and sugar, or of mere by-products of metabolism which are soon excreted after their formation, either to the outside or into the vacuole.

What kind of work will result? it depends upon the configuration \* and the specific chemical structure of these protein molecules on the one hand, and upon the specific construction of the machinery on the other. Thus, the protoplasts of the various vegetable and animal glands resemble just as

\* The relative position of atoms in space.

many different chemical laboratories; the protoplasm of the muscular fibers severs molar motions; that of the nerves is especially adapted to conduct impressions by irritation to considerable distances. But the most complicated differentiation governs the structures of the nuclei of the generative cells, the most intricate laws rule the genetic differentiations in the development of an organism.\*

From the chemical standpoint our first inquiry is directed, as already mentioned, to the question : What causes the respirationprocess of the cells ? What enables the proteins to cause the active oxidations of fat and glucose as long as the cells are alive, and why do these oxidations cease as soon as the cells are killed? Oxidation is a purely chemical phenomenon; hence, this question is of a plain chemical nature. Some might claim that by the death of the cells the organization is destroyed and this is the cause of the stoppage of the oxidation. But this view cannot be upheld, since even the most complicated machinery cannot produce work without the impelling energy. There must also be a certain amount of energy at the bottom of the respiration itself, there must be some energy for kindling the fire of the locomotive. What is this energy that leads to respiration? There remains no other answer than this: It is chemical energy caused by the specific nature of the proteids of the living protoplasm which nature changes in the process of dying. + Nu-

\* Nucleo proteids form the framework of the nucleus and of the cytoplasm and may exist in innumerable isomeric forms, of which the stereo-isomeric forms probably are of great importance as regards the differences between species. The word *proteid* is used here to designate the complicated compounds of proteins, such as nucleins, hæmoglobin, mucin, while the word *protein* comprises all kinds of albuminous matter in a general sense.

† A chemical change in the proteins of the living matter in the dying process was assumed as early as 1837 by John Fletcher and again in 1875 by E. Pflüger. But even at the present day many physimerous compounds are known which very easily undergo a chemical change; modern chemistry defines them as *labile* (unstable) compounds. Sometimes influences of a very subtle nature suffice to cause migration of the atoms in labile position to a more stable position whereby an isomeric and more stable product results.

I have repeatedly pointed out that we must distinguish between potentially labile and kinetically labile compounds; in other words, between static labile and dynamic labile.\* To the former belong for example, the explosive organic compounds, as nitroglycerole or certain diazo compounds, to the latter aldehydes and ketones.<sup>†</sup> While the former are destroyed by chemical changes either totally or partially, the latter furnish numerous derivatives with great readiness, or change easily by atomic migration or by polymerization into isomeric or polymeric compounds. Many highly interesting cases of chemical change by atomic migration within labile molecules are known, but reference needs here be made only to one of the simpler instances-the change of ammonium cyanate into urea which is accomplished by merely heating the aqueous solution of the former.

Ammonium cyanate. Urea.  

$$O:C:N.NH_4$$
 becomes  $O:C: NH_2$ .

This transformation is also of interest from another point of view, as being the first synthesis of an organic substance, accomplished by Woehler in 1828.

ologists adhere to the old opinion of chemical identity of proteins in the living and dead protoplasm.

\* The potential chemical energy in this discussion does not refer to the energetic relation of the compound to others, as in combustion, but to the intramolecular relation between the atoms of the compound itself. We may distinguish this as intramolecular potential chemical energy.

† The enzymes also belong to the dynamically labile compounds, as I have pointed out in SCIENCE, December, 1899. The analogy of living matter to dynamically labile compounds is also elucidated by the action of many poisons. Prussic acid, diamidogen, hydroxylamine, have in moderate dilutions at the ordinary temperature no action whatever on dead protoplasm or on the ordinary proteins, while they change living protoplasm very easily to dead protoplasm, a change induced by a chemical attack, labile compounds being more easily attacked by chemical agents than stable ones.\*

The principle of chemical lability has not yet been the object of close investigation even by chemists, while physiologists have ignored it altogether, and this may be the reason that the necessity for assuming a chemical difference between the proteins of the living and of the dead protoplasm has not found due consideration, although this distinction is absolutely necessary for comprehending the *chemical* properties of living protoplasm. The free chemical energy due to the labile character of the proteids in the living protoplasm leads to respiration and since this energy cannot be produced after those labile proteins have changed to stable ones, respiration must cease also at the moment of death. The heat produced by respiration increases still further the oscillations of the labile atoms in the plasma proteins, in other words, it increases the charge of chemical energy, and the most complicated chemical work can now be carried out, the specific construction of a protoplast determining the kind and direction of the work. The maintenance of the respiration process is just as little due to a con-

<sup>\*</sup> A systematic toxicological review shows us among other things that all compounds acting upon aldehydes and all that easily attack labile amido-groups are poisonous for all kinds of living protoplasm which fact led me to infer that the lability of the plasma proteids is caused by the presence of aldehyde and amido-groups within the same molecules. Compare: A natural system of poisonous actions, Munich, E. Wolff, publisher.

tinuous self-decomposition and regeneration of protoplasm, as Pflüger and Detmer assume, as to a previous activifying of oxygen as various other authors had supposed; but, as I have repeatedly pointed out, to an 'activifying of the thermogens' by a charge with chemical energy from the protoplasm. An activified oxygen (ozone) or also hydrogen peroxid would kill the protoplasm sooner than it would burn up fat and sugar to carbonic acid and water. Indeed, nature has provided every living cell with a special enzyme which decomposes rapidly any trace of hydrogen peroxid that might make its appearance as a by-product in the active course of the cellular respiration.

Other functions than chemical ones, as for instance the intricate phenomena of karyokinesis or the remarkable differentiation into ectoderm, entoderm, and mesoderm, or the differentiation of nervous fibers, although they are still very mysterious, also depend primarily upon the labile nature of the proteins. Not the slightest advance towards an understanding of these phenomena can be expected when this is disregarded.

The primum movens in the living protoplasm must be defined as a mode of motion of labile atoms in the plasma proteins; that is, as a special case of chemical energy. Living protoplasm has often been compared to a watch and dead protoplasm to a watch whose machinery has been destroyed by crushing, but this comparison is not a proper analogy, for, while the chemical character of the watch material remains unchanged after pulverizing, that of the dying protoplasm does not, but it undergoes a chemical change.

These deductions must necessarily lead to the further question: Is there any evi-

dence of labile proteins existing before the organization into living matter is accomplished? Is any not yet organized forerunner of living protoplasm found in cells? Investigations of Dr. Th. Bokorny and myself carried on for a number of years have demonstrated beyond a doubt that there indeed occurs in many plants a reserve protein matter of highly labile nature, different from all other reserve proteins. It undergoes a great change under the same conditions that cause the death of the protoplasm itself, although more slowly. It seems logical to conceive this as the material which, by being converted into organized nucleo proteids, forms the living matter. The ordinary proteins must be brought first into the labile easily changeable condition before they can serve this purpose. We designated that labile reserve protein as active albumin or proto-protein, in contradistinction to the passive reserve proteins.

This peculiar, easily changeable body is met with in certain groups of plants very frequently, as in Julifloræ, Cystofloræ, Æ-Saxifragineæ, Myrtifloræ, and sculineæ, Rosifloræ, while in others rarely, as in Compositæ, Labiatæ, Leguminosæ, and Gramineæ, in fungi and in algæ. Spirogyra forms an exception with the algæ, inasmuch as this special group contains often very large quantities of the labile reserve protein. The widespread occurrence of this substance may be inferred from the fact that of 250 species examined by Th. Bokorny, G. Daikuhara, and myself, fully 120 were found to contain it in one part or other.

Of special objects rich in this proto-protein may be enumerated: Leaves of Prunus, Rosa, Quercus, Alnus, Mimosa, Pæonia, Saxifraga, Sedum, and Cephalotus, the bark of Prunus, Quercus, and Fagus; petals of Gentiana, Primula, Sorbus, Cyclamen, Hotteia, and Cornus; stamens of Eugenia, Drosera, and Melaleuca; pistils of Crocus, Salix, Euphorbia, and Rhododendron; nectaria of Passiflora;

<sup>&</sup>lt;sup>†</sup>A detailed description of this enzyme will be given in a special Bulletin of the U.S. Dep't of Agriculture. A preliminary note appeared in SCIENCE recently.

roots (epidermis) of Saxifraga, Enothera, Xanthoxylon, and Thesium; fruits (epidermis) of Punica and Camellia. In the roots and fruits it seems to occur less frequently than in leaves and flowers, especially frequent is the occurrence in the epidermis and the fibro-vascular tissue.\* Leaves in the shade contain less than those exposed to light, while leaves with partial albinism may contain in the white parts as much as in the green. In plants exposed to starvation by being kept in darkness it is gradually consumed with production of amido compounds.

It is generally stored up in the vacuole, but in some cases also in the cytoplasm. The method of proving its presence consists in the application of dilute solutions (0.5%)of weak organic bases as caffeine and antipyrine. These can easily enter the cells without killing the protoplasm immediately and can separate the active albumin in form of little globules which coalesce gradually

to larger bright droplets whose changes by various reagents can easily be followed under the microscope.

Generally it will suffice to place small pieces of vegetable tissue in a few drops of caffeine solution and then tear them up into finer fragments by the aid of dissecting needles. In other cases it is preferable to let the caffeine solution act for a number of hours.

These globular formations were designated by us as proteosomes. When the objects soon after the formation of the proteosomes are replaced in water the droplets will grad-

\* Especially noticeable is the large amount present in insectivorous plants, *Utricularia* alone being devoid of it. In Cephalotus the large amount is especially remarkable. *Drosera* shows it not only in the leaf but also in stem and flower. ually disappear again in proportion as the caffeine or antipyrine leaves the cells again by osmosis. Return of the objects to the former solutions makes the droplets re-When, however, the cells die appear. gradually or are killed by iodine solution or acids in high dilution, or by formaldehyde, hydroxylamine, diamidogen. prussic acid, free cyanogen, or salts of copper, or by vapors of ether, these droplets change their properties, becoming vacuolized, insoluble, and solid. Generally they become at first turbid from innumerable little vacuoles which in most cases unite soon and form one large vacuole, thus producing a hollow sphere representing itself under the microscope as a ring. If now the objects are placed again in water these changed proteosomes will not dissolve as they did before. The coagulation by heat is easily observed on dipping the objects in boiling water containing 5 per cent. of sodium chlorid. A change somewhat different is



FIG. 1.

FIG. 2.

FIG. 1. Subepidermal cells of the lower side of the leaf of Echvera, after treatment with caffeine.

FIG. 2. Cells of Spirogyra treated with caffeine. The proteosomes produced show beginning vacuolization.

> brought on by highly dilute ammonia of 1 per mille or less, inasmuch as the proteosomes thereby shrink and solidify but generally do not vacuolize as in the above cases.\*

> \*A full description of the proteosomes is given in Chapters 9 and 10 of my treatise: 'Die chemische Energie der lebenden Zellen,' Munich, 1899. E. Wolff, publisher.

In dead cells caffeine never produces proteosomes. If we treat Spirogyra, which is an excellent object for studying the behavior of the proteosomes, for one minute with a dilute solution of iodine in potassium iodide the globules may still be produced immediately afterwards but not after ten minutes. It can easily be shown that the substance has not passed to the outside by osmosis, since the liquid surrounding the treated algæ does not show any reaction with caffeine. Various tests proved that the proteosomes consist of protein matter, but in most cases there are impurities present, especially tannin, a fact which has misled Pfeffer and some of his students so far as to assume these proteosomes to be merely compounds of tannin with common albumin and with caffeine. It is evident that such compounds would not exist in two different modifications and would not change their entire behavior with the death of the cells as above described. Pfeffer's objections are untenable, as repeatedly demonstrated. He has, for example, assumed that on the death of the cells certain compounds leave the protoplasm and upon entering into the vacuole cause there a change of the proteosomes. But it is easy to convince one's self that proteosomes can also often be produced in the cytoplasm itself, especially in the case of Spirogyra. Since these proteosomes remain in the cytoplasm also unchanged so long as the cells are alive, the assertion of He has also argued Pfeffer is groundless. that the phenomenon in question, viz, the production of proteosomes, may be due to the neutralization of the acid cell sap, but we have shown that the cell sap of Spirogyra has no acid reaction\* and nevertheless it yields frequently numerous proteosomes.†

It is to be regretted that many plant phy-

\* Botanische Zeitung, 1884.

<sup>+</sup>A careful observer will not confound these easily changing proteosomes produced only in living cells (as Dr. Albert F. Woods has suggested) with other glosiologists rely upon the declarations made by some 'authority' instead of forming their own opinion from an unbiased critical investigation. The history of science shows that erroneous conceptions are often sustained for a long time in scientific circles simply because a man of a certain influence has defended them. The recognition of the genuine respiration of green plants furnishes a good illustration to this remark. Liebig, by weight of his authority, wiped out this truth for 20 years from science.

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THE NEW YORK BOTANICAL GARDEN.\*

THE corporate body known as the New York Botanical Garden was created by an act of the legislature approved by the governor April 28, 1891, and amended March 7, 1894. This association was called into existence "for the purpose of establishing and maintaining a botanical garden and museum and arboretum therein, for the collection and culture of plants, flowers, shrubs and trees, the advancement of botanical science and knowledge, and the prosecution of original researches therein and in kindred subjects, for affording instruction in the same, for the prosecution and exhibition of ornamental and decorative horticulture and gardening, and for the entertainment, recreation and instruction of the people."

By the same act the Board of Commissioners of the Department of Public Parks were authorized to set aside two hundred and fifty acres of Bronx Park, and erect suitable museum and other buildings at a cost

\* Written by the request of the Editor of SCIENCE. See also article on same subject by author in the *Popular Science Monthly* for June, 1900.

bular masses produced by hypochlorite of soda upon the protoplasm of dead cells. Such formations and their distinction from proteosomes were described by Woods in SCIENCE, April, 1899.