THE MICROSCOPICAL STUDY OF LIVING MATTER.

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I.—METHODS.

SCIENTIFIC apparatus are, broadly defined, of two classes—quantitative and qualitative; or, in other words, they are either instruments to increase the accuracy of the work of scientific observation, or else to increase the range, or the number of possible kinds, of observations. In each class there is one apparatus which rightfully holds the first place, *facile princeps* —among instruments of precision, the balance; among instruments extending the possibilities of observation, the microscope. Yet it must be at once added that it is only since the microscope has had the co-operating advantage of the microtome that its full value has been developed. The microtome is in certain respects the most perfect instrument of precision we possess, and works with greater exactitude than most fine apparatus of the physicist. Before proceeding to the special subject of this article, a few words in regard to both the microscope and the microtome are necessary.

The microscope has been very slowly evolved and is the creation of no one man. In its present form it is, like a living species according to Darwin, the outcome of the survival of the fittest of innumerable variations, the majority of which have been discarded. Indeed to one interested in microscopes and familiar with the present model, nothing can seem quainter than the old forms, which prevailed during the earlier half of this century and have since become extinct. In the evolution of the microscope two factors have been dominant, the demand for optical improvement and the demand for mechanical convenience. Both of these demands have been well met, so that there appears little left for the future to achieve, until an entirely new direction is opened for further evolution. It need hardly be premised that the optical part is the essential part of a microscope. The optical performance of the best microscopes is to-day nearly perfect, having become so very slowly by numerous small improvements. Although magnifying glasses were invented, it is said, in the twelfth century, compound microscopes with achromatic lenses have been in use barely three-quarters of a century, while the introduction of homogeneous immersion lenses dates from 1878, and of the perfected apochromatic lenses from 1886.

The history of the microtome is much briefer, only about twenty-five years. About 1865 the habit of making thin slices of plants and of certain parts of animals for microscopical examination was becoming general, for it was found to be very advantageous for the study of the minute elementary constituents of organs. By means of sections taken in various planes it was found possible to gain a profound insight into the intimate structure of parts far too small and delicate for ordinary dissection of them to be possible. Another discovery increased the scientific importance of microscopical sections. The microscopically visible elements of living bodies are found to include a variety of constituents, which in their natural condition can be identified only with difficulty, or not at all. The discovery referred to was that some of these constituents can be artificially colored so that they become conspicuous when magnified; it was accidentally made in 1858 by Professor Gerlach, who used carmine to prepare a colored injection of blood vessels. Since then very numerous coloring agents have been employed and the methods of applying them to sections to bring out this or that fine detail of organization have been enormously developed. Thus it has come about that our present conceptions of the structure and functions of living matter are so largely based on the knowledge derived from the study of sections artificially stained, that without the art of preparing them half of the progress made by biology during the last quarter century would certainly have been impossible. From this standpoint it is easy to understand the importance, to investigators and students of biological sciences, of the section-cutting machine or microtome.

The first microtomes were invented by Frenchmen, one form by the botanist Gabriel Rivet,* the other by the anatomist, Léon Ranvier, in whose laboratory at the *Collége de France* in Paris I had the advantage of working shortly after his microtome was first introduced (1872). A very simple affair it was, nevertheless at that time a great boon. Until then when a section for the microscope was needed, the object to be cut was held in one hand and a sharp razor in the other—we did not know then that a razor is a very poor tool for delicate cutting—and one cut as thin a slice as one's skill permitted. To make any section at all required great natural dexterity and much practice; and to make a tolerable section was the privilege of only the exceptionally skilful; to make what would now be considered a good section was altogether impossible. The idea which Rivet and Ranvier applied was that of mechanical movement, on the one hand for the knife, on the other for raising the object so as to regulate the thickness of the sections. Their microtomes gave us sections of even and known thickness, their best performance being at the rate of from thirty to forty sections per millimetre. It is true that microtomes had been made earlier by Oschatz and Welcker and perhaps others, but nothing available resulted. The instruments were failures, and the credit for the invention is properly divided between Rivet and Ranvier.

During the next few years a rapid succession of minor improvements in microtome construction was effected, by which the possibility was achieved of securing sections about one-half as thick as before. It then became clear that the principal difficulty lay with the tissues of plants and animals, and that any further advance in the art of preparing very thin sections must depend upon so treating the living material that it would be better suited for slicing. After innumerable trials of methods the most diverse, we have learned by experience to look upon paraffine as our chief resource. It is superfluous to enter upon details of technique, but by a series of delicate manipulations it is possible to permeate a vegetable or animal organ with paraffine, so that it becomes absolutely uniform in consistency, especially as regards elasticity and hardness, and may therefore be readily cut into much thinner sections than otherwise, so that at present it is an easy matter to make sections at the rate of 150 to 200 per millimetre. This again puts greater requirements upon the

* First announced in "*Bulletin Soc. Botanique de France*," Vol. XV., p. 31 1868).

microtome. Experience has here been instructive. When a section is made which is only one-two-hundredth of a millimetre thick, its perfection depends upon its having two parallel surfaces, both cut by the knife. If the surfaces are not parallel the sections will not be good, in other words the error of the cutting motion must be only a fraction of the diameter of the section, which means that the accuracy of the microtome must be far beyond the utmost visible precision. To attain this result two important rules have been learned. *First,* that the microtome must be built very heavy and entirely of metal; the finer the sections it is expected to make, the more solid and rigid must the construction be. *Second,* the knife must be made not thin-bladed, like a razor, but very thick and more like a chisel. The best knives are now about three-eighths of an inch thick at the back and their cross-section is wedge-shaped. The object of these rules is to eliminate the error due to the elasticity of the metals, for a bending of a metallic part of a microtome far too minute to be seen by the naked eye would suffice to ruin the quality of a section. I have been testing a new microtome, invented and manufactured in this country, in which especial attention has been given to securing the utmost delicacy of action, and it now seems probable that with this machine a successfully prepared specimen can be cut into perfect sections of one five-hundreth of a millimetre, which is at the rate of nearly 13,000 to an inch. Indeed, I hope sections of one-thousandth of a millimetre will be readily made. The special value of such sections to the biologist is owing to the size of the microscopic elements of animals and plants. These elements are named *cells;* each cell consists of a central mass or *nucleus,* which is completely enveloped in a peculiar substance, known as protoplasm. In the human body the diameter of a single cell averages perhaps fourteen-thousandths of a millimetre (0.014 mm.) and the diameter of a nucleus perhaps six-thousandths of a millimetre (0.006 mm.). It is not until our sections made with a microtome are less than one-two-hundredth of a millimetre that they are sufficiently thin to take in not more than a single nucleus. These thin sections are therefore indispensable for the study of nuclei, and from the study of nuclei, many of the most remarkable and fundamental conclusions of modern biology are derived, such, for example, as our current theory of heredity.

Some conception of the minute dimensions within which the biologist actually carries on his manipulations may be gathered from the fact that, whereas he makes sections of two thousandths of a millimetre and which probably do not vary one five-thousandth in thickness, the astronomer, as I learn through the Director of the Harvard Observatory—Professor Pickering—does not carry his finest accurate measurements beyond about the same value:—0.002 mm.*

The progress of biology depends to a greater extent than the progress of any other branch of science upon the application of the microscrope. The increase in the efficiency of the microscope as an instrument of research during the past dozen years has been great and is due to the improvement of the optical quality of the lenses, and is due still more to improvements: *First,* in the art of section cutting, and, *second,* in the art of artificially coloring sections, so as to render clearly visible details of microscopical structure, which cannot be otherwise definitely recognized. Every microscopist anticipates immediate progress because the use of coloring matters for his work is in its infancy only and the possibilities of the near future in this regard have aroused the keenest hopes.

II.—THE THEORY OF LIFE-UNITS.

THE existence of cells in plants was demonstrated by Schleiden in 1838, their existence in animals by Schwann in 1839. Cells have been termed by the Viennese physiologist, Ernst Brücke, "elementary organisms" and this appellation still indicates the prevalent conception of biologists to-day. But there has been a growing reaction, which has found its culminating expression in Professor Whitman's utterances on the "inadequacy of the cell-doctrine." This new view seeks for material units much smaller than a single cell, units which carry on vital functions very much as molecules carry on chemical functions. I propose to designate this view as the "Theory of life-units." The theory is protean, but despite its forms and modifications

*Professor Pickering writes me that Nobert's nineteenth band has been resolved and is ruled on glass at the rate of about 4,436 lines to a millimetre. He adds: "A graduate circle 1 m. in diameter (such as are in common use for meridian circles) allows $\frac{1,000\,\pi}{1,296,000}$ mm. to every second of arc; that is 0.0024 mm. The readings are usually made to tenths of seconds, and the graduation should be correct to within a few tenths of a second. The successive lines of the graduation are usually 5′ apart.

its invariable foundation is the hypothesis that the living matter (protoplasm and nucleus) includes, *first*, a non-living portion, and, *second*, a number of discrete particles which are very much smaller than cells and carry on some or all of the distinctively vital functions, especially hereditary transmission and the production of organization. The authorities are agreed as to the existence of such particles for which I have adopted the name of "Life-units," but they are nowise agreed as to the exact size, number or functions of life-units, upon which many different names have been bestowed, whereof more later. As to size the particles are held to be somewhere near the limits of microscopic vision, that is either too small to be seen, or else identical with certain visible granules of minute dimensions.

The theories of life-units were foreshadowed in the last century by Buffon and Bonnet, and in this century by Richard Owen, in his article on Parthenogenesis, published in 1849, but it was reserved for that Englishman, who stands next to Shakespeare in world-wide fame, to create the first scientifically available form of the theory with which we are here concerned. Darwin called his view the "Theory of Pangenesis," and named the life-units "*gemmules*." Their existence was purely hypothetical with him, and he made no attempt to determine their size. *Gemmules* are thrown off from every part of the body and circulate freely through the system ; they are transmitted from parent to offspring ; in their dormant state they have a mutual affinity for each other, leading to their aggregation either into buds or into sexual elements ; when supplied by proper nutriment they multiply by self-division, subsequently becoming developed into cells like those from which they were derived. Such in brief was Darwin's theory, stated almost in his own phrases. Although we now know definitely that the theory is untenable, I can repeat what I have said elsewhere:* "This hypothesis is the suggestion of a masterly mind, and as a succinct and comprehensive expression of the facts of heredity must command admiration."

The second of the theories of life-units was formulated by Herbert Spencer in his *Principles of Biology*, 1864. It was an original and valuable hypothesis and a distinct advance beyond the theory of Pangenesis. Spencer's arguments may be found in the fourth and eighth chapters of Part II., of his *Biology*. He

**Human Embryology*, p. 86. (New York, 1892.)

terms his hypothetical particles "*physiological units,*" fixes their size as larger than chemical units, and smaller than cells, and assigns to them especially what would now be called the morphogenetic function or production of organization, in other words he attributes to their activities the phenomena of repair, regeneration of lost parts, and of reproduction (hereditary transmission). Spencer's theory is historically so important that I will quote a few lines. "If then, this organic polarity can be possessed neither by the chemical units nor the morphological units, we must conceive it as possessed by certain intermediate units, which we may term physiological. . . . We shall hereafter find various reasons for inferring that such physiological units exist, and that to their specific properties, more or less unlike in each plant and animal, various organic phenomena are due."

There have been several modifications of the theory of pangenesis. One of the first illustrates the grotesque vagaries of ignorance, and may interest the psychologically curious, though scientifically it is worthless. It was entitled the "perigenesis of the plastidules" and was produced by Ernst Hæckel, a sensational popular writer, whose statements have been found to be usually erroneous. *Plastidules* were life-units, and the same term has been used by the late Dr. Elsberg of New York. The botanist Nägeli, in 1884, uses the term *idioplasmatheilchen,* de Vries, in 1889, the term *pangenes,* Wiesner *plassomes,* Whitman, in 1893, *idiosomes,* Haacke *gemmaria.* August Weismann has a much more complete scheme; his life-units are *biophores,* which in groups form *determinants,* groups of which form *ids,* groups of which form *idants.* Each one of these authors has his special set of opinions regarding the sizes, shapes, relations, qualities and functions of his particular kind of life-unit. Together they have made a gay tournament of hypotheses.

In all the theories above alluded to the life-units are purely hypothetical; but there is another set in which the life-units are identified as certain visible minute granules found in cells. The first to propound a theory of visible life-units was Lionel Beale, who maintained that all organisms consist of formative material, which was living, and of formed material, or dead matter. The visible grains of formative matter were his life-units and were designated by him with the name *biolasts*. Essentially similar are the theories of *granuli* (Bioblasten) of the German Altmann and his Italian follower, Zoja. Again, under the influence of the prevailing tendency, various writers have found the agents of hereditary transmission in certain granules in the nucleus, which are thus made into life-units with restricted functions. These granules are termed chromosomes, on account of their special affinity for coloring matters of various kinds, but by the theorizers are variously christened.

The theory of life units, whatever its precise form, is closely connected with the conception of definite structure in living matter. Now structure, as biologists currently conceive it, implies the presence of at least a framework of solid material. When we examine living matter it is usually after it has been subjected to various re-agents and profoundly altered. Studying protoplasm in this way, investigators have been led to consider it to be made up of a network of true protoplasm, the meshes of which are filled with fluid. Since the fluid is merely water with certain substances in solution, it cannot be alive, but life resides properly in the threads of protoplasm, which contain accordingly the hypothetical life-units.

III.—THE THEORY OF THE LIVING FLUID.

THIS theory is new, though hints and suggestions of it are not lacking in older publications. It may be briefly formulated thus: *The physical basis of life is protoplasm; protoplasm consists of two fluids, intimately commingled, yet separate, and which may include various granules of solid organic substances, more or less complex, and also include globules of various liquids.* This theory in its best form has been termed the foam theory, because foam offers the most familiar illustration of the kind of structure conceived by this theory as characteristic of living matter. As in foam, air and water are commingled, so in protoplasm are cell-sap and the proteid or albumenoid fluid commingled. The latter it is, which, when coagulated by our so-called preserving re-agents, gives under the microscope the familiar appearance of a network of solid threads. This theory I consider by far the best theory of the nature of protoplasm yet advanced. Professor Otto Bütschli of Heidelberg has been the leading advocate of the theory. I will mention only two of the kinds of phenomena, which the foam theory accounts for better than any other. The first is the fusing of two masses of protoplasm, so that they are absolutely confluent. The second is the incessant chemical change, which would be, there are reasons for thinking, more perfectly favored by the foam structure than by any other disposition of the multifarious materials collected in protoplasm.

The point which interests us in the present connection is that the acceptance of the *Theory of the Living Fluid* involves the rejection of all the theories of life-units. The former requires us to conceive that the phenomena of life are due to the interaction of numerous substances, many of which are present in sufficient bulk to be visible under the microscope. Hence the smallest volume in which vital phenomena can show themselves must be larger than the particles which have been assumed to be life-units. It has been shown experimentally that there is a limit to the mechanical division of living matter. It seems to me that we have now reached a point when we need no longer divide protoplasm into its living and not living constituents. It is all living, the water and salts as much as the proteids and other organic compounds. Its phenomena are displays of energy resulting, so far as we at present know, from chemical actions, the possibility of which is given by the commingling of substances in the foam-structure.

The perfected machinery and methods which we discussed in the first part promise to give us a vastly more profound insight into the minute composition of protoplasm. It is a problem of composition, not of structure, as ordinarily understood, nor of life-units, which protoplasm presents to us.

The conception of protoplasm above advocated seems at first to involve a complete materialism, but against this conclusion I must protest, for I hold that an opposite interpretation of life best accords with our knowledge—namely, that since there appear to be vital phenomena, which do not occur without life, it is legitimate to assume that there is a special vital power, which is not necessarily identical with any form of physical energy, though it may be conceived to cause the transformation of energy. Indeed, it is perfectly thinkable that the universe would come to rest, were not the balance of the forms of energy disturbed by the life-power.

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