THE STUDY OF THE INVISIBLY SMALL.*

ULTRAMICROSCOPES AND ULTRAMICROSCOPICAL OBJECTS.

BY A. COTTON AND H. MOUTON.

Of all our senses, seeing gives us the most extensive knowledge, and we have the greatest confidence in what we have learned by the eye; consequently, when we try to increase our perceptions by the aid of instruments, we pay especial attention to those that enable us to see new objects. Hence it is that the telescope on the one hand, and the microscope on the other are, perhaps, the instruments most familiar to those who do not frequent the laboratory. The microscope in particular owes a large number of its improvements to the constant desire of makers to satisfy their public, which is made up not only of regular scientists but also of amateur scientists. In some cases the latter have been found more exacting than the former. Thus, these amateur students of nature wished to study the marks and ornaments on the shells of the diatomaceæ. Scientifically, this work was of no great importance, either practical or philosophical, and its only reward was the vanquishing of a difficulty. But from a mechanical point of view, it was of real use, for it called for still greater improvements in the microscope, which improvements have been utilized by the regular scientists in more than one field.

But, notwithstanding all the care taken by the manufacturers of microscopes, the result obtained is limited. It is true that greater magnifying power might be attained, but this is without real interest, for the ideal microscope would be the one in which each point in the object under view would be imaged forth by a point of inappreciable dimensions. But what really happens is this: Even in the best instruments, the image of a point is always a small spot whose dimensions increase with the increase of the magnifying power of the instrument, so that when two points are too near one another in the object under examination, their images lap over one another a little and the eye is no longer able to separate them. The smallest resolvable distance, though this depends somewhat on the nature of the object that is under examination, is never less than a quarter of a thousandth of a millimeter, even when the very best instruments are used. The explanation of this limitation of the powers of the microscope has been found in the nature of light itself. Considering light to be a result of vibratory motion and taking into account the phenomenon of diffraction which is peculiar to the microscope as to all optical instruments, we can calculate the diameter of the spot forming the image of a point and show that this diameter will be smaller if, in the first place, the cone of rays which the instrument receives from the point under consideration is more open, and, in the second place, if this point is placed in a medium of higher index and sends out rays of shorter wave-length, viz., nearer the violet in the spectrum, if we consider only visible rays. But with the instruments now in use, permitting homogeneous immersion and great numerical aperture, we may say that the limit assigned theoretically to the resolving power of the microscope has been practically reached. We may hope, however, to extend it a little further by employing ultra-violet light, to which, though the eye is not sensitive, the photographic plate is sensitive. Yet, even by this method, we can hardly hope to do more than double the resolving power of the apparatus and this only after surmounting great technical difficulties.

But the ultramicroscope makes it possible to study much smaller objects than could be studied in the old way, these objects in certain exceptional cases having the millionth part of a millimeter. There is no contradiction between this statement and the one made a few paragraphs above, for this problem differs entirely from the one previously stated. That one was the distinguishing from one another, points set very closely together, or, which comes to the same thing, determining the form of a very small detail. But we refer now to ascertaining the existence, in a homogeneous medium, of extremely small objects which are far enough apart so that their pictures do not lap over one another.

It may be asked how the presence of ultramicroscopic objects is revealed in a homogeneous medium. The answer is by using with great care a method employed by amateurs of the microscope, often in a rather imperfect manner, but with good results. We refer to the lighting up of a dark background. Consequently, an ultramicroscope is not a microscope whose optical parts have been improved, but an instrument provided with a special mode of lighting. The rules to be observed in this lighting have been laid down by Sieden-

topf and Zsigmondy, who were the first to make known this method to the scientific world. Light must be thrown as intensely as possible on the little objects which are to be studied. They then appear luminous and can therefore be observed if care has been taken to place the apparatus in such a position that no luminous ray of the lighting shaft can penetrate into the object-glass. The particles then appear luminous on the absolutely black field of the microscope; in fact, they are made visible just as are particles of dust when a ray of sunshine penetrates a dark room.

Siedentopf and Zsigmondy themselves made their first apparatus for the observation of ultramicroscopic objects, by whose use a convergent horizontal ray of light brightens under the microscope a very thin layer of the body that is being examined. Since then a somewhat different plan has been adopted by us. We introduce a ray of light very obliquely under the body which is being examined, and the ray is then wholly reflected on the upper surface, and we thus prevent any direct rays from penetrating into the apparatus. Recently Siedentopf has invented still another kind of apparatus and other scientists have done the same. Each of these inventions has certain advantages and certain faults, and it is best to use one or the other according to circumstances.

Now a word about the lighting of an ultramicroscope. You get the brightest and cheapest light by utilizing ordinary sunlight. This gives a wonderful aspect to the object under view and enables the observer to distinguish very minute particles which would not come out with any other sort of light. But this source of light is unfortunately too inconstant, and you are apt to use an electric arc, if one is at hand, and if you are not trying to determine the presence of exceedingly small objects. It is not necessary to make use of a too powerful arc provided the rays strike squarely the preparation, that is, the body under examination; for the light emitted by the little objects which you perceive depends on the brilliancy of the source of the light, this brilliancy being the same for all arcs, and not on the total intensity of this light. In the vast majority of our experiments we have always used an arc of only three amperes, which can be bought at a reasonable price.

When you examine a transparent body containing little ultramicroscopical objects, you are reminded of the starry heavens when seen through a telescope. If you examine certain glasses colored by traces of metal, gold or silver, for instance, you perceive on the dark background of the field a mass of brilliant starlike objects, the reflection of the small metallic particles. These reflections differ in their brilliancy, the most brilliant ones being the reflections of the larger particles. Their appearance is all the more beautiful from the fact that they are not all of the same color. By examining in this way the colors of certain natural crystals—particularly those of crystallized salt, which are sometimes pink or white—one has been able to prove that this coloration is due to small ultramicroscopical crystals lodged in the finest fissures of the crystal and which we have reason to believe are formed by metallic sodium. This is rather a surprising fact if we remember that this substance is so easily oxidized that it is found nowhere else in a metallic state and that its preparation and conservation have long been considered by chemists as a very difficult problem.

If instead of a solid body, we examine a liquid holding little ultramicroscopical bodies in suspension, the same phenomenon is still more striking. But in this case the brilliant points are all animated by the "Brownian movement," which affects all small particles, even when they are but microscopic and are held in suspension in a liquid. But in this particular instance, this constant agitation sometimes becomes extraordinarily animated, the whole field of the microscope being marked by a sort of general swarming, often accompanied, in the case of the most brilliant particles, by scintillations, when the luminous points change color, are extinguished and then reilluminated by turns. One may enjoy this really admirable sight by employing for the experiment a very diluted solution of colloidal silver sold under the name of argyrol. Or one may examine one of those pretty "solutions" of gold, red or blue in color, easily prepared by reducing with a drop of formol a trace of gold chloride in some very pure water containing a little sodium carbonate. Under the most favorable conditions of light—sunshine at noon in July—and observation, you can discover in such liquids metallic parti-

cles whose average diameters have been estimated to have a minimum size of one-sixth millionth of a millimeter! Under less advantageous conditions, and especially when the little bodies which are being observed have not the optical qualities peculiar to metals you cannot of course expect to see anything except where the particles are considerably larger than this and yet far smaller than those seen in the ordinary microscope.

Ultramicroscopes have so far been used in very different sorts of research work, but the most important results have been obtained where colloidal liquids were experimented with. These liquids play a very important part in chemistry and in biology, especially; and thanks to the ultramicroscope, we now know for certain that colloidal bodies are not really dissolved in their "solutions," but remain there in a state of stable suspension. It has not been possible in every case to observe the little particles of these bodies, but we have good ground for believing that even the colloids which it has not been possible to resolve have a discontinuous structure like the other ones. This special state of the colloidal group helps to explain their particular properties, which have long been under observation, as, for instance, their coagulation and their electric transformation, this last phenomenon being especially observable through the ultramicroscope.

In a recent work—"Ultramicroscopes and Ultramicroscopical Bodies," Paris: Masson—we have given the results of our experiments in this field and have also tried to show how one can get at the composition and structure of colloids by combining our conclusions with those obtained by other students in this same branch of scientific work. Among the recent contributions to this problem, we have found especially useful those of Malfitano and J. Duclaux, who, by separating in certain cases ultramicroscopical bodies from the medium in which they are bathed, have been able to give us some idea of their composition. We are now led to consider these particles to be "granules" charged with electricity and englobed in a zone of matter carrying charges which are equal, but of differing sign, the whole forming an electrically neutral micella.

Not a few biologists have employed the ultramicroscope for the study of living things. By this means they have been able in some cases to follow the movements which take place in the interior of vegetable cellules through the displacement of the little bodies which they contain and which appear as luminous points. It has also been possible to make more apparent in their living state certain micro-organisms existing in water or humors. With this apparatus it is possible even to see very easily a microbe, in pleuropneumonia of oxen, for instance, whose dimensions are a little inferior to the limit of vision. The difficulties lying in the way of determining the form of ultramicroscopical objects have made it impossible up to the present to use the ultramicroscope in the discovery of numerous pathogenic microbes whose dimensions are inferior to those which can be seen under present lighting methods. It is much to be hoped that in some way we may be able to determine the specific optical character of these various small beings, some of which are the causes of terrible diseases.

We have just seen that the greatest service which, up to the present, has been rendered to biology by the ultramicroscope is in the field of colloidal liquids. If, on the one hand, we bear in mind how imperfect the studies in this department of science still are, and if, on the other hand, we remember that all living matter seems to be in a colloidal state, then we will fully grasp the great value to biology of this new apparatus.

The proportion of ash in leaves increases regularly with their age, but this rule does not apply to every ingredient of the ash. In May, July, and September of the years 1907 and 1908 Vandewele collected leaves of a great variety of plants in three localities, several miles apart, in Belgium, for the purpose of determining the proportion of chlorine in the leaves. He found every possible variation in the course of the chlorine content, including regular increase from May to September, maximum in September with minimum in July, maximum in July with minimum in September, regular decrease from May to September, and maximum in May with minimum in July. In many plants the chlorine content fluctuated irregularly. The cause of these variations is entirely unknown.

* The Independent.