hours, when their sterility was proven by inoculating tubes of beef tea with material from each flask. After this these flasks were treated as follows:

No. 1 was inoculated with *Bacillus pyocyaneus;* No. 2 was treated with 100 mg. of morphin and then inoculated with *Bacillus pyocyaneus;* No. 3 was treated with 200 mg. of morphin and then inoculated with *Bacillus pyocyaneus;* No. 4 was inoculated with *Bacillus coli communis;* No. 5 was inoculated with *Bacillus coli communis* and treated with 100 mg. of morphin; No. 6 was treated with 200 mg. of morphin and inoculated with *Bacillus coli communis.*

All of these flasks were then placed in the incubator at 37 C., where they were kept for two weeks. At the expiration of this time they were all autoclaved at 120 C. for forty-five minutes. A 2 per cent. solution of tannic acid in glycerin was poured into each flask in an amount equal to the mass therein contained. The flasks were then placed in an incubator and kept for forty-eight hours at 40 C. Then the flasks were removed from the incubator, the contents strained through muslin, and the filtrate heated on a water bath at 60 C. for one-half hour in order to bring down the coagulable proteids. After filtration through paper the filtrates were shaken with twice their volumes of petroleum ether. After separation of the ether the material was heated in order to drive off traces of the solvent, and then rendered slightly alkaline with sodium hydrate and shaken with chloroform. The chloroform extract from each flask promptly reduced iodic acid and potassium permanganate. Like results were obtained with amylic alcohol extracts from an alkaline mixture. None of these residues gave either the ferric chlorid or the Froehde test for morphin in a satisfactory manner. Great difficulty was experienced in obtaining clean pure residues. This was true with both the chloroform and amylic alcohol extracts, and it was found to be necessary to repeatedly take up the residues with water slightly acidulated with acetic acid, and again render alkaline and shake with the solvent. Crystalline residues were finally obtained from only two flasks, one of which contained morphin, while the other did not. The crystals in both of these cases seemed identical microscopically with those obtained from an alkaline solution of morphin shaken with chloroform, and the chloroform extract evaporated. The crystalline residue was so small in amount that the possibility of determining the melting point was excluded.

It must be evident from these results that a satisfactory method of extracting morphin in medicolegal examinations is not furnished by Kippenberger.

THE EXTRACTION OF A TOXIN FROM LIVER CELLS.

A PRELIMINARY REPORT.*

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The liver of an ox, just after the animal had been slaughtered, was obtained and passed through a sausage machine, finely dividing the structure and removing a considerable portion of the connective tissue. This finely divided material was stirred up with five times its volume of 1 per cent. sulphuric acid, and the mixture was heated for three hours and thirty minutes in the water bath at a temperature of 81 C. Then it was heated for three hours longer over the direct flame at 100 C. and filtered. The filtrate proved to be a clear, light amber-colored fluid, which was added drop by drop to three volumes of 95 per cent. alcohol, with constant stirring. The precipitate which formed was collected on a hard filter, washed with alcohol, and then dissolved in a minimum amount of water. This solution was again precipitated by adding it drop by drop to three times its volume of 95 per cent. alcohol. This precipitate was washed with alcohol and ether, then redissolved in distilled water. This process was repeated until the aqueous solution failed to give a test for sulphuric acid with dimethylamidoazobenzol. The substance thus obtained was dried in vacuo and ground, first in a porcelain and then in an agate mortar.

The cleavage product thus obtained, when dissolved in water and injected intraperitoneally into guinea-pigs and rabbits, kills the animal when the proportion is 1 part of the extract to 500 parts of body weight. When used in smaller quantities there is more or less marked emaciation, depending on the quantity injected, from which the animal recovers very slowly. So far we have not been able to secure any marked degree of immunity by beginning with small doses and gradually increasing the quantity.

After acute poisoning with the quantity above mentioned, postmortem examination shows the liver of the dead animal to be deeply congested. The spleen is soft and mottled, with dark and pale red spots. The kidneys show no gross changes, but the adrenals are markedly congested. The gastric and mesenteric vessels are greatly dilated, and there are frequently found small hemorrhagic areas under the peritoneum. We are now engaged in studying the effects of very small doses frequently repeated on the structure of the liver and other organs.

* See note to paper of Dr. MacIntyre.

A METHOD OF MICROSCOPIC OBSERVATION BY MEANS OF LATERAL ILLUMINATION.

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INTRODUCTION.

Last year two German physicists, Siedentopf and Zsigmondy, published an article[1] in which they described an apparatus for the observation of ultramicroscopic particles. The object which they selected for examination consisted of a piece of glass through which was diffused uniformly particles of metallic gold in a very fine state of division. By means of a powerful light—either the sun or an electric arc—passed through a complicated system of condensers, a very small but exceedingly intense focus of illumination was obtained. The piece of glass containing the gold was placed in this focus so that the light entered the glass from the side. It was then observed with an ordinary microscope from above. By this arrangement none of the light entered the microscope directly. To the naked eye, or with the ordinary microscope, the glass appeared perfectly homogeneous. But by this special illumination the individual particles of gold so reflected and refracted the light that they could be observed as distinct luminous points. This being the only light that entered the microscope, the particles appeared as bright points against a dark background.

1. Annalen der Physik., 1903, x, 1.

Three conditions are necessary for seeing particles: First, they must not be beyond the limits of visibility of the apparatus; second, they must not be too near together, about 0.25 micron being the necessary distance in order to distinguish them from one another; third, they must have an index of refraction different from that of the surrounding media.

Siedentopf and Zsigmondy were able to calculate the size of the particles observed. Knowing the amount of gold in the glass, and then counting the number of particles seen under the microscope in a definite volume of the glass, the size of the particles could readily be calculated. They found that they could see particles as small as 0.005 micron and even smaller. This is within the limits of the size of molecules, as calculated by Van der Waals and others. Since the limit of observation had been placed by Helmholtz at about 0.2 micron, a tremendous stride was taken in reducing the limits of visibility. These authors also made observations on colloidal solutions. Mouton[2] later suggested the application of this method to bacteriologic problems.

Since then Raehlman[3] has been continuing the work. He has reported observations on colloids, sugars, glycogen, dyes, etc., in which in high dilutions numerous small particles with characteristic movements may be observed. Also he has been able to see organisms with progressive motion in filtered putrefying liquids.

Mouton and Cotton,[4] following the work of Siedentopf and Zsigmondy, suggested a modification of the method described above. This consisted in passing a concentrated pencil of light, emanating from a Nernst lamp, through a suitable piece of glass in such a way that the light is totally reflected from the upper surface of the glass or the upper surface of a cover-slip placed on it. When such a piece of glass is placed under the microscope no light will pass directly into the tube of the instrument, and any particles in the glass or between the glass and cover-slip will become illuminated and can be directly observed. They very briefly reported a few preliminary observations made at that time.

Last fall, at the suggestion of Professor Hektoen, I attempted to set up an apparatus following the fundamental idea suggested by Mouton and Cotton.

DESCRIPTION OF APPARATUS.

The source of light used is a Nernst lamp. This is a non-vacuum electric lamp, which can be screwed into an ordinary incandescent light socket. The current passes through a short filament composed largely of metallic oxids, such as magnesium, zirconium, cesium, and which consequently emits a very intense white light when raised to the proper temperature. The glass globe of the lamp is covered with lampblack on the inside, except a small round area about 0.5 cm. in diameter at its vertex. This black is used to prevent all reflection of light from the inside of the globe. In this manner we have a pencil of rays passing through the clear area at the vertex of the globe and coming from a small but intense source of light. This pencil is now passed through a condenser. For this purpose a low-power objective is very suitable and convenient. I have used one the focal distance of which is about 1 cm.

The essential part of the apparatus consists of a triangular glass prism properly supported and so placed beneath the objective of a microscope that its upper surface is horizontal. The light coming from the condenser is directed from below obliquely upward and enters the prism at right angles to one of its lateral surfaces. It passes to the upper surface of the prism and is there totally reflected and passes out through the other side of the prism. A clear conception of this arrangement is easily obtained by reference to the diagram (Fig. 1). In order that total reflection shall occur at the upper surface of the prism, it is necessary that the angle x made by the incident ray and the perpendicular drawn to the surface at the same point, be of a certain magnitude. Otherwise the rays will simply be refracted at the upper surface. For glass and air the magnitude of this angle, known as the critical angle, is about 41 degrees. If it is greater than this total reflection occurs; if less, the rays pass through the upper surface and are simply refracted. Therefore, for our purpose, the angle x must always be greater than 41 degrees. This is very important, for if there is not total reflection some of the light directly enters the microscope. Now it is also necessary that the light should enter the surface bc at right angles, or approximately so, for otherwise there will be troublesome refraction here. It can be seen, therefore, that the magnitude of the angle b of the prism is very important, since it is always equal to angle x, as can be readily shown geometrically, provided the ray of light enters the surface bc at right angles. It therefore follows that if angle b is less than 41 degrees total reflection will not occur.

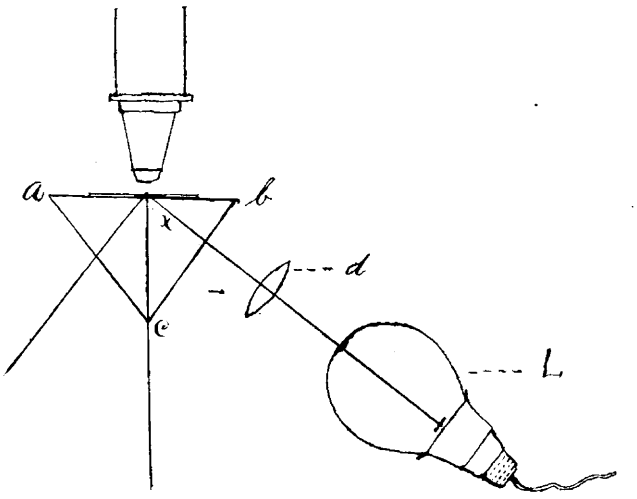

Fig. 1.—a, b, c, prism; d, condenser; L, Nernst lamp.

At first I used a right-angled isosceles prism, angle b being in this case 45 degrees. This was found unsatisfactory, because the converging rays on the lower side of the pencil of light were not all reflected, some passing through the surface and into the microscope above. I have, therefore, resorted to the use of an equilateral triangular prism, each angle being 60 degrees. This fulfills the necessary conditions.

For the examination of any fluid a drop or two is placed directly on the prism and a cover-slip applied. Or the fluid can be mounted with a cover-slip on a glass slide in the ordinary way, and the slide placed on the prism and separated from it by cedar oil or any substance with about the same index of refraction as the glass. The former method is much more convenient. Under these conditions the light passes through the fluid to the upper surface of the cover-slip and is then totally reflected. Any particles in the fluid in the path of the light will be made visible by the light which they reflect and refract, enlarged also by diffraction. Also

2. Bull. de l'Institut Pasteur, 1903, i, 97.
3. Münchener med. Woch., No. 48, 1903, also No. 2, 1904.
4. Comptes Rendus de l'Acad. des Sciences, No. 26, 1903.

the phenomenon of irradiation operates here to cause the luminous particles to appear larger than they really are. It is only this light, therefore, which enters the microscope, and it can be readily seen why it is so necessary to exclude all other light. We see in the field of the microscope the particles as bright points of light against a dark background; hence the appearance which has been likened to the stars in the heavens.

Attention should be called to the fact that it is necessary to have the prism surface and the surfaces of the cover-glass as nearly perfect as possible. Unfortunately a perfect surface can not be obtained. Every defect in the glass surfaces or in the glass itself, however slight, scatters the light, and, therefore, interferes with the observations. With the best cover-slips carefully cleaned and the surface of the prism polished, this difficulty is reduced to a minimum and is not serious.

SOME PRELIMINARY OBSERVATIONS.

I have made a number of preliminary observations on different substances largely to obtain some idea of the possibilities of the apparatus and also to become familiar with the appearances of various fluids and particles in order to be better able to interpret them when seen under various conditions. I have gone through exactly the same experience a novice goes through when first learning to use an ordinary microscope. It is necessary first to become familiar with the appearance of ordinary air bubbles, slight defects in the cover-glass and débris of different kinds, cells, etc. However, with a little experience, it is remarkable how easily such objects may be differentiated, considering the fact that they are only seen by lateral illumination.

Metallic colloidal solutions afford an interesting study. It is ordinarily stated that the particles suspended in these solutions are too small to be observed with the highest power of the microscope. Ehrenhaft[5] says that such particles are about 0.5 micron in diameter. I have used solutions of platinum and silver made according to the method of Bredig by forcing the metal from the cathode by means of a powerful electric spark under double distilled water. Such solutions when examined in the light show numerous bright particles, all undergoing a rapid vibratory motion. They are by no means all of the same size, varying from particles large enough to be seen with a one-twelfth oil immersion to particles so fine that they can be just observed as the faintest points of light. Colloidal silver particles in the solution which I have examined are, on the whole, larger than platinum particles and slightly more active. The silver particles also vary in color. Three distinct tints are seen—red, white and green. I have examined three different platinum solutions and I have found the different solutions to vary somewhat in the size of the particles. This may depend on the strength of the current used in making the solutions, though I have made no observations to test this.

Many of the particles are held back by filtering through a porcelain filter. The particles are then less numerous, and, on the whole, are finer. Colloidal solutions of arsenic sulphid are also very interesting. I prepared solutions of the α, β and γ sulphids as described by Picton and Linder.[6] The particles in the α and β solutions could be seen, but not those of the γ solution. On account of the greenish-yellow color of the particles and their vibratory motion a field of exquisite beauty is presented to the eye.

5. Zeitschrift für physik. Chem., Oct. 31, 1903.
6. Trans. Chem. Soc., London, 1892 and 1895.

Of great interest is the study of animal fluids, such as serum, exudates, milk, cerebrospinal fluid, etc.

Perfectly clear blood serum which has been centrifugated shows an immense number of particles. They vary in size and have a rapid vibratory motion. Differences in the number and general appearance of the particles have been observed in sera obtained from different sources. The significance of such variations is entirely undetermined; they are certainly very suggestive.

After filtering serum through a porcelain filter the number of particles is very considerably diminished. On heating serum to about 56 C. for a few hours, a noticeable increase in the number of the particles occurs. They also appear slightly larger. This may be interpreted as a process of coagulation slowly occurring under these conditions. At room temperature also the serum changes in much the same way as at 56 C., but very much slower, the serum at 56 C. changing as much in a few hours as that at room temperature in several days. Serum kept in the ice box for two days showed no changes. These observations must be made with the serum much diluted with salt solution.

Exudates such as pericardial and ascites fluid, etc., vary very much in their appearance according to their nature. They show numerous particles—often large flakes of albumin and cells of various kinds. I have made only a few observations on such fluids.

ORGANISMS.

Micro-organisms furnish the most attractive field of any yet suggested for study by the method. Bacteria are seen as bright points of light and present an interesting sight as they tumble about in the fluid or appear in rapid motion. The method suggests itself at once as a favorable one for observing and determining motility of organisms. A few observations on typhoid bacilli have been made. They appear in rapid motion as bright points of light traversing the field in all directions, and often remind one of a display of fireworks.

The method can be applied also for observing the decrease in motility and the clumping of organisms in agglutinative tests, and I believe that it is superior to the ordinary methods used in determining the motility of bacteria.

Where the flagella are numerous, as in typhoid bacilli, they, on account of their rapid motion, appear as an indefinite flurry about the body of the organism. I have not succeeded in getting a clear picture of the flagella of the organisms when at rest.

In considering the application of this method to the study of organisms, one naturally thinks at once of the possibilities of observing the ultramicroscopic organisms for the existence of which very strong evidence has accumulated. One serious difficulty in this direction is to differentiate an organism from other particles which are usually present. If it is motile then the difficulty disappears at once. Here an excellent field opens up for the study of a number of diseases, the virus of which is known to pass through porcelain filters. For those organisms which are not motile the problem is not so easy. For example, the peripneumonia organism is just at the limit of visibility for the highest power of our best microscopes. It grows well in beef-serum bouillon, as shown by the gradual clouding of the medium after a few days. A culture of this organism was obtained from the Pasteur Institute of Paris and the organisms studied in growing culture. There was a gradual increase in the number of particles from day to day, and

undoubtedly most of these were the peripneumonia organisms. But some of the particles I am sure were not organisms, for they were too large and varied too much in size. These particles unquestionably were formed as a result of changes occurring in the medium due to the growth of the microbe. The organisms, not being motile, can not be distinguished clearly from other particles which may be present. However, uniformity in the size of the particles and distinctness in their appearance speak in favor of their being organisms rather than particles of proteid or other material. In dealing, then, with these extremely small organisms, the observations must be controlled most carefully.

Sections of tissue are not suited for the application of this method. In teased specimens, however, one can observe striation in muscle and the course of fibers in brain tissue. I could observe nerve fibers ramifying between cells, and in several instances I was able to see clearly the axis cylinder coming from the nerve cell and to trace it for some distance. The method may be of some value in studying the branching of the nerve fibers.

POSSIBILITIES AND LIMITATIONS.

The possibilities and limitations of the apparatus described here can only be determined by a large number of observations. New methods usually fail to meet all our predictions, yet they often become of service in some manner entirely unforeseen at first. The progress of science depends very largely on the development of new methods of technic, and consequently all such methods should be most thoroughly investigated before being discarded.

There is undoubtedly a large field for the application of this method in studying the structure of that important class of substances known as colloids, both organic and inorganic. This will include such substances as toxins, antitoxins, ferments and the like. The work already done by Raehlman is extremely suggestive, and indicates a most important field for further study.

Emphasis must be placed on the fact that the value of the method is by no means limited to the study of ultramicroscopic particles. One of its greatest uses will be to observe particles or objects, not too small to be seen by our ordinary microscopes, but which, owing to their slight refractive properties, can not now be seen by our present methods. Because of the property of this method of bringing out slight differences of refraction, I am inclined to believe it will replace, for many purposes, at least, the hanging-drop. That we can see objects not seen in the hanging-drop is illustrated nicely by the fact that red blood corpuscles in dilute acetic acid can not be seen either in hanging-drop or under a cover-slip when unstained, but under lateral illumination the stroma of the corpuscles is rendered plainly visible. Now it may be well that there exist important bodies whose refractive powers are too slight to enable them to be seen in hanging-drop, and which can not be stained by our present methods. In such cases this method of observation will be of positive value.

Since the method is applicable only to particles or small bodies in suspension, it apparently can be of no service in determining structure of tissues. The form of larger objects may be observed, but since the method does not magnify, the form of the smaller particles can not be determined.

In respect to the simplicity of the apparatus and to ease of examination of any fluid, this new method surpasses even the familiar hanging-drop.

Finally, I would call attention also to the fact that the few observations reported here were made with an imperfect apparatus.

A much more perfect illuminating system is desired, so as to obtain a smaller and more intense focus of light. This applies particularly to the study of ultramicroscopic particles, for it is generally true that the stronger the light the smaller are the particles which may be seen.

I wish here to express my thanks to Dr. Hektoen for suggestions.

THE DANGERS OF POTASSIC CHLORATE.

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Every obscure subject in medical literature should be reviewed from time to time on principles broader and more general than can usually be employed in text-books or special articles; and particularly when such obscurity is caused by a want of precision of language, by errors in the use of terms—faulty or loose terminology—and, what is more serious, by a failure to distinguish between two objects, which, though alike, are chemically and physiologically speaking, separate agencies.

For instance, a frequent and widespread source of error—and consequently of danger—arises from a confusion in the nomenclature. That is to say, potassic chlorate has been confounded, both in medical writings and in prescriptions, with potassic chlorid (KCL). Secondly, by a false analogy, the chlorate has been thought to have properties and effects identical with the chlorid, and not to be any stronger. For the belief that potassic chlorate and potassic chlorid are interchangeable, or at least as alike as twins, it is customary among some writers to blame the pharmacopeias. Our own, as it will be seen, is not liable to this criticism; but the German offends heavily. Thus a high authority[1] says: "In the pharmocopeia by the term potassic chlorate is understood potassic chlorid (KCL), while chemists apply the former name solely to the chlorate." A similar remark is made by Kobert.[2] On the inelegance of this ambiguous use of scientific terms, Virchow[3] comments with severity. In spite of these strictures from such good authority, it is possible to find in medical literature, numerous instances where the term "chlorid" is used where the "chlorate" is meant; and, sometimes in the course of the same paper both terms are employed so loosely that it is frequently difficult to tell from the context which is to be understood.[4] Finally, not only must the chlorid and chlorate be clearly separated, but both also must be carefully distinguished from the "hypochlorites" (KCLO, CaCLO2), often improperly called "chlorids," and in this false character responsible for some cases of poisoning.[5]

Into the error of confusing terms a writer in the *Therapeutische Monatshefte*[6] falls, while pointing out another blunder. Speaking of the danger of mixing potassic chlorate with organic or combustible substances, he cites as an instance of the non-observance of this rule the formula of a French surgeon where potassic chlorate is prescribed in conjunction with saccharin. The result was a violent detonation.[7] This mistake, absurd as it

1. Das Chlorsaure Kali, J. von Mering, Berlin, 1885, p. 1.
2. Pharmakotherapie, vol. i, p. 55.
3. Barbarismen in der med. Sprache.
4. Podcopaew: Archiv f. path. Anat., vol. xxxiii, p. 510.
5. Dict. de Thér., vol. ii, p. 1.
6. 1894.
7. Rev. de Thér. med. Chir., 1894, p. 241.