Assuredly, the man who can serve as a pharmacist the people of Missouri, can equally well serve those of Indiana, Colorado or Illinois.

We, as individuals, are transient, but the cause and the public we represent are permanent. We are here today and replaced tomorrow. But let us, in our allotted time, at least, keep the cause moving. In order that there may be no mistakes and no regrets, let us adopt a uniform standard of examinations. Make it as high as the majority wills. Let it be such that it *can* and *will* be recognized from Arizona to Alabama, from Minnesota to Maine. Leave it not to those who follow us to say, "They halted when they should have marched. They saw their duty and they heeded not."

H. C. CHRISTENSEN, Chairman,
E. L. BRANDIS,
CHARLES GIETNER,
Committee.

THE MICROSCOPIC EXAMINATION OF OINTMENTS.*

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The value of an ointment depends upon many factors, including such things as amount of active ingredient present, absorbability of the vehicle, etc., but not the least important of these factors is the degree or fineness of subdivision of the active ingredient in the vehicle, generally, though erroneously, called the base. No doubt, the finer this subdivision, the better the ointment will be, the more quickly will it be absorbed, and we have as the ultimate limit of fineness of subdivision those preparations in which the active principle is in actual molecular solution, when on the one hand it is soluble in the vehicle, and those in which the active principle is in colloidal solution or suspension, when on the other hand it is insoluble in the vehicle. Only a small proportion of the ointments, official and unofficial in present day use, however, approach these ideal conditions.

Every maker of an ointment, therefore, should endeavor to subdivide his active ingredients as finely and as evenly as possible throughout the mass, and he should therefore have a means of determining when he has reached the desired limit, or when he can conscientiously consider his ointment fine and even enough to insure satisfactory results. Chemical analysis will of course not suffice, for the active ingredient may be present in ample and correct proportion and yet be present in such a rough suspension, as to be useless or even dangerous, as for example, in the case of an improperly prepared yellow oxide of mercury ointment for eye medication.

The only satisfactory method for determining whether or not the proper degree of subdivision has been secured is by means of the microscope. The U. S. P. requires that mercurial ointment shall be rubbed until the individual globules of mercury are no longer visible under a lens magnifying ten diameters. But if still better and more nearly uniform results are desired, and who shall

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say that what we have is good enough, that there shall not be further progress, the microscope must be used. In the course of many years' use of the microscope in the examination of hundreds of samples of ointments, the writers thought it might prove of interest to point out some of the precautions that must be observed even in so apparently simple a task as this. It is quite easy to obtain erroneous results if the conditions under which the samples are taken and under which the observations and comparisons are made, are not watched very carefully. The part of the work to which we wish to call particular attention is the preparation of the slide, which we shall illustrate with micro-photographs of mercury ointments.

The method usually employed, and which naturally suggests itself to the operator, is simply to spread a layer of the ointment on a slide, thinning it out to the necessary degree by rubbing the finger, or another slide, over its surface in

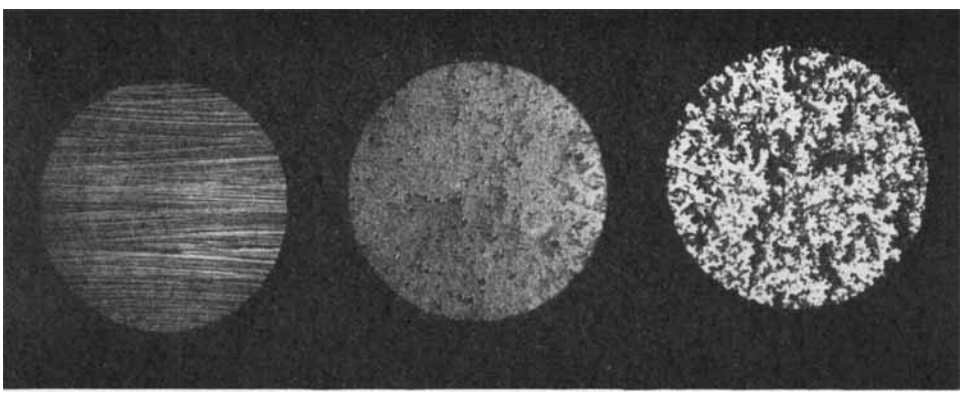

FIGURE No. 1.
Blue Ointment magnified 100 diameters, showing ordinary method of preparing slide.

FIGURE No. 2.
Same Ointment magnified 100 diameters, showing improved method of preparing slide.

FIGURE No. 3.
Same slide as No. 2, showing effect of too much light.

the manner employed in making a blood smear. Unfortunately, the appearance of the globules, in the case of mercury ointments, will depend largely upon the pressure which is exerted in running the finger or slide over the specimen. Sometimes the larger globules are rubbed up finer by this method and oftentimes the large globules are pushed to one side, leaving only the smaller globules to appear in the field of vision, and in either case causing the ointment to appear better than it really is. On the other hand, the pressure sometimes causes a number of the smaller globules to unite to form larger ones, while if not enough pressure be used, a slide insufficiently transparent to permit of good judgment of the ointment results. Moreover, different fields on the same slide prepared in this manner differ considerably in the average size of their particles. Figure No. 1 is typical of the appearance of a slide prepared in the above manner.

In striking contrast is Figure No. 2, which is a micro-photograph of a specimen of the same ointment shown in Figure No. 1, magnified to the same extent (about 100 diameters), but prepared by the following method which we have found, after long experience, to give the most satisfactory and uniform results.

We take a small amount of the ointment—the amount which is held in a small platinum wire loop is sufficient—and place it on the center of a slide. The latter is then gently heated on the water bath until the specimen of ointment is just melted. A cover glass is now gently dropped on the softened ointment on the slide, when, owing to capillary attraction, the ointment will spread itself between the glass and the slide in a very thin film in such a way as to have formed in the center of the disc an area which tends to retain the heaviest globules, while the finer particles will sometimes follow the capillary movement of the melted vehicle toward the circumference. The heavier particles therefore, if they still be contained in the ointment, in this way remain concentrated, and the judgment of the ointment is very easy, since the central area should show particles rather uniform in size, and but few, if any of them, should be very large in comparison with the average.

The question naturally suggests itself as to how we can be sure that the particles in such a slide have not to a certain extent coalesced during the softening

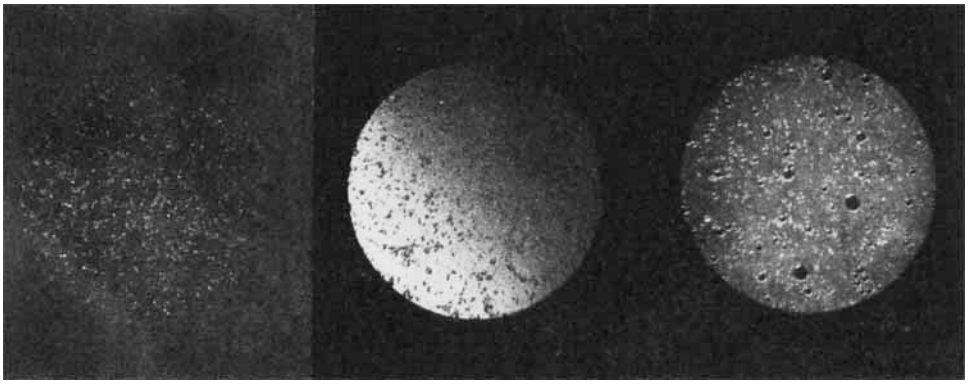

FIGURE No. 4.

Blue Ointment on market, magnified 100 diameters.

FIGURE No. 5.

Another Blue Ointment on market, magnified 100 diameters.

FIGURE No. 6.

Another Commercial Blue Ointment, magnified 100 diameters.

of the ointment. We are sure that coalescence does not occur, because we have observed the melting down of the ointment under the miscroscope, and have also prepared slides repeatedly from the same ointment with varying conditions as to time and temperature of the melting, and have in this way assured ourselves, that if the above directions are followed, no coalescence of particles occurs and concordant results will be obtained. The same method is used for preparing slides from other kinds of ointments.

The best size of enlargement in our opinion is obtained by a magnification of about 100 diameters, as obtained by using a ⅔ Baush & Lomb objective, and No. 1 ocular with a tube length of 160 mm. The light should be regulated in such a way that the mercury particles appear as silvery globules on a dark background. This can be easily done by using partly the mirror of the microscope and partly the hand to cut off some of the light. Under these conditions the mercury globules will have the metallic appearance and luster as mercury ordinarily has

in transfused light, and in this form it is much easier to differentiate between the smaller and larger particles, as well as to distinguish a mercury globule from a casual air bubble. If the mirror be used so that the full amount of light enters the field, the contrast between the dark mercury and the light background is so

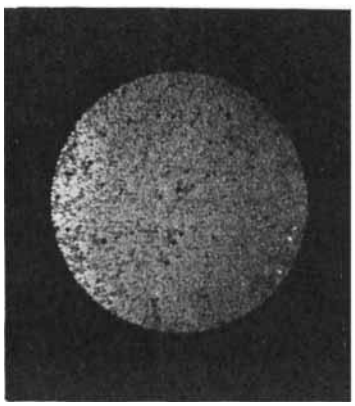

FIGURE No. 7.
Blue Ointment (X100) after 5 hours' grinding.

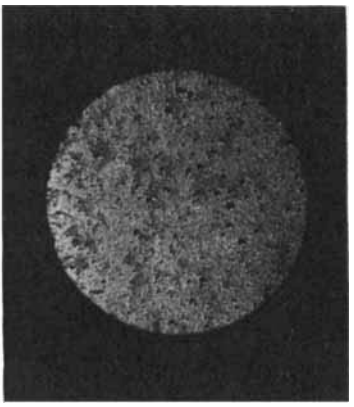

FIGURE No. 8.
Blue Ointment (X100) after 8 hours' grinding.

great that it is difficult to see the individual particles. (See Figure No. 3, which is a photograph of the same field shown in Figure No. 2, but differing in the amount of light admitted in taking the picture.)

As for the standards to be adopted, these are of course arbitrary. It would be difficult to fix a standard for the average size of the particles, as a few very

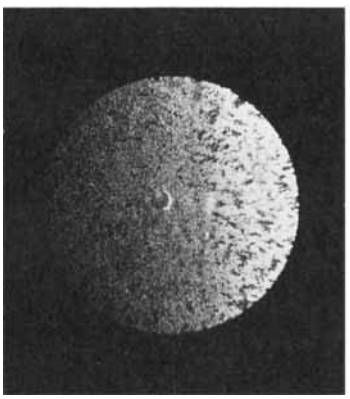

FIGURE No. 9.
Blue Ointment (X100) after 21 hours' grinding.

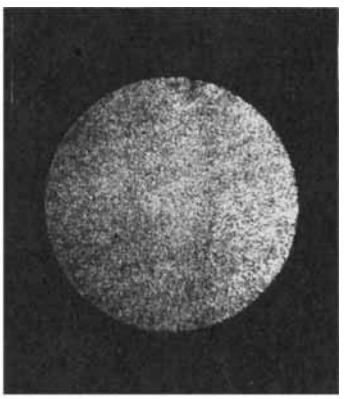

FIGURE No. 10.
Blue Ointment (X100) after 37 hours' grinding.

large particles might contain more material than hundreds of other particles taken together. The best plan to adopt is to take a certain specimen as a satisfactory example and make comparisons of new lots with the sample taken as a standard.

As proof that some attention should be paid to the fineness and uniformity of mercury ointments on the markets, Figures 4, 5 and 6 are shown. These figures represent three samples of Blue Ointment, made by manufacturing pharmacists, as found on the market, magnified about 100 diameters. The question may be raised as to why it should be necessary to reduce the mercury to so fine a state of subdivision. We have observed that if the particles are not very finely subdivided, they will easily coalesce to form larger globules when rubbed into the skin, and thus a part of the material is wasted. We have found, however, that after the particles have become quite finely subdivided, they do not reunite, but are ground still finer on rubbing.

To control the amount of grinding necessary to produce a uniform ointment, the following experiment was made. A lot of Blue Ointment was rubbed up

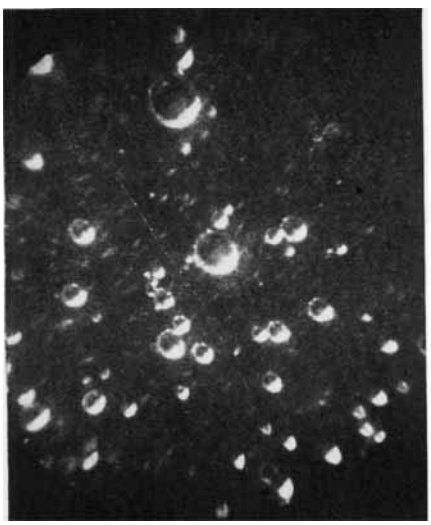

FIGURE No. 11.
Ultra-Micro-photograph (X1000) of same Blue Ointment shown in Figure No. 10.

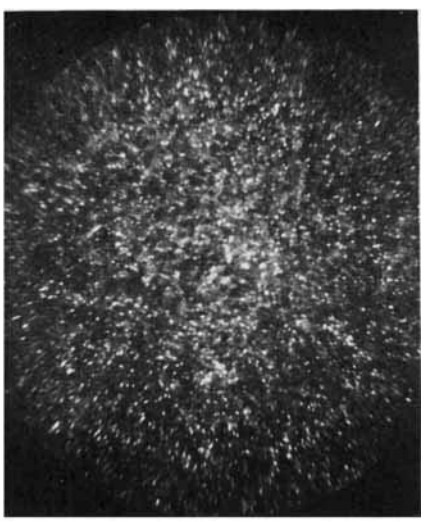

FIGURE No. 12.
Ultra-Micro-photograph (X1000) of Colloidal Mercury Ointment, 10%.

in a ball mill for 37 hours and samples were taken out for examination at intervals during this time. Figures No. 7, 8, 9 and 10, show the condition of this ointment after 5, 8, 21 and 37 hours, respectively. It will be noticed that by using the above described method of preparing slides, it is very easy to observe the gradual diminution of the size of the globules.

Special attention is called to Figure No. 9, in which a large air bubble, being perfectly transparent, may be very readily distinguished from the mercury globules. Figure No. 10 shows the great uniformity in appearance of the particles under a magnification of 100 diameters after 37 hours' grinding, at which stage it was considered finished.

That the apparent uniformity of the ointment is only relative to the degree of magnification, however, can be seen by still further magnifying the same slide to about 1000 diameters. Figure No. 11 was made from the same slide as was

Figure No. 4, but by the aid of the ultra-microscope, using dark field illumination. That the particles are after all, not actually uniformly subdivided is apparent at a glance.

As suggested in the opening paragraphs of this paper, the only way to obtain the ultimate and highest degree of uniformity combined with the finest subdivision, in the case of a substance like mercury, insoluble in the vehicle, would be to prepare it in colloidal form. This we have succeeded in doing, and we are now engaged in collecting clinical data as to whether these colloidal ointments and oil suspensions possess therapeutic advantages over the ordinary metallic ointments and over the Grey Oil used for intramuscular injection. Figures No. 12, 13, 14 and 15, show ultra-micro-photographs of colloidal mercurial ointment. The individual particles, though theoretically magnified only about 1000 diameters, are actually many times smaller than this, due to the fact that to the observer

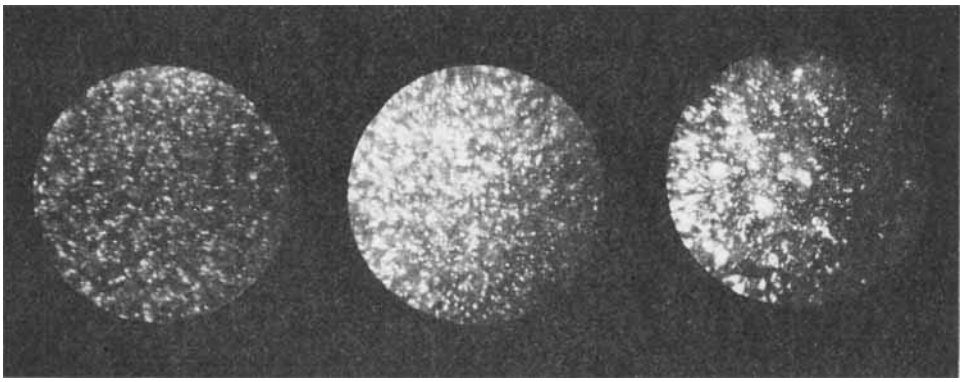

FIGURE No. 13.
Ultra - Micro-photograph (X1000) of Colloidal Mercury Ointment with lowest possible illumination.

FIGURE No. 14.
Same as No. 13, but with increased illumination.

FIGURE No. 15.
Same as Nos. 13 and 14, but with more intense illumination.

the particles themselves are luminous and sources of light. Consequently, the brighter the illumination (a high power arc light is required) the larger the particles will appear. This is well shown in Figures No. 13, 14 and 15, which represent the same ointment with gradually increasing intensity of illumination. It is of interest to note how the particles appear to coalesce into kinds of luminous nebulae with increase in luminosity, while if the latter be decreased, the individual character of the particles again becomes discernible. Moreover, no adequate conception of the actual size of the particles can be had because they are in indescribably rapid motion similar in appearance to the so-called Brownian movement of floating dust particles. Thus each bright spot, as shown particularly well in Figure No. 12, represents not the size of one colloidal particle magnified 1000 times, but rather the entire circumference of the glow of a particle rapidly vibrating throughout a space many times its own actual dimensions.

RESEARCH LABORATORY OF H. K. MULFORD COMPANY, June 20, 1913.