SCIENTIFIC APPARATUS AND LABORATORY METHODS

THE ULTRA-VIOLET MICROSCOPE AS EMPLOYED BY BARNARD IN HIS CANCER RESEARCHES

ACCOUNTS in the daily press regarding the work of Gye and Barnard emphasize that the discovery of the reported organism would not have been possible without the aid of a special microscope for the use of ultra-violet light, and the original article in the issue of *The Lancet* for July 18 which has just been received shows that Barnard relied chiefly on this microscope for ascertaining the appearance of the organisms under investigation.

The advantage of employing short wave lengths of light as a means of enhancing the resolving power of an objective has long been recognized and is patent upon an examination of Abbe's well-known formula for resolving power: size of smallest particle visible = $\frac{\frac{1}{2} \text{ wave length of light}}{\text{numerical aperture}}$. Without here again entering into a detailed discussion of this formula and its implications, it is to be noted the upper practical limit for numerical aperture has apparently been reached at 1.4 in the apochromatic objectives devised by Abbe. And even this aperture is not available for living objects in aqueous media, where the upper limit of the available numerical aperture is about 1.25.

The only line of progress then open towards greater resolving power would seem to lie in the direction of employing light of shorter wave lengths.

Amici already had found that a slight but appreciable gain can be achieved by employing green light. The still further gain which should be obtained by employing blue light is largely lost because of decreased visual acuity in blue. Photographic plates, however, are still sensitive at the blue end of the spectrum and even in the ultra-violet. Assuming an average wave length of 550μμ for ordinary visual light, it would be possible to practically double the resolving power of an objective by employing ultra-violet light with a wave length of 275μμ.

A. Köhler aided by M. v. Rohr, members of the scientific staff of the Zeiss Works, set about to construct a microscope for the use of ultra-violet light. Serious difficulties had to be overcome (glass is practically opaque to waves of less than 300μμ), but in 1904 Köhler published a detailed description of the completed instrument. This microscope for microphotography by the use of ultra-violet light at once received great attention and a considerable number of institutions as well as private investigators provided themselves with an equipment. For the most part, however, the results obtained did not come up to what the performance of the objectives gave a right to expect. The explanation lies close at hand. Twenty years ago microphotographic knowledge was less widely disseminated than to-day. The ultra-violet equipment not only requires familiarity with microphotographic methods but presents several additional complications which, while not difficult or troublesome to one acquainted with them, are more than most biologists were prepared to handle. Since then much has changed. Biologists have become accustomed to handling elaborate equipment, and it is to be expected the ultra-violet microscope before long will be yielding results in the hands of biologists commensurate with its capacity.

It is essentially the Köhler ultra-violet microscope which Barnard employed in his researches. Mechanically he made some modifications in the equipment which were intended to increase the rigidity of the microscope and to provide for more delicate focusing. In this Barnard followed the well-known British penchant towards massive and complicated microscope stands. Without questioning the good services which Barnard's stand is rendering its inventor, it may well be doubted whether its employment in other hands would be of advantage. There is greater opportunity for getting the parts out of alignment in this modified microscope. It was precisely one of Köhler's aims in constructing his equipment to have the arrangements such that as little opportunity as possible be given for dis-adjusting the parts while at the same time providing convenient means for such movements as are necessary. As for stability and precision of focusing, the writer from long-continued use of several of Köhler's outfits can testify that he never encountered the slightest need of greater refinements in these directions.

Barnard also employed an ingenious combination condenser, used alternately for darkfield illumination with visible light and for microphotography with ultra-violet light. For general use, however, it would seem a sounder procedure to employ for darkfield studies a separate darkfield outfit. As a finder for minute objects to be photographed by means of ultra-violet light, the darkfield combination is of questionable value. It is probably simpler and fully as effective to employ as a guide markings or objects of an appropriate size for ready detection scattered among the more minute structures. Barnard, himself, has called attention to and used this method (droplets of mercury sublimated on to the quartz slide).

Finally, a word as to the prospects for using light of still shorter wave length. There is no special difficulty in correcting objectives for wave lengths between

275 and about 200μμ (where the absorption of air and quartz becomes troublesome). It is not easy, however, to obtain a sufficiently intense and at the same time sufficiently monochromatic source of light for these shorter wave lengths and it is questionable whether the theoretical gain in resolution achieved by passing from 275μμ to say 225μμ would be realized in practice. Barnard employed an objective with light of wave length 257μμ and reproduces a photograph taken with this combination. From a comparison of this photograph with another taken with 275μμ, it is not at all certain that the differences are due to increased resolution on account of the shorter wave length rather than to adventitious circumstances.

If a further step is to be taken, it may well be best to pass directly into the region of Schumann rays. But for the present the possibilities of the equipment for ultra-violet light of 275μμ have not yet been fully utilized and the most promising results seem to be in sight through its more thorough use. Much is to be expected in this respect in the near future. A number of biologists are at present successfully using the apparatus and its use has also been extended to metallography (opaque objects) where it is opening up an entirely new field.

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SPECIAL ARTICLES

THE HYDROPHILIC EFFECT OF IONS ON AGAR AND PROTOPLASMIC COMPONENTS

PROFESSOR L. MICHAELIS says in his recently published American lectures on the effects of ions on colloidal systems:[1] "One can not talk of *the* hydrophilic effect of ions. In different cases different kinds of effects become manifest." This pronouncement was made with especial reference to conclusions of Loeb, based chiefly on experiments with gelatine, that the differential action of ions as expressed in the Hofmeister series does not hold. Michaelis was led to make this cautionary statement because numerous experiments in his own laboratory have shown that the series is valid in the hydration of agar in neutral salt solutions through a certain range of concentration. Scores of workers have shown that the lyotropic series *is* apparent when dealing with living cell-masses and the results of the senior author prove that it runs through the hydration reactions of agar and agar-protein mixtures, and that the differential action of univalent anions as well as kations is demonstrable in the artificial cell constructed of these materials.

It is to be noted that even Professor Michaelis does not realize the full force of his cautionary statement. Relying upon results of van Kruyt, de Jong and Dokan, he says:

> When a piece of agar jelly is put into water or an aqueous electrolyte solution, swelling occurs and the weight attains a constant value after about one day's swelling. The degree of swelling can be measured very exactly, and the swelling is found to be most pronounced when agar is exposed to pure water.[2]

Specifically this statement may be connected with some recent results by Dokan,[3] whose method was to dry a 2 per cent. agar gel to one third its original thickness, then allow it to swell twenty-four hours. Changes were determined by weight. Obviously only a narrow sector of the hydrating action was measured, and the data thus obtained might be expected to yield no fine distinctions. These, however, were sufficient to show that Loeb's generalizations as to the invalidity of the Hofmeister series would not hold. By this gross method univalent kations were equivalent in their effects in concentrations below 0.1 N, but the Hofmeister series was evident above this. A more exact method would have extended the series to extremely dilute solutions.

This has been done repeatedly in this laboratory during the last decade by a method in which 2.5 per cent. warm agar solutions, which cast as plates cool, set as a firm jelly and dry down to a thickness of 0.1 to 0.5 mm, according to the thickness of the original, and which in a freshly air-dried condition at 15° have a water content of about 25 per cent. Trios of small sections with surfaces of 8 to 10 sq. mm, and a volume of 2 to 5 cu. mm, were placed in a Stender dish covered with a triangular piece of glass plate on the center of which a vertical arm of an auxograph had a bearing. About 50 ml. of solution was poured in each dish and resultant swelling was recorded for a week or as much longer as desirable with daily replacement of the solution. Determination of losses by solution from the sections showed a loss of one seventh of the dry weight of such sections immersed in water during the first twenty-four hours. Such losses by solution do not appear to have been taken into account by workers who obtain data as to swelling by weighing the sections. It will be noted that if auxographic records were corrected for such

[1] Baltimore, 1925, Williams and Wilkins Company (see p. 97).

[2] *Ibid.*, p. 88 and Fig. 2.

[3] Dokan, Von S.: Die Wirkung der Elektrolyte auf die Quellung der Agar. Kolloid. Zeitschrift, *34*, 155 (1924).