

plane on one surface. In practice these wafers may be cut as thin as 0.2 mm, using a jeweler's circular saw 0.2 mm thick. Water makes an excellent lubricant for this process. It is thus possible to get about three sections per millimeter. The polished surface is next attached to a "Plexiglass" slide, using the partial polymer as a cement. The whole system is placed in a 40° C. oven until polymerization of the cement is complete, about six hours. The thick section adhering to the slide is now ground to the desired thinness, using the same abrasives as before. Parallel grinding is made easier and excessive thinness avoided if the two ends of the slide are wrapped with paper-thin copper foil. This grinding of the second face takes slightly longer than the first and may consume ten or fifteen minutes, in the course of which the section is viewed from time to time under a low power microscope. With very little practice a thinness and evenness of the section equalling that produced with a microtome may be attained. Following this grinding a cover glass is affixed by the usual method.

Mass staining of tissues sectioned by this technique is usually quite satisfactory. However, it is surprising to note that staining after section is also possible. The stains used should be relatively strong, for example, safranin or methylene blue, and the times necessary are somewhat longer than usual. The results are nevertheless excellent. If this method is to be used it is advisable to stain the surface exposed by the first grinding, in order that the color of the section may be utilized to judge its thickness during the second grinding.

By this technique one can, with simple equipment, obtain excellent sections not only of ordinary tissues but also of those not easily treated by the standard microtome technique. The time required to get a single section is somewhat longer than that demanded by the paraffin method, due to the time occupied in polymerization; however, the time spent by the operator in manipulation is nearly the same. We hope to amplify this bald description with further details in a more complete publication elsewhere.

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THE STAINING OF ACID-FAST TUBERCLE BACTERIA

MORE bacteria in a given sample are revealed by the fluorescence technique¹ than by the Ziehl-Neelsen method. Our observations confirm the reports of the German investigators, especially Didion.² The increased number is a real difference beyond the in-

creased visibility of the bacteria with the fluorescence technique. Of the many compounds isolated from tubercle bacilli by Professor Rudolph Anderson and his associates, only mycolic acid³ was found to be acid-fast. Professor Anderson very kindly furnished me with some pure mycolic acid.

A small amount was melted on clean slides and stained in carbol-auramin and in carbol-fuchsin. The auramin stained the mycolic acid intensely yellow at room temperature, and the combination resisted destaining in acid alcohol. After three days and nights destaining, most of the auramin remained in the sample. On exposure to ultraviolet light an intense bright yellow fluorescence was observed.

The mycolic acid was stained with difficulty by the carbol-fuchsin of the Ziehl-Neelsen method and easily destained in acid-alcohol (0.5 per cent. HCl) to about one third of the original intensity. Slightly better staining occurred when the acid was stained with steaming carbol-fuchsin, but the mycolic acid is not strongly acid-fast to fuchsin.

Mycolic acid itself is weakly fluorescent, showing pale blue-white and may have caused the fluorescence observed by Kaiserling⁴ and by Arloing *et al.*⁵ in tubercle bacteria. Melting and cooling the acid markedly reduces the autofluorescence of the acid.

The observations suggest that the acid-fast staining of the tubercle bacilli may be due to mycolic acid. The firmer combination of mycolic acid with carbol-auramin than with carbol-fuchsin may explain why more bacteria are revealed by the fluorescence technique, using the former, than by the Ziehl-Neelsen method, using the latter.

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³ F. H. Stodola, A. Lesuk and R. J. Anderson, *Jour. Biol. Chem.*, 126: 505-513, 1938.

⁴ C. Kaiserling, *Ztschr. Tuberk.*, 27: 156-161, 1917.

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¹ O. W. Richards and D. K. Miller, *Am. Jour. Clin. Path., Tech. Suppl.*, January, 1941.

² H. Didion, *Klin. Wschr.*, 18: 1315-1318, 1939.