of the plants on plot No. 8L, none of which were diseased (I), further towards the summit apex (N = 100) and away from the right base apex (K_2O = 100), indicating a higher relative proportion of N and a lower proportion of K_2O in the composition of the respective *NPK-units*.

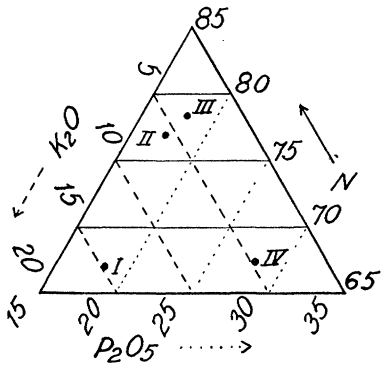

FIG. 1. Relative positions of coordinates of the 16th leaf from plants growing on plot No. 8L on which no disease appeared (I) and of those from plants on plot No. 12L showing no visible symptoms of disease (II), from plants showing slight infection (III), and from badly infected plants (IV). Only a portion of the triangle of which each side is 100 is shown.

The coordinate point (II) of leaves from plants on the diseased plot, which showed no visible symptoms of infection at this time, is displaced, however, further toward the left base apex (K_2O = 100) than that of leaves from the plants on this plot which showed visible manifestations of disease (III and IV).

The intensities of nutrition—the sum of the percentages of N, P_2O_5 and K_2O in the dried foliage—for the plants from the diseased plot were 4.86 (II), 4.43 (III) and 5.65 (IV), and for the healthy plants, 7.90.

The infection by the virus was associated with a type of nutrition having quantitatively a lower intensity with respect to the plastic elements, and qualitatively with a disequilibrium with respect to these elements, characterized predominantly by higher values for N and much lower values for K_2O in the composition of the *NPK-unit* of the susceptible compared with resistant plants.

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SCIENTIFIC APPARATUS AND LABORATORY METHODS

HISTOLOGICAL SECTIONING OF HARD TISSUES BY A NEW TECHNIQUE

VARIOUS concretions, bone and other hard or brittle tissues are not easily sectioned on the microtome. Since fluid methacrylates, the basis of "Lucite" and "Plexiglass," can be polymerized to solids *in situ*, it is possible to imbed such tissues in a solid medium firm enough to allow the grinding of very thin sections, using the well-known methods of petrography. We outline below some of the details of such a technique which we have used with good success.

The specimen, which we shall suppose to be about 1 cm on a side, is dehydrated, using the alcohol, acetone or dioxane technique, and is then cleared in xylol. Some care must be taken in the choice of a clearing agent, as some of these are not miscible with methyl methacrylate; while chloroform, for example, may decompose during the ensuing polymerization giving rise to bubbles. With dry substances, of course, these steps may be omitted. The specimen is next immersed in monomeric (unpolymerized) methyl methacrylate, a liquid having a sufficiently low viscosity to allow of complete penetration in twelve hours, using three changes. The monomer, as supplied commercially, contains a trace of hydroquinone in inhibit polymerization; and .05 per cent. benzoyl peroxide should be added to act as a catalyst for polymerization and consequent solidification upon heating. In the case of dry substances reduced pressures may advantageously be used to insure complete penetration of the liquid medium. A test-tube, of about 17 mm diameter, is prepared by polymerizing a 4 cm rod of solid methacrylate in its bottom, this to act as a temporary handle. The impregnated specimen is placed in the test-tube on top of this rod and covered with methacrylate, previously polymerized to the consistency of molasses.

The partial polymer is prepared by heating the catalyzed monomer to 80° C. until thick, about twenty minutes. The use of the partial polymer in this step helps to reduce the overall heat liberated by the reaction, while its presence catalyzes the polymerization of the monomer permeating the tissue. The syrup containing the specimen is now caused to become an integral extension of the solid rod by completing its polymerization in an oven at 40° C. The test-tube should be corked to prevent undue evaporation. This step requires about twenty-four hours, after which the test-tube is cracked away from the rod, which now contains the specimen firmly imbedded near one end. This rod is next cut through the specimen, normal to the rod axis, and the exposed surface, containing the tissue, ground flat and polished. For this grinding process successive grades of emery paper and mild polishing powders suffice.

If serial sections are desired the tissue-containing rod may be cut into wafers, each of which is polished

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 technique[1] 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 increased 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 acid[3] 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 Kaiserling[4] 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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