ture of the wax should be but slightly above its melting-point.

This method of reducing desiccation in slant cultures and in tubed media, as used for several years by the writer, has been uniformly successful. The method is very useful when a long period of incubation is required. Cultures can be kept in a suitable condition for study for several weeks, and, by completely sealing, sterile tubed media may be kept ready for use during a long period.

The method has a decided disadvantage. When the tubes are sealed the cotton stoppers are kept moist by water of condensation, permitting fungi to grow through them and contaminate the cultures. Contamination in this way is almost entirely avoided, however, when the work of preparing the waxed paper disks, the storing of them and the sealing of the tubes is done under aseptic conditions.

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A METHOD FOR DETECTING ACID-FAST BACTERIA IN THE SOIL

IT has been assumed that saprophytic acid-fast bacteria are rather wide spread in nature and that their appearance in soil is not an uncommon occurrence. There has been, however, no definite evidence to substantiate this assumption.

In view of these facts experiments have been under way for several months attempting to identify these organisms under various natural conditions and more especially in the soil.

The technique used is as follows. Soil samples in approximately one gram portions are mixed with about 50 to 60 cc of modified Büttner's[1] medium in 200-cc flasks. This medium which has previously been sterilized in the flasks has the following composition:

Tap water	1000 cc
K_2HPO_4	0.5 gm
NH_4Cl	0.5 gm
$MgSO_4$	0.2 gm
$CaCO_3$	0.2 gm

It will be noticed that this medium contains no available carbon. To supply this carbon, paraffin-coated pebbles are placed in the flasks. These pebbles are large enough so that they will extend above the surface of the medium. Supplying carbon in this way seems to be quite effective in keeping down contamination since a great many organisms can not thrive under these conditions.

Incubation is carried out at 47.5° C. It was found that at lower temperature there were gross contaminations by molds so that the acid-fast bacteria were so completely covered as to render detection of the organism very difficult and their isolation virtually impossible. When the high temperature was used there was no evidence of contamination by molds.

Up to the present time this technique has been applied to some thirty soil samples, and acid-fast bacteria have been found in every instance after from two to seven days' incubation.

In the incubation process some of the paraffin melts from the coated pebble and forms a thin pellicle on the fluid. The organisms are found on the under side of this pellicle and on the paraffined surface of the pebble. They can also be found, in many cases, adhering to the sides of the flask just above the surface of the medium. Under this latter condition we frequently get large masses of the organism which are comparatively free from contamination. After prolonged incubation it is usually possible to detect masses of the organisms with the naked eye. These have the appearance of opaque bodies on the surface layer of paraffin, on the pebble or on the side of the flask. In chromogenic species the color can be detected in these areas.

The organisms when isolated show numerous variations both morphologically and culturally. A discussion of these variations does not seem necessary at this time. When the organisms are stained with hot carbol fuchsin all are highly resistant to decolorization by 3 per cent. HCl in 95 per cent. alcohol.

A number of these organisms have been isolated by making dilution plates using Conn's[2] medium to which is added gentian violet in a dilution of 1 to 10,000. The composition of this medium is as follows:

Water	1000 cc
Agar	15 gm
Glycerin	10 gm
K_2HPO_4	1 gm
Sodium Asparaginate	1 gm

While the number of soil samples examined is rather small, the writer feels that the method described is a relatively simple way of detecting these organisms in soil. The collecting of samples from various parts of the United States is now under way, and these will be subjected to this technique. A more detailed account of this problem will appear at a later date.

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[1] Hans Büttner, "Zur Kenntnis der Mykobakterien insbesondere ihres quantitativen Stoffwechsels auf Paraffinnährboden," *Arch. f. Hyg.*, 97: 12, 1926.

[2] H. J. Conn, "The Use of Various Culture Media in Characterizing Actinomycetes," N. Y. Ag. Exp. Sta. Tech. Bul. 83, April, 1921.