The difficulties met in endeavoring to obtain chemically pure products have incited us to try to develop a micro-technique for the study of carbohydrate fermentation by bacteria. By this technique, used by us since 1941 and involving over five hundred different strains, we have found that we gained much room in our incubators, made a notable saving of time in the preparation of the material, in the inoculating of the bacteria, and in the reading of the data. Furthermore, we obtained a practical constancy of results which makes possible comparison of the action on the different materials used. This is not always the case when using current methods. Other bacteriologists, such as Hauduroy (1938), have noticed the inconsistencies in the customary techniques employed.

The saving of purified carbohydrates by this method is considerable; in fact, a research on the fermentation of twenty different glucides can be made with a total weight of only 40 cg, which is only two cg apiece.

THE APPARATUS

As a container, one may use a simple glass-covered dish 14 cm in diameter and 5 cm high, the cover being 15 cm in diameter and 4 cm high. Into this container is placed a frame containing 20 numbered divisions in 4 rows of five compartments each. There is a space equal to the width of two rows between the upper two and the lower two rows of compartments (see fig. 1). The outside dimensions of this frame are 10.5 x 8.6 cm, and each compartment is 16 x 16 mm, inside measurements, with walls 1 mm thick. Empty neosalvarsan boxes make useful material when they have been waxed and the bottoms removed. These compartments are numbered horizontally, and in such a manner as to be visible from the outside of the container. The frame could be made from aluminum sheets or any other rust-proof material.

Into each compartment place a small tube 1.5 cm in diameter and 4.5 cm high, with a round bottom. For lack of better material, use old neosalvarsan ampoules. The bottom of these tubes must rest directly on the bottom surface of the glass container which is to receive the sugar solutions.

Ten such boxes are required, and should be sterilized at 180 C in a Pasteur sterilizer.

PREPARATION AND ARRANGEMENT OF THE GLUCIDES

During this time the sugar solutions are being prepared by dissolving 0.2 g in 5 ml of doubly distilled water. The glucides which are difficult to dissolve are placed in a water bath.
With a graduated pipette of 1 ml divided into tenths place one-half ml in each micro-tube according to number, and put each back in its proper place without further marking.

The number of these compartments are arbitrarily arranged. In our work, we used the following arrangement:

<table>
<thead>
<tr>
<th>4th row:</th>
<th>21 Xylose</th>
<th>20 Erythritol</th>
<th>19 Inulin</th>
<th>18 Adonitol</th>
<th>17 Dextrin</th>
</tr>
</thead>
<tbody>
<tr>
<td>3rd row:</td>
<td>12 Galactose</td>
<td>13 Arabinose</td>
<td>14 Sorbitol</td>
<td>15 Raffinose</td>
<td>16 Inositol</td>
</tr>
<tr>
<td>2nd row:</td>
<td>11 Amygdaline</td>
<td>10 Salicine</td>
<td>9 Rhamnose</td>
<td>8 Dulcitol</td>
<td>7 Glycerol</td>
</tr>
<tr>
<td>1st row:</td>
<td>1 Glucose</td>
<td>3 Lactose</td>
<td>4 Maltose</td>
<td>5 Mannitol</td>
<td>6 Sucrose</td>
</tr>
</tbody>
</table>

No. 2 is omitted because in our classification this is fructose and this sugar cannot be used where sterilization by heat must be employed. Sterilize in a steam-pressure sterilizer at 110 C. By using doubly distilled water in the neutral glasses, the danger of hydrolysis is avoided, and none of the abnormal reactions attributable to it have ever been noticed in our experience.

The preparation of these ten micro-glucide dishes requires no more than two hours, including the necessary sterilization. If one desires to prepare only part of the series, the sugar solution at 4 per cent, unsterilized, may be kept in a refrigerator, but must be hermetically sealed. If one pours two tubes of distilled water into the unnumbered compartments placed in the center space of the frame (see fig. 1), these can be preserved for several weeks without any sign of desiccation.

**THE INOCULATION OF THE BACTERIA INTO THE MICRO-GLUCIDE DISHES**

The germ chosen for the study is first isolated, then inoculated into 12 ml of a 2 per cent aqueous peptone solution to which is added 1 per cent of Andrade's indicator. With germs difficult to grow ascitic fluid is added. According to the rapidity of growth of the culture, the tube of peptone water is placed in the incubator from 24 to 48 hours at a temperature of 30 to 37 C, as
necessary. The germ itself consumes all traces of fermentable sugar which may contaminate the peptone water. After this period the culture is examined fresh and after gram-staining to check its purity.

Then this culture is inoculated into the micro-tubes, 0.5 ml in each. To do this, the apparatus is placed at an angle of 10° to 15° on a convenient support, and the assistant raises the cover on one side only, exposing, as little as possible, the two rows of micro-tubes in the compartments of that side (see fig. 2). A 5 ml pipette, graduated in 0.5 ml intervals and having a nipple, is used for the distribution of the culture. After the first ten tubes, the pipette is refilled. During this refilling, the cover is replaced, the apparatus turned, and when the pipette is refilled, the cover is again carefully lifted, and the remaining com-

![Fig. 2](http://jb.asm.org/)

partments are filled in the same manner. This operation requires but 45 seconds. Gently shake to and fro to mix the germs with the sugar solutions. Micro-glucide dishes are marked with a crayon and placed in the incubator.

**READING OF THE RESULTS**

In the afternoon of the same day, some of the carbohydrates already begin to turn red. The reading is made from top to bottom against a white background, as illustrated in fig. 1. The following day, most of the sugar fermented by the strain under study has taken a turn. These observations are carefully made for a period of one week.

With experience, one quickly obtains a mental image of the strain under study. The color changes become so familiar that one can tell at a glance the different types of carbohydrates that are involved with the specific cultures. It is this visible demonstration, as well as the number of different glucides used, that compensate for the fault of our technique which gives no information on the gases produced. We believe, however, that it could be adapted for that purpose.

Reading of the results is easily and quickly made because the number on the frame is always the same and easily visible.
Cleansing of the micro-tubes is quickly done. One simply removes them with tweezers and plunges them into an antiseptic solution which quickly sterilizes them. Then they are boiled in a carbonate solution, rinsed, and passed through a sulfuric solution of potassium bichromate, without the necessity of washing each one individually.

RESEARCH FOR ANAEROBIC BACTERIA

In our work, which is chiefly against the plague, we find little need to work with anaerobic bacteria. It seems to us, however, that this box of micro-glucides could easily be adapted for such a study in any one of the following manners:

1. The dish can be hermetically sealed by a rubber bag 2 cm high and 3 mm thick, applied on the bottom of the box on which the glass cover fits tightly.
2. It is possible to add a reducer to the peptone water with Andrade’s indicator. One may use sodium pyruvate (Berthelot, 1923) or ascorbic acid (Fauvert, 1939).
3. The oxygen in the box can be absorbed by the use of a mixture of pyrogallol and potash (Buchner, 1888) contained in two inclined tubes which are emptied into the container after it has been closed, or by an aerobic culture (Fortner, 1928) inoculated in broth and emptied into the bottom of the container before it is closed.

These are simply suggestions which we have not yet been able to test by experiment.

REFERENCES