One-Tube Test for Determining Oxidative or Fermentative Metabolism of Bacteria

WILLIAM J. BROWN

Hutzel Hospital and Department of Immunology and Microbiology, Wayne State University School of Medicine, Detroit, Michigan 48201

Received for publication 21 December 1973

A one-tube test for distinguishing oxidation from fermentation in the metabolism of carbohydrates was examined on 1,200 gram-negative bacilli. There was a 100% correlation between the one-tube method and the standard two-tube procedure.

Hugh and Leifson (2) first reported the taxonomic significance of fermentative versus oxidative metabolism of carbohydrates by various gram-negative bacteria. This characteristic has since become an essential part of identification protocols (1, 3). The standard procedure for determining if an isolate is metabolizing either by an oxidative or a fermentative pathway is to inoculate two tubes of identical composition and to cover the medium in one tube with a substance such as sterile melted paraffin or mineral oil to restrict oxygen diffusion into the medium. The uncovered tube reflects oxidative reactions and the covered tube is for the fermentative reactions.

By use of a Beckford constricted tube, we evaluated a one-tube test for distinguishing the type of carbohydrate metabolism. These tubes were obtained from Research Diagnostic, Inc., Roslyn, N.Y., with either BBL or Difco OF base medium plus 1.0% of the respective carbohydrate. It was observed early in the testing that the reactions were difficult to read when the BBL OF base was used. Therefore, that company’s OF base medium is not available in the commercial constricted tubes and all further tests were performed using only the Difco OF base. For comparative experiments, Difco OF bases with 1.0% of each of the following carbohydrates were also prepared in conventional test tubes: glucose, sucrose, maltose, lactose, xylose, and mannitol. The organisms tested were either ATCC strains or clinical isolates identified by the method of Weaver et al. (3). Each of the organisms was inoculated into one constricted tube of each sugar and two conventional tubes of each sugar. One of each of the latter tubes was overlaid with 3.0 ml of sterile mineral oil for the fermentation reactions. The medium above the stricture in the Beckford tube corresponds with the uncovered conventional tube for oxidation reactions, and the area below the stricture in the Beckford tube corresponds with the covered conventional tube. The inoculated tubes and non-inoculated controls were incubated at 35°C for 7 days and examined daily.

Figure 1 shows an example of the reactions obtained. The constricted tubes with both reactions are labeled OF and the conventional tubes are labeled as either O for oxidative uncovered medium, or F for fermentative covered medium. Tube 1 is a non-inoculated OF glucose and tube 2 a non-inoculated O glucose. Tubes 3, 4, and 5 have been inoculated with Serratia marcescens and shows the reaction of a bacterium with a
fication metabolism. Tubes 6, 7, and 8 are an example of an oxidative metabolism as produced after inoculation with Pseudomonas aeruginosa with acid only above the stricture (tube 6) and in the uncovered conventional tube (tube 7). Acinetobacter lwoffi was inoculated into tubes 9, 10, and 11. No acid was detected in any of these three tubes.

Although only glucose needs to be tested to determine the ability of an isolate to carry out fermentation, many carbohydrates are tested for identification purposes. In this study, all organisms were tested in each of the carbohydrates. Table 1 shows the results obtained with the organisms tested in comparative reactions in constricted and conventional tubes. P. aeruginosa isolates were the most frequently tested organisms, comprising 54% of the strains tested. Strains of two members of the Enterobacteriaceae were included to evaluate the fermentative reaction. However, the great majority of isolates tested were nonfermenting bacteria since it is these latter organisms for which the oxidation or fermentation reactions are mostly needed for identification. Although 27 of the isolates gave reactions which were not typical of characterized organisms, they did produce identical reactions in both systems. Tubes were held for one week. All 1,200 strains tested in the six carbohydrates produced reactions in the constricted tube identical with the reactions obtained in the corresponding two conventional tubes. Furthermore, we have observed that commercially prepared OF medium in conventional tubes tends to give unreliable reactions, particularly with false positive fermentative reactions by nonfermenting organisms. This problem has not been observed in fresh medium prepared in our laboratory or in the prepared medium in constricted tubes.

In summary, a one-tube system for determining if an organism utilizes carbohydrates by an oxidative or fermentative metabolism or not at all is described. The results in the constricted tube were in 100% agreement with the two-tube conventional system. Since only one tube is needed, and thus one inoculation, and there is no need to overlay with an oxygen retarding substance, this constricted tube system can be substituted for the conventional tubes of OF medium in identification schemes.

This study was supported by Public Health Service General Research Support grant RR05844.

**LITERATURE CITED**
