

NOTES

Radiometric Detection of Carbohydrate Catabolism by Pathogenic *Neisseria*

F. R. COX,^{1*} M. E. COX,² J. R. MARTIN,¹ AND U. H. STOER¹

Department of Pathology, Schumpert Medical Center, Shreveport, Louisiana 71101,^{1} and Department of Medicine, Louisiana State University School of Medicine, Shreveport, Louisiana 71130²*

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A liquid scintillation procedure for the catabolism of D-[1-¹⁴C]glucose and [U-¹⁴C]maltose by pathogenic *Neisseria* was tested. Definitive results were obtained within a 30-min incubation period.

The classical carbohydrate utilization test for the species identification of *Neisseria gonorrhoeae* (4) and the many modifications of the test (3, 5) are dependent upon a change in color of an indicator dye caused by acid production. Incubation periods of 18 to 24 h for the conventional test and 6 h for reported modifications are required. We initiated this study to test a simple and rapid liquid scintillation procedure (1) for glucose and maltose catabolism by pathogenic *Neisseria*. Briefly, Whatman no. 40 filter paper was cut into strips (5 by 10 cm) and dipped in Aquasol (New England Nuclear), and the solvent was allowed to evaporate. Liquid scintillation vials (Rochester Scientific no. 7494 plastic vials) were lined with the fluor-impregnated paper strips and stored at room temperature. Prior to use, 0.3 ml of 1 N NaOH was pipetted onto the impregnated filter strips, and sterile inner sample vials (Rochester Scientific no. 7499) were inserted in the fluor-containing vials. One-half microcurie of D-[1-¹⁴C]glucose (3.9 mCi/mmol) and 0.5 μ Ci of [U-¹⁴C]maltose (719 mCi/mmol; Amersham/Searle) contained in 0.1 ml of distilled water were pipetted into insert vials.

Conventionally identified clinical isolates of *N. gonorrhoeae* and *Neisseria meningitidis* were the test organisms. Eighteen- to 28-h subcultures on chocolate agar were used for radiochemical studies. Several colonies were picked and suspended in a 1.2-ml solution of NaCl-tris(hydroxymethyl)aminomethane-hydrochloride minimal medium (2) to a density of approximately the MacFarland no. 3 barium sulfate standard. One-half milliliter of the standardized inoculum was pipetted to each carbohydrate-containing inner vial, and the outer vials were tightly capped and incubated at 35°C for 30 min. An *o*-nitrophenyl- β -D-galactopyrano-

side disk was placed in the remaining 0.2 ml of inoculum for detection of any lactose-positive *Neisseria lactamica*. The minimal medium contained per liter: MgSO₄·7H₂O, 0.1 g; NH₄Cl, 1.5 g; FeSO₄·7H₂O, 0.001 g; MnSO₄·H₂O, 0.001 g; CaCl₂·2H₂O, 0.1 g; Na₂S₂O₃, 0.025 g; and NaCl, 8.7 g. The medium was buffered at pH 7.3 by 0.01 M tris(hydroxymethyl)aminomethane-hydrochloride.

The results of radiochemical tests are given in Table 1. Qualitative data were desired in the current study; therefore, no attempt was made to stop the enzymatic reactions at the end of a 30-min incubation. Thus because of the cumulative nature of the test, vials toward the end of the counting sequence had 30 min of incubation at 35°C and up to 15 min of incubation in a refrigerated scintillation counter. This incubation change would be a contributing factor in the observed range of values obtained. A minimum of a 160-fold difference in radioactivity was observed between the [¹⁴C]glucose and [¹⁴C]maltose substrates with *N. gonorrhoeae*, with a mean difference of 630-fold. The minimum counts per minute for *N. meningitidis* and [¹⁴C]maltose were 22-fold greater than the highest value of 130 cpm for *N. gonorrhoeae* in the presence of [¹⁴C]maltose. The presence or absence of enzymes for the catabolism of these carbohydrate substrates was quite definitive. Radiometric results corresponded with those obtained with CTA carbohydrates (4) and Minitek disks (3). All isolates were *o*-nitrophenyl- β -D-galactopyranoside negative. In addition to these three carbohydrates, fructose and/or sucrose must be incorporated into schemes for the species identification of *Neisseria*, whether conventional or radiometric. The usually saprophytic species of *Neisseria* were not tested in the current study; however, well-established

TABLE 1. Radiochemical detection of [1-¹⁴C]glucose and [U-¹⁴C]maltose utilization by *N. gonorrhoeae* and *N. meningitidis*

| Organism | No. tested | [1- ¹⁴ C]glucose (cpm) | | [U- ¹⁴ C]maltose (cpm) | |
|------------------------|------------|-----------------------------------|---------------|-----------------------------------|--------------|
| | | Mean | Range | Mean | Range |
| <i>N. gonorrhoeae</i> | 35 | 55,848 | 17,795-96,934 | 88 | 55-130 |
| <i>N. meningitidis</i> | 20 | 23,882 | 5,925-64,379 | 10,512 | 2,946-23,848 |

procedures exist that aid in the exclusion of these species (i.e., lack of growth on modified Thayer-Martin medium, growth at 22°C on enriched media, growth on salt-free nutrient agar, KNO₂ reduction, and amylosucrase production). This preliminary study indicates that glucose and maltose reactions for the pathogenic *Neisseria* may be determined by a rapid liquid scintillation procedure applicable to routine clinical laboratory use.

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