RADIOMETRIC IDENTIFICATION OF STREPTOCOCCUS GROUP A IN THROAT CULTURES

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Streptococcus Group A may be identified in simulated and patients' throat cultures by the selective inhibition of Group A streptococcus carbohydrate metabolism in the presence of specific antisera.

Radiometric studies of bacteria have been concerned mainly with the detection of pathogens in clinical samples (*1–3*). We have recently begun to evaluate the possibility of extending the radiometric concept to the identification of particular bacteria. We have developed a technique for rapid speciation of Neisseria in pure culture (*4*), based on the differential oxidation of ^{14}C-labeled sugars to $^{14}CO_2$.

The use of specific metabolic patterns to identify a micro-organism in mixed culture with other organisms is difficult since the ability to oxidize a given substrate is frequently shared by a number of quite disparate species. We have therefore begun to explore the possibility of using immune inhibition of metabolism—neutralization of bacteria by specific antibody —as a way to identify bacteria in mixed culture.

We have previously shown that the carbohydrate metabolism of streptococci could be profoundly and selectively inhibited by specific antisera when the organisms were grown in pure culture (*5*). In the present paper we report our preliminary experience with the inhibition by Group A antiserum of Group A streptococci growing in mixed culture with normal mouth flora.

MATERIALS AND METHODS

We began by studying simulated cultures with varying proportions of streptococcus Group A and normal flora. The bacteria were prepared as follows: normal mouth flora were obtained by throat swab from one of us (MC) and the organisms were grown overnight at 37°C in peptone-supplemented broth (BBL No. 4955). The organisms were then spun down and resuspended in trypticase soy broth without glucose (BBL No. 11774). A final concentration of 10^5 organisms/cc was obtained by reference to 1:1000 dilution of a McFarland standard. Streptococcus Group A (Maryland Department of Health Laboratories, Microbiology Division, Baltimore, Md.) was taken from stock culture on a chocolate agar slant, streaked on a chocolate agar plate, and incubated at 37°C. After overnight growth, 10^5 organisms/cc in trypticase soy broth (TSB) without glucose (BBL No. 11774) was again prepared by reference to the appropriate standard. Subsequently, the two suspensions were mixed so that in the total concentration of 10^5 organisms/cc there was 0%, 5%, 25%, 50%, and 100% Group A streptococcus.

An additional series of pilot experiments was performed using throat cultures from patients. Patients suspected of having beta streptococcus throat infection had throat swabs taken and streaked onto a 5% sheep blood agar plate with a bacitracin disk. The next day the plate was inspected to check the zone of beta hemolysis and inhibition by bacitracin. Organisms were then scraped off the plate into TSB without glucose (BBL No. 11774) and prepared in a suspension of 10^8 organisms/cc by reference to a McFarland standard. Appropriate dilutions were made with TSB to obtain a concentration of 10^5 organisms/cc.

To measure bacterial metabolism radiometrically, 10^5 bacteria were inoculated into a sealed 50-cc culture vial that contained 10 cc of glucose-free TSB to which had been added 1.5 μCi of ^{14}C-labeled glucose (Amersham/Searle, uniformly labeled 200 mCi/mmole). Radioactive $^{14}CO_2$ produced by bacterial metabolism was measured hourly in a semi-automated instrument that is capable of sampling 25 vials in sequence (Bactec 225, Johnson Laboratories, Cockeysville, Md.). This device utilizes an ionization chamber to determine the radioactivity, and the results are expressed in terms of "index units" where 100 = 0.25 μCi.

Received March 9, 1975; revision accepted April 20, 1975.

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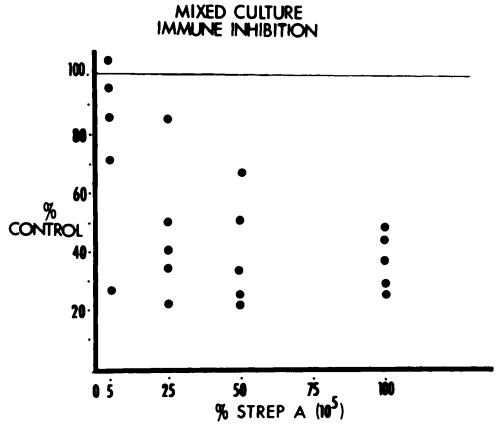

FIG. 1. Inhibition of $^{14}CO_2$ production from ^{14}C-labeled substrates in mixed cultures of mouth flora and streptococcus Group A, the inhibiting agent being streptococcus Group A antiserum. Inhibition increases with increasing proportion of streptococcus Group A (see text for details).

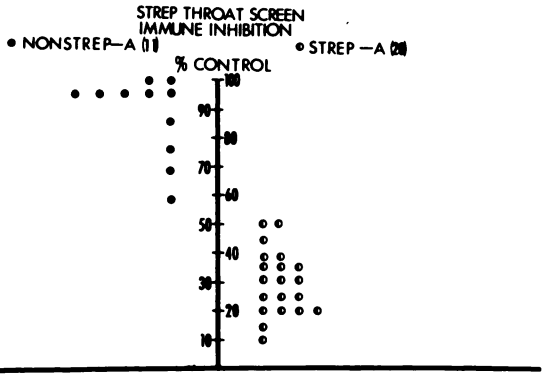

FIG. 2. Inhibition of $^{14}CO_2$ production from ^{14}C-labeled substrates in cultures from patients suspected of having strep throat. Designations "nonstrep" and "strep" refer to whether or not beta streptococcus was isolated from these throats.

The effect of streptococcus Group A antiserum on the metabolism of the mixed suspensions was studied as follows. The vials were incubated in duplicate at 37°C and hourly readings were taken. After 3–4 hr, when a metabolic rate of 40–50 index units was observed, 1 cc of a 1:1280 dilution of streptococcus Group A antisera (BBL No. 40687) was added to the incubation mixture. Thirty minutes after addition of the antiserum, the samples were measured radiometrically and the effect of the antiserum was determined by reference to a control culture to which no antisera had been added. The metabolic rate of the inhibited sample was expressed as a percent of the metabolic rate of a control vial.

The effect of streptococcus Group A antisera on the carbohydrate metabolism of the incubation mixtures derived from patients' throat cultures was measured in the same way with one minor exception. In the mixed suspensions studies, it was observed that the most profound depression of metabolism occurred when the antiserum was added relatively early to the metabolizing cultures. For this reason the antiserum was added after 1–2 hr of incubation, when the growth index was 15–30. All samples were run in duplicate and the results were calculated as before.

The results from the mixed culture suspensions are shown in Fig. 1. There is a prompt suppression of the metabolism of the organisms, which is evident even at the relatively low percentages of streptococcus Group A. The average suppression is a function of the percent streptococcus Group A in culture, the inhibition being greatest for the 50–100% range. In the incubation vials taken from suspensions of patient cultures, 11 cultures from patients without beta streptococcus showed a percent activity that was always greater than 60% of the control cultures, and the majority of cultures showed little inhibition by the antiserum. In the patients with documented streptococcus Group A, all 20 showed pronounced immune inhibition, with growth indices of only about 10–50% of the control (Fig. 2). In this limited series there was no overlap between the "strep" and "nonstrep" cultures.

This paper represents the first description of the radiometric identification of bacteria in mixed culture. The technique is relatively rapid: in these pilot studies a reliable determination of the presence of Group A streptococcus could be obtained in 2½ hr.

We performed these experiments to evaluate the feasibility of using the principle of immune inhibition to develop a rapid screening test for the presence of Group A streptococcus in throat cultures. Based on these data, comparative studies are currently under way to determine the applicability of the radiometric technique in the clinical setting.

ACKNOWLEDGMENT

This work was supported by USPHS Grants GM-10548 and GM-1496 from the National Institutes of General Medical Sciences and by USPHS Research Career Development Award 5K04-A108610 to Dr. Charache.

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