Inhibition of the Metabolism of Streptococci and Salmonella by Specific Antisera

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Streptococcal and salmonella antisera inhibited carbohydrate metabolism for groups A, B, C, and D streptococci and group E salmonella, as measured by the formation of [14C]dioxide from [14C]glucose metabolism. For salmonella, the inhibition was type specific since group E salmonella were inhibited only by salmonella E antisera and not by anti-salmonella A or C1. For streptococci, quantitative differences were demonstrated, but major cross-reactivity was observed. At high concentrations, the antisera were bactericidal; at more dilute concentrations, for both salmonella and streptococci, carbohydrate metabolism was suppressed, but subculture on chocolate agar showed abundant growth. Cross-reacting antibodies could be absorbed by incubation with either antigen, e.g., streptococcal antisera versus heat-killed salmonella. The results suggest that the radiometric technique can be more sensitive than either capillary flocculation or visual detection of bacterial growth for detecting the inhibition of streptococci and salmonella by specific antibodies. The use of specific antisera may prove useful for bacterial species identification in an automated system for detection of bacterial growth.

The presence of bacteria in biologic samples can be detected radiometrically by measuring the 14CO2 produced by the bacterial metabolism of 14C-labeled glucose and other substrates (4). Utilization of radiometric techniques for detecting bacteremia was originally reported in 1970 (2, 3). Subsequently, this principle has been successfully applied to antibiotic sensitivity testing (1). Studies so far completed indicate that the radiometric method can provide a rapid and sensitive means for bacterial detection.

We have recently observed that the metabolism of streptococci and salmonella can be profoundly inhibited by specific antisera. We propose that these observations may provide a basis for a significant extension of the radiometric detection technique to include the rapid automated speciation of bacteria.

MATERIALS AND METHODS

Radiometric measurement. To measure bacterial growth radiometrically, bacteria are inoculated into sealed aerobic culture vials containing 1 μCi of 14C-labeled glucose (Amersham-Searle, uniformly labeled, >200 mCi/mmol) in glucose-free trypticase soy broth (TSB) (BBL no. 11774). Radioactive 14CO2 produced by bacterial action is measured at hourly intervals in Bactec-225 that automatically samples 25 incubation vials in sequence (Johnston Laboratories, Inc., Cockeysville, Md.). The details of this system have been published previously (2).

Experimental procedure. The intent of these experiments was to evaluate the effect of various dilutions of antisera on the metabolism and growth of salmonella group E and streptococci group A, B, C, and D. Salmonella group E was obtained from the Johns Hopkins bacteriology laboratory. Streptococci groups A, B, C, and D were obtained from the Maryland State Department of Health. The salmonella and streptococci were grown overnight at 37°C on a trypticase soy agar slant and chocolate agar slants, respectively. On the morning of the study, organisms were scraped off the agar into TSB without glucose, in order to achieve a turbidity of organisms in the broth which corresponded to a twofold dilution of McFarland no. 1 standard (1.5 × 108). Serial dilutions were made to obtain a concentration of 108 organisms/ml; these concentrations were subsequently confirmed by pour plates after overnight growth at 37°C. Approximately 108 organisms were added to a mixture which contained 1 μCi of [U-14C]glucose and the appropriate antisera. The mixture was made up to a final volume of 10 ml with TSB in a sealed aerobic culture vial. The following antisera were used: for salmonella—group E, C1, and A; for streptococci—group A, B, C, and D and anti-streptolysin O. Dilutions of antisera in glucose-free TSB were made so that the final concentration in each test vial was 1:200 to 1:25,600.
The radioactivity in each vial was measured at 2-h intervals for the next 12 h. The measurements were then summed, and the results were compared with a control vial, to which no antisera had been added. Results were expressed as a percentage of the radioactivity produced in the control vial. All vials were run in duplicate, and the data presented are from single examples drawn from 10 to 12 experiments. The effect of varying dilutions of these antisera on the metabolism and the growth of the streptococcal strains shown in Table 1 were determined.

Non-immune sera. To evaluate the effect of non-specific serum factors on the metabolism of these bacteria, sera were obtained from non-immune rabbits. These sera were concentrated in 1:10 to an inoculum of salmonella group E and streptococcus group A.

Streptococcus grouping tests. Extracts of the streptococci were prepared by autoclaving, and the supernatant was used in a capillary tube precipitation test according to specifications (6). Groups A, B, C, and D antisera were used, and control extracts (BBL grouping sera) were observed for precipitation in parallel (6).

Antibody absorption. To study the nature of the antibody which caused cross-reactivity between streptococci and salmonellae, antibody absorption studies were performed as follows: (i) Inoculate a loopful of salmonella group E in TSB (30 ml) and grow overnight; (ii) Centrifuge and remove the supernatant; (iii) Boil 20 ml of distilled water in a boiling water bath; (iv) Take precipitate from step 2 and suspend in 2 ml of hot distilled water; (v) Add to 18 ml of distilled water and boil for 20 min. (vi) Subculture to be certain bacteria are dead; (vii) Centrifuge and decant the supernatant; and (viii) Add bacteria to an equal volume of streptococcal group A antisera and mix. Leave overnight at 4 C.

The above procedure was then repeated by using the same absorbed antisera, except that the bacterial-antisera mixture was incubated at 4 C for 48 h, instead of 24 h. The effect of this doubly absorbed antisera on the metabolism of 10^6 streptococcal group A and salmonella group E was then determined for serial dilutions of antisera of 1:200 to 1:25,600. All concentrations were studied in duplicate. The data are presented in Fig. 4.

**RESULTS**

Effect of nonimmune rabbit sera. In comparison to a culture without any sera added, the salmonella group E was minimally inhibited by the addition of nonimmune sera as measured by a delay in peak radioactivity (9 versus 6 h) for the culture with the nonimmune rabbit antisera of 1:10 dilution. There was no inhibition for the streptococcal cultures. All of the nonimmune sera showed much less inhibition against both salmonella group E and streptococcus group A than the hyperimmune sera, even though relatively concentrated nonimmune sera (1:10) were used.

Streptococcus. The effect of streptococcal antisera on the metabolism of [14C]glucose by streptococcus group A is shown in Fig. 1. The metabolism of [14C]glucose to 14CO2 is greatly inhibited by the anti-A, but not the anti-streptolysin O. Inhibition of metabolic activity by specific dilutions of the antisera is shown in Table 1. There was inhibition of all four groups by all four grouping sera even though the precipitin reactions were group specific. On subculture, the bacteria grew, although the number of colonies was low (40 to 50) compared to the size of the inocula (10^5). With the addition of more concentrated antisera, there were fewer colonies with progressively more abundant growth at dilute mixtures. This suggests a predominantly bactericidal activity of the antisera at high concentrations with bacteriostatic activity of the low concentrations of antibody. The titer at which metabolism was first observed was always much lower than the titer at which growth was observed, and within the groups, the inhibition titer was characteris-

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**Table 1. Inhibition by antisera**

| Streptococcus type and identification no. | Dilution at which metabolism first observed | Dilution at which growth* first observed | Capillary tube grouping test* |
|------------------------------------------|-------------------------------------------|----------------------------------------|-----------------------------|
|                                          | A  B  C  D                                | A  B  C  D                            | A  B  C  D                  |
| A 331                                    | 1:25,600 1:3200 1:25,600 1:25,600         | 1:200 1:200 1:200 1:200               | +  0  0  0                  |
| A J049                                   | 1:25,600 1:12,800 1:25,600 1:25,600       | 1:200 1:800 1:200 1:200             | +  0  0  0                  |
| B 37765-72                               | 1:6400 1:3200 1:6400 1:3200             | 1:200 1:800 1:200 1:200             | 0  +  0  0                  |
| B 9961                                   | 1:6400 1:6400 1:6400 1:12,800           | 1:200 1:200 1:200 1:200             | 0  +  0  0                  |
| C 40470                                  | 1:25,600 1:12,800 1:12,800 1:12,800     | 1:200 1:6400 1:200 1:3200         | ±  0  +  0                  |
| D B1-011                                 | 1:3200 1:800 1:3200 1:1600             | 1:200 1:200 1:200 1:100           | 0  0  0  +                  |

* Active growth (50 or more colonies).
* Symbols: 0, suppressed growth; ±, weakly positive.
tic. An example of the relative strength of the streptococcal group A antisera is shown in Fig. 2; group A and group C were sensitive to the effects of the antisera, being inhibited by concentrations of 1:25,000. Group B was less sensitive to the same antisera, being inhibited by 1:3,000 to 1:6,000 dilutions. The group D organisms were more resistant.

*Salmonella.* Figure 3 shows the specific inhibition of carbohydrate metabolism of group E salmonella by the group E salmonella antiserum, but not the antisera directed against group A or C1. These results were in contrast with the streptococcus where the grouping reactions did not predict whether an antisera would be inhibitory.

*Comparison of cross-reactivity for streptococcus and salmonella.* The response of salmonella and streptococci to the grouping antisera is shown in Table 2. There is cross-reactivity for salmonella group E antisera against streptococcus group A with depression of metabolism of the streptococcus. Both group A antisera and group B anti-streptococcal antisera inhibit group E salmonella. However, salmonella group C1 antisera does not inhibit the growth of salmonella group E, whereas the streptococcus group A is inhibited. Note that with this particular streptococcal C antisera, the group A streptococcus was not inhibited. This result was consistently observed with individual batches of C antisera, even though the grouping reaction seemed to be anti-C specific.

In Fig. 4, the relative inhibitory capacity of streptococcal A antisera is shown for salmonella group E and streptococcus group A. There is cross-reactivity with inhibition of salmonella growth by streptococcal antisera. The serum is a much more potent inhibitor for streptococcal metabolism by a factor of about 10. A dilution of 1:3,200 or less will have no salmonella inhibition, whereas streptococci is still greatly inhibited. In Fig. 5, the effect of a doubly absorbed antisera on the metabolism of streptococcus group A and salmonella group E is shown. By adsorption, the amount of inhibitory antibody is reduced for both salmonella group E and streptococcus group A. The absorbed an-
tiserum inhibits but does not totally suppress the metabolism of salmonella even though the streptococcus metabolism is suppressed down to a dilution of 1:3,200. Note that although a reduction in the concentration of salmonella antibody was obtained, a reduction in the concentration of streptococcal antibody was also seen, when compared with the usual titer of streptococcus antibody (see Fig. 4) of about 1:25,000.

**DISCUSSION**

This paper is, as far as we are aware, the first report of the detection of inhibition of bacterial carbohydrate metabolism by specific antisera. For the antisera studied, the effect on metabolism was practically immediate. Although the reason for this effect was not determined, one possible explanation could have been bactericidal, cell wall-directed antibodies.

The commerically available antisera used in these studies were the results of an immunization with bacterial cell wall extracts, and as such might be expected to be directed against a number of cell wall antigens. Therefore, it is not surprising that such antisera were heterogeneous. For example, for the streptococcus Lancefield group A, the critical antibody for metabolic inhibition is apparently not the anti-polysaccharide antibody of importance to the grouping reaction, since sera directed against other groups also inhibited streptococcus group A metabolism. Not all grouping sera inhibited the metabolism of individual bacteria, but this property seemed to be a characteristic of the individual antisera used. For example, one group A antisera was tested which did not inhibit the metabolism of a streptococcus group A and one group C, even though the sera gave a group specific precipitin reaction. For the anti-salmonella antisera, depression of metabolism was exclusively a type-specific reaction and possibly determined by the grouping somatic antigens.

### Table 2. Cross-reaction between salmonella and streptococcus

| Organism                  | Metabolism  | Growth                  |
|---------------------------|-------------|-------------------------|
|                           | Anti-streptococcus | Anti-salmonella | Anti-streptococcus | Anti-salmonella |
|                           | A  B  C  E  A  Cₐ |            | A  B  C  E  A  Cₐ |
| Salmonella, group E       | 0  0  +  0  +  +  | ND  0  +  0  +  +   |
| Streptococcus, group A    | 0  0  +  0  +  0  | ND  0  ND  0  +  0 |

*All antisera were at a concentration of 1:200. Symbols: 0, suppressed metabolism or growth; +, active metabolism or growth; ND, not done.*

**Fig. 4. Streptococcus A antisera versus salmonella group E and streptococcus group A.**

**Fig. 5. Inhibition of carbohydrate metabolism by streptococcal antisera absorbed with salmonella group E antigen.**
Because of the uncertainties about the active constituents of most antisera, any scheme for identification of these microorganisms which is based on inhibition of metabolism must be empirical, in which, cross-reactions are used to advantage to "finger-print" organisms with standard sera of known reaction pattern. Cross-reactions between antigenically related species can be used in an identification process as follows: broad grouping antibodies are used in a "polyvalent" step as a guide to selection of subsequently more "monospecific" antisera for discrete species identification. For example, at the present time, we can distinguish between salmonella group E and streptococcus group A based on a characteristic pattern of inhibition of $^{14}$CO$_2$ release in the presence of type specific antisera. Both group A streptococcus and salmonella group E are inhibited by salmonella group E antisera. The group E antisera is the polyvalent antibody in this example. At the monospecific step, the antibodies used would be salmonella A and C; antisera. Group A streptococcus will be distinguished from group E salmonella since the group E salmonella will not be inhibited by any of the antisera, where the streptococcal group A will be inhibited only by the anti-salmonella C$_1$ antisera. The time required for such a two-step procedure would be about 4 h. We have recently observed that bacterial metabolism is also greatly inhibited by addition of antisera to an actively growing culture of sensitive microorganisms. Significant depression in metabolism, as measured by a greater than 50% drop in the bacterial growth index, is seen by 30 min after addition of antisera. This greatly shortens the time of identification, so that a two-step procedure (polyvalent-monospecific) could be performed within 1 h.

It should be emphasized that cross-reactivity between salmonella and streptococcus was only relative, and the streptococcus was much more sensitive to the effect of the homologous antisera. Therefore, it is possible to choose a dilution of antisera for which there is no cross-reactivity (e.g., 1:3,200 in Fig. 4). This feature of the antisera may be exploited to enhance the specificity of the antibody inhibition technique.

At the present time there is insufficient data to warrant a conclusion regarding the nature of the cross-reacting antibody of antibodies observed in these experiments. The cross-reactivity of group C streptococci and group A streptococcus was expected, because these two organisms seem to be closely related immunologically and frequently cross-react in the standard tube precipitation technique. With regard to the cross-reaction of the salmonella and streptococcus, the data, although inconclusive, suggests that the same antibody is involved, because a reduction of the titer by absorption with salmonella organisms caused both a decrease in the titer of inhibitory antibody for salmonella and a parallel decrease in the inhibitory titer for streptococcus group A. In preliminary experiments, we have also observed suppression of metabolism for Shigella with Salmonella antisera, but no suppression of Escherichia coli.

Streptococcus and salmonella antibodies are commonly present in normal serum, in relatively low amounts (5). The low concentrations of hyperimmune antisera in this study inhibited the carbohydrate metabolism of the bacteria but did not kill the bacteria. Normal rabbit serum did not significantly suppress bacterial metabolism. However, it is conceivable that enough antibodies may be present in human serum in individuals with previous infections to prevent the radiometric detection, but not the growth of microorganisms on routine culture media. Thus, naturally present antibodies in human serum may theoretically interfere with the radiometric detection of bacteremia. At present, this had not been a major problem in comparative studies of conventional and routine techniques.

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