Inhibition of the Indole Test Reaction by Sodium Nitrite

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Received for publication 13 October 1971

Sodium nitrite formed by nitraten reduction produced a false-negative reaction in the detection of indole formation when both tests were conducted in one medium.

The production of indole has been a useful test in the identification of bacteria, including the gram-negative enteric bacilli. Some laboratories may choose to use media which combine the indole test with nitrate reduction. Although this dual test medium works satisfactorily with some bacteria, some difficulties were noted with enteric bacilli yielding false-negative indole tests in indole-nitrite medium. This report describes the results of a study to find the cause of this problem.

Five strains of *Escherichia coli* isolated from separate urine specimens were grown in Trypticase soy broth (BBL) overnight and inoculated into the following media: 1% tryptone broth (Difco), 2% Trypticase broth (BBL), nitrate broth (Difco), and indole-nitrite semisolid medium (BBL). The latter medium was also prepared without agar. Cultures were incubated for 24 and 48 hr at 37 C. One milliliter of each broth culture was transferred to Wasserman tubes, and two drops of either Ehrlich or Kovac indole reagent were added. Both reagents were tested in parallel, adding the reagents directly to broths and to broths that were first extracted with 0.1 ml of xylene. The nitrate reduction test was also conducted on 1 ml broth samples. All reagents were prepared by standard recommended formulas (2). Filter-sterilized sodium nitrite was added to indole test media in some experiments.

The five strains of *E. coli* produced strong indole reactions in media free from nitrate (Table 1). In indole-nitrate medium with or without agar, indole was detected only when the broth was first extracted with xylene before adding the indole reagent. Since 1 mg of sodium nitrate per ml is the usual concentration of substrate added to media to test bacteria for nitrate reduction, various quantities of sodium nitrate were added to indole test broths (Table 2). Sodium nitrate concentrations of 0.25 mg/ml or greater completely inhibited detection of indole when the reagent was layered directly over broth. This occurred in broths incubated for 24 and 48 hr tested by either the Ehrlich or Kovac reagent. Sodium nitrite in concentrations of 0.75 mg/ml or greater partially or completely blocked detection of indole, although xylene was first used to extract the indole. Simulated nitrate reduction in the test media was tested to determine the end point in the colorimetric test for nitrite. This experiment indicated that at least 25% of the sodium nitrate substrate would need to be reduced before reduction could be detected with any certainty by the standard colorimetric test.

These results indicate that an organism's relative rates of tryptophan and nitrate metabolism could greatly affect two important diagnostic tests. An indole-producing organism which rapidly reduced nitrate could form enough nitrite to cause a false-negative indole test even with xylene extraction. The mechanism of nitrite interference requires study. Many laboratories probably use separate media for indole and nitrate reduction, espe-

| Table 1. Detection of indole production by *E. coli* |
|-----------------------------------------------|
| Medium | Ehrlich | Kovac | Nitrate reduction |
|        | Di-rect | Xy-lene | Di-rect | Xy-lene |
| Indole broth | +     | +     | +     | +     | - |
| Indole-nitrate | -     | +     | -     | +     | + |
| Nitrate broth | -     | -     | -     | -     | + |

Plus indicates a positive reaction; negative indicates no reaction.
Table 2. Effect of sodium nitrite on detection of indole production by E. coli

| Sodium nitrite (mg/ml) | Indole reaction | Nitrite reaction |
|-----------------------|----------------|-----------------|
|                       | Direct | Xylene |        |        |
| 1.00                  | -      | -      | +      |        |
| 0.75                  | -      | or W   | +      |        |
| 0.50                  | -      | +      | +W     |        |
| 0.25                  | -      | +      | +W     |        |
| 0.10                  | +      | +      | -      |        |
| 0.00                  | +      | +      | -      |        |

*Plus indicates a positive reaction; negative indicates no reaction; W indicates a weak positive reaction.

*Tryptone (1%) and 2% Trypticase broths.

Additionally with the enteric gram-negative bacilli. The manufacturer of the combined indole nitrite medium does not recommend it for the indole test with enteric bacilli (1). Consideration of separate media for each test should be extended to other groups of bacteria which may form indole but rapidly and strongly reduce nitrate to nitrite. In this instance, xylene extraction would not guarantee a valid indole determination.

This study was supported by funds from the Shriners of North America.

LITERATURE CITED
1. Baltimore Biological Laboratory, Inc. 1968. Manual of products and laboratory procedures, 5th ed., p. 114. Baltimore Biological Laboratory, Inc., Baltimore, Md.
2. Blair, J. E., E. H. Lennette, and J. P. Truant (ed.). 1970. Manual of clinical microbiology. American Society for Microbiology, Bethesda, Md.