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In previous studies it was observed that the thioglycollate, peroxidase, neutral red, and serpentine cord tests were useful for group differentiation of M. tuberculosis, atypical acid-fast bacilli, and saprophytic mycobacteria. The present investigation was undertaken to determine further the differential potential of these procedures, and to ascertain whether the niacin test could also be used to help in the differentiation of these groups of organisms.

Materials and Methods

Fifty-five strains of recently isolated human tubercle bacilli, 45 strains of atypical acid-fast bacilli, and seven strains of saprophytic mycobacteria were selected for study. The majority of the organisms were supplied through the courtesy of other workers, as indicated.

Tubercle Bacilli: Strains A-23 through A-44 were obtained from Mr. Frank Clifton, Veterans Administration Hospital, Alexandria, Louisiana. Strains CDC-1 through CDC-3 were obtained from Dr. George P. Kubica, Communicable Disease Center, Public Health Service, Chamblee, Georgia. Strain H37Rv was obtained from Dr. William Steenken, Jr., Trudeau Laboratory, Saranac Lake, New York.

Atypical Mycobacteria: Strains AP-9, AP-10, and AS-14 through AS-16 were obtained from Mr. Frank Clifton. Strains AP-11 and AS-17 were obtained from Miss Gloria E. Veach, Veterans Administration Center, Wadsworth, Kansas.

Saprophytic Mycobacteria: Strains M. smegmatis-a, M. smegmatis-b, M. phlei-a, and M. butyricum were obtained from Dr. George P. Kubica. Strain M. phlei-b was obtained from Dr. William Steenken, Jr.

The tubercle bacilli and atypical organisms were isolated from various clinical specimens such as sputa, bronchial washings, gastric lavages, resected lung lesions, pleural fluids, lymph nodes, and catheterized ureteral urines. Of the 45 atypical strains, 17 were photochromogens, and 28 were scotochromogens. To avoid the use of patients' names and for convenience in arrangement, most of the strains have been redesignated.

All the tests were performed by the methods described elsewhere using five-week old stock cultures of Proskauer-Beck solid and Löwenstein-Jensen media. Each test was repeated three times. The final readings represent average results.

Results

The data in Table 1 summarize typical findings from each group of organisms. The thioglycollate medium failed to support the growth of

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TABLE 1 — RESULTS OF THIOGLYCOLLATE, PEROXIDASE, NEUTRAL RED, SERPENTINE CORD, AND NIACIN TESTS WITH M. TUBERCULOSIS, ATYPICAL ACID-FAST BACILLI, AND SAPROPHYTIC MYCOBACTERIA

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P = pellicle formation. Numbers = the day growth was first observed.
any of the tubercle bacilli, but did support the growth of 88 per cent of the atypical strains (8 photochromogens and 14 scotochromogens), and 100 per cent of the saprophytes. Growth of the atypical mycobacteria was slow and dysgonic, requiring from 12 to 34 days for initial development of the photochromogens, and from 15 to 41 days for the scotochromogens. The saprophytes grew rapidly and luxuriantly in one day with pellicle formation.

All the tubercle bacilli gave positive results with the peroxidase, neutral red, serpentine cord, and niacin tests, whereas all of the atypical and saprophytic strains, with light exceptions, gave negative results with these tests. As observed previously, slight to moderate degrees of cord formation were exhibited by three of the saprophytic organisms. However, their morphologic patterns were sufficiently characteristic to distinguish them from the type of cording found in the tubercle bacilli.

One of the photochromogens, two of the scotochromogens, and two of the saprophytic strains gave positive niacin tests, but the reactions were slight compared with the reactions of the tubercle bacilli which were either moderate or intense. In repeat niacin tests, in which one to four week old stock cultures of Proskauer-Beck and Löwenstein-Jensen media were used, all of the atypical and saprophytic mycobacteria gave either negative or doubtful reactions, while the tubercle bacilli, in most instances, exhibited lesser degrees of positivity compared with the results obtained with cultures from five to six weeks old. Two strains of tubercle bacilli gave negative niacin reactions with cultures one week old, doubtful to slightly positive results with cultures from two to four weeks old, and moderately to intensely positive reactions with cultures five weeks old. Because of these observations, it was decided to use cultures at least five weeks old before testing the strains for niacin content. That niacin concentration increases with the age of the culture has been pointed out previously by Gilani and Selkon, and Konno. Regardless of the methods employed, it has been observed that human type tubercle bacilli, in most instances, give more marked niacin reactions than other mycobacteria. Accordingly, the niacin test has proved helpful for distinguishing human strains of tubercle bacilli from other mycobacteria.

**SUMMARY**

From these results it can be seen that the peroxidase, neutral red, serpentine cord, and niacin tests are able, in most instances, to distinguish human tubercle bacilli from atypical and saprophytic mycobacteria, but they cannot differentiate the atypical from the saprophytic group of organisms. On the other hand, the thioglycollate test is able, in most instances, to distinguish between all three groups, and when used in conjunction with the other tests, differentiation is further facilitated. All these tests are easy to perform, and have proved useful for group differentiation of *M. tuberculosis*, atypical acid-fast bacilli, and saprophytic mycobacteria.

**RESUMEN**

Según estos resultados puede verse que las reacciones de la peroxidasa, de la cuerda serpentina y de la nacina, son capaces de distinguir en la mayoría de los casos, el bacilo tuberculoso humano de las microbacterias atípicas y saprofitas, pero no pueden diferenciar las atípicas de las saprofitas. Por otra parte, la reacción del tioglicolato es capaz en la mayoría de los casos, de distinguir entre estos tres grupos y cuando se usa en combinación con las otras reacciones, la diferenciación es más fácil aún.

Todas estas reacciones son fáciles de realizar y se han demostrado útiles para la diferenciación del grupo de *M. tuberculosis*, bacilos atípicos ácido-resistentes y microbacterias saprofitas.
RESUMÉ

D'après les résultats rapportés par l'auteur, on peut voir que la peroxydase, le rouge neutre, la structure en cordon sinueux, et les tests à la niacine sont capables, dans la plupart des cas, de distinguer les bacilles tuberculés ils ne peuvent différencier les microbes atypiques des germes saprophytiques. D'un autre côté, le test au thioglycollate est capbael, dans la plupart des cas, de distinguer entre les trois groupes et lorsqu'il est utilisé en association avec les autres tests, la différenciation est alors facilitée. Tous ces tests sont faciles à pratiquer, et se sont montrés utiles pour différencier du groupe des M. tuberculosis les bacilles atypiques acido-résistants, et les mycobactéries saprophytes.

ZUSAMMENFASSUNG


REFERENCES