The Effect of Anticontamination Agents in Media for the Isolation of Mycobacteria*

VICTOR LORIAN, M.D. AND STEPHEN MADDOCK, M.D.

Boston, Massachusetts

The problem of overgrowth of saprophytic organisms troubles every bacteriologic laboratory. This is particularly important in isolating mycobacteria. Although this factor does not present too much difficulty in hospitalized patients, it can be extremely important where sputum specimens are submitted by mail in which a time lapse of 48 hours or more occurs before the specimen can be examined and cultured. Since this laboratory receives a large number of specimens through the mail, an attempt was made to find a proper method which would inhibit contaminating organisms, but not interfere with the growth of M. tuberculosis.

Materials and Methods

Preliminary experiments were performed with antibiotics which were supposed to have no inhibitory effect on the growth of mycobacteria. The antibiotics tested were tetracycline 5 μ/ml.; chloramphenicol 6 μ/ml.; erythromycin 5 μ/ml.; oleandomycin 4 μ/ml.; spiramycin 4 μ/ml.; colimycin 3 μ/ml.; amphotericin B 10 μ/ml. and penicillin 10 μ/ml.

It was found that contrary to expectation, only colycin, spiramycin, and amphotericin B did not inhibit the growth of mycobacteria. Penicillin was found to be inactivated in 7H10 medium (by the oleic acid-albumin complex) to 90 per cent in the first 24 hours and almost completely at 72 hours.

Twenty mycobacteria strains of human type, two bovine, five atypical group I, five III and one M. Fortuitum (collection strains) as well as 1625 sputa received by mail from the Boston Health Department were used in this investigation.

*From the Sanatorium Division, Boston City Hospital, Mattapan, and Boston University School of Medicine, Infectious Disease Section.

Media used: Middlebrook 7H10 agar (Difco). The same media with streptomycin 2.5 μ/ml. or INH 0.2 μ/ml. or PAS 3.5 μ/ml. was used for the susceptibility tests of mycobacteria.

All strains were subcultured in 7H9 broth for seven days, diluted 10⁵ in bovine albumin 0.5 per cent and planted on (a.) 7H10 medium in plastic quadrant plates (I was control, II streptomycin, III PAS and IV INH) and (b.) in the same media to which colimycin** 3 μ/ml., spiramycin† 4 μ/ml., and amphotericin B‡ 10 μ/ml. had been added to prevent contamination.

The 1625 sputa were also planted (according to the CDC method††) in two groups of plates (a.) 7H10 and (b.) the same media to which the three above mentioned anticontamination antibiotics were added.

Air contamination was investigated on both types of media totaling 800 plates. These plates were opened four times. The work was done in a non-sterile room without hoods. All plates were wrapped in plastic bags and incubated for 21 days at 37°C. in an atmosphere of 5 per cent CO₂ and examined for mycobacteria growth and contamination at the 12th and 21st day.

Results

At 21 days, all the mycobacteria collection strains presented an equal growth on 7H10 media with or without the addition of colimycin, spiramycin, and amphotericin B. All the sensitivity tests to INH, streptomycin and PAS were identical on both

**Coly-mycin Diagnostic— Warner-Chilcott
†Rovamycin—Specia, Paris
‡Fungisone—Squibb
††The Communicable Disease Center, Atlanta, Georgia
types of media. The contamination of the 800 plates containing 7H10 media with or without the three anticontamination agents presented: 6 plates (0.7 per cent) contaminated where the three anticontamination drugs were added, and 36 plates (4.5 per cent) where they were not added.

The 1625 sputa presented 193 (10.8 per cent) contaminated plates with 17 (1 per cent) acid-fast positives on 7H10 medium and 146 (8.9 per cent) contaminated plates with 17 (1 per cent) acid-fast positives on the same medium to which colimycin, spiramycin, and amphotericin B was added (Table 1).

**DISCUSSION**

The results show that the addition of colimycin, spiramycin, and amphotericin B has no inhibitory effect on the human, bovine, and atypical mycobacteria tested. The antibiotic susceptibility tests were equal; this shows no additive, synergistic or antagonist effect on streptomycin, PAS or INH.

The positive results of the 1625 sputa were practically equal in both media. The low percentage of positives is due to the fact that these sputa are sent from health units of the city of Boston from non-tuberculous patients just for incidental examination and are generally assumed to be negative.

Although the difference in contamination is relatively small and the final positive results were similar for the sputa, the differences of air-contamination in the experimental work are more significant.

The addition of these anticontamination agents is recommended for experimental work.

**SUMMARY**

A comparative study for mycobacterial growth, sensitivity tests, and contamination was done on collection strains of mycobacteria and for 1625 sputum specimens planted on 7H10 agar and the same medium to which colimycin, spiramycin, and amphotericin B were added. The collection strains showed equal growth and equal sensitivity results on 7H10 media with or without the addition of the three mentioned anticontamination agents. The 7H10 agar containing colimycin, spiramycin, and amphotericin B compared to the 7H10 agar alone showed 10 per cent less contamination on the sputum cultures and 70 per cent less contamination for the experimental work where air contamination was tested.

**Resumen**

El autor presenta un estudio comparativo de la germinación, pruebas de susceptibilidad a las drogas e índice de contaminación en cepas de colección de microorganismos y para 1625 esputos sembrados en agar 7H10 y en el propio medio con adición de colimicina, espiramicina, y amfotericina B. Las cepas de colección mostraron el mismo grado de germinación y los mismos resultados en pruebas de sensibilidad en el medio 7H10 con o sin adición de los tres agentes de anticontaminación mencionados. En los cultivos de esputos se comprobó un 10 por ciento menor de contaminación en el 7H10 con colimicina, espiramicina o amfotericina B, comparado con el 7H10 simple.

Hubo también en 70 por ciento menos de contaminación aérea en los cultivos experimentales en los que se investigó dicha posibilidad.

**Résumé**

Les auteurs rapportent une étude comparative portant sur la culture des bacilles, les tests de sensibilité et la contamination du milieu 7H10 agar et du même milieu additionné de colimycine, de spiramycine et d’amphotéricine B. Ils ont utilisé pour cette comparaison des souches de collection et l’ensemencement de 1625 crachats. Pour les souches de collection on constata un développement semblable, une sensibilité semblable que le milieu ait été ou non additionné par les 3 antibiotiques qui viennent d'être cités. Le milieu 7H10 agar contenant de la colimycine, de la spiramycine et de l’amphotéricine donna 10 pour cent de moins de contamination des cultures de crachats et 70 pour cent de moins de contamination pour l’étude de la présence de germe dans l’atmosphère par comparaison au même milieu utilisé seul.
Es wurde eine vergleichende Untersuchung unternommen, das Wachstum der Mycobakterien betreffend, ferner die Empfindlichkeitsteste und die Verunreinigung und zwar bei einer Familie von verschiedenen Mycobakterien und bei 1625 Sputen, die auf 7H10 agar verimpft waren und ein Medium außerdem, dem Colymycin, Spiramycin und Amphotericin B hinzugefügt war. Die erwähnte Auswahl von Erregern zeigte ein gleichmäßiges Wachstum und gleichmässige Sensibilitäts-Ergebnisse auf dem Nährboden 7H10 mit oder ohne Zusatz der 3 gegen Verunreinigung verwendeten Stoffe. Der 7H10 agar, der Colymycin, Spiramycin und Amphotericin B enthieilt, zeigte im Vergleich zu dem 7H10 agar allein 10 prozent weniger Verunreinigungen bei den Spu-
tumkulturen und 70 prozent weniger Verunreini-
gungen bei den experimentellen Untersuchungen, bei denen eine aerogene Verunreinigung getestet wurde.

For reprints, please write: Dr. Lorian, 249 River Street, Mattapan, Mass.

EXPERIMENTAL BRONCHITIS IN DOGS

The authors describe the bronchoscopic and biopsy findings of experimental bronchitis induced in dogs that had been sensitized parenterally to horse serum, then submitted to local shock through endotracheal inoculation of the same serum.

The bronchoscopic findings ranged from hyperemia after the first shock to edema after two shocks: some time after the third and last endotracheal inoculation, the bronchial mucous membrane lost the characteristics of acute inflammation and revealed aspects of chronic bronchitis, generally hypertrophic of the pseudopolypous type, or even, but more seldom, atrophic. Biopsy findings showed after the second and third local interventions, acute edema, congestion, exudation, frequent eosinophilia and infiltration of the bronchial wall. Some time after the local shocks, the attenuation of the more acute phenomena is accompanied by pavement-like metaplastic transformations of the bronchial mucous membrane, from calciform hyperplasias and muciparous metaplasias, and from sclerosis of the connective tissue to chronic bronchitis. These studies showed the possibility of inducing experimentally in animals bronchial alterations that are most similar to those described in human pathology as inflammatory processes of the bronchial system augmented by allergic mechanisms.


INTRALOBAR SEQUESTRATION IN A CHILD

A case of intralobar sequestration in the left lower lobe of an eight-year-old girl is reported. Chest roentgenogram revealed a round, circumscribed tumor-like shadow in the left lower lung field on her admission with a chief complaint of dry cough. On thoracotomy, a mass was palpated in the left lower lobe and three abnormal arteries were found entering the lesion directly from the lower part of the thoracic aorta. Histologic findings of the tumor were quite similar to the infected bronchogenic cyst.


THYMOLIPOMA

The occurrence of a thymolipoma and Graves' disease in a 27-year-old woman is reported. The clinical features of the 34 reported thymolipomas are analyzed and the tumor's pathogenesis is discussed. The significance of the coincidence of thymolipomas and Graves' disease at present is not understood.