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A SELECTIVE OLEIC ACID ALBUMIN AGAR MEDIUM FOR TUBERCLE BACILLI

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MEDIA in which selective growth is obtained by the incorporation of antibacterial drugs have been developed for the isolation of many bacterial genera (Lowbury and Lilly, 1955; Jones and Morgan, 1958; Jeffries, 1959; Finegold and Sweeney, 1961; Lowbury, Kidson and Lilly, 1964; Thayer and Martin, 1964). The addition to oleic acid albumin agar medium of antibacterial drugs that did not inhibit tubercle bacilli but were inhibitory to a wide range of other organisms was therefore investigated. The main purpose was to allow counting of the numbers of viable tubercle bacilli in sputum without the use of decontamination procedures which kill a proportion of the bacilli present.

MATERIALS AND METHODS

Antibacterial drugs. The antibacterial drugs studied and their spectrum of activity (Garrod and O'Grady, 1971) were as follows: polymyxin B sulphate (Aerosporin, Burroughs Wellcome, London), which inhibits most Gram-negative bacteria, including *Pseudomonas aeruginosa* but not *Proteus* species at concentrations of 5–10 units per ml; carbenicillin (Pyopen, Beecham, Brentford), which inhibits Gram-positive and Gram-negative organisms including *Proteus* species and *Ps. aeruginosa*, but is susceptible to some bacterial penicillinases; nalidixic acid (Negram, Winthrop, Newcastle-on-Tyne), which inhibits most Gram-negative bacilli other than *Pseudomonas* species and has been used by Gruft (1965) at a concentration of 35 µg per ml in Löwenstein-Jensen medium to reduce the contamination rate in the culture of mycobacteria; bacitracin (Burroughs Wellcome, London), which inhibits Gram-positive organisms; cloxacillin (Orbenin, Beecham, London), which inhibits Gram-positive organisms and is resistant to staphylococcal penicillinase; trimethoprim (Burroughs Wellcome, London), which inhibits Gram-positive organisms and most Gram-negative organisms other than *Pseudomonas* species at concentrations usually under 5 µg per ml (stock solutions were prepared from trimethoprim lactate: 1.3 g = 1.0 g trimethoprim); nystatin (Mycostatin, Squibb,

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New York), which inhibits most fungi including *Candida* species; and amphotericin B (Fungizone, Squibb, New York), which inhibits fungi including *Candida* species at a concentration of *c.* 5 µg per ml, and is more heat-stable than nystatin. The tissue culture grade was used except where otherwise stated.

None of these antibacterial drugs in the highest concentrations tested is reported as inhibiting the growth of *Mycobacterium tuberculosis*.

Mycobacterial strains. The origins of the strains of mycobacteria used are as follows: from Dr J. Marks, Cardiff: *Myco. scrofulaceum*, strain 26, *Myco. gordonae*, strain 10267, *Myco. avium*, type I, strain 15769, *Myco. gastri*, strain 25319, *Myco. ulcerans*, strain 411; from Dr J. Stanford, Bland-Sutton Institute: *Myco. ulcerans*, strain 411; from our own laboratory collection: *Myco. tuberculosis*, strains H37 Rv and R1 Rv; the remaining strains of *Myco. tuberculosis* were isolated from British or Hong Kong patients; *Myco. bovis*, strain BCG (Glaxo), four strains from cattle and one strain from a British patient; *Myco. kansasii* and *Myco. intracellulare* (Davis serotype) from British patients; *Myco. xenopi*, NCTC no. 10042; *Myco. rhodochrous*, NCTC no. 10210; *Myco. phlei*, *Myco. smegmatis* and *Myco. fortuitum* were our own laboratory strains.

Inhibition of organisms other than Myco. tuberculosis. Specimens of sputum were obtained from patients with non-tuberculous chest conditions, frequently chronic bronchitis. The sputum was digested with pancreatin (Oxoid Ltd, London) and one loopful spread on 5 per cent. horse blood nutrient agar plates containing no drugs or the drugs under study. The plates were incubated at 37°C and examined at 1 and 7 days after inoculation.

Media. Cultures of mycobacteria were grown in 7H9 Tween-albumin liquid medium (Bacto, Difco Laboratories, Detroit) or on Löwenstein-Jensen (LJ) medium slopes. From the fully grown cultures in 7H9 medium or from bacterial suspensions, obtained by grinding growth from slopes in 1 per cent. aqueous gelatin in a polytetrafluorethylene (PTFE) grinder, serial ten-fold dilutions were made in sterile distilled water and inoculated on to segments of plates containing 7H10 oleic acid albumin medium (Bacto, including 0.0025 per cent. w/v malachite green) with or without the drugs under study.

Growth of Myco. tuberculosis from sputum. The effect of drugs on the early growth rate of *Myco. tuberculosis* was studied in slide culture of sputum obtained from patients in Hong Kong before the start of chemotherapy by the method of Stritch and Dickinson (1958). The growth medium was equal parts of citrated human bank blood and distilled water and microcolonies were examined by fluorescence microscopy.

Viable counts were done on sputum obtained before the start of chemotherapy from British patients. The specimens were liquefied by shaking with an equal volume of dithiothreitol solution (Sputolysin, Calbiochem, California) and leaving at room temperature for 15 min. After centrifuging for 15 min. at 3500 r.p.m., the deposit was suspended in sterile water and divided into two equal portions. One portion was treated with an equal volume of 1M NaOH for 15 min. and then neutralised with 1M HCl. The remaining portion was made up to the same volume as the first portion with sterile water. From each portion, five serial ten-fold dilutions were made in sterile distilled water. From the neat suspension and each dilution, 0.1 ml was added to a segment of one-third of each of two 7H10 plates with or without the drugs under study and one loopful (0.0109 ml) from the NaOH-treated dilution series was placed on each of two slopes of LJ medium.

Growth of Myco. tuberculosis from guinea-pig spleens. In an experiment similar to that described by Dickinson, Ellard and Mitchison (1968), spleens were removed with aseptic precautions from infected animals that had been either treated with isoniazid or had received no treatment. Portions of the spleens were homogenised with water in a PTFE grinder and serial dilutions were inoculated into two bottles of 7H10 medium, one containing the selective drugs, for viable counting by the method of Selkon and Mitchison (1957).

Cultural conditions. Plates were incubated at 37°C in polyethylene bags containing an additional plate seeded with *Myco. phlei* (Cohn and Middlebrook, 1963) and colonies were counted at 2–3 wk for rapidly growing mycobacteria and at 4 wk for slowly growing species. Counts are expressed as viable units (v.u.) per ml of neat suspension.

RESULTS

Development of the selective medium

The suppression of organisms other than mycobacteria. This was examined on 248 sputum specimens in eight experiments. In each experiment the sputum concentrate was seeded on two to eight plates containing different combinations of antibacterial drugs. Although each plate usually contained three or

TABLE I
Suppression of sputum flora by various drugs

Flora to be suppressed	Antibacterial drugs*	Sputum specimens		Organisms growing
		Number examined	Percentage with growth	
Gram-negative bacilli	POLY 25-200	8	43	<i>Escherichia coli</i> , <i>Proteus</i> <i>Escherichia coli</i> , <i>Proteus</i> <i>Escherichia coli</i> , <i>Pseudomonas</i>
	CARB 100-200	17	6	
	POLY 50-100+ CARB 100-200	157	1	
	POLY 50+NAL 30	17	0	
Gram-positive bacteria	BAC 25-100	123	0	<i>Staphylococcus</i> , <i>Streptococcus</i> , diphtheroid <i>Staphylococcus</i> , <i>Streptococcus</i> <i>Staphylococcus</i> <i>Streptococcus pneumoniae</i>
	CARB 100-200	50	10	
	CARB 50+CLOX 5	50	8	
	CARB 100+CLOX 10	50	2	
Fungi	CARB 100+TRIM 2	40	2	
	NYST 200-500	50	4	<i>Candida</i> <i>Candida</i>
	NYST 1000	90	3	

* POLY = Polymyxin B, CARB = carbenicillin, NAL = nalidixic acid, BAC = bacitracin, CLOX = cloxacillin, TRIM = trimethoprim, NYST = nystatin. Concentrations in µg per ml (or units per ml for polymyxin B, bacitracin and nystatin).

four drugs with the aim of suppressing all growth, it is easier to appreciate the stages in developing the medium by separate consideration of the effects on Gram-negative and Gram-positive bacterial growth and on fungal growth (table I).

Gram-negative bacteria: neither polymyxin nor carbenicillin alone was satisfactory in preventing the growth of Gram-negative rods, but combinations of polymyxin and carbenicillin, or polymyxin and nalidixic acid, allowed only the very occasional growth of a few colonies, often sensitive to polymyxin on subculture. Carbenicillin was preferred to nalidixic acid because of its wider activity on Gram-positive as well as on Gram-negative flora.

Gram-positive bacteria: initial experiments indicated that bacitracin was highly effective in suppressing all Gram-positive bacterial growth. However, in viable counts on nine strains of *Myco. tuberculosis*, colonies appeared much

more slowly on plates containing bacitracin. In view of this result, and because Rieber, Imaeda and Cesari (1969) found bacitracin to be bactericidal to tubercle bacilli in medium with a low citrate concentration, a search was made for other drugs to suppress the Gram-positive flora. Carbenicillin alone often allowed growth of organisms that were still sensitive to it on subculture, suggesting that it might have been inactivated by penicillinase-producing organisms in the flora. The addition to carbenicillin of cloxacillin 10 μg per ml, but not 5 μg per ml, or

TABLE II
Viable counts of four strains of Myco. tuberculosis of medium containing various drugs

Drugs incorporated in 7H10 medium*	Mean viable count (log ₁₀ v.u. per ml)
None	7.81
POLY 100, 200, 500	7.90–8.03
CARB 100, 200	7.92–8.00
CARB 500	7.69†
TRIM 2, 10, 50	7.92–7.94
CLOX 10	7.61
CLOX 50	7.72
AMPH 5, 50	7.72–7.88
NYST 1000	8.00
POLY 100+CARB 100+TRIM 2+AMPH 5	7.93
POLY 200+CARB 200+TRIM 10+AMPH 50	7.70
POLY 200+CARB 200+CLOX 10+AMPH 50	7.65
POLY 100+CARB 100+TRIM 2+NYST 1000	7.70
POLY 200+CARB 200+TRIM 10+NYST 1000	7.62
POLY 200+CARB 200+CLOX 50+AMPH 5	...‡

* POLY = Polymyxin B, CARB = carbenicillin, TRIM = trimethoprim, CLOX = cloxacillin, AMPH = amphotericin B, NYST = nystatin. Concentrations in μg per ml (or units per ml for polymyxin B and nystatin).

† Values (underlined) of the mean viable count of less than log₁₀ 7.70 v.u. per ml are likely to indicate a true reduction from the viable count on drug-free medium.

‡ One of four strains completely inhibited.

trimethoprim 2 μg per ml, reduced the contamination rate to about 2 per cent., but the growth of scanty colonies of *Staphylococcus albus* from one specimen and *Streptococcus pneumoniae* from another suggested that higher concentrations of these drugs should be used.

Fungi: nystatin, even at 1000 μg per ml, suppressed all fungal growth at 24 hr, but colonies of *Candida albicans* sometimes appeared on plates incubated for 7 days. It was therefore replaced by amphotericin B, which is more stable at 37°C.

Inhibition of Myco. tuberculosis by drugs. Viable counts were made on four strains of *Myco. tuberculosis*, comprising three strains recently isolated from sputum and strain H37 Rv, on plates with various combinations of drugs (table II). A count of less than log₁₀ 7.70 v.u. per ml probably indicated some inhibition. Carbenicillin, at a concentration of 500 μg per ml but not at 100–200 μg per ml, appeared slightly inhibitory. Cloxacillin 10–50 μg per ml also appeared inhibitory when used alone or in combination with other drugs.

There was some suggestion that counts on medium containing polymyxin 200 units per ml, carbenicillin 200 μg per ml and trimethoprim 10 μg per ml were slightly lower than those on medium containing lesser concentrations of each drug. Pure cultures were also attached to silicone-treated slides and incubated in medium containing the same drug combinations as are set out in table I. Medium containing carbenicillin 500 μg per ml slowed the growth of microcolonies, but the growth rate was not affected by any other drug combination.

The selective medium

The drugs finally chosen for the selective medium were polymyxin B 200 units per ml, carbenicillin 100 μg per ml, amphotericin B 10 μg per ml and trimethoprim 10 or 20 μg per ml.

Inhibition of organisms other than tubercle bacilli. Of 175 sputum specimens inoculated on selective medium containing 10 μg per ml trimethoprim, the cultures of six (3 per cent.) were contaminated, all with less than ten colonies. *Strep. pneumoniae* was obtained from four specimens and *Staph. aureus* from two specimens; four of these organisms were sensitive to carbenicillin. In the later part of the experiment, parallel plates containing trimethoprim 20 μg per ml were included, and two (2 per cent.) of the 109 specimens inoculated on this medium were contaminated, one with two colonies of *Staph. aureus* and the other with one colony of a coliform bacillus. *Strep. pneumoniae* grew from two specimens on medium containing trimethoprim 10 μg per ml but not on medium containing 20 μg per ml.

Isolation of Myco. tuberculosis from sputum. Early growth rate: triplicate slide cultures were prepared from 51 specimens of sputum that contained acid-fast bacilli on direct examination. One set was decontaminated with HCl ("acid"); the second set was placed, immediately after the sputum had dried on the slides, into medium containing the same drug concentrations as in the selective medium, including trimethoprim 10 μg per ml ("drug"), and a third set was treated with acid and then placed in drug-containing medium ("acid+drug"). The relative sizes of the microcolonies in the three sets of slides for each specimen were compared after 3 and 7 days' incubation (table III); the slides were examined blind in a random order. At 3 days, microcolonies were larger on drug than on acid slides and were of similar size on acid and on acid+drug slides. At 7 days, the drugs were inhibitory, evident from larger colonies on acid than on acid+drug slides, but probably less so than after acid treatment.

Colony counts: specimens of sputum were divided into two portions. From the portion treated with NaOH, viable counts were set up on slopes of LJ medium without drugs (NaOH-LJ), on 7H10 medium plates without drugs (NaOH-7H10), and on 7H10 medium with drugs including trimethoprim 20 μg per ml (NaOH-7H10+drug). From the portion not treated with NaOH, a similar viable count was set up on 7H10 medium with drugs (7H10+drug). Of the total of 59 specimens examined, four specimens have been excluded from analysis since they yielded no growth in one or more of the counts and up to four colonies in the remaining counts. The remaining 55 specimens were

obtained from 29 patients. Correction was made for six further missing counts by a covariance method (Cochran, 1957).

TABLE III

Size of microcolonies in slide cultures (1) treated with acid ("acid"), (2) incubated in medium containing drugs ("drug") or (3) both ("acid+drug")

Relative size of microcolonies	Number of sets of slides showing the stated relation between colony sizes (and values of P) after incubation for	
	3 days	7 days
Drug > acid Drug = acid Drug < acid	36 } (<0.001) 9 6 }	25 } (>0.1) 11 15 }
Acid > acid + drug Acid = acid + drug Acid < acid + drug	16 } (>0.3) 23 12 }	36 } (<0.001) 5 10 }
Drug > acid + drug Drug = acid + drug Drug < acid + drug	40 } (<0.001) 7 4 }	40 } (<0.001) 5 6 }

TABLE IV

Viable counts of Myco. tuberculosis and contamination rates in 55 specimens of sputum

Method of treatment and culture*	Geometric mean count of tubercle bacilli (v.u. per ml)	Slopes or plates		
		Number examined	Number (and percentage) showing	
			major contamination*	minor contamination*
NaOH-LJ	5.23×10^5	648	2 (0.3)	0 (0.0)
NaOH-7H10	1.42×10^6	215	22 (10.2)	9 (4.2)
NaOH-7H10 + drug	1.28×10^6	220	1 (0.5)	1 (0.5)
7H10 + drug	3.40×10^6	219	0 (0.0)	1 (0.5)

* See text.

The results are summarised in table IV. The geometric mean counts of tubercle bacilli were 5.23×10^5 v.u. per ml in the NaOH-LJ series and 1.42×10^6 v.u. per ml in the NaOH-7H10 series, a significant difference ($P < 0.001$). The LJ slopes were inoculated with a standard loop and the 7H10 medium plates with a pipette. The content of the loop, when used during the counts, could

have varied from the content estimated in a separate experiment and used for calculating the counts. Nevertheless, 7H10 medium probably yielded higher counts than LJ medium. The effect of the drugs on the tubercle bacilli that survived treatment with NaOH can be estimated by comparing the mean counts in the NaOH-7H10 series with the NaOH-7H10+drug series. The drugs reduced the counts on average by 10 per cent. (range: 40 per cent. increase to 79 per cent. decrease due to drugs) from 1.42×10^6 v.u. per ml to 1.28×10^6 v.u. per ml. This difference just attains statistical significance ($P \simeq 0.02$), but the probability of such a result occurring by chance is greater as there were several non-orthogonal comparisons on the same body of data. The effect of NaOH treatment can be estimated by comparing the mean count of 3.40×10^6 v.u. per ml on the 7H10+drug series with the mean count of 1.28×10^6 v.u. per ml in the NaOH-7H10+drug series, a significant difference ($P < 0.001$). Thus, NaOH treatment reduced the counts on average by 62 per cent. (range: 13 per cent. increase to 95 per cent. decrease due to NaOH). Finally, the counts in the NaOH-7H10 series were on average 58 per cent. lower (range: 21 per cent. increase to 98 per cent. decrease due to NaOH) than those in the 7H10+drug series ($P < 0.001$), indicating that far fewer organisms were prevented from growing by the selective medium than by treatment with NaOH.

The three sets of differences were further examined by analysis of variance. For each difference, the variation from patient to patient was no greater than the variation between replicate specimens from the same patient. Thus the effects of the drugs or of treatment with NaOH were similar in the strains from the 29 patients.

Contamination was considered major if it prevented the reading of any of the counts on a slope or plate, and minor otherwise. The contamination rate was considerably higher on 7H10 plates without drugs than on other media. Major contamination was mainly due to fungal growth. The few colonies responsible for minor contamination on the selective 7H10 medium in these and other experiments were usually actinomycetales or occasionally pseudomonas or aeromonas species.

Growth of Myco. tuberculosis from guinea-pig spleens. Viable counts were carried out on spleens from guinea-pigs infected with *Myco. tuberculosis*, strain 175. There were 100 spleens that yielded more than six colonies. The mean viable counts on these spleens were 5.46×10^4 v.u. per ml in the normal counting medium and 5.32×10^4 v.u. per ml in the medium containing the selective drugs, including trimethoprim 10 μ g per ml; the difference is not statistically significant. Of the 39 spleens that yielded six colonies or less, ten were positive and 19 were negative in both types of medium. Colonies were obtained from six spleens in normal but not in selective medium, and from four spleens in selective but not in normal medium.

Growth of various species of mycobacteria. Viable counts of various species of mycobacteria were set up on plates of 7H10 medium with and without the selective drugs (trimethoprim 20 μ g per ml). As is evident from table V, the selective drugs had little effect on the counts on ten strains of *Myco. tuberculosis*, including three resistant respectively to isoniazid only, to streptomycin only

and to *p*-aminosalicylic acid only, or on six strains of *Myco. bovis*. Strains of *Myco. kansasii*, *Myco. avium* and *Myco. fortuitum* were also little affected, though one of the three strains of *Myco. kansasii* yielded small colonies on the selective medium. All other strains of mycobacteria were partially or completely inhibited by the selective drugs.

Antifungal cover. Assay of the content of amphotericin in the selective medium during incubation at 37°C showed that the period of antifungal cover could be increased from about 14 days to about 28 days by increasing the initial

TABLE V

Growth of various species of mycobacteria on 7H10 medium with and without drugs

Species	Number of strains	Geometric mean viable count (v.u. per ml) on 7H10 medium		
		without drugs	with drugs	Difference (per cent.)
<i>Myco. tuberculosis</i> (drug-sensitive)	7	4.32×10^6	3.42×10^6	-21
<i>Myco. tuberculosis</i> (drug-resistant)	3	1.22×10^7	1.32×10^7	8.2
<i>Myco. bovis</i>	6	2.67×10^7	2.16×10^7	-19
<i>Myco. kansasii</i>	3	1.06×10^7	7.90×10^6	-25
<i>Myco. scrofulaceum</i>	1	3.35×10^9	2.40×10^6	-99.93
<i>Myco. gordonae</i>	1	1.90×10^8	NG	...
<i>Myco. avium</i>	1	5.60×10^8	3.60×10^8	-36
<i>Myco. intracellulare</i>	1	1.75×10^9	1.90×10^7	-98.91
<i>Myco. xenopi</i>	1	1.85×10^9	1.50×10^5	-99.99
<i>Myco. gastri</i>	1	1.80×10^8	3.30×10^7	-82
<i>Myco. fortuitum</i>	1	9.00×10^8	9.50×10^8	5.6
<i>Myco. phlei</i>	1	1.15×10^8	3.15×10^5	-99.73
<i>Myco. smegmatis</i>	1	1.54×10^9	PI	...
<i>Myco. rhodochrous</i>	1	4.70×10^8	NG	...
<i>Myco. ulcerans</i> *	1	2.50×10^7	1.25×10^4	-99.95

NG = No growth on selective medium.

PI = Partial inhibition on selective medium.

* Incubated at 32°C.

concentration from 10 µg per ml to 50 µg per ml. The geometric means of 41 viable counts of *Myco. tuberculosis* from sputum were similar on selective medium containing amphotericin 10 µg per ml (5.02×10^5 v.u. per ml) or 50 µg per ml (5.32×10^5 v.u. per ml). The means of 28 counts on medium with tissue grade and with intravenous grade amphotericin were also similar. Plates containing amphotericin 100 µg per ml partially inhibited *Myco. tuberculosis*, due to the deoxycholate present as a dissolving agent in the ampoules of the drug.

Löwenstein-Jensen medium

Attempts to incorporate the same drugs in LJ medium before inspissation were unsuccessful, because polymyxin B was not detectable by microbiological assay (Garrod and O'Grady, 1971, p. 482) in the water of condensation after

inspissation. Replacement of the polymyxin with nalidixic acid 30 μg per ml was not satisfactory, as about one-third of the cultures inoculated directly with dithiothreitol-treated sputum were contaminated, mainly with species of *Pseudomonas*. When 2 ml of a solution of polymyxin B 3000 units per ml was added to each slope, left in contact for 24 hr, and then removed with a pipette, the final concentration in the water of condensation was about 200 units per ml. Limited experience with selective medium slopes to which polymyxin was added in this way suggested that they were much less often contaminated than the medium containing nalidixic acid.

DISCUSSION

The selective oleic acid albumin agar medium described proved very effective in allowing growth of tubercle bacilli and preventing the growth of all other organisms found in sputum. The assessment by the slide culture method suggested that the early multiplication of *Myco. tuberculosis* was slightly slowed by the selective drugs, but to a less extent than by treatment with acid. Assessments by viable counts on sputum or on the spleen of tuberculous guinea-pigs indicated that the selective drugs reduced the counts by, at most, 10 per cent. In contrast, minimal decontamination with sodium hydroxide reduced the counts by about 62 per cent. Thus, the bacillary content of sputum can be more accurately estimated by plating directly on to the selective medium than by conventional decontamination with sodium hydroxide.

The plates used in our studies were poured in a laminar-flow cabinet, which minimised the risk of airborne contamination. Under these circumstances, the contamination rate on the selective medium was very low. However, under less satisfactory conditions of medium preparation, as in East Africa (E. Edwards, personal communication), plates may become contaminated with fungi. An increase of the amphotericin B concentration to 50 μg per ml provided anti-fungal cover for almost the entire incubation period with no reduction in the viable count of *Myco. tuberculosis*.

Attempts to use the same drugs in Löwenstein-Jensen medium were not successful, mainly because polymyxin B was completely inactivated during inspissation of the medium. It is possible to add this drug to the medium after inspissation, but such a procedure is tedious to perform.

The use of the selective medium for the routine diagnosis of tuberculosis should be of value in laboratories that already use oleic acid albumin agar medium for this purpose. However, many laboratories prefer the use of LJ medium for three reasons: the high contamination rate of plate cultures, the increased hazard of handling plates rather than the screw-capped bottles in which LJ medium is often dispensed, and the high cost of the albumin in 7H10 medium. Although the high contamination rate would be greatly reduced by the use of the selective medium, the disadvantages of additional risk to laboratory workers and high cost still remain. Safety might be improved by dispensing the medium in screw-capped bottles, but these would probably require additional CO_2 , which is considered essential for diagnostic culture on 7H10 medium (Middlebrook and Cohn, 1958). If a liquid medium, such as those of

Kirchner or Sula, is already used for diagnostic isolation of mycobacteria, addition of the selective drugs would reduce contamination, a major disadvantage of this type of medium, and might also increase the isolation rate by allowing culture without any decontamination of the specimen.

Among the species of mycobacteria other than *Myco. tuberculosis* or *Myco. bovis* that are pathogenic in man, the selective medium allowed the growth of *Myco. kansasii*, *Myco. avium* and *Myco. fortuitum*, but inhibited *Myco. intracellulare*, *Myco. xenopi* and *Myco. ulcerans*. Little is known about the bactericidal activity of conventional decontamination procedures against these organisms, but it would be unwise to replace conventional decontamination procedures and media entirely by the selective medium without further investigation.

SUMMARY

A selective medium for *Mycobacterium tuberculosis* and *Myco. bovis* has been developed containing polymyxin B 200 units per ml, carbenicillin 100 μ g per ml, amphotericin B 10 μ g per ml and trimethoprim 10 or 20 μ g per ml in 7H10 oleic acid albumin agar. When the centrifuged deposit from sputum, liquefied by treatment with dithiothreitol, was plated directly on to the selective medium, less than 1 per cent. of plates were contaminated and the viable counts were about 240 per cent. higher than parallel counts after treatment of the deposit with NaOH and plating on 7H10 medium without drugs. A comparison of results on medium with and without the selective drugs showed that the drugs slowed the early growth of *Myco. tuberculosis* in slide culture and reduced viable counts by, at most, 10 per cent. Improved anti-fungal cover could be obtained with no reduction in viable counts by increasing the concentration of amphotericin B to 50 μ g per ml.

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