sence of detoxin D, approaching a maximum rate of zero order with respect to the concentration of blasticidin S (figure 2, b). In the presence of 10 mM N-ethylmaleimide, a SH-blocking agent of proteins, the amount of blasticidin S taken up within the first 10 min was reduced in the presence and in the absence of detoxin D to 9–9.5% of the control value, which was lower than in the presence of detoxin D only. Preincubation of the cells with detoxin D or poisons of energy metabolism led to a strong reduction of the amount taken up; the reduced uptake was 27.5% with the addition of 10 µg/ml detoxin D, 31.8% with 30 mM sodium azide, 36.3% with 20 mM 2-thenoyltrifluoroacetone and 35.9% with 20 mM 2.4 dinitrophenol (data not shown). The effect of 30 mM sodium azide on the time course of blasticidin S uptake was the same as that obtained with 10 µg/ml detoxin D (figure 1). These results suggest that blasticidin S is taken up by *B. cereus* both by a carrier-mediated passive transport, namely facilitated diffusion, and active transport, and that detoxin D interferes only with the latter.

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2 This is Part VII of 'Studies on Detoxin Complex, the Selective Antagonists of Blasticidin S'. For Part VI, see the preceding report.

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Differential medium for the isolation and identification of *Mycobacterium fortuitum* complex

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Summary. A single medium efficiently selects for and identifies *Mycobacterium fortuitum* complex on the basis of bile tolerance and arylsulfatase activity.

Mycobacterium fortuitum and *M. chelonei* are 2 potentially pathogenic species among the rapidly growing mycobacteria[1]. Because of the similarity of the type of infection caused by them, the 2 species are usually combined under the term *M. fortuitum* complex[2]. Growth on MacConkey agar and arylsulfatase activity are the key tests in identification of *M. fortuitum* complex[3,4].

We have developed a medium which selects for and permits the identification of bile salts tolerant, rapidly growing mycobacteria on the basis of arylsulfatase activity. The medium can be regarded as a modified MacConkey agar in which peptone and bile salts were replaced by tryptose and sodium desoxycholate, respectively. Lactose, neutral red and crystal violet were omitted, and arylsulfatase substrate was incorporated into the medium.

The tryptose-arylsulfate-desoxycholate (TAD) medium was prepared by dissolving 1 g tryptose (Difco Laboratories, Detroit, Mich.), 0.3 g beef extract (Difco), 0.05 g sodium desoxycholate (Difco), 0.5 g NaCl and 1.5 g agar (Difco) in 100 ml distilled water and autoclaving for 15 min at 121 °C. After cooling to 50 °C, 1.5 ml of 0.08 M sterile arylsulfatase substrate solution were added and plates were poured. The complete medium was transparent and colorless. In TAB medium, bile salts No. 3 (Difco) 0.15 g/100 ml medium, were used instead of sodium desoxycholate.

The 0.08 M solution of arylsulfatase substrate was prepared by dissolving 2.6 g phenolphthalein disulfate tripotassium salt (Eastman Kodak, Rochester, NY) in 50 ml distilled water and sterilizing the solution by membrane filtration (Millipore Corp., Bedford, Mass.)[5].

Altogether 45 strains of rapidly growing mycobacteria were used. The various strains were kindly provided by Dr H. Gruft of the New York State Department of Health, Dr I. Weitzman of the New York City Department of Health and Drs McClatchy and Tsang of the National Jewish Hospital, Denver, Colorado. The following type and neo-

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Species	No. of strains	TAD agar Growth	TAD agar Arylsulfatase	MacConkey agar Growth	MacConkey agar Arylsulfatase*
Mycobacterium fortuitum	18	+	+	+	+
M. chelonei	17	+	+	+	+
M. smegmatis	4	–	–	–	–
M. phlei	3	–	–	–	–
M. vaccae	3	–	–	–	–

* Arylsulfatase test was performed by the CDC method which utilizes liquid substrate medium[5].

type strains were obtained from the American Type Culture Collection: *M. fortuitum* ATCC 6841, *M. chelonei* ATCC 19977, *M. smegmatis* ATCC 19420, *M. phlei* ATCC 11758 and *M. vaccae* ATCC 15483. Rapidly growing mycobacteria isolated in the microbiology laboratory of the Methodist Hospital as well as strains from the Center for Disease Control and College of American Pathologists survey isolates were also tested.

All cultures had been grown for 5-7 days at 36 °C in 5% CO_2 atmosphere and were inspected for growth. Both the TAD and TAB media supported growth of all strains of *M. fortuitum* and *M. chelonei* capable of growing on MacConkey agar, while inhibiting the growth of all other rapidly growing mycobacteria (table). The growth of *M. fortuitum* and *M. chelonei* on TAD media was more luxuriant than on TAB media.

Detection of arylsulfatase activity was performed by flooding the plate with 2.0 M solution of sodium carbonate. Colonies with arylsulfatase activity become pink, indicating the release of phenolphthalein from the substrate. All strains of *M. fortuitum* and *M. chelonei* were positive for arylsulfatase.

Various representatives of bile tolerant Enterobacteriaceae, including Salmonellae, capable of growing on MacConkey agar also grew on TAB and TAD agar plates, but their colonies became visible after overnight incubation and they were invariably arylsulfatase negative.

The tryptose-arylsulfate-desoxycholate and tryptose-arylsulfate-bile salts media can be used for the isolation and simultaneous identification of *M. fortuitum* complex from processed sputum specimens. The use of TAD or TAB media for this purpose is economical because it obviates the need for subculturing and reduces the total time required for the identification of pathogenic, rapidly growing mycobacteria.

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Préparation des acides desoxyribonucléiques de foie de rat par filtration sur Ultrogel A 2

Preparation of DNA from rat liver by gel filtration on Ultrogel A 2

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Summary. Rat liver DNA was prepared by 2 successive filtrations on Ultrogel A 2 (LKB, Sweden). The method gives a high yield (2.33 mg/g of fresh liver) of pure ($A_{260}/A_{230} = 2.44$, $A_{260}/A_{280} = 1.88$) HMW-DNA (s = 26.2 S).

Matériel et méthodes. Le foie de rat est conservé à −25 °C. L'organe, encore congelé, est coupé en tranches minces. 2 g de tissu sont homogénéisés à 20 °C à l'aide d'un homogénéiseur Polytron dans 10 ml de LiCl 2 M, tris 0,01 M, pH 7,5. L'homogénat est laissé 30 min à 2 °C, et agité doucement à la main 2 ou 3 fois. Il est ensuite centrifugé 30 min à 25000 × g à 0 °C. Le surnageant est soumis à 2 filtrations successives sur Ultrogel A 2. Le haut de chacune des colonnes C1 et C2 est muni d'un entonnoir servant de réservoir et restant à demeure. Le débit des colonnes, assuré par 2 pompes péristaltiques P1 et P2, est de 3 ml/$cm^2 \cdot h$. La filtration est descendante et s'effectue à 20 °C.

L'Ultrogel A 2 de la colonne C1 (2,5 × 24 cm) est équilibré avec NaCl 2 M, EDTA 2,5 mM, pH 7,5. Nous déposons 5 ml de surnageant directement à la surface du gel. Lorsque l'échantillon a entièrement pénétré dans le gel, nous remplissons l'entonnoir de C1 de tampon NaCl 0,1 M, SDS 0,1%, tris 0,01 M, pH 7,5. L'absorbance à 260 nm de l'éluat est enregistrée. Tant qu'aucun composé absorbant à cette longueur d'onde n'est détecté, l'éluat est éliminé.

L'Ultrogel A 2 de la colonne C2 (2,5 × 48 cm) est équilibré avec NaCl 0,1 M, SDS 0,1%, tris 0,01 M, pH 7,5. Après avoir déposé le surnageant sur la colonne C1, on dépose 30 mg de protéase VI (Sigma) dans 30 ml de NaCl 0,2 M, tris 0,05 M, SDS 0,2%, pH 7,5 à la surface du gel de la colonne C2. Lorsque la protéase a entièrement pénétré dans le gel, la pompe P2 est arrêtée.

Au moment où un pic commence à apparaître dans l'éluat de C1, on fait couler ce dernier dans l'entonnoir de C2, et l'on règle P2 de façon à ce que son débit soit égal à celui de P1. On désolidarise de nouveau les 2 colonnes après le passage du premier pic de C1, et l'on remplit l'entonnoir de C2 avec son tampon d'équilibration. L'absorbance des fractions recueillies à la sortie de C2 est lue au spectrophotomètre à 230, 260 et 280 nm.

Pour une utilisation ultérieure, le gel de C1 est rééquilibré avec NaCl 2 M, EDTA 2,5 mM, tris 0,01 M, pH 7,5, après avoir fait passer au moins 100 ml de NaCl 0,1 M, SDS 0,1%, tris 0,01 M, pH 7,5, déposé après le surnageant, puis au moins 100 ml d'eau distillée. Les 2 colonnes peuvent servir un grand nombre de fois sans qu'il soit besoin de recouler le gel.

Résultats et discussion. La figure donne un exemple de diagramme des absorbances des fractions du premier pic de C2. Le spectre UV de ces fractions est caractéristique des ADN. En particulier les rapports (moyennes de 16 valeurs ± écart-type) $A_{260}/A_{230} = 2{,}44 \pm 0{,}12$ et $A_{260}/A_{280} = 1{,}88 \pm 0{,}10$ montrent que la contamination par les protéines est faible (par les méthodes de la littérature, A_{260}/A_{230} varie de 2,23[1] à 2,32[2] et A_{260}/A_{280} de 1,80[1] à 1,86[2,3]). Le dosage colorimétrique des protéines[4] est négatif. Dans nos conditions d'expérience, cela correspond à une contamination protéique inférieure à 3%. La réaction colorimétrique à l'orcinol[5] et une chromatographie sur colonne MAK[6] mon-