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Effect of Delays in Processing on the Survival of *Mycobacterium avium-M. intracellulare* in the Isolator Blood Culture System

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Concentrations of *Mycobacterium avium-M. intracellulare* ranging from 10^{-1} to 10^3 CFU/ml were added to blood, placed in Isolator tubes, and held at room temperature for intervals ranging from 4 h to 56 days before being processed (centrifugation and culture on Middlebrook 7H10 agar). At all concentrations tested, *M. avium-M. intracellulare* was recovered after hold times ranging from 4 h to 7 days; the number of final CFU actually increased progressively for hold times of 8 h or more. Hold times of up to 7 days did not increase the time from processing to the first appearance of visible colonies. At an inoculum of 10^2 CFU/ml, *M. avium-M. intracellulare* was recovered from Isolator tubes processed 56 days after inoculation. Two Isolator blood cultures were drawn from a patient with AIDS; *M. avium-M. intracellulare* was recovered from the sample processed immediately and from the sample processed after a hold time of 7 days. Since *M. avium-M. intracellulare* survives for prolonged periods in Isolator tubes, blood cultures may be collected in outpatient settings or in hospitals without mycobacterial culture facilities and shipped to reference laboratories for processing without loss of viability.

Disseminated infection with organisms of the *Mycobacterium avium* complex (MAC) occurs in 30 to 50% of patients with AIDS in the United States, usually among patients with CD4 counts of less than 100/mm^3 (7, 8). Disseminated infection with MAC may produce disabling symptoms, including fever, malaise, weight loss, and diarrhea (7). Carefully conducted analytical epidemiologic studies have demonstrated that disseminated MAC shortens survival by 5 to 7 months among patients with AIDS (9, 10). Treatment with multiple drug regimens has been shown to clear systemic symptoms, reduce bacteremia, and improve survival (3, 10, 11). With more effective antiviral therapy for human immunodeficiency virus infection and with prolonged survival, the number of cases of disseminated MAC is likely to increase substantially, and its relative contribution to AIDS mortality is likely to increase concomitantly. As a result of these developments, there will be an increased demand for diagnostic testing for MAC in both referral and community hospitals.

Disseminated *M. avium* is confirmed when the organism can be cultured from blood, bone marrow, or multiple tissues. The most sensitive method for the detection of mycobacteremia is the lysis-centrifugation blood culture system (Isolator, Wampole Laboratories, Cranbury, N.J.) (13). Lysis of leukocytes with saponin releases intracellular mycobacteria and produces colony counts 1.9 to 5.2 times higher than in unlysed blood (15). Pretreatment Isolator cultures demonstrate 10^2 to 10^4 CFU/ml of blood (15).

Because the Isolator system requires special centrifugation and handling procedures and because smaller laboratories may not be equipped to isolate and identify mycobacteria, there is need for a method to refer patient samples for processing. Shipment of Isolator tubes to distant laboratories has not been a consideration, because available data have shown a delay in the time needed to recover bacteria when Isolator tubes are held for more than 8 h before processing (2, 6). In one clinical study, delays of >9 h in processing Isolator tubes was associated with a fivefold reduction in the number of cultures positive for MAC (14). However, environmental studies of MAC have shown that organisms are viable for up to 30 days in natural water samples (5), and MAC is known to grow on detergents such as saponin (4).

The present study was conducted to determine whether prolonged storage of MAC-infected blood in Isolator tubes before processing would lead to quantitative reductions in recovery rates of MAC or delays in recovery of MAC.

MATERIALS AND METHODS

Preparation of blood for simulated blood cultures. Four units of whole blood anticoagulated with CPD (sodium citrate, dextrose, sodium phosphate solution) were obtained from healthy volunteers, stored for 1 day to 1 week at 4°C, and then used. Before each experiment, blood was pooled, mixed well, and divided into sterile beakers for further use.

***M. avium-M. intracellulare* strains and culture conditions.** Isolator blood cultures were performed on patients with AIDS, plated on Middlebrook 7H10 agar, and incubated at 35°C in 5% CO_2 (12). The organism used in experiments A and B was initially recovered after 18 days of incubation. Organisms were identified as *M. avium-M. intracellulare* complex (Mayo Medical Laboratories, Rochester, Minn.) by gas liquid chromatography (MIDI Microbial Identification System, Newark, Del.). Serotyping was performed by Anna Tsang, National Jewish Center for Immunology and Respiratory Medicine (Denver, Colo.), by both enzyme-linked immunosorbent assay (16) and thin-layer chromatography (1). Isolates were saved for the present study by transferring them to Middlebrook 7H10 agar at 35°C in 5% CO_2. To prepare inocula, a loopful of this growth was inoculated into Middlebrook 7H9 broth, which was then transferred by using a 1% inoculum at 5- to 15-day intervals at 35°C.

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TABLE 1. Experiment A: *M. avium-M. intracellulare* (serovar 6) recovered from Isolator tubes inoculated in vitro and held at room temperature before processing

Isolator tube no.	CFU/ml at hold time of:							
	0 h	4 h	8 h	16 h	24 h	48 h	72 h	7 days
1	710	440	590	650	790	840	810	800
2	470	580	570	650	700	760	780	880
3	630	680	610	660	730	720	760	850
4	520	580	530	650	700	780	780	940
5	500	460	650	590	670	770	750	880
Mean	566	548	590	640	718	774	776	870

Preparation of inocula for simulated blood cultures. Inocula were prepared by obtaining a log-phase broth (Middlebrook 7H9 plus 0.5% Tween) culture (5 days of growth) of *M. avium-M. intracellulare* and adjusting it to a 1.0 McFarland standard (11). This is referred to as the initial suspension.

Dilution of inocula. Experiments A and B were as follows. Serial 1:10 dilutions of an initial suspension of *M. avium-M. intracellulare* (serovar 6) were made in the broth to yield suspensions of approximately 10^4 and 10^5 CFU/ml. Preliminary plating experiments were conducted to predict the approximate number of CFU per milliliter in these initial experiments. One milliliter of each *M. avium-M. intracellulare* broth suspension was added to 100 ml of continuously stirred pooled blood to yield a final concentration of approximately 10^2 CFU/ml (experiment A) or 10^3 CFU/ml (experiment B). The contents of each beaker were then continuously stirred with a magnetic stir bar.

Experiment C was as follows. Serial dilutions of an initial suspension of *M. avium-M. intracellulare* (serovar 8) were made to yield concentrations of approximately 10^1, 10^2, 10^3, and 10^4 CFU/ml. Two milliliters of the 10^1, 10^2, and 10^3 concentrations were added to 200-ml aliquots of blood to yield final concentrations of 10^{-1}, 10^0, and 10^1 CFU/ml. Four milliliters of the 10^4 broth was added to 400 ml of blood to yield a final concentration of 10^2 CFU/ml.

Patient Isolator blood culture. Blood from a 37-year-old man with AIDS who was being treated with rifampin, clofazimine, and ethambutol for known disseminated *M. avium-M. intracellulare* (serovar 6) infection was drawn into two Isolator tubes. One tube was processed immediately as outlined below and the second tube was held at room temperature for 7 days before being processed.

Inoculation and processing of Isolator tubes. For the simulated cultures (experiments A, B, and C), sterile needles and syringes were used to fill Isolator tubes with 10 ml each of blood-broth suspension (after cleaning the stopper with alcohol). All Isolator tubes (experiments A, B, C, and D) were inverted several times and allowed to stand at room temperature for 30 min (time 0) and for the subsequent hold times. At time 0 and at each of the subsequent room temperature hold times (4 h, 8 h, 16 h, 24 h, 48 h, 72 h, 7 days, 21 days, 28 days, and 56 days), Isolator tubes of each *M. avium-M. intracellulare* concentration were centrifuged for 30 min at 3,000 × *g*, the supernatant was withdrawn, and the sediment was removed for culture according to the manufacturer's instructions (Wampole Laboratories). The entire sediment from each tube (approximately 1.5 ml) was inoculated onto 5 Middlebrook 7H10 agar plates (approximately 0.3 ml per plate) and incubated at 35°C in 5% CO_2. In experiments A and B, the numbers of CFU were recorded after 10, 18, and 40 days of incubation. In experiment C, the number of CFU was recorded once after 5 to 10 weeks of incubation. In experiment D, the number of CFU was recorded once after 4 to 5 weeks of incubation.

RESULTS

Experiment A (inoculum, 10^2 CFU/ml). Delays in processing of up to 1 week did not reduce the number of *M. avium-M. intracellulare* CFU/ml recovered (Table 1). For tubes held 24 h or more, there was actually an increase in CFU compared with those processed immediately (time 0), with maximum CFU per milliliter recorded for tubes processed after a 7-day hold time. For tubes held 7 days before processing, the numbers of CFU increased 54% (from 566 to 870 CFU/ml) compared with those of tubes processed immediately (time 0).

Visible growth of individual colonies was apparent on all plates (including those processed immediately and those processed at 7 days) after incubation at 35°C for only 10 days, but colony counts were not maximal until 18 days of incubation. Increasing hold times led to no differences in the time to the first appearance of visible colonies. The numbers of CFU recorded were 24 to 79% higher at 18 days of incubation than at 10 days of incubation.

Experiment B (inoculum, 10^3 CFU/ml). More than 2,000 CFU/ml were recovered at all processing intervals (4 h to 7 days). Because growth on these plates was confluent, it was not possible to ascertain small differences in recovery rates after various processing times.

Experiment C (inocula, 10^{-1} to 10^2 CFU/ml). Delays in processing of up to 1 week did not reduce recovery rates or the time needed for visible growth even at an inoculum of 10^{-1} CFU/ml (Table 2). As in experiment A, the numbers of CFU per milliliter were higher in tubes held 7 days at room temperature than in tubes processed immediately. At the 10^2

TABLE 2. Experiment C: *M. avium-M. intracellulare* (serovar 8) recovered from Isolator tubes inoculated at various concentrations in vitro and held at room temperature before processing[a]

Inoculum	CFU/ml (± SEM) at hold time of:						
	0 h	8 h	24 h	7 days	21 days	28 days	56 days
10^{-1}	0.6[b] (0.3)	0.7 (0.1)	0.6 (0.1)	1.0 (0.3)	ND[c]	ND	ND
10^0	4.6 (0.6)	4.6[b] (0.2)	6.3 (0.1)	7.2 (0.5)	ND	ND	ND
10^1	45.0 (1.0)	45.9 (1.5)	54.9 (1.2)	67.8[b] (2.1)	ND	ND	ND
10^2	334 (31.8)	414 (10.1)	416 (17.8)	461 (25.3)	495 (59.4)	468 (35.5)	31.4 (8.54)

[a] Five tubes were inoculated and processed for each mean number of CFU per milliliter shown.
[b] Mean number of CFU per milliliter for four inoculated Isolator tubes (overgrowth of contaminant in fifth tube).
[c] ND, Not done.

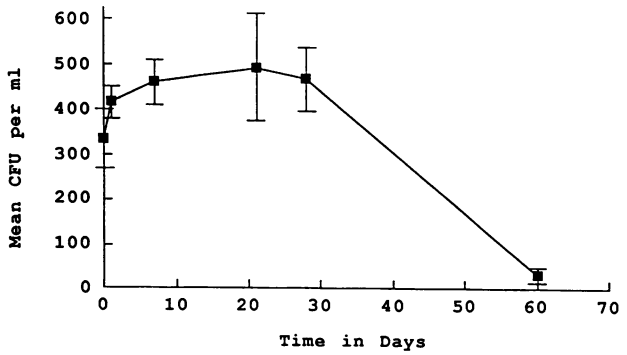

FIG. 1. Experiment C. Mean number of *M. avium-M. intracellulare* CFU per milliliter (± 2 standard errors of the mean) recovered from Isolator tubes inoculated with 10^2 CFU/ml in vitro and held at room temperature before processing.

CFU/ml inoculum, *M. avium-M. intracellulare* was still recovered when the Isolator tubes were processed after being held for 8 weeks at room temperature; the numbers of CFU per milliliter began to fall when processing was delayed longer than 4 weeks (Fig. 1).

Experiment D (patient blood culture). The Isolator tube taken directly from a patient with AIDS and processed immediately grew to a concentration of 11 CFU/ml, and the tube processed after being held for 7 days grew to a concentration of 14 CFU/ml.

DISCUSSION

The present study demonstrates that MAC survives in Isolator tubes for at least a week at concentrations encountered in disseminated human infection and suggests that Isolator tubes may be shipped from one laboratory to another at room temperature without loss of viability during this interval. At an inoculum of 10^2 CFU/ml, organisms survive as long as 8 weeks in the Isolator system before being cultured on Middlebrook 7H10 agar. Since the numbers of CFU per milliliter were actually shown to increase with hold times of up to 4 weeks, the Isolator system serves as a suitable transport medium for MAC. Because the numbers of CFU per milliliter begin to increase after hold times of ≥8 h, Isolator blood cultures should be processed within 8 h for quantitative bacteremia studies (e.g., drug treatment studies). The present study also shows that delays in processing of up to 1 week do not cause a delay in the time required to detect visible colonies after plating on agar.

Previous studies on the delayed processing of Isolator tubes either have not examined mycobacteria specifically or have used a design different from that of the present study. The studies of Hamilton et al. (6) and Cashman et al. (2) showed that rapidly growing bacteria were viable in the Isolator system after hold times of 8 to 24 h but that delayed processing led to an increase in the time needed to recover certain species (e.g., streptococci and members of the family *Enterobacteriaceae*) and decreased quantitative recovery rates for certain species (*Streptococcus pneumoniae*, *Haemophilus influenzae*). Only one mycobacterial strain (identified only as atypical mycobacterium) was studied. Another study compared the recovery of bacteria and fungi from 5,125 Isolator blood cultures held either less than 9 h or between 9 and 20 h before being processed (14). *M. avium-M. intracellulare* was recovered from 34 cultures, and most of these were in the <9-h group. However, different processing times were not applied to the same patient samples. Rather, recovery of *M. avium-M. intracellulare* was compared for patients whose blood was processed the same day versus patients whose blood was drawn after 4:00 p.m. and processed the next day. With this study design, the recovery rates may have reflected lower *M. avium-M. intracellulare* infection rates among the patients whose blood was cultured in the evening or night.

The principal finding of the present study, that *M. avium-M. intracellulare* survives for long periods in the Isolator system, is in accord with the known ability of the organism to survive for prolonged periods in natural waters (5) and the ability of detergents such as saponin to serve as a growth medium for the organism (4).

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