

Radiometric Method for Detection of Bacteremia

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A study was performed with simulated blood cultures to evaluate the production of $^{14}\text{CO}_2$ as an index of bacterial growth. With a range of inoculum sizes from 4 to 4,250 colony-forming units, it was not possible to detect $^{14}\text{CO}_2$ within 6 hr after inoculation in 59 separate experiments. In a limited trial with patients' blood cultures, the radiometric method failed to provide any earlier evidence of bacteremia than did routine broth cultures.

In view of the usual delay, 18 to 24 hr at a minimum, in detecting the presence of bacterial growth in blood cultures, the development of more rapid and practical techniques to demonstrate bacteremia would be highly desirable. DeLand and Wagner (1, 2) described a method in which ^{14}C -glucose is the sole carbohydrate in a liquid medium and the production of ^{14}C -labeled carbon dioxide is the index of bacterial growth. In the present study, we evaluated the influences of inoculum size and incubation time in simulated cultures on the production of detectable levels of $^{14}\text{CO}_2$.

MATERIALS AND METHODS

Simulated blood cultures were prepared by adding 1.0 ml of sterile whole blood from normal donors to 10 ml of Trypticase Soy Broth containing 0.5 μCi of ^{14}C -glucose (36 μg of glucose) in a 50-ml serum bottle (Contemporary Science, Mt. Prospect, Ill.) and then adding a freshly prepared bacterial inoculum. Laboratory control strains of *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and group D *Streptococcus* were inoculated into 2 ml of Mueller-Hinton broth (BBL), incubated at 35 C for 3 hr, and then diluted in Mueller-Hinton broth to provide final inocula for the blood culture bottle; these inocula ranged from approximately 4 to 4,250 colony-forming units (CFU) per ml. The inoculum size in each experiment was determined by performing colony counts, in pour plates, of the Mueller-Hinton broth dilutions used to inoculate the bottles. The simulated blood cultures were incubated in a rotary shaker at 35 C (Gyrotomy Shaker, New Brunswick Scientific Co., Inc., New Brunswick, N.J.).

Blood samples from approximately 65 patients with suspected bacteremia were also studied by inoculating 1.0 ml of the blood into the serum bottles containing ^{14}C -glucose. Additional blood, drawn concurrently, was inoculated, in 10-ml volumes, into 100 ml of thiol and 100 ml of tryptic soy broths (Difco). The ^{14}C -glucose bottles were incubated on a rotary shaker and monitored at 2, 4, 6, 18, 48, and 72 hr. The thiol and

tryptic soy broths were incubated at 35 C and examined daily for 2 weeks before being discarded as negative.

The production of $^{14}\text{CO}_2$ was determined by aspirating air periodically from the bottle through a 0.5- μm filter (Gelman Instrument Co., Ann Arbor, Mich.) and an electrostatic precipitator into an ionization chamber of a monitor (Triton 755 C; Johnston Laboratories, Inc., Cockeysville, Md.). In this instrument, gaseous radioactivity induces a current which is amplified and detected on a meter. The addition of blood alone to the serum bottle containing medium and ^{14}C -glucose was noted to yield readings minimally above base line. Only readings above this minimal background were considered to be significant.

RESULTS AND DISCUSSION

The results of 59 experiments are shown in Table 1. In none of the 33 initial experiments was there any detectable $^{14}\text{CO}_2$ within 6 hr after inoculation of the media; therefore, readings were subsequently made at 6 and 18 hr after inoculation. In no instance was there detectable $^{14}\text{CO}_2$ before the 18-hr reading. According to DeLand and Wagner (2), the time required for $^{14}\text{CO}_2$ detection when the cultures were continuously agitated was 1.5 to 3.5 hr, a finding we have been unable to substantiate.

All simulated blood cultures of *S. aureus* and *E. coli* were positive at 18 hr, but four cultures with small inocula of *P. aeruginosa* and five with small inocula of group D *Streptococcus* were negative at 18 hr. In all experiments with detectable $^{14}\text{CO}_2$, the cultures were also visibly positive, and the presence of viable bacteria was corroborated by subculture. *Bacteroides fragilis* (100 to 1,000 CFU) in 10 ml of thioglycolate broth containing 0.5 μCi of ^{14}C -glucose in a 50-ml serum bottle also failed to produce detectable radioactivity within 96 hr of observation, despite repeated positive subcultures.

Although Finegold et al. (3) found that average

TABLE 1. Results with simulated blood cultures

Organism	Inoculum (CFU)	No. of expt	Cumulative no. positive at intervals after inoculation			
			2 hr	4 hr	6 hr	18 hr
<i>Staphylococcus aureus</i>	4	8	0	0	0	8
	15	1			0	1
	76	1			0	1
	168	1			0	1
	371	1			0	1
	608	1			0	1
<i>Escherichia coli</i>	4	7	0	0	0	7
	8	3	0	0	0	3
	24	1			0	1
	157	1			0	1
	542	1			0	1
	750	1			0	1
<i>Pseudomonas aeruginosa</i>	4	7	0	0	0	3
	10	3			0	3
	16	2			0	2
	32	1			0	1
	188	1			0	1
	1,547	1			0	1
	3,237	1			0	1
	4,250	1			0	1
Group D <i>Streptococcus</i>	4	8	0	0	0	7
	11	1			0	0
	16	2			0	0
	73	1			0	0
	365	1			0	1
	892	1			0	1
	1,167	1			0	1

and median colony counts per milliliter of blood in bacteremic patients varied widely (155 and 7, respectively), a large number of low-level bacteremias (≤ 10 CFU/ml) were encountered. Any rapid detection system of bacteremia must therefore have sufficient sensitivity to detect a small inoculum.

Of the 65 patients with suspected bacteremia studied, 6 had positive routine cultures and 2 had positive cultures with the radiometric method (Table 2). The failure to isolate *B. fragilis* from the ^{14}C -glucose bottles was not surprising, since these bottles were not prepared anaerobically, but it is of interest that the negative results with the radiometric method included bacteremias due to organisms yielding no or inconsistent results with small inocula in the simulated blood culture studies. Although the studies of patients with suspected bacteremia are too small to be conclusive, it also is of interest that in no instance

TABLE 2. Results with blood cultures from patients

Patient	Routine culture		$^{14}\text{CO}_2$	
	Organism	Time to detection (hr)	Present	Time to detection (hr)
1	Group D <i>Streptococcus</i>	24	No	To 72
2	<i>Candida albicans</i>	72	Yes	72
	<i>Escherichia coli</i>	72	Yes	72
3	<i>Staphylococcus aureus</i>	48	Yes	48
	<i>Bacteriodes fragilis</i>	48	No	To 72
5	<i>B. fragilis</i>	96	No	To 72
6	<i>Pseudomonas aeruginosa</i>	72	No	To 72

was there a detectable $^{14}\text{CO}_2$ level in advance of visible signs of growth in the routine cultures. Further patient studies were not undertaken in view of the results of the simulated blood culture experiments.

The striking differences between our results in studies of simulated blood cultures and those of DeLand and Wagner (1, 2) are not readily explained. In their first publication (2), it is not clear that blood was added to the medium at all. In their second report (1), the addition of bacteria to the media was followed successively by the addition of 1.0 ml of blood and 0.5 μCi of ^{14}C -D-glucose (36 μg of glucose); however, it is not stated what the time intervals were between the additions and whether the measured incubation time started with the addition of bacteria or with the addition of ^{14}C -glucose. At any rate, it is clear from our studies that this system of early detection of bacteremia lacks the sensitivity to be a suitable alternative to conventional broth culture techniques. DeLand and Wagner (1) found that slight agitation of the culture medium resulted in earlier detection of $^{14}\text{CO}_2$ and used a magnetic stirrer to accomplish the agitation. It is questionable whether our use of a rotary shaker instead of a magnetic stirrer could be responsible for the differences in results, because results comparable to our own were obtained by J. D. MacLowry (*personal communication*) with a magnetic stirrer.

LITERATURE CITED

- DeLand, F., and H. N. Wagner, Jr. 1970. Automated radiometric detection of bacterial growth in blood cultures. *J. Lab. Clin. Med.* 75:529-534.
- DeLand, F. H., and H. N. Wagner, Jr. 1969. Early detection of bacterial growth, with carbon-14-labeled glucose. *Radiology* 92:154-155.
- Finegold, S. M., M. L. White, I. Ziment, and W. R. Winn. 1969. Rapid diagnosis of bacteremia. *Appl. Microbiol.* 18:458-463.