Microcalorimetry as a Tool for Evaluation of Blood Culture Media

K. TORVALD RIPA,* PER-ANDERS MÅRDH, BIRGITTA HOVELIUS, AND KARIN LJUNGHOLM

Institute of Medical Microbiology, University of Lund, S-223 62 Lund,* and Thermochemistry Laboratory, Chemical Center, University of Lund, S-220 07 Lund, Sweden

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Evaluation of optimal compositions of blood culture media has called for extensive and laborious work in comparative studies of large series of clinical specimens. Bacterial growth is accompanied by heat production, and calorimetry provides an analytical tool for its detection and quantification. A twin microcalorimeter of the heat conduction type was used to register heat effects in experimentally infected blood cultures. When studying *Escherichia coli* and *Staphylococcus aureus*, larger heat effects were produced with 0.05% sodium polyanetholsulfonate than with 600 IU of heparin per ml, which was also the case when using 10% sucrose. The addition of IsoVitaleX (BBL) increased the heat effects produced by the two species mentioned, whereas it had the opposite effect in cultures of *Neisseria meningitidis*. The present study indicates that microcalorimetry is a valuable and time-saving tool for the evaluation of optimal compositions of bacterial culture media.

A variety of media have been devised for the detection of bacteremia (for review see references 16, 21).

The use of sodium polyanetholsulfonate (SPS) in blood culture media has been found superior to heparin or the omission of anticoagulants (2, 4, 5, 14). The addition of sucrose to such media has in some series of clinical specimens (8, 13, 14, 18), but not in others (12, 22), been found to increase the positive culture rate. In order to enrich blood culture media, other additives such as amino acids, hemin, and vitamins are also used.

The evaluation of various blood culture media and additives, has called for extensive work in which double or triple culture sets have been used in large clinical series (4, 7, 8, 13-15, 18, 22).

Microcalorimetry constitutes a general analytical tool for characterizing microbial growth processes (17). By this technique, the heat effects evolved by a bacterial culture can be registered continuously without disturbing the processes by sampling procedures. Microcalorimetry is a nonselective analytical technique; that is, it registers the heat effects produced by all metabolic processes taking place in a bacterial culture. With this technique the inhibitory (11) or stimulatory influence on bacterial growth of the addition of various compounds to culture media may be registered.

In the present investigation microcalorimetry was used as an analytical tool to estimate

the value of various additives to blood culture media, i.e., of SPS, heparin, sucrose, and IsoVitaleX. Fresh human blood was experimentally infected with *Escherichia coli*, *Staphylococcus aureus*, and *Neisseria meningitidis* and cultured in media containing one or combinations of the above-mentioned compounds.

MATERIALS AND METHODS

Organisms. Five strains of *E. coli*, four strains of *S. aureus*, and two strains of *N. meningitidis* isolated from blood of patients with clinical evidence of septicaemia were tested. To standardize the inoculum used in each test, portions of cultures of the strains were frozen to -80°C in a medium consisting of 50% (vol/vol) fetal calf serum (Flow Labs. Ltd.), 49% (vol/vol) Trypticase soy broth (TSB) (BBL), and 1% (wt/vol) fresh yeast extract. The pH of the medium was 7.2. The cultures were diluted to contain 10⁴ bacteria/ml. Each portion was checked by viable count before use in the calorimetric experiments.

Blood culture medium. TSB, pH 7.2, was used as basic culture medium.

Additives. SPS (Roche), 0.05% (vol/vol); heparin (Vitrum, Sweden), 600 IU/ml; sucrose (Merck), 10% (wt/vol); and IsoVitaleX (BBL), 1% (vol/vol) were used. The compounds, or combinations thereof, were used as described below.

Calorimetric equipment. A twin-batch microcalorimeter of the heat conduction type (a slightly modified version of the LKB calorimeter) (20) was used. The samples were enclosed in stainless-steel ampoules, 4-ml volume. The calorimeter was thermostated at 37.00°C. The voltage signal was amplified with a Keithley 150B microvolt ammeter. The

base-line stability of the instrument was $1.5 \mu\text{W}$ over 24 h.

Calorimetric measurements. All tests with the same bacterial strain were made with blood drawn from one and the same person. After thorough skin cleansing with 70% ethanol, which was allowed to dry, the blood was taken by venapuncture. Blood and culture medium were mixed in the proportion 1:10. The mixtures were infected with the organism under study to give a final concentration of 10^2 bacteria/ml. The calorimetric ampoules were heat-sterilized before use. One ampoule (the test ampoule) was charged with the infected mixture, whereas the other (the reference ampoule) was charged with the same, but sterile, mixture. The ampoules were filled with 3 ml of the samples to be tested. The gas phase in the ampoules was 1 ml of air. At the start of the experiment, the medium was in equilibrium with the atmosphere. The media in the ampoules were thermostated for 5 min at 37°C before being introduced into the calorimeter. They were equilibrated for 3 plus 7 min in the two heat-exchange positions and were then inserted into the ampoule holder in the thermopile zone. When the ampoules were withdrawn from the calorimeter after the test, the broth medium in the test ampoule was tested for the presence of contaminating bacteria, and the broth medium in the reference ampoule was tested for sterility. In no case was any contamination found.

RESULTS

Representative thermograms recorded when testing *E. coli*, *S. aureus*, and *N. meningitidis* in TSB with various additives are shown in Fig. 1-3.

Effects of SPS and heparin. In tests with four strains of *S. aureus* and two strains of *E. coli*, the time until measurable heat effects ($\mu\text{W}/\text{ml}$) were registrable was shorter when SPS was used as anticoagulant instead of heparin. The heat effects produced were greater

when using the SPS-containing broth than the heparin-containing broth for three out of four strains of *S. aureus* and for one out of two strains of *E. coli*, whereas there was no difference in this respect between the two remaining strains. In tests using two strains of *N. meningitidis*, detectable heat effects were recorded more rapidly and the amount of heat produced was greater when heparin, instead of SPS, was used.

Effect of sucrose and IsoVitaleX when using SPS as anticoagulant. When sucrose was added to the TSB medium, registrable heat effects occurred more rapidly with three of four strains of *S. aureus* and with four of five strains of *E. coli* than with corresponding noncarbohydrate-containing cultures. IsoVitaleX also shortened the same period of time in experiments made with two strains of *S. aureus*, whereas it was longer for all three strains of *E. coli* studied. The addition of sucrose as well as of IsoVitaleX to the culture medium resulted in increased heat effects for all five strains tested.

Effects of IsoVitaleX when using heparin as anticoagulant in cultures of *N. meningitidis*. The addition of IsoVitaleX to TSB was found to be nonfavorable for the growth of *N. meningitidis*, as judged from the heat effects recorded when testing two strains of meningococci, i.e., the periods of time before any measurable heat effects occurred were longer and the heat effects produced were smaller than in the meningococcal cultures in which this additive was not used.

DISCUSSION

The purpose of the present investigation was to demonstrate that microcalorimetry can be

FIG. 1. Batch calorimetric experiments showing the heat effects produced by a strain of *E. coli* cultured in a 1:10 dilution of freshly drawn blood in TSB with the addition of 0.05% SPS (·····); 0.05% SPS and 10% sucrose (---); and 600 IU of heparin per ml (—).

FIG. 2. Batch calorimetric experiments showing the heat effects produced by a strain of *S. aureus* cultured in a 1:10 dilution of freshly drawn blood in TSB with the addition of 0.05% SPS (· · · · ·); 0.05% SPS and 1% IsoVitaleX (—); and 600 IU of heparin per ml (— —).

FIG. 3. Batch calorimetric experiments showing the heat effects produced by a strain of *Neisseria meningitidis* cultured in a 1:10 dilution of freshly drawn blood in TSB with the addition of 600 IU of heparin per ml (—); and 600 IU of heparin per ml and 1% IsoVitaleX (— —).

used to evaluate bacterial growth in complex bacterial culture systems, such as blood cultures. By this technique it may be possible to avoid the laborious task of establishing the value of various additives to blood culture media by comparative studies in large series of clinical specimens.

The use of blood experimentally infected in vitro for the evaluation of blood culture media carries the potential error of testing bacteria which, before the culture, have not been exhibited to various defense mechanisms in the host. The possible interference of such factors could not be evaluated in the present study. However, the results of our in vitro study on the influence of the addition of various additives of blood culture media correspond well to observations made in comparative studies of clinical blood specimens.

SPS not only acts as an anticoagulant but also inhibits bactericidal activities of serum (1, 9, 10, 19). The differences, as judged by the heat

effects evolved, in the growth of *E. coli* and *S. aureus*, and as found in our experimental blood culture system when using SPS and heparin as additives, correspond to findings in clinical blood specimens (4). SPS has been claimed as toxic for certain bacterial species, i.e., *N. meningitidis* (2, 3) and *Peptostreptococcus anaerobius* (6). When testing *N. meningitidis* and SPS, the heat effects that developed were smaller and occurred later than when using heparin. It can be noted that Eng (2) found *N. meningitidis* susceptible to SPS when used in blood culture media in the same concentrations as when used for testing in our microcalorimetric experiments.

Sucrose in concentrations ranging from 10 to 30% has been claimed to increase the rate of positive blood cultures (8, 13, 14, 18). However, Washington et al. (22) found that the addition of 15% sucrose to blood culture media reduced the isolation rate of *S. aureus* and of *Haemophilus* and *Bacteroides* species. It has been sug-

gested that the favorable effect of sucrose in clinical blood cultures may be attributable to its stabilizing effect on cell wall-deficient bacteria formed in the blood of patients with bacteraemia (8, 13, 14). We found that 10% sucrose increased the heat production. It may be wondered whether this observation made in our microcalorimetric experiments could be ascribed to sucrose simply being a nutrient factor.

In the diagnostic laboratory, blood is often obtained from patients undergoing antibiotic therapy, which can interfere with the recovery rate of bacteria. The influence of the presence of antibiotics on bacterial growth in blood culture media was not evaluated in our study. However, it can be noted that SPS exhibits an inhibitory effect on aminoglycoside antibiotics (9). In blood cultures of patients given penicillins, sucrose might increase the recovery rate not only by facilitating the survival of cell wall-deficient bacteria, but also by neutralizing the antibacterial effect of such antibiotics (22).

IsoVitaleX had a stimulatory effect on the growth of *E. coli* and *S. aureus*. However, in the concentrations used by us, this compound was inhibitory for both strains of *N. meningitidis* tested. This finding was unexpected, since IsoVitaleX is supposed to facilitate the growth of organisms belonging to genus *Neisseria*.

LITERATURE CITED

- Belding, M. E., and S. J. Klebanoff. 1972. Effect of sodium polyanetholsulfonate on antimicrobial systems in blood. *Appl. Microbiol.* 24:691-698.
- Eng, J. 1975. Effect of sodium polyanethol sulfonate in blood cultures. *J. Clin. Microbiol.* 1:119-123.
- Eng, J., and H. Iveland. 1975. Inhibitory effect in vitro of sodium polyanethol sulfonate on the growth of *Neisseria meningitidis*. *J. Clin. Microbiol.* 1:444-447.
- Evans, G. L., T. Cekoric, Jr., and R. L. Searcy. 1968. Comparative effects of anticoagulants on bacterial growth in experimental blood cultures. *Am. J. Med. Technol.* 34:103-112.
- Finegold, S. M., I. Ziment, M. L. White, W. R. Winn, and W. T. Carter. 1968. Evaluation of polyanethol sulfonate (Liquoid) in blood cultures, 692-696. *Antimicrob. Agents Chemother.* 1967.
- Graves, M. H., J. A. Morello, and F. E. Kocka. 1974. Sodium polyanetholsulfonate sensitivity of anaerobic cocci. *Appl. Microbiol.* 27:1131-1133.
- Hall, M., E. Warren, and J. A. Washington II. 1974. Detection of bacteremia with liquid media containing sodium polyanetholsulfonate. *Appl. Microbiol.* 27:187-191.
- Henrichsen, J., and B. Bruun. 1973. An evaluation of the effects of a high concentration of sucrose in blood culture media. *Acta Pathol. Microbiol. Scand. Sect. B* 81:707-710.
- Kocka, F. E., T. Magoc, and R. L. Searcy. 1972. Action of sulfated polyanions used in blood culture on lysozyme, complement and antibiotics. *Ann. Clin. Lab. Sci.* 2:470-473.
- Lowrance, B. L., and W. H. Traub. Inactivation of the bactericidal activity of human serum by Liquoid (sodium polyanetholsulfonate). *Appl. Microbiol.* 17:839-842.
- Mårdh, P. -A., T. Ripa, K. -E. Andersson, and I. Wadsö. 1976. Kinetics of the actions of tetracyclines on *Escherichia coli* as studied by microcalorimetry. *Antimicrob. Agents Chemother.* 10:604-609.
- Roberts, G. D., C. D. Horstmeier, and D. M. Ilstrup. 1976. Evaluation of a hypertonic sucrose medium for the detection of fungi in blood cultures. *J. Clin. Microbiol.* 4:110-111.
- Rosner, R. 1970. Comparison of a blood culture system containing Liquoid and sucrose with systems containing either reagent alone. *Appl. Microbiol.* 19:281-282.
- Rosner, R. 1972. A quantitative evaluation of three blood culture systems. *Am. J. Clin. Pathol.* 57:220-227.
- Rosner, R. 1974. Evaluation of four blood culture systems using parallel culture methods. *Appl. Microbiol.* 28:245-247.
- Sonnenwirth, A. C. 1973. Bacteremia. Laboratory and clinical aspects. Charles C Thomas, Publisher, Springfield, Ill.
- Spink, C., and I. Wadsö. 1976. Calorimetry as an analytical tool in biochemistry and biology, p. 1-159. In D. Glick (ed.), *Methods in biochemical analysis*, vol. 23. Wiley-Interscience, New York.
- Sullivan, N. M., V. L. Sutter, W. T. Carter, H. R. Attebery, and S. M. Finegold. 1972. Bacteremia after genitourinary tract manipulation: bacteriological aspects and evaluation of various blood culture systems. *Appl. Microbiol.* 23:1101-1106.
- Traub, W. H., and B. L. Lowrance. 1970. Anticomplementary, anticoagulatory, and serum-protein precipitating activity of sodium polyanetholsulfonate. *Appl. Microbiol.* 20:465-468.
- Wadsö, I. 1974. A microcalorimeter for biological analysis. *Sci. Tools* 21:18-21.
- Washington, J. A., II. 1975. Blood cultures. Principles and techniques. *Mayo Clin. Proc.* 50:91-98.
- Washington, J. A., II, M. M. Hall, and E. Warren. 1975. Evaluation of blood culture media supplemented with sucrose or with cysteine. *J. Clin. Microbiol.* 1:79-81.