Effect of Osmotic Stabilizers on $^{14}$CO$_2$ Production by Bacteria and Blood

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Received for publication 11 January 1973

Evidence is accumulating which shows the advantages of hypertonic medium in detecting bacteremia when compared to medium containing no osmotic stabilizers. Increased survival of pathogenic organisms in hypertonic medium may be attributed either to greater stability of bacterial cell walls and membranes (3, 4), to the suppression of bactericidal action of complement (2), or, perhaps, to both of these factors.

In developing a hypertonic medium, care must be taken to ensure that osmotic-stabilizing agents not only enhance survival of osmotically fragile cells, but also that they do not suppress growth of cells which are not osmotically fragile. This report presents data on the effect of several osmotic-stabilizing agents on $^{14}$CO$_2$ evolution from blood and also from bacteria which are not very sensitive to osmotic pressure changes.

**MATERIALS AND METHODS**

Stock culture bacteria were grown overnight on chocolate agar slants at 35 C. The organisms tested were *Diplococcus pneumoniae*, *Haemophilus* sp., *Pseudomonas aeruginosa*, *Pseudomonas diminuta*, and *Streptococcus pyogenes*. The growth on the slants was suspended with 1 ml of tryptic soy broth and diluted with tryptic soy broth to yield approximately 10$^4$ colony-forming units/ml, and 1 ml of each suspension was inoculated into the test media. For the *Haemophilus* culture, 1 ml of whole blood was added to the culture media. The control medium was JLI no. 6A blood culture vial (Johnston Laboratories, Cockeysville, Md.) containing 30 ml of enriched tryptic soy broth with 1.5 $\mu$Ci of $^{14}$C-labeled substrates and a magnetic stirring bar. The hypertonic media consisted of various osmotic-stabilizing agents added to the control medium. These agents were 1 and 3% NaCl, 10% sucrose, and 5, 10, and 15% dextran. (Sigma Chemical Co., St. Louis, Mo.; approximate average mol wt = 15,000-20,000). All percentages are expressed on a weight to volume basis.

The evolution of $^{14}$CO$_2$ by these cultures was monitored by using the automated BACTEC model 225 system (Johnston Laboratories). This radiometric technique has been demonstrated to be very sensitive in detecting $^{14}$CO$_2$ production by all levels of bacterial inocula (5) and is used in clinical laboratories for routine blood culturing. The instrument, which incubates culture vials at 35 C while providing continuous agitation through the magnetic stirring bar, analyzed each culture for $^{14}$CO$_2$ production every 2 h with 90% air—10% CO$_2$ as the flushing and culture gas.

A similar experiment was performed with, instead of bacteria, 1 ml of whole blood obtained from a healthy donor. These blood cultures were tested in the same manner on a 4-h cycle.
RESULTS

Table 1 provides some chemical characteristics of the osmotic-stabilizing agents and the resultant media. The 3% NaCl medium was the treatment with the highest osmotic pressure, assuming 100% dissociation of NaCl in the medium. The lowest osmotic pressures were from the dextran treatments since these polymers are of high-molecular weight and do not dissociate into smaller units upon dissolving. Dextran lowered the pH of the medium, and the 15% dextran treatment was difficult to dissolve since this concentration approached the saturation level.

Table 2 presents the effects of the various osmotic-stabilizing agents on the test bacteria. Detection time of the cultures was taken when the growth index (GI) reached a value of 20 or higher. The GI is an arbitrary scale of 0 to 100 which is linearly proportional to the amount of carbon-14 detected. A full-scale reading of 100 corresponds to 0.025 μCi of carbon-14 liberated. The maximum GI indicated in the data is the highest GI produced by a culture after its detection. For example, if 2-h readings yielded values of 5, 4, 5, 12, 25, 75, 63, 41, 20, 8, then the detection time would be 10 h since this is the hour at which the GI reached or surpassed 20, and the maximum GI would be 75 since this is the highest value produced by the culture.

No osmotic agent appeared to affect P. aeruginosa. Detection times and maximum GI values were the same for all treatments (detection time differences of 2 h were not considered significant). The 10% sucrose and 15% dextran treatments prolonged the detection time of D. pneumoniae, whereas 1% NaCl and 10% sucrose reduced the maximum GI values as compared to the control. The 3% NaCl treatment stopped the growth of this organism. While the detection time of P. diminuta was increased by 1% NaCl, 10% sucrose, and the 10 and 15% dextran treatments, none of these treatments significantly lowered the maximum GI. However, the 3% NaCl treatment was detrimental to the organism resulting in very poor growth and no 14CO₂ production. The growth of Haemophilus was prevented by 3% NaCl, whereas 1% NaCl and 10% sucrose delayed detection times. All osmotic-stabilizing agents reduced the maximum 14CO₂ output of this organism. The detection time of S. pyogenes was delayed by 15% dextran, whereas 10% sucrose reduced the 14CO₂ evolution by this organism below the detection level of 20 without inhibiting the growth of this organism.

Since blood by itself can metabolize the 14C-substrates in the medium and produce small quantities of 14CO₂, the effects of osmotic agents on 14CO₂ evolution by 1 ml of sterile blood were also investigated (Table 3). Normal peak blood background GI values range between 15 and 20. The only osmotic agent which reduced the maximum GI of blood was 10%

| Osmotic stabilizer | Percent (wt/vol) | Molarity | Molecules/liter | pH of medium |
|--------------------|-----------------|----------|----------------|--------------|
| Control            | 1               | 0.17     | 0.34           | 7.4          |
| NaCl               | 3               | 0.51     | 1.02           | 7.3          |
| Sodium             | 10              | 0.29     | 0.29           | 7.3          |
| Sucrose            | 5               | 0.0025   | 0.0025         | 7.25         |
| Dextran            | 10              | 0.006    | 0.005          | 7.15         |
| Dextran            | 15              | 0.0075   | 0.0075         | 7.05         |

Table 2. Effect of hypertonic media on 14CO₂ production by bacteria

| Medium            | Diplococcus pneumoniae | Pseudomonas aeruginosa | Pseudomonas diminuta | Haemophilus | Streptococcus pyogenes |
|-------------------|------------------------|------------------------|----------------------|-------------|------------------------|
|                   | Detection time (h) | Max GI* | Detection time (h) | Max GI | Detection time (h) | Max GI | Detection time (h) | Max GI | Detection time (h) | Max GI |
| 6A (control)      | 6                      | 93        | 2                   | 100   | 14                    | 49    | 6                      | 100   | 4                      | 32    |
| 6A + 1% NaCl      | 8                      | 76        | 2                   | 100   | 18                    | 40    | 10                     | 65    | 4                      | 34    |
| 6A + 3% NaCl      | NG                     | 4         | 100                  | PG    | 12                    | 45    | 6                      | 66    | 6                      | 37    |
| 6A + 10% sucrose  | 10                     | 55        | 4                   | 100   | 18                    | 39    | 12                     | 33    | 4                      | 34    |
| 6A + 5% dextran   | 8                      | 100       | 4                   | 100   | 16                    | 45    | 6                      | 66    | 6                      | 37    |
| 6A + 10% dextran  | 10                     | 100       | 4                   | 100   | 18                    | 44    | 6                      | 67    | 8                      | 28    |

* Tested every 2 h at 35 C with 90% air-10% CO₂ as a flushing gas. Symbols: NG, no growth; PG, poor growth; +, good growth, no detection.

* Maximum growth index.
The sucrase in the medium was being used in preference to the 14C-substrates with the consequent release of predominantly unlabeled CO₂.

The control medium did not hemolyze blood, even after extensive agitation. Of the hypertonic treatments tested, only 10% sucrase produced a reduction in 14CO₂ evolution by blood. Consequently, it was also concluded that this effect was not due to osmotic changes, but due to 14C-substrate dilution.

Sucrose appears to be the agent of choice since it did not significantly inhibit the growth of the bacteria tested. However, it did reduce the amount of 14CO₂ produced by bacteria. This agent could cause erratic performance of radiometric systems which rely on 14CO₂ production for detection of bacteria. However, alternatives may be employed to compensate for this reduction in bacterial 14CO₂ evolution. For example, since 14CO₂ evolution from blood is reduced, the sensitivity of a radiometric instrument can be increased to a level where small amounts of 14CO₂, previously produced by blood, could become adequate for bacterial detection. A 10% sucrase concentration, therefore, might give acceptable performance in radiometric detection systems, provided the critical GI value used to signify detection of a positive culture was reduced from the value used with normal culture medium. These and other alternatives, as well as the effect of 10% sucrase on 14CO₂ production by osmotically sensitive bacteria, are currently under investigation.

**DISCUSSION**

Of the six osmotic treatments tested, the most deleterious appeared to be 3% NaCl. Although this treatment would produce the highest osmotic pressure in a medium, its injurious effects may not be totally due to osmotic effects. Reduction of the water potential (a_w) in the medium could also have been damaging to the bacteria (1). It appears that 3% NaCl cannot be used as an osmotic-stabilizing agent since it not only prevents 14CO₂ production by bacteria, but also inhibits growth of certain organisms.

The dextrans probably are not good osmotic-stabilizing agents since they are of high-molecular weight and yield relatively few osmotically active particles when compared to lower molecular-weight-stabilizing agents on a unit weight basis. They also lower the pH and impart a dark color to the medium.

The 10% sucrase treatment reduced the maximum 14CO₂ produced by three of the five bacteria tested and also by the blood. It also delayed detection times for four bacteria. This does not appear to be due to osmotic effects since growth was prolific in these cultures and since 1% NaCl, which yields a higher osmotic pressure than 10% sucrase, did not produce similar results. Most likely, the delay in detection times and the reduction of maximum GI were due to a 14C-substrate dilution effect, i.e.,

| Medium            | Max GI |
|-------------------|--------|
| 6A (control)      | 17     |
| 6A + 10% sucrose  | 8      |
| 6A + 1% NaCl      | 15     |
| 6A + 3% NaCl      | 15     |
| 6A + 5% dextran   | 20     |
| 6A + 10% dextran  | 22     |

* Tested every 4 h at 35 °C with 90% air-10% CO₂ as a flushing gas.

* Maximum growth index.

sucrose. The other treatments appeared to have no effect on blood 14CO₂ production.

**LITERATURE CITED**

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