Inhibitory Effect of Glycerin on Vibrio parahaemolyticus and Salmonella

DOKI CHUN, SUNG YONG SEOL, RYUNBIN TAK, AND CHEONG KYU PARK

Department of Bacteriology, School of Medicine, and Division of Veterinary Medicine, Agricultural College, Kyungpook National University, Taegu, Korea

Received for publication 22 August 1972

In a study of the effect of glycerin in transport media on Vibrio parahaemolyticus and Salmonella, it was found that a concentration of 30% glycerin was highly inhibitory for V. parahaemolyticus and to a lesser degree for Salmonella. The incorporation of peptone or human feces in media did not reduce the inhibitory effect of glycerin. In media with 15% glycerin, viable counts of V. parahaemolyticus and Salmonella increased after 24 hr of incubation both in the presence and absence of feces. Due to the concurrent increase in the total bacterial count in the media containing feces, no enrichment effect was noted.

Since the report of Taegue and Clurman (9), media containing 30% glycerin have been widely used for the preservation of enteric pathogens in clinical specimens (3, 6). Buffered glycerin saline solution is a classical transport medium employed for specimens suspected of containing Salmonella and Shigella. Buffered salt solution and buffered sea water containing 30% glycerin have been recommended as transport media for Vibrio parahaemolyticus (4, 7, 8). However, DeWitt et al. (2) reported that buffered glycerin saline solution was toxic for V. cholerae. Marshall et al. (5) found glycerin inhibitory for other relatively salt-tolerant bacteria (5). In this paper, we present data showing the inhibitory effect of transport media containing high concentrations of glycerin for V. parahaemolyticus and Salmonella.

MATERIALS AND METHODS

Cultures. Strains of V. parahaemolyticus isolated from food poisoning patients were obtained from H. Zen-Yoji, Tokyo-to Laboratories for Medical Sciences, Bureau of Health, Tokyo, Japan, and Salmonella strains were obtained from G. J. Hermann, Center for Disease Control, Atlanta, Ga. Identification of and cultural characteristics for each strain were determined prior to use in this study.

Media. Buffered glycerin salt solution for V. parahaemolyticus was prepared according to the formula of LeClair et al. (4). Buffered glycerin saline solution reported by Sachs (6) was employed for Salmonella. These media contain 30% glycerin USP, pH 7.4, after sterilization. Media of the same formulas containing either 0 or 15% glycerin were used as controls. Brom Thymol Blue (BTB) Teepol agar was prepared as described by Sakazaki (7) for the colony count of V. parahaemolyticus. MacConkey agar (Difco) was used for the colony count of Salmonella. Differentiation of Salmonella from other lactose-negative enteric flora was not difficult; however, slide agglutination tests using specific antisera were carried out when necessary.

Procedures. One-tenth milliliter of appropriate dilutions of 24-hr cultures of V. parahaemolyticus grown in nutrient broth, supplemented with 2% sodium chloride, was inoculated into 5 ml of test media. After varying periods of incubation, 0.1 ml of decimal dilutions of media inoculated with test organisms was spread on solid media, and colonies were counted after 24 hr at 37 C. Counts of total bacteria in media inoculated with feces were made on nutrient agar plates. All counts were carried out in triplicate for each medium, and mean values were calculated.

RESULTS

Effect of glycerin on V. parahaemolyticus and Salmonella. Table 1 shows the viable counts of V. parahaemolyticus after incubation at 28 C in glycerin-containing media. All strains showed a marked decrease in viable counts after 6 hr of incubation in 30% glycerin, and four of five strains failed to produce a single colony after 24 hr of exposure. In media containing 15% glycerin, cultures remained essentially static during the first 6 hr, and an additional 18 hr of incubation resulted in limited multiplication of all strains. The basic media supported the growth of all strains tested.

In a second experiment with large inocula
and shorter exposure times, it was revealed that 30% glycerin and, to a lesser extent, 15% glycerin were toxic for *V. parahaemolyticus* during the first 30 min of exposure (Table 2). In 15% glycerin, the cultures appeared to stabilize between hour 3 and 6.

A similar trend was noted for five species of *Salmonella*. However, with the exception of *S. paratyphi* A, all strains survived a 24-hr exposure to 30% glycerin. The effect of 15% glycerin on *Salmonella* was similar to the results obtained with *V. parahaemolyticus* (Table 3). These results clearly indicate that 30% glycerin is highly toxic for *V. parahaemolyticus* and to a lesser degree for *Salmonella*.

**Table 1. Effect of glycerin on Vibrio parahaemolyticus in phosphate-buffered salt solution**

| Strain | Glycerin (%) | Viable counts |
|--------|--------------|---------------|
|        | 0            | 6             | 24            |
| 1      | 30           | 114           | 8             | 0             |
|        | 15           | 108           | 130           | 1,300         |
|        | 0            | 122           | 1,200         | 60,000        |
| 2      | 30           | 145           | 12            | 6             |
|        | 15           | 150           | 250           | 1,800         |
|        | 0            | 141           | 1,150         | 38,000        |
| 11     | 30           | 159           | 0             | 0             |
|        | 15           | 187           | 65            | 1,500         |
|        | 0            | 162           | 1,400         | 55,000        |
| 51     | 30           | 133           | 4             | 0             |
|        | 15           | 125           | 160           | 3,500         |
|        | 0            | 106           | 1,360         | 53,000        |
| 52     | 30           | 122           | 4             | 0             |
|        | 15           | 114           | 220           | 4,200         |
|        | 0            | 135           | 1,140         | 78,000        |

*a* Numbers in our list.

*b* Number of viable cells per 0.1 ml.

*c* Hours of incubation at 28 C.

**Table 2. Decrease in viable counts of Vibrio parahaemolyticus in phosphate-buffered salt solution during a 6-hr period**

| Strain | Glycerin (%) | Viable counts |
|--------|--------------|---------------|
|        | 0            | ½ | 1 | 3 | 6 |
| 1      | 30           | 2,635 | 137 | 93 | 49 | 18 |
|        | 15           | 2,827 | 1,520 | 1,390 | 650 | 680 |
| 52     | 30           | 1,500 | 360 | 291 | 57 | 52 |
|        | 15           | 1,736 | 1,556 | 1,440 | 422 | 920 |

*a* Numbers in our list.

*b* Number of viable cells per 0.1 ml.

*c* Hours of incubation at 28 C.

**Table 3. Effect of glycerin on Salmonella in phosphate-buffered saline solution**

| Strain               | Glycerin (%) | Viable counts |
|----------------------|--------------|---------------|
|                      | 0 | 6 | 24 |
| *S. paratyphi A*     | 30 | 450 | 7 | 0 |
|                      | 15 | 420 | 600 | 160 |
|                      | 0 | 460 | 1,200 | 8,000 |
| *S. paratyphi B*     | 30 | 530 | 300 | 220 |
|                      | 15 | 560 | 1,100 | 7,000 |
|                      | 0 | 520 | 2,500 | 28,000 |
| *S. typhimurium*     | 30 | 460 | 260 | 150 |
|                      | 15 | 500 | 900 | 7,000 |
|                      | 0 | 480 | 4,000 | 15,000 |
| *S. cholerae suis*  | 30 | 290 | 35 | 5 |
|                      | 15 | 200 | 600 | 4,500 |
|                      | 0 | 200 | 1,200 | 22,000 |
| *S. typhi H901*      | 30 | 500 | 95 | 25 |
|                      | 15 | 530 | 450 | 1,100 |
|                      | 0 | 510 | 2,500 | 31,000 |

*a* Number of viable cells per 0.1 ml.

*b* Hours of incubation at 28 C.

**Table 4. Toxic effect of 30% glycerin on Vibrio parahaemolyticus and Salmonella at 20 C**

| Organisms                  | Viable counts |
|---------------------------|---------------|
|                           | 0 | 6 | 24 |
| *V. parahaemolyticus*     |   |
| Strain 1                  | 1,400 | 34 | 0 |
| 2                         | 1,100 | 60 | 5 |
| 11                        | 1,500 | 150 | 0 |
| 51                        | 2,250 | 25 | 0 |
| 52                        | 1,100 | 20 | 0 |
| *S. paratyphi A*          | 350 | 290 | 40 |
| *S. paratyphi B*          | 500 | 410 | 210 |
| *S. typhimurium*          | 550 | 670 | 250 |
| *S. cholerae suis*        | 240 | 290 | 55 |
| *S. typhi H901*           | 340 | 360 | 120 |

*a* *V. parahaemolyticus*, in buffered salt (NaCl 2%) solution with glycerin. *Salmonella*, in buffered saline solution with glycerin.

*b* Number of viable cells per 0.1 ml.

*c* Hours of incubation.

**Effect of temperature and peptone on the activity of glycerin.** To determine the influence of temperature on the toxic effect of glycerin, the experiments were repeated at 20 C (Table 4). The results were essentially the same as those at 28 C, indicating that temperature does not play a major role in the toxic effect of glycerin. Similarly, 1% peptone in media did not influence the inhibitory effect of glycerin.
TABLE 5. Effect of glycerin in peptone water* on growth of Vibrio parahaemolyticus and Salmonella

| Organism              | Glycerin (%) | Viable counts* |
|-----------------------|--------------|----------------|
|                       | 0'           | 6              | 24             |
| **V. parahaemolyticus** |             |                |                |
| strain 1              | 30           | 350            | 2              | 0              |
|                       | 15           | 300            | 450            | 40,000         |
|                       | 0            | 320            | 50,000         | 2,300,000      |
| **S. typhimurium**    |              |                |                |
|                       | 30           | 850            | 650            | 260            |
|                       | 15           | 770            | 2,600          | 120,000        |
|                       | 0            | 810            | 6,500          | 1,500,000      |

*Peptone, 1%, pH 7.2; NaCl concentrations, 2% for V. parahaemolyticus and 0.5% for S. typhimurium.

**Number of viable cells per 0.1 ml.

***Hours of incubation at 28 C.

TABLE 6. Effect of glycerin in phosphate-buffered saline containing feces on survival of Vibrio parahaemolyticus and Salmonella

| Organisms counted | Glycerin (%) | Viable counts* |
|-------------------|--------------|----------------|
|                   | 0'           | 6              | 24             |
| **V. parahaemolyticus** |             |                |                |
| strain 1          | 30           | 520            | 20             | 0              |
|                   | 15           | 550            | 3,200          | 20,000         |
|                   | 0            | 620            | 6,500          | 250,000        |
| **Total**         | 30           | 12,000         | 10,000         | 11,000         |
|                   | 15           | 11,000         | 21,000         | 3,600,000      |
|                   | 0            | 11,000         | 46,000         | 10,000,000     |
| **S. typhimurium** |              |                |                |
|                   | 30           | 1,000          | 1,000          | 450            |
|                   | 15           | 1,100          | 2,000          | 9,000          |
|                   | 0            | 1,000          | 5,000          | 50,000         |
| **Total**         | 30           | 14,000         | 13,000         | 13,000         |
|                   | 15           | 13,000         | 15,000         | 3,600,000      |
|                   | 0            | 17,000         | 32,000         | 5,200,000      |

*V. parahaemolyticus, in buffered salt (NaCl 2%) solution with glycerin and feces; Salmonella, in buffered saline solution with glycerin and feces.

**Number of viable cells per 0.1 ml.

***Hours of incubation at 28 C.

Effect of feces on the activity of glycerin.

As one of the most common specimens to be transported is infected feces, test organisms were inoculated into 5 ml of media containing 0.1 g of feces free from V. parahaemolyticus and Salmonella, and specific and total cell counts were determined. Representative results (Table 6) indicated that the decrease in numbers of V. parahaemolyticus and S. typhimurium in media containing 30% glycerin were similar to those in media without feces (Table 1). In media with 15% glycerin, specific counts of both organisms increased more rapidly than the total counts during the first 6 hr; however, after 24 hr this partial enrichment phenomenon was reversed. In the absence of glycerin, both the specific and the total counts increase rapidly during the entire incubation period. The results indicate that glycerin does not appear to offer any specific favorable effect to the pathogenic species as compared to species normally occurring in the feces.

DISCUSSION

Even though a limited number of strains was used, our results clearly indicate that buffered salt solution containing 30% glycerin is highly inhibitory for V. parahaemolyticus, and we supposed that media reported by LeClair et al. (4) and Sakazaki (7, 8) are not suitable for the preservation of this organism even for short periods of time. This finding is in agreement with the result of DeWitt et al. (2) who reported the inhibitory effect of buffered glycerin saline solution on V. cholerae and ascribed the effect to glycerin. Our results are also in agreement with the experiments of Marshall et al. (5) who reported the inhibitory effect of glycerin on other relatively salt-tolerant organisms. Our experiments were carried out at 28 C which is the ambient mean temperature during the summer months in the temperate zone. Lowering the incubation temperature to 20 C, the mean ambient temperature occurring during the months at the beginning and end of the epidemic season did not influence the survival of V. parahaemolyticus.

It is essential that a transport medium maintain the specific pathogens in essentially the same ratio to the normally occurring species that is found in the fresh specimen during the period of time required for the shipment of the specimen to the laboratory. While 30% buffered glycerin saline can be used as a transport medium for specimens containing large numbers of Salmonella for short periods of time as shown by Sachs (6), the medium is not adequate if periods of time exceeding 24 hr are encountered. In the case of V. parahaemolyticus, 30% glycerin in buffered sea water is inhibitory in as little as 30 min.

Preliminary studies have indicated that Cary-Blair medium (1), modified to contain 2% NaCl, is not inhibitory to V. parahaemolyticus and is adequate for the shipment of specimens suspected of containing this organism.

ACKNOWLEDGMENTS

This research was supported by the U.S. Army Research and Development Group (Far East), Department of the Army, under grant DA-CRD-AFE-S92-544-71-G175.
We thank E. Neter, Children's Hospital, Buffalo, N.Y., and J. D. Marshall, Jr., U.S. Army Research and Development Group (Far East), for helpful criticism and advice in preparing the manuscript.

LITERATURE CITED
1. Cary, S. G., and E. B. Blair. 1964. New transport medium for shipment of clinical specimens. I. Fecal specimens. J. Bacteriol. 88:96-98.
2. DeWitt, W. E., E. J. Gangarosa, I. Huq, and A. Zarifi. 1971. Holding media for the transport of Vibrio cholerae from field to laboratory. Amer. J. Trop. Med. Hyg. 20:685-688.
3. Hajna, A. A. 1955. A new specimen preservative for gram-negative organisms of the intestinal group. Pub. Health Lab. 13:59-62.
4. LeClair, R. A., H. Zen-Yoji, and S. Sakai. 1970. Isolation and identification of Vibrio parahaemolyticus from clinical specimens. J. Conf. Public Health Lab. Direct. 28:92-92.
5. Marshall, B. J., D. F. Ohye, and J. H. B. Christian. 1971. Tolerance of bacteria to high concentrations of NaCl and glycerol in the growth medium. Appl. Microbiol. 21:363-364.
6. Sachs, A. 1939. Difficulties associated with bacteriological diagnosis of bacillary dysentery. J. Royal Army Med. Corps 73:235-239.
7. Sakazaki, R. 1956. Vibrio parahaemolyticus. Isolation and identification. Nihon Eiyo Kagaku Co., Ltd., Tokyo, Japan.
8. Sakazaki, R. 1967. Isolation and identification of Vibrio parahaemolyticus, p. 119-137. In T. Fujino and H. Fukumi (ed.), Vibrio parahaemolyticus (In Japanese) II. Naya Book Co., Tokyo, Japan.
9. Taegue, O., and A. W. Clurman. 1916. A method of preserving typhoid stools for delayed examination and comparative study of the efficacy of eosin brilliant-green agar, eosine methylene-blue agar and endo agar for the isolation of typhoid bacilli from stools. J. Infect. Dis. 18:653-671.