A CAMP PHENOMENON BETWEEN *VIBRIO CHOLERAE* BIOTYPE EL TOR AND STAPHYLOCOCCAL B-HEMOLYSIN

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INTRODUCTION

Although hemolysin production by the El Tor vibrios was originally considered as a feature in differentiating this biotype from classical *V. cholerae*, there is no technical uniformity for demonstration of the hemolytic activity. Furthermore, there is a difference of opinion about the hemolytic properties of vibrios. In recent years some investigators found that the El Tor biotype lost its typical hemolytic characteristic (Felsenfeld, 1964; Gangarosa *et al.*, 1967; Roy *et al.*, 1963) whereas others (Sakazaki, 1970) maintained the hemolytic activity was the essential characteristic of the El Tor vibrio.

The tube hemolysin test for hemolytic activity as described originally by Greig (1914) required alkaline broth and had some disadvantages; it required 4 to 5 days to complete and results were inconsistent (Barua, 1974). Later, Feeley and Pittman (1963) used brain heart infusion broth and claimed that this medium gave more consistent results. Barua and Mukherjee (1964) added 1% glycerol to heart infusion broth (HIBG) and found it superior to heart infusion broth alone. Several investigators confirmed the finding but a number of El Tor vibrio strains still failed to show hemolytic activity in HIBG (Barua *et al.*, 1964; Sakazaki *et al.*, 1971).

It was found that B-lysin producing *S. aureus* was able to enhance the hemolytic activity of Lancefield group B streptococci (Christie *et al.*, 1944) and of pathogenic *Listeria monocytogenes* (Groves *et al.*, 1977). This characteristic has since been called the CAMP test. Our recent studies showed that the hemolytic activity of El Tor vibrios could also be demonstrated in 5% sheep red blood cells-tryptic soy agar medium by the CAMP phenomenon. This method appeared to eliminate the subjective interpretation of questionable hemolysis by the tube method. The results are reported herein.

MATERIALS AND METHODS

Bacterial strains: A total of 394 *V. cholerae* biotype EL Tor strains included in this study were isolated from human clinical specimens submitted to our laboratory during 1978-1980. The El Tor vibrios included Ogawa (369) and Inaba (25) serogroups. A total of 7 classical *V. cholerae* strains were kindly supplied by Dr. P. Echeverria, Armed Forces Research Institute for Medical Sciences, Bangkok, Thailand (1), Dr. Julius Surjawidjaja, Faculty of Medicine, Trisakti University, Jakarta, Indonesia (2), Dr. V. Basaca-Sevilla, Bureau of Research and Laboratories, Manila, Philippines (2) and Bio Farma, Bandung, Indonesia (2). All El Tor vibrios were typically resistant to Polymyxin B, 50 μg (PB-50).

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Media: Blood agar (BA) medium was prepared by adding 5% sterile saline X3 washed sheep red blood cells to sterile trypticase soy agar base (BBL). Heart infusion broth (BBL) with (HIBG) and without (HIB) 1% glycerol (Sigma) was also prepared, adjusted to pH 7.4 and 1 ml dispensed to screw cap tubes. Sterilization was completed at 15 p.s.i.g. for 15 minutes.

CAMP test: The test was performed according to the procedure described elsewhere (Christie *et al.*, 1944; Darling, 1975; Facklam; 1976; Groves *et al.*, 1977). B-lysin producing *S. aureus* was streaked in a straight line across the center of a BA plate dividing the plate into two equal parts. El Tor vibrio strains were streaked perpendicular to the line of the *S. aureus* inoculum leaving a 2-4 mm space between the two organisms.

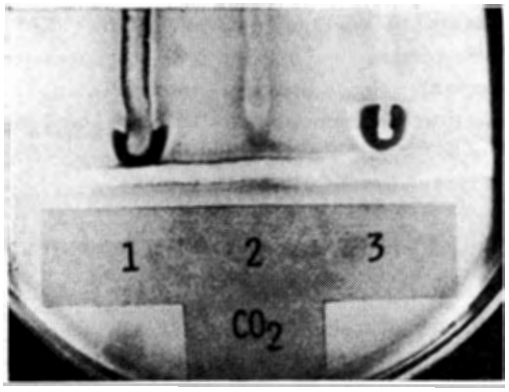

Fig. 1—CAMP reaction between *Vibrio cholerae*, El Tor, No. 1, *V. cholerae*, classical, No. 2, *Streptococcus* group B, No. 3 and *Staphylococus aureus* (perpendicular streak) incubated in candle jar.

Duplicate BA plates were inoculated with 10 vibrio strains in the above manner, 5 on each half of the plate. As a control to verify the CAMP test, Lancefield group B *Streptococcus* and the classical *V. cholerae* strain were streaked on each BA plate in a manner similar to that for the El Tor vibrios. One plate was incubated aerobically and the other in a candle jar at 37°C for 18-20 hours. A positive CAMP reaction was recognized as a crescent-shaped zone of complete hemolysis at the juncture of the growth of *S. aureus* and the El Tor vibrio strain.

Tube hemolysis tests: The test in HIBG was performed according to the method developed by Barua and Mukherjee (1964). One ml of the broth was inoculated with a loopful of an overnight *V. chloreae* broth culture. After 24 hours incubation at 37°C, an equal volume of a 1% suspension (v/v) of X3 washed sheep red cells in saline was added to the broth, mixed by gentle shaking, incubated at 37°C for 2 hours and then kept overnight in the refrigerator.

The test in HIB was done by the method described by Feeley and Pittman (1963). Ten ml HIB were inoculated with the *V. cholerae* strain, incubated at 37°C for 24 hours and 0.5 ml of the broth culture was then mixed with an equal volume of 1% washed sheep red cells. The mixture was incubated at 37°C for 2 hours and held overnight in the refrigerator.

Interpretation of the tube hemolysis tests was made the next morning without shaking the tubes.

RESULTS

A total of 394 El Tor vibrio strains were tested by the CAMP method and 382/394 (97%) of strains incubated in the cadle jar gave a strong positive CAMP reaction. The positive reaction appeared as a large crescent-shaped hemolysis at the juncture of the growth of El Tor vibrios and *S. aureus*. Only 12 (3%) of the isolates showed moderate hemolysis but still in the shape of a readily discernable crescent. The aerobic CAMP test was strongly positive with 204 (52%) and moderately positive with 60 (15%) of the El Tor vibrio strains. The HIBG tube hemolysin test was weakly or moderately

positive with 283 (72%) and the HIB tube test with 35 (9%) of the El Tor vibrio strains. The 12 strains showing moderate hemolysis in BA cultures grown in the candle jar were included in those that were negative in both HIB and HIBG cultures. Likewise, the 130 isolates that were negative in BA cultures incubated aerobically included the corresponding negative in BA cultures incubated aerobically included the corresponding negative isolates in HIB and HIBG tube tests. The CAMP and tube hemolysin tests were negative with the 7 classical *V. cholerae* strains tested.

DISCUSSION

The hemolytic activity of El Tor vibrios has been studied by many investigators using different modifications of the tube hemolysin test but with inconsistent results. Because of this, the test has been replaced by other identifying features and has lost its historical importance in the differentiation of El Tor vibrios from classical *V. cholerae*.

Feeley and Pittman (1963) mentioned several factors that affected the results of the tube hemolysin test. Among those were inadequate media, the importance of standard incubation conditions and the loss of hemolysin production by the strain as it aged. Wake and Yamamoto (1966) also reported a hemolysin - destructive factor produced simultaneously with hemolysin by EL Tor vibrios.

We found that the B-hemolysin of *S. aureus* appeared to have a synergistic action with the hemolysin or some other factor produced by the El Tor vibrios and caused hemolysis of sheep red blood cells which produced a strong to moderately positive CAMP test. Unlike the tube test which required 4 to 5 days the CAMP test was able to demonstrate the hemolytic activity of El Tor vibrios within 24 hours after growth on the primary plating agar medium. The CAMP reaction was positive with 100% of the El Tor vibrios incubated in a candle jar but only 67% when incubated aerobically. Not only was there a significant difference of the results between aerobic and CO_2 incubation, there was also a difference in the size of the zone of hemolysis. We also found that washed sheep blood cells were much more sensitive to the CAMP phenomenon than defibrinated sheep blood.

Finkelstein (1966) found that all El Tor vibrio strains from a 1961 cholera outbreak in Southeast Asia had hemolytic activity. Barua and Gomez (1967) noted that the number of hemolytic El Tor vibrios isolated in the Philippines decreased in 1962 and 1963 and when those isolated in 1964-65 were tested, found that less than 1% of the isolates cultured in nutrient broth were hemolytic. However, when the same isolates were tested using heart infusion broth containing 1% glycerol, 85% were shown to be hemolytic. None of the El Tor vibrios that we tested were non-hemolytic by the CAMP test although 28% in HIBG and 91% in HIB were non-hemolytic. We had only 7 isolates of classical *V. cholerae* and more should be tested to qualify the sensitivity and specificity of the negative CAMP reaction that we found in our study.

Further study with the CAMP test may help resolve the problems of "non-hemolytic" El Tor vibrios that have been found in increasing numbers during recent years. Once additional classical *V. cholerae* strains are tested and if a consistent non-hemolytic pattern is obtained, then the CAMP test may be useful for routine use in the differentiation of EL Tor vibrios from classical *V. cholerae*.

SUMMARY

A CAMP phenomenon was demonstrated by *Vibrio cholerae* biotype El Tor and B-lysin

producing *Staphylococcus aureus* in 5% sheep red blood cells-tryptic soy agar medium. All 394 El Tor vibrio strains tested, all showed a crescent-shaped hemolysis (positive CAMP) when the cultures were incubated in a candle jar whereas 67% were CAMP positive when incubated aerobically. Only 9% of the isolates produced detectable hemolysin in a standard tube test using heart infusion broth and 72% in a tube test using heart infusion broth containing 1% glycerol. Seven classical *V. cholerae* tested were CAMP negative. The CAMP reaction is easy to perform and may be useful for routine use in the differentiation of *V. cholerae* biotype El Tor from classical *V. cholerae*.

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