Sensitivity of Cholera and El Tor Vibrios to Cold Shock

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INTRODUCTION

Cold shock causes appreciable loss of viability of a number of Gram-negative organisms, for example, *Escherichia coli* (Sherman & Albus, 1923; Hegarty & Weeks, 1940; Meynell, 1958; Houghtby & Liston, 1965), *Pseudomonas pyocyanea* (*aeruginosa*) (Gorrill & McNeil, 1960; Farrell & Rose, 1968), *Salmonella typhimurium* (Gorrill & McNeil, 1960), *Aerobacter aerogenes* (Strange & Dark, 1962) and *Serratia marcescens* (Strange & Ness, 1963). Ring (1965) observed a similar phenomenon in a Gram-positive bacterium, *Streptomyces hydrogenans*, and Jansz & Maclean (1973) reported that it occurred in the blue-green alga, *Anacystis nidulans*. The decrease in viability due to cold shock depends on the age of the cultures (Meynell, 1958; Gorrill & McNeil, 1960; Strange & Dark, 1962; Houghtby & Liston, 1965; Jansz & Maclean, 1973) and the nature of the suspending medium (Gorrill & McNeil, 1960; Strange & Dark, 1962). Cold shock usually releases intracellular materials from the organisms (Strange & Dark, 1962; Farrell & Rose, 1968; Jansz & Maclean, 1973). The major effect of cold shock may be damage to the permeability properties of the bacterial cytoplasmic membrane (Meynell, 1958; Strange & Dark, 1962; Ring, 1965; Jansz & Maclean, 1973).

This communication records the effect of cold shock on cholera and El Tor vibrios.

METHODS

Organisms. *Vibrio cholerae*, Inaba 569B was obtained from Dr N. K. Dutta, Haffkine Institute, Bombay, India; *V. cholerae*, Inaba C 16/71; *V. cholerae*, Ogawa 154; *V. cholerae*, 447/70; *V. cholerae*, In Taki; *V. eltor*, OE/27; *V. eltor*, OE/64; *V. eltor*, OE/65; *V. eltor*, E 373/69 and *V. eltor*, E 435/70 were obtained from Dr K. N. Neogy, Department of Bacteriology of this institute; *V. eltor*, Mak 757 was obtained from Dr S. Mukerjee, Cholera Research Centre, Calcutta.

Test procedure. Bacteria were grown in the alkaline peptone water, nutrient broth or syncase medium described by Finkelstein, Atthasampunna, Chulasamaya & Charunmethee (1966). After the desired period of growth with shaking at 37 °C, they were harvested by centrifugation at room temperature (20 °C), washed in 0.15 M-NaCl adjusted with 1 M-NaOH to pH 7.6, in tris buffer (0.03 M, pH 7.6) or in sodium phosphate buffer (0.1 M, pH 7.6) and then resuspended in the respective medium. In experiments on the effect of cold shock on the viability of the bacteria, the washed suspensions were diluted with the respective medium at room temperature with or without 0.3 M-sucrose, 5 mM-CaCl₂ or 5 mM-MgCl₂. Small portions (5 ml) containing the equivalent of 0.03 mg dry wt/ml were quickly dispensed into Erlenmeyer flasks (50 ml) which had been cooled for 1 h at 0 °C; it took less than 2 min for the temperature of the sample to fall to 0 °C. Immediately after dispensing, and at suitable intervals thereafter, samples (1 ml) were withdrawn and their viabilities relative to

controls were determined after serial dilution in the respective medium; 0.1 ml volumes of suitable dilutions were spread on peptone agar plates and colonies were counted after incubation at 37 °C for 18 to 24 h.

To study the release of intracellular materials after cold shock, dense washed suspensions containing the equivalent of 3.0 mg dry wt/ml were similarly subjected to chilling at 0 °C. After the desired period of time, the suspensions were removed from the bath and rapidly filtered through membrane filters (BAC-T-FLEX type B-6, Schleicher and Schull Co., Keene, New Hampshire, U.S.A.). The extinctions of these solutions were measured at 260 nm in a Beckman DU spectrophotometer with a blank of the suspending medium. Protein in the filtrates was estimated by the method of Lowry, Rosebrough, Farr & Randall (1951) with crystalline serum albumin as the standard. Carbohydrate in the filtrates was determined by the method of Dubois *et al.* (1956) with glucose as the standard. Polyacrylamide gel electrophoretic studies of protein spectra in vibrio cells and cell-free filtrates were made according to the method of Takayama, MacLennan, Tzagoloff & Stoner (1966). Filtrates separated from similar suspensions at 20 °C were analysed as controls.

RESULTS AND DISCUSSION

After cold shock at 0 °C both *V. cholerae* and *V. eltor* underwent considerable loss of viability. Viability decreased gradually with increasing duration of cold shock. This depended on the nature of the diluent, losses being higher in phosphate buffer than in tris buffer or 0.15 M-NaCl. Losses of viabilities of *V. cholerae* after 100 min exposure to 0 °C were 32 % in 0.15 M-NaCl, 43 % in tris buffer, and about 65 % in phosphate buffer. Gorrill & McNeil (1960) reported that in *Pseudomonas pyocyanea* the simpler the composition of the diluent, the more lethal it was; distilled water was the most effective. Strange & Dark (1962), however, observed higher losses in viability of *Aerobacter aerogenes* in tris buffer than in saline buffer or distilled water. It thus appears that the lethality of diluents varies with different organisms. Susceptibility of both cholera and El Tor vibrios to cold shock was much more pronounced in vibrios from exponential-phase cultures than from stationary-phase cultures. Vibrios grown in different media showed no significant differences in their sensitivity to cold shock.

When the vibrios were subjected to chilling at 0 °C, increased amounts (as compared with controls) of protein and carbohydrate were released extracellularly. With increasing duration of cold shock, more protein than carbohydrate was released from the vibrios. For example, protein in a filtrate from *V. cholerae* after 100 min exposure to 0 °C in phosphate buffer was 76 µg/ml, while carbohydrate was only 10 µg/ml. Compared with controls, there was a considerable leakage of 260 nm absorbing materials from the vibrios after cold shock. Gel electrophoresis revealed the presence of 19 protein bands in the control *V. cholerae* (of mobility values 0.10, 0.13, 0.17, 0.18, 0.20, 0.22, 0.27, 0.29, 0.32, 0.36, 0.43, 0.48, 0.51, 0.54, 0.62, 0.65, 0.68, 0.74 and 0.82) and only 10 in the cell-free filtrates (of mobility values 0.10, 0.17, 0.18, 0.20, 0.22, 0.27, 0.29, 0.36, 0.43 and 0.48). The protein spectrum of cold shocked bacteria indicated that release of individual proteins was not complete.

Incorporation of 0.3 M-sucrose, 5 mM-CaCl₂ or 5 mM-MgCl₂ in the suspending medium completely or almost completely protected vibrios from loss of viability during cold shock. The protective action of these compounds was reported by Strange & Dark (1962) in *Aerobacter aerogenes* and that of Mg²⁺ by Farrell & Rose (1968) in pseudomonads.

Vibrio cholerae and *V. eltor* differed in their susceptibility to cold shock. For example, after 100 min exposure to 0 °C in phosphate buffer, *V. cholerae* were 30 to 38 % viable and

Table 1. *Effect of chilling on the viability of Vibrio cholerae and V. eltor suspended in phosphate buffer*

Bacteria were grown at 37 °C, harvested and washed and suspended in 0.1 M-phosphate buffer at room temperature. Portions (5 ml) were rapidly chilled to 0 °C and after 100 min viabilities relative to controls were determined.

Strain No.	Percentage of original viability
<i>V. cholerae</i>	
Inaba 569B	30.0
Inaba C16/71	38.0
Ogawa 154	34.0
447/70	36.4
In Taki	37.7
<i>V. eltor</i>	
Mak 757	69.1
OE/27	79.3
OE/64	72.8
OE/65	76.6
E373/69	75.2
E435/70	79.0

V. eltor 69 to 79 % (Table 1). However, when *V. cholerae* were grown at 24 °C, the loss of viability was only about 10 % after chilling for 100 min in 0.1 M-phosphate buffer. Farrell & Rose (1968) investigated cold shock in a mesophilic and a psychrophilic pseudomonads to discover whether the differences in the properties of their cytoplasmic membranes affected the susceptibility of the bacteria to cold shock. They reported that the decrease in viability of the psychrophile due to cold shock was much less than that of the mesophile. They correlated the susceptibility of the Pseudomonads to cold shock with the concentrations of unsaturated fatty acids in their lipids. Growth of micro-organisms at temperatures below the optimum for growth favours increased synthesis of unsaturated fatty acids (Kates, 1964; Farrell & Rose, 1967) and the results obtained here may indicate a difference in the properties of the cytoplasmic membranes of the two vibrio biotypes.

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