MICROBIOLOGY

Effect of Chilling on Bacteria in Aqueous Suspension

CERTAIN bacteria in the logarithmic growth phase become non-viable when chilled in aqueous suspension. The phenomenon has been observed with strains of *Escherichia coli*[1], *Pseudomonas pyocyanea*[2] and *Aerobacter aerogenes*[3]. Loss of viability of logarithmic phase *A. aerogenes* suspended in chilled phosphate-saline is accompanied by the leakage of endogenous solutes, and the leakage products, in sufficient concentration, protect the bacteria against the lethal effect[3]. Thus, loss of viability is greater the sparser the population and is insignificant in chilled suspensions containing more than 5×10^9 organisms/ml. Correlation between loss of viability and leakage of endogenous solutes has now been obtained by observing the effects of chilling on three Gram-negative bacteria.

Escherichia coli strain Jepp, *Aerobacter aerogenes* NCTC 418 and a laboratory strain of *Serratia marcescens* were used. Cultures growing exponentially were obtained by seeding flasks (1·5 litre) containing 100 ml. of either a defined mannitol–ammonia–salts medium[4] or tryptic meat broth with 4 ml. of a 16-h culture in the same medium, and shaking for 2–3 h at 37°. Bacteria were separated from the culture and washed twice with phosphate-saline (0·11 M sodium chloride and $K_2HPO_4 + KH_2PO_4$ (0·02 M —PO_4), *p*H 6·5) by centrifugation, then resuspended in this diluent (about 10^{10} bacteria/ml.). The effect of chilling on viability was assessed by adding washed bacterial suspension (0·1 ml.) to cold phosphate-saline (10 ml.) held in a temperature-controlled bath at 0° ± 0·25°; samples were removed at intervals for determination of viability by slide culture[5] on tryptic meat broth agar. Denser suspensions were used to examine the leakage of substances from chilled bacteria: washed suspension ($4–5 \times 10^9$ bacteria/ml.) was rapidly cooled to near 0° by contact with brine at −10° and placed in the bath at 0°; at intervals, samples (5 ml.) were quickly filtered through well-washed Oxoid filter membranes (grade *AP*). Filtrates were analysed for ultra-violet absorption at 260 mμ, adenosine triphosphate with the firefly luminescence technique[6] and ninhydrin-reacting substances[7]. As controls, filtrates separated from similar suspensions at 20° were analysed.

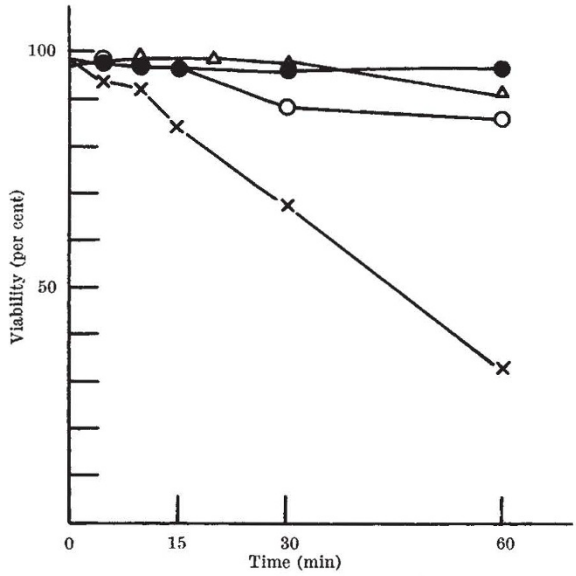

Fig. 1. Changes in viability of bacterial suspensions at 0°. Washed exponential phase bacteria were held in cold phosphate-saline, *p*H 6·5 (about 10^8/ml.). *A. aerogenes* NCTC 418 grown in a defined medium (×) and tryptic meat broth (○); *E. coli* Jepp grown in both media (●); *S. marcescens* grown in tryptic meat broth (△). Viabilities were determined by a slide culture technique (ref. 5)

Fig. 1 shows changes which occurred in the viability of suspensions containing about 10^8 exponential phase bacteria/ml. during 1 h at 0°. When grown in the defined medium, *A. aerogenes* were markedly susceptible to chilling whereas the organisms were more resistant when grown in tryptic meat broth; *E. coli* grown in either medium or *S. marcescens* grown in tryptic meat broth were almost completely resistant. The viabilities of similar suspensions of these organisms at 20° remained unchanged during 1 h.

At concentrations of about 5×10^9 bacteria/ml. of phosphate-saline no loss of viability of these organisms occurred during 1 h at 0°. However, when compared with similar suspensions at 20°, there was a considerable leakage of ultra-violet-absorbing substances (including adenosine triphosphate) and ninhydrin-reacting substances from *A. aerogenes* grown in the defined medium, much less from this organism grown in tryptic meat broth (Table 1). Leakage from *E. coli* at 0° was less than 20° (Table 1) and about the same at these temperatures from *S. marcescens*. Thus, on chilling bacteria in relatively dense suspension, the extent of leakage was related to the loss of viability which occurred in more dilute suspension (Fig. 1). Since the adenosine triphosphate concentration in filtrates of chilled *A. aerogenes* reached a maximum in 30 min and then decreased (Table 1), resorption, adsorption or breakdown of this substance must have occurred.

Loss of endocellular constituents on chilling bacterial suspensions may be a cause but is not an effect of bacterial death since leakage occurs from bacteria in relatively dense suspensions which remain viable. Presumably, in dilute suspension, the concentration of leakage products in the suspending fluid is too low for resorption to occur and loss of bacterial viability results. It is of interest that exponential phase *Aerobacter aerogenes* grown in a complex medium were more resistant to chilling than organisms grown in a defined medium.

Table 1. LEAKAGE OF ENDOGENOUS CONSTITUENTS FROM WASHED EXPONENTIAL PHASE *Aerobacter aerogenes* NCTC 418 AND *Escherichia coli* STRAIN JEPP HELD IN PHOSPHATE-SALINE AT 20° AND 0°

Results refer to filtrates from suspensions containing 2 mg bacterial dry weight/ml.

Organism and growth medium	Temperature of suspension	Ultra-violet absorption of filtrate (log I_0/I at 260 mμ 1 cm light path). Time (min)				ATP μg/ml. filtrate Time (min)				Ninhydrin-reacting substances as alanine (μg/ml. filtrate) Time (min)				% Viability after 60 min (slide culture. ref. 5)
		0	15	30	60	0	15	30	60	0	15	30	60	
A. aerogenes from defined medium (ref. 4)	20°	0·12	0·17	0·23	0·37	0	0	0	0	2·8	3·3	4·1	5·4	99
	0°	0·12	0·49	0·63	0·65	0	0·92	0·95	0·05	2·8	10·7	12·6	13·0	99
A. aerogenes from tryptic meat broth	20°	0·09	0·12	0·13	0·22	0	0	0	0	2·3	3·4	3·8	5·7	98
	0°	0·09	0·15	0·16	0·24	0	0·02	0·07	0	2·3	4·0	5·7	5·0	98
E. coli Jepp from defined medium (ref. 4)	20°	0·11	0·17	0·25	0·37	0	0	0	0	2·8	3·9	5·0	6·3	99
	0°	0·11	0·09	0·11	0·10	0	0	0	0	2·8	3·5	4·7	2·8	98

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[1] Sherman, J. M., and Albus, W. R., *J. Bact.*, **8**, 127 (1923). Hegarty, C. P., and Weeks, O. B., *ibid.*, **39**, 475 (1940). Meynell, G. G., *J. Gen. Microbiol.*, **19**, 380 (1958).
[2] Gorrill, R. H., and McNeil, E. M., *J. Gen. Microbiol.*, **22**, 437 (1960).
[3] Strange, R. E., and Dark, F. A., *J. Gen. Microbiol.*, **29**, 719 (1962).
[4] Strange, R. E., Dark, F. A., and Ness, A. G., *J. Gen. Microbiol.*, **25**, 61 (1961).
[5] Postgate, J. R., Crumpton, J. E., and Hunter, J. R., *J. Gen. Microbiol.*, **24**, 15 (1961).
[6] Strehler, B. L., and Totter, J. R., *Arch. Biochem. Biophys.*, **40**, 28 (1952).
[7] Yemm, E. W., and Cocking, E. C., *Analyst*, **80**, 209 (1955).