A NOTE ON "DEFENCE RUPTURE" AND THE ACTION OF ELECTROLYTES.

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The etiology of tetanus and gas gangrene presents a biological problem of an unusual nature. When the organisms which cause these diseases are deprived of toxin, by washing in tap-water saline or by washing and then heating, they are non-pathogenic to laboratory animals. In a recent investigation of the problem[1] we have shown that soluble ionisable calcium salts enable detoxicated *B. tetani, B. Welchii, B. oedematiens* and *vibrion septique* to exert their pathogenic powers. We concluded that calcium owes this property to its local action on the tissues.

Our conclusions have been criticised by Shearer[2], who offers an alternative explanation of the facts. Shearer has shown that the unbalanced Na ion rapidly kills the meningococcus, and has brought forward evidence to prove that the pneumococcus and *B. anthracis* lose their pathogenicity when treated with a solution of pure NaCl in distilled water. His explanation of our work is that by washing in saline the gas gangrene bacilli are robbed of their pathogenicity which is restored by adding Ca. According to Shearer our experiments are examples of the interaction of bivalent and monovalent ions on the normal stability of the cell wall. We think our previous papers contain sufficient evidence to nullify Shearer's view of our work. The following experiments show it to be incorrect.

Experiment 1.

A large tube of meat broth was inoculated with spores of *vibrion septique* which had been kept in tap-water saline at room temperature for 15 months. In 24 hours there was a vigorous growth with evolution of gas. The culture was kept at 37° C. for 48 hours and was then washed twice in Ringer's solution[3]. The final thick deposit of particles of meat and spores was heated in vaccine phials to 80° C. for 25 minutes. On the 3rd December, equal quantities of the spores were added to (*A*) 5 c.c. of Ringer, and (*B*) 2·5 c.c. of Ringer to which 2·5 c.c. of a 1 per cent. solution of $CaCl_2$ was added. Ten mice were then inoculated with suspension (*A*) and ten with suspension (*B*).

[1] *Proc. Roy. Soc.* Ser. b, xc. 513; also *6th Scientific Report, Imperial Cancer Research Fund.*

[2] *Journ. of Hygiene,* xviii. 337.

[3] Composition: NaCl 0·77 %; KCl 0·024 %; $CaCl_2$ 0·0208 %.

	Inoculum	Dose and site	Result
(*A*) Mice 1 to 10.	Suspension of v. s. spores in Ringer.	0·5 c.c. right flank.	4 Dec. All alive and well. The animals remained in good health. On 10 Dec. each was injected with 0·5 c.c. of 1 % $CaCl_2$: 11 Dec. all were dead of gas gangrene.
(*B*) Mice 11 to 20.	Suspension of spores in equal quantities of Ringer and 1 % $CaCl_2$.	0·5 c.c. right flank.	4 Dec. 9 dead of gas gangrene; 1 alive and well. This animal remained in good health. On 10 Dec. it was injected with 0·5 c.c. of 1% $CaCl_2$: 11 Dec. dead of gas gangrene.

EXPERIMENT 2.

4th December. A tube of serum agar was inoculated with spores of *vibrion septique* and the tube incubated anaerobically. 6th December a thick growth of a pure culture of *vibrion septique*. The culture was taken off from the surface of the serum agar by means of a platinum loop and was emulsified in 1 c.c. of Ringer; 0·5 c.c. of the emulsion was added to (*A*) 3 c.c. of Ringer and 0·5 c.c. to (*B*) a mixture of 1·5 c.c. of Ringer and 1·5 c.c. of 1 per cent. $CaCl_2$.

Six mice were inoculated with the suspension in Ringer and six animals with the suspension in Ringer + $CaCl_2$.

	Inoculum	Dose and site	Result
(*A*) Mice 1 to 6.	Suspension of organisms in Ringer.	0·5 c.c. right flank.	7 Dec. Five alive and well. One dead of gas gangrene. The five mice remained in good health. 10 Dec. Inoculated with 0·5 c.c. 1 % $CaCl_2$. 11 Dec. All dead of gas gangrene.
(*B*) Mice 7 to 12.	Suspension of organisms in Ringer + $CaCl_2$.	0·5 c.c. right flank.	7 Dec. All dead of gas gangrene.

EXPERIMENT 3.

4th December. A culture of *B. Welchii* in meat broth was made.

5th December. Vigorous growth. Culture pure. 50 c.c. of culture centrifuged and broth pipetted off from deposit, which was then washed thrice with sterile Ringer. The final deposit was emulsified in 1 c.c. Ringer; 0·5 c.c. of the emulsion was added (*A*) to 3 c.c. of Ringer; (*B*) 0·5 c.c. to a mixture of 1·5 c.c. Ringer and 1·5 c.c. 1 per cent. $CaCl_2$.

The following inoculations were made:—

	Inoculum	Dose and site	Result
(*A*) Mice 1 to 6.	Suspension of *B. Welchii* in Ringer.	0·5 c.c. right flank.	All alive and well; the mice have remained in good health.
(*B*) Mice 7 to 12.	Suspension of *B. Welchii* in Ringer and $CaCl_2$.	0·5 c.c. right flank.	All the mice died within 36 hours of gas gangrene.
(*C*) Mice 13 to 18.	Whole meat broth culture of *B. Welchii*.	0·25 c.c. right flank.	All mice died within 36 hours of gas gangrene.