RESISTANCE OF VEGETATIVE CELLS OF *CLOSTRIDIUM WELCHII* TO LOW pH

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CLOSTRIDIUM WELCHII is now firmly established as a major cause of food poisoning, at least in England, the USA and Japan. It is generally agreed that the minimum infecting dose is high; Dische and Elek (1957) concluded that a dose of $c. 5 \times 10^8$ viable organisms was necessary to produce symptoms.

The present paper deals with attempts to determine quantitatively the effect of low pH (1.5–3.6) on the survival of *Cl. welchii*, and to relate the results to the possible survival during passage through the stomach of the large number of organisms needed to initiate infection.

MATERIALS AND METHODS

Technique. 10-ml volumes for 5- and 24-hr cultures of *Cl. welchii* in cooked-meat medium (CMM) were centrifuged, and the organisms were washed three times with quarter-strength Ringer's solution and resuspended in 10-ml volumes of 1 per cent. peptone water. Millilitre samples of these suspensions were inoculated into 100 ml of peptone water of the required pH (1.5–3.6) or into human gastric juice in 150-ml screw-capped containers, and the diluted material was mixed by inversion. Millilitre samples were immediately pipetted into $6 \times \frac{3}{8}$ in. (15×1.5 cm) test-tubes, which were placed in a 37°C waterbath for short-term tests or in anaerobic jars if the test was to last for more than 2 hr. Results from tests under aerobic conditions did not differ from those obtained under anaerobic conditions.

The reaction was timed from the moment the organisms were suspended in the low-pH material; duplicate 1-ml samples were immediately placed in buffer to provide a zero reading. After a range of time-intervals, duplicate samples were removed from the waterbath or incubator and neutralised by the addition of 9 ml of buffered quarter-strength Reinforced Clostridial Medium (RCM). Serial dilutions were made in quarter-strength RCM, and the number of survivors was counted on RCA by the surface plate technique. A drop from the 1 in 10 dilution was examined microscopically; the results were disregarded if clumping was prevalent. Fortunately there was little clumping with the strains used.

Organisms. The following strains were used: *Clostridium welchii* F 2063 and F 9191, both non-haemolytic and heat-resistant; F 8352 and NCTC8246, both β -haemolytic and heat-sensitive. All were isolated at the Food Hygiene Laboratory from samples connected with food-poisoning incidents.

RESULTS

Tests in peptone water

Survivor curves were drawn for vegetative cells of *Cl. welchii* derived from 5- and 24-hr CMM cultures of all four strains of *Cl. welchii* suspended in peptone water at each pH level from 1.5 to 3.6; the figure shows the curve obtained with strain F 8246. As the death rate was not always linear, decimal reduction times (D values) were not calculated. From the survivor curves the times taken to reduce the initial counts to 0.01 per cent. (i.e., an inactivation factor of 10^4) were calculated (table I). Rubbo, Gardner and Webb (1967) used a similar inactivation factor when they compared the death rates of bacterial spores in various concentrations of glutaraldehyde.

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At lower pH values (1.5 and 2.0), when death was rapid, the death rate was primarily linear, but as the pH increased the survivor curves became sigmoid, with an initial lag period in which little change occurred in the number of survivors. Smith (1923) computed survivor

FIGURE.—Effect of low pH on the survival of vegetative cells of *Clostridium welchii* F8246 grown in CMM for 5 hr (—) and 24 hr (---) at 37°C.

curves for the disinfectant action of a range of phenol concentrations and obtained similar results. He felt that the sigmoid curve was probably more representative of the true death-rate, because when the death-rate was high the initial lag period, though not eliminated, was so short that it was not detectable by normal counting methods.

For any given strain of *Cl. welchii* the death rate increased with decreasing pH . Above pH 3.6 the death rate was small, and at pH 4.0 and 4.5 little lethal action occurred within 24 hr.

The β -haemolytic strains (F 8246, F 8352) survived for longer periods than the non-haemolytic, heat-resistant strains (F 2063, F 9191).

The death rate was dependent on the age of the culture. The 5-hr cultures were more susceptible to low pH than the 24-hr cultures of the same strain. Although the incubation period was controlled at 5 and 24 hr, it was not possible to assume that all cultures were in exactly the same place of the growth cycle. Therefore, to obtain reproducible results with different cultures of the same strain, it was necessary to test at all pH levels with a single suspension of each culture.

TABLE I

Effect of low pH on the survival of vegetative cells of Clostridium welchii suspended in peptone water

Strain (and year)	pH of suspending medium	Time (min.) at 37°C for reduction of viable count to 0.01 per cent. in a	
		5-hr culture	24-hr culture
F2063 (1967)	1.5	6	35
	2.0	25.5	105
	2.5	68	181
	3.0	100	262
F9191 (1966)	1.5	11	29
	2.0	15	56
	2.4	22	109
	3.1	60	210
	3.6	185	360
F8352 (1966)	1.5	280	405
	2.0	450	750
	2.5	950	>1000
NCTC8246	1.5	30	84
	2.0	150	205
	2.6	300	560

Tests in gastric juice

The procedure was repeated with five samples of gastric juice (pH 1.7–2.5) collected from healthy students, and with one sample (pH 7.0) from a patient with achlorhydria. Because of their small volume, each sample could be tested with only one strain of *Cl. welchii*. Table II gives the results, together with those for suspensions of the same strains, in peptone water at the same pH. Vegetative cells of *Cl. welchii* were evidently more susceptible to gastric juice than to peptone water at the same pH, and the 24-hr cultures were more resistant to the bactericidal effects of low pH than the 5-hr cultures.

DISCUSSION

Results from this work indicate that the acid resistance of an organism, and therefore its ability to survive passage through the intestine, is dependent on the age of the culture; young, actively growing cultures are more susceptible to low pH than cultures in the stationary phase of the growth cycle. We suggest that this may have an important effect on the results of feeding experiments. Hauschild and Thatcher (1967) produced diarrhoea in volunteers fed

with $(4-6) \times 10^9$ vegetative cells from an 18-hr CMM culture, but Hauschild, Hilsheimer and Thatcher (1967) were unable to produce symptoms in patients fed with a similar dose of organisms grown for only 1-2 hr in medium CP-2V. The latter authors found that the young cells were more susceptible to low pH and synthetic gastric juice than the older cells from the CMM culture. Dische and Elek (1957) stated that the variation in susceptibility of volunteers to *Cl. welchii* cultures depended on the survival of the bacteria in the stomach; this may be extended by saying that the survival within the stomach, and therefore the ability to cause food poisoning, is dependent on the physiological state of the host organism. This should be remembered when carrying out feeding experiments, either with human volunteers or experimental animals.

TABLE II

Survival of Cl. welchii in samples of gastric juice and of peptone water at the same pH

Strain	pH	Time (min.) at 37°C for reduction of viable count to 0.01 per cent. in suspensions in			
		gastric juice of a		peptone water of a	
		5-hr culture	24-hr culture	5-hr culture	24-hr culture
F8352	1.7	45	90	250	480
	2.2	90	200	240	804
	2.5	120	...	625	...
F2063	2.5	6.5	22	65	170
F8246	2.3	23	...	180	...
	7.0	>1440	>1440	>1440	>1440

Duncan and Strong (1971) and Strong, Duncan and Perna (1971), in their work on the effect of *Cl. welchii* on the rabbit ileum, used monkeys and human volunteers for feeding experiments. They observed that the effect on persons fed with viable cultures was increased if the cultures were taken with large amounts of food which, they suggest, buffered the acidity of the stomach.

SUMMARY

The resistance of vegetative cells of *Cl. welchii* to low pH was tested in gastric juice and in peptone water of pH 1.5-3.6. The results showed that, for any given strain the death rate increased with decreasing pH; the death rate was dependent on the age of the culture—for cells grown in cooked-meat medium a 5-hr culture was more susceptible to low pH than a 24-hr culture of the same strain; the cells were more susceptible to gastric juice than to peptone water of the same pH.

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