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The Growth of *Brucella abortus* Strain 19 in Aerated Dialysed media

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SUMMARY: An apparatus for growing *Brucella abortus* in a dialysed medium in an aerated cellophan tube is described from which 500 ml. of culture of strain 19 having a density of 7 to 10×10^{11} organisms/ml., equivalent to 50-70 g. dry weight of organisms/l. could be harvested every 3 days.

The production of vaccine from *Brucella abortus* strain 19 grown on the surface of solid media is extremely costly because of the high rate of contamination, and of the low yield. Little interest has been shown in deep culture methods for the routine production of *Brucella* vaccine in spite of promising experimental results by a number of workers (Gerhardt & Gee, 1946; Glassman & Elberg, 1946; Saunders & Huddleson, 1950). Sterne & Wentzel (quoted by van Drimmelen, 1952) obtained *c.* 150×10^9 viable *Brucella abortus*/ml. in shake flasks in volumes of 3-4 l. This method was further developed by van Drimmelen (1956) and is used in South Africa for routine vaccine preparation. Dr G. P. Gladstone in 1947 (personal communication) obtained large harvests of *Salmonella typhi* in media aerated in cellophan tubes, and later (Gladstone, 1948) used a similar technique for preparing anthrax immunizing antigen. Gorelik, Mead & Kelly (1951) showed that the growth of *Brucella suis* in shake flasks could be increased by enclosing the medium in cellophan sacs. Anderson (1953, 1954, 1955) at the South African Institute for Medical Research has obtained yields of *S. typhi* up to 1.2×10^{11} organisms/ml. in aerated cellophan tubes, and Dr J. H. Mason of the same Institute suggested that the method should be tried for growing *B. abortus*. The apparatus described below is virtually identical with that used by Fredette & Vinet (1952) for growing *Clostridium tetani*. It is simple to operate and has yielded up to 1.8×10^{12} viable organisms/ml. with *B. abortus* strain 19.

METHODS

Apparatus. Figure 1 shows a drawing of the apparatus used. The culture vessel *K* is 780 mm. long and 80 mm. diam. The constricted ends *H* and *H*₁ are 48 mm. diam. and 40 mm. long. Tubes *I*₁ and *I* are for inflow and outflow of medium respectively. *L* is a cellophan tube (Visking's regenerated cellulose tubing, diam. $1\frac{7}{8}$ in.). This tubing is well soaked in water, threaded through *K*, and the ends reflected over *H* and *H*₁ where they are held in position by rubber pads (25 mm. thick) *F* and *F*₁ clamped between metal disks *E* and *E*₁ by means of three equally spaced $\frac{1}{4}$ in. threaded metal rods *B* furnished with wing nuts; only two of these rods are shown in the figure. *M* is a sparger

formed of a 40 mm. no. 3 sintered disk which is connected to an air supply through a humidifier and a filter (not shown). *N* is a pyrex Y-piece, one branch of which is connected to a reservoir containing physiological saline, while the other branch is used for sampling and harvesting the culture and for steaming the apparatus (see below). *A* is a 500 ml. boiling flask with a tube *C*, 25 mm. diam., fused into the bottom. This is connected to a 25 mm. diam. tube *D* which pierces *F*. *G* is a stainless steel spiral to prevent deformation of the cellophan tube *L* during aeration.

The apparatus is assembled as shown and furnished with appropriate rubber connexions for attachment to the reservoirs for medium and saline, the air-line, and a reservoir for spent medium. About 50 ml. distilled water are introduced into the cellophan tube *L*, the wing nuts tightened sufficiently to make a good seal between the ends *H*, the cellophan tube *L* and the rubber pad *F*, and the whole apparatus then autoclaved. After sterilization, the apparatus can be left for long periods, assembled and ready for use, without risk of contamination.

Medium. The culture medium used was virtually the same as that suggested by van Drimmelen (1952): 3% (w/v) 'peptone' (the peptone was a papain digest of horse muscle containing 17 g. total-N/100 g.); 3% (w/v) glucose; 1% (w/v) Marmite; 0.15% (w/v) anhydrous Na_2HPO_4 . The pH was adjusted to 6.4 and the medium sterilized by filtration through Seitz pads.

Operation of the apparatus. The cellophan tube *L* is filled through *N* with *c.* 1000 ml. physiological saline from a reservoir and the surrounding annular space filled with *c.* 1500 ml. medium from another reservoir through *I*₁. After diffusion has proceeded for about 4 hr. the diffusate in *L* is inoculated with the washings of an agar slope culture of *Brucella abortus* strain 19 together with antifoam (Dow-Corning silicone antifoam A in technical light oil) and air is passed through at the rate of 250 ml./min. The whole apparatus is incubated in a hot room at 37° C. After 24 hr. growth a drip of 400 ml. of medium/24 hr. is started. The culture is harvested after 3–4 days, the cellophan tube then refilled with saline and fresh medium run into the annular space. Growth is more rapid in the second and subsequent cycles because of the large residue of actively growing organisms left from the previous run.

In order to avoid the development of mutants, the apparatus is steamed after four to six cycles by attaching a wash bottle of sterile distilled water to

Fig. 1. Apparatus for deep culture of *Brucella abortus*.

the harvesting nozzle *N* and boiling the water over a gas ring. In practice, there has been little tendency for rough variants to appear so long as there is no interruption to the air supply.

RESULTS

The results of eight successive batches are given in Table 1. Clearly, the number of organisms/ml. varied inversely as the volume harvested. This was due to concentration by dialysis since there was virtually no increase in the

Table 1. *Details of eight successive batches (28 harvests) of Brucella abortus strain 19 grown in aerated dialysed medium*

Batch	Cycle	Volume harvested (ml.)	Opacity by Brown's tubes	Yield of organisms (equivalent g. dry weight/l.)		No. of viable organisms/ml. harvest ($\times 10^9$)	Total no. viable organisms in harvest ($\times 10^{12}$)	No. viable organisms/g. dry weight organisms ($\times 10^{12}$)
				Yield of organisms (equivalent g. dry weight/l.)	Total yield of organisms in harvest (equivalent g. dry weight)			
4	1	850	350	28.9	24.6	496	422	17.2
	2	750	450	45.8	34.4	—	—	—
	3	400	800	77.2	30.9	1015 $\pm$ 32	406 $\pm$ 13	13.1 $\pm$ 0.4
5	1	510	350	36.5	18.6	448	228	12.8
	2	350	650	67.9	23.8	960 $\pm$ 64	336 $\pm$ 23	14.1 $\pm$ 1.0
	3	425	500	54.6	23.2	640 $\pm$ 48	272 $\pm$ 21	11.6 $\pm$ 0.9
	4	600	400	34.7	20.8	547 $\pm$ 48	328 $\pm$ 29	15.8 $\pm$ 1.4
	5	600	500	48.2	23.9	1080 $\pm$ 90	648 $\pm$ 54	22.4 $\pm$ 1.9
6	1	375	600	72	27	1156 $\pm$ 56	434 $\pm$ 21	16 $\pm$ 0.8
	2	500	500	43.9	22	509 $\pm$ 18	255 $\pm$ 9	11.6 $\pm$ 0.4
	3	500	640	—	—	824 $\pm$ 58	412 $\pm$ 29	—
	4	370	700	76.3	28.2	1021 $\pm$ 54	378 $\pm$ 20	13.4 $\pm$ 0.7
	5	580	350	29.5	17.1	562 $\pm$ 32	325 $\pm$ 19	19.0 $\pm$ 1.1
	6	350	750	75.9	26.6	1616 $\pm$ 93	565 $\pm$ 33	21.2 $\pm$ 1.2
7	1	500	520	58.3	29.2	858 $\pm$ 48	429 $\pm$ 24	14.7 $\pm$ 0.8
8	1	550	320	34.7	19.1	536 $\pm$ 39	295 $\pm$ 22	15.4 $\pm$ 1.1
	2	600	300	38.2	22.9	389 $\pm$ 32	233 $\pm$ 19	10.2 $\pm$ 0.9
9	1	460	450	48.9	22.5	691 $\pm$ 43	318 $\pm$ 20	14.1 $\pm$ 0.9
	2	460	500	49.4	22.8	698 $\pm$ 45	321 $\pm$ 22	14.1 $\pm$ 0.9
	3	500	480	46.2	23.1	909 $\pm$ 38	455 $\pm$ 19	19.7 $\pm$ 0.8
	4	420	500	50.1	21	811 $\pm$ 43	341 $\pm$ 18	16.2 $\pm$ 0.9
10	1	530	420	40.4	21.4	749 $\pm$ 58	397 $\pm$ 32	18.5 $\pm$ 1.5
	2	430	630	57.7	24.8	1280 $\pm$ 141	550 $\pm$ 52	22.2 $\pm$ 2.1
	3	455	480	45.4	20.7	883 $\pm$ 64	393 $\pm$ 29	18.9 $\pm$ 1.4
	4	430	500	54	23.2	672 $\pm$ 58	289 $\pm$ 25	12.5 $\pm$ 1.1
11	1	600	250	—	—	—	—	—
	2	450	540	64	28.8	970 $\pm$ 74	437 $\pm$ 34	15.1 $\pm$ 1.2
	3	240	1200	117	28.1	1797 $\pm$ 168	431 $\pm$ 40	15.3 $\pm$ 1.4

total number of viable organisms between 72 and 96 hr. An average expectation was a harvest of 500 ml. containing 7 to 10×10^{11} viable organisms/ml. equivalent to 50–70 g. dry wt. organisms/l. The average total harvest was $c. 400 \times 10^{12}$ viable organisms/batch. The fluctuation in the proportion of viable

organisms (column 9) was more apparent than real and reflected mainly variations in the efficiency of the medium on which the viable counts were made.

A harvest of 400×10^{12} organisms makes 6000–7000 doses of vaccine, and an average of six cycles/month can be maintained comfortably, so that 400,000 to 500,000 doses/year can be produced by one such apparatus which, with all its accessories, occupies only 4.5 sq.ft. of incubator floor space. The labour required is a fraction of that needed for producing similar amounts of vaccine by conventional methods.

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