Brucella abortus Strain 19 cultivated in Aerated Liquid Medium

AERATED liquid culture methods[1] have been evolved to produce *Brucella* vaccine for cattle at greatly reduced cost. The shake-flask technique was employed. Rectangular flasks of four-litre capacity, containing 450 ml. liquid culture, were agitated on a shaking machine at 64 excursions per min. of 12 cm. each. The medium[2] consisted of 3 per cent peptone, 3 per cent glucose, 1 per cent 'Marmite' (yeast extract), 0·16 per cent disodium hydrogen phosphate (mol. wt. 141·98), 0·03 per cent 'Antifoam' (polyoxethylene derivative of ricinoleic acid supplied by I.C.I., Ltd.) in tap-water; *p*H 6·4.

The medium was cleared through 'Speedex' filter powder over blotting paper and sintered glass (porosity No. 2), and sterilized by filtering through a 'Ford Sterimat' (Seitz *EK*) asbestos pad. The seed, a 48-hr. potato agar culture, suspended in buffered saline (0·021 *M* Na_2HPO_4; 0·045 *M* $NaH_2PO_4.2H_2O$; 0·017 *M* NaCl; *p*H 6·4) was introduced by siphoning through a hooded pipette over a flame to produce a viable count of 500,000,000 organisms per ml. (see Fig. 1).

The growth was harvested after shaking for 66 hr. at 37·5° C. in the dark with the flasks fixed crosswise at a slant of 1 : 50 upwards to the neck to allow for a semicircular reversing agitation. A bent hooded pipette was used to siphon the culture, containing at least 150 × 10^9 organisms per ml., into buffered saline diluent to make a vaccine suspension of about 30 × 10^9 organisms per ml. This was stored at 4° C. in 10-litre flasks while samples from each flask were being tested for *p*H, purity on culture, density, viable count, *S*- to *R*-variation and tetrazolium reduction. Bulked samples of each group of batches were tested biologically for safety and immunizing quality. After bottling, purity tests were repeated in a separate laboratory.

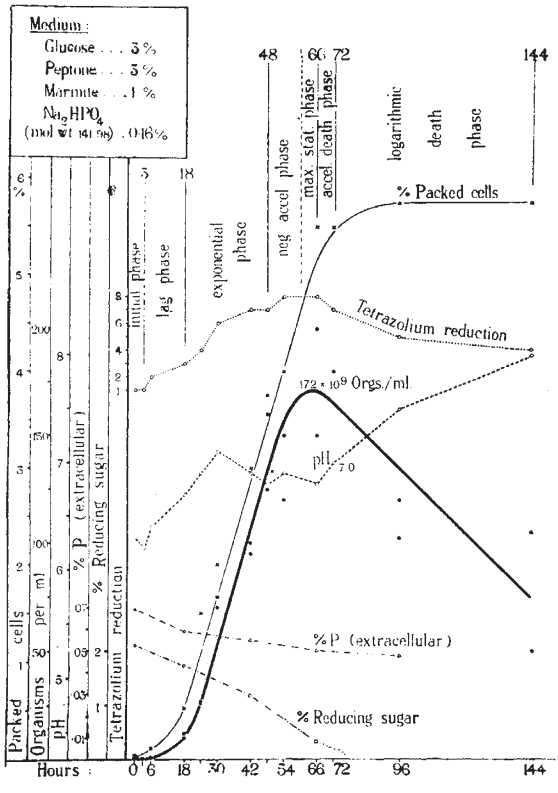

Fig. 1. Growth curve of *Brucella abortus* *S*.19 in shake-flask culture

The exponential phase of growth in this culture occurred between the twentieth and sixtieth hour of incubation (Fig. 1). *S*- to *R*-variation was not observed, and antigenicity tests consistently gave results identical with those of surface culture vaccine. Reactions produced in vaccinated animals were also identical. The output of the laboratory was increased tenfold by the adoption of this technique[3].

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Production of Plaques with Influenza Viruses

THE demonstration of the production of plaques, that is, necrotic foci, in tissue culture with the viruses of Eastern equine encephalomyelitis[1], Western equine encephalomyelitis[2], Newcastle disease[2], vaccinia[3], and poliomyelitis[4], offers additional quantitative applications to the study of these viruses. A necessary condition for the development of a plaque in tissue culture has been shown to be a destructive activity of the virus on the tissue employed. With the demonstration of a highly destructive effect of the *WSE* strain of influenza *A* virus on various chick embryonic tissues (9-day chorio-allantoic membrane, and 14-day lung and liver in roller tube cultures, unpublished results), experiments were undertaken to show production of plaques with this virus. Using the technique of Noyes[3], plaques averaging 0·5 mm. in diameter were obtained in five days on chick embryonic layer tissue cultures with a high dilution of the allantoic fluids used. Attention was then directed to a less heterogeneous source of tissue, chick embryo lung. Plaque formation was shown to occur readily with this tissue not only with the *WSE* strain of influenza *A* virus, but also with the other influenza virus strains tested.

Lungs from thirty-six 14-day chick embryos were removed, washed twice with Earle's salt solution (*BSS*) and pressed into approximately 30 ml. of *BSS* through a wire mesh strainer having 10 strands per cm. After standing for 10 min. at room temperature, the supernatant containing cell debris was discarded. The sediment was resuspended in 18 ml. *BSS* (0·5 ml. *BSS* per one set of lungs) and rapidly pipetted twenty times with a 10-ml. pipette. The suspension was allowed to stand for 10 min. at room temperature, during which time the large particles settled into the bottom 1–2 ml. This was discarded, and the procedure repeated twice. The resulting preparation consisted mostly of a single-cell suspension. The cell concentration was determined in a hæmocytometer and the suspension diluted with nutrient fluid (40 per cent horse serum heated to 60° C. for 30 min.; 20 per cent embryo extract (1 : 2 in *BSS*) made from 9-day