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Factors affecting the growth of virus of foot-and-mouth disease on surviving explants of bovine tongue epithelium

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INTRODUCTION

The large-scale cultivation of the virus of foot-and-mouth disease on explants of bovine tongue epithelium is limited, almost exclusively, to the method devised by Frenkel (1947) and modified by Frenkel & Ribelin (1956). Experience of this method led the authors to believe that the full potential of the system as a source of virus was not being realized. The yield of infective material often varied from culture to culture because many of the physical variables were neither clearly defined nor controlled.

Examination of published data shows that, in developing this method for production, small vessels were used which in no way resembled those subsequently employed for large-scale cultivation, so it could be argued that some reservation should have been made before applying the results of one to the other. Since a stirred system was in use at Pirbright for large-scale cultivation of the virus, attempts were made to develop a laboratory-sized experimental apparatus having a similar system of agitation and, proportionally, of the same dimensions so that it might be easier to simulate the conditions of the larger vessels.

Some years have elapsed since the following experiments were attempted. The findings were subsequently successfully applied to large-scale procedures and are now in regular use for preparation of inactivated vaccine.

APPARATUS AND MATERIALS

Culture equipment

A 2 lb. size straight-sided domestic fruit bottling jar* (Kilner Jar Improved Pattern—United Glass Ltd., London, S.W. 1) was used as the culture vessel. The vessel had a total volume of 850 c.c. and was of such dimensions that the ratio of the depth of liquid to the diameter of vessels was approximately unity (Cooper, Fernstrom & Miller, 1944) when the jar held 400 ml. of medium.

A $\frac{3}{8}$ in. hole was drilled in the bottom of the jar to admit the shank of a metal-mounted $\frac{5}{8}$ in. $\times$ $\frac{5}{8}$ in. Kieselguhr filter candle (British Berkefeld Ltd., Kent. Size 9a). The jars were annealed at 500° C. to eliminate stresses set up in the glass by drilling.

The glass cap was replaced by one of stainless steel, or cadmium plated brass,

* The Improved Pattern Kilner Jar has been superseded by the 'Dual Purpose' jar which is of slightly different shape. The alterations in dimensions are such as not to change significantly the ratio of depth of liquid/diameter when used as described in this paper.

machined so as to locate it centrally within the neck of the jar and fitted with a $\frac{1}{16}$ in. rubber gasket. The stirrer was flat bladed, a stainless steel strip 3 in. × $\frac{3}{8}$ in., fitted to a bush screwed to the end of a $\frac{1}{4}$ in. shaft. The shaft passed first through a graphite impregnated Ferrobestos journal bearing (J. W. Roberts, Lostock, Bolton), thence through a 3 in. × $\frac{1}{2}$ in. i.d. tube tightly packed with superfine glass wool (Fibreglass Ltd., St Helens, Lancs) and finally through a second graphite impregnated bearing, which was a press fit in the top of the glass-wool packed tube. The whole was located and held in position by a distance piece assembly. Two $\frac{1}{4}$ in. internal diameter stainless steel Schrader nipples were screwed into the lid to provide exhaust and inoculating or sampling ports (Pl. 1*a*).

A metal framework held three such bottles immersed in a thermostatically controlled water-bath 18 in. × 18$\frac{1}{2}$ in. × 8 in. (Grant Instruments Ltd., Cambridge). The bath was set in a simple scaffold constructed from slotted angle framework (Messrs Dexion Ltd., London, N.W. 6) on which was mounted a single-phase fractional horse-power motor geared to 60 r.p.m. (Hillman Electric Motors Ltd., London, N. 7), clips to locate small sterile glass-wool packed filters and a manifold to conduct away the exhaust gases to a pair of acid traps (Pl. 1*b*).

The assembled culture vessel together with the gas inlet filter and vent tubing was sterilized in an autoclave at 15 p.s.i. for 15 min.

Culture medium

The standard medium was Tyrode solution modified by increasing the sodium bicarbonate concentration from 0·1 % (w/v) to 0·2 % (w/v). Lactalbumin hydrolysate was added to a final concentration of 0·5 % (w/v). The medium was sterilized by E. K. filtration. Penicillin 450 i.u./ml., Neomycin 45 i.u./ml. and Nystatin 20 i.u./ml. were added immediately before use.

When aeration was in excess of 60 cm.^{3}/l./min., 5 ml. of 10% (v/v) suspension of antifoam (MS Antifoam R.D., Midland Silicones Ltd.) in sterile saline was added per 400 ml. of culture medium to suppress foaming.

Bovine tongue epithelium

Whole cattle tongues were obtained from local abattoirs within 2 hr. of slaughter. Preparation of the tongues and method of removal of the epithelial tissue was as described by Frenkel (1954).

Keratinous tissue was removed in a slice approximately 100 μ thick and was discarded. The underlying tissue was collected in slices 75–100 μ thick and stored in normal Tyrode solution at 4° C. until required for use.

Using this technique, 20–25 g. of epithelial tissue slices was obtained from each tongue.

Virus

Except where otherwise stated, the strain of virus used was 'Dutch O' type O, which originated from the Dutch State Veterinary Laboratories where it is used as a strain for vaccine production. It was well adapted to tongue epithelial culture, having been passaged serially over 32 passages when received and a further 40 times during the current work.

Virus assay

Tissue/liquid extracts from cultures were diluted by fivefold steps in 0·04 M phosphate buffer at pH 7·6. Each dilution was inoculated intraperitoneally (Skinner, 1951) into groups of six unweaned Pirbright strain mice (Subak-Sharpe, 1961). The Spearman–Kärber method of computation was used to calculate the 50 % end-point dilution. The fiducial limits of the test described above are $\pm 10^{0.36}$ (Subak-Sharpe, 1961).

Standard culture procedure

For comparative experiments, epithelial tissue slices from several tongues were pooled, finely minced with scissors and weighed out into equal portions of 32 g. Each culture vessel was charged with 400 ml. of the standard culture medium containing antibiotics, with or without antifoam, followed by the minced tissue. The vessels were immersed in the water-bath at 37° C., stirred at 60 r.p.m. and aerated with a mixture of 5 % carbon dioxide in oxygen at a rate of 50 cm.^{3}/min. (125 cm.^{3}/l./min.) for 30 min. A measured volume of seed virus of known infectivity was added to each vessel through the inoculation port. The cultures were usually incubated for 16–18 hr.

Tissue harvested from a culture was ground with sand in a mortar, resuspended in its own culture medium, centrifuged, E. K. filtered and diluted for assay of infectivity.

Estimation of glucose

Harding's modification (Harding, Nicholson, Grant, Hern & Dowes, 1932; Harding & Downs, 1933) of the Schaffer–Hartmann method was used. It consists of re-oxidation of the glucose reduced alkaline copper with iodine and estimating the amount of iodine used with sodium thiosulphate.

Two ml. of a centrifuged sample of the culture medium was used for estimation. A 'blank' test was always included.

RESULTS

Hydrogen-ion concentration

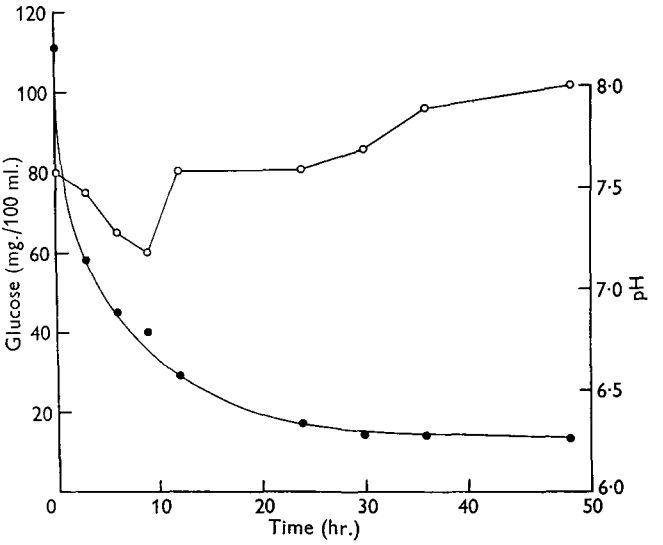
Using the culture technique as described by Frenkel & Ribelin (1956), considerable variation of the reaction of the medium was possible. Values as low as pH 6·8 have been recorded (Frenkel & Ribelin, 1956), pH 7·0 has been reported by Henderson (unpublished data) and pH 7·0–7·2 has been found by us. It is, however, recognized that the pH range for maximal survival of infective virus is as narrow as pH 7·5–7·7 and that, particularly at 37° C., relatively small changes in hydrogen-ion concentration are of great importance.

When oxygen was substituted for 5 % carbon dioxide/oxygen, the pH of the cultures at 18 hr. was increased from approximately pH 7·0 to pH 7·2–7·3, resulting in slightly better yields of infective material; but, if the flow rate of oxygen were increased beyond 40 cm.^{3}/l./min., the pH value rose above pH 8·0 and the yield of infective material was low.

Examination of a system aerated with oxygen at 25 cm.^{3}/l./min. (Text-Fig. 1) showed a rapid fall in pH during the first 9 hr. of cultivation followed by a rise to

pH 7·6 in 24 hr., thence to pH 8·0 in 48 hr. Simultaneously, there was rapid utilization of glucose during the early part of culture—55 % utilized during the first 5 hr., whereas only a further 10 % was used during the next 5 hr. After 24 hr. the amount of glucose remaining was 17 % of the original value.

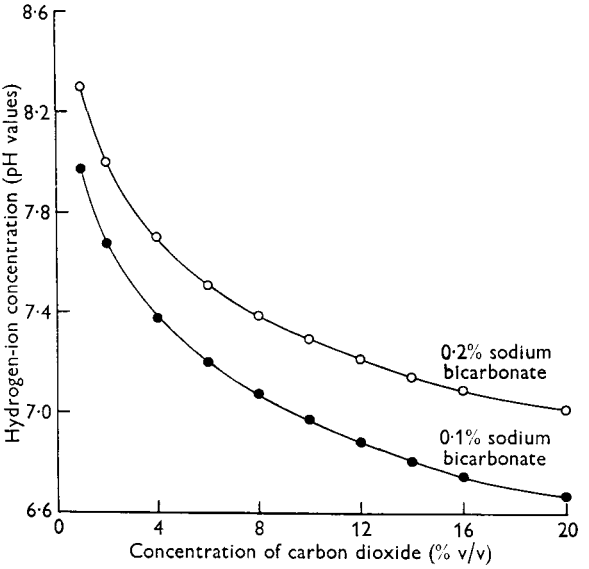
The initial rapid fall in pH was attributed to rapid evolution of carbon dioxide by respiring cells. Agitation and aeration at this stage was apparently insufficient to control the reaction of the medium.



Text-fig. 1. Rate of glucose utilization and pH change in culture medium containing 0·1 % sodium bicarbonate aerated with oxygen. pH, O—O; glucose, ●—●.

The hydrogen-ion concentration of a bicarbonate-carbon dioxide buffered system such as Tyrode solution at stated temperature and pressure is dependent on the relative concentration of bicarbonate and carbon dioxide. A 0·1 % solution of sodium bicarbonate ($1{\cdot}19 \times 10^{-2}$ M) aerated with 5 % carbon dioxide at 37° C., as defined by the Henderson–Hasselbalch equation, is pH 7·3, and the reaction of the system is fixed so long as these conditions are maintained. Since a value within the range pH 7·5–7·7 was required, the concentration of sodium bicarbonate only was adjusted to 0·2 % (w/v) ($2{\cdot}38 \times 10^{-2}$ M), thus giving a smaller swing of pH in the face of increased concentrations of carbon dioxide (Text-fig. 2). It was also decided to aerate vigorously with 5 % carbon dioxide in oxygen to attempt to carry off carbon dioxide evolved in the medium and maintain values close to 5 %, particularly when glucose was being most actively metabolized.

When the standard culture medium containing 0·2 % (w/v) sodium bicarbonate and 8 % (w/v) tongue epithelial fragments was aerated with 5 % carbon dioxide in oxygen at 250 cm.^{3}/l./min. at 37° C., a hydrogen-ion concentration of pH 7·6 was maintained throughout the 7 hr. of incubation (Table 1), indicating that the concentration of carbon dioxide may be controlled within acceptable limits without major alteration to the existing culture technique.



Text-fig. 2. Hydrogen-ion concentration of solutions of bicarbonate at 37° C. aerated with varied concentrations of carbon dioxide.

Table 1. *Utilization of glucose by tongue epithelial fragments* 8 % (*w/v*)

Time (hr.)	pH	Conc. glucose (mg./100 ml.)	Corrected glucose values (mg./100 ml.)
0	7·6	95	100
0·5	7·6	96	96
1·0	7·6	83	89
1·5	7·6	89	85
2·0	7·6	79	80
2·5	7·6	78	74
3·0	7·6	72	69
3·5	7·6	70	64
4·0	7·6	60	62
4·5	7·6	64	58
5·0	7·6	52	54
5·5	7·6	55	50
6·0	7·6	44	47
6·5	7·6	41	45
7·0	7·6	38	42

Aeration

Efficient aeration will depend upon the geometry of the vessel, degree of agitation and gas flow rate (Cooper *et al.* 1944). In a vessel of standard design increased gas solution rates are obtained by shear action of a high-speed impeller in a baffled vessel or by use of a device to reduce bubble size when slow stirrer speeds are involved (Finn, 1954). Equipment already available was of the second type so that aerators examined included single holed spargers of approximately 1·0 mm.

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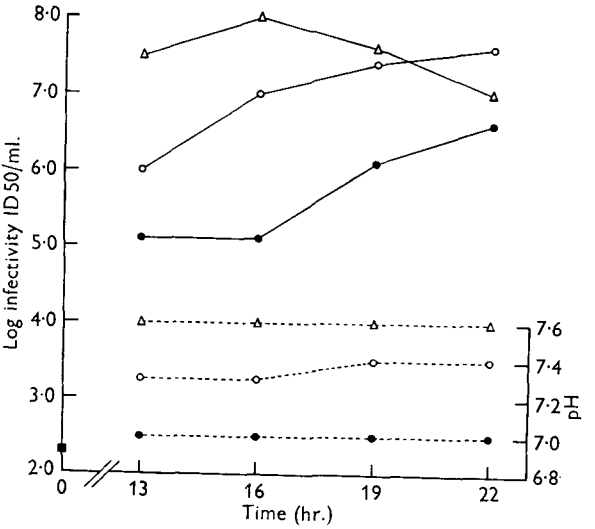
orifices, sintered stainless steel gauze 8–10 μ porosity (Hixon & Gaden, 1950) and standard grade Berkefeld bacteriological filter candles. The latter proved to be superior, providing a sulphite oxidation rate of 30 mmoles O_2/l./hr. at 20° C. from an oxygen flow rate of 125 cm.³/l./min. Both of these conditions were found to be consistent with control of hydrogen-ion concentration and increased virus production.



Text-fig. 3. Effect of bicarbonate concentration and aeration upon the growth of Dutch 'O' virus. △—△, 0·2 % sodium bicarbonate, 5 % CO_2/O_2 125 cm.³/l./min. Berkefeld candle aerator; ○—○, 0·2 % sodium bicarbonate, 5 % CO_2/O_2 17·5 cm.³/l./min., 0·5 mm. sparger; ●—●, 0·1 % sodium bicarbonate, oxygen 17·5 cm.³/l./min. 0·5 mm. sparger; ---, pH values; ■, infectivity of starting material.

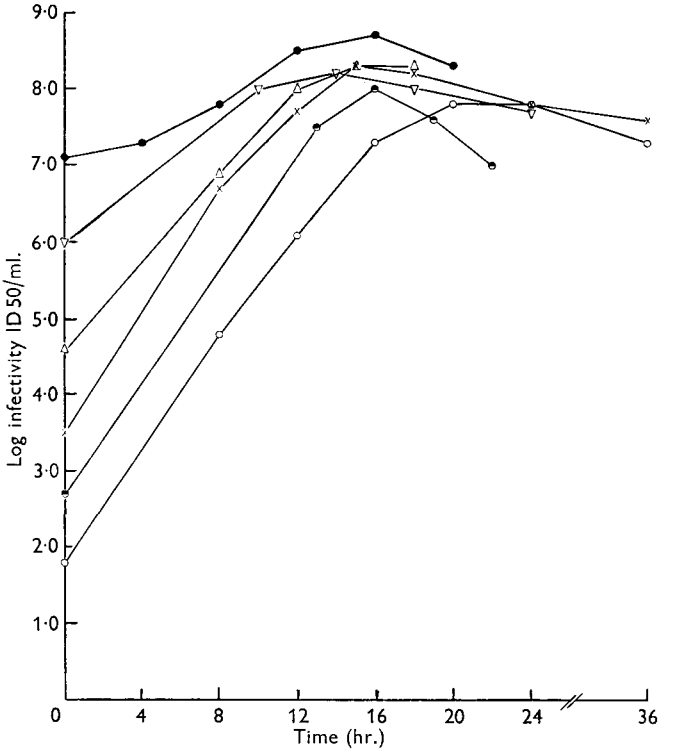
The effects of hydrogen-ion concentration and aeration upon infective virus yield is shown in a series of cultures inoculated with the same amount of seed virus (Text-fig. 3) contrasting the older method of cultivation and the modified technique with higher gas solution rate. As may be expected, the yield of virus is enhanced when the hydrogen-ion concentration is maintained at a level more conducive to its survival. It is also seen that the peak titre appears sooner under improved conditions of aeration and pH control.

It is difficult to separate the effects due to pH control and supply of oxygen but it is likely that the rate of oxygen utilization is independent of the rate of supply, since the yield of infective material may be maintained by substitution of 5% carbon dioxide in air. When nitrogen is substituted for air or oxygen in the gas mixture, virus does not multiply and the hydrogen-ion concentration is not controlled to the same extent as in aerobic cultures.

The difference may be due to fermentation on the one hand and respiration on the other, with predominant production of lactic acid and carbon dioxide respectively (Dickens, 1959). This avenue has not been investigated but rather used as a case for good aerobic conditions within the culture system (Baron, Porterfield & Isaacs, 1961).

Table 2. *Anaerobic cultivation of the virus of foot-and-mouth disease*

Time (hr.)	Glucose (mg./100 ml.)	pH	Infectivity (ID 50/ml.)
0	100	7·6	$10^{4·6}$
16	17	7·3	$10^{9·5}$
24	13	7·2	$10^{8·8}$
40	12	7·3	$10^{8·5}$



Text-fig. 4. Effect of inoculum upon rate of growth of Dutch 'O' virus.

Inoculum

The peak of infectivity has been reported as occurring after 18–24 hr. of culture (Henderson, 1953); Frenkel reports a mean incubation period of 19·2 hr. (Frenkel & Ribelin, 1956). After this point the infectivity titre wanes at a rate depending upon the strain of virus (Henderson, 1953). In our experience the infectivity approximates closely to its peak value after 16–18 hr. incubation (Text-fig. 4), although the range may be as wide as 14–20 hr. in cultures originating from inocula at the higher and lower extremities of the inoculum scale, respectively.

Under standard conditions of culture and time of harvest, the relationship between the infectivity of the starting and final materials is quantitative (Text-fig. 5).

This relation between titres of inoculum and harvested material applies to other strains of virus adapted to epithelial culture. Table 3 shows a number of stock

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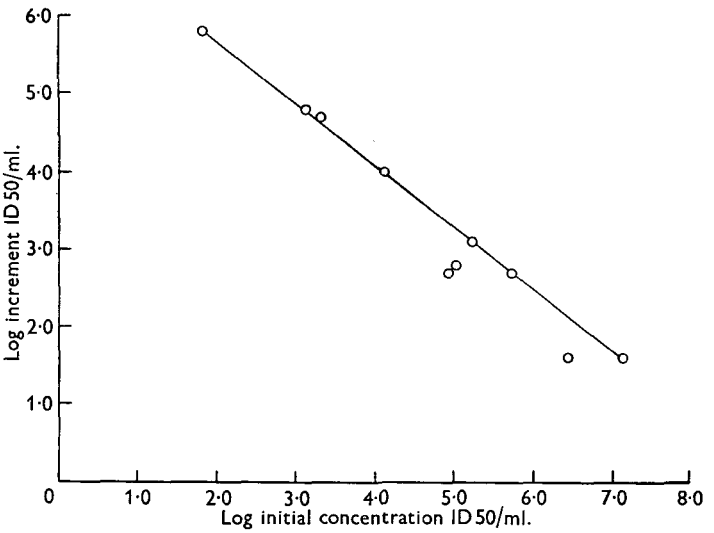
strains passaged routinely for use in the laboratory. Although, with one exception, the initial concentrations of infective material are of a similar order, the amount actually harvested is closely related in each case to the predicted amount calculated from Text-fig. 5.

The ability to be able to forecast the amount of infective material which could be obtained from a particular culture was used to indicate whether a virus had been adapted to this form of cultivation. Any disparity suggested that the virus strain was not so modified.



Text-fig. 5. Relationship between inoculum and material harvested after 16–18 hr. incubation at 37° C.

Table 3. *Relationship between inoculum and harvest infectivity titres*

Virus type	Titre at 0 hr. (ID 50/ml.)	Culture period (hr.)	Titre of harvest (ID 50/ml.)
O ('Dutch O')	$10^{6\cdot1}$	16	$10^{8\cdot3}$
C (C 997)	$10^{6\cdot4}$	16·5	$10^{8\cdot3}$
C (C 997)	$10^{5\cdot7}$	16	$10^{8\cdot1}$
C (U/C/54)	$10^{6\cdot0}$	18	$10^{8\cdot2}$
SAT 1 (RV. 11)	$10^{6\cdot1}$	15	$10^{8\cdot1}$
SAT 1 (Bec. 19/60)	$10^{6\cdot0}$	16·5	$10^{8\cdot0}$
SAT 2 (Ken. 3/57)	$10^{6\cdot8}$	17	$10^{8\cdot5}$
ASIA 1 (Pak 1)	$10^{5\cdot8}$	15	$10^{8\cdot3}$
ASIA 1 (Pak 1)	$10^{6\cdot1}$	16	$10^{8\cdot3}$

The volume of inoculum to produce a high initial infectivity is relatively easily obtained for small-scale cultures but in serial large-scale cultures the same proportional volume would involve material which might represent a large amount of inactivated vaccine. In the latter case, experience has shown that the most economical starting virus titre is approximately $10^{6\cdot0}$ID 50/ml., which will yield

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$10^{8\cdot2}$ID 50/ml. in 16–18 hr. of culture. Assuming serial batch cultures, this would mean reservation of 1 % of the harvested material in each successive passage or 1 l. of seed at $10^{8\cdot0}$ID 50/ml. to a culture of 100 l.

Tissue

Slices of epithelium removed from bovine tongues by means of a Berkel bacon slicer vary in the number and size of susceptible cells, depending upon the level in the epithelial layer from which they are removed. Tissue immediately above the connective tissue layer is most susceptible (Brooksby & Wardle, 1954) and keratinous tissue not at all. It has therefore been general practice to discard a slice of keratinous tissue some 100 μ thick and to collect the underlying tissue in slices 75–100 μ thick down to, but not including, the musculature.

In small-scale experiments the effect of thickness is not always evident, since the epithelial slices are finely minced with scissors to make them more manageable in culture (Table 4).

Table 4. *Cultivation of virus of foot-and-mouth disease in finely clipped tissue*

Average thickness (μ)	Titre at 0 hr. (ID 50/ml.)	pH	Titre of harvest (ID 50/ml.)
Thin, 100	$10^{3\cdot4}$	7·6	$10^{7\cdot7}$
Medium, 500	—	7·6	$10^{7\cdot9}$
Thick, 1000	—	7·6	$10^{7\cdot7}$

Table 5. *Cultivation of the virus of foot-and-mouth disease in coarsely clipped tissue*

Average thickness (μ)	Titre at 0 hr. (ID 50/ml.)	pH	Titre of harvest (ID 50/ml.)
Thin, 100	$10^{3\cdot4}$	7·6	$10^{7\cdot7}$
Medium, 500	—	7·5	$10^{7\cdot3}$
Thick, 1000	—	7·5	$10^{7\cdot1}$

But when the sliced tissue is coarsely minced, approximately $\frac{1}{4}$ in. squares, the difference is more apparent (Table 5).

In large-scale cultivation it is not easy to reduce the tissue slices to small pieces by clipping because a machine of special design (Frenkel, 1951) is required. For cultures of the order of 100 l., considerable time is consumed in clipping a batch of tissue slices and added to this there is the risk of bacterial contamination during the process. It has been shown (Frenkel, 1954) that thinly sliced tissue, in long strips as removed from bovine tongue, is as effective in promoting virus growth as thicker slices when clipped. Strips of tissue, despite their thinness, may clog exit ports of large-scale culture vessels during harvesting procedures. Because of this we examined the effect of mincing epithelial slices by means of a domestic mincing machine. In Table 6 the virus yield from tissue in various states of division is shown; in these experiments tissue was sliced and then bulked before

further manipulation. Despite probable additional cell damage caused by compression as well as cutting, the relative yields of virus justify the method. Mincing tissue through a sterile, electrically operated butcher's mincing machine (Hobart Ltd., London N.) has been adopted here, using a $\frac{5}{16}$ in. cutting die. Tissue treated by this method will not block the outlet port and is still of such a size as to be separated from the culture medium during harvesting by means of a stainless steel gauze sieve (18-mesh).

Table 6. *Cultivation of virus of foot-and-mouth disease in tissue of various states of division*

(Infectivity titres of tissue/liquid extracts after cultivation for 16 hr. expressed in ID 50/ml.)

	Whole slices	Clipped slices	Minced slices
1	$10^{8\cdot2}$	$10^{8\cdot9}$	—
2	—	$10^{8\cdot1}$	$10^{7\cdot9}$
3	$10^{7\cdot8}$	$10^{6\cdot9}$	$10^{7\cdot8}$
4	$10^{7\cdot9}$	—	$10^{7\cdot8}$
5	$10^{7\cdot7}$	$10^{8\cdot0}$	$10^{8\cdot0}$

The time which elapses between removal of the epithelium from cattle tongues and its use in culture may be of importance in yielding material of high infectivity, particularly in large-scale culture. Tissue for small-scale cultures can be kept for 24 hr. or so under favourable conditions with respect to its concentration in the suspending fluid and reaction of the medium (Table 7).

Table 7. *Effect of storage of bovine tongue epithelial tissue (small scale) upon virus yield, 8 % suspension (w/v) in normal Tyrode solution*

(Infectivity titres of tissue/liquid extracts after cultivation for 16 hr. expressed in ID50/ml.)

Temp. of storage (° C.)	Length of time stored (hr.)			
	0	18	24	72
37	$10^{8\cdot3}$	—	$10^{7\cdot3}$	$10^{6\cdot4}$
37	$10^{8\cdot0}$	$10^{8\cdot3}$	—	—
4	$10^{8\cdot0}$	$10^{7\cdot8}$	—	—

In the case of tissue for large-scale cultivation where considerable distance may separate supplier and user (Frenkel, 1953) and where, for economy of space and transport, the tissue is collected in jars of Tyrode's solution at many times the concentration it would be used at during cultivation of virus, there is evidence of reduced viability after 24 hr. storage at 4° C. (Table 8).

Table 8. *Effects of storage of bovine tongue epithelial tissue (large scale) upon virus yield, 50–60 % suspension (w/v) in normal Tyrode solution*

(Temperature of storage 4° C.)

Virus type and strain	Age of tissue (hr.)	Initial virus titre (ID 50/ml.)	Titre of harvest (ID 50/ml.)
SAT 1 (Bec. 19/60)	24	$10^{5\cdot8}$	$10^{7\cdot3}$
SAT 1 (Bec. 19/60)	3–8	$10^{6\cdot0}$	$10^{8\cdot1}$
SAT 1 (RV. 11)	24	$10^{5\cdot8}$	$10^{7\cdot5}$
SAT 1 (RV. 11)	4–12	$10^{6\cdot0}$	$10^{8\cdot3}$

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Adaptation

Adaptation of virus from a field sample is not a difficult problem if a sizeable sample of infected tissue is available. More commonly, field material may consist of a very small quantity of material of low infectivity.

The procedure adopted with such a sample is to prepare an EK filtrate of a $\frac{1}{2}$–$\frac{1}{5}$ (w/v) suspension in 0·04 M phosphate buffer, pH 7·6. The filtrate is inoculated intraperitoneally into unweaned mice 5–7 days old and also used as seed for a tongue tissue culture. The virus is taken from one or the other and is passaged serially in culture. If after 4–6 passages material of $10^{7\cdot5}$–$10^{8\cdot0}$ ID 50/ml. has not been obtained, the culture material should be passaged once again through mice and thence to culture. Normally one mouse passage is sufficient to render the virus amenable to culture but in a few cases a second may be found necessary.

DISCUSSION

Using a laboratory-sized culture equipment of particular design to improve gas solution rates and gas 'hold up' (Finn, 1954) in an examination of the bicarbonate–carbon dioxide buffer system, it has been possible to control the hydrogen-ion concentration throughout the period of culture and, by suitable adjustment in conjunction with other defined variables, to increase the output of infective material approximately tenfold.

There was no evidence of major variation between batches of tissue when freshly collected since it was possible to predict, fairly accurately, the expected yield of virus under defined conditions of culture and within experimental error. Although it was possible to store tissue for a considerable length of time without apparent deterioration in susceptibility, when large amounts of tissue are transported over long distances it is difficult to arrange optimum conditions. Reduction of the time interval between collection and use may be necessary to compensate for these difficulties.

The higher concentration of bicarbonate in conjunction with 5 % carbon dioxide in oxygen provided good pH control during cultivation of the virus, in addition to damping the pH swing at higher concentrations of carbon dioxide. But it is seen that the slope of the curve increases steeply as the values of carbon dioxide fall below 5 %. The increase towards the alkaline side (i.e. > pH 7·8) is not desirable when harvesting large-scale cultures because of the length of time the virus may be exposed to unsuitable pH conditions. We have minimized such a rise in routine large-scale procedure by emptying the culture vessel under pressure of 5 % carbon dioxide in oxygen and by bulking with the filtrate the filter washings, consisting of about one-third of its volume of 0·04 M phosphate buffer, pH 7·6. The pH of harvested medium and the virus filtrate is thus maintained at approximately pH 7·8.

Aerobic conditions of culture appear to be necessary for virus multiplication. This has been shown in the case of other viruses (Baron *et al.* 1961; Taylor-Robinson, Zwartow & Westwood, 1961). The oxygen gradient within the thicker slices (Minami, 1923) may be partly responsible when such slices are employed.

The concentration of glucose had little effect on virus production but did indicate cell viability. Current experiments indicate that similar virus yields may be obtained, under certain conditions, in the absence of glucose.

In trying to assess whether epithelial tissue produced virus to maximal capacity, it was estimated, by repeated trypsinization of sliced and minced tissue, that there were $10^{8\cdot0}$ cells/g. Under optimal culture conditions the virus output was $10^{9\cdot6}$ ID 50/g. of tissue which, if attributable to the whole tissue mass, amounted to 40ID 50/cell. This figure is lower than that found when cultivating the virus in other cell systems (Sellers, 1959; Patty, Bachrach & Hess, 1960). If it is assumed that most of the virus is produced by cells immediately above the musculature (Brooksby & Wardle, 1954), which is about one-fifth of the total weight used in this technique, a value of similar order to that obtained in the case of cell cultures is achieved. The technique may thus be considered to be no less efficient than others and the inclusion of so much relatively inert matter is justified only by the simplicity and rapidity with which the whole may be collected and the uncertainty of how much epithelial matter may be discarded without detriment to the virus yield.

The standard culture procedure outlined earlier in this text has been applied to large-scale culture procedures for production of inactivated vaccines. It was possible to prepare two million doses of SAT 1 vaccine during the winter of 1961 for an outbreak of foot-and-mouth disease in South-West Africa (Galloway, 1962).

SUMMARY

A simple, laboratory scale, stirred system has been developed to examine conditions of culture necessary for optimum return of infective virus from explants of surviving bovine tongue epithelium, with particular bias towards large-scale procedures.

Control of hydrogen-ion concentration and aeration of the culture are emphasized.

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EXPLANATION OF PLATE

(*a*) The 400 ml. Kilner jar culture vessel assembled ready for sterilization. Note the gas inlet filter fitted to the open-ended rubber tube.

(*b*) Three 400 ml. culture vessels housed in the complete culture assembly. The individual gas metering system is seen on the left and the acid traps for dealing with the exhaust gas on the right.