TITRATION OF THE VIRUS OF FOOT-AND-MOUTH DISEASE IN CULTURE

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(With Plates 1—3 and 3 Figures in the Text)

The method devised by Frenkel (1947) for the growth of the virus of foot-and-mouth disease in surviving epithelial tissue from the tongues of cattle has been applied mainly to the propagation of virus for routine vaccine production. The technique, however, has a large number of possible applications to research on the virus. For example, Henderson (1953), in this Institute, has used the culture method, with slight modifications, in studies on the growth and multiplication of the virus. The present paper describes a method of titration of the virus of foot-and-mouth disease in which tissue collected by Frenkel's method is used to support the growth of virus to a stage when a complement-fixation test can serve as an indicator of virus multiplication.

This technique is similar to those which have been applied recently to some other viruses (Huang, 1943; Fulton & Armitage, 1951; Ledinko, Riordan & Melnick, 1951). All are based on the inoculation of serial dilutions of a virus suspension into suitably large numbers of small samples of tissue in a buffered salt solution. Multiplication of virus is indicated by alterations in the culture medium such as changes in pH, by pathological changes in the tissue or by the results of haemagglutination or complement-fixation tests. Calculation of the titre, in terms of the 50% positive end-point dilution, is then based on the numbers of positive and negative observations at the various dilutions tested.

In the early experiments the virus cultures were incubated in 30 ml. screw-capped bottles and complement-fixation tests were made on samples of the supernatants from each bottle. When it was found that the complement-fixation test could be carried out on the whole culture without removing the tissue from the medium, bottles were abandoned in favour of cups in Perspex plates. The plate technique is much less laborious and saves the washing-up of culture bottles and the tubes used in the complement-fixation tests. It is therefore the method of choice for routine titrations, and, although some of the earlier experiments described below were made using bottles, only the plate technique will be described in detail.

METHODS

Perspex plates and lids. The Perspex plates* used are from the same mould as those made for the W.H.O. influenza centre at the National Institute for Medical Research at Mill Hill, where they are used for haemagglutination tests. They

* The Perspex plates are obtainable from Messrs Prestware Ltd., Lombard Road, London, S.W. 19.

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contain eight rows of ten round-bottomed cups each 1·5 cm. in diameter and 1 cm. deep. Under the conditions of shaking used, 0·6 ml. can be safely accommodated in each cup without spilling, but the total volume during the culture of virus is 0·3 ml. Several methods of preventing evaporation during incubation have been tried. It is necessary to leave a gap between any covering and the surface of the plates to prevent movement of the condensate by capillarity. Perspex lids have been made from $\frac{1}{4}$ in. sheet with an overhanging ridge of Perspex strip at the edge to locate the lid on the plate. A rubber washer $\frac{1}{8}$ in. thick and $\frac{1}{8}$ in. wide serves to keep the lid from the surface of the plate. The plates can then be sealed by suitable metal clamps and thumb screws, but a more convenient arrangement is to hold the lid in position with rubber bands and insert the plate in a bag made from polythene tubing ('Layflat' polythene tubing 8 in. wide and 0·003 in. thick, BX Plastics Ltd., Chingford, London) which is readily sealed by heat. As a further precaution against evaporation a small piece of wet filter-paper can be included with the plate in the sealed bag (Plate 1). After incubation the bags are opened by cutting away the sealed end with scissors. If the bags are cut to a larger size than necessary they can be re-used many times. They can be sterilized by boiling. This method of sealing is less cumbersome than the use of metal clamps and allows the plates to be stacked more easily in the incubator or refrigerator.

After use the plates and lids are sterilized in a tank containing 1·0 % HCl. They are then washed in tap water, rinsed with distilled water and dried in a desiccator over H_2SO_4. Before use the plates and lids are exposed for 30 min. to ultraviolet light in a cabinet. The necessary solutions and minced tissue are added in the same cabinet and the lids placed on the plates. Inoculation of virus dilutions is made on the laboratory bench using a flat metal box open at the ends and with a central slit wide enough to expose one row of ten cups. The plates are inserted at one end of the box, and each row is inoculated in turn as the plates are pushed through (Plate 2). Exposure of the plates for the minimum time during inoculation appears to be an important factor in reducing chance contamination with moulds. When moulds do occur the plates are treated with 10 % HCl before being used again.

Shaking machines. The machine used for agitation has been designed to hold twelve plates in a rack which oscillates horizontally through a stroke of 1 in., at 120 cyc./min. This movement is preferred to rocking as all cups are subjected to the same agitation. When bottles were used for the titration, rotators driven at speeds of from 1 to 12 rev./min. were found to be satisfactory, and such rotators are still used for the growth of the stock virus in culture. It would seem that the degree of agitation is not critical. Incubation and agitation are continued for about 65 hr. at 37° C.

Medium. The modification of glucosol (Parker, 1938) used by Fulton & Armitage (1951) in their work on the titration of influenza virus has been successfully adopted for the virus of foot-and-mouth disease. It consists of: NaCl, 8·0 g.; $CaCl_2$, 0·2 g.; $MgCl6H_2O$, 0·5 g.; glucose, 1·0 g.; glass-distilled water to 1000·0 ml. This solution is buffered by the addition of an equal volume of M/25 phosphate buffer, pH 7·6 which contains: (i) $Na_2HPO_42H_2O$, 7·126 g./l.; (ii) KH_2PO_4, 5·446 g./l. These solutions are mixed in the proportion of six parts of (i) to one of (ii). Both solutions are

autoclaved at 15 lb. pressure for 20 min. and stored at 4° C. The glucosol-phosphate mixture is prepared before each experiment and sufficient penicillin (benzyl-penicillin B.P.) is added to give a concentration of 1000 units/ml.

One ml. pipettes are used to add 0·2 ml. of glucosol-phosphate to each cup on the plates, but for the smaller volumes (0·1 ml.) of virus dilutions, serum, complement and sensitized cell suspension, 0·05 ml. dropping pipettes are used.

Tissue. The tissue is collected from bovine tongues obtained from a slaughter-house 5 miles distant. Less than 2 hr. elapse from the death of the animal till the collection of the tissue and it is essential that rigor mortis should not have occurred. Indeed the fresher the tongue, the easier the subsequent operation. The method of removal of the outer keratinized layer of epithelium is that described by Frenkel (1947) in which the layer is held in a clamp and rolled off from the surface. From the exposed surface, which is covered with cells of the basal layers of epithelium, tissue is then sliced off by means of a safety-razor blade in a holder.

The tissue slices removed on the razor blade are placed in a sterile Petri dish and the pooled material from two or more tongues is minced with sterile scissors to give pieces of not more than about 1 mm. cube. The minced tissue is washed once with glucosol-phosphate on the centrifuge, and then distributed among cups on the plates. From 12 to 16 mg. of tissue are placed in each cup. Some variation (twofold in either direction) in the amounts of tissue does not appear to affect the result. About 3 g. of tissue can be obtained from one tongue and this is sufficient for approximately 200 cups.

Virus dilutions. The dilutions of virus are prepared in glucosol-phosphate solution. The tenfold series used in most of the experiments is prepared by pipetting 0·4 ml. into 3·6 ml. of the diluent. A fresh pipette is used to mix the diluted sample and to transfer the required 0·4 ml. to the next bottle in the series. The dilutions are always prepared within 30 min. of the inoculation of the cultures, except in the case of serum-neutralization tests, when time is allowed for the neutralization to proceed.

Complement, antiserum, sensitized cells. These reagents are all as described in a recent report from this laboratory (Brooksby, 1952). A suitable dilution of complement sufficient to give 100% haemolysis in the presence of serum or tissue is used. Usually 0·1 ml. of a 1/50 dilution of the lyophilized complement prepared in this laboratory is suitable. The antiserum appropriate to the type of virus being titrated is added as 0·1 ml. of a 1/8 dilution. The plate is then laid on a tray in a water-bath at 37° C. for 30 min. 0·1 ml. of 1·7% suspension of maximally sensitized sheep cells is then added, and incubation continued for a further 45 min.

The reading of the results can be made immediately the plate is removed from the bath (Plate 3) or, alternatively, after holding it at 4° C. for 4 or more hours, when sedimentation of the non-haemolysed cells makes the detection of haemolysis easier. There are few doubtful reactions when the dose of complement has been well chosen, and negative cups showing haemolysis are readily distinguished from the clear positives.

RESULTS

Many of the earlier experiments directed at establishing the method throw light on the culture of virus in general, and they are therefore reported in some detail before the results obtained with the method as currently employed.

Tissue. The collection of the tissue is probably the most important factor governing the validity of the results. In general, Frenkel's (1947) procedures have been employed in removing the tissue from the tongue. The actual layer of tissue used, however, has been varied in a number of experiments. When the flap of epithelium is rolled from the tongue, the lower layer remaining on the tongue is papillated and brownish pink in colour, unless the tongue is naturally pigmented. This layer is sliced off leaving a surface of fibrous tissue overlying the musculature. The layer sliced off will be referred to as the inner layer. The flap rolled off can be spread out and a similar layer of tissue removed from its under side—the inner layer of the flap. The remaining portion of the flap will also support virus growth.

Table 1. *Susceptibility of layer of tissue used*

Expt.	Tissue	Apparent titre of filtrate used
1	(*a*) Inner layer and inner layer of flap	$10^{-3\cdot2}$
	(*b*) Flap after removal of inner layer	$10^{-0\cdot5}$
2	(*a*) Inner layer	$10^{-4\cdot0}$
	(*b*) Inner layer of flap and fibrous tissue below (*a*)	$10^{-3\cdot4}$
3	(*a*) Inner layer	$10^{-5\cdot0}$
	(*b*) Inner layer of flap	$10^{-3\cdot5}$

Three experiments have been made to compare the suitability of these layers for titrations. In each case the tissue was obtained from two tongues and the samples from the appropriate layers pooled and minced together. Titrations were then set up using ten observations per dilution in a tenfold series, and the apparent titres of the virus filtrates used in each experiment were calculated. The results are shown in Table 1.

From these experiments it appears that the more superficial layer of the flap is of low susceptibility and that the inner layers representing the samples of epithelium nearest the basal membrane is of greatest susceptibility. There is some correlation between this observation and the macroscopic appearance of the minced tissue. The inner layer and the portion of the flap immediately adjacent are much more friable than the parts nearer the surface of the tongue. The mincing of this friable tissue can be done more efficiently than that of the hard outer layers, and it is reasonable to assume that, apart from probable differences in the susceptibility of the individual cells, the exposure of the cells to the virus is more readily achieved in the softer tissues. The results obtained agree in general with those of Frenkel & Dunne (1952) in which the maximum titres reached in the various layers were measured.

Use of stored tissue. In three experiments, the same virus filtrate has been titrated on successive occasions in samples of the same tissue. Deterioration of the filtrate would not be expected in the first 4 days after preparation, but it is

possible that the sample titrated at 7 days might have decreased in titre. The tissue was in each case a sample of minced inner layer and it was stored as packed tissue in glucosol-phosphate at 4° C. The results of the titrations are shown in Table 2. Although in the first experiment there was no fall in titre and in the second the fall was not great enough to be statistically significant, there is an indication from the third that the use of stored tissue may decrease the sensitivity of the test. It has therefore been the practice in all other experiments to use tissue as soon as possible after collection, and certainly within 6 hr. Improved methods of storage may render it unnecessary to use fresh tissue.

Table 2. *Use of stored tissue*

(All titrations were made with ten observations at each dilution tested.)

Expt.	Strain of virus	Duration of storage (days)	Apparent titre
1	Strain 119 (Vallée A type)	0	$10^{-3\cdot5}$
		1	$10^{-3\cdot7}$
		2	$10^{-3\cdot6}$
2	Strain 997 (Waldmann C type)	0	$10^{-4\cdot6}$
		1	$10^{-4\cdot0}$
		2	$10^{-4\cdot0}$
3	Strain 997 (Waldmann C type)	0	$10^{-4\cdot8}$
		2	$10^{-3\cdot6}$
		4	$10^{-3\cdot5}$
		7	$10^{-3\cdot3}$

Table 3. *Titrations of virus of strain* 997 *in bottles and on plates*

(All titrations were made with ten observations at each dilution tested.)

Sample	Apparent titre (1) In bottles	Apparent titre (2) On plates
1	$10^{-4\cdot6}$	$10^{-4\cdot5}$
2	$10^{-4\cdot8}$	$10^{-5\cdot5}$
3	$10^{-3\cdot6}$	$10^{-3\cdot7}$

Medium. Following the initial success with the glucosol-phosphate medium, little work has been done on varying the composition of the salt solutions. Since Frenkel (1947, 1952) has recommended the inclusion of bovine serum, or a selection of amino-acids, to enrich the medium, several experiments have been made with and without varying concentrations of serum. In a typical experiment with strain 997 (Waldmann C type) identical results were obtained in three titrations with the same filtrate, made in the presence of 10 %, 2 % and no bovine serum respectively.

The pH of the medium was fixed at 7·6 since this value is optimal for survival of virus. In one experiment with strain 119 (Vallée A type) growth was demonstrated at pH 7·6 and none at pH 6·8.

Culture in bottles compared with Perspex plates. Three titrations of filtrates of strain 997 virus culture supernatants were made with duplicate sets of observations on cultures in 1 ml. amounts in 30 ml. screw-capped bottles and 0·3 ml. amounts in the cups on plates. The results shown in Table 3 demonstrate good agreement between the two methods.

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The presence of the tissue does not appear to affect the complement-fixation test if allowance is made for a possible slight anticomplementary effect. Identical observations were obtained in three experiments when, after incubation of the plates for growth of the virus, a sample of fluid from each cup was transferred to a fresh plate without tissue and a complement-fixation test performed on the two plates.

Influence of amount of complement on apparent titre. The amount of complement used can be varied within quite wide limits without influencing the result. Five titrations using a tenfold series of virus dilutions inoculated into ten cups per dilution were made on the same sample of virus. In the subsequent test 0·1 ml. amounts of different dilutions of complement were used. The 1/80 dilution was estimated to give 100% haemolysis. The calculated virus titres corresponding to each complement dilution were as follows:

Complement dilution	1/80	1/65	1/60	1/40	1/20
Apparent virus titre	$10^{-5\cdot4}$	$10^{-5\cdot4}$	$10^{-5\cdot6}$	$10^{-5\cdot5}$	$10^{-5\cdot5}$

The uniform result suggests that when multiplication has once taken place in the individual cup, it proceeds to a point when sufficient complement-fixing antigen is produced to fix a large amount of complement. In this experiment four 100% haemolytic units have been fixed.

Table 4. *Alternative methods of determining the result of tissue culture titrations*

Sample of virus	Dilution tested	Observations on complement fixation by individual cultures	Log complement fixed by pooled samples for each dilution inoculated
1	10^{-1}	+ + ± + + + + + + +	0·23
	10^{-2}	+ + + + + ± + + + +	0·20
	10^{-3}	− ± ± + − − + + + ±	0·11
	10^{-4}	± ± ± − ± ± ± − ± −	0·02
2	10^{-1}	+ + + ± + + + ± + +	0·24
	10^{-2}	+ ± ± + + + + + + −	0·26
	10^{-3}	+ ± + + ± + + + ± +	0·23
	10^{-4}	± − ± + ± + + + ± +	0·15

An alternative method of estimation of the result from a titration in culture would be to determine the total complement-fixing activity of a pooled sample from the cultures inoculated with each of the dilutions of virus. This method is more laborious and would only be worthwhile if difficulty were experienced in reading the results of the plate tests on an 'all-or-none' basis due to anticomplementary effects of culture or antiserum. This difficulty, however, is usually readily overcome by adjustment of the complement dilution. An example of a test in which, due to incorrect adjustment of complement dilution, many indeterminate results were obtained, is shown in Table 4. A pooled sample of 0·2 ml. amounts from the groups of individual cultures at each serial dilution inoculated had been obtained and the complement-fixing activity of each was determined. From the results in column 4 of the table it is apparent that sample 2 is of approximately tenfold higher titre

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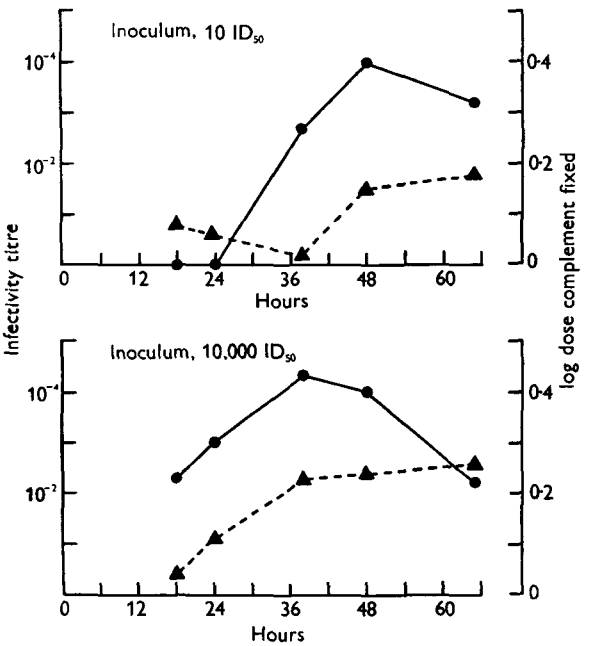
than sample 1, a result which would have been difficult to deduce from the observations on individual cultures in this case.

Duration of incubation. Early in these experiments it became apparent that a more prolonged incubation was necessary for the development of maximal complement-fixing activity in cultures inoculated with small amounts of virus than in those inoculated with larger amounts. In Text-fig. 1 is shown the rise in infectivity and in complement-fixing activity in cultures incubated in bottles. Ten cultures were pooled to give each point. With an inoculum of 10,000 ID_{50} virus growth could



Text-fig. 1. Development of infectivity (solid line) and complement-fixing activity (broken line) in culture after inoculation with 10,000 ID_{50} and 10 ID_{50} of virus respectively.

be detected from the time of the first observation at 18 hr. while with an inoculum of 10 ID_{50} growth was first demonstrated at 38 hr. The complement-fixing activity reached a significant level (log 0·1 complement fixed) by 24 hr. in the case of the stronger inoculum, but not until 48 hr. for the weaker. Two intermediate sizes of inoculum gave intermediate figures. In Text-fig. 2 with a 100-fold difference in virus dose inoculated, the difference is less marked but the complement-fixing activity is not detectable at 24 hr. and only minimal at 36 hr. with the weaker inoculum. With the other series, the 24 hr. sample gives complement-fixation and a high level is reached by 36 hr. A further demonstration of the earlier initiation of virus growth with larger inocula is given in Text-fig. 3. In this case no significant complement-fixation was obtained in any series before 30 hr. In general, maximal complement-fixing activity appears to be reached, even with weak inocula, by about 48 hr., but since no loss of activity has been seen in a further 24 hr., it has been the rule to incubate for approximately 65 hr.

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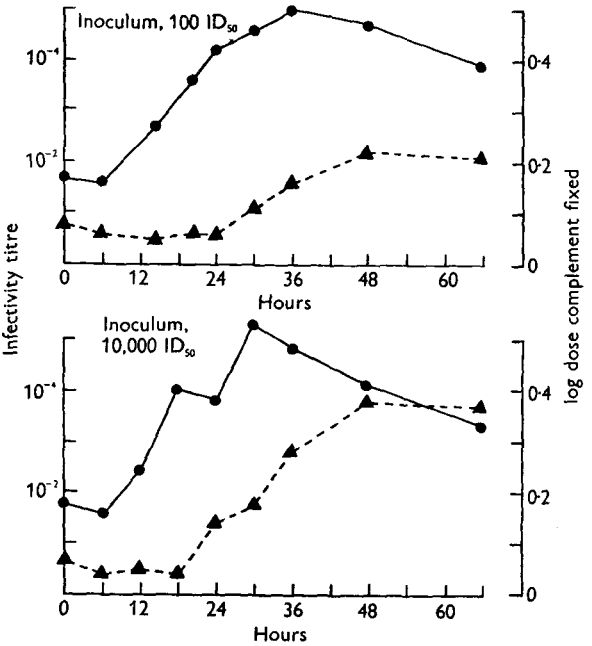
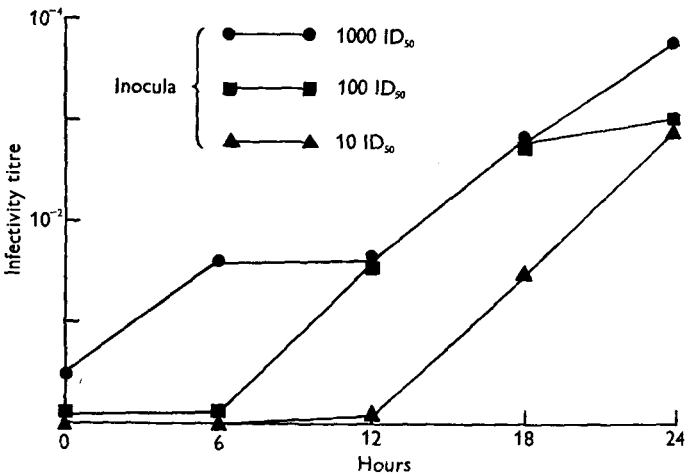
In the three experiments described in this section, all infectivity titres were measured by titrations performed in cultures. The data obtained without the use of live animals demonstrates the practical value of the method of titration in culture in research on the growth and multiplication of the virus.



Text-fig. 2. Development of infectivity (solid line) and complement-fixing activity (broken line) in culture after inoculation with 10,000 ID_{50} and 100 ID_{50} of virus respectively.



Text-fig. 3. Development of infectivity in culture inoculated with 1000, 100 and 10 ID_{50} of virus respectively.

Comparison of titre as estimated in culture and in cattle. The results of the titration in cattle and in culture of seven samples of virus of five different strains are presented in Table 5. The observations in cattle were obtained by the method of

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Henderson (1949), and we are in fact indebted to Dr Henderson for the results of the three titrations of strain Ven 1 in cattle which were made by him. One observation is of course provided by each of twenty sites inoculated on the tongue of a steer. The culture observations were obtained from tests on plates made within 24 hr. of the cattle test. Only in one instance (Strain M 14) does the difference between the two methods exceed 0·9 log units estimated by Henderson as the

Table 5. *Comparative data from titrations in culture and cattle*

Strain and origin	Dilutions of virus tested	Culture		Cattle	
		No. of positives: total observed	Apparent titre	No. of positives: total observed	Apparent titre
119 (cattle)	10^{-6}	2 : 20	$10^{-5\cdot0}$	1 : 10	$10^{-5\cdot1}$
	10^{-5}	9 : 20		5 : 10	
	10^{-4}	20 : 20		10 : 10	
	10^{-3}	20 : 20		10 : 10	
Syria 116 (cattle)	10^{-5}	2 : 30	$10^{-4\cdot5}$	1 : 10	$10^{-4\cdot5}$
	10^{-4}	26 : 30		9 : 10	
	10^{-3}	30 : 30		10 : 10	
997 (culture)	10^{-6}	12 : 30	$10^{-5\cdot6}$	0 : 10	$10^{-5\cdot2}$
	10^{-5}	21 : 30		6 : 10	
	10^{-4}	30 : 30		10 : 10	
	10^{-3}	30 : 30		10 : 10	
M 14 (cattle)	10^{-6}	54 : 80	$10^{-6\cdot3}$	1 : 10	$10^{-4\cdot6}$
	10^{-5}	80 : 80		1 : 10	
	10^{-4}	80 : 80		10 : 10	
	10^{-3}	80 : 80		10 : 10	
Ven 1 (culture)					
20 hr.	10^{-7}	5 : 30	$10^{-6\cdot2}$	1 : 10	$10^{-5\cdot8}$
	10^{-6}	17 : 30		24 : 60	
	10^{-5}	30 : 30		10 : 10	
	10^{-4}	30 : 30		.	
48 hr.	10^{-6}	1 : 30	$10^{-4\cdot7}$	.	$10^{-4\cdot1}$
	10^{-5}	7 : 30		3 : 10	
	$10^{-4\cdot6}$	.		9 : 40	
	10^{-4}	30 : 30		18 : 20	
	10^{-3}	30 : 30		10 : 10	
72 hr.	10^{-5}	0 : 30	$10^{-2\cdot8}$	.	$10^{-3\cdot5}$
	10^{-4}	1 : 30		.	
	$10^{-3\cdot6}$	.		19 : 40	
	10^{-3}	12 : 30		6 : 10	
	10^{-2}	29 : 30		20 : 20	
	10^{-1}	.		10 : 10	

significant difference between the results of two titrations each based on ten observations for each of four tenfold dilutions in two cattle. No explanation of the discrepancy in the case of M 14 can be found. In all other cases there is good agreement between the two results, suggesting that the culture method is as sensitive for the titration of virus as the bovine tongue. In Table 5, all results are calculated by the method of Reed & Muench (1938) since the numbers of observations are fewer and the intervals between dilutions tested greater than would warrant analysis by more accurate methods. An example of a titration result in

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which not less than fifty-five observations were made on each of eight dilutions of a virus suspension in a twofold series is given in Table 6. In this instance the method of probit analysis was used in the calculation of the result. The line fitted to the data by the method described by Fisher & Yates (1948) has a slope (b) 1·76 and gives a 50 % end-point at $10^{-5\cdot54}$ with 95 % fiducial limits at $10^{-5\cdot45}$ and $10^{-5\cdot63}$. This result indicates the precision which may be attained by the increase in numbers of observations at each dilution. Such increase in numbers of observations can be more readily achieved in the culture technique than in the use of living animals.

Table 6. *Example of titration in twofold series of strain* 997 *virus*

Dilution inoculated 10^-	Observations		Percentage positive
	Positive	Negative	
6·4	9	54	14
6·1	12	57	20
5·8	16	45	26
5·5	22	35	39
5·2	38	17	69
4·9	60	5	92
4·6	68	2	96
4·3	69	1	99

Table 7. *Titration of strain* 997 (*Waldmann C type*) *culture virus in different samples of tongue tissue, inoculating four dilutions in a tenfold series*

Breed of animal	Approximate age (years)	Class of animal	No. of observations per dilution	Apparent titre 10^-
Shorthorn	Over 4	Steer	30	5·6
Shorthorn	Over 4	Cow	30	5·6
Shorthorn	3·5	Heifer	30	5·8
Hereford	3·5	Steer	30	5·85
Shorthorn	Over 4	Steer	40	5·9
Pooled tissue from above			60	5·5

Variation in susceptibility of tissue from different tongues. Since in the routine application of the culture method of titration, it has been the practice to pool tissue from two or more tongues, it is not likely that slight variation in individual susceptibility will affect the results. The collection of tongues from the slaughter-house, however, implies less uniformity in the source of the tissue than in the laboratory, where cattle of one breed and approximately the same age are used for titration (Henderson, 1949). A deliberate attempt was therefore made to select tongues from a number of different types of animal and the relative susceptibilities were measured by performing a titration in tissue of each and a pooled sample of all five tongues. The results are shown in Table 7. Within this small group there appears to be no greater variation than might have been met in five titrations on a pooled sample of tongue tissue. Further observations on these lines are planned.

Serum neutralization tests. Only preliminary experiments have been made, but the results indicate the potential value of the culture method in the titration of antibody. The technique employed has been to mix with dilutions of virus, dilutions

of the sera to be tested. Controls with normal bovine sera are included. The virus-serum mixtures after an hour at room temperature are inoculated into cultures which are then incubated for the normal time of 65 hr. The usual complement-fixation test is then performed using a dose of complement sufficient to give 100 % haemolysis in the presence of the concentration of bovine serum included in the test. Readings are made in the usual way. The result of a test of this kind, in which the serum neutralization was directed at the determination of immunological type is shown in Table 8. The end-point in the presence of the heterologous and normal sera were not determined in respect of their upper limit, but the result of the test as a whole is in little doubt.

Table 8. *Serum neutralization test with strain M* 14 *Vallée A type*

Serum	Dilution of serum	Dilution of virus 10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}
Nil	.	.	10	10	10	6
Normal bovine	1/10	10	10	10	10	.
Type O bovine	1/10	10	10	10	10	.
Type A bovine	1/50	10	10	7	2	.
Type A bovine	1/10	9	0	0	0	.
Type C bovine	1/10	10	10	10	10	.

The figures indicate the number of positive observations in the ten made at each dilution.

DISCUSSION

The culture method described has been applied successfully to the titration of the virus of foot-and-mouth disease of cattle or culture origin. In the comparison of end-points obtained from cattle and culture titration of the same samples of virus, good agreement has been found in six out of seven cases in which five strains of virus were used. This does not obviate the necessity, however, of determining for any new strain of virus the correlation between culture and cattle titration before using observations on culture as an accurate index of titre. The estimation of relative potencies of two or more samples of the same strain is a simpler matter since no absolute value for the titre of any one sample of virus is involved, and by making suitably large numbers of observations the accuracy of such estimates can be greatly increased. Such accurate comparisons would be valuable in research on multiplication of the virus and in studies of sedimentation of the virus in the ultracentrifuge and of the concentration of the virus.

The outstanding advantage of the culture technique, as compared with the titration in the live animal, is economic, both in respect of initial cost in material and subsequent facilities required. It is therefore possible to contemplate more extensive and detailed investigation of virus titre in different circumstances. The disadvantage of the method in respect of determination of titres of strains of virus of unknown properties in relation to those determined in cattle are mentioned above. Two other factors may also, in certain circumstances, be reckoned against the use of the method. They are the time necessary to obtain a result, which is prolonged to 72 hr as compared with 24 hr in the cattle titration, and the necessity to have

a supply of type-specific guinea-pig antiserum of good titre. None of these objections is likely to weigh heavily against the use of the method in a wide range of circumstances.

SUMMARY

A technique is presented for the titration of the virus of foot-and-mouth disease in culture in surviving epithelial tissue from the tongues of cattle. The cultures are incubated in cups on Perspex plates, and the detection of virus multiplication is by a complement-fixation test made on the culture in each cup.

On the basis of comparative titrations in culture and in cattle, the method has been found to be as sensitive for the detection of virus as the titration by intradermal inoculation of the tongue of cattle. The method can also be applied in the detection of antibody in neutralization tests.

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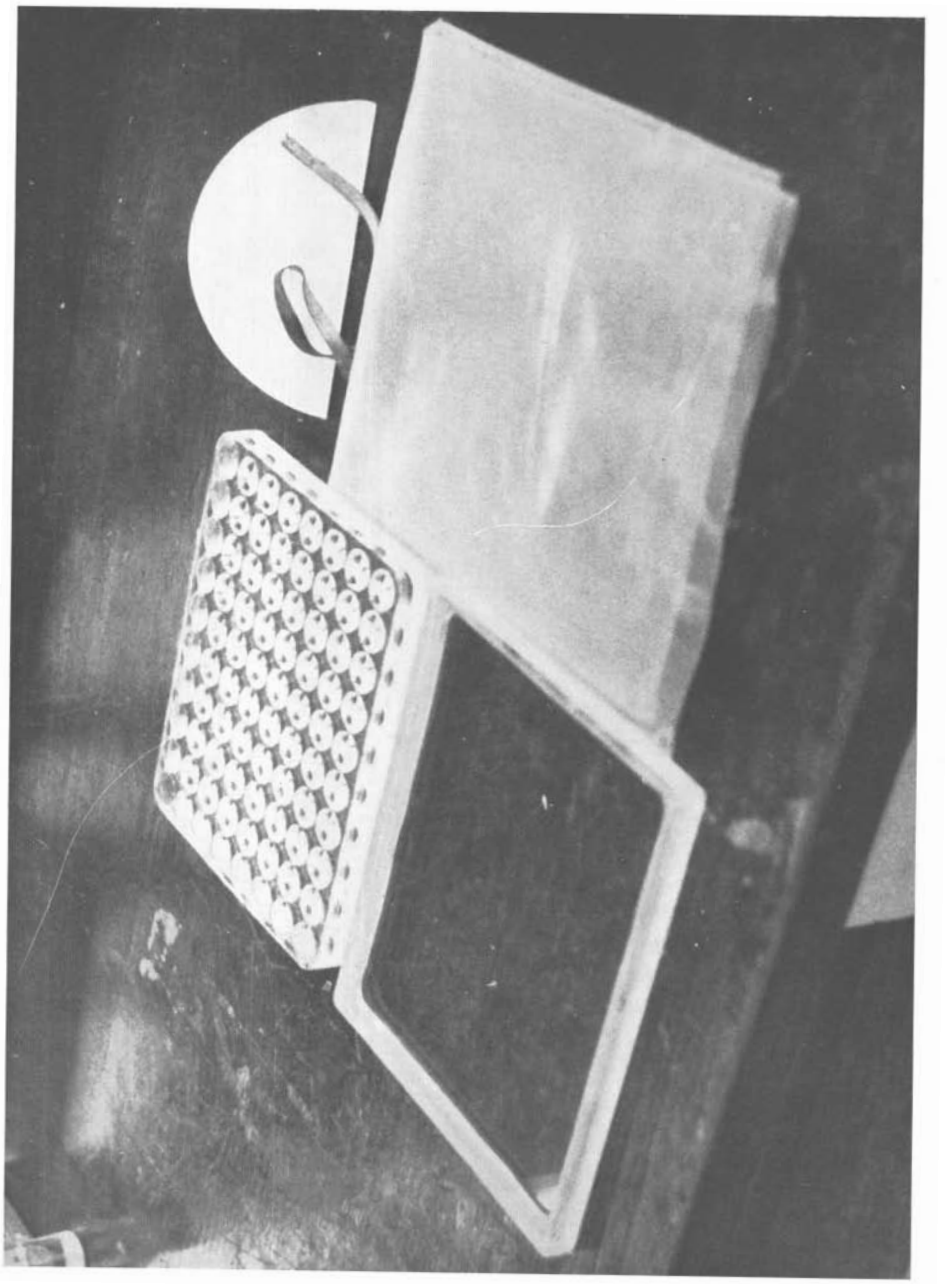

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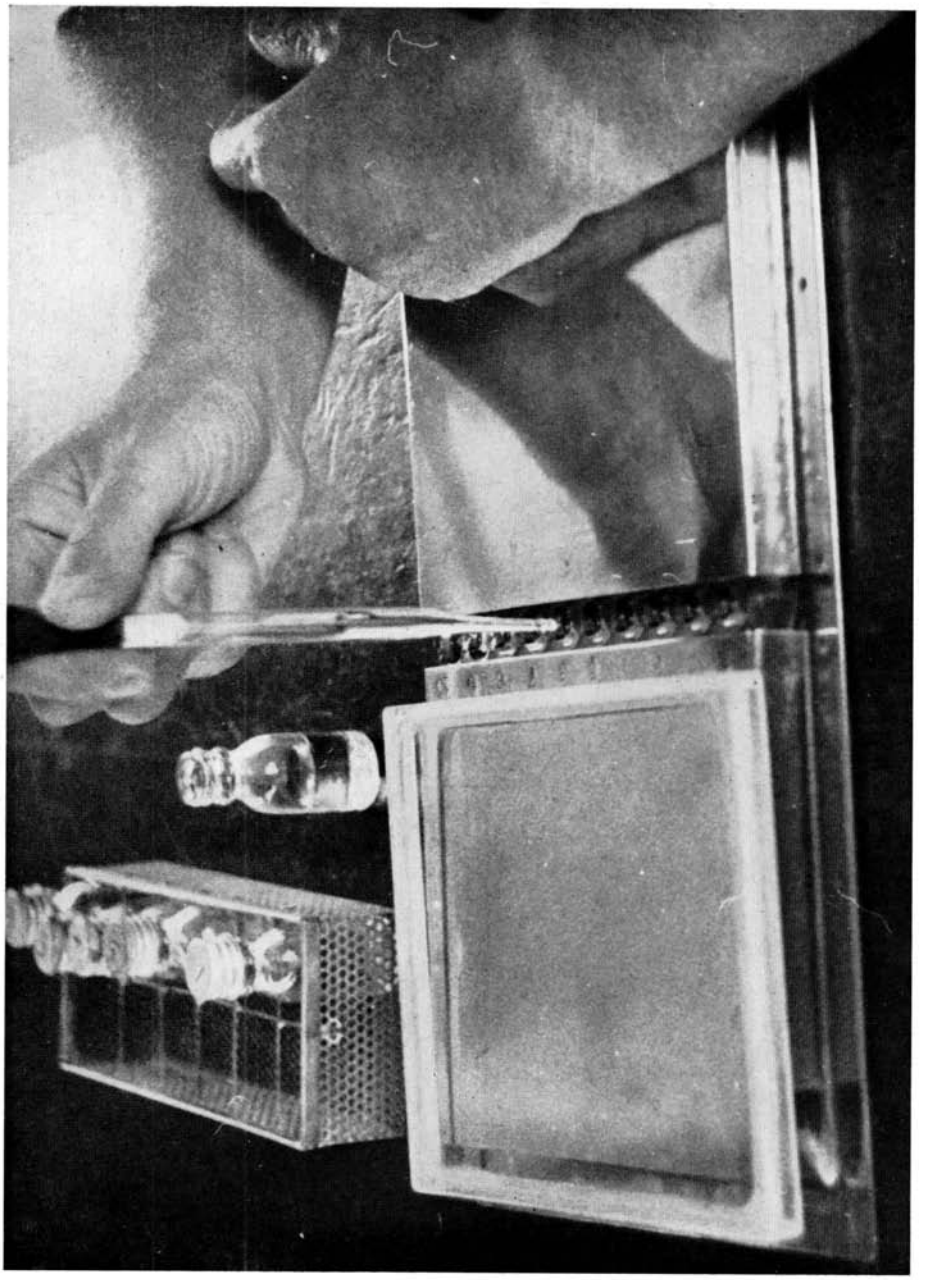

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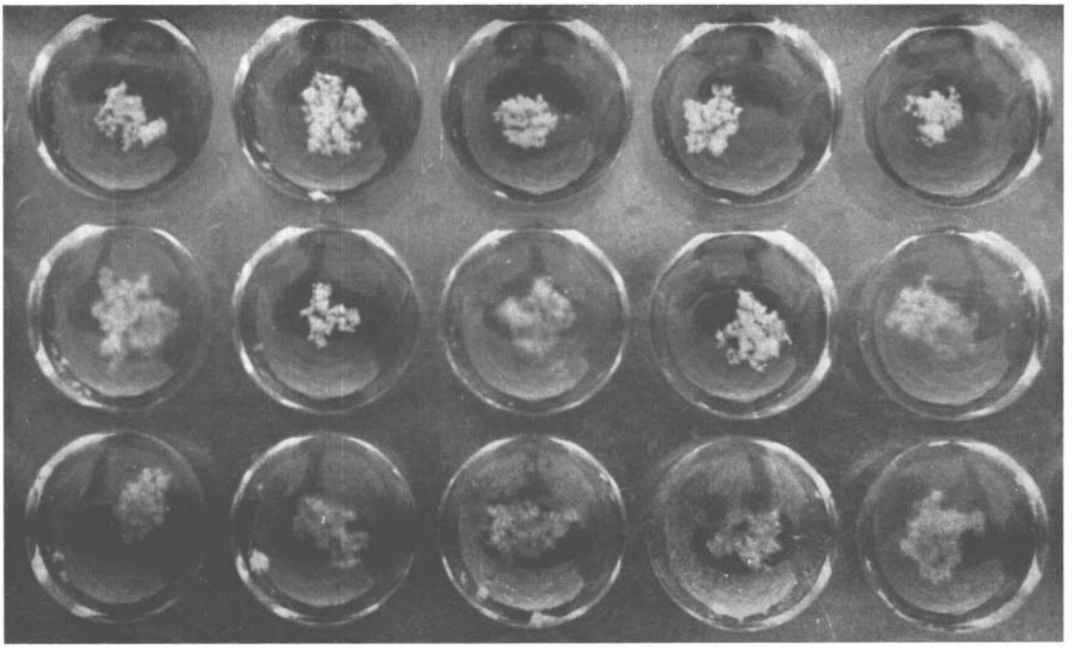

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EXPLANATIONS OF PLATES 1—3

Plate 1

Perspex plate, lid, polythene bag, rubber band and moist filter-paper used for titration in culture.

Plate 2

Inoculation of virus dilutions.

Plate 3

Observations on cups in Perspex plates. Key:

Top row	−	−	−	−	−
Middle row	+	−	+	−	+
Bottom row	+	+	+	+	+

In the positive cups the erythrocytes in suspension can be seen as a faint turbidity which obscures the outlines of the tissue fragments in the bottom of the cup. In the negative cups the tissue fragments are more clearly seen.

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