THE USE OF CULTURE VIRUS IN THE PREPARATION OF FOOT-AND-MOUTH DISEASE VACCINE

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(With 3 Figures in the Text)

The source of virus for preparation of vaccines for the immunization of cattle against foot-and-mouth disease has been limited, almost exclusively, to material collected from cattle reacting to inoculation with the virus. The chief disadvantages of using cattle for this purpose are their cost, the necessity for having buildings suitable for housing large numbers of diseased cattle under conditions that will prevent dissemination of infection, and the difficulty of obtaining susceptible cattle in countries where the disease is prevalent or where the use of vaccine is widespread.

Since Maitland & Maitland (1931) and Hecke (1930, 1931) reported the successful culture of the virus of foot-and-mouth disease in guinea-pig embryo tissue, a number of *in vitro* methods of propagation of the virus have been studied with the object of replacing the living animal as a source of virus for vaccine production. The method described by Frenkel (1947), in which epithelial tissue of the cattle tongue is used, is of most general practicability. Before it can be assumed, however, that the virus produced in culture is suitable for use in the routine preparation of vaccine, it must be shown that culture virus is as effective an antigen as cattle virus, that the virus content of culture tissue is sufficiently high, and that no modification in antigenic behaviour of the virus is likely to occur during culture. These requirements are in addition to having to overcome the practical difficulties of the large-scale culture that would be necessary for routine vaccine production.

A considerable amount of information relevant to these points has been accumulated in the course of work at this Institute with culture virus. In particular, three experiments have been performed in which a comparison was made under controlled conditions in cattle of the efficacy of vaccine prepared from virus passaged in cattle and vaccine prepared from virus passaged in culture.

METHODS

The cattle used in these experiments

The cattle used were Devon steers from 1½ to 2 years old. It can be assumed that these animals were of high initial susceptibility. The favourable conditions in this country for the supply of susceptible cattle have been described previously, for example, see Henderson (1952*a*).

Passage of virus strains in cattle

Devon steers were inoculated intradermally into the tongue with a suspension of cattle tongue epithelium from a previous reactor. Epithelium was collected

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from the resulting tongue lesions about 20 hr. after inoculation and stored, until required, at 4° C. in equal parts of glycerine and M/25-phosphate buffer solution, pH 7·6.

Passage of virus strains in culture

The method of virus culture was essentially that described by Frenkel (1947). Cattle tongues were obtained from an abattoir and within 2 hr. of slaughter the superficial layers of the mucous membrane were removed by Frenkel's method of traction. The exposed deeper layers of the mucous membrane and the similar tissue on the inner surface of the superficial portion first removed, were then collected with aseptic precautions and minced with scissors in Tyrode's solution, pH 7·6. The minced tissue in Tyrode's solution was stored at 4° C. until required. The longest period of storage in these experiments was 5 days.

The starting material for the series of culture passages of each strain was a Seitz EK filtrate of a 1:25 suspension of virus-infected epithelium from cattle tongue lesions, in a mixture at pH 7·6 of equal parts of M/25-phosphate buffer solution and Hartley's digest broth. Subsequent passages were made with filtrates prepared in the same way from the tissue of the preceding culture. Before each culture passage, minced tongue tissue was added to the virus filtrate and stored at 4° C., usually for 3–4 hr. but, occasionally, for 18 hr. The tissue was then transferred from the filtrate to 725 ml. flasks containing 45 ml. Tyrode's solution, 5 ml. normal cattle serum and 50,000 units of penicillin (sodium salt). About 2 g. of tissue was added to each flask. Instead of aerating the flasks continuously during incubation, as described by Frenkel (1947), the air in the flasks was replaced with 95 % oxygen and 5 % carbon dioxide and the flasks were sealed. The sealed flasks were then incubated at 37° C. for 18–24 hr., being gently rocked during this time. Under these conditions the maximum infectivity of the tissue is reached at about 18–24 hr. After incubation, the tissue was separated from the culture liquid by centrifugation, the tissue from each flask was pooled and, if not immediately required, stored at $-20°$ C.

Estimation of the virus content of infective material

The tissue suspension filtrates used in the preparation of vaccines or in the testing of the immunity of vaccinated cattle were titrated in cattle by the multiple inoculation technique (Henderson, 1949). The 50 % positive end-point dilution was calculated by the method of Reed & Muench (1938), and the virus content of the filtrates expressed in ID_{50}, the 50 % Infective Dose. In the particular conditions of inoculation used in the titration method, 1 ID_{50} is the amount of virus producing 50 % of positive reactions on multiple inoculation of the tongues of cattle with volumes not exceeding 0·1 ml. at each site. The number of ID_{50} per 0·1 ml. of the undiluted filtrate is, therefore, the reciprocal of the 50 % positive end-point dilution. Thus, if this end-point dilution of a filtrate was 10^{-5} then the undiluted filtrate would contain 10^5 ID_{50} per 0·1 ml. or 10^6 ID_{50} per ml.

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Preparation of vaccines

The vaccines used in these experiments were of the Schmidt-Waldmann formalinized aluminium hydroxide type. Our method of preparation was to dilute one part of virus filtrate with one part of distilled water, to mix this with three parts of aluminium hydroxide gel, to add formalin (40 % solution of formaldehyde) to a final concentration of 1 in 2000, and to keep the mixture in a water-bath at 26° C. for 3 days; which treatment was expected to give a non-infective product. The virus filtrate was obtained by preparing a suspension of vesicle epithelium or culture tissue in 25 % M/25-phosphate buffer solution, pH 7·6; 25 % Hartley's digest broth, pH 7·6 and 50 % distilled water. The suspension was clarified by centrifugation and the supernatant fluid passed through a Seitz EK filter pad. The aluminium hydroxide gel had an equivalent Al_2O_3 content of 1·48 %. This preparation of aluminium hydroxide gel was the same as that currently used in the Danish Government's foot-and-mouth disease vaccine, and it was supplied through the courtesy of Dr E. Fogedby, Director of the State Veterinary Research Institute for Virus Diseases, Lindholm, Denmark.

The culture virus vaccines were prepared from the culture tissue collected after 18–24 hr. incubation, when infectivity was maximum. No culture liquid was included so that the vaccine would be comparable to that made from the epithelium of infected cattle.

Test for non-infectivity of vaccines

Four or six cattle for each vaccine were inoculated intradermally into the tongue with volumes not exceeding 0·1 ml. at twenty sites each. The susceptibility of the tongue tissue of these cattle was tested subsequently, and the significance of the test for non-infectivity assessed, see Henderson (1952*b*).

Vaccination of cattle

Vaccine was injected subcutaneously in the dewlap in the region of the brisket.

Test of immunity of vaccinated cattle

In the third week after vaccination, the vaccinated and unvaccinated control cattle were inoculated intradermally into the tongue with 10,000 ID_{50} of the appropriate strain of virus at each of ten sites. In such a test of immunity, although primary lesions may develop at the sites of inoculation, complete protection against the development of secondary lesions corresponds to complete protection against infection by contact exposure with reacting cattle. At least two doses of vaccine were tested of approximately threefold difference in volume, preferably a dose protecting more than half and a dose protecting less than half of the group of cattle being tested. Eight cattle were vaccinated with each dose, and at least eight unvaccinated control cattle were included for each virus strain. The 50 % protection dose or the dose of vaccine required to protect half of a group of cattle was calculated by the method of Reed & Muench (1938). In this calculation, only those animals with no secondary lesions were classed as 'protected' and an animal with any secondary lesions, no matter how small, was classed as 'not protected'.

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RESULTS

Comparisons between foot-and-mouth disease vaccines prepared from tongue epithelium of reacting cattle and vaccines prepared from culture tissue

(1) Strain Ven 1, Vallée O type; a strain of recent field origin recovered from material received from an outbreak of foot-and-mouth disease in cattle at Villa de Cura, Venezuela, June 1950

Cattle virus vaccine	Vac/Al/O Ven 1	121
Culture virus vaccine	Vac/Al/O Ven 1	122

Both vaccines were prepared and tested in the same experiment.

A filtrate of a 1 in 15 suspension of tongue epithelium, freshly collected from reactors of the 3rd cattle passage, was used for preparation of the cattle virus vaccine, and a filtrate of a 1 in 40 suspension of tissue of the 12th culture passage was used for the culture virus vaccine. The culture series had been started with virus of the 2nd cattle passage.

Preliminary infectivity titrations were made on two cattle for each filtrate. The two 50% positive end-point dilutions so determined were then inoculated simultaneously into the tongues of four cattle, at ten sites for each dilution on each tongue. The 50% positive end-points calculated from the accumulated observations provided by the two titrations were 10^{-6} for the cattle virus filtrate and $10^{-5\cdot6}$ for the culture virus filtrate. In preparing the vaccines, the filtrates were diluted 1 in 2 with distilled water and a further 1 in 2·5 with the aluminium hydroxide gel. A filtrate of titre 10^{-6} thus diluted would provide a product with an estimated 50% end-point of $10^{-5\cdot3}$. The original virus content of the cattle virus vaccine would be, therefore, $10^{5\cdot3}$ ID_{50} per 0·1 ml. or $10^{6\cdot3}$ ID_{50} per ml. Similarly, the original virus content of the culture virus vaccine was estimated to be $10^{5\cdot9}$ ID_{50} per ml. The suspension of culture tissue had been made weaker than the suspension of cattle epithelium in an attempt to have two vaccines of the same virus content, the result of an earlier titration having suggested that the titre of the epithelium might be lower than that of the culture tissue. In fact, the final estimates of the virus content of the two tissues were the same, 1 in 15 : 10^{-6} being approximately equal to 1 in 40 : $10^{-5\cdot6}$.

The pH of each vaccine at the completion of incubation was 7·04.

No active virus was detected in the tests for non-infectivity in which four cattle for each vaccine were inoculated into the tongue at twenty sites, each to provide eighty observations. The percentage of infective 0·1 ml. samples would be unlikely ($P = 0\cdot05$) to exceed 3·7 and highly unlikely ($P = 0\cdot01$) to exceed 5·6, Henderson (1952*b*).

Three groups of eight cattle were vaccinated with each vaccine with 3, 10 and 30 ml. doses respectively. Their immunity was tested 17 days later using strain Ven 1 virus of the 3rd cattle passage. The result is shown in Fig. 1. The unvaccinated control cattle all developed extensive primary lesions followed by secondary lesions on all four feet. The 50% protection doses were 3·5 ml. for the cattle virus vaccine and 6·2 ml. for the culture virus vaccine, see Table 1. As the original virus content

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of the cattle virus vaccine was $10^{6\cdot3}$ ID_{50} per ml., this 50% protection dose of 3·5 ml. corresponds to an original virus content of $10^{6\cdot8}$ ID_{50}. The 50% protection dose of the culture virus vaccine, 6·2 ml., corresponds to an original virus content of $10^{6\cdot7}$ ID_{50}.

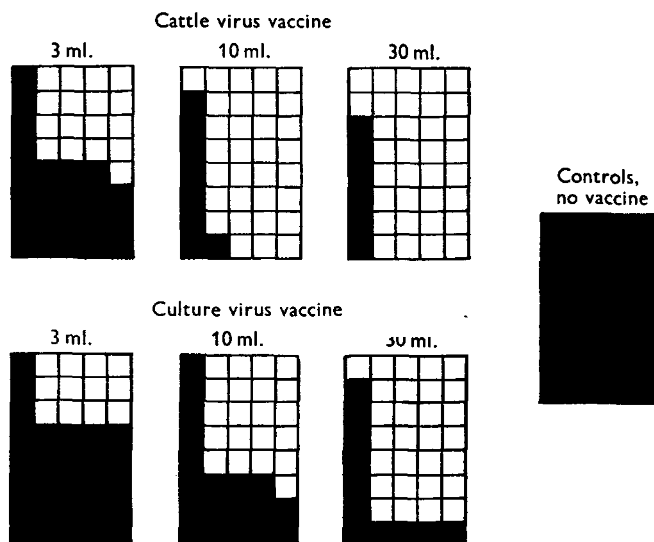

Fig. 1. Strain Ven 1. Test of vaccinated cattle by tongue inoculation with cattle virus. In this and the subsequent figures, each large rectangle depicts a group of eight cattle with a horizontal line of five squares for each animal. The first square represents the tongue and lips and the next four squares the four feet. A lesion at any particular site is denoted by blackening the appropriate square except that a lesion on the lips is shown by a diagonal white stripe when no secondary lesions occurred elsewhere, see Figs. 2 and 3. The criterion of 'protection' is the absence of lesions on the lips and feet.

Table 1. *Calculations of 50% protection doses of strain Ven 1 cattle and culture virus vaccines*

	Dose (ml.)	Log dose	Observed results: Protected	Observed results: Not protected	Accumulated results: Protected	Accumulated results: Not protected	% protected
Cattle virus vaccine	30	1·48	8	0	19	0	100
	10	1·0	7	1	11	1	92
	3	0·48	4	4	4	5	44
Culture virus vaccine	30	1·48	7	1	15	1	94
	10	1·0	5	3	8	4	67
	3	0·48	3	5	3	9	25

Cattle virus vaccine: log 50% protection dose $= 1\cdot0 - \left(\frac{92-50}{92-44} \times 0\cdot52\right)$, therefore 50% protection dose = 3·5 ml.

Culture virus vaccine: log 50% protection dose $= 1\cdot0 - \left(\frac{67-50}{67-25} \times 0\cdot52\right)$, therefore 50% protection dose = 6·2 ml.

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(2) Strain 119, Vallée A type; recovered from an outbreak of foot-and-mouth disease in cattle in Great Britain in 1932 and used since that date as a stock A type strain

Citing the original virus content of the 50 % protection dose of the vaccines in these experiments presupposes that the degree of protection varies in relation to the amount of potent antigen in the vaccine and that this depends on the original virus content of the vaccine. This hypothesis was tested in a strain 119 experiment by preparing and testing a second cattle virus vaccine having a virus content one-third that of the vaccine prepared by the routine method.

Cattle virus vaccines	Vac/Al/A 119	127
	Vac/Al/A 119	128
Culture virus vaccine	Vac/Al/A 119	129

The cattle virus vaccines were prepared and tested in one experiment and the culture virus vaccine was prepared and tested in another experiment carried out 2 months later.

A filtrate of a 1 in 15 suspension of tongue epithelium, freshly collected from reactors of the 75th cattle passage, was used to prepare vaccine no. 127 and a 1 in 3 dilution of the filtrate, keeping the same proportions of buffer solution, broth and distilled water, was used to prepare vaccine no. 128. A filtrate of a 1 in 15 suspension of tissue of the 14th culture passage was used to prepare the culture virus vaccine. The culture series had been started with virus of the 74th cattle passage.

These filtrates were prepared at different times, so that a simultaneous titration was not possible. The cattle virus filtrate was titrated on six cattle, five sites on each tongue for the 10^{-5} dilution and fifteen sites on each tongue for 10^{-6} dilution. The 50 % end-point of the filtrate was $10^{-6\cdot 1}$, thus vaccine no. 127 had an original virus content of $10^{6\cdot 4}$ ID_{50} per ml. and vaccine no. 128, $10^{5\cdot 9}$ ID_{50} per ml. Six cattle were used for titration of the culture virus filtrate, five, ten and five sites on each tongue for the 10^{-5}, 10^{-6} and 10^{-7} dilutions respectively. The 50 % end-point was $10^{-5\cdot 9}$, thus the original virus content of the culture vaccine was $10^{6\cdot 2}$ ID_{50} per ml.

The pH values of the vaccines were: no. 127, 7·21; no. 128, 7·10; no. 129, 7·32.

No active virus was detected in the tests for non-infectivity, in which six cattle for each vaccine were inoculated into the tongue at twenty sites each to provide 120 observations. In a subsequent test of the susceptibility of the tongue tissue of these cattle, one animal in the group used to test vaccine no. 127 was apparently insufficiently susceptible for the detection of minimal quantities of virus and its contribution in the test for non-infectivity was disregarded. For vaccine no. 127, therefore, it is only justifiable to cite 100 observations from five cattle. The significance of this result is that the percentage of infective 0·1 ml. samples of the vaccine would be unlikely ($P = 0\cdot 05$) to exceed 3·0 and highly unlikely ($P = 0\cdot 01$) to exceed 4·5. In the case of vaccines nos. 128 and 129 with 120 observations, the percentage of infective 0·1 ml. samples would be unlikely to exceed 2·5 and highly unlikely to exceed 3·8, Henderson (1952*b*).

In the expectation with this strain and with this type of vaccine that an original virus content of about $10^{6\cdot 8}$ ID_{50} would be required for 50 % protection, the doses

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selected in an attempt to bracket the 50% protection dose were 2 and 6 ml. for vaccine no. 127, 6 and 18 ml. for vaccine no. 128 and 3 and 10 ml. for vaccine no. 129. In both experiments the cattle were inoculated 15 days after vaccination with freshly passaged virus of the 75th cattle passage of strain 119. A group of eight unvaccinated control cattle was included in each case. The results are shown in Fig. 2.

The cattle virus vaccine no. 127 had a 50% protection dose of 2·3 ml. corresponding to an original virus content of $10^{6\cdot8}$ ID_{50}.

The cattle virus vaccine no. 128, with one-third of the original virus content of no. 127, had a 50% protection dose of 6 ml., corresponding to an original virus content of $10^{6\cdot7}$ ID_{50}.

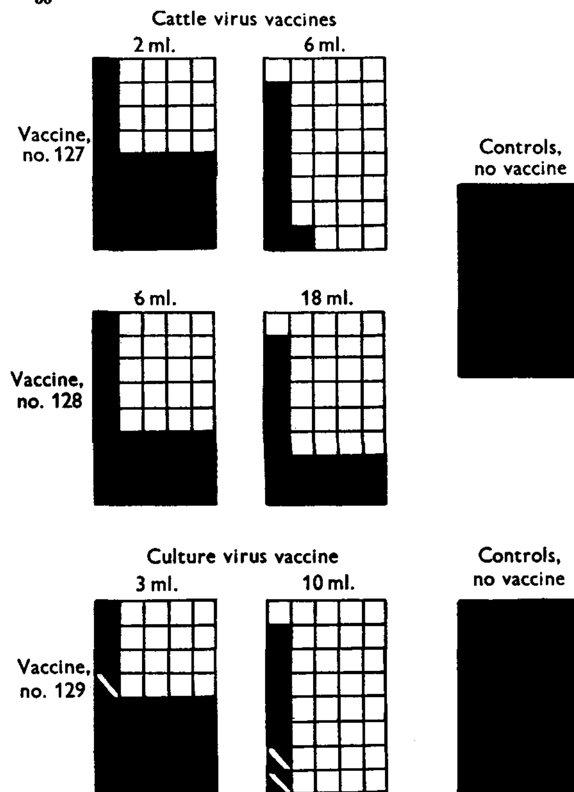

Fig. 2. Strain 119. Test of vaccinated cattle by tongue inoculation with cattle virus.

The culture virus vaccine no. 129 had a 50% protection dose of 4·8 ml. corresponding to an original virus content of $10^{6\cdot9}$ ID_{50}.

(3) Strain H 2200, Vallée A type; a strain of recent field origin recovered from an outbreak of foot-and-mouth disease in cattle in Holland, October 1951

Cattle virus vaccine	Vac/Al/A H 2200	132.
Culture virus vaccine	Vac/Al/A H 2200	135.

Both vaccines were prepared and tested in the same experiment with the exception that the sequence of vaccination and test of eight cattle receiving 3 ml. doses of the culture virus vaccine was started 1 month later.

A filtrate of a 1 in 15 suspension of tongue epithelium, freshly collected from

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reactors of the 4th cattle passage, was used for preparation of the cattle virus vaccine, and a filtrate of a 1 in 15 suspension of tissue of the 10th culture passage was used for preparation of the culture virus vaccine. The culture series had been started with virus of the 2nd cattle passage.

Preliminary titrations were made using two cattle for each filtrate. The 50% positive end-point dilutions, as determined by these preliminary titrations, were then inoculated into the tongues of two cattle for each filtrate at twenty sites on each tongue. The 50% end-points calculated from the accumulated results of both titrations were $10^{-6 \cdot 3}$ for the cattle virus filtrate and $10^{-5 \cdot 6}$ for the culture virus filtrate. The original virus contents of the two vaccines were, therefore, $10^{6 \cdot 6}$ ID_{50} per ml. for the cattle virus vaccine and $10^{5 \cdot 9}$ ID_{50} per ml. for the culture virus vaccine.

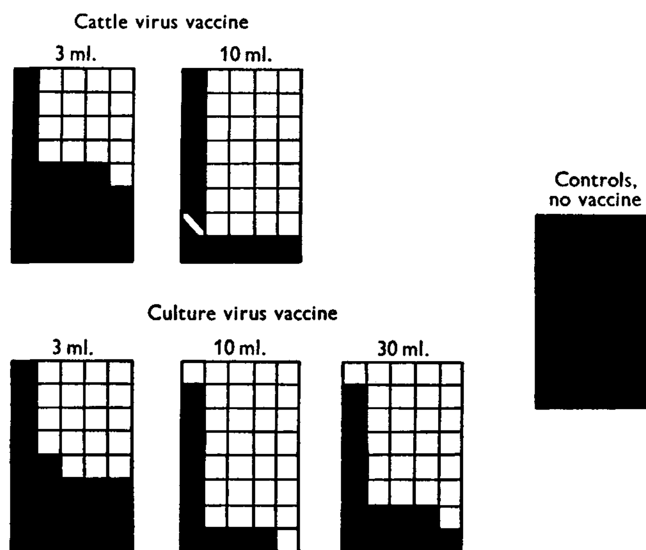

Fig. 3. Strain H 2200. Test of vaccinated cattle by tongue inoculation with cattle virus.

The pH values of the vaccines were: no. 132, 7·62; no. 135, 7·52.

No active virus was detected in the tests for non-infectivity in which six cattle were used for each vaccine. A subsequent test of susceptibility resulted in discarding the contribution of one of the cattle of the group used to test vaccine no. 135. The significance of the results of the non-infectivity tests of vaccines nos. 132 and 135 is the same as that of the results of the tests of vaccines nos. 128 and 127 respectively of the previous experiment.

The protection afforded by 3 and 10 ml. doses of the cattle virus vaccine and 3, 10 and 30 ml. doses of the culture virus vaccine was tested using groups of eight cattle. The virus inoculations were made 14 days after vaccination using cattle virus of the 4th passage of strain H 2200. The results are shown in Fig. 3.

The cattle virus vaccine had a 50% protection dose of 4 ml., corresponding to an original virus content of $10^{7 \cdot 2}$ ID_{50}.

The culture virus vaccine had a 50% protection dose of 4·5 ml. corresponding to an original virus content of $10^{6 \cdot 6}$ ID_{50}.

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The virus content of culture tissue

A comparison of the titres of the filtrates used in preparation of the cattle and culture virus vaccines shows little or no appreciable difference in the infectivity of the two sources of virus in the case of strains Ven 1 and 119. Other titrations have been made in the course of passaging these strains during the period of these experiments. The mean virus contents in ID_{50} per g. of tissue for the two sources of virus are given in Table 2. There is no indication of any difference in the virus content of the strain Ven 1 materials, and although the strain 119 result shows a threefold difference between the virus content of the two sources, further samples would have to be examined before this difference, based on the comparison of only three paired values, could be regarded as being significant. Nevertheless, the virus content, $10^{7.9}$ ID_{50} per g. of the culture tissue is adequate for vaccine preparation, and it may be noted that the virus content from the accumulated results of a larger number of other titrations of strain 119 cattle tongue epithelium was, in fact, $10^{7.8}$ ID_{50} per g. (Henderson, 1949).

Results were less satisfactory with strain H 2200. Eleven culture passages were made before the infectivity of the tissue was tested. The virus content of the 11th passage tissue was $10^{7.0}$ ID_{50} per g. which was lower than any passage tested of strains Ven 1 or 119, and was lower than desired for vaccine preparation. The $10^{-5.3}$ dilutions of filtrates of 1 in 15 suspensions of stored culture tissue of the 3rd, 6th, 8th and 10th passages were then prepared, and each was inoculated at five sites on the tongue of each of two cattle. The number of positive observations was 0, 3, 6 and 0 for these $10^{-5.3}$ dilutions of the 3rd, 6th, 8th and 10th passage filtrates respectively. The estimated 50 % positive dilution was 10^{-5} and $10^{-5.5}$ for passages 6 and 8, corresponding to $10^{7.2}$ and $10^{7.7}$ ID_{50} per g. of tissue. The 8th passage material was used to continue the series, and the 10th passage tissue, i.e. of the 2nd subsequent passage, had a virus content of $10^{7.8}$ ID_{50} per g. This was the tissue used for the preparation of the strain H 2200 culture virus vaccine. In other work with culture virus it has become evident that the degree of infectivity of the culture tissue after a set period of incubation is closely associated with the virus content of the starting material. The higher the concentration of virus available to the tissue in the period at 4° C. prior to incubation, the greater is the amount of virus acquired by the tissue and the greater is the virus content of the tissue after a set period of incubation. With some strains, therefore, it may be necessary to determine the degree of infectivity of the tissue of each passage in order to ensure that a succession of passages of low titre does not yield a product of inadequate virus content for the preparation of potent vaccine.

Table 2. *The virus content of culture tissue*

	Cattle tongue epithelium		Culture tissue	
Virus strain	No. of samples	Mean virus content ID_{50} per g.	No. of samples	Mean virus content ID_{50} per g.
Ven 1	6	$10^{8.1}$	5	$10^{8.1}$
119	3	$10^{8.4}$	3	$10^{7.9}$

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The antigenic specificity of virus strains passaged in culture

In the three vaccination experiments, the virus used to test animals vaccinated with either cattle or culture vaccines was of the first or second cattle passage following that used to start the culture series. There is no indication that the antigenic specificity of either cattle or culture virus had changed, for both vaccines gave similar protection.

Additional evidence of the antigenic stability in culture of strains 119 and H 2200 was provided by the results of the following two tests:

(1) Three cattle, injected with cattle virus vaccine, which had not reacted when inoculated intradermally in the tongue with strain 119 cattle virus, were similarly inoculated 3 weeks later with a 1 in 15 suspension of the 16th culture passage virus of the same strain. Again no reaction occurred except slight superficial erosion of the epithelium at some of the sites of inoculation on two of the cattle. Filtrates of similar suspensions of the same culture passage virus were shown to have titres of 10^{-5} in unvaccinated cattle.

(2) Eight cattle, 2–3 weeks after reaction to strain H 2200 cattle virus were inoculated intradermally into the tongue with a 1 in 25 suspension of the 11th culture passage tissue of the same strain. No reaction was observed in these cattle. A similar suspension of the same culture tissue in a titration in guinea-pigs was infective at a dilution of 10^{-5}.

DISCUSSION

Routine production of vaccines or comparison of the antigenic behaviour of strains of virus in cross-vaccination experiments demand only evidence of a certain minimum of active virus in the material used to prepare the vaccines. Comparison of different vaccines, however, demands a more exact knowledge of the virus content of the material used in their preparation. For this reason the filtrates from which the vaccines tested in the present experiments were made were titrated on four or six cattle instead of the two usually used in routine titrations. Moreover, extra inoculations increased the number of observations in the region of the expected 50 % positive end-point, at the expense of higher and lower dilutions. The standard deviation, on a log scale to the base 10, of the end-points in the strain Ven 1 titrations was estimated to be $\pm 0{\cdot}13$. In the titrations of the other strains, in which simultaneous comparisons on the same tongues were not made, the standard deviation was estimated to be about $\pm 0{\cdot}2$.

In comparing different vaccines, the dose providing most information for any particular number of observations is the 50 % protection dose. In the experiments reported here, using a group of eight cattle for each of two or three doses on a scale of threefold differences in volume, the 50 % protection dose was bracketed in each case. Consideration of the significance of such tests of vaccine potency (unpublished) indicated that the standard deviation of the log dose was $\pm 0{\cdot}13$. This level of accuracy corresponds well with that achieved in determining the virus content of the starting material used in preparation of the vaccines.

The degree of protection afforded by a vaccine prepared from a particular strain of virus might be expected to vary with the amount of potent antigen in the

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vaccine and thus with the original virus content of the vaccine. The result of the strain 119 experiment, in which two vaccines of a threefold difference in original virus content had the expected threefold difference in the 50 % protection dose, confirmed this expectation.

The comparisons of cattle and culture passaged virus as sources of material for vaccine production, summarized in Table 3, are expressed as the calculated original virus content of each 50 % protection dose. The figures provide the fundamental information for a comparison in vaccine preparation of different sources of virus of the same strain. The precision of the results on which they are based is subject to the sum of the errors of the virus titrations and the errors of the vaccine protection tests. From the estimations given above, the standard deviation of the values in

Table 3. *The effectiveness in vaccine preparation of virus passaged in cattle and virus passaged in culture*

Virus strain	Source of virus	Original virus content of 50 % protection dose of vaccine (ID_{50})
Ven 1	Cattle	$10^{6.8}$
	Culture	$10^{6.7}$
119	Cattle	$10^{6.8}$
	Cattle	$10^{6.7}$
	Culture	$10^{6.9}$
H 2200	Cattle	$10^{7.2}$
	Culture	$10^{6.6}$

Table 3 is about ± 0.3. No significant difference was, in fact, detected between the effectiveness of cattle and culture virus in vaccine preparation as far as strains Ven 1 and 119 were concerned. With strain H 2200, however, the difference observed between the effectiveness of the two sources of virus is just significant, the culture virus being the better. There is no doubt, therefore, that culture passaged virus of these strains is as effective an antigen in vaccine as is the virus passaged in cattle.

Although a number of authors have mentioned the use of culture virus for preparation of foot-and-mouth disease vaccines, the published (Mace, Dunne, Eichhorn & Camargo, 1951; Fogedby & Johnson, 1952) data for comparisons between cattle and culture virus vaccines are inadequate to relate the degree of protection produced by the vaccines to their original virus content. In the absence of this information, conclusions that vaccines prepared from virus of one source are as good as, or better than, vaccines prepared from virus of another source may reflect merely the virus content of the sources and not the relative effectiveness of the antigens.

Evidently, with some strains of foot-and-mouth disease virus, no difficulty is likely to be experienced in obtaining cultures of sufficiently high virus content for vaccine production and, so far, there has been no indication of any modification in antigenic behaviour as a result of passage in culture. Other strains may, however, give lower titres in culture, as did strain H 2200. Similar findings have been

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reported by Fogedby & Jacobsen (1950), who had no difficulty in passaging four strains but failed to get adequate growth with two strains. In the experiments reported here, strains were maintained by serial passage in culture but this may be unnecessary. With strains which show a drop in titre on culture passage it may be better to make vaccine from first-passage cultures. Although one or two animals would be needed to provide the seed for each batch of vaccine, preparation of the vaccine from first-passage culture material would still be more economical and convenient than resorting to cattle passaged virus.

The culture virus in these experiments was grown on a small laboratory scale in 50 ml. of medium in 725 ml. flasks. Successful experiments have since been made on a scale 100 times larger, in which 5 l. of medium in a 50 l. container were employed. Thousand-fold increases in virus were obtained in 18 hr., yielding tissue of about 10^8 ID_{50} per g. The Dutch workers, however, have developed suitable techniques and apparatus by which the tissue from some hundreds of tongues can be used (Frenkel, 1953). Work on a scale necessary for routine vaccine production is therefore possible.

SUMMARY

In three cattle vaccination experiments, with three strains of the virus of foot-and-mouth disease, it was shown that virus passaged in culture in cattle tongue epithelial tissue was as effective an antigen as virus passaged in cattle.

With two virus strains, the virus content of the culture at the peak of infectivity was about the same as that of vesicle epithelium from the tongues of reacting cattle. Variable and less satisfactory results were obtained with the third strain, but one culture passage yielded tissue of sufficiently high virus content for the preparation of vaccine of adequate potency.

No modification in the antigenic behaviour of the strains was detected as a result of passage in culture.

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