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In vitro measurement of the potency of inactivated foot-and-mouth disease virus vaccines

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INTRODUCTION

An *in vitro* test which measures the potency of inactivated foot-and-mouth disease (FMD) virus vaccines would be of considerable value because the currently available animal potency tests are extremely costly. In addition, the tests are lengthy, requiring 4 weeks to complete, so that any emergency requirements cannot be met with vaccines of known potency unless these have been tested prior to storage.

Randrup (1954) and Brown & Crick (1959) have shown that the 25 mμ component of FMD virus suspensions possesses immunizing properties after suitable inactivation of its infectivity, whereas the accompanying 7 mμ component is not immunogenic. It seemed worthwhile, therefore, to examine the relationship between the amount of the inactivated 25 mμ component in different FMD virus suspensions and the potency of the vaccines prepared from them.

MATERIALS AND METHODS

Virus preparations and titration

Strain 1 (type O) was used in all the experiments described. This is a strain which has been maintained for more than 30 years by passage in guinea-pigs by intradermal inoculation of the hind pads and subsequent harvesting of the pads and vesicle fluid 24 hr. after inoculation. In these experiments the pads and vesicle fluid were harvested separately. Virus suspensions were prepared from the pad epithelium by grinding in a mortar with 0·04 M phosphate buffer, pH 7·6. Mouse virus was obtained by one passage of the guinea-pig vesicle fluid (intraperitoneal inoculation of 10^{-6} dilution) in 7-day-old unweaned mice and the leg muscles were collected from the mice immediately after death. The virus was extracted by grinding the mouse tissue in 0·04 M phosphate buffer, pH 7·6. Virus was also grown in monolayers of pig kidney and baby hamster kidney cells (BHK 21—MacPherson & Stoker, 1962) by infecting each type of monolayer with guinea-pig vesicle fluid and incubating in nutrient medium at 37° C. until the cell sheet was destroyed by the action of the virus. For the pig kidney cells, Earle's saline containing 0·5 % lactalbumin hydrolysate and 0·01 % yeast extract was used; a modified Eagle's medium (MacPherson & Stoker, 1962) was used for the baby hamster kidney cells. The cell debris was removed from the tissue culture harvests by centrifugation at 2000 r.p.m. All the virus titrations were carried out by intraperitoneal inoculation of 7-day-old unweaned mice (Skinner, 1951).

Estimation of complement-fixing antigens

Comparison of the amounts of complement-fixing antigens present in the different suspensions was made by using Brooksby's method (1952), in which the virus and antiserum are allowed to react with different amounts of complement and the 50 % haemolytic end-point calculated by the use of probits.

Treatment of suspensions with Arcton

Virus suspensions were homogenized at 13,000 r.p.m. with one-half volume of Arcton 113 (I.C.I. Ltd.), maintaining the suspension at 0° C. The homogenate was then centrifuged at 2000 r.p.m. and the aqueous layer treated in the same way until homogenization no longer produced an interface between the aqueous and solvent layers.

Inactivation and potency testing

Acetylethyleneimine (I.C.I. Ltd.) was used for virus inactivations (Brown & Crick, 1959). As a standard procedure, all virus suspensions were incubated for 6 hr. at 37° C. with 0·05 % acetylethyleneimine. The reaction was then stopped with 2 % sodium thiosulphate and the preparations tested for innocuity by intraperitoneal inoculation of mice. Appropriate dilutions of the vaccines were then mixed with equal volumes of aluminium hydroxide gel and inoculated subcutaneously into groups of guinea-pigs. Twenty-one days after vaccination, the animals were challenged by intradermal inoculation of one hind pad with 10^4 ID 50 of infective virus (prepared from infected guinea-pig pads) and the animals examined daily for 10 days for the development of lesions on the feet and tongue. From the number of animals showing lesions on the feet and tongue, the dilution of vaccine which protected 50 % of the animals from generalized infection was calculated.

RESULTS

Concentration of 25 mμ component in different virus suspensions

Since previous work by Randrup (1954) and Brown & Crick (1959) has indicated the importance of the 25 mμ component in FMD virus vaccines, a method was sought which would allow the rapid and accurate estimation of this component in several virus suspensions. The relative amounts of the 25 mμ component in five different virus suspensions, prepared from guinea-pig vesicle fluid, guinea-pig pad epithelium, infected mice and cultivated pig kidney and baby hamster kidney cells were compared by estimating the amount of complement which they fixed after treatment with Arcton 113. This procedure removes the 7 mμ component of FMD virus suspensions without lowering their infectivity (Mussgay, 1959; Brown & Cartwright, 1960). The data in Table 1 show that the proportion of the complement-fixing activity due to the 25 mμ component of the virus is about 50 % for the vesicle fluid and tissue culture viruses, but considerably lower for the pad epithelium and mouse tissue suspensions.

Table 1. *Concentration of 25 mμ component in FMD virus suspensions*

Virus source	Complement-fixing activity (ml. 1/30 C′/ml. antigen)	
	Initial suspension	Arcton-treated suspension
Vesicle fluid	144·0	72·0
Pad epithelium	4·12	0·80
Mouse muscle	15·4	2·0
Pig kidney cells	0·23	0·11
Baby hamster kidney cells	1·23	0·49

Comparison of potencies of different vaccines

The potencies of vaccines prepared from the five different sources of the virus were compared in experiments with guinea-pigs. The results of these experiments show that the amount of 25 mμ component required to protect 50% of the vaccinated animals against generalized infection was fairly constant for different batches of a given source of virus, but varied considerably between sources (Table 2). The values obtained for any of the five individual sources of virus used here varied by a factor less than 3. The difference in the amounts of 25 mμ component in the guinea-pig and tissue culture vaccines which were required for 50% protection was greater than this factor, suggesting a difference between the virus suspensions which was not measurable by this test.

Table 2. *Potency of FMD vaccines prepared from different sources of the virus*

Source	Complement-fixing activity of 25 mμ component (ml. 1/30 C′/ml.)	Amount of 25 mμ component protecting 50% of animals (ml. 1/30 C′/ml.)
Guinea-pig vesicle fluid	38·5	0·08
	60·0	0·17
	72·0	0·11
	55·0	0·11
Guinea-pig pad epithelium	1·6	0·16
	0·60	0·30
	1·1	0·20
Mouse muscle	0·63	0·08
	1·1	0·16
	2·3	0·07
	1·3	0·06
Pig kidney cells	0·095	0·02
	0·05	0·01
	0·11	0·01
	0·045	0·015
Baby hamster kidney cells	0·49	0·01
	0·32	0·01
	1·1	0·02

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Physical properties of the immunizing antigen

In view of the differences between the amounts of 25 mμ component (measured by complement-fixation tests) in the different suspensions which were required to produce immunity, the nature of the immunizing antigen in guinea-pig vesicle fluid and in pig kidney tissue culture virus was examined in more detail. With each suspension, the immunizing antigen was deposited by centrifuging at 30,000 r.p.m. for 150 min. (Spinco L 40 head), survived extraction with Arcton 113 (which removes all the 7 mμ component of the virus), and was unaffected by incubation at 25° C. or 37° C. for periods which reduced the infectivity to less than 0·1 % of the initial value. The immunizing antigen was completely destroyed on heating to 56° C. and considerably reduced by lowering the pH to 6·5. These properties are also possessed by the 25 mμ infective component of the virus, thus confirming the earlier conclusion reached for guinea-pig vesicle fluid that the immunizing antigen is associated with the 25 mμ component of the virus.

DISCUSSION

The lengthy and costly tests in cattle which are currently used to assess the potency of inactivated FMD vaccines make it worthwhile to explore the possibility of devising an *in vitro* test for this purpose. The prime requirement of such a test is that it should correlate reliably with the *in vivo* test. A serological test of this sort has been devised by Beale (1961) for polio vaccines.

The method most extensively used for the production of commercial FMD vaccines is based on the inactivation of the virus following its adsorption to aluminium hydroxide gel. *In vitro* testing of such vaccines would require prior elution of the inactivated virus from the aluminium hydroxide. Elution is difficult so that efforts to correlate the potency of FMD vaccines with other properties of the virus suspensions from which they were prepared must, at this stage, be made with the virus suspensions before inactivation.

In the experiments described here, the physical properties of the immunizing antigen have been studied in some detail. In accordance with the previous reports of Randrup (1954) and Brown & Crick (1959) the 25 mμ component of the virus has been shown to be immunogenic. Further, the 25 mμ component and immunizing antigen appear to behave identically under a variety of conditions, suggesting that the entire immunogenicity is associated with the 25 mμ component. Of particular interest in connexion with the growth of virus for the preparation of FMD vaccines is the fact that the immunizing properties of the virus suspensions are unaltered by incubation at 37° C. for periods long enough to reduce their infectivity titre by more than 99 %. This means that the stability of the immunizing antigen at 37° C. is much greater than that of the infective component of the virus. Brown, Cartwright & Stewart (1963) have shown that the effect of incubating FMD virus suspensions at 37° C. is to destroy the infectivity of the ribonucleic acid component of the virus without significantly altering the viral protein.

When FMD virus is grown in cultured cells at 37° C., virus released into the

medium during the early part of the growth cycle will decrease in infectivity (*ca.* 1 log in 8 hr.) although its immunogenicity will be unaffected. Unless the virus is released from all the susceptible cells over a short time interval, however, the times at which maximum infectivity and immunogenicity are reached will not coincide. This has been clearly demonstrated by Henderson (1953) and Ubertini, Nardelli, Barei & Santen (1956) in experiments with virus grown in surviving

Table 3. *Relationship between infectivity, complement-fixing activity and immunogenicity of two FMD virus suspensions*

Treatment or fraction	Infectivity (log ID50/ml.)	Complement-fixing activity (ml. 1/30 C′ fixed by 1 ml.)	Dilution of vaccine protecting 50 % of animals
Initial virus (guinea-pig vesicle fluid)	9·2	144·0	1/750
Heated at 25° C. for 72 hr.	6·0	140·1	1/500
Heated at 37° C. for 48 hr.	3·2	144·0	1/750
Acidified to pH 6·5	5·9	267·2	1/20
Heated at 56° C. for 0·5 hr.	4·4	202·0	No protection with 1/5
Arcton-extracted	9·2	72·5	1/500
Fractions from centrifuge 30,000 r.p.m. for 1 hr.			
Top 7 ml.	5·5	47·5	1/5
Bottom 4 ml.	6·3	27·3	1/20
Pellet	8·9	93·5	1/350
Initial virus (pig kidney tissue culture suspension)	6·0	0·23	1/10
Heated at 37° C. for 24 hr.	3·0	0·22	1/5
Heated at 56° C. for 0·5 hr.	< 2·0	0·34	No protection with 1/1
Acidified to pH 6·5	5·0	0·37	No protection with 1/1
Arcton-extracted	6·0	0·11	1/10
Fractions from centrifuge 30,000 r.p.m. for 1 hr.			
Top 7 ml.	2·8	0·04	No protection with 1/1
Bottom 4 ml.	4·2	0·06	No protection with 1/1
Pellet	5·9	0·10	1/4

tongue epithelium fragments. Experiments at this Institute with several strains of the virus have shown that it is possible to assess the potency of a vaccine from its infectivity titre if the growth cycle of the particular strain is well characterized (Henderson & Galloway, unpublished observations). Differences in the multiplication of different virus strains, even in the same tissue system, make it essential to study the growth cycle under precisely controlled conditions before an assessment of its potency can be made from its infectivity titre. Even with these precautions, a relationship between infectivity and potency which is applicable to all virus strains grown in a variety of tissues or cells would be difficult to establish.

It is known that the complement-fixing activity of FMD virus suspensions which have been extracted with Arcton is a measure of both the infective and non-infective 25 mμ components of the virus. Consequently, the relationship between the complement-fixing activity of the 25 mμ component in several virus suspensions and their potencies as vaccines for guinea-pigs has been examined. For different preparations of virus from one kind of animal tissue or cell, the amount of 25 mμ component required to protect 50% of the guinea-pigs varied by less than threefold. Larger differences were obtained, however, between the various sources used here. For example, the 50% protective dose of 25 mμ component was about 10 times greater for guinea-pig vesicle fluid than for pig kidney or baby hamster kidney virus. This may be due to masking of the 25 mμ component in the tissue culture viruses by a cellular component. Masking of this sort is known to occur with FMD virus (Brown, Cartwright & Stewart, 1962) and also with poliomyelitis virus (Holland & McLaren, 1959). While, therefore, the complement-fixing activity of the 25 mμ component of suspensions can be used as an index of their potency as vaccines if they are all derived from similar animal or culture systems, the relation between vaccines prepared from different cell systems has still to be determined.

SUMMARY

Inactivated vaccines have been prepared from one strain of FMD virus grown in guinea-pig pad epithelium, unweaned mice and cultured pig kidney and baby hamster kidney cells. The potencies of these vaccines in protecting guinea-pigs against challenge with inoculated infective virus of the same strain have been compared and related to the amounts of 25 mμ component present in the different virus suspensions. Although it was possible to obtain a relationship between the content of 25 mμ component and potency for an individual source of virus, this relationship does not hold for all the different sources of virus used. It is suggested that the reason for this failure is the partial masking of the 25 mμ component by a cell constituent present in some of the virus suspensions so that the component is incompletely estimated by the complement-fixation test.

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