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Antigenic differences between strains of foot-and-mouth disease virus of type SAT 1

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INTRODUCTION

Strains of foot-and-mouth disease (F.M.D.) virus of types O, A and C, isolated during the past 15 years from outbreaks of the disease in the field, have exhibited marked differences of invasiveness, of virulence and of pathogenicity for various animal species. Tests *in vitro* reveal that many strains show preferential fixation of complement in the presence of antiserum against the identical strain, and a quantitatively inferior degree of fixation with antisera produced by other strains of the same type. Furthermore, there is now considerable experimental evidence that differences in antigenic constitution can be demonstrated not only by serological methods, complement fixation and serum neutralization tests, but also by cross immunity experiments in cattle.

Traub & Möhlmann (1946) and Galloway, Henderson & Brooksby (1948), in studies of type A strains, were the first to demonstrate the significance, in relation to immunization, of antigenic differences between strains within a main immunological type by serological tests and experimental cross immunity tests in cattle.

From 1947 onwards the investigators at Pirbright have made comparisons of eight pairs of strains belonging to four different type groups—O, A, SAT 1 and SAT 2—by complement fixation and serum neutralization tests and by cross vaccination experiments in cattle.

Type A strains: 119 and MP; 119 and MI; MI and MP (referred to above, Galloway *et al.* 1948)).

Type O strains: Ven 1 (Venezuela 1950) and H_2 (Holland). H_{2200} (Holland 1951) and Ca 1 (Canada 1952). H_{2200} and 734 (Great Britain 1951).

Type SAT 1 strains: RV 11 (Southern Rhodesia 1937) and Bec 1 (Bechuanaland 1948).

Type SAT 2 strains: Rho 1 (Northern Rhodesia 1948) and RV 1 (Southern Rhodesia 1931).

The results obtained with three of the pairs—Rho 1 and RV 1, H_{2200} and Ca 1, H_{2200} and 734—indicated that the two strains compared in each case were identical in their antigenic behaviour. With the other five pairs, the results indicated that the strains in each pair differed in their antigenic structure and could be classified as variant strains (subtypes). In the case of the similar pairs, a vaccine prepared from one strain protected quantitatively equally well against infection with either strain, whereas, in the case of dissimilar pairs, a vaccine prepared from one strain

protected better against the homologous strain than against the heterologous strain.

Martin, Davies & Smith (1962) found that a mouse-adapted vaccine strain (Rho 1/48) of type SAT 2 evoked good protection against challenge with the homologous strain but only incomplete protection against challenge with the heterologous strain SA 106/59. Complement fixation (C.F.) tests indicate that these strains are of different subtypes (Davie, 1962).

Girard & Mackowiak (1950) reported strain differences in strains of type O. Ubertini (1951) recorded similar observations in investigations on strains of type A and O recovered from outbreaks in the Po valley. These authors stressed likewise that demonstrable differences in antigenic structure of strains of the same immunological type were correlated with the results of vaccination, though Röhrer, Möhlmann & Pyl (1951) considered these differences to be of little practical importance.

Although antigenic differences between strains of type C and type SAT 3 have been detected by serological methods, cross-immunity tests with these strains have not been made. As regards type ASIA 1 no information is available as yet on subtype differences between strains. However, there is no reason to suppose that the results of investigations on strains of these three types would be at variance with those reported on the other four main immunological type-groups.

A recent outbreak of F.M.D. in South-West Africa gave impetus to investigations on several strains of type SAT 1. The following report demonstrates the effect of immunological differences between two strains which were revealed by cross protection tests *in vivo* and confirmed *in vitro* by C.F. tests and by neutralization of virus with serum from immunized cattle. The paper also indicates that it is possible to prepare potent inactivated vaccines against F.M.D. virus of type SAT 1.

MATERIALS AND METHODS

Virus

Type SAT 1 F.M.D. virus of strain RV 11, isolated in 1937 from an infected kudu in Southern Rhodesia, and of strain SA 13/61, recently isolated from an ox during an outbreak in the Northern Transvaal, were each grown in tongue epithelium tissue cultures and used for the production of formalin-inactivated vaccines. Virulent virus of both strains, serially passaged in susceptible cattle, was employed to challenge the resistance of vaccinated animals. Guinea-pig adapted strains of the same isolates were maintained for similar tests in guinea-pigs.

Cattle

All cattle used in these experiments were Devon steers, about 18 months old, which were purchased through a dealer who collected them from various parts of south-west England. During the experiments all cattle were housed in isolation.

Guinea-pigs

Groups of young guinea-pigs (about 500 g. body weight) were obtained from the small-animal breeding unit at this Institute. Guinea-pigs infected with different strains were housed in separate rooms.

Mice

Infectivity titrations were made by inoculating serial dilutions of virus suspensions into 4–6-day-old albino mice of the Pirbright 'P' strain.

Vaccine production

The method employed at Pirbright is a modification of that described by Frenkel (1949, 1951, 1953), virus being propagated, inactivated and stored in large mechanically stirred stainless steel vessels, the temperature of which is accurately determined by thermostatically controlled water-jackets.

In the production of a typical batch of vaccine, 7·5 kg. of freshly harvested bovine tongue epithelium fragments were coarsely minced and then suspended in 200 l. of a medium consisting of Tyrode's solution containing 0·5 % lactalbumin hydrolysate and antibiotics (Penicillin, Neomycin and Mycostatin).

'Seed' virus was then inoculated and the culture was incubated for 16 hr. at 37 °C., pH being maintained at about 7·6 by aeration with a mixture of 5 % CO_2/95 % O_2 (7·5 l./min.).

Tissue fragments were then strained from the fluid, milled into a paste and resuspended in the culture fluid to extract residual virus. After clarification by continuous-flow centrifugation, the extract was filtered through Seitz K and EK grade asbestos pads mounted in series, and was then pumped into jacketed vessels maintained at 26 °C. Samples were withdrawn for infectivity titrations in mice.

The suspension was mixed with 250 l. of aluminium hydroxide gel and 5 l. of 2 M glycine buffer (pH 9·0) and exposed to the action of 250 ml. of 40 % formaldehyde solution. Inactivation continued for 48 hr. at 26° C., after which the temperature was reduced to 4° C. to inhibit further action of any residual formalin.

Storage

Vaccine batches were bottled mechanically and stored in cold rooms at 4° C. Stock strains of virulent virus were stored either at −20° C. or at 4° C. in other refrigerators.

Vaccine assay

Innocuity. Volumes of 0·1 ml. from pooled samples of each batch of vaccine used in these experiments were inoculated into the lingual epithelium at 20 sites on the tongue of each of 6 susceptible steers; no F.M.D. lesions were observed. The validity of such tests was discussed by Henderson (1952*a*, *b*).

Potency. The potency of each batch of vaccine was determined by inoculating a group of steers with the usual 15·0 ml. dose of undiluted vaccine and a further group with the same volume of vaccine diluted 1/3 with inert vaccine base. After 3 weeks the cattle were challenged, together with unvaccinated controls, by inoculating 10,000 ID 50 of virulent cattle-passaged virus into the epithelium at each of 10 sites on the tongue. All animals were bled for serum before vaccination and again before challenge. Cattle which showed no secondary lesions at sites other than in the oral cavity were considered to be resistant to infection.

Samples of tongue epithelium collected from 'virus donor' cattle (in which the virus was passaged immediately before the challenge experiment), and also from representative reacting cattle in each group, were examined for virus specificity against all seven types of F.M.D. antiserum by the C.F. test.

Serum-neutralization tests

Pre-vaccination and pre-challenge samples of serum were titrated for neutralizing antibody by the tissue culture colour test technique described by Martin & Chapman (1961) with a slight modification. All sera were titrated in serial twofold dilutions against 100 T.C. ID50 of virus of each of the strains used in the cattle experiments; the usual controls were included in each test. Results were expressed as the final dilution in the serum-virus mixture.

Complement-fixation tests

Virus of each of the strains under investigation was adapted to growth on the plantar pads of guinea-pigs, and strain-specific hyperimmune sera were produced. Complement-fixation tests employing these sera followed the method described by Brooksby (1952) and the results were assessed in accordance with the principles described by Bradish, Brooksby & Tsubahara (1960) and by Bradish & Brooksby (1960).

Each virus was tested with each of the strain-specific sera, and the amount of complement required for 50% haemolysis was determined. The results were expressed as the cross-fixation ratios (C.F.R.) of the systems, i.e.

$$\frac{\text{amount of complement fixed in the heterologous system}}{\text{amount of complement fixed in the homologous system}}.$$

The product of the C.F.R. for two strains was used to indicate their degree of antigenic relationship.

RESULTS

1. *Tests in guinea-pigs*

A group of 10 guinea-pigs was infected by inoculation of the left plantar pad with guinea-pig passaged F.M.D. virus of strain RV 11. A similar group was infected with strain SA 13/61. The disease generalized in all animals. After a recovery period of 28 days, 5 guinea-pigs from each group and 4 uninoculated controls were inoculated on the right plantar pad with virus strain RV 11; the remaining animals of the convalescent groups and 4 susceptible controls were inoculated with strain SA 13/61 virus.

The results of this experiment, which are shown in Table 1, indicated that the antigenic constitutions of the two strains were dissimilar, severe infection with one strain stimulating good resistance to reinfection with the same strain but evoking only poor protection against the other strain.

Another cross-protection experiment (Table 2) demonstrated that intradermal inoculation of virulent suspensions of strain RV 11 on two occasions, with an interval of 19 days between, failed to protect 1 of 2 guinea-pigs reinoculated 22 days later with virus of strain SA 13/61.

2. *Tests in cattle*

Groups of cattle were inoculated with vaccine of batches 613 (RV 11), 614 and 615 (SA 13/61). The virus content of these vaccines, as determined by infectivity tests before inactivation, was $10^{6.8}$, $10^{6.9}$ and $10^{7.1}$ mouse ID 50/ml., respectively.

Innocuity tests by inoculation into the epithelium of the tongue did not reveal infectivity in any of the batches, and all the cattle vaccinated by the subcutaneous route remained normal during the 3 weeks' period between vaccination and challenge.

Table 1. *Reinfection of guinea-pigs convalescent from experimental infection with* F.M.D. *virus of type SAT* 1

Recovered from	Reinfected with	Lesions	
		Primary	Generalized
RV 11	RV 11	2/5	0/5
SA 13/61	SA 13/61	0/5	0/5
SA 13/61	RV 11	5/5	2/5
RV 11	SA 13/61	5/5	3/5
Controls	RV 11	5/5	5/5
Controls	SA 13/61	5/5	5/5

Table 2. *Reinfection of guinea-pigs hyperimmunized with* F.M.D. *virus of type SAT* 1

Immunization strain	Guinea-pig lesions after challenge	
	RV 11	SA 13/61
RV 11	0/2	1/2
SA 13/61	0/2	0/2
Controls	2/2	2/2

(A) *Challenge with virus of the homologous strain*

In a group of 10 steers inoculated with undiluted vaccine of strain RV 11 (batch 613), none developed secondary lesions after challenge with 10^5 ID 50 of virulent virus of the same strain. In a similar group inoculated with Batch 613 diluted 1/3 in aluminium hydroxide-buffer base, 3 showed some degree of generalization. Generalization to all feet occurred in 3 unvaccinated controls.

Further groups of 10 steers were vaccinated with undiluted vaccine of strain SA 13/61 from batches 614 and 615, and a 3rd group was inoculated with vaccine of batch 615 diluted 1/3. All animals which had received undiluted vaccine withstood the challenge without generalization of infection. Incomplete generalization occurred in 2 of the 10 steers which were inoculated with diluted vaccine. In contrast, lesions generalized in all of 10 unvaccinated controls.

Columns 8 and 16 of Figs. 1 and 2 show the clinical results for individual steers. The black symbol in the first square represents the extent of the primary lesions on the tongue, and a diagonal band in this square indicates development of vesicles

in parts of the buccal cavity other than the tongue (usually the lips, gums or dental pad). Black symbols in the succeeding squares represent lesions on 1 or more feet.

(*B*) *Challenge with virus of the heterologous strain*

The satisfactory results obtained with both strains, when the immunity of vaccinated cattle was challenged with the homologous strain, were not observed when cattle vaccinated with either strain were exposed to challenge with the heterologous strain.

Of 10 steers inoculated with undiluted vaccine of strain RV 11 and challenged 22 days later with strain SA 13/61, only 5 were protected completely. In the similar group for which the vaccine was diluted 1/3, none was protected completely although 2 developed lesions on the dental pad only (Fig. 1). Generalization was also complete in all of 6 unvaccinated steers.

The difference between strains RV 11 and SA 13/61 was revealed even more clearly when the immunity of cattle inoculated with strain SA 13/61 vaccine was challenged with virus of strain RV 11.

As in the corresponding experiment, in which the cattle were challenged with strain SA 13/61, 10 steers were inoculated with undiluted vaccine of batch 614, 10 steers received undiluted vaccine of Batch 615 and a further 10 steers were inoculated with vaccine of Batch 615 diluted 1/3. Among the groups which received undiluted vaccine a significant difference between vaccine batches was not observed, the strain RV 11 challenge virus generalizing to 1 or more feet in each case. Of the 10 cattle inoculated with vaccine diluted 1/3, all developed foot lesions. Infection generalized to all the feet of 4 unvaccinated control animals (Fig. 2).

To provide additional evidence of the identity of the challenge material, part of the virus filtrate used to infect the cattle was subjected to a C.F. test against reference antisera of Types O, A, C, SAT 1, SAT 2, SAT 3 and ASIA 1. The test was positive for SAT 1 only. Another part of the filtrate was inoculated into mice, and suspensions of the triturated carcasses of mice dying from these inoculations were pooled; samples from this pool were mixed with equal volumes of serial dilutions of SAT 1 antiserum before subinoculation into further mice. Carcasses of mice dying at the end-point dilution, when used as antigen for C.F. tests against the 7 reference antisera, produced a positive result with SAT 1 antiserum only.

3. *Serum neutralization tests*

The results shown in Figs. 1 and 2 indicate that there was an increase in virus-neutralizing antibody in the sera of the majority of cattle during the 3 weeks interval between vaccination and challenge.

For various reasons, pairs of serum samples collected before vaccination and before challenge were not available for all of the 100 vaccinated cattle. When samples were tested against virus of the strain incorporated in the vaccine, only 3 of the 97 animals for which pairs of sera were available failed to show an increase in titre. When the same sera were tested against virus of the alternative strain,

Challenge strain RV 11

Antigen strain RV 11			Serum titre				
Vaccine			Day 0		Day 22		
No.	Dilution	Animal No.	RV 11	13/61	RV 11	13/61	Lesions
613	0	DL 18	1/3	1/16	1/256	1/3	
		19	1/11	1/22	1/90	N.A.	
		20	1/11	1/22	1/45	1/11	
		21	1/8	N.A.	1/355	N.A.	
		22	1/3	1/3	1/90	1/22	
		23	1/11	1/22	1/64	N.A.	
		24	1/16	1/16	1/64	1/16	
		25	1/11	1/16	1/90	1/22	
		DD 26	1/3	1/3	1/64	1/22	
		27	N.A.	1/11	1/64	1/22	
613	1/3	DD 28	1/3	1/22	1/64	N.A.	
		29	1/3	1/8	1/90	1/22	
		30	1/16	1/3	1/128	1/22	
		31	1/16	1/3	1/45	N.A.	
		32	1/11	1/3	1/45	N.A.	
		33	1/16	1/3	1/45	N.A.	
		34	1/22	1/3	1/90	N.A.	
		35	1/22	1/3	1/90	1/45	
		36	1/22	1/3	1/22	1/22	
		37	1/3	1/3	1/45	1/16	
Unvaccinated controls		DL 26					
		27					
		28					

Challenge strain 13/61

Antigen strain RV 11			Serum titre				
Vaccine			Day 0		Day 22		
No.	Dilution	Animal No.	RV 11	13/61	RV 11	13/61	Lesions
613	0	DK 72	1/8	1/3	1/90	1/45	
		73	1/3	1/3	1/45	N.A.	
		74	1/3	1/3	1/45	1/8	
		75	1/22	1/32	1/90	1/45	
		76	1/11	1/3	1/128	N.A.	
		77	1/8	1/3	1/90	1/11	
		78	1/11	1/8	1/90	1/45	
		79	1/22	1/11	1/178	1/22	
		80	1/11	1/3	1/178	1/45	
		81	1/8	1/8	1/128	1/22	
613	1/3	DK 82	1/11	1/3	1/32	1/11	
		83	1/8	1/4	1/64	1/11	
		84	1/8	1/11	1/90	1/22	
		85	1/8	1/3	1/178	N.A.	
		86	1/8	1/3	1/45	1/3	
		87	1/22	1/3	1/90	1/16	
		88	1/11	1/3	1/64	1/3	
		89	1/16	1/16	1/32	1/8	
		90	1/6	1/3	1/178	1/22	
		91	1/11	1/3	1/90	1/22	
Unvaccinated controls		DL 12					
		13					
		14					
		15					
		16					
		17					

Fig. 1. Neutralizing antibody responses of cattle to RV 11 antigen, and reactions to challenge after 22 days. N.A., Result not available.

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24 of 87 pairs of sera did not show a rise in titre, and of these 6 showed a slight (but probably non-significant) fall.

Individual cattle showed well-marked differences in the response to the antigenic stimulus of the vaccines and, for this reason, groups of cattle will be considered as a whole. Table 3 shows the geometric mean ($n\sqrt{(a.b.c. \ldots)}$) of the reciprocal titre for each group at the times of vaccination and of challenge. In each case the mean for the 'homologous' serum-virus system reveals a satisfactory level of antibody immediately before challenge, whereas in the 'heterologous' system the antibody level was much lower at this time. Because the pre-vaccination mean antibody titres are not similar for all the groups, the logarithmic index of neutralization has been calculated (Table 3, columns 5 and 8). These indices demonstrate that, 3 weeks after vaccination, irrespective of the antigen used,

Antigen strain 13/61			Challenge strain 13/61				
			Serum titre				
Vaccine		Animal No.	Day 0		Day 22		Lesions
No.	Dilution		RV 11	13/61	RV 11	13/61	
614	0	DN 6	1/3	1/16	1/22	1/178	
		7	1/11	1/16	1/64	1/256	
		8	1/3	1/3	1/22	1/90	
		9	1/3	1/8	1/32	1/256	
		10	1/3	1/11	1/45	1/178	
		11	1/3	1/6	1/8	1/90	
		12	1/3	1/3	1/45	1/512	
		13	1/22	1/3	1/90	1/512	
		14	1/22	1/45	1/64	1/512	
		15	1/3	1/3	1/32	1/64	
615	0	DM 86	1/3	1/8	1/16	1/128	
		87	1/3	1/22	1/11	1/128	
		88	1/6	1/3	1/11	1/90	
		89	1/22	1/22	1/11	1/64	
		90	1/11	1/3	1/8	1/90	
		91	1/11	1/16	1/11	1/128	
		92	1/8	1/45	1/3	1/90	
		93	1/8	1/11	1/8	1/64	
		94	1/16	1/22	1/16	1/90	
		95	1/22	1/45	1/22	1/256	
615	1/3	DM 96	1/8	1/11	1/32	1/128	
		97	1/3	1/22	N.A.	1/45	
		98	1/3	1/3	1/6	1/45	
		99	1/3	1/16	1/3	1/64	
		DN 0	1/3	1/11	1/22	1/64	
		1	1/3	1/8	1/45	1/90	
		2	1/8	1/16	1/64	1/64	
		3	1/3	1/3	1/22	1/22	
		4	1/3	1/3	1/16	1/64	
		5	1/3	1/16	1/11	1/178	
Unvaccinated controls		DN 41					
		42					
		43					
		44					
		DM 80					
		81					
		82					
		83					
		84					
		85					

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Antigen strain 13/61			Challenge strain RV 11				
			Serum titre				
Vaccine			Day 0		Day 22		
No.	Dilution	Animal No.	RV 11	13/61	RV 11	13/61	Lesions
614	0	DN 65	1/3	1/8	1/22	1/178	
		66	1/3	1/3	1/11	1/178	
		67	N.A.	N.A.	1/22	1/90	
		68	1/3	1/3	1/22	1/90	
		69	1/3	1/11	1/3	1/64	
		70	1/3	1/3	1/3	1/90	
		71	1/3	1/11	1/3	1/32	
		72	1/3	1/3	1/22	1/90	
		73	1/3	1/3	1/22	1/90	
		74	1/3	1/3	1/8	1/64	
615	0	DN 45	1/8	1/3	1/22	1/90	
		46	1/11	1/22	1/32	1/90	
		47	1/6	1/11	1/3	1/32	
		48	1/3	1/3	1/3	1/90	
		49	1/8	1/3	1/22	1/90	
		50	1/3	1/3	1/8	1/22	
		51	1/4	1/8	1/16	1/64	
		52	1/3	1/3	1/8	1/45	
		53	1/8	1/8	1/45	1/90	
		54	1/8	1/16	1/16	1/11	
615	1/3	DN 55	1/3	1/8	1/22	1/16	
		56	1/3	1/3	1/22	1/3	
		57	1/11	1/8	1/11	1/90	
		58	1/8	1/11	1/8	1/45	
		59	1/3	1/3	1/11	1/22	
		60	1/11	1/22	1/11	1/45	
		61	1/3	1/4	1/8	1/45	
		62	1/6	1/6	1/11	1/22	
		63	1/3	1/11	1/3	1/90	
		64	1/3	N.A.	1/3	1/90	
Unvaccinated controls		DO 1					
		2					
		3					
		4					

Fig. 2. Neutralizing antibody responses of cattle to SA 13/61 antigen and reactions to challenge after 22 days. N.A., Results not available.

Table 3. *Geometric means of the reciprocal neutralization titres of sera from vaccinated cattle tested against virus of strains RV* 11 *and SA* 13/61

		Mean reciprocal neutralization titre against virus strains					
		RV 11			SA 13/61		
Experiment no.	Antigen	day 0 (*a*)	day 22 (*b*)	N.I.* log *b*–log *a*	day 0 (*c*)	day 22 (*d*)	N.I.* log *d*–log *c*
1	RV 11	7·2	93·3	1·11	11·7	14·3	0·09
2	RV 11	8·9	96·3	1·04	5·3	25·4	0·68
3	SA 13/61	6·7	19·2	0·46	10·1	146·4	1·16
4	SA 13/61	4·1	11·3	0·44	5·2	66·9	1·11

* Neutralization index.

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the mean serum-neutralization titre has increased approximately threefold for the 'heterologous' serum-virus system and approximately 12-fold for the 'homologous' system.

Despite the wide variations of individual response revealed by serum neutralization tests, Figs. 1 and 2 demonstrate a good correlation between the serum

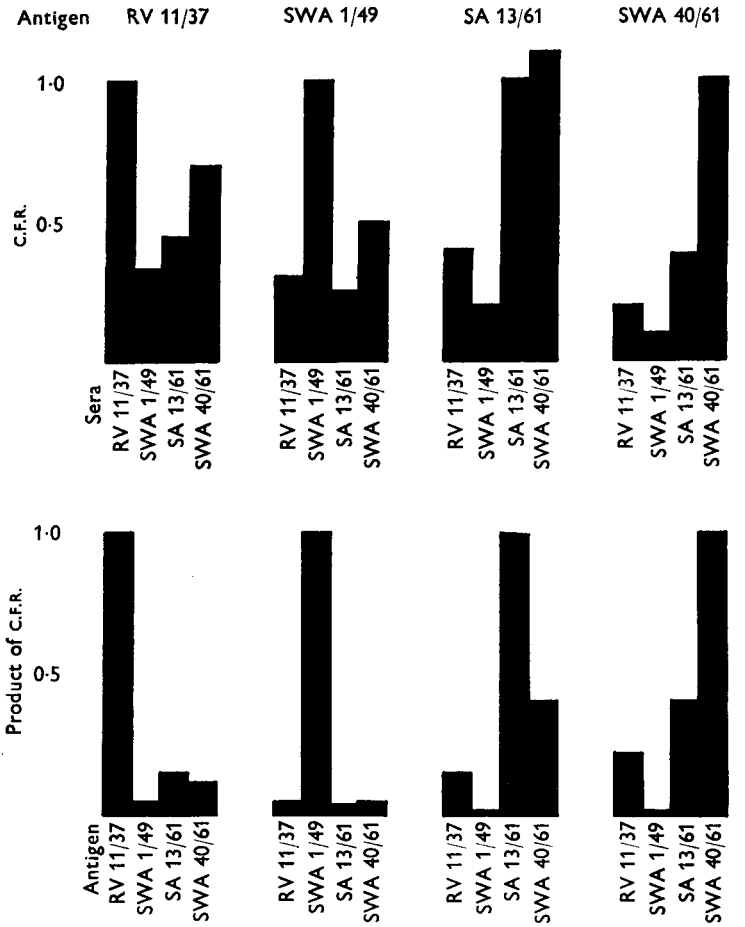

Fig. 3. Cross fixation ratios and their products for four strains of F.M.D. virus with strain-specific sera.

titre at the time of challenge and the clinical results of the challenge for each animal. Notwithstanding a few exceptions, it may be stated that cattle in which a titre of 1/45 is evoked are unlikely to develop a generalized infection as a result of the inoculation of 10^4 ID 50 of virus at each of 10 sites on the tongue; generalization is very likely to occur, however, in cattle whose neutralization titres are 1/22 or less.

4. *Complement-fixation tests*

During a survey of virus strains of type SAT 1, C.F. tests revealed (Davie, 1962) that the strains investigated could be classified into at least six distinct subtype groups. The strains RV 11, SA 13/61 and the stock type-specific strain SWA 1/49,

used for routine diagnostic tests in the World Reference Laboratory, each represent a separate subtype group.

The relationships of these strains and of strain SWA 40/61, isolated from the recent outbreak in South-West Africa, are illustrated by the histograms in Fig. 3. Because the antiserum of strain SWA 40/61 is of very high titre and the strain possesses some antigenic similarity to strain SA 13/61, its C.F.R. with strain SA 13/61 antigen is greater than unity. The cross-fixation product of the two antigens is 0·42.

The C.F.R. of strains RV 11 and SA 13/61 are small and the cross-fixation product is 0·17. This evidence of a wide antigenic difference between the strains is confirmed by the other tests described in this paper.

DISCUSSION

Although it seems at present that most strains of the virus of F.M.D. can be grouped into distinct types by serological and cross immunity tests, there is an increasing volume of evidence of wide variation within these type groups. Doubt may even exist if two strains at the extremes of the spectrum of variation within a type can be classified as of the same type. When, however, two such strains are related serologically to the intermediate strains within the type, and when tests reveal some cross immunity, albeit not complete, it would appear best to continue to refer to the strains as of the same type. Evolutionary progress may result in even greater subtype variation and so may make type definition even more difficult.

In the work reported here, the two strains concerned failed to give complete cross immunity in tests in guinea-pigs; and this may be considered to indicate an unusually great degree of dissimilarity between the strains. Shortage of cattle accommodation has precluded further examination, by cross-protection tests, of the antigenic relationship between these strains and other strains of type SAT 1, but the results of vaccination trials in the field would suggest that the classification of both strains as type SAT 1 is justified.

The complete protection of cattle evoked by vaccination with two strains of type SAT 1 against a severe experimental infection with the homologous strain is amply demonstrated in the foregoing experiments. This immunity contrasts remarkably with the almost complete lack of resistance to virus of the heterologous strain, although the animals in these groups all possessed serum antibody titres against virus of the vaccine strain which might have been expected to protect them (Figs. 1 and 2) against the homologous strain. The serum antibody titres against the heterologous strain were uniformly low and were consistent with the clinical results. Whether such low mean neutralizing antibody levels would be of any effect against *natural* exposure, which might be less severe than our experimental intradermal inoculation, remains a matter for speculation. It is unlikely, however, that the protection resulting from these liminal values would be of very long duration unless it was quickly reinforced by 'field virus'.

Figure 4 illustrates (diagrammatically only) the probable effect of strain differences on the performance of vaccines in the field; the regression curve for

this diagram is derived from data relating to a similar vaccine of type ASIA 1. If natural exposure occurred during the period represented by the shaded area of the graph, herd resistance might be reinforced without the appearance of clinical disease and thus a strain of considerable antigenic dissimilarity from the field strain would appear to evoke satisfactory immunity. Outside this period only the homologous strain, or a strain very similar to it, would produce satisfactory results.

The results of the use of vaccines under field conditions may be modified considerably by the interval between vaccination and exposure and by a variety of other extraneous factors which may mask or exacerbate the effects of antigenic differences between the vaccine strain and the wild strain of virus. Nevertheless, if C.F. tests demonstrate that antigenic dissimilarity is not very great, it appears that vaccination may provide some protection against natural exposure to a fully

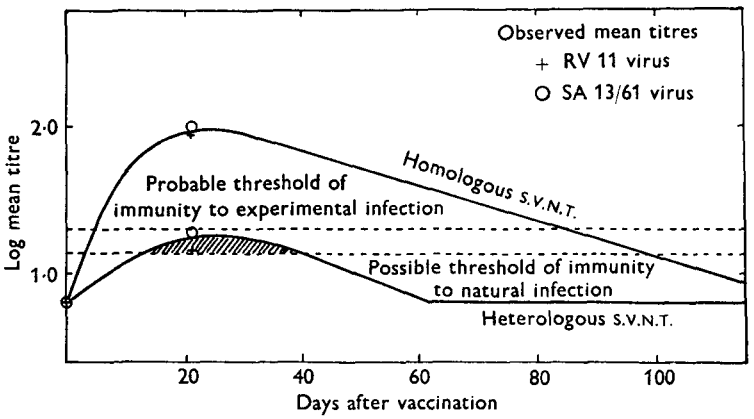

Fig. 4. Diagram illustrating the effect of strain differences on the resistance of cattle to experimental and natural infection.

invasive field strain of a somewhat different antigenic constitution. Thus, preliminary reports indicate that an inactivated vaccine of strain SA 13/61, which is being used currently against the outbreak of F.M.D. from which strain SWA 40/61 was isolated, has given generally satisfactory results (about 95% protection) under African conditions in which exposure to infection probably occurred fairly soon after vaccination. The relatively close antigenic relationship between the two strains is shown in Fig. 3. These field trials have been the subject of a preliminary report (Galloway, 1962). Figure 3 also illustrates the relationship between these strains, strain RV 11, and the standard SAT 1 diagnostic strain SWA 1/49. Although the use for vaccines of strains which, though not identical, are shown to be antigenically related closely may be dictated by practical considerations, the controlled experiments suggest that *optimum* results from vaccination may be expected only when the homologous strain is incorporated in the vaccine.

As a consequence of experience gained with a pilot-scale plant at this Institute, little difficulty has been encountered in culturing F.M.D. virus of many strains to a high titre (10^7—$10^{8\cdot5}$ ID 50/ml.). Furthermore, it has been possible to produce virus of some strains on a 500 l. batch scale within 2 weeks of primary culture.

This ability to produce quickly large quantities of strain-specific vaccine suggests that, in the parts of the world where vaccination is acceptable as a control measure, future vaccination campaigns might commence with vaccine from existing stocks of the strain shown by C.F. tests to resemble the field strain most closely, but, if a substantial difference existed between stock and field strain, as soon as possible thereafter the use of this vaccine would be superseded by vaccine prepared from the local strain of virus of adequate antigenicity.

SUMMARY

Antigenic differences between the strains RV 11 and SA 13/61 of foot-and-mouth disease virus (type SAT 1) were studied *in vivo* by cross-protection tests. Cattle inoculated with formolized antigen of either strain developed good immunity to experimental infection with the identical strain but little resistance to the other strain.

In vitro the results of complement fixation tests and of serum-virus neutralization tests in tissue culture were consistent with the observations made *in vivo*. The results of studies on the serological relationships between four strains of type SAT 1 are presented.

The importance of strain differences in the epizootiology and control of the disease is discussed briefly.

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