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The evaluation of hypersensitivity tests in cattle after foot-and-mouth disease vaccination

By L. BLACK and T. W. F. PAY
The Wellcome Foot and Mouth Disease Vaccine Laboratory, Pirbright, Surrey

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SUMMARY

The response to passive cutaneous anaphylaxis, dermal hypersensitivity and intravenous provocation tests has been compared in 30, 40, 31 and 24 cattle injected with foot-and-mouth disease vaccine 0, 1, 2 and 3 times respectively, using vaccine components and other substances as test materials. Reaginic antibodies, demonstrated by passive cutaneous anaphylaxis in goats, were directed against BHK 21 cell extracts (20), hydroxypropylmethylcellulose (3) and an unidentified vaccine component (3), and distributed in 0, 5, 19 and 75% of the cattle vaccinated 0, 1, 2 and 3 times. None of the animals showed clinical signs of allergy after vaccination. When BHK 21 cell extract was injected intradermally a significant correlation was noted between the development of large weals and the presence of reagins although the size of the weals was not correlated with the reagin titres. In the case of hydroxypropylmethylcellulose a similar trend was evident. The majority of cattle with large dermal weals possessed reagins but the number of reactions was too small for statistical evaluation. Dermal reactions to sodium penicillin, sodium carboxymethylcellulose, saponin and whole vaccine occurred in both unvaccinated and vaccinated cattle but BHK 21 cell lysate and normal bovine serum provoked weals which increased in frequency according to the number of vaccinations experienced. Intravenous hydroxypropylmethylcellulose elicited a response in all the animals previously injected with certain batches of vaccine but none in those injected with other batches or in unvaccinated controls. BHK 21 cell extract intravenously produced a clinical response in half the tested animals which was uncorrelated with the results of the passive cutaneous anaphylaxis or dermal hypersensitivity tests.

INTRODUCTION

Allergic reactions of the immediate (Mayr *et al.* 1969; Ubertini & Barei, 1970; Beadle, 1971) and delayed types (Bauer, Kaaden & Mussgay, 1970; Bahnemann, Gielhausen & Schweckendiek, 1971; Mayr, 1971) have been reported after foot-and-mouth disease (FMD) vaccination. Although the total incidence has been low (*ca.* 0·12 per 1000 in the Federal Republic of Germany during 1967 to 1970) (Lorenz & Straub, 1973) alarming increases have been reported on certain occasions (Capstick *et al.* 1970; Mayr *et al.* 1969; Mussgay, 1970). Comparisons between different vaccines used in Germany have yielded conflicting results. Frenkel

vaccines provoked more untoward reactions (immediate and delayed allergies and abortions) than vaccines produced on cell cultures in the 1970 vaccinations of some 7·97 million cattle (Lorenz & Straub, 1973) though the reverse was true in the 1967 and 1968 vaccination campaigns (Mayr & Mussgay, 1970; Baljer & Mayr, 1971).

The response of allergic subjects to intradermal (i/d) injections has been used in efforts to identify the allergens in vaccine (Bahnemann *et al.* 1971; Ballarini, Zannetti & Scandiani, 1971; Ubertini & Barei, 1970) but this test gives inconsistent results. Interpretation is complicated because reactions occur to allergens which are not responsible for the clinical signs (Lindblad & Farr, 1961; Capstick *et al.* 1970; Mayr & Mussgay, 1970; Lieberman, Patterson, Metz & Lucena, 1971) and there is the difficulty of fixing a threshold of positivity in presence of variations in skin sensitivity (Bachofen, Scherrer, Trautlein & Wyss, 1967; Van Arsdel, 1971). Furthermore test substances which are irritant cause non-specific reactions (Vanselow, 1967) and responses are dependent on both the dose of antigen and technique of administration (Biliotti, Passaleva, Romagnani & Ricci, 1972; Stenius, 1973). Using the passive cutaneous anaphylaxis test, reaginic antibodies to vaccine components have been demonstrated in clinically allergic and non-allergic cattle (Capstick *et al.* 1970; Beadle & Pay, 1975) and provocation tests have also been used to identify the substances eliciting a clinical response (Eyal & Mayer, 1971; Ginanni & Maglione, 1972).

In atopic human subjects correlations have been demonstrated between skin sensitivity and serum IgE concentrations (Stenius *et al.* 1971; Fitch, Turner & Morton, 1972; Loeffler, Cawley & Moeder, 1973), between skin and provocation tests (Hosen, 1965) and between provocation tests and serum IgE (Wide, Bennich & Johansson, 1967; Berg, Bennich & Johansson, 1971). None the less the difficulty of quantitating reactions is illustrated by failures to correlate the sensitivity of shock organs with the size of skin weals (McAllen, Assem & Maunsell, 1970; Stenius, 1973), the response to passive sensitization (McAllen *et al.* 1970) or the serum IgE levels as measured by radioallergosorbent test (Stenius, 1973). Furthermore the relation between the tests and the clinical response depends on the nature of the test substance (Aas & Johansson, 1971). This paper describes the results of performing dermal hypersensitivity (DH), passive cutaneous anaphylaxis (PCA) and intravenous provocation (IVP) tests in cattle vaccinated 0, 1, 2 and 3 times, with the object of evaluating the relation of the tests one to another and to the incidence of clinical allergy.

MATERIALS AND METHODS

One hundred and twenty-five cattle, of which 114 were 18-month- to 2-year-old Devon steers and the rest milk cows of undetermined ages and breeds, were housed under quarantine conditions and divided into lots of 30, 40, 31 and 24, vaccinated 0, 1, 2 and 3 times respectively. All, other than 24 of the unvaccinated controls, were subjected to a DH test 3–5 weeks after the last vaccination. Some were also subjected to IVP tests by injection of sodium carboxymethylcellulose, Grade B50 (Edifas B, ICI) (CMC), hydroxypropylmethylcellulose (Methocel HG, Dow Chemicals) (MC) or BHK 21 cell extract. Serum taken from the cattle immediately

Table 1. *Schedule of tests*

	No. of cattle	Ear tags	Vaccine used	Test	Vaccination† interval	Test† interval IVP	Test† interval DH
›t 0 (0 × 'accinated)	24	KA 15–38	None	*IVP-CMC/MC	N.A.	N.A.	N.A.
	6	JW 10–15	None	DH only	N.A.	N.A.	N.A.
›t 1 (1 × 'accinated)	24	JT 25–48 JT 76–83	Batch 49	IVP-CMC/MC DH	N.A.	30	35
	12	JT 88–91	Batch 55	DH only	N.A.	—	33
	‡4	JT 84–87	Batch 56	DH only	N.A.	—	33
›t 2 (2 × 'accinated)	11	JT 7–18	Batch 54	IVP-CMC/MC DH	42	56	63
	12	JW 68–79	Batch 60	IVP-BHK DH	24	58	48
	‡4	JW 80–83	Batch 61	IVP-BHK DH	24	58	48
	‡4	JW 84–87	Batch 62	IVP-BHK DH	24	58	48
›t 3 (3 × 'accinated	24	JW 36–59	Batch 57	IVP-CMC/MC DH	29; 53	75	74

N.A. Not applicable.
* Intravenous provocation test with CMC, MC or BHK extract.
† Vaccination and test intervals refer to the number of days between the first vaccination and subsequent ccination/s or test/s.
‡ At first vaccination, vaccines diluted 1/50, 1/10 and 1/2 were injected into equal numbers of animals except in oups marked ‡ where 1/2 dilution was used in all the animals. Subsequent vaccinations were full strength.

before the DH tests or, in the case of 24 of the controls, before the IVP test, was used for a PCA test. Details of the vaccination and test schedule are given in Table 1. Goats were 7- or 18-month-old white Saanen male castrates obtained from the Animal Research Council Institute for Research in Animal Diseases, Compton, Berks., and were housed under quarantine conditions.

Vaccinations

Vaccines (Wellcome Foot and Mouth Disease Vaccine, Batches 49, 54, 55, 56, 57, 60, 61 and 62) were prepared from BHK cell suspension virus harvests and inactivated with acetyl ethyleneimine, as described previously (Telling *et al.* 1972). Three ml. doses were injected subcutaneously.

Baby hamster kidney cell extract

Two litres of a BHK 21 cell suspension grown in Eagle's medium with 10% added normal bovine serum (NBS) containing 10^6 cells/ml. was centrifuged (3000 *g* for 7 min.) and washed 3 times in veronal buffered saline (VBS). The cells were resuspended in 50 ml. distilled water, frozen and thawed three times by immersing in liquid nitrogen, centrifuged at 6000 *g* for 15 min. to remove debris and diluted with distilled water to give a 0·5%, w/v, final protein concentration.

Dermal hypersensitivity test

Cattle were injected intramuscularly with 2 ml. of 2-(2,6-xylidino)-5,6 dihydro-4H-1,3 thiazine hydrochloride (Rompun, Bayer) and the skin at the side of the neck was clipped and marked out in seventeen 35 × 35 mm. squares with a felt-tipped marker. The test substances, some of which were vaccine components, were coded, randomized to avoid bias, and injected i/d 0·1 ml. to a square. The

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Table 2. *Dermal hypersensitivity: response to 0·1 ml. i/d injections*

	Percentage of animals in each lot responding with weals ⩾ 14 mm. diameter							
No. of vaccinations (Lot no.) ...	0		1		2		3	
No. cattle in each lot tested for DH ...	6		40		31		24	
* Early (E) or late (L) reactions ...	E	L	E	L	E	L	E	L
Test substance								
A. Serum-free antibiotic-free Eagle's medium	0	0	0	0	0	0	4	42
B. Na-penicillin† 500000 i.u./ml. in distilled water	100	0	85	0	100	0	96	0
C. 0·1 % CMC (Edifas, ICI) in VBS	0	0	2·5	5	0	3	4	4
D. 0·5 % CMC (Edifas, ICI) in VBS	0	17	7·5	17·5	0	26	0	4
E. 1 % CMC (Edifas, ICI) in VBS	0	33	0	25	3	29	4	21
F. 0·1 % MC (Methocel, Dow Chemical Co.) in VBS	0	0	10	0	13	0	0	0
G. 0·5 % MC (Methocel, Dow Chemical Co.) in VBS	0	0	10	0	10	0	8	0
H. 1 % MC (Methocel, Dow Chemical Co.) in VBS	0	0	15	2·5	10	6	0	0
I. Whole vaccine (the same batch as that used for vaccination)	83	0	90	10	93	7	100	0
J. $Al_2(OH)_3$ 0·5 % in distilled water (Algel, Cooper-Zeltia, Spain)	0	33	7·5	20	10	23	4	12·5
M. VBS	0	0	0	0	0	0	0	0
O. BHK extract	0	0	20	5	97	3	100	0
P. NBS	0	0	20	7·5	64·5	0	68	0
Q. 5 % saponin in distilled water	100	0	100	0	100	0	Not done	
R. Vaccine without saponin (the same batch as that used for vaccination)	0	17	27·5	52·5	85	7	Not done	

* Early reactions measuring ⩾ 14 mm. diameter at 1½ hr.
Late reactions measuring < 14 mm. at 1½ hr. but ⩾ 14 mm. at 24 hr. or later.
† ICI, Glaxo and Dista Products Na-penicillin used at different trials. All gave similar results.

diameters of weals which resulted were measured with the skin flat at 1·5, 24, 48 and 72 hr. The intravenous (i/v) injection of 20 ml. 2 % Evans blue before the i/d injections (Leeman, de Weck & Schneider, 1969) was not found to be helpful in reading the results and was discontinued after initial trials. Details of the injected substances are given in Table 2.

Passive cutaneous anaphylaxis test

The method used was similar to that described by Beadle (1971). Goats were injected with 0·4 or 0·45 ml. Rompun according to size and, after clipping the hair, 50 squares were marked with a felt-tipped marker on each of the chest walls and flanks. The cattle sera were randomized and injected i/d 0·1 ml. to a square and each serum was injected into 6 goats. In addition, each goat was similarly injected with a doubling dilution series, neat to 1/128 of a serum known to contain anti-BHK reagins. After 3 or 4 days 10 ml. 2 % Evans blue was injected i/v followed by 3·5 ml. BHK extract, 1 % MC, 1 % CMC, sodium penicillin 500,000 i.u./ml., serum-free and antibiotic-free Eagle's medium, NBS and whole vaccine, all i/v at

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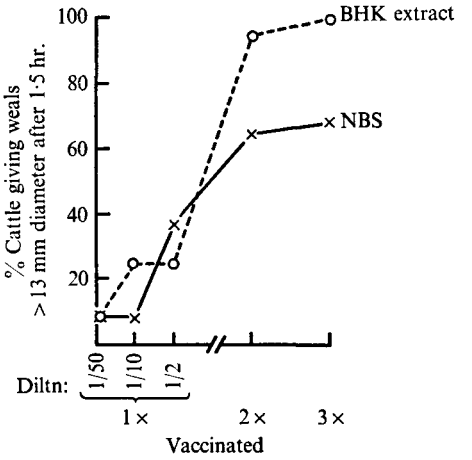

Fig. 1. Frequency of large weals in response to dermal hypersensitivity tests in cattle after 1, 2 or 3 FMD vaccinations.

approximately 60 min. intervals in different combinations and sequences in each of the goats.

The diameters of the blue stains caused by extravasation of dye at the reaction sites was noted 55–60 min. after each injection. The person reading the results was unaware at the time of the site of any individual serum.

Intravenous provocation tests

Five ml. 0·1 % CMC or MC or 2 ml. BHK extract was injected i/v into cattle and their behaviour observed for 30 min.

RESULTS

DH tests

Table 2 shows that in both vaccinated and unvaccinated cattle weals $\geqslant$ 14 mm. diameter were observed at 1·5 hr. after the injection of sodium penicillin, whole vaccine and saponin, while late reactions, first exceeding 13 mm. at 24 hr. or later, occurred in the case of CMC and aluminium hydroxide. Only BHK extract, NBS, MC and serum-free and antibiotic-free Eagle's medium provoked reactions in vaccinated but not in unvaccinated animals and Fig. 1 shows that the BHK extract and NBS weals increased in frequency according to the number of vaccinations experienced.

PCA test

Reactions were usually clearcut and appeared about 20 min. after i/v challenge (Plate 1). A small proportion came up as doubtful blotches or appeared up to an hour later. The skin of one goat became diffusely blue after i/v injection of the BHK extract and this animal has been excluded from the results because the reactions were unclear. Table 3 shows the PCA responses as a function of the

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Table 3. *PCA responses: influence of the number of vaccinations and/or vaccine batch*

	No. vaccinations and batch	Proportion of cattle producing reagins to: BHK extract	MC	Whole vaccine
Lot 0	Unvaccinated	0/30	0/30	0/30
Lot 1	1 × batch 49	0/24	0/24	0/24
	1 × batch 55 or 56	0/16	2/16	0/16
Lot 2	2 × batch 54	0/11	0/11	0/11
	2 × batch 60, 61 or 62	3/20	1/20	2/20 (?)
Lot 3	3 × batch 57	17/24	0/24	1/24 (?)

(?) = Doubtful reactions.

vaccine batch used and/or the number of vaccinations. Reagins directed against BHK extract and MC were demonstrated in 20 and 3 of the sera respectively, while whole vaccine, which was injected last in all cases, caused reactions with three additional sera. The last mentioned reactions were all doubtful, but none the less it is possible that they were caused by reagins to vaccine components other than BHK extract, MC or any of the previously injected substances. Table 4 shows that the sensitivity of different goats varied widely but such variations were consistent with their response to the positive control serum. Two goats (A and B) reacted with the control serum to a dilution of 1/128, two goats (C and D) to a dilution of 1/64 and goat E to a dilution of 1/32. In Tables 4(*a*), (*b*) and (*c*) the goats have been arranged from left to right in order of sensitivity and it can be seen that with few exceptions the diameters of the reactions varied in conformity with this. The sera which caused stains of the largest diameter appear at the top of each section and these caused reactions on most goats, while the sera at the bottom gave small reactions on the most sensitive goats and none on any of the others. It has been noted previously that the areas of PCA stains are logarithmically related to the antiserum titres (Stanworth, 1973).

Correlation between the DH and PCA responses

In Tables 4(*a*), (*b*) and (*c*) the diameters of the DH weals on the cattle can be compared with those of the PCA responses evoked by their sera. Most of the animals producing reagins developed dermal weals exceeding 25 mm. at 1·5 hr. although, other than this, no correlation was evident between the magnitude of the PCA and DH reactions. Fig. 2 shows the frequency of reagin production in relation to the diameters of the DH weals. With BHK extract there was a significant correlation ($P < 0{\cdot}0001$) between the two parameters when weals 25 mm. diameter (mean of measurements at 1·5, 24, 48 and 72 hr.) were regarded as positive. With MC the number of reactions was too small for statistical evaluation but a similar trend was evident. Two of the three animals with large weals (30–60 mm.) produced

Table 4. *PCA and DH responses compared*

	Animal no.	PCA test: Diameter of reactions on goats (mm.) A	B	C	D	E	DH test: diameter (mm.) of weals on cattle 1·5 hr. after i/d injections
		(*a*) Reactions to BHK extract					
Lot 0	—	—	—	—	—	—	—
Lot 1	—	—	—	—	—	—	—
Lot 2	JW 72	25	14	—	15	—	34
	JW 71	16	20	—	—	—	30
	JW 77	22	—	—	14	—	23
Lot 3	JW 51	37	28	24	22	21	32
	JW 48	27	26	24	20	21	28
	JW 45	27	27	23	21	19	28
	JW 40	24	30	23	16	11	—
	JW 55	27	27	25	11	5	50
	JW 46	24	23	23	20	3	34
	JW 47	24	29	25	22	—	32
	JW 37	25	27	16	21	—	24
	JW 58	24	28	19	17	—	24
	JW 36	26	26	21	—	13	40
	JW 49	21	22	11	13	—	24
	JW 38	—	29	15	—	—	30
	JW 44	17	20	—	—	—	30
	JW 57	17	—	—	—	—	25
	JW 56	16	—	—	—	—	34
	JW 54	14	—	—	—	—	60
	JW 50	10	—	—	—	—	33
Limiting titre of response to control serum 2364		⩾ 1/128	⩾ 1/128	1/64	1/64	1/32	
		(*b*) Reactions to MC					
Lot 0	—	—	—	—	—	—	—
Lot 1	JT 80	32	24	21	19	17	45
	JW 69	30	20	19	20	13	58
Lot 2	JT 89	17	11	—	—	—	0
Lot 3	—	—	—	—	—	—	—
		(*c*) Reactions to whole vaccine					
Lot 0	—	—	—	—	—	—	—
Lot 1	—	—	—	—	—	—	—
Lot 2	JW 73	14	—	—	—	—	30
	JW 84	15	12	—	—	—	26
Lot 3	JW 41	14	—	—	—	—	18

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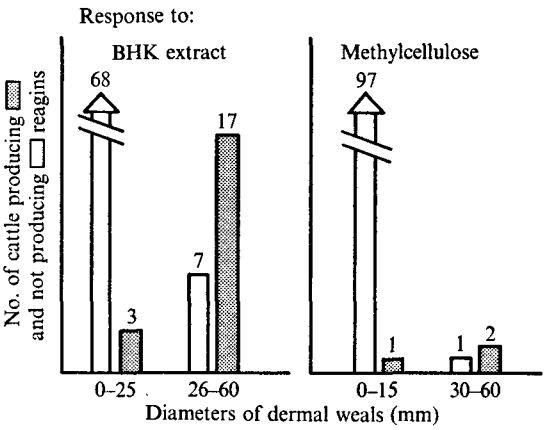

Fig. 2. Relationship between the production of serum reagins and the size of weals after dermal hypersensitivity tests.

Table 5. *IVP response as a function of vaccine batch and/or number of vaccinations*

	No. vaccinations and batch	Proportion of cattle responding to IVP with		
		CMC	MC	BHK extract
Lot 0	Unvaccinated	0/12	0/12	ND
Lot 1	1 × batch 49	1/12	12/12	ND
Lot 2	2 × batch 54	2/5	6/6	ND
	2 × batch 60, 61 or 62	ND	ND	10/20
Lot 3	3 × batch 57	0/12	0/12	ND

reagins while 97 of the remaining 98 tested both failed to produce reagins and had weals $\leqslant$ 15 mm. mean diameter.

IVP tests

Signs generally appeared within 10 min. and subsided approximately 15 min. later. In the steers they usually consisted of itchiness (restlessness, head shaking, licking at the coat, rubbing against objects and scratching with a hind limb), and occasionally coughing and dyspnoea in addition. Severe cases exhibited periorbital and anal fold oedema. The cows showed signs of respiratory distress (laboured breathing, cyanosis, frothing at the muzzle, lacrimation and cough) and sometimes tremors, but no itchiness. Table 5 shows the frequency of IVP responses to BHK extract, CMC and MC as a function of the vaccine batch and/or number of vaccinations.

Reactions to MC occurred regularly after one vaccination with Batch 49 or two of Batch 54 but none were observed after three Batch 57 vaccinations. The substance responsible for the anti-MC reactions has recently been identified and details will be published shortly (Black, Menard, Beadle & Pay). On the other hand a

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Table 6. *Comparison of the DH, PCA and IVP responses to BHK extract in twice vaccinated animals*

Animal no.	Diameter of DH weals (mm.) at (hr.) 1·5	24	48	72	PCA* response	IVP† response
JW 68	21	0	0	0	.	+
JW 69	23	20	13	13	.	+ +
JW 70	17	0	0	0	.	±
JW 71	30	16	10	0	2/5	−
JW 72	34	17	12	0	3/5	+ + +
JW 73	27	22	15	15	.	+
JW 74	28	15	8	0	.	+
JW 75	30	15	0	0	.	±
JW 76	30	30	18	16	.	+ + +
JW 77	23	0	0	0	2/5	±
JW 78	28	0	0	0	.	+ + +
JW 79	24	0	0	0	.	±
JW 80	26	30	23	18	.	±
JW 81	22	10	10	10	.	+ + +
JW 82	28	24	15	0	.	+
JW 83	20	0	0	0	.	−
JW 84	28	0	0	0	.	±
JW 85	30	12	15	0	.	+ +
JW 86	21	12	13	0	.	±
JW 87	17	0	0	0	.	Not done

* Proportion of goats giving PCA reactions.
† ± to + + + + + = Intensity of signs judged subjectively.

different pattern of response was noted when BHK extract was used for IVP test and only 10 of the 20 animals tested showed a reaction. Table 6 shows that these reactions were not correlated with the results of DH or PCA tests.

DISCUSSION

The results reported here gave an indication of the magnitude, frequency and inter-relations of the responses to three commonly used hypersensitivity tests in vaccinated cattle. In performing the PCA test a wide variation was noted in the sensitivity of different recipients but the chance of overlooking a positive reaction was minimized by repeating each experiment on a number of goats, the sensitivities of which were determined according to their responses to a standard antiserum. The reactivity of different recipients has been found to be inversely related to their own IgE levels (Bazarel, Orgel & Hamburger, 1973) and it was suggested that the recipients' IgE antibodies compete with those in the test serum for the available tissue binding sites.

The development of serum reagins and enhanced skin sensitivity appears to be a usual response to foot-and-mouth disease vaccination in cattle. Reagins were demonstrated in 0, 5, 19 and 75% of the animals vaccinated 0, 1, 2, and 3 times respectively, and the proportion with enhanced skin sensitivity increased *pari passu*. However the presence of serum reagins and dermal hypersensitivity did not,

per se, portend the development of clinical signs and none of the cattle showed untoward reactions after vaccination. The same is seen in human subjects where weal and flare skin reactivity is increased in non-atopic individuals after parenteral protein and polysaccharide immunization (Leskowitz & Lowell, 1961).

If it is permissible to interpret the PCA and DH tests as reflecting the amounts of serum and cell bound reagins respectively, the results obtained suggest that the fixed and circulating reagins bore no constant relation to one another. Although PCA reactions were often noted in the animals with large skin weals the magnitude of the PCA reactions were not correlated with those of the DH tests. It seems possible that once the available tissue binding sites are occupied serum reagins can fluctuate within broad limits independent of the fixed antibody titres. The role of fixed and circulating reagins in the causation of clinical signs remains unclear. Some cattle which gave DH and PCA reactions to BHK extract failed to respond to provocation tests and vice versa (Table 6).

The IVP test produced anaphylactic signs resembling those seen experimentally and in the field (Aitken & Sanford, 1969; Mussgay, 1970). The cows suffered respiratory distress and the steers predominantly skin itchiness. However, it was not possible to relate the difference in syndrome to any special factor because, apart from sex, the two groups differed in age, breed and immunological experience. It is noteworthy that widely different allergic syndromes of the immediate type have been described in cattle, including abortion (Lorenz & Straub, 1973), tympany (Code & Hester, 1939), laminitis (Nilsson, 1963), respiratory embarrassment (Dungworth, 1965; Aitkin & Sanford, 1969), itchiness and urticaria (Gerlach, 1922; Beadle, 1971).

On evidence of one or more of the allergic tests a number of foot-and-mouth disease vaccine components have been implicated in the causation of immediate or delayed hypersensitivity. These include BHK cell extracts (Mussgay, Kaaden & Bauer, 1970), modified bovine serum (Capstick *et al.* 1970; Jensen, 1969; Beadle, 1971), viral proteins (Mayr *et al.* 1969; Bauer *et al.* 1970), carboxymethylcellulose (Leeman *et al.* 1969; Eyal & Mayer, 1971), and antibiotics (de Quiroz, Sutmöller & Barroeta, 1964; Palacios, 1964; Makarova & Intizarov, 1966; Michi, 1966; Mayr & Bibrack, 1969; Arostegui, Caggiano & Gatto, 1963; Ubertini, 1970). Although BHK extract, methylcellulose and normal bovine serum provoked frequent responses to the allergic tests investigated here, their role in the causation of the clinical allergy syndrome was not demonstrated.

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L. BLACK AND T. W. F. PAY (*Facing p.* 181)

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EXPLANATION OF PLATE

Passive cutaneous anaphylaxis reactions on a goat.