*J. Hyg., Camb.* (1975), **75**, 79
*With 1 plate*
*Printed in Great Britain*



# Hypersensitivity in cattle after foot-and-mouth disease vaccination: response to hydroxypropylmethylcellulose

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(*Received* 15 *January* 1975)

SUMMARY

Intravenous provocation (IVP) tests demonstrated that hydroxypropylmethylcellulose (MC) was able to elicit anaphylactic signs in cattle vaccinated with foot-and-mouth disease (FMD) vaccine produced at one centre but not with similar vaccine produced at another. The former vaccine also provoked serum reagins which were demonstrated by passive cutaneous anaphylaxis (PCA) tests in goats.

Reaginic sera which reacted specifically with MC were used in PCA tests to screen samples taken serially from the vaccine production lines. The reactions observed suggested that a substance with MC or similar specificity was present in the antifoaming agent routinely added to vaccines.

## INTRODUCTION

Allergy ascribed to carboxymethylcellulose (CMC) has been reported after penicillin injections in Swiss cattle previously vaccinated against foot-and-mouth disease (FMD) (Leeman, de Weck & Schneider, 1969). CMC, which is a common additive to injectable preparations, has also proved allergenic in guinea-pigs and mice (Platt, Bicanovsky, Dallow & Thonard, 1966; Leeman *et al.* 1969) but not so far in horses (Straub *et al.* 1972), rats (McCollister & Oyen, 1954) or man (Leeman *et al.* 1969). FMD vaccine has been implicated in the causation of CMC hypersensitivity in Israeli cattle (Eyal & Mayer, 1971). In trials on FMD vaccine produced at Pirbright vaccinated cattle often showed allergic signs after intravenous (i/v) hydroxypropylmethylcellulose (MC) (Methocel HG, Dow Chemicals Co.) (Beadle, 1971). This was unexpected as the source and nature of the immunogen in the vaccine were unknown. Furthermore, similar vaccine produced in Germany failed to sensitize cattle in the same way. This paper describes investigations carried out to identify the component in Pirbright vaccine which was capable of reacting in PCA tests with specific anti-MC sera and which might be regarded as allergenic.

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https://doi.org/10.1017/S0022172400047082 Published online by Cambridge University Press

## MATERIAL AND METHODS

### *Animals*

Four hundred and twelve Devon steers aged 18 to 24 months, eleven milk cows of indeterminate age and breed and twenty-four 6- to 12-month-old white male castrate Saanen goats, never previously vaccinated against or in contact with FMD, were obtained from a dealer and housed under quarantine conditions.

### *Allergenicity of whole vaccines tested by the intravenous provocation (IVP) test*

One hundred and fourteen cattle were injected subcutaneously with 3 ml. FMD vaccine produced as described by Telling *et al.* (1972) at the Wellcome Foot and Mouth Disease Vaccine Laboratory, Pirbright, Surrey and 24 were injected with vaccine produced similarly in Germany (Impfstoffwerk Wellcome GmbH, Grossburgwedel). All cattle were challenged 3 weeks after vaccination with 5 ml. 0·1 % MC or sodium CMC (Edifas B, ICI) i/v as shown in Table 1. Twenty-four unvaccinated cattle were also challenged. The animals were kept under observation for 30 min. for signs of anaphylaxis (dyspnoea and/or pruritis).

### *Passive cutaneous anaphylaxis (PCA) test*

PCA tests were performed as previously described (Capstick *et al.* 1970; Beadle & Pay, 1975; Black & Pay, 1975). Briefly, 0·1 ml. of each bovine serum was injected intradermally (i/d) into 6 goats and three days later, after injecting 10 ml. of 2 % Evans blue i/v, the putative allergens were injected i/v one after the other at hourly intervals in various combinations and sequences in each of the goats. Stains caused by the extravasation of dye at the positive reactions sites were measured 55 min. after the i/v injection of each test substance.

### *Preparation of specific anti-MC reaginic sera*

Two hundred and twenty-five cattle were injected subcutaneously 1, 2 or 3 times at 3 week intervals with 3 ml. Pirbright-produced FMD vaccine and bled 3 to 5 weeks after the last vaccination for purposes unrelated to this experiment. The sera were stored at $-20°$ C. and screened by PCA tests for activity against MC and other possible vaccine allergens. Thirty-two sera reacted with MC and of these two were selected which gave negative results with all the other substances tested, viz. sodium penicillin (ICI) 500,000 iu/ml., normal bovine serum, BHK 21 cell lysate (0·5 % w/v protein) prepared as previously described (Black & Pay, 1975), 1 % CMC, 0·1 % saponin and 0·25 % aluminium hydroxide gel.

### *Identification of vaccine components reacting in PCA tests with specific anti-MC sera*

Doubling dilutions of the two anti-MC sera described above 1/1 to 1/256 in 0·15 M saline, were injected i/d into goats. Samples taken from the vaccine production lines at various stages of the process were used for sequential i/v injections three days later. The cell growth medium provoked reactions so further trials were carried out in a similar fashion to test the reactivity of the components of cell growth medium. Details of the substances tested are given in Table 2*b* together with the results.

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Table 1. *Intravenous provocation test. Response of cattle to* 5 ml. 0·1 % *CMC or MC i/v*

| | Unvaccinated | | Vaccinated 21 days previously: vaccine produced in Pirbright | | Vaccinated 21 days previously: vaccine produced in Germany | |
|---|---|---|---|---|---|---|
| Challenged i/v with | MC | CMC | MC | CMC | MC | CMC |
| Proportion reacting | 0/12 | 0/12 | 64/102 | 1/12 | 0/12 | 0/12 |

Table 2*a*. *PCA test in goats. Response to cell growth medium* i/v after injecting anti-MC sera i/d*

| | | Dilutions of anti-MC sera | | | | | |
|---|---|---|---|---|---|---|---|
| | | 1/1 | 1/2 | 1/4 | 1/8 | 1/16 | 1/32 |
| Goat no. 1. Antiserum | (i) | + + + | — | — | — | — | — |
| | (ii) | — | — | — | — | — | — |
| No. 2. | (i) | + + + + | — | — | — | — | — |
| | (ii) | — | — | — | — | — | — |
| No. 3. | (i) | + + + + + | + + | — | — | — | — |
| | (ii) | + + + + | — | — | — | — | — |
| No. 4. | (i) | — | — | — | — | — | — |
| | (ii) | — | — | — | — | — | — |
| No. 5. | (i) | + + + + + | + + + + + | + + + + | — | — | — |
| | (ii) | + + + + | + + + | + | — | — | — |
| No. 6. | (i) | + + + + + | + + + + | + + + + | + + | + | — |
| | (ii) | + | — | — | — | — | — |

Each + represents 5 mm. diameter reaction stain.

* Modified Eagle's balanced salt solution (MacPherson & Stoker, 1962) with 10 % added bovine serum.

## RESULTS

### *IVP tests. Allergenicity of whole vaccines*

Table 1 shows that 63 % of cattle vaccinated with Pirbright-produced vaccine experienced anaphylactic reactions after i/v MC injections but only 8 % responded to injections of CMC. Unvaccinated cattle and those injected with German-produced vaccine showed no reactions to either CMC or MC.

### *PCA tests. The reactivity of vaccine fractions with anti-MC sera*

Table 2*a* shows that in the first trial cell growth medium provoked PCA reactions with both the anti-MC sera on 3/6 goats and with one of the sera on a further 2/6. The more potent antiserum (i) reacted to a limiting dilution of 1/1, 1/1, 1/2, 1/4 and 1/16 on the different goats. Plate 1 is a photograph of the reactions on one of these animals.

Table 2*b* shows that, of the cell growth medium components tested in the



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Table 2*b*. *PCA test in goats. Response to cell growth medium components after injecting anti-MC sera i/d*

| Goat | Substance injected i/v | Antiserum | Dilutions of anti-MC sera 1/1 | 1/2 | 1/4 | 1/8 | 1/16 | 1/32 |
|---|---|---|---|---|---|---|---|---|
| No. 7 | Polymixin 500,000 iu/ml. | (i) | — | — | — | — | — | — |
| | | (ii) | — | — | — | — | — | — |
| | Amino acids* | (i) | — | — | — | — | — | — |
| | | (ii) | — | — | — | — | — | — |
| | Normal bovine serum | (i) | — | — | — | — | — | — |
| | | (ii) | — | — | — | — | — | — |
| | Antifoam 10 ml. 1% | (i) | + + + | + + + | — | — | — | — |
| | | (ii) | + | — | — | — | — | — |
| No. 8 | Neomycin 500,000 iu/ml. | (i) | — | — | — | — | — | — |
| | | (ii) | — | — | — | — | — | — |
| | Antifoam 10 ml. 1% | (i) | + + + + | + + + + | + + + | + + + | — | — |
| | | (ii) | + + + + | + + + + | + + + | + | — | — |
| No. 9 | Polymixin | (i) | — | — | — | — | — | — |
| | | (ii) | — | — | — | — | — | — |
| | Vitamins† | (i) | — | — | — | — | — | — |
| | | (ii) | — | — | — | — | — | — |
| | Normal bovine serum | (i) | — | — | — | — | — | — |
| | | (ii) | — | — | — | — | — | — |
| | Antifoam | (i) | + + + + | + + + | + + + | + + | + + | — |
| | | (ii) | + + | + | — | — | — | — |
| No. 10 | Sodium penicillin 500,000 iu/ml. | (i) | — | — | — | — | — | — |
| | | (ii) | — | — | — | — | — | — |
| | Amino acids | (i) | — | — | — | — | — | — |
| | | (ii) | — | — | — | — | — | — |
| | Normal bovine serum | (i) | — | — | — | — | — | — |
| | | (ii) | — | — | — | — | — | — |
| | Antifoam | (i) | + + + + + | + + + + | + + + + | + + + | + + + | — |
| | | (ii) | + + + + | + + + + | + + + | — | — | — |

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Table 2*b* (*cont.*)

| Goat | Substance injected i/v | Antiserum | Dilutions of anti-MC sera 1/1 | 1/2 | 1/4 | 1/8 | 1/16 | 1/32 |
|---|---|---|---|---|---|---|---|---|
| No. 11 | Sodium penicillin | (i) | — | — | — | — | — | — |
| | | (ii) | — | — | — | — | — | — |
| | Vitamins | (i) | — | — | — | — | — | — |
| | | (ii) | — | — | — | — | — | — |
| | Normal bovine serum | (i) | — | — | — | — | — | — |
| | | (ii) | — | — | — | — | — | — |
| | Antifoam | (i) | + + + + + + | + + + + + | + + + + | + + + | + + + | + + |
| | | (ii) | + + + + | + + | + + | — | — | — |
| No. 12 | Neomycin | (i) | — | — | — | — | — | — |
| | | (ii) | — | — | — | — | — | — |
| | Antifoam | (i) | — | — | — | — | — | — |
| | | (ii) | — | — | — | — | — | — |
| | Normal bovine serum | (i) | — | — | — | — | — | — |
| | | (ii) | — | — | — | — | — | — |

* At concentration 50 × that in modified Eagle's balanced salt solution (MacPherson & Stoker, 1962).
† At concentration 100 × that in modified Eagle's balanced salt solution (MacPherson & Stoker, 1962).

Table 3. *The response of vaccinated cattle to vaccine i/v followed* 30 *min. later by MC i/v*

| | Proportion reacting | % |
|---|---|---|
| 3 ml. vaccine i/v | 0/36 | 0 |
| 5 ml. 0·1% hydroxypropylmethylcellulose i/v | 25/36 | 69 |

second trial, only antifoam caused reactions and both the anti-MC sera reacted with this product on 5/6 goats. In this case antiserum (i) reacted to a limiting dilution of 1/2, 1/8, 1/16, 1/16 and 1/32 on the different goats.

## DISCUSSION

The demonstration that cellulose derivatives are able to act allergenically in cattle (Leeman *et al.* 1969) may have far-reaching implications as these compounds are widely used in the production of both human and veterinary preparations. Their elimination from vaccines is desirable from the point of view of reactions after repeated vaccination and also of cross-reactions after the use of other products.

CMC (Edifas B) has been described as a component of the medium for cell growth (Telling & Stone, 1964; Telling & Ellsworth, 1965) but no cellulose derivatives as such have been employed in production of FMD vaccine at any Wellcome Laboratories. Nor have there been reports of clinical hypersensitivity to MC after the use of Wellcome FMD vaccines in the field. Nonetheless the identification of this potential allergen in a vaccine component raises the possibility that reactions could have occurred undiagnosed. In a recent report Huenermund (1974) described exudative skin lesions in cattle in Kenya which he ascribed to allergic reactions to FMD vaccine. On intuitive grounds and in absence of substantiation he implicated CMC as probably responsible for the condition, claiming that reports of CMC anaphylaxis led to withdrawal of the vaccine in 1972 and its replacement with a CMC-free product. However, this is not the case. The formulation of Wellcome Foot and Mouth Disease vaccine, which is used exclusively in Kenya, has not been changed since 1969 and even then the change involved replacing one inactivating agent (formalin) with another (acetylethelineimine). Furthermore, the clinical signs described in this paper (skin lesions first appearing some days after vaccination) resemble a delayed hypersensitivity syndrome (Mayr *et al.* 1969) whereas all known cases of CMC hypersensitivity (Leeman *et al.* 1969; Eyal & Mayer, 1971) have been characterized by signs of immediate hypersensitivity (urticaria, pruritis and/or dyspnoea occurring within an hour or two after vaccination).

The IVP and PCA tests have been used in the work described here to identify a potentially allergenic vaccine component but reactions to these tests give no indication of the importance of MC in provoking allergic reactions in the field. It was shown for example that reagins to BHK 21 cell lysates developed in the sera of most vaccinated cattle although none of these animals showed signs of

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allergy after vaccination (Black & Pay, 1975). The IVP test may also have little significance in context of field reactions. The test is highly sensitive and i/v injections are often able to evoke an allergic response in cattle which tolerate the usual subcutaneous injections with no ill effects. Furthermore, the dose of MC in the i/v test injections used here (5 mg.) was some 67 times greater than that in 3 ml. of vaccine (0·075 mg.) as calculated from data supplied by the antifoam producer. The result of a small trial which illustrates the relative potentialities of MC and vaccine to evoke an allergic response when given to vaccinated cattle by the same route is shown in Table 3. When 36 vaccinated cattle were challenged i/v with 3 ml. Pirbright-produced vaccine and 30 min. later with 5 ml. of 0·1 % MC none reacted to vaccine but 69 % reacted to the MC.

So far efforts to provoke serum reagins in cattle by injection of MC and adjuvants alone have failed and it seems possible that MC acts in an immunogenic capacity only when associated with some other substance present in vaccine. It is noteworthy that the reaginic antisera produced by vaccinated cattle reacted in PCA tests with MC but failed to do so with CMC. This supports the observations of Mayer & Eyal (1972) who noted the narrow specificity of the reactions to various cellulose derivatives.

The antifoam in German 'Wellcome' vaccine was of different manufacture from that of the Pirbright product and this may account for the difference in the MC sensitizing propensities of the vaccines. The antifoam in 'Wellcome' FMD vaccine produced at Pirbright has been changed and the manufacturers of the antifoam originally used have since confirmed its MC content at the time of the investigations described above.

It is a pleasure to thank Mrs E. Pink for excellent technical help and Messrs A. Smith, L. Collyer and I. E. Buttle for invaluable help with the animals.

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## EXPLANATION OF PLATE

PCA reactions on a goat. The bottom row of sites was injected i/d with a doubling dilution series of serum containing anti-MC reagins. The i/v injection of Evans blue and cell growth medium 3 days later elicited the stains which can be seen on the photograph.

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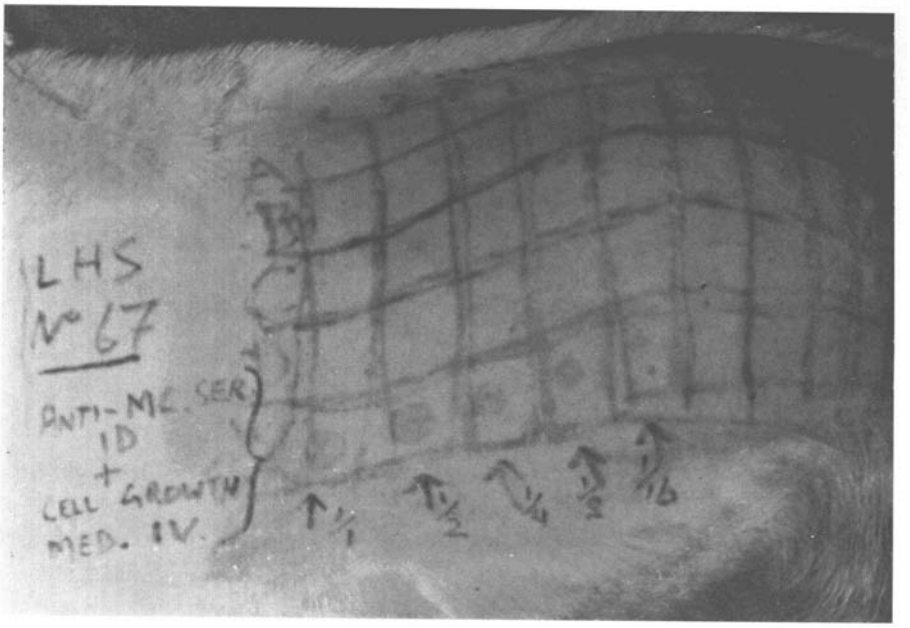


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