antiserum did, in fact, give the result that all the strains fixed complement in the presence of GB antiserum, although there were at least 3 distinct variants in the group of strains used.

Discussion. The occurrence of variant strains has imposed new conditions on the successful application of the complement fixation test in foot-and-mouth disease, as well as emphasizing the importance of precision in carrying out the test. In the light of the results reported here the necessity for standardization of reagents is clear. Definition of units for antiserum and antigen must await more extended observations. The other essential would appear to be the use of a wider range of antisera, including in most cases, one which is strain homologous. As mentioned earlier, only further work will show what differences in respect of complement fixation must be demonstrated before the differences between strains are of practical moment in immunization.

Summary. Two strains of the virus of foot-and-mouth disease, of Vallée A type, have been found to differ antigenically from each other and from the stock strain of this type used in this Institute.

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Strains of Virus of Foot-and-Mouth Disease Recovered from Outbreaks in Mexico. Serum Neutralization Tests.

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The neutralization of the virus of foot-and-mouth disease by specific antisera has not been studied extensively. Bedson, Maitland and Burbury[1] described a technic in which guinea-pigs were used as test animals. They referred to the absence of cross neutralization between virus and antiserum of different immunological types. In examining 2 strains of the same type, they observed, in one experiment, that although the antiserum prepared against one strain neutralized to some extent virus of both strains, there was greater neutralization in the homologous mixtures. The errors implicit in their test, however, were great enough to make the validity of their conclusions somewhat doubtful. The development of a neutralization test in cattle using the method of inoculation of multiple sites on the tongues of cattle has the advantage of greater accuracy and also makes it possible to deal with both antiserum and virus from the naturally susceptible species. Preliminary investigations suggested that this method might be used for the identification of immunological types of virus in cattle and for the detection of even finer antigenic differences. Again, as in the use of the complement-fixation test in this sphere, caution must be applied to the interpretation of results and transference of conclusions to the field of vaccination experiments. Only more extensive studies can establish the extent of the correlations to be expected between this and the other methods of measurement of antigenic differences.

Methods. The strains of virus used have been described.[2,3] The antisera were from

[1] Bedson, S. P., Maitland, H. B., and Burbury, Y. M., Foot-and-Mouth Disease Research Committee, 2nd Progress Report, 1927, London, p. 95.

[2] Brooksby, J. B., Henderson, W. M., and Galloway, Ian A., PROC. SOC. EXP. BIOL. AND MED., 1948, **69**, 64.

[3] Brooksby, J. B., Galloway, Ian A., and Henderson, W. M., PROC. SOC. EXP. BIOL. AND MED., 1948, **69**, 70.

cattle convalescent from 14 to 21 days after the earliest lesion of the disease had appeared. Pooled samples of serum from 4 or more animals were used. The details of technique followed, in general, those described earlier.[4] In all but one of the observations, mixtures of diluted virus and excess bovine serum were prepared, so that, for example one volume of a 10^{-2} dilution of virus filtrate was added to 9 volumes of the antiserum concerned, giving a 10^{-3} dilution in antiserum.

From 30-90 minutes after preparing the dilutions of virus in antiserum, the series were inoculated into the tongues of cattle. Four groups of 5 sites were inoculated in one animal, each group with one serial dilution. Two animals were used for each series so that 10 observations were available on each serial dilution. The calculation of the 50% end-point dilution is therefore justified. Inoculations were made when the animal was narcotized with Pentothal Sodium (Abbott),[5] and observations on the occurrence of lesions at the sites inoculated were made at approximately 20 and 28 hours after inoculation.

The amount of virus neutralized was arrived at by deducting the titre (*e.g.* $10^{-2.5}$) of the virus in the presence of antiserum from the titre (*e.g.* $10^{-5.2}$) of the virus diluted in Hartley's Broth, giving for this example $10^{-2.7}$. The results of comparative titrations of virus in normal serum showed that little error could be attributed to non-specific effects.

An alternative method was used in one experiment described below. Dilutions of antiserum were prepared and each was mixed with an equal volume of a virus filtrate suitably diluted, as estimated on the basis of an earlier titration. In this case the dilution of antiserum corresponding to the 50% end-point was calculated from the result.

Results. The results of 4 experiments are represented diagrammatically in Fig. 1-4. On Fig. 1-3 are presented the data on the inter-

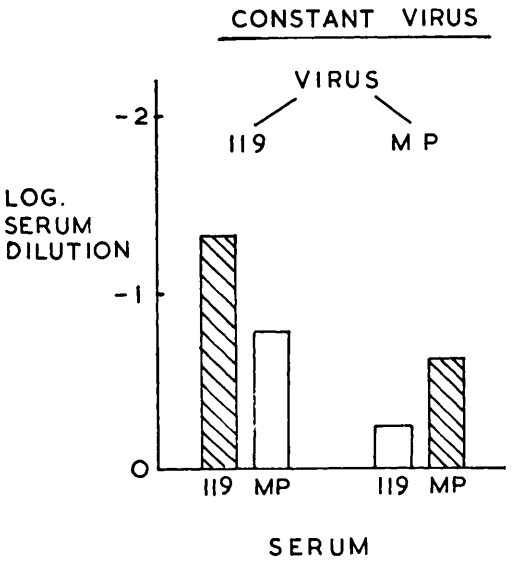

FIG. 1.
Neutralization test with viruses and antisera of two Vallée A type strains.

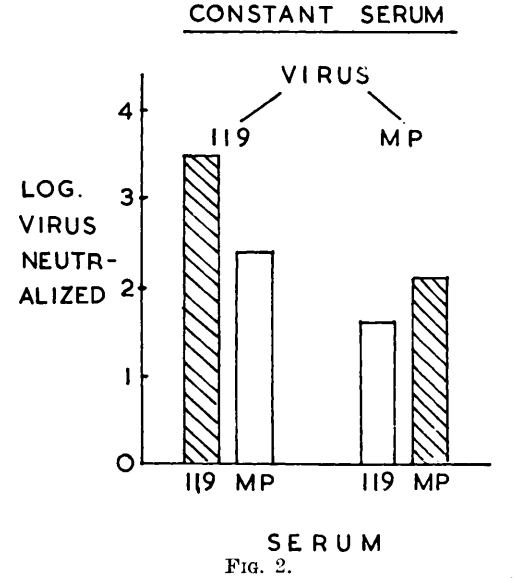

FIG. 2.
Neutralization test with viruses and antisera of two Vallée A type strains.

relationship between the Pirbright stock strain of Vallée A type, 119, and the 2 Mexican strains MP and M.1. It will be noted that for the comparison of strains 119 and MP, the observations for both serum-dilution and virus dilution methods are given. In both cases the neutralizing powers of the antisera were greatest against the corresponding strains.

[4] Brooksby, J. B., 1946, Thesis, University of London, (in press as an Agricultural Research Council Report.)

[5] Henderson, W. M., *J. Comp. Path.*, 1944, **54**, 245.

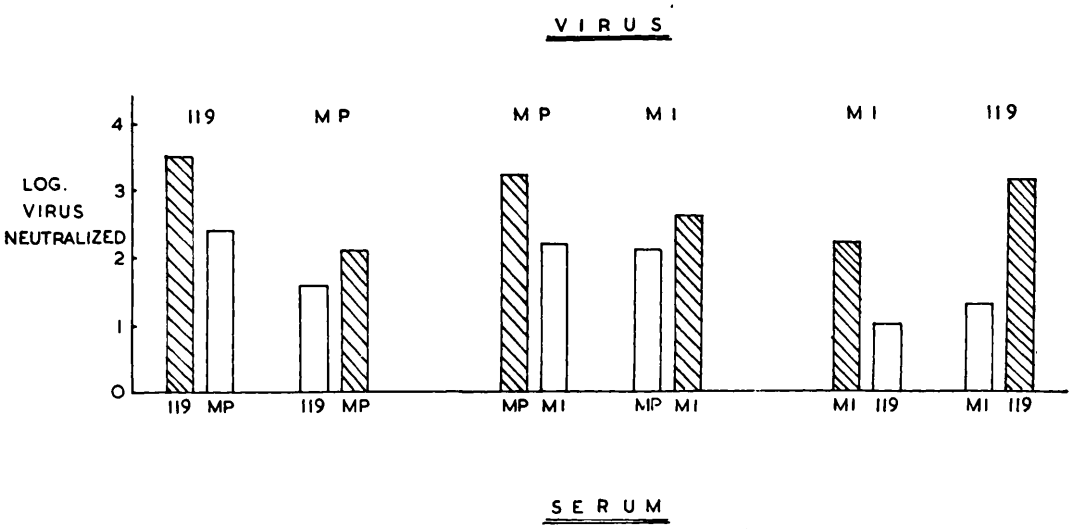

FIG. 3.
Neutralization tests with viruses and antisera of three Vallée A type strains.

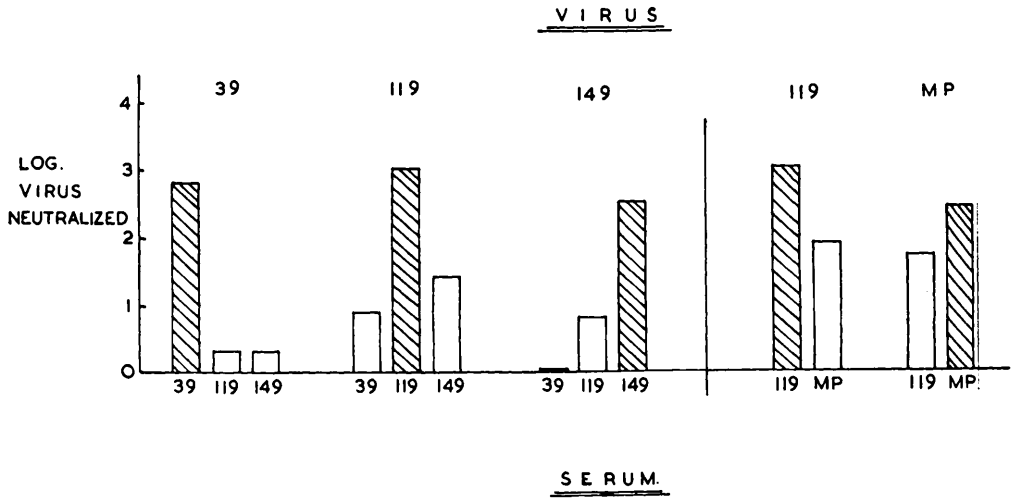

FIG. 4.
Neutralization tests with viruses and antisera of strain 39 (Vallée O type) strains 119 and MP (Vallée A type) and strain 149 (Waldmann C type).

A useful expression of the difference in relative activity of two antisera is given by the sum of the logarithmic differences in the amounts of virus neutralized by them when they are tested against first the strain corresponding to one of them and secondly the strain corresponding to the other. Thus in Fig. 2 the logarithmic difference between the amount of virus of strain 119 neutralized by strain 119 and strain MP antisera was 1.1, while for virus of strain MP, the logarithmic difference was 0.5. The sum of logarithmic differences for these 2 antisera is therefore 1.6, and this value may be called the "differential neutralization" as between strains 119 and MP. From Fig. 3, similar calculation gives the value of the differential neutralization between strains MP and M.1 as 1.5 and between strains M.1 and 119 as 3.0.

The high figure obtained with strains M.1 and 119 led to an investigation of the differences between strains of different immunological type. The results of this experiment are represented in Fig. 4, which also repeats

the observations on strains 119 and MP. The figures for differential neutralization derived from these results are

Between strains 39 and 119 (Vallée O and A types)	4.6
Between strains 39 and 149 (Vallée O and Waldmann C types)	5.0
Between strains 119 and 149 (Vallée A and Waldmann C types)	3.3
Between strains 119 and MP	1.9

Thus only in the case of strains 119 and M.1 does the magnitude of a strain difference nearly equal a type difference.

Discussion. The antibody produced in response to infection appears to differ qualitatively from strain to strain of those examined. The importance of these differences in immunization may depend on a number of factors. The antibody response of an animal to an injected vaccine may not reproduce qualitatively the response to an infection even if the preparation of vaccine from a particular strain has preserved the antigenic peculiarities of that strain. Again the quantitative factors influencing immunization may render strain specificity less important than it would at first appear. Further comment must be deferred until observations have been accumulated on these aspects. Strains shown by cross-immunity tests in vaccination experiments to be more closely related than those mentioned in this paper require to be examined, and work on these lines is continuing.

Summary. (1) Differences between strains of the virus of foot-and-mouth disease of one type have been demonstrated by cross-neutralization tests in cattle. (2) The magnitude of such differences in these experiments is not so great as that between immunological types.

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Strains of the Virus of Foot-and-Mouth Disease Recovered from Outbreaks in Mexico. Vaccination Experiments.

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Experiments on the immunization of cattle against infection with strains of virus from Mexico were made to determine whether vaccines prepared from the highly immunogenic Pirbright stock cattle strain 119 of the Vallée A type would protect them against infection with these strains which are of the same type. The results of these early experiments showed a difference in the antigenic behavior of strain 119 and the Mexican strain MP. This observation led to a study of these and other Mexican strains by methods described elsewhere.[1,2] Observations were made also on the immunizing value of vaccines prepared from the Mexican strains themselves. The problem became complicated later by failure of these Mexican strains to infect susceptible cattle, in control groups included in contact infection tests, with the regularity associated with highly invasive strains.

The pattern of the experiments was the same in each case. Vaccines were prepared from different strains, groups of cattle were injected with these vaccines and 2 to 3 weeks later, the protection established was tested with the same strain or another strain of the same immunological type.

Methods. Particulars of the strains of virus

[1] Brooksby, J. B., Galloway, Ian A., and Henderson, W. M., PROC. SOC. EXP. BIOL. AND MED., 1948, **69**, 70.

[2] Brooksby, J. B., Galloway, Ian A., and Henderson, W. M., PROC. SOC. EXP. BIOL. AND MED., 1948, **69**, 74.