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Strains of Virus of Foot-and-Mouth Disease Recovered from Outbreaks in Mexico. Complement Fixation Tests.

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(Introduced by R. E. Shope.)

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On the basis of cross-immunity tests, the great majority of strains of the virus of foot-and-mouth disease can be classified as belonging to one of 3 immunological types, known as Vallée O, Vallée A, and Waldmann C. It has been suspected, however, almost since the types themselves were established, that differences of a lesser degree existed in the antigenic behavior of some strains within one immunological type. Such suspicion arose from observations on cross-immunity in cattle and guinea pigs, but owing to the difficulties associated with experiments in large animals with this highly infective virus, and the deficiencies of experiments in laboratory animals, arising from possible alterations in strains during passage, precise observations are lacking. The differences between such strains within a type in any case appeared to be of small significance as compared with the very clear-cut demarcation of the types themselves.

When work was proceeding at this Institute, during 1947, on strains of virus recovered from the present epizootic in Mexico, this phenomenon of "variant" strains was encountered in a new and important connection. It was found that, although a particular strain (MP), from Mexico, could be classified as of Vallée A type, vaccination of cattle with a potent vaccine prepared from the Pirbright stock cattle strain 119 of the same type, gave poor protection against the Mexican strain. This result is reported in detail elsewhere.[1]

To obtain further information on this discrepancy, opportunity was taken to apply one of the more recent methods of quantitative study of the virus and its antibody. Cross-neutralization tests were carried out as described in a subsequent paper of this series, and the results confirmed the existence of an antigenic difference between strains MP and 119.

While this work was in progress, our attention was drawn to papers by Traub and Rodriguez[2] and Traub and Möhlmann.[3] The authors had, in the course of examination of field strains by the complement fixation method encountered 2 classes of "variant" strains of the virus, one of them being within the Vallée A type group. Some of these strains were recovered from vaccinated cattle which had later developed the disease. They could be shown to be different in their complement fixing properties from the stock vaccine strain of Vallée A type by cross tests with guinea pig antisera prepared against the individual strains. Traub and Möhlmann drew attention to the complications introduced into vaccination against the disease by the occurrence of these variant strains and it is unfortunate that the war has prevented them from publishing in full their protocols.

The development of complement fixation as a method of "typing" the virus of foot-and-mouth disease dates from the observations of Ciuca[4] in which guinea pig antigen and antiserum were used. Traub and Möhlmann[5] produced reliable and consistent results with bovine virus as antigen, an advance which increased greatly the usefulness of the test. Rodriguez, Prado, and Palacios,[6] working

[1] Henderson, W. M., Galloway, Ian A., and Brooksby, J. B., Proc. Soc. Exp. Biol. and Med., 1948, **69**, 77.

[2] Traub, E., and Rodriguez, R., *Zbl. Bakt. Abt.*, Orig., 1944, **151**, 380.

[3] Traub, E., and Möhlmann, H., *Berl. und Münch. tierärztl. Wschr.*, 1946, **1**, 1.

[4] Ciuca, A., *J. Hyg. Camb.*, 1929, **28**, 325.

[5] Traub, E., and Möhlmann, H., *Zbl. Bakt. Abt.*, Orig., 1943 **150**, 289, 300.

independently, also showed the value of the method. Work at this Institute has been directed at increasing the precision of measurement of the reaction, using methods already described.[7] Application of these methods to the Mexican strains, together with the use of strictly homologous antisera, soon confirmed the existence of differences between them and strain 119, the stock Vallée A strain. The data on these observations are presented below.

Methods. The technic used follows that described in the differentiation of vesicular stomatitis and foot-and-mouth disease,[7] and the results are presented in the same way, all end-points being referred to the dose of complement necessary for 50% hemolysis. For the differentiation of variants, it is essential to prepare a strain-homologous antiserum. So far, no success has attended experiments with bovine antisera and all results were obtained with antisera prepared against the guinea pig adapted strains.

Serum from convalescent guinea pigs was used in one or two cases but in the remainder a course of intramuscular inoculations with virus of the strains concerned was given to the convalescent guinea pigs. The antigens were 1:4 or 1:10 suspensions of infective bovine tongue epithelium, ground in M/25 phosphate buffer solution with sand. They were prepared from 4-24 hours before use and centrifuged for 20 minutes at 2,000-3,000 r.p.m. Complement and the hemolytic system were the same as in the earlier experiments.[7] The strains used were:

TABLE I.
Complement Fixation with Vallée A Type Variants.

Antiserum	Virus strain			
	119	MP	M.1	Nil
119a	.76	.62	.62	.53
119b	.80	.61	.62	.53
MP	.51	.69	.56	.53
M.1	.52	.49	.79	.53
Nil	.50	.39	.36	.53

[6] Rodriguez, R., Prado, M., and Palacios, R., *Bol. Soc. Paul. Med. vet.*, 1945, **7**, 94.

[7] Brooksby, J. B., PROC. SOC. EXP. BIOL. AND MED., 1948, **67**, 254.

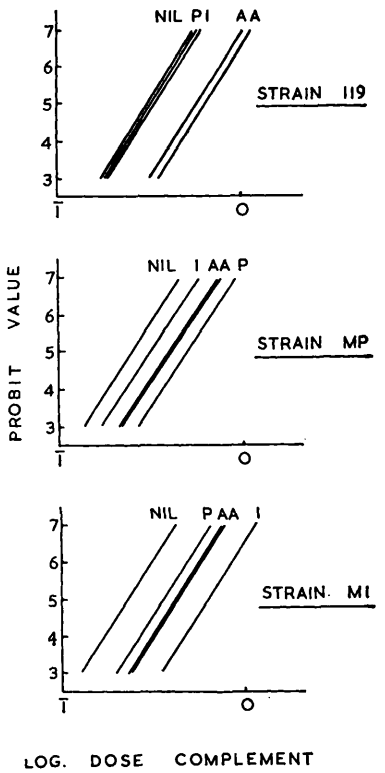

FIG. 1.
Complement-fixation with Vallée A Type Variants. The antisera used are indicated thus: A, A—Two batches of strain 119 antiserum. P—Strain MP antiserum. I—Strain M.1 antiserum.

Vallée O Type. Strain 1. A strain from cattle recovered from a field outbreak in 1924 and since maintained in guinea pigs.

Strain 39. A strain from cattle recovered from a field outbreak in 1927 and since passaged in cattle.

Vallée A Type. Strain GB. A guinea pig adapted strain received from the island of Riems, Germany, in 1929.

Strain 119. A strain from cattle recovered from a field outbreak in 1932 and since passaged in cattle.

Strain MP. A strain from cattle recovered in Mexico, December, 1946.

Strains M.1 and M.2. Strains from cattle recovered in Mexico, October, 1947.

Strain AX. A cattle-passaged strain received from the Argentine in 1944.

Waldmann C Type. Strain GC. A guinea

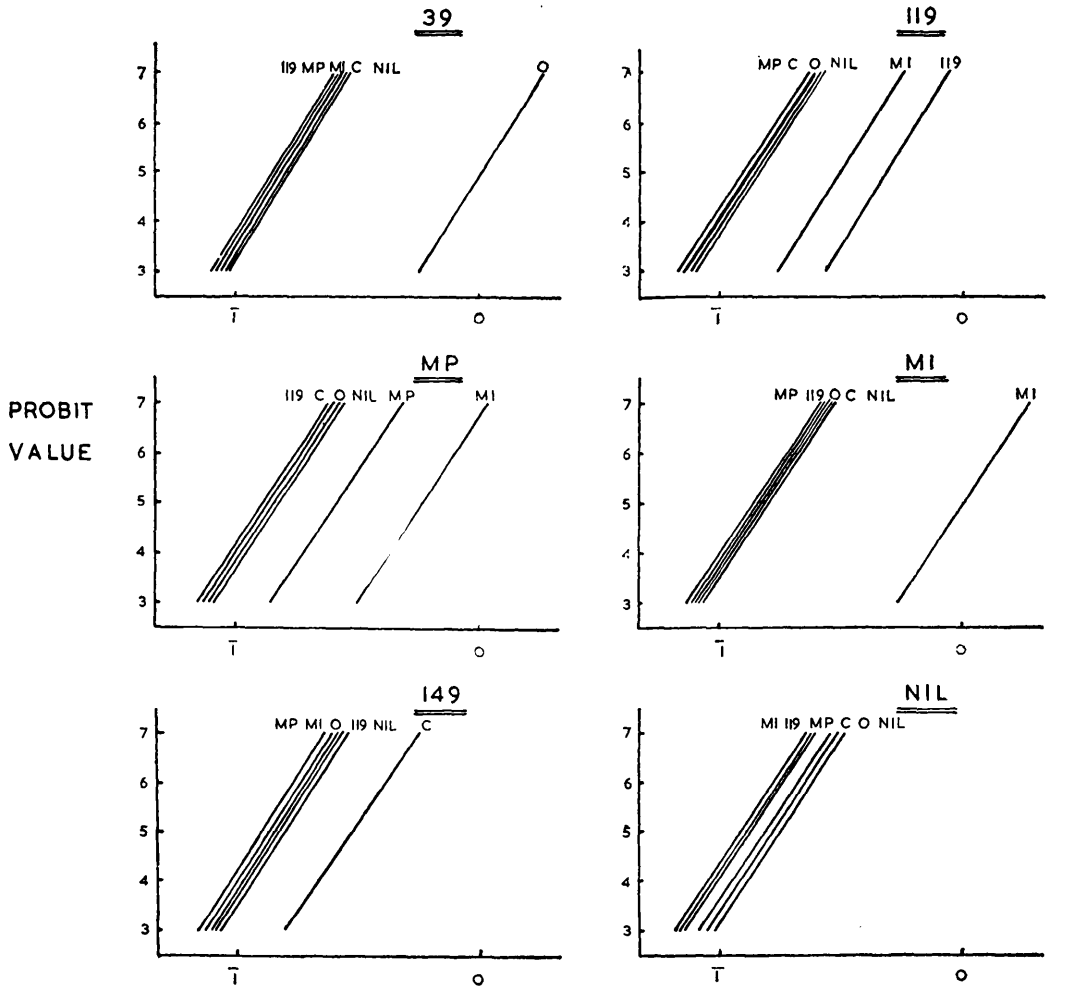

FIG. 2.

The relationship of Vallée A type variants to other strains. The antisera are indicated thus: O—Strain 1 antiserum, Vallée O type. 119—Strain 119 antiserum, Vallée A type. MP—Strain MP antiserum, Vallée A type. M.1—Strain M.1 antiserum, Vallée A type. C—Strain GC antiserum, Waldmann C type.

pig adapted strain received from the island of Riems, Germany, in 1933.

Strain 149. A strain from cattle recovered from a field outbreak in 1934 and since passaged in cattle.

Results. The result of a simple experiment illustrating the detection of variants is presented in Table I and shown graphically in Fig. 1. Three antigens, from strains 119, MP and M.1, prepared as 1:10 suspensions of infective bovine epithelium, were tested against antisera from guinea pigs convalescent from infection with these strains. Two samples of strain 119 antiserum were included. They behaved consistently. In the table, the values are the logarithmic doses of complement corresponding to the 50% hemolysis end-points in the presence of the various antigen-antiserum mixtures. The effect of the various sera on complement has been allowed for by applying a correction to all the figures in each horizontal row in the table so that the serum control series (without antigen) give the same value. From the

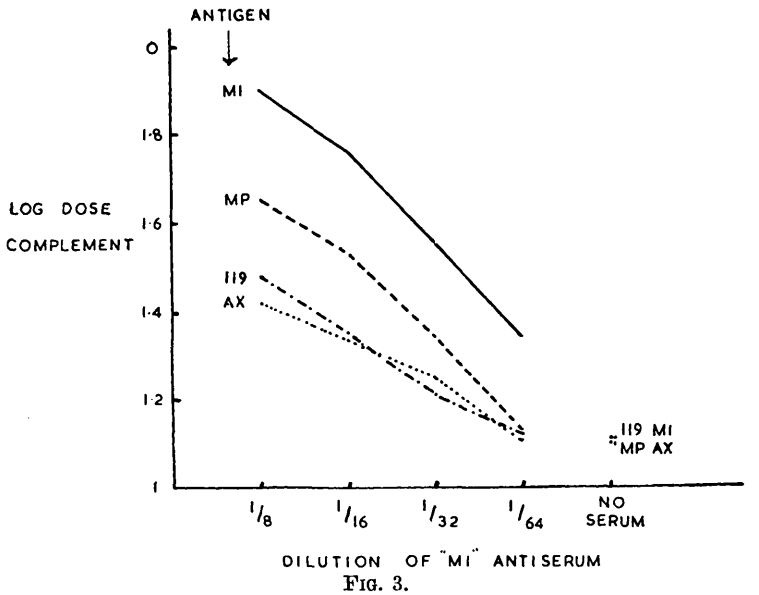

FIG. 3.
The effect of dilution of antiserum on cross-fixation with Vallée A type variants.

graphs in Fig. 1, it is clear that the homologous mixtures show greater fixation in each case, or, as is equally important, the relative efficacy of fixation by the various antisera depends on the antigen present. Fixation is indicated by the line concerned being further to the right.

Further observations on the relation of the variants to each other and to the immunological types are presented in Fig. 2. The result is shown of tests of strains 39, 119, MP, M.1, and 149 with the guinea pig antisera against strains 1, 119, MP, M.1, and GC. In the case of strains 39 and 149 the result is clear cut in that no serum other than the homologous shows any fixation. In the Vallée A group, strain 119 virus shows fixation, better with 119 antiserum but also with M.1 antiserum; MP virus fixes best with M.1 antiserum but also with MP antiserum, and M.1 virus shows very strong fixation with M.1 antiserum. This result would suggest that M.1 might be a "universal" A antiserum; but it is more likely to be due to a high concentration of antibody. The MP antiserum was from convalescent guinea pigs, as opposed to the other sera involved which were obtained from hyperimmunized guinea pigs. The result with MP antigen was therefore, in all probability an expression of the low titer of the MP antiserum and high titer of the M.1 antiserum. Such anomalous effects could be counteracted by titration and suitable dilution of the serum before use in the test. This view is supported by the result shown in Fig. 3, where dilutions of the same M.1 antiserum were prepared and tested against the strain homologous virus and 3 other Vallée A antigens, 119, MP, and AX. The log. doses of complement giving 50% hemolysis are plotted against the dilutions of serum and a curve obtained by joining the points for each antigen. It is apparent from this graph that if a 1/64 dilution of serum were used in this test, fixation would be shown with only the homologous strain, the reaction with other strains of the same type having disappeared. This finding agrees well with that shown in Traub and Möhlmann's paper relating to the variants within the Vallée A immunological groups encountered by them.

In carrying out the routine type tests, the stock antisera used are from hyperimmunized guinea pigs. The aim here is to ensure fixation when testing with any strain of the same type, and for this purpose a high titer is an advantage. A test of the several variant strains of Vallée A type with the stock GB

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.