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Vesicular Stomatitis and Foot-and-Mouth Disease Differentiation by Complement-Fixation.

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It is generally admitted that the differential diagnosis of vesicular stomatitis and foot-and-mouth disease on clinical grounds alone is virtually impossible. Methods of differentiating between the two diseases which have been suggested include: (a) determination of the particle size of the infective agent (Galloway and Elford[1]); (b) determination of infectivity for the developing egg by the chorioallantoic route (Burnet and Galloway,[2] Galloway and Elford[3]); (c) study of the pH stability range of the infective agent (Pyl and Klenk,[4] Pyl,[5] Reppin, and Pyl[6]); (d) tests of susceptibility of several species of animal (*e.g.*, horses, cattle, swine, sheep, guinea pigs) by a variety of routes; (e) cross-immunity tests in experimental animals (Wagener).[7]

In addition may be included epidemiological criteria, such as spreading power and degree of generalization of the disease in infected animals. These are of little use in countries such as the United States of America and Great Britain, where the first object of policy is to limit the possible spread of the infective vesicular diseases of animals by the slaughter of infected stock, and destruction of all material likely to harbour the viruses.

All the methods listed above depend, in the last analysis, on the susceptibility of the test animals, and the virulence of the material used. It is therefore doubtful if too great reliance can be placed on any one method. For example, if, in a test of species susceptibility, one or two horses do not react to inoculation with an unknown virus, which has however infected a similar number of cattle and swine, a diagnosis of foot-and-mouth disease cannot be given with confidence. Similarly, the criterion tentatively adduced by Shahan, Frank, and Mott,[8] depending on the intramuscular inoculation of cattle, has already been found by the same workers to be unreliable, in that an animal inoculated with the virus of vesicular stomatitis has reacted, while observations at this Institute with some strains of the virus of foot-and-mouth disease show that the result of intramuscular inoculation of foot-and-mouth disease virus is not always regular.

In these circumstances, any data which can be gathered rapidly, on an infective agent causing vesicular lesions in cattle and swine, are of great value. The virtues of an *in vitro* test, in respect of speed as much as of economy, are especially attractive. These are the considerations which have stimulated publication of the present paper. Of necessity, since the work has been done in a country where the virus of vesicular stomatitis has not so far been identified in the field, the observations are based on strains which have been passaged in the laboratory; but the well-defined results obtained—better than those usually encountered under comparable conditions with the virus of foot-and-mouth disease—suggest that the method could be of great value with material from field outbreaks. The results presented below support the view that complement-fixation should be added to the list of methods of differential diagnosis,

[1] Galloway, I. A., and Elford, W. J., *Brit. J. Exp. Path.*, 1933, **14**, 400.

[2] Burnet, F. M., and Galloway, I. A., *Brit. J. Exp. Path.*, 1934, **15**, 105.

[3] Galloway, I. A., and Elford, W. J., *Brit. J. Exp. Path.*, 1935, **16**, 588.

[4] Pyl, G., and Klenk, L., *Zbl. Bakt. Abt.*, 1933, 1, Orig. **128**, 161.

[5] Pyl, G., *Hoppe-Seyler's Z.*, 1934, **226**, 18.

[6] Reppin, K., and Pyl, G., *Arch. wiss. prakt. Tierhlk.*, 1934, **68**, 183.

[7] Wagener, K., *Arch. wiss. prakt. Tierhlk.*, 1933, **66**, 173, 301, 363.

[8] Shahan, M. S., Frank, A. H., and Mott, L. O., *J. Am. Vet. Med. Assn.*, 1946, **108**, 5.

and included in any study of an outbreak of a vesicular disease of cattle or swine.

The methods used are still under investigation in a study of the complement-fixation reaction in foot-and-mouth disease. Refinement of technic, however, should lead only to greater delicacy in the detection of immunological differences, a goal greatly desired in respect of foot-and-mouth disease and perhaps not without significance for vesicular stomatitis.

Methods. Some details of the strains of virus used are given since other experiments with the same strains have been published by American workers.

Vesicular Stomatitis (V.S.) type Indiana Strain USCB, received from Dr. Schoening of the U. S. Bureau of Animal Industry in 1944. It was derived from bovine material from an outbreak in Colorado. It is believed that this strain is identical with that described by Shahan, Frank, and Mott[9] as Ind C. It has been maintained at this Institute by serial passage in cattle, and the material used in the experiments below was of the 8th passage. Epithelium was collected from the tongue of an infected steer on 10th June, 1947, cut up, and dried over sulphuric acid and, later, P_2O_5. It was stored in sealed ampoules at 4°C.

V.S. type New Jersey Strain USKP, received as above, and derived from an outbreak in Kansas City, Mo. Later described by the workers cited above as NJM. Two batches of material were used, one of the 3rd passage stored frozen at −20°C from 25th May, 1947, and the other from the 5th passage, dried and stored as the Indiana type material. Guinea pig material of the two type strains was also included, in each case as the 13th serial passage of these strains in guinea pigs, stored as frozen guinea pig pads at −20°C.

Foot-and-Mouth Disease (F. & M.) type Vallee "O" Strain ASJ, an Argentine strain of bovine origin. Passaged in cattle at Pirbright from 1944 till 10 April, 1946. Bovine tongue epithelium was collected and stored in 50/50 glycerine and M/25 phosphate buffer at pH 7.6.

F. & M. type Vallee "A" Strain 119, a bovine strain, originating from an outbreak in Great Britain in 1932, and since given 68 serial passages in cattle. Material collected and stored as for "O" type, from 14th October, 1947.

F. & M. type Waldmann "C" Strain 149. Also a bovine field strain, recovered in Great Britain in 1934, and since given 33 serial passages at this Institute. Epithelium was collected on 10th November, 1947, and stored frozen.

Three strains of foot-and-mouth disease, passaged in guinea pigs, were also included in the work. They were: Strain 1, "O" type, passaged at Pirbright since 1924, Strain GB, "A" type passaged since 1929, and Strain GC, "C" type, passaged since 1933.

For use in the complement-fixation test, an approximately 1/10 suspension (calculated on wet weight) was prepared, in 0.9% NaCl for V.S. and in M/25 phosphate buffer for F. & M. The suspensions were prepared from 4-18 hours before use, and then centrifuged at 2,000 r.p.m. for 10 minutes immediately before test. The specific sera were all obtained from guinea pigs which had passed through the disease. In the case of V.S. the infecting strains were those described above, while for F. & M. the strains used were the stock guinea pig strains used in this Institute. The guinea pigs used for V.S. (Indiana) serum had received a subsequent intramuscular inoculation of virus before use as serum donors and those for F. & M. (O, A, and C) serum, a course of 4 inoculations by the same route. The F. & M. sera were lyophilized, but the V.S. sera were stored as fluid sera at 4°C. The complement was a stock lyophilized guinea pig serum prepared in batches for use in routine complement-fixation tests in F. & M. The hemolytic system was obtained from a reputable supplier of biological products.

Details of the test methods will be published in full when the data on F. & M. are presented. Briefly, the procedure is to carry out an abbreviated titration of complement

[9] Shahan, M. S., Frank, A. H., and Mott, L. O., 1946, *loc. cit.*

TABLE I.
Type-specific Fixation of Complement in Vesicular Stomatitis and Foot-and-Mouth Disease.

		Antiserum					
		Vesicular stomatitis		Foot-and-mouth disease			
Antigen	Type	Indiana	New Jersey	"O"	"A"	"C"	Nil
Vesicular stomatitis	Indiana	1.44	.23	.24	.22	.24	.14
	New Jersey	.21	.74	.30	.32	.30	.17
Foot-and-mouth disease	"O"	.15	.17	.37	.19	.23	.18
	"A"	.18	.20	.23	.39	.24	.21
	"C"	.17	.19	.21	.20	.44	.16
Nil	—	.20	.16	.27	.26	.26	.21

N.B. The figure in each case represents the amount of complement, as ml of 1/25 guinea pig serum, necessary for 50% hemolysis.

in the presence of the various reagents, combined, and with suitable controls. The doses of complement are arranged in a logarithmic series, and the end-point used is 50% hemolysis, calculated from the readings obtained with the centrifugalized tests on a photo-electric absorptiometer ("Spekker" by Hilger, London). The amounts of the various reagents used are (1) virus, 0.2 ml of 1/10 suspension; (2) serum, 0.4 ml of 1/8 dilution in 0.9% NaCl; (3) complement, 1 ml of a dilution of guinea pig serum in 0.9% NaCl, calculated to give the appropriate dose of a series, *e.g.*, 0.2, 0.3, 0.45, 0.67, and 1.0 ml of a 1/25 dilution. Where a reagent is omitted, the volume is made up with 0.9% NaCl.

Fixation of complement is allowed to take place in a water bath at 37°C for 30 minutes. The hemolytic system is then added (0.5 ml of 3.3% cells with hemolytic included) and incubation continued for a further 30 minutes. The tubes are then removed from the water bath, and centrifuged at 1,500 r.p.m. for 10 minutes. Readings are made directly on the tubes, held in a special mount. From these readings, the per cent hemolysis for each tube is obtained from a graph, and hence the amount of complement for the 50% hemolysis end-point is calculated.

Alternatively, the results may be presented graphically, plotting the per cent hemolysis against the log. dose of complement. Titration of complement alone and in the presence of virus and specific sera gives a sigmoid curve in such graphs, and as it is more convenient to deal with straight lines, the transformation evolved by Gaddum[10] and Bliss[11] is used. The values for per cent hemolysis are converted to probit values, using the tables of Fisher and Yates,[12] and these values are plotted against the log dose of complement. The probit value 5.0 corresponds to 50% hemolysis and the end-points are easily obtained from these graphs.

Results. Type specific complement-fixation has been obtained using virus from cattle or guinea pigs in foot-and-mouth disease and from both these sources and also from infected eggs in vesicular stomatitis. Since the results are substantially the same with different antigens, the results of a representative test in which virus of bovine origin was employed is shown in Table I, and, graphically in Fig. 1. The fixation of complement by the homologous virus-serum mixture is demonstrated by the increased amount of complement necessary for 50% hemolysis. In the figure, this is shown by the shift to the right of the appropriate graph.

The result with V.S. Indiana virus is particularly clear-cut. The degree of fixation exceeds by two- or three-fold that hitherto encountered in F. & M. disease, under optimum conditions. The poorer fixation with V.S. New Jersey virus may be explained by the fact that the homologous serum was ob-

10 Gaddum, J. H., No. 183, Spec. Rep. Ser., Med. Res. Coun., H. M. Stationery Office, London, 1933.

11 Bliss, C. I., *Ann. Appl. Biol.*, 1935, **22**, 134.

12 Fisher, R. A., and Yates, F., *Statistical Tables*, 2nd edit., Edinburgh, Oliver & Boyd, 1943.

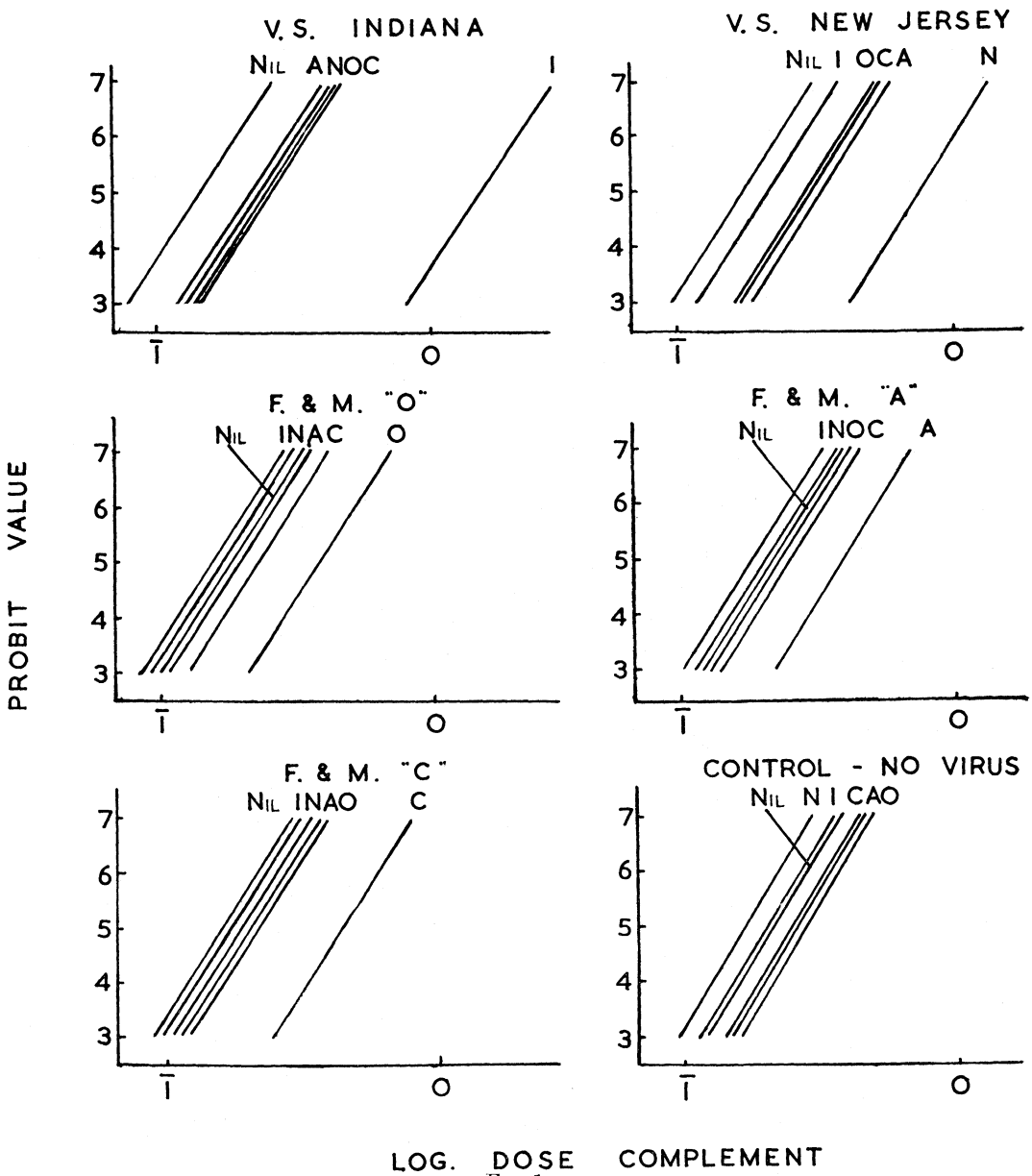

FIG. 1.

Complement fixation in vesicular stomatitis and foot-and-mouth disease. Antisera used are indicated thus: I = V.S. Indiana; N = V.S. New Jersey; O = F. & M. "O"; A = F. & M. "A"; C = F. & M. "C"; Nil = No antiserum (antigen control).

tained from guinea pigs convalescent from the disease, whereas the Indiana anti-serum came from guinea pigs which had had an additional inoculation with virus, by the intramuscular route. That the difference between the results was dependent on the sera was suggested by an experiment where guinea pig and bovine virus of the two types were tested against the same two sera. The results here showed that the degree of fixation recorded with each serum ran parallel in the presence of the different antigens.

The F. & M. type-immune sera were all from guinea pigs which had been hyper-

immunized, and yet the difference in the amount of complement necessary for 50% hemolysis, in the presence of homologous as compared with heterologous serum and virus, is only about two-fold. The quantitative difference between V.S. and F. & M. in respect of the complement fixing properties may be correlated with the behavior with neutralizing antibodies. Studies on neutralization of the virus of V.S. by its antisera show that the degree of neutralization recorded for typical sera greatly exceed the corresponding values for F. & M. and its antisera. Thus a 1:10 dilution of V.S. serum may neutralize 10^5 $I.D._{50}$ of virus (Skinner[13]) while no similar dilution of F. & M. serum has yet been found to neutralize as much as 10^3 $I.D._{50}$.

Discussion. It must be emphasized that the results recorded were obtained with material of only 2 strains of the virus of vesicular stomatitis. Confirmation using other strains is greatly to be desired, in view of the difficulties which have attended the examination of a large proportion of strains of the virus of foot-and-mouth disease from field outbreaks. The bovine V.S. material used was epithelium about 4-6 months old, and 2 of the samples had been dried. It is not known how fresh material, collected, perhaps, at a time when the virus was not fully active, would behave in the test. Nevertheless, in view of the clear-cut results recorded here, an optimistic view of the value of the test is probably justified, and the addition of complement fixation to the armory of tests to which a virus of this group can be submitted would seem to be well worth while.

Summary. (1) The fixation of complement by vesicular stomatitis virus and its antiserum is described. (2) This fixation is type specific for the 2 strains of vesicular stomatitis virus of Indiana and New Jersey type available. (3) There is no cross-fixation with any of the three types of the virus of foot-and-mouth disease.

[13] Skinner, H. H., personal communication, 1947.