

intradermally into the tongue (M.2, M.3 and M.4) with 4 other Mexican strains of the virus, none developed any lesions.

Furthermore a limited number of tests were made by inoculating Mexican strains of virus into cattle recovered from infection with Vallée O and Waldmann C type strains of the virus of foot-and-mouth disease and in all of these a generalized foot-and-mouth disease infection resulted.

On perusal of the accumulated evidence it will be seen that none of it would suggest that any infective agent other than the virus of foot-and-mouth disease was present in any of the 13 samples of material collected from

cattle in Mexico and forwarded to Pirbright for examination. Moreover each of the 13 strains could be clearly classified in the main Vallée A immunological type group of the virus under discussion. The examination of these Mexican strains of virus involved inoculation of cattle, horses, donkeys, swine, guinea-pigs and developing hen eggs, fractional filtration experiments, complement fixation tests and cross immunity typing tests in guinea-pigs and cattle against stock type strains of the virus of foot-and-mouth disease and it was therefore much more exhaustive than that usually made of "unknown" samples of virus.

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Strains of Virus of Foot-and-Mouth Disease Recovered from Outbreaks in Mexico. Pathogenicity and Invasiveness.

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(Introduced by R. E. Shope.)

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A report on the identification of samples of virus received from Mexico has been made.¹

Before referring to the general effects of the Mexican strains of virus in cattle exposed to infection by inoculation, by contact with diseased cattle, or by the nasal instillation of virus, something should be said about the animals employed in these experiments.

Foot-and-mouth disease is not endemic in Great Britain but is introduced at irregular intervals, outbreaks occurring sporadically. Since the policy of control is by "stamping-out," that is, elimination of infected and "in-contact" animals by slaughter and burning (or under the exigencies of war, by burial) along with the rigorous application of other disinfection and "stand-still" orders, it is possible only to purchase cattle with a "clean" history as regards foot-and-mouth disease. The cattle used in these experiments

were Devon steers between 1½ and 2 years old, in good condition and weighing between 600-800 lbs, and they could be considered as relatively "standard."

1. *Intradermal inoculation into the tongue.*

In all 296 cattle have been inoculated with centrifuged suspensions or stock filtrates of the 13 strains of Mexican virus. 184 were inoculated with MP virus, 80 with M.1 virus, and the remaining 32 with the other 11 strains. 293 of these 296 reacted well, with local lesions at the sites of inoculation and generalized or secondary lesions at some or all of the usual sites of predilection, feet, and lips, in all cases except 2. These 2 were control animals inoculated with filtrates in an experiment described elsewhere.²

There were 3 cattle which failed to show lesions at the sites of inoculation. These had been inoculated with similar virus material

¹ Brooksby, J. B., Henderson, W. M., and Galloway, Ian A., *PROC. SOC. EXP. BIOL. AND MED.*, 1948, **69**, 64.

² Henderson, W. M., Galloway, Ian A., and Brooksby, J. B., *PROC. SOC. EXP. BIOL. AND MED.*, 1948, **69**, 77.

to that which had produced the disease in other cattle. One of these however subsequently developed lesions at sites other than those inoculated, probably resulting from infection by contact with its box companion which had reacted. The other 2 developed local lesions on the tongue when re-inoculated with the Pirbright stock cattle strain 119 of the Vallée A type.

Such occasional failures to react to intradermal tongue inoculations of relatively large doses of virus have been observed with other strains of the virus of foot-and-mouth disease.

In general therefore Mexican strains of virus produce infection in the Pirbright cattle with the same degree of regularity as other strains when inoculated intradermally into the tongue.

However, there appears to be a tendency for delayed appearance of the secondary lesions. Moreover, although the presence of these secondary lesions on all 4 feet and sometimes the lips has been observed in a considerable number of cattle inoculated intradermally into the tongue with Mexican strains of virus, in many animals generalization has been incomplete and limited as regards the degree of involvement of the feet.

2. *Intramuscular inoculation.* Only 10 cattle have been inoculated by the intramuscular route, 3 with strain MP, 2 with MPB, 1 with MC, 2 with MSL, and 2 with M.1, and all of these reacted with a severe generalized foot-and-mouth disease infection.

3. *Titration of Mexican strains of virus in cattle.* Observations have been limited so far mainly to the MP and M.1 strains of virus and it has been found from the accumulated results of titrations of suspensions of epithelium from tongue lesions that the mean 50% end-point for Mexican strains of virus, $10^{-6.1}$ (414 observations) for strain MP and $10^{-6.2}$ (394 observations) for strain M.1, is not appreciably different from that recorded for other strains of the virus of foot-and-mouth disease, e.g., $10^{-6.8}$ for stock cattle strain 119 of the Vallée A type.

However, it has been noted that a number of titrations of Mexican strains, e.g., MP and M.1, have been noteworthy for the difference in reactions between cattle in pairs used

for this purpose. It seemed that the effect of the virus was impeded to a greater extent by any increased inherent resistance of the inoculated animal than was the case with other strains of the virus which have been studied. This would appear to be a more likely explanation than that the cattle used in these titrations were exhibiting greater variation in susceptibility than when other virus strains have been examined. It is interesting that although the mean 50% end-point of titrations of virus suspensions prepared from tongue lesions was not widely different from that observed with other strains of virus, yet the mean 50% end-point of titrations of defibrinated blood collected from cattle at the height of infection was somewhat lower, $10^{-2.4}$ (120 observations) for strain MP and $10^{-2.9}$ (60 observations) for strain M.1 as compared with $10^{-3.5}$ for our stock strain 119. Incidentally although an impression was received that individual comparative titrations of strains MP and M.1 showed in some cases a higher titer for the M.1 as compared with the MP strain, the accumulated results of all observations did not confirm this impression. The generally lower titer of the blood of cattle infected with Mexican strains as compared with that of animals infected with other strains of virus seems to fit into the picture of the lower invasiveness of Mexican virus strains examined. This lesser invasiveness was demonstrated in the first instance by a tendency to slower generalization after intradermal tongue inoculation but it is apparent to a greater degree in the experiments about to be described.

4. *Exposure of cattle to infection by contact.* As will be seen from Fig. 1, of 48 untreated susceptible cattle exposed in different experiments to virus infection with Mexican strains by contact with cattle showing well developed lesions, 20 failed to show any reactions. All the 48 cattle had been placed in contact with the virus donors from the time the latter were infected by intradermal inoculation of the tongue with a strong dose of virus, and had been left with them for a period of about 3 weeks. The important point is that they were with the virus donors throughout the whole of the acute stage of the infection.

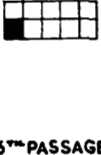
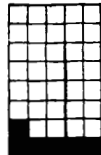
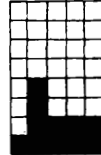
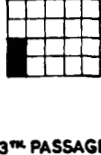
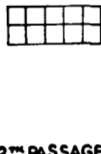
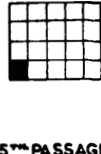
CONTACT EXPOSURE	1 DONOR PER ANIMAL. EACH PAIR IN A SEPARATE BOX	LATER TONGUE INOCULATION OF NON-REACTORS	1 DONOR PER 8 ANIMALS. ALL IN ONE BOX	LATER TONGUE INOCULATION OF NON-REACTORS
STRAIN MP VIRUS		 6 TH PASSAGE		 6 TH PASSAGE
STRAIN MP VIRUS		 16 TH PASSAGE		 13 TH PASSAGE
STRAIN MI VIRUS		 12 TH PASSAGE		 5 TH PASSAGE

FIG. 1.

Contact exposure of susceptible cattle to Mexican strain infection. Each large rectangle depicts a group of cattle with a horizontal line of five squares for each animal. The first square represents the tongue and/or lips and the next four squares the four feet. A lesion at any particular site is denoted by blackening the appropriate square. A diagonal stripe is used when lesions occurred only on the lips and not on the tongue.

When these 20 non-reactors were inoculated subsequently into the tongue with at least 10,000 ID₅₀ doses of homologous virus, 17 were shown to have developed a high grade of immunity, 11 having no reactions and 6 having reactions only at the sites of inoculation. The other 3 behaved in this severe test of resistance like unexposed fresh control cattle. It is to be noted that such development of an "inapparent infection" accompanied by a high grade of resistance to reinfection characterizes the foot-and-mouth disease pathological picture to a greater degree than is appreciated. In the writers' experience the phenomenon is much more pronounced in the case of the Mexican strains of virus under discussion than when contact exposure tests are made with other strains of virus.

In marked contrast observations may be cited on similar contact exposure tests with Pirbright cattle in which strain 119, the stock

cattle strain of the Vallée A type was investigated.

Of 72 untreated susceptible control cattle (groups of 8 in 9 different experiments) placed in contact separately in boxes with single virus donors infected with strain 119 only 2 failed to develop visible lesions. These two were proved to have contracted an "inapparent infection" by the fact that they resisted the subsequent inoculation of 10,000 ID₅₀ doses of the homologous virus. Again of 52 cattle (5 different experiments, four groups of 8 and one of 20) exposed to infection by contact with one virus donor, all developed a severe generalized infection.

It would appear to be justifiable to refer to the Mexican strains of virus as strains of reasonably good spreading power but of low invasiveness and strain 119 as one of good spreading power and high invasiveness.

Our experiments with other strains of virus have shown that some strains, *e.g.*, 39 and 336,

may be intermediate in their invasiveness. This was evidenced in experiments in which susceptible cattle are exposed to infection for a short time or individually to single virus donors. They differ from the Mexican strains in that when groups of susceptible cattle are exposed to an accumulation of infection in one large box then they have all developed a severe generalized disease. An impression has been gained also that while certain strains of virus may exhibit lability in their spreading power and invasiveness from time to time others have greater stability in this respect. There is as yet no reliable information available on what factors, *e.g.*, method of storage, method of passage in animals, etc., are likely to affect the invasiveness of a strain. One of the difficulties lies in the setting up of an adequate organization for studies of this sort, and many strains may have been manipulated in the laboratory in one way or other to a considerable degree before being submitted to investigation. It is certain however that some strains, *e.g.*, 119 and the WA strain (a Vallée O strain which was recovered from cattle during the epizootic which spread rapidly across Europe during the period 1937-40), maintain their spreading power and invasiveness even after repeated passage in cattle by intradermal tongue inoculation or after storage in 50% glycerine for periods of from 4 to 7 years.

An attempt was made to enhance the invasiveness of the Mexican strain MP by infection of cattle by contact exposure passages but the series was broken by some of

the cattle failing to develop lesions. Another attempt was made by rapid passages in cattle by intradermal tongue inoculations (6th to 13th passage) but as will be seen from Fig. 1 the invasiveness was not increased in the tests made after the 13th and 16th passages.

Let it be mentioned also that strain M.1 recovered from cattle in a field outbreak in Mexico in October, 1947, when the disease had been spreading for about a year exhibited the same low invasiveness as strain MP recovered from cattle in Mexico in December, 1946.

5. *Intranasal instillation of virus.* It is noteworthy that this low invasiveness of strain 39 (*vide supra*) and the more pronounced low invasiveness of the Mexican strains MP and M.1 is exhibited also when single or repeated large doses of highly active virus are administered by intranasal spray and inapparent infections may develop in the same way under such conditions of experiment.

The result of one experiment with the Mexican virus strain M.1 may be cited. Four susceptible cattle received 3 doses of a gradocol membrane (Elford) virus filtrate with a titer of 10^{-4} at 24 hours interval. One animal developed lesions on the 5th day followed by a severe generalized infection involving the mouth and all four feet by the 6th day. The three others remained free of lesions and after a fortnight they were inoculated intradermally into the tongue with a filtrate of a titer of 10^{-4} . Two showed no lesions and the third had local lesions at the site of inoculation only which regressed rapidly.