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Pathogenesis of foot-and-mouth disease: the lung as an additional portal of entry of the virus

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SUMMARY

Donor cattle infected with foot and mouth disease (FMD) virus subtype O_1 were used to expose experimental cattle. The pharyngeal virus growth and viraemia patterns after contact exposure were quite different from those obtained after intranasal inoculation and suggested that the lower respiratory tract might provide an additional portal of entry for the virus. A tracheotomy was performed on experimental cattle to let the respiration bypass the pharynx, followed by exposure to FMD virus by different routes. The results confirmed that FMD virus can enter the bloodstream via the lung, followed by haematogenic infection of the pharynx and other replication sites simultaneously. These observations led to further experiments in which the intravenous route of infection was used to study the interaction of virus growth in the phayrnx, in other sites, and in viraemia.

INTRODUCTION

In a companion paper (McVicar & Sutmoller, 1976), we describe the growth of foot and mouth disease (FMD) virus in the upper respiratory tract of cattle after intranasal inoculation with the virus. General virus growth patterns were established relative to the dose of infecting virus and the pre-exposure immune status of non-immunized, immunized, and recovered cattle. These studies also covered the onset and development of viraemia relative to the virus growth in the pharyngeal area. We have also explored the mechanisms of entry and removal of virus from the vascular system (Sutmoller & McVicar, 1976).

For the objectives of the studies described in the companion paper (McVicar & Sutmoller, 1976), the intranasal route was chosen because it enabled us to control the dose of virus and the exact moment of virus exposure.

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Earlier we proposed a working hypothesis for the pathogenesis of FMD (Sutmoller, 1971). Briefly, infection started with local multiplication of virus in the pharynx, followed by drainage to the circulatory system with subsequent spread to epithelial sites; the result was formation of vesicles. The results of our present series of experiments suggest the need to expand the hypothesis and to include the lung as an additional portal of entry of the virus.

MATERIALS AND METHODS

Cattle

Hereford steers approximately 18 months old were housed in isolation units as described elsewhere (Callis & Cottral, 1968).

Virus

FMD virus, type O, subtype 1, strain CANEFA-2 was used after the 7th passage in primary bovine kidney cell (BK) cultures.

Virus inoculation

For intranasal inoculation, 5 ml. of virus suspension was slowly instilled into the ventral part of the nostrils (2·5 ml. on each side) while the head was held slightly elevated. For intravenous inoculation, the virus was injected into the jugular vein through a polyethylene catheter.*

Collection of samples

Samples of oesophageal-pharyngeal fluid (OPF) were taken with a cup probang (McVicar, Graves & Sutmoller, 1970). A polyethylene catheter placed in the jugular vein was used to collect heparinized blood samples. Approximately 50 cm of tubing was threaded through a 13-gauge bleeding needle previously inserted in the vein. The needle was withdrawn over the tubing and a blunted 18-gauge needle was inserted into the free end. A 5-ml. plastic syringe filled with phosphate-buffered saline (PBS) was attached to the fitting and fastened to the halter of the animal. A new syringe was used for each sample, and the catheter was flushed with PBS before and after a sample was taken. OPF and blood samples were held at $-20°$ C. until tested.

Virus assay

Titrations of viral infectivity in OPF and blood samples were made by plaque assay in secondary BK cultures in plastic plates† under gum tragacanth overlay as described previously (McVicar, Sutmoller & Andersen, 1974). All infectivity titres were expressed in plaque forming units (p.f.u.)/ml.

Tracheal intubation

Tracheotomy was performed under local anaesthesia. A section of two tracheal rings was removed, and a tracheal tube‡ with inflatable cuff was inserted approxi-

* PE-190 Intramedic polyethylene tubing, ID 0·047 in, OD 0·067 in. Clay Adams Co., Parsippany, N.J.

† Linbro Chemical Co., New Haven, Conn.

‡ Magill's endotracheal tubes $1\frac{1}{8}$ in. OD × $\frac{7}{8}$ in. ID Arnold-Nasco Ltd., Guelph, Ontario, Canada.

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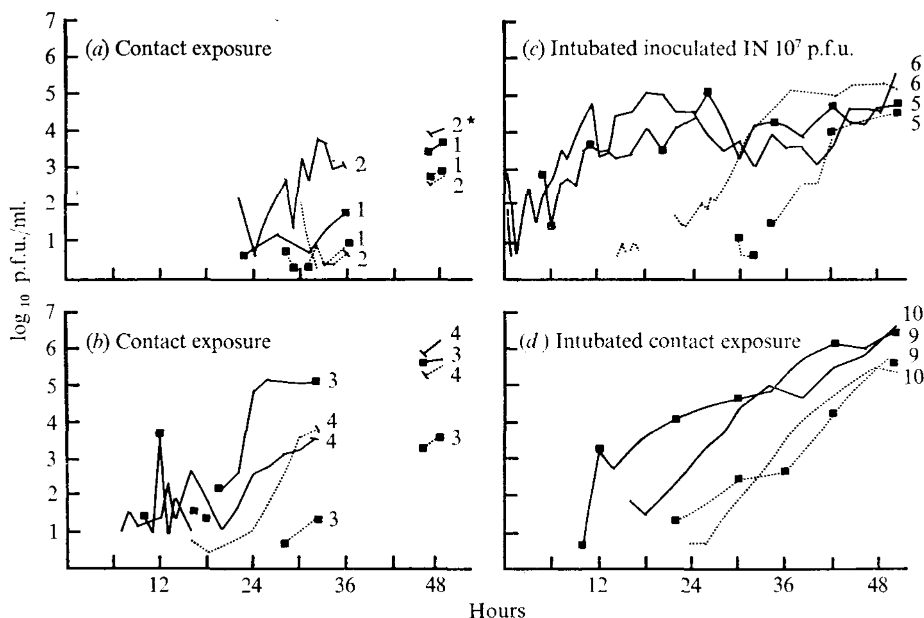

Fig. 1. Virus titre of blood (······) and oesophageal-pharyngeal fluid (——) of cattle exposed to foot-and-mouth disease virus. IN, intranasal; *, steer number.

mately 40 cm. into the posterior end of the trachea. After inflation of the cuff, the animal breathed through the tube that protruded approximately 25 cm. from the skin incision.

EXPERIMENTS AND RESULTS

Contact exposure

Two pairs of steers were exposed by contact with donor steers that had been inoculated intranasally 48 hr. earlier. The donor for steers 1 and 2 (Fig. 1*a*) did not have fever at the start of the contact but became clinically ill during the first day of contact. The donor for steers 3 and 4 (Fig. 1*b*) had fever without lesions at the start of contact and probably was at its peak of infectivity (Graves, McVicar, Sutmoller & Trautman, 1971). OPF and blood samples were collected every hour from all contact steers until 14 hr. after the start of contact and every 2 hr. up to 34 hr. from steers 1 and 2 and up to 30 hr. from steers 3 and 4. The next samples were taken at 40 hr. The growth curves of the two pairs of steers are presented in Fig. 1. The curves of steers 1 and 2 are clearly different from those of the other pairs; this difference most likely is a reflexion of the differences in infectiousness of the donor steers at the time of exposure.

The curves of steers 2–4 show a series of sharp peaks and valleys preceding the actual growth phase. We do not know whether these peaks are caused by growth and intermittant release of virus in the pharnyx or by accumulation of aerosolized virus trapped in the nasal mucus. Viraemia was found rather early relative to the detection of virus in the OPF. In steer 4, positive blood samples were obtained at 7 and 8 hr., which is much sooner than the time observed after intranasal inoculation (McVicar & Sutmoller, 1976).

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Tracheal intubation

We felt that the most likely route for virus aerosolized by the donor to gain early entry to the circulation would be through the lung. Experiments were designed to test this possibility with cattle in which the upper and lower respiratory tract were separated by means of an inflated cuff of a trachea tube. The animals breathed through the tube and thus minimized infection of the pharyngeal area by contaminated air. Because base line information was available for intranasally inoculated cattle (McVicar & Sutmoller, 1976), this route was chosen to assess the influence of the operation and procedure on the virus growth and viraemia.

Steers 5 and 6 (Fig. 1*c*) were intubated and inoculated intranasally with 10^7 p.f.u. of virus. OPF was collected at 20-min. intervals between 0 and 2 hr. after inoculation, every hour until 12 hr. and thereafter every 2 hr. until 48 hr. after inoculation. Blood samples were collected at 30-min. intervals until 32 hr. and at 2-hr. intervals until 48 hr.

The growth curves of steers 5 and 6 presented in Fig. 1*c* cannot be classified as typical of either a high or a low growth rate (McVicar & Sutmoller, 1976), as described earlier for intranasally inoculated cattle. However, because of a slightly retarded development of clinical disease, the growth rates probably were low. An impeded pharyngeal clearance due to the intubation could have resulted in somewhat elevated titres of the OPF.

The viraemia patterns of steers 5 and 6 were very similar to those observed in nontracheotomized, intranasally inoculated steers (McVicar & Sutmoller, 1976). Thus, tracheal intubation did not substantially alter the virus growth pattern in the pharyngeal region, viraemia, or clinical response. The results substantiate the earlier contention (Sutmoller, 1971) that during local multiplication in the pharynx, virus drains via the lymphatics to the bloodstream. The present experiment also confirmed earlier observations (McVicar & Sutmoller, 1976) that after intranasal inoculation, a low-level viraemia exists before the actual rise of virus titre of the blood. Virus titres of the OPF of steer 5 were decreasing during the rising virus titre of the blood, probably indicating release of virus from other replication sites.

Tracheal intubation, aerosol exposure

Introductory to an experiment with contact exposure, steers 7 and 8 were intubated and infected by aerosol. Five ml. of virus suspension containing 10^8 p.f.u. was aerosolized with a glass nebulizer* and blown through a rubber tube inserted into the trachea tube to the tracheal bifurcation. Blood samples were collected at 5-min. intervals for 30 min. From 30 min. to 8 hr. after inoculation, blood and OPF samples were collected at 30-min. intervals.

The 30-min. blood sample and the 6- and 6·5 hr. OPF samples of steer 8 contained trace amounts of virus. Unfortunately, samples were not obtained from 8 to 20 hr. after inoculation; but when sampling was resumed, both steers had rising virus titres of the blood. Steer 8 also had rising virus titres of the OPF at this time, but the OPF of steer 7 already contained high concentrations of virus.

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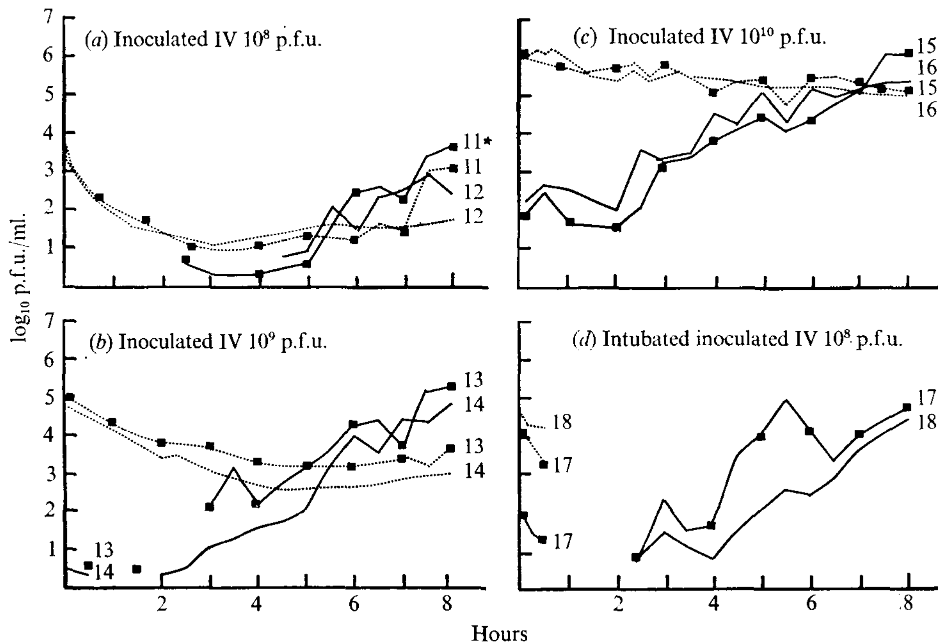

Fig. 2. Virus titre of blood (------) and oesophageal-pharyngeal fluid (——) of cattle inoculated intravenously with foot-and-mouth disease virus. IV, intravenous; *, Steer number.

Even though data on these steers are fragmentary, the treatment apparently produced pharyngeal growth patterns and viraemia comparable with those seen after contact exposure of intact cattle.

Tracheal intubation, contact exposure

Steers 9 and 10 (Fig. 1*d*) were intubated and exposed by placing them in contact with an infected donor steer that had fever but no lesions and thus was comparable with the donor of steers 3 and 4 (Fig. 1*b*). OPF and blood samples were collected from steers 9 and 10 at hourly intervals up to 12 hr. and at 2-hr. intervals up to 48 hr. The early part of the OPF curves of steers 9 and 10 differ from those of steers 3 and 4 (Fig. 1*b*) because of the absence of peaks and valleys in the virus titres. This observation strengthens the suggestion that the peaks and valleys seen in steers 3 and 4 were caused by virus trapped in the mucus of the upper respiratory tract. The rest of the curves of steers 9 and 10 are quite similar to those of steers 1–4. Thus, bypassing the upper respiratory tract does not substantially change the growth pattern in the pharynx nor the viraemia in animals exposed to FMDV by contact with an infected donor animal.

Intravenous inoculation

The previous experiments suggested that during contact exposure, virus entered the circulation via the lung. This virus in the bloodstream then efficiently seeded not only the pharyngeal area, but also other sites that subsequently released sufficient virus in the circulating system to cause the rising titre of the blood.

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The next series of experiments was designed to study the haematogenic infection of the pharyngeal area, as well as the ensuing growth pattern in that area and the course of the viraemia.

Steers 11–16 (Figs. 2*a–c*) were inoculated intravenously with FMD virus in doses ranging from 10^8 to 10^{10} p.f.u. Blood samples were collected at 10-min. intervals for the first 90 min. and then every 30 min. until 8 hr. after inoculation. The clearance of FMD virus from the circulation of these steers during the first 90 min. will be discussed elsewhere (Sutmoller & McVicar, 1976).

At 3·5–4 hr. after inoculation, the viraemia curves of steers 11 and 12 given 10^8 p.f.u. showed an upward trend, which indicates that the input of virus into the circulation exceeded the clearance of virus from the blood. This early upward trend was not observed in the cattle inoculated with higher doses (Figs. 2*b*, *c*), but the residual virus in the circulation probably obscured the input of lower doses of virus.

The OPF of steers 13–16 (Figs. 2*b*, *c*), inoculated with the higher doses, contained virus very shortly after the intravenous inoculation and before virus growth in the pharynx is to be expected. The virus titre of the OPF of steers 15 and 16 given 10^{10} p.f.u. increased during the first 30 min., indicating that during that period virus from the circulation entered the pharyngeal cavity in amounts that exceeded the clearance of the virus from that area. At 2·5–3 hr. after inoculation, the OPF titres of these animals increased. This increase indicated that the input of FMD virus in the pharyngeal cavity again exceeded the clearance and that the virus had multiplied.

The first indication of growth of virus in the pharyngeal area after the intravenous inoculation of 10^8 p.f.u. was observed at 2·5 hr. for steer 11 and at 4·5 hr. for steer 12.

The data from these two steers show that feedback of virus progeny into the bloodstream may precede virus growth in the pharyngeal area and thus that the rise of virus titres in the blood is caused by the release of virus from as yet unknown replication sites and not primarily by input of pharyngeal virus.

Trachael intubation, intravenous inoculation

Virus in the pharyngeal cavity shortly after intravenous inoculation or at a later stage of the infectious process may have come directly from the circulation into the pharynx. Another possibility is that the virus was aerosolized in the lung and trapped in the pharyngeal mucus at exhalation. Steers 17 and 18 (Fig. 2*d*) were intubated and inoculated intravenously with 10^8 p.f.u. of FMD virus to test these possibilities.

Virus titres of blood collected during the first 30 min. were slightly above the expected range. The OPF of steer 17 contained relatively large amounts of FMD virus shortly after inoculation. In both steers, the first detectable virus growth in the pharynx was between 2·5 and 3 hr. after inoculation. The virus titres decreased sharply between 3 and 4 hr., but the start of a second growth peak was observed between 4 and 4·5 hr. Thereafter virus input varied similarly to that of steers 11–16. The general growth patterns were much like those of steers 13 and 14

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inoculated intravenously with 10^9 p.f.u. (Fig. 2*b*). Thus, bypassing the upper respiratory tract does not interfere with the virus growth in the pharynx after intravenous inoculation. The elevated titres of the OPF shortly after inoculation and during the growth phase may have been caused by an interference with the normal pharyngeal clearance mechanism due to the intubation. This experiment further confirms that after intravenous inoculation, the infection of the pharynx is haematogenic.

DISCUSSION

In a companion paper (McVicar & Sutmoller, 1976), we reported on the growth of FMD virus in the pharyngeal area of intranasally exposed cattle. The present studies show that the pharyngeal area also can be readily infected via the bloodstream. This same finding was reported by Burrows *et al.* (1971), who detected FMD virus in the pharynx within hours after introduction of the virus into the udder through the teat canal.

The sequence of virus growth in the pharynx and development of viraemia after intranasal inoculation was quite different after aerosol or contact exposure. With intranasal infection virus growth in the pharynx preceded viraemia by several hours; whereas, in animals exposed by aerosol or contact, virus growth in pharynx and a rise in virus blood titres were practically simultaneous. Aerosol exposure and contact transmission experiments showed that virus entered the bloodstream via the lung and that this type of entry gave rise to an early low-level viraemia.

The experiments further showed that once virus entered the blood, it was transported to replication sites other than the pharynx. The release of virus from those as yet unknown replication sites and not the virus growth in the pharynx caused the rising blood virus titres. Earlier we presented a working hypothesis for the pathogenesis of FMD (Sutmoller, 1971) in which the upper respiratory tract was considered as the portal of entry for the virus. The present results require revision of this hypothesis to include the role of the lung, particularly because a substantial amount of evidence has established the importance of airborne transmission of FMD (Donaldson, Herniman, Parker & Sellers, 1970; Hyslop, 1965; Sellers & Parker, 1969, Sellers, Donaldson & Herniman, 1970; Sellers *et al.* 1973). FMD-infected animals generate aerosols with various droplet sizes (Sellers & Parker, 1969), and recipient animals will be exposed to all of them. Depending on the droplet size of the aerosol, this virus may be trapped in the upper or in the lower respiratory tract. However, for the sake of simplicity, each area will be considered separately.

Upper respiratory tract

Some factors have been indicated or identified as possible determinants for the course of the local infection in the nasopharynx. These include the concentration of virus in the air or of initial infective dose (McVicar & Sutmoller, 1976), other resident viruses (Graves, McVicar, Sutmoller, Trautman & Wagner, 1971; Sutmoller, Graves & McVicar, 1970), the proportion of defective interfering particles or of variants in the viral population (Sutmoller, in preparation); Sutmoller & McVicar, 1972), and local antibody (McVicar & Sutmoller, 1974). Any one or

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combination of these factors could determine whether the infection would proliferate. Once the infection is established, virus would drain to the regional lymph nodes where much of it would be eliminated. However, failure of this filtration system would allow virus to enter the circulation. Blood clearance of such small amounts of virus has been shown to be very efficient (Sutmoller & McVicar, 1976), supposedly by macrophages of the reticuloendothelial system (Mims, 1964). However, circulating virus or virus transported by phagocytic macrophages (Mims, 1964) could infect target areas. Replication and massive release of virus from such additional areas would cause rising virus titres of the blood.

Lower respiratory tract

Virus penetrating deeply into the lung would immediately enter the bloodstream by means of phagocytic alveolar macrophages returning to the circulation (Mims, 1964). Non-phagocytosed virus would be cleared similarly to virus draining from the pharyngeal lymph nodes. In any case, some of the virus from the lung would reach target areas soon after inhalation. In non-immunized animals, the infection via the lung would circumvent many hurdles that the virus would encounter in the upper respiratory tract.

Thus, both after upper respiratory tract infection and after virus passing through the lung, a low-level viraemia would establish foci of infection in one or more target areas. Virus replication in these foci would result in a feed-back of large amounts of virus into the bloodstream and high virus titres of the blood for 2–3 days. The early low-level viraemia usually goes unrecognized but appears to be more significant for the disease process than the late stage with high virus titres. The target areas that cause the feedback could be the lung (Eskilden, 1969) but more likely are the germinative layers of the skin or mucus membranes (Gailiunas & Cottral, 1966; Korn, 1957; Seibold, 1963). In this connexion, it is of interest to recall some early research of British workers (reviewed by Skinner & Knight, 1964) demonstrating that FMD skin lession could be stimulated by a low degree of local trauma.

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REFERENCES

BURROWS, R., MANN, J. A., GREIG, A., CHAPMEN, W. G. & GOODRIDGE, D. (1971). The growth and persistence of foot-and-mouth disease virus in the bovine mammary gland. *Journal of Hygiene* **69**, 307.

CALLIS, J. J. & COTTRAL, G. E. (1968). Methods for containment of animal pathogens at the Plum Island Animal Disease Laboratory. *Methods in Virology* (Ed. K. Maramorosch and H. Koprowski), p. 465, Academic Press.

DONALDSON, A. I., HERNIMAN, K. A. J., PARKER, J. & SELLERS, R. F. (1970). Further investigations on the airborne excretion of foot-and-mouth disease virus. *Journal of Hygiene* **68**, 557.

ESKILDSEN, M. K. (1968). Experimental pulmonary foot-and-mouth disease infection of cattle. *Presented: European Commission for the Control of Foot-and-Mouth Disease. Report of the Meeting of the Research Group of the Standing Technical Committee, State Veterinary Institute for Virus Research,* 93 Rome, Food and Agriculture Organization of the United Nations, 124 (1969).

https://doi.org/10.1017/S0022172400024669 Published online by Cambridge University Press

GAILIUNAS, P. & COTTRAL, G. E. (1966). Presence and persistence of foot-and-mouth disease virus in bovine skin. *Journal of Bacteriology* **91**, 2333.

GRAVES, J. H., MCVICAR, J. W., SUTMOLLER, P. & TRAUTMAN, R. (1971). Contact transmission of foot-and-mouth disease from infected to susceptible cattle. *Journal of Infectious Diseases* **123**, 386.

GRAVES, J. H., MCVICAR, J. W., SUTMOLLER, P., TRAUTMAN, R. & WAGNER, G. G. (1971). Latent viral infection in transmission of foot-and-mouth disease by contact between infected and susceptible cattle. *Journal of Infectious Diseases* **124**, 270.

HYSLOP, N. St G. (1965). Airborne infection with the virus of foot-and-mouth disease. *Journal of Comparative Pathology* **75**, 119.

KORN, G. (1957). Experimentelle Untersuchungen zum Virusnachweis im Inkubationsstadium der Maul – und Klauenseuche und zu ihrer Pathogenese. *Archiv für experimentelle Veterinaermedizin* **11**, 637.

MCVICAR, J. W., GRAVES, J. H. & SUTMOLLER, P. (1970). The growth of foot-and-mouth disease virus in the bovine pharynx. *United States Animal Health Association Proceedings* **74**, 230.

MCVICAR, J. W. & SUTMOLLER, P. (1974). Neutralizing activity in the serum and oesophageal-pharyngeal fluid of cattle after exposure to foot-and-mouth disease virus and subsequent re-exposure. *Archiv für die gesamte Virusforschung* **44**, 173.

MCVICAR, J. W. & SUTMOLLER, P. (1976). The growth of foot-and-mouth disease virus in the upper respiratory tract of non-immunized, vaccinated, and recovered cattle after intranasal inoculation. *Journal of Hygiene* **76**, 467.

MCVICAR, J. W., SUTMOLLER, P. & ANDERSEN, A. A. (1974). Foot-and-mouth disease virus: Plaque reduction neutralization test. *Archiv für die gesamte Virusforschung* **44**, 168.

MIMS, C. A. (1964). Aspects of the pathogenesis of virus diseases. *Bacteriological Reviews* **28**, 30.

SEIBOLD, H. R. (1963). A revised concept of the lingual lesions in cattle with foot-and-mouth disease. *American Journal of Veterinary Research* **24**, 1123.

SELLERS, R. F., BARLOW, D. F., DONALDSON, A. I., HERNIMAN, K. A. J. & PARKER, J. (1973). Foot-and-mouth disease: A case study of airborne disease. In: *International Symposium of Aerobiology* IV, 405, Utrecht: Oosthoek Publishing Company.

SELLERS, R. F., DONALDSON, A. I. & HERNIMAN, K. A. J. (1970). Inhalation, persistence, and dispersal of foot-and-mouth disease virus by man. *Journal of Hygiene* **68**, 565.

SELLERS, R. F. & PARKER, J. (1969). Airborne excretion of foot-and-mouth disease virus. *Journal of Hygiene* **67**, 671.

SKINNER, H. H. & KNIGHT, E. H. (1964). Environmental factors influencing the response of guinea-pigs to modified strains of foot-and-mouth disease virus. *Bulletin of the Office International des Epizooties*. **61**, 1523.

SUTMOLLER, P. (1971). Persistent foot-and-mouth disease virus infections. In: *International Symposium on Medical and Applied Virology*, III. Ft. Lauderdale, Fla. 1970; *Viruses Affecting Man and Animals;* 295, compiled and edited by Murray Sanders, and Morris Schaeffer. St. Louis, Missouri, Green, xvii, 459.

SUTMOLLER, P., GRAVES, J. H. & MCVICAR, J. W. (1970). Influence of enterovirus on foot-and-mouth disease virus infection. A hypothesis. *United States Animal Health Association Proceedings* **74**, 235.

SUTMOLLER, P. & MCVICAR, J. W. (1972). Three variants of foot-and-mouth disease virus, type O: III. Exposure of cattle. *American Journal of Veterinary Research* **33**, 1641.

SUTMOLLER, P. & MCVICAR, J. W. (1976). Pathogenesis of foot-and-mouth disease: clearance of virus from the circulation of cattle and goats during experimental viraemia. *Journal of Hygiene*, **77**, 245.

https://doi.org/10.1017/S0022172400024669 Published online by Cambridge University Press