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Pathogenesis of foot-and-mouth disease: clearance of the virus from the circulation of cattle and goats during experimental viraemia

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SUMMARY

Viraemia is an important aspect of the pathogenesis of infectious diseases, but the mechanisms of entry and removal of virus from the vascular system particularly in natural hosts are poorly understood. The results of this study showed that the clearance of foot and mouth disease virus (FMDV) from the circulation of cattle and goats followed the general rules for the clearance of inert particulate materials and other viruses from the circulation. High doses of infused FMDV were cleared less efficiently than low doses, probably as a result of a depletion of the reticulo-endothelial system by the higher doses. FMDV was cleared from the circulation of cattle at a considerably slower rate than from the circulation of goats, but in both species significant individual variation in clearance was observed. These results could explain individual as well as species variations relative to the onset and duration of viraemia.

INTRODUCTION

Foot and mouth disease virus (FMDV) replicates in the upper respiratory tract of susceptible ruminants and pigs without producing gross lesions (McVicar, Graves & Sutmoller, 1970). This growth is usually followed by a period of viraemia and generalized infection, with vesicle formation on the oral mucosa, interdigital skin, or the udder.

A number of reports describe the onset and duration of viraemia and virus titres in the blood relative to the exposure dose, virus strain, or route of exposure (Cottral & Bachrach, 1969). The bloodstream, however, not only provides virus with a means of reaching tissues distant from the site of initial growth, but also exposes it to adverse conditions such as antibodies and phagocytic macrophages

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(Mims, 1964). Thus, these undoubtedly are important factors influencing the course of a disease.

The experiments reported in this paper are a study of the clearance of FMDV from the circulation of cattle and goats after intravenous infusion of the virus.

Clearance of the virus from the bloodstream of mice has been studied for Rift Valley fever virus (Mims, 1956*a*, *b*), ectromelia virus (Mims, 1959), vesicular stomatitis virus, and Newcastle disease virus (Brunner, Hurez, McCluskey & Benacerraf, 1960). The most common method to study clearance is to inject known amounts of virus into the circulation and measure its removal as a function of time. Schultz & Neva (1965) found a blood clearance of $10^{1.8}$–$10^{2.6}$ TCID 50 of poliovirus during a 2-hr. period after intravenous injection. Nathanson & Harrington (1966) used repeated intravenous infusions to study the clearance of Langat virus in monkeys. The virus was given at a constant rate and they calculated its mean survival as circulating virus particles by determining the steady state of passive viraemia or that level of infusion that equalled removal. Results of the studies in monkeys and in mice suggested that virus, similar to inert particulate matter, was cleared from the blood by the reticulo-endothelial system (RES) and, in particular, by the Kupffer cells in the liver. Larger particles were removed more efficiently than small particles. Below a certain critical dose, the efficiency of clearance was great, and the clearance rate was directly proportional to the concentration of virus in the blood. Above that critical dose, the clearance of particulate matter of a uniform size was an exponential function of time, and the clearance rate was inversely proportional to the dose rather than to the concentration of virus in the blood (Benacerraf *et al.* 1957; Biozzi, Benacerraf & Halpern, 1953). Some researchers suggested that the critical dose was determined by the availability of phagocytic macrophages or specific or non-specific opsonins or a combination of these factors (Benacerraf *et al.*, 1957; Jenkin & Rowley, 1961).

MATERIALS AND METHODS

Animals

Grade Hereford cattle, approximately 18 months old, weighing 250–300 kg. were used. Goats of mixed breeding, 1–2 years old, weighing 25–30 kg., were used.

Virus

FMDV, subtype O_1, originated from infectious tongue epithelium from cattle with FMD in Argentina. Six passages were made in primary cultures of bovine kidney (BK) cells grown in 4-oz. prescription bottles. Stock virus of one additional passage was made in secondary BK cell cultures grown in 2-l. Baxter rolling bottles. The infectious culture fluid was clarified by centrifugation and stored at $-20°$ C., thawed 24 hr. before use, and then kept at 4° C. The plaque titre of this stock virus in secondary cultures of BK cells was 10^8 plaque-forming units/ml. (p.f.u./ml.).

Inactivated virus was prepared from this stock virus by addition of acetylethyleneimine and incubation at 37° C. for 72 hr. as described by Graves McKercher, Farris & Cowan (1968).

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Inoculation and sampling

Polyethylene catheters* placed in the jugular veins were used for virus infusions and for the collection of blood samples. The 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. disposable plastic syringe filled with phosphate-buffered saline (PBS) was attached to the fitting. The syringe was then clamped to the halter of the animal. For virus infusions a catheter was inserted approximately 50 cm. into the vein of cattle and 20 cm. into the vein of goats. The tube was flushed with PBS before and after each infusion. Blood samples were drawn from a similar catheter in the opposite jugular vein. A new syringe was used for each sample, and the catheter was flushed with PBS before a sample was taken.

Blood samples were collected, from animals given a single dose of virus, every 2 min. for 10 min. after the infusion, every 5 min. from 10–30 min. and at 10-min. intervals from 30–90 min. By 90 min. newly formed virus could be released (McVicar, *et al.* 1970). The inoculation or the sampling did not appear to interfere with the normal physiological activities of the experimental animals.

Virus assay

The virus content of blood samples was determined by placing 0·1 ml. of dilutions on 5- to 6-day old cultures of secondary BK cells grown in 35-mm. dishes of disposable plates.† Six cultures were used per dilution. After 1 hr. at 37° C. in a 3 % CO_2 atmosphere, the cells were overlayered with 0·6 % gum tragacanth in Hanks' balanced salt solution containing 0·5 % lactalbumin hydrolysate and 4 % horse serum (Mirchamsy & Rapp, 1968). After a further 24-hr. incubation, the plates were stained by submerging them for 20 min. in a 20 % formalin solution containing 1/2000 crystal violet. Plaques were counted after the plates were rinsed and dried.

RESULTS

Cattle: single infusion of virus

Ten cattle were given single intravenous infusions of FMDV, ranging in amount from 10^7 to 10^{10} p.f.u. to correlate blood clearance with the dose of virus (Fig. 1). The virus titres in the blood were similar for each of the doses. Steers A and B which received 10^{10} p.f.u. had blood virus titres of 10^6 p.f.u./ml. by 2 min. after the termination of the infusion. The mean clearance was 0·005 log p.f.u./min. The blood samples from steer C and D that had received 10^9 p.f.u. had titres of $10^{4·9}$ and $10^{4·6}$ p.f.u./ml. in blood samples taken at the same time. The mean clearance was 0·008 log p.f.u./min. The virus titres of the blood of steers E, F, and G at 2 min. after infusion and which had received 10^8 p.f.u. ranged between $10^{3·3}$ and $10^{3·6}$ p.f.u./ml. In these three animals 90 % of the virus was removed from the circulation by 30 min. From 30 to 90 min. the mean clearance was 0·012 log p.f.u./min.

* PE-190 Intramedic polyethylene tubing, ID 0·047 in., OD 0·067 in. (7436) Clay Adams Co., Inc., Parsippany, N.J. 07054.

† Linbro multi dishes 35-mm. FB-6-TC. Linbro Chemical Co., New Haven, Ct. 06511.

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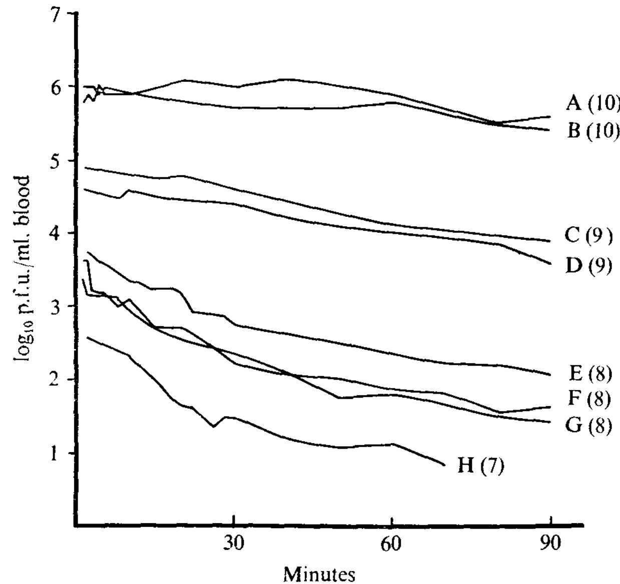

Fig. 1. Virus clearance from the circulation of cattle after single doses of foot-and-mouth disease virus. Steers are identified by letters. The dose ($\log_{10}$ p.f.u.) of virus given is in parentheses.

Steer H infused with 10^7 p.f.u. also had an initial high rate of removal of virus from the blood followed by slower clearance after 30 min. However, 2 other steers (I and J, Fig. 2), given this same amount of virus did not have this initial high rate. The total clearance was 0·01 log p.f.u./min. which was similar to that of the 30–60 min. period of steers E, F, and G.

Cattle: single infusion of virus following an infusion of inactivated virus

Steers K and L (Fig. 3) were given an intravenous infusion of chemically inactivated virus to explore the possibility of depletion of the RES by large doses of virus. They were given the equivalent of a total of 10^{10} p.f.u. and 10^9 p.f.u. of live virus, respectively. Immediately following these infusions, each animal was given 10^8 p.f.u. of live virus intravenously. The results of the virus clearance are plotted in Fig. 3. Three significant observations are: (1) the initial virus titre of live virus in the blood was higher than that of cattle given 10^8 p.f.u. without prior infusion of inactivated virus; (2) there was no initial fast clearance; and (3) the clearance was similar to those of the cattle infused with 10^9 and 10^{10} p.f.u. of live virus without prior inactivated virus treatment.

Goats: single infusion of virus

Goats were infused with a single dose of 10^7, 10^8, or 10^9 p.f.u. of FMDV. Two goats were used for each dose. Because the body weight of the goats was about 1/10 of that of the cattle, we hypothesized that these virus doses would produce passive viraemia of the same order as the 10^8, 10^9 and 10^{10} p.f.u. per dose did in cattle. The results shown in Fig. 4 support this; however, a much faster clearance

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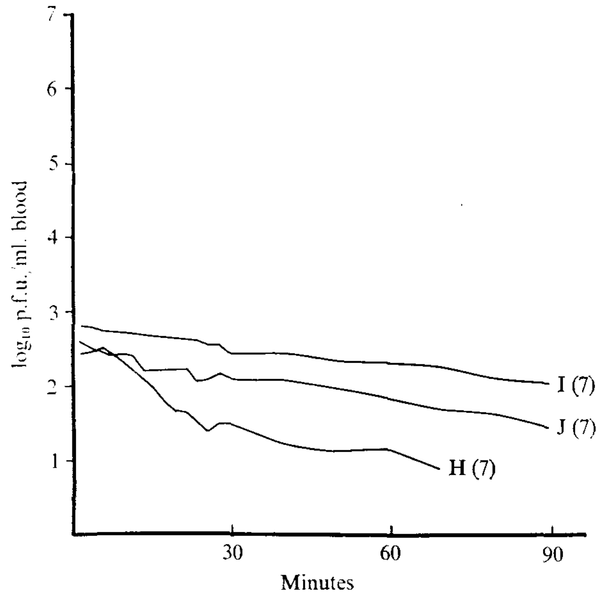

Fig. 2. Virus clearance from the circulation of cattle after single doses of 10^7 p.f.u. of foot-and-mouth disease virus. Steers are identified by letters. The dose of virus ($\log_{10}$ p.f.u.) given is in parentheses.

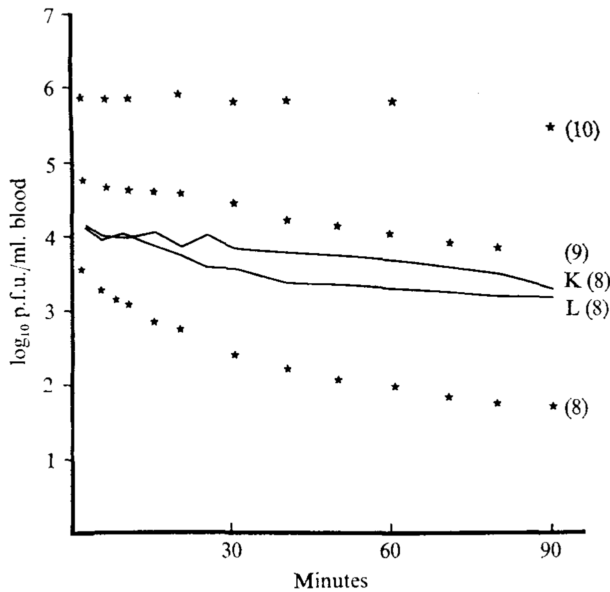

Fig. 3. Virus clearance from the circulation of cattle after single doses of inactivated foot and mouth disease virus followed by 10^8 p.f.u. of live virus. Steers are identified by a letter. The dose of live virus ($\log_{10}$ p.f.u.) given is in parentheses. The expected curves (*) are taken from the data for Fig. 1.

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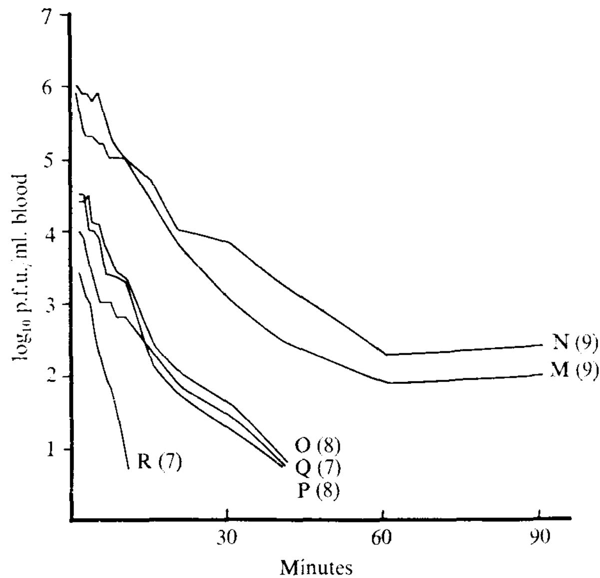

Fig. 4. Virus clearance from the circulation of goats after single doses of foot and mouth disease virus. Goats are identified by a letter. The dose (log_{10} p.f.u.) of virus given is in parentheses.

was observed than in cattle and only goats M and N, infused with the highest dose of virus, were viraemic beyond 30 min. Goat Q, infused with the lowest dose, cleared the virus much more slowly than goat R, similarly treated.

Cattle: multiple infusions of virus

Cattle were given infusions of FMDV, ranging from 10^6 to 10^9 p.f.u. every 10 min. to investigate whether a steady-state passive viraemia could be obtained and to study the eventual depletion of the RES by such repeated infusions.

Blood samples were collected every 2 min. for 20 min. and immediately before any viral infusions. Additional 2-min. blood samples were collected from steers S, T, V, and X, as indicated in Fig. 5. The passive viraemia reached a steady state sooner with high doses of virus than with lower doses, and virus infused later was cleared less efficiently than virus infused earlier. Steer V repeatedly infused with 10^7 p.f.u. of virus cleared the virus substantially slower than steer W, similarly treated.

DISCUSSION

In these studies, plaque assay was used for measurement of virus. For FMDV, the virus particle to p.f.u. ratio ranges from 10^2 to $10^{3 \cdot 4}$ (Bachrach, 1968). Thus, the plaque titres represent only a relatively small part of the total virus mass. Some of the viral infectivity may have been reduced by thermal inactivation during the experiments, but, *in vitro*, FMDV in whole heparinized blood is quite stable when held at 37° C. for 90 min. Therefore, as with other virus systems used in studies of this kind (Brunner *et al.* 1960; Nathanson & Harrington, 1966) this portion of clearance was not considered to be of importance for the results of this study.

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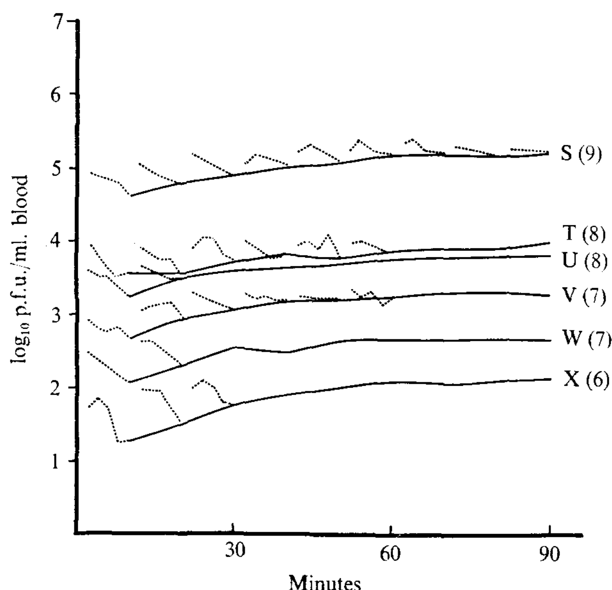

Fig. 5. Viraemia titres of cattle given repeated doses of foot and mouth disease virus. Steers are identified by letters. The dose of virus ($\log_{10}$ p.f.u.) given 9 times at 10-min. intervals is in parentheses. Dotted lines show the results of blood samples taken at 2-min. intervals after each virus infusion; solid lines show the results of the blood samples immediately before the next virus infusion.

Heparinized blood was tested for simplicity because no differences could be found on a volume basis between whole blood, serum or plasma.

The dynamics of clearance of virus from the blood is best visualized in the curves of steers E, F, G, and H (Fig. 1) given the lower virus doses. Clearance during the first 20–30 min. was fairly fast but then became slower. During the early clearance period, the RES probably was depleted or saturated and, from that time on, the more gradual exponential clearance phase ensued. Brunner *et al.* (1960) in their study of the kinetics of blood clearance of vesicular stomatitis virus and Newcastle disease virus in mice found that these viruses were phagocytosed by the Kupffer cells in the liver with a high degree of efficiency. They also found that depletion of the RES by thorotrast slowed the clearance of the virus from the blood, whereas the presence of specific antibody significantly accelerated the clearance of virus. Studies by Murray (1962) and Jenkin & Rowley (1961) suggest that this so-called RES blockade is due to the depletion of serum factors (opsonins) and not to saturation of phagocytic cells.

There are two likely reasons why the early rapid clearance rate was not seen in steers A, B, C, and D (Fig. 1). First, there was sufficient virus available to deplete or saturate the RES before the 2-min. sample was taken; secondly, the amount of virus removed was insignificant in relation to the total amount of virus present. Therefore, in these steers, the virus titre of the blood sample at 2 min. for all practical purposes might have been due to dilution of the virus in the total blood volume. This 'dilution' factor was about 10^{-4} for cattle and 10^{-3} for goats; these

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values agreed with the values given for blood volumes (Swenson, 1970). The starting virus titres in the blood of animals given lower doses of virus were lower than would be expected from dilution alone, probably because the amount of virus removed before depletion or saturation of the RES caused a measurable decrease in titre even before the 2-min. samples were collected.

When steers were infused with high doses of inactivated virus immediately before infusion of a low dose of virus there was no early fast clearance and the titres of the virus in the blood at 2 min. could be accounted for by dilution alone indicating that the RES had been depleted or saturated by the infusion of inactivated virus. Thus, as with other viruses (Brunner *et al.* 1960; Mims, 1956*b*) two dose values can be distinguished: (1) a lower, when clearance rates are high and independent of the dose; and (2) a higher, where the RES quickly becomes saturated and the clearance rates are inversely proportional to the dose of virus given. It can only be surmised that the RES of the steers I and J, which did not show early fast clearance after an infusion of 10^7 p.f.u. of virus, must have been depleted or saturated at the time of infusion.

The influence of the clearance rate on steady-state viraemia was illustrated by steers V and W (Fig. 5) which were given multiple infusion of 10^7 p.f.u. The virus titre in the blood of steer V at 2 min. ($10^{3\cdot0}$ p.f.u./ml.) could be due to dilution alone as a result of prior depletion or saturation of the RES. In this steer, the clearance during the 10-min. interval between the first and the second infusion was 0·03 log p.f.u./min. The virus titre in the blood of steer W at 2 min. was $10^{2\cdot4}$ p.f.u./ml. Steer W, therefore, showed early fast clearance before the 2-min. blood sample was taken. The initial clearance was 0·06 log p.f.u./min. which is twice as fast as that in steer V. We can see that differences in clearance rates of animals receiving the same amounts of virus may result in quite different degrees of steady-state viraemia. Individual variation in clearance rates would be of considerable importance for the onset and degree of viraemia in the natural disease.

The present studies indicate that once FMDV stops entering the circulation, viraemia would end as a result of clearance in less than 10 hr. The appearance of antibody with the formation of larger virus-antibody complexes would speed up clearance and thus lower the viraemia titres even faster. If the depletion of opsonins is the main factor responsible for the depletion of RES (Jenkin & Rowley, 1961; Murray, 1962), a stimulation of the release of opsonins would also increase the clearance and thus lower the titre and persistence of the viraemia.

Interestingly, clearance of FMDV from the circulation of goats was found to be much faster than that from cattle. It was hoped that goats would provide a cheaper and easier to handle experimental animal but the results show that generalization of conclusions from goats to cattle must be done cautiously even if both species are natural hosts of the virus.

Cattle with FMD almost invariably have viraemia for 3–5 days after infection. The blood virus titre is usually between 10^4 and 10^5 p.f.u./ml. for several days (Cottral & Bachrach, 1969). Goats with FMD may have viraemia for a period that ranges from a short time with low virus titres to as long as 5 days. In one study, circulating virus could not be demonstrated in 8 % of infected goats (McVicar &

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Sutmoller, 1972). Such differences between viraemia in cattle and in goats may be a result of the relatively faster virus clearance in goats.

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