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THE INHIBITORY ACTIVITY OF SECRETIONS IN CATTLE AGAINST FOOT AND MOUTH DISEASE VIRUS

Thesis presented for the Degree of Doctor of Philosophy

by

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ABSTRACT

Natural and induced factors inhibiting foot and mouth disease virus were investigated in bovine secretions, especially in those from the upper respiratory and oro pharyngeal areas. Techniques were devised to collect lachrymal, nasal, buccal and pharyngeal fluids from normal, convalescent and passively or actively immunised steers.

The pH and total protein content of secretions were established in normal cattle. Immunoglobulin types IgA and IgG_1 predominated. Interferon was not detected. Normal tears exhibited no antiviral activity but nasal secretion, oral saliva and pharyngeal fluid were inhibitory due to their alkaline pH and, in the case of salivary fluids, to the presence of an additional anti viral factor which was partially characterised. Virus lost infectivity *in vitro* due to natural, non specific factors at rates which varied with the strain of virus to a maximum of 1.25 log units per hour.

Clinical disease, viral excretion, interferon and antibody were studied following infection with virus of types O, A and C. Interferon was detected for up to 4 days at the period of maximum viral excretion. Secretory neutralising antibody developed 3 - 5 days after exposure, reached a peak within 21 - 28 days and was associated principally with IgA and IgG_1. Serum levels were consistently greater than those in secretions. Passive immunisation studies showed that some secretory antibody was derived from serum. Antibody persisted for at least $5\frac{1}{2}$ years after infection.

Secretory antibody levels increased with successive subcutaneous doses of inactivated vaccine. A single dose elicited good humoral but

poor secretory responses. Levels in serum and secretions rose after a second dose and approached convalescent levels after a third. Secretory antibody was principally IgG_1 but after a third vaccination IgA was also detected.

The relation between secretory antibody and the outcome of exposure to infection was studied. In steers vaccinated once or twice and exposed 14 days after the last vaccination, no lesions were observed and little virus was recovered from their secretions excepting pharyngeal fluid. After three vaccinations much less pharyngeal virus was recovered following exposure. Convalescent animals reexposed $5\frac{1}{2}$ years after primary infection were immune and did not become carriers, pharyngeal samples remaining negative. These observations correlated well with the presence of neutralising IgA in the secretions.

OBJECT OF THE RESEARCH

Little is known about the local defence mechanisms of the bovine species against foot and mouth disease (FMD) and an investigation was considered desirable for the following reasons:

1. The main portal of entry of foot and mouth disease virus (FMDV) is thought to be via the infection of superficial epidermal and mucosal surfaces particularly those of the naso and oro pharynx.
2. The clinical lesions of FMD are found mainly in external locations.
3. FMDV is excreted in quantity in secretions and excretions before, during and after the development of clinical lesions.
4. FMDV spreads via the agency of infected secretions which come into contact, directly or indirectly, with susceptible animals.
5. The carrier state is regarded as a normal sequel to exposure to FMDV in cattle and is equally readily established in fully susceptible animals or in vaccinated animals immune to the development of clinical lesions. Virus is perpetuated by multiplication in the superficial tissues of the oro and naso pharynx and is recoverable in secretions from this area for months or years after infection.
6. Recent studies on a number of diseases of man and animals have indicated the protective importance of local immunity.

A study of the local defence mechanisms conducted in normal, infected and immunised cattle could therefore be expected to provide fundamental information on the factors which determine host resistance or susceptibility, the pathogenesis of the disease, the maintenance and spread of the disease in nature and the protection of animals against FMD.

3. LAYOUT OF THE THESIS.

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5. LITERATURE REVIEW

5.1. Foot and Mouth Disease

The literature is reviewed with particular reference to cattle under four headings:-

5.1.1. General Information

5.1.2. Pathogenesis of Foot and Mouth Disease

5.1.3. The Carrier State

5.1.4. Host Defences

5.1.1. General Information

Foot and mouth disease is at once a condition of great antiquity and a major problem of modern livestock production. General reviews of the subject have been contributed by Brooksby (1958), Henderson (1960), Shahan (1962), Bachrach (1968) and Hyslop (1970).

The typical clinical disease is acute, febrile and characterised by painful vesiculation of the mouth, feet and udder. The resulting annorexia, lameness and mastitis leads to loss of weight and milk yield. Sequellae may include abortion, sterility, chronic lameness and mastitis and metabolic disorders.

The condition is perhaps the most infectious of all animal diseases and morbidity may attain 100% in susceptible cattle, sheep, goats and pigs. Mortality is generally less than 5% and the importance of the disease lies in the suffering which it causes and the resultant loss of productivity which has been estimated at 25%.

FMD has a world wide distribution with only the Australian, Central

and North American continents remaining free of infection for long periods of time. The striking clinical signs were probably first described by the Italian author Fracastorius in 1546 and the filterable nature of the aetiological agent was demonstrated by Loeffler and Frosch in 1897 when it became the earliest identified animal virus and only the second viral agent to be discovered, Ivanowsky having shown the filterability of Tobacco Mosaic Virus in 1892.

The epizootiological complexity of the disease became somewhat less baffling in 1922 when Vallee and Carre showed the plurality of FMD viruses, a phenomenon hitherto unknown in virology, and by 1954 the disease was known to exist in seven clinically identical but immunologically distinct types. Work on subtypes began with the cross immunity studies of Waldmann and Trautwein (1926) and was extended by the application of serum neutralisation and complement fixation techniques. The World Reference Laboratory for FMD currently recognises some 65 subtypes comprising 11 subtypes of type O, 32 of type A, 5 of type C, 7 of type SAT_1, 3 of type SAT_2, 4 of type SAT_3 and 3 of type $Asia_1$ (Arrowsmith 1974 personal communication.)

Types O, A, and C are most widely prevalent having been found in Europe, South and Central America, Africa and Asia. The distribution of the South African Territories (SAT) and Asia types is broadly as suggested by the names but movement of virus types from endemic to non endemic areas poses a continual threat to livestock as does the emergence of new virus strains. Thus the spread of exotic virus into the Middle East threatened Europe in 1962 when a serious epizootic of type SAT_1 occurred and again in 1973 when type $Asia_1$ appeared in Turkey for the first time. The A_{22} subtype also emerged in this sector in 1965 and spread to become the dominant type A strain in the Middle East and Russia.

The existence of multiple antigenic types, the occurence of antigenic drift and the extreme infectiousness of the agent make control complex and expensive. Nevertheless the development of efficient vaccines and the careful prosecution of prophylactic vaccination schemes has been attended by dramatic reduction in outbreaks in many parts of the world. The scale of the problem is indicated by the fact that over a thousand million doses of vaccine were administered in 1973.

FMDV was one of the first for which estimation of size was made by ultrafiltration (Galloway and Elford 1931) and by ultracentrifugation (Elford and Galloway 1937). Since then a wealth of biophysical and biochemical information has accumulated and the virus has been studied to the level of its component polypeptides and amino acids. These aspects have been extensively reviewed by Brown (1962, 1973) and Bachrach (1968).

FMDV has a naked capsid with icosahedral symmetry measuring 23 ± 2 nanometres and containing 31% single stranded ribonucleic acid with a molecular weight of 2.6×10^6 daltons. These features, together with the fact that the virus is resistant to a variety of organic solvents including ether and that viral synthesis and maturation takes place in the cytoplasm of infected cells, serve to classify the virus as a member of the family Picornaviridae while its acid sensitivity places it in the Rhinovirus genus.

5.1.2. Pathogenesis of FMD

The pathogenesis of FMD has been studied at the level of the cell, the organ and the individual animal.

When virus randomly encounters a susceptible cell the particle attaches to a receptor site and is engulfed. Experiments in tissue culture systems have shown that adsorption and penetration of FMDV are temperature and calcium ion dependent and that, given optimum conditions, the half time of penetration at 37°C is 30 seconds (Thorne and Cartwright 1961, Thorne 1962, Brown et al 1962). The exact mechanism by which FMDV attains the interior of the cell is unknown but studies with poliovirus, a fellow member of the picornavirus froup, have shown that an irreversible eclipse occurs at the surface of susceptible cells (Holland 1962) and that viropexis (Mandel 1962,1967a and b, Dales 1973) or direct penetration of the plasma membrane (Dunnebacke et al 1969) are involved.

Once within the cell the virus eclipses and the redirection of cellular biosynthesis proceeds. In BHK - 21 cells the rate of cellular protein synthesis decreased by 50% within the first half hour of infection and then showed three periods of accelerated activity, the first occurred an hour after infection and was associated with the synthesis of FMDV specific RNA polymerase, the second was of an unidentified nature while the third, at two hours post infection, was associated with the maturation of progeny virus (Brown et al 1966). In calf kidney cells new intracellular virus was detected 100 minutes after infection and the rate of viral synthesis increased logarithmically for the next 50 minutes when virus began to be released from the cell. The rate of production of new virus then decreased but persisted at a lower level for up to 5 hours post infection at which time cell death ensued (Polatnick and Bachrach 1960, Pledger 1961).

The isolated FMDV specific RNA polymerase has been used in the synthesis of RNA in a cell free system and the reaction has been extensively

studied (Arlinghaus et al 1966, Polatnick and Arlinghaus 1967). At least 10 different FMDV specific proteins are synthesised in the infected cell (Ascione et al 1971) only some of which are found in the mature virion. A replicative mechanism has been proposed based on a circular minus strand of viral RNA on which the plus strand grows as a tail being cleaved at various points to yield different molecular species of RNA (Brown and Martin 1965) but the exact mechanism of viral replication remains unknown.

The virus replicates in a wide variety of dedifferentiated tissues in vitro and growth is usually accompanied by the development of typical enterovirus cytopathic effect, the rounding up and shrinkage of refractile cells, pyknosis of nuclei, fragmentation of chromatin, loss of structure and ultimately cell death. Some infected cells may survive and chronically infected cultures have been serially passaged through many generations (Seibold 1966). The Fluorescent antibody technique has been used to show that replication and maturation of virus are restricted to the cytoplasm of infected cells (Mussgay 1958).

In vivo the virus shows a high degree of tissue tropism with predilection for superficial mucosae and epithelia, particularly that of the mouth and pharynx and the coronary band and interdigital space of the foot. Other superficial areas such as the udder (Burrows et al 1971), the teats (Gailiunas et al 1964), the pillars of the rumen (Geiger and Otte 1958) or the traumatised skin of the pig hock may show symptoms and microscopical lesions can be found even in hairy skin of cattle (Gailiunas and Cottral 1966), an area which very rarely exhibits clinical lesions. The virus may also replicate in musculature, especially in immature animals where the damage to cardiac muscle can prove fatal (Potel 1958), and occasionally in parts of the central nervous system - particularly the pituitary body (Scott et al 1965) - where its effects have been

blamed for subsequent endocrine disturbances.

Clinical observations suggest that the distribution and severity of clinical lesions is related to mechanical stress and trauma. This effect was investigated in the guinea pig by Skinner and Knight (1964) who found that foot lesions following intramuscular inoculation of virus were more severe in animals kept on wire grid floors than in animals kept on solid floors with sawdust bedding and that prior injury of the plantar pads was associated with greater lesion severity even in pigs maintained on solid floors. Using the same species Jones (1968) demonstrated that mild mechanical injury of the pinna of one ear inflicted up to 4 days before or 2 days after metatarsal pad inoculation of virus resulted in higher concentrations of virus in the tissues of the damaged ear than in those of the normal control ear. Cottral et al (1965) showed that exposure of the dorsal surface of the bovine tongue to $10^{7.8}ID_{50}$ of FMDV for 7 minutes was not followed by the development of clinical disease whereas lesions did ensue if the epithelium was lightly scratched before exposure to virus. The intact integument can therefore protect a predilection site while any defect in the barrier may predispose to infection.

Platt (1961, 1963a and b) has postulated that phagocytosis is an important factor in determining the penetration of virus into susceptible cells and that the distribution of lesions in FMD is partly attributable to enhanced phagocytosis and cell membrane activity at predilection sites due to continual low grade trauma. In addition, injury leads to increased vascular permeability which might allow the extravasation of virus during the viraemic phase of the disease and to other complex cellular changes (Platt 1966) which affect the epidermal susceptibility to FMDV in ways which are not yet clear. The general increase in

cellular metabolism following injury may provide a favourable milieu for viral infection and multiplication since it is known that trauma is rapidly followed by an increase in DNA synthesis (Hell and Cruikshank 1963), by bursts of mitotic activity (Sullivan and Epstein 1963) and by an increase in RNA and protein synthesis (Baden and Pearlman 1964).

The histopathology of superficial vesicles has been intensively studied by light and electron microscopy in cattle, pigs and guinea pigs (Kablov 1958, Platt 1961, Seibold 1963, Gailiunas et al 1964, Vetterlein 1967, Krasinkov 1968 etc.) as has the damage to cardiac muscle (Potel 1958, Lubke 1960) and the skeletal muscle and pancreas of the mouse (Platt 1956, Krasnikov 1966 etc.). The basic epithelial lesion has been described as 'Balloon degeneration' and it commences with swelling of the nucleus and cytoplasm of infected cells in the stratum spinosum of the epidermis, especially in the prickle cells. Vacuolation, separation, necrosis and disintegration ensue with the liberation of virus and the development of intercellular oedema and granulocytic infiltration. Pathological changes also occur in the endothelial cells of dermal blood vessels and probably account for the leakage of lymph into damaged tissues during the inflamatory process. The separation of tissue and the accumulation of fluid progresses with the formation of macroscopic vesicles. In some areas the degenerative process is of a more necrotic character and epithelia or mucosa may die and strip away without vesiculation.

The primary sites of FMDV infection have not been exhaustively studied but much evidence has accumulated to incriminate the pharyngeal area as an important predilection site in cattle (Burrows 1966a, 1968a and 1972, Burrows et al 1971, Sellers et al 1968b and 1969, Sutmoller 1970, Mohanty and Cottral 1970, McVicar et al 1970 and 1971). There are also indications that the lower respiratory tract constitutes an important

portal of entry (Eskildsen 1969), while local inoculation of the nasal passages (Korn 1957), the udder (Burrows et al 1971) and the foot may also provide routes for infection. A number of experiments have indicated that exposure of the alimentary tract to virus in food and water is a relatively inefficient and variable method of infection for pigs (Henderson and Brooksby 1948, Nathans 1965) or for cattle (Burrows 1972). Terpstra (1972) has shown that pigs may be infected with low doses of aerosolised virus, when primary multiplication occurs in the lung, or with larger doses by the oral instillation of virus when multiplication occurs primarily in the tonsils and cervical lymph nodes. Burrows (1968) has also presented data to support the early involvement of the pharyngeal area in pigs.

The establishment of infection depends upon a number of interrelating variables, one of which is the amount of virus which is required to initiate disease. In FMD this amount may show considerable differences between types and strains of virus. A second variable, the innate resistance of the animal, may also exhibit wide differences between individuals which in turn creates difficulties of a practically insurmountable nature in attempts to establish minimum infective dose levels for any particular species, virus or route. Nevertheless, within these limitations, much useful data has accumulated and Sellers (1971) has prepared an extensive review of the amounts of FMDV required to infect cattle, sheep and pigs by various routes.

In cattle the lowest reported amounts of virus required to initiate infection is $10^{1.0}ID_{50}$ for the intramuscular (Cottral et al 1966) or intranasal (Eskildsen1969) route, $10^{1.4}$ for subcutaneous (Henderson 1952), $10^{1.5}$ for intratracheal (Eskildsen 1969) and $10^{2.0}$ for tonsillar sinus routes (Sutmoller et al 1968). The intraperitoneal route required $10^{2.2}ID_{50}$ (Gailiunas and Cottral 1970), the intravenous route $10^{2.8}$

(Henderson 1952), artificial insemination $10^{3.4}$ (Cottral et al 1968) and retropharyngeal spray $10^{5.0}$ (Mowat and Sellers 1967). The oral administration of $10^{5.8}$ - $10^{6.8}ID_{50}$ resulted in the infection of less than half the animals exposed within 72 hours (Burrows 1972).

Exposure to virus, even in high concentrations, does not necessarily result in the production of the disease. This effect may be explained in part by the existence of virus strains which show a high degree of natural adaptation to a particular species and many workers have reported on strains which were extremely virulent for pigs but avirulent or only mildly virulent for cattle (Andrews et al 1937, Henderson et al 1948, Brooksby 1950, Graves and Cunliffe 1960).

Once infection has been established in superficial areas the disease typically progresses with the development of viraemia, the distribution of virus throughout the body, the onset of pyrexia and the appearance of generalised disease. There have been many studies of the disease process in animals but an important distinction must be made between experiments performed before and after 1968. Those from the earlier era used direct inoculation (of the tongue or musculature) as the main method of infection while latterly infection has more often been established by means of direct or indirect contact with infected donor animals. The earlier approach has the advantage of allowing more precise timing and quantit - ation of the virus dose but the present shift in emphasis has provided information which is more directly applicable to the disease situation in the field and has increasingly focussed infection on the prodromal phase of pathogenesis, an area which is virtually bypassed by the method of direct inoculation.

When virus is introduced by intradermolingual inoculation, the first detection of infectivity in tongue tissue may be as early as 6 hours

after infection with increasing activity to a peak at 12 hours when the vesicles are still immature (Brooksby 1952). Tongue vesicles develop at the inoculation site within 18 - 30 hours, viraemia within 5 - 15 hours and generalised disease within 36 - 96 hours (Burrows 1972). Intravenous inoculation of virus in cattle effectively anticipates the earlier events of pathogenesis and can result in generalised disease within 24 hours of inoculation (Mann and Garland unpublished data). The intranasal instillation of $10^{8.0}$ p.f.u. of FMDV resulted in the pharyngeal production of new virus within 2 hours of infection with the titre rising to a maximum at ten hours and persisting at a fairly uniform level for at least 24 hours. Viraemia ensued at 24 hours and lesions were detected at 72 hours post instillation (Graves et al 1971a). Exposure to lower intranasal doses ($10^{3.0}$ p.f.u.) gave variable results and while one steer so infected developed lesions within 96 hours, three others failed to develop clinical disease or show any serological response to the virus (Graves et al 1971b).

McVicar et al (1971) also followed the development of viral infectivity in bovine pharyngeal fluid by sequential sampling after the intranasal instillation of FMDV. The titre increased sharply after 2 to 6 hours, depending on the strain of virus used, until about 12 hours after infection when the production of virus levelled out at a titre determined by the initial dose. Viraemia was detected at 24 hours and a second rise in the viral content of pharyngeal fluid preceded the appearance of tongue lesions at 48 hours after infection. One animal showed a much slower pathogenesis with low levels of pharyngeal virus which persisted for 3 days before the titre rose on the 4th day, when viraemia was detected. Lesions appeared on the 6th day.

Attempts to simulate natural infection by direct and indirect contact have shown that virus may be recovered from external situations, espec-

ially the mouth and pharynx, prior to the development of clinical lesions for up to 9 days in cattle and sheep and up to 10 days in pigs (Burrows 1968a and 1972, Sellers et al 1968b and 1969, Graves et al 1971a). The average time for which pharyngeal virus could be recovered before the onset of clinical disease following indirect contact infection was 2.5 days in cattle and sheep (range 0 - 5 days) and 5 days in pigs (range 2 - 10 days) (Burrows 1972).

In an extensive study involving all seven types of FMD Cottral and Bachrach (1968) found that viraemia was detectable in cattle following tongue inoculation as early as two hours and as late as 120 hours post inoculation, the rapidity of development being proportional to the amount of virus introduced. Intramuscular inoculation or aerosol infection delayed the onset of viraemia by 24 - 48 hours. The development, duration and amount of virus in the blood varied with the individual animal but the peak concentration of virus was found on average at 40 - 42 hours after tongue inoculation while individuals showed peaks between 24 and 72 hours post inoculation. In another report viraemia persisted for up to 4 days in cattle following intramuscular or tongue inoculation (Scott et al 1966). Cottral and Bachrach (1968) summarised earlier work on FMD viraemia, most of it conducted in animals infected by direct inoculation, and quoted the following times for the duration of viraemia: cattle 18 - 103 hours, pigs 12 - 96 hours, sheep 12 - 72 hours, and goats 16 - 92 hours. An early report of the intermittent recovery of virus from the blood of steers 7 - 58 days convalescent to FMD (Waldmann et al 1931) has not been confirmed. Viraemia developed in steers within 24 hours of the intranasal instillation of $10^{8.0}$ p.f.u. of virus or within 1 to 3 days after the demonstration of virus in pharyngeal fluid following contact infection. Lesions invariably appeared within three days of the first detection of virus in the blood (Graves et al 1971a). Viraemia was detected in cattle following contact infection 1 - 2 days (Burrows

1968a) or 1 - 6 days (Sellers et al 1968) before the onset of lesions.

The minor discrepancies which exist between authors are explainable by their use of different virus strains, dose rates and methods of infection but the general pattern is clear; viraemia is rarely detectable for more than 6 days and has almost invariably subsided within 2 to 3 days of the development of clinical lesions, the clearance of virus from the blood coinciding with the rise in serum neutralising antibody.

Attempts have been made to determine the distribution of virus throughout the body during the course of disease. The methods used have included the sampling of the natural secretions and excretions following infection and the 'routine titration' approach of Mimms (1964) whereby animals are slaughtered at intervals after exposure to infection and the amount of virus present in their tissues at the time of death is determined. During the prodromal phase in cattle following contact infection virus has been most consistently recovered from the pharyngeal area (Burrows 1968a and b, and 1972, Sellers et al 1968).

Predictably, once virus has attained the blood stream it can be recovered from the majority of tissues and its presence in any particular location is then of dubious significance. Conclusions may be safely drawn however, where the infectivity of tissues exceeds that found at the same time in blood. Using these criteria a number of internal organs and tissues have been shown to support the growth of virus in vivo and to retain their infectivity after the viraemic phase. In cattle the lymph nodes may contain virus for up to 15 days post infection (Cottral et al 1963). Internal sites of viral multiplication include the rumen, thryoid, adrenal, pineal body and pancreas which may harbour virus for at least 8 days and the kidney and bone marrow in which virus may persist for up to 6 days after tongue inoculation (Hess et al 1960, Cottral 1969).

Using fluorocarbon purification and polyethylene glycol concentration, Tsetkova and Sobko (1972) were able to recover virus from the bone marrow, thyroid and lung of a steer slaughtered 8 days after infection. Skin contains persistent virus for up to 7 days (Gailiunas and Cottral 1966) and Cottral and co-workers (1968) found that testes could contain virus for up to 10 days. Waldmann's claim in 1931 to have recovered virus from the kidneys and urinary bladder of cattle for up to 94 days after infection has not been confirmed.

Virus has been found in other internal, non vascular tissues and fluids, possibly as a result of transcapillary escape during viraemia. Thus FMDV has been recovered from parts of the bfain (Kunter 1958) and the brain, spinal chord and cerebro spinal fluid (Scott et al 1965). Similarly synovial fluid may contain virus for as long as 5 days after tongue infection (Gailiunas and Cottral 1964) and fluid from the pleural and peritoneal cavities may be infective for up to 6 days (Cottral and Gailiunas quoted by Cottral 1969).

Following contact infection most secretions and excretions contain virus for several days prior to the development of clinical disease. In cattle, pharyngeal fluid may be infective for 0 to 9 days, saliva for 1 - 7 days, semen and milk for 1 - 4 days, vaginal swabs for 0 - 1 day, rectal swabs for 1 - 6 days and preputial swabs for 2 - 4 days before the first recognition of clinical signs (Burrows 1968a and 1972, Sellers et al 1968). Virus is therefore disseminated in the secretions well before the eruption of vesicles.

The wide spectrum of clinical FMD is well known (Graham 1959) as is the fact that the incubation period and extent of lesions in the experimental disease is related to the virus strain, dose and route of infection employed. In the most severe bovine case there may be extensive

vesiculation of the tongue, gums, hard palate, dental pad, lips, muzzle, nares, teats, udder, prepuce/vagina, coronary bands and interdigital space. Milder cases may show only small lesions, possibly of an erosive character, on less than four feet and at a few sites in the nose and mouth, often without tongue involvement. In some instances sub clinical infection occurs as demonstrated by the recovery of virus from blood and pharyngeal fluid and by the rise of antibody (Sutmoller et al 1968). In others pharyngeal virus may be recovered in the absence of circulating antibody (Hedger 1968, Auge de Mello et al 1966). Yet another form has been described in cattle concurrently infected with bovine enterovirus (Graves et al 1970, 1971b) in which a transient pyrexia and viraemia was seen in 1 of 8 animals as the only clinical sign following the intranasal instillation of FMDV and neither symptoms nor antibody could be detected until 40 - 120 days after infection when generalised disease suddenly developed. These observations were explained on the basis of a) Competition between FMDV and the preexisting bovine enterovirus and b) phenotypic transencapsidation. In this phenomenon the FMDV ribonucleic acid core is enclosed in an enterovirus coat in which form it persists until, by means as yet unknown, FMD is able to develop (Sutmoller et al 1970). Independent confirmation and irrefutable proof of the existence of this latent form has yet to be provided.

Several natural secretions are altered during the clinical course of FMD. In particular the stomatitis often results in increased salivation in cattle and the saliva may assume a viscid, mucoid nature. Bilateral nasal discharge, initially serous but often becoming muco-purulent, is also a common feature while conjunctivitis with ocular discharge may occasionally complicate the syndrome. Early reports recognised that lymph and epithelium from vesicles of the mouth or feet were particularly dangerous sources of virus as was saliva during the clinical disease (Waldmann and Reppin 1927, Olitsky 1927, Olitsky et al 1928, British Foot

and Mouth Disease Research Committee 4th Progress Report 1931).

Saliva collected from cattle following tongue inoculation contained $10^{4.0}$ Mouse ID_{50} per ml at 16 hours post infection before the development of vesicles and the amount of virus increased to a maximum of $10^{8.5}$ ID_{50} at 38 hours when ripe vesicles were rupturing (Hyslop 1965). Waldmann and Reppin (1927) found virus in saliva between 9 hours and 11 days after infection while Wittmann and Eissner (1966) stated that virus could be recovered from bovine saliva between the 4th and 7th day from cattle infected by virus rubbed over the oral mucosa. Scott et al (1966) found virus for up to 9 days after infection with peak titres of $10^{8.0}$ p.f.u. per ml of saliva occurring on the day after tongue inoculation. Many reports therefore chronicle the disappearance of FMDV from bovine saliva 7 to 11 days after infection. However it is important to differentiate between saliva which has been derived from the mouth and saliva which has been obtained from the pharynx since oral saliva loses infectivity within a few days of infection but pharyngeal fluid may retain infectivity for months or years. The chronic residence and excretion of virus in the bovine carrier state is reviewed in section 5.1.3.

Virus may also be present in nasal discharge for up to 6 days post inoculation to a maximum titre of $10^{7.7}$ p.f.u. per ml at the time of rupture of nasal vesicles (Scott et al 1966).

The highest titres of virus have been found in vesicle epithelium which may contain up to 10^{10} p.f.u. per g. Virus has been recovered from tongue epithelium for as long as 8 days, from palate lesions for up to 11 days and intermittently from foot epithelium for up to 11 days post infection (Scott et al 1966). Burrows (1966a) was able to recover type A virus at low titre from the tongue of one or two steers sampled at post mortem 23 days after infection. Olitsky et al (1928) found virus

in hoof scrapings from only 1 of 23 convalescent cattle sampled between 20 to 180 days after infection and then only on one occasion on the 34th day. Brandt(1928) carried out a similar search in cattle but was unable to recover virus between the 5th and 159th day. In this area, as in others, the work carried out between 1920 and 1950 is remarkable in its scale and thoroughness but it suffers from the lack of sensitive assay systems, much of it having been carried out in guinea pigs, and consequently quantitative results are scarce in studies made during this period.

It is not surprising that most secretions and excretions contain virus, particularly at the height of clinical disease. Bovine urine may be contaminated by the overspill of virus during viraemia or by the shedding of virus during growth in the kidney or urinary bladder. Hess et al (1960) studied the renal multiplication of FMDV in calves and showed that virus was present in kidney tissue for up to 6 days after tongue inoculation whereas viraemia lasted for only 2 days. Virus was recovered from 17 of 111 urine samples derived from cattle 7 to 103 hours after infection by tongue scarification (Trautwein et al 1928). Voinov (1955) detected virus in urine for only up to 15 hours after tongue infection. Cottral et al (1968) reported intermittent recovery of virus from the urine of cattle from 12 hours to 7 days post tongue inoculation although all but 1 of 10 animals were free of detectable urinary virus after 3 days. Maximum titres of $10^{4.9}$ Mouse ID_{50} per ml of urine were recorded 78 hours after infection. Waldmann et al (1931) used absorption and concentration techniques to study the excretion of virus in cattle successively exposed to several types of FMDV and claimed that virus could be recovered from urine for as long as 246 days and from tissues of the urinary bladder for up to 94 days post infection. Burrows (1966a) failed to recover virus from the urinary bladder of 2 steers at post mortem 9 to 11 weeks after infection. Swabs taken from the bovine

prepuce following contact infection may contain virus for 2 to 4 days before the appearance of lesions (Burrows 1968a, Sellers et al 1968). The origin of this virus may have been local multiplication in the prepuce or contamination during the passage of infected urine.

Although virus derived from swallowed saliva and oral lesions, rumenal vesicles, viraemic blood and bile must contaminate the contents of the alimentary tract during clinical FMD, the faecal excretion of virus is minimal. The acid pH of the gut contents (Kay 1966) is one factor which presumably contributes to the low levels of virus found in faeces. Since the original report by Bielang (1923) many workers have recorded the inconsistent recovery of low levels of FMDV from the faeces of infected animals. Voinov (1955) was unable to demonstrate infectivity in faecal samples derived from cattle infected by tongue inoculation. Wittmann and Eissner (1966) produced clinical disease in cattle by rubbing a suspension of virus over the tongue, oral cavity and nostrils and recovered virus from the faeces on the 1st but not on the 4th day after infection. Trautwein et al (1928) recovered virus from 7 out of 111 faecal samples taken from cattle during the first 5 days after tongue inoculation. Guinea pig inoculation was used to detect virus and no quantitative determinations were made.

Parker (1971) showed that faeces which were allowed to accumulate on the floor of a loose box housing 2 steers infected with FMD could attain levels of up to $10^{5.5}$ Mouse ID_{50} per g. by the 6th day after tongue inoculation. Virus was recovered in considerable quantity between the 1st and 12th day but the levels were considered to be a reflection of the total excretion of virus by all routes rather than of excretion by the rectal route alone. Following contact infection Burrows (1968a) found virus in rectal swabs from 2 of 4 cows one day before lesions were detected while Sellers et al (1968) made intermittant

recoveries of virus from the faeces of 3 of 4 bulls on the 6th, 4th and 3rd days before vesicles were observed. Sellers et al (1969) also recovered virus from 8 of 48 rectal swabs when 4 steers were sampled daily for 12 days following contact infection, clinical disease being first detected on the 5th, 6th and 7th days.

Lactating cattle infected with FMD excrete virus abundantly in the milk. Lebailly (1920) first drew attention to the presence of virus in milk during the viraemic stage of the disease prior to the development of lesions and German workers amply confirmed his observations, finding virus as early as 13 hours and as late as 5 days after the recognition of clinical disease (Trautwein et al 1928, Terbruggen 1932). Fluckiger (1940) also observed that the virus may be excreted in the milk before the disease becomes clinically obvious. The epidemiological studies of Hedger and Dawson (1970) showed the massive dissemination of virus in milk and indicated that virus was excreted for at least 33 hours before the onset of clinical disease. Burrows and co-workers (1971) studied the pathogenesis of FMD in the mammary gland and recorded virus concentrations of up to $10^{7.0}$ p.f.u. per ml of milk within 8 - 32 hours of the instillation of virus in one quarter. Virus was recovered from other distant sites such as the pharynx, mouth, nose and vagina within 4 - 24 hours of instillation and although signs of mastitis were often detected during the early multiplication of virus, the classical signs of FMD did not appear for 52 - 117 hours. Following contact infection, virus was present in the milk for 0 - 4 days (type A) or 1 - 4 days (type O) before the appearance of clinical lesions and persisted for as long as 3 weeks (type A) or 7 weeks (type O). The extreme susceptibility of mammary tissue was shown by the rapid multiplication of virus following primary infection and by the fact that attempts to reinfect convalescent cows by the intramammary instillation of homologous virus resulted in at least one cycle of viral replication in the udder, despite

the presence of high levels of neutralising antibody in serum and milk.

The infected cow may also excrete virus in the vaginal secretions and Burrows (1968) was able to find virus in the order of $10^{3.0}$ p.f.u. per sample in vaginal swabs on the day before clinical lesions appeared.

FMDV multiplies in the testes of the infected bull and may be present within 12 - 20 hours of parenteral injection, before lesions have developed, and for up to ten days afterwards (Cottral et al 1968). Sellers et al (1968) found virus in semen for 1 - 4 days before clinical lesions appeared in cattle infected by indirect contact. Maximum amounts of virus were found at the height of clinical infection with titres of up to $10^{6.2}$ ID_{50} per ml of semen.

Another means, of crucial importance, by which FMDV is disseminated from the infected animal is by the creation of infective aerosols. The airborne spread of virus has been suspected for many years (5th Progress Report of the FMD Research Committee 1937) and several authors have reviewed epidemiological incidents which pointed to the excretion and transmission of virus by this route (Michelsen 1968, Sellers and Parker 1969, Hyslop 1970).

The initial experiments in this field brought controversy since some workers succeeded in transmitting infection by the airborne route (Fogedby et al 1960, McKercher et al 1966) while others were unable to do so (Moosbrugger 1948, Traub and Wittmann 1957). The subject remained obscure until Hyslop (1965c) commenced the quantitative study of excretion and was able to recover virus from the air before the appearance of clinical lesions and for up to 14 days after tongue inoculation. Kiryukin and Pasechnikov (1966) recovered 6.3 to 630 ID_{50} of virus per litre of exhaled air from infected calves. Sellers and Parker (1969)

found that airborne virus was released in the greatest amounts by pigs (up to $10^{4.7}$ ID_{50} per animal per hour) and in lesser quantity by cattle and sheep (up to $10^{3.2}$ ID_{50} per animal per hour). The excretion totalled $10^{6.0}$ ID_{50} per pig over 5 days and $10^{4.5}$ ID_{50} per steer or sheep over 4 days following tongue inoculation. Peak recoveries occurred at 41 hours post inoculation in cattle and pigs, when disease had generalised, and at 17 hours in sheep, prior to the development of lesions. Further work showed the considerable influence of virus type and strain on the amount of airborne virus which could be recovered (Donaldson et al 1970) and provided some explanation for the conflicting results obtained by other workers.

Sellers and Parker (1969) measured the distribution of viral infectivity in droplets of various sizes in the aerosol from infected pigs. They showed that 65 - 71% of virus was recovered at an aerosol size of larger than 6μ, 19 - 24% at 3 - 6μ and 10 - 11% at smaller than 3μ. By analogy with man these droplets might be expected to be deposited principally in the mouth, nose and pharynx (6μ), the bronchi (3 - 6μ) and the alveoli (3μ) (Hatch 1961). Droplets of greater than 5μ sediment fairly rapidly but particles of a smaller size may remain in aerial suspension for long periods and, given favourable atmospheric conditions, can remain viable during dissemination over long distances (Hyslop 1970, 1972).

To summarise, virus is disseminated in large amounts for several days before and after clinical FMD with maximum excretion occurring by most routes at the height of the syndrome. Virus can rarely be recovered from tissue, secretions or excretions later than a few days after the acute phase except from milk, the soft palate, the pharynx and secretions from this area in cattle and sheep. With regard to natural contact infection Graves et al (1971a) have shown that a steer infected

by the intranasal instillation of $10^{8.0}$ p.f.u. of FMDV remained infectious for susceptible animals for 7 to 8 days after the instillation of virus. The most efficient transmission occurred 72 - 96 hours after infection of the donor when clinical disease was at its peak.

Immunity, both natural and acquired, can greatly modify the pathogenesis of FMD. Vaccination can effectively prevent the development of clinical disease in animals challenged with homologous virus but will not prevent the acquisition of the carrier state (see section 5.1.3.). Potent inactivated vaccines can however markedly reduce the excretion of virus by all other routes, thus Sellers et al (1969) exposed two groups of cattle to indirect contact infection; one group vaccinated 19 days previously and the other not vaccinated, and compared the daily recovery of virus from each group between the 7th and 10th day after exposure. Viraemia and lesions were detected only in the control animals. Viral recoveries were made from pharyngeal secretion, oral saliva and swabs from the prepuce and rectum in 38, 7.3, 5.5 and 1.8% respectively of samples from vaccinated cattle and in 71, 41, 33 and 16% of samples from non vaccinated cattle. Immune animals also excreted virus at much lower titre. Weyhe (1966) examined the milk and saliva of vaccinated cattle challenged by oral swabbing or contact infection. The animals remained clinically normal and virus was recovered in the saliva of 40 out of 156 cattle and in the milk of 2 out of 8 cows. In saliva, FMDV was found most frequently between 4 and 6 days after exposure and could not be detected beyond the 13th day. Milk contained virus only on the 6th and 9th day after challenge. Wittmann and Eissner (1966) were unable to recover virus from the saliva, urine or faeces of convalescent cattle after reexposure to homologous virus, even when samples were concentrated 10 - 30 times prior to assay. Cottral et al (1963) found virus in the regional lymph nodes of cattle for up to 15 days after tongue inoculation. In contrast, virus could

not be recovered from the lymph nodes of convalescent cattle slaughtered 2 days after reinoculation of the tongue with the same virus type.

It will be seen from the foregoing that, despite the existence of a vast body of information, no single systematic study has been made of the excretion of FMDV in normal and vaccinated cattle before, during and after clinical disease following contact infection.

5.1.3. The Carrier State

The carrier state in FMD had been suspected on epidemiological grounds since the turn of the century but proof of its existence eluded investigators for over 50 years. Attempts to recover virus from the natural secretions and excretions after the acute disease had subsided were generally unsuccessful (see section 5.1.2.). Then in 1959, Van Bekkum and his colleagues published their account of the intermittent recovery of virus from the pharyngeal fluid of convalescent cattle which they sampled by means of a special probang cup (Plate 3). Other laboratories confirmed their findings and a new chapter opened in FMD research. Reviews of this topic have been collated by Sutmoller et al 1967b, Henderson 1966/67, Singh 1969, Hyslop 1970 and Sutmoller 1970.

It is now recognised that a high proportion of cattle will become carriers as a sequel to infection with FMD. In the original observations of Van Bekkum et al (1959), 10 of 13 convalescent cattle carried type A virus for up to 5 months after infection. Sutmoller and Gaggero (1965) found 14 out of 25 animals positive six months after infection in the field. Burrows (1966a), working with 3 types and 4 strains of FMDV, recovered virus from 23 of 30 steers following experimental infection. Virus was detected in 68.8% (208/302) of pharyngeal fluid samples taken between 2 and 26 weeks after tongue inoculation. Van Bekkum et

al (1966) demonstrated persistence for periods of 7 weeks (type A), 8 weeks (type O) and 14 weeks (type C) in cattle following intradermo-lingual infection. McVicar and Sutmoller (1968) reported that 19 of 32 steers became carriers after intranasal or contact infection. In Africa Hedger (1968) surveyed an enzootic area and was able to isolate type SAT_3 virus from 20% of the animals sampled between 7 and 12 months after natural infection. Virus persisted for up to $2\frac{1}{2}$ years after infection (Hedger and Stubbins 1971).

Not all cattle exposed to a similar challenge become carriers (Burrows 1966a and b, Sutmoller 1970, Kaaden et al 1970 etc.) indeed, considerable variation exists between individual animals in the establishment and course of the carrier state.

The amount of virus and the route by which it is presented influence the acquisition of chronic pharyngeal infection. At low virus dose rates (100 p.f.u.), more carriers were produced by the instillation of virus into the tonsillar sinus and pharyngeal region (8/10) than were by intranasal instillation (4/18). A dose of 10,000 p.f.u., however, established the carrier state in 5/6 and 6/6 animals following pharyngeal and nasal infection respectively.(Sutmoller et al 1968).

Some evidence exists to suggest that virus type and strain may be an important determinant in the carrier state. Thus Burrows (1966a and b) found that the bovine carrier state was less frequently detected and of shorter duration following infection with types SAT_1 and SAT_3 than with type A Turkey or A119 while the titre of pharyngeal virus was much lower after infection with types A Turkey or SAT_3 than after type A119. The significance of these results should not be overemphasised since they were derived from small numbers of cattle. Kaaden et al (1970) were able to recover type O virus quite frequently from 8 cattle sampled

between 1 and 9 months after infection whereas type A virus could not be recovered from a similar group sampled one month after infection. The effect must be related to strain rather than type since persistent excretion has been reported for strains of type A, e.g. A119 and A4691 (Van Bekkum et al 1959, Burrows 1966a, Sutmoller and McVicar 1972). It is possible that preferential adaptation of strains of FMDV to particular species may have a bearing on the carrier state. Young et al (1972) described an outbreak of FMD in African buffalo following natural infection with type SAT_2 and showed that disease could be transmitted to cattle held in an adjoining pen. Both species developed clinical lesions but only the buffalo became carriers.

Species which can be infected with FMD differ in their susceptibility to the carrier state. Cattle readily become carriers as does the African buffalo (Hedger et al 1969, Hedger 1971, Young et al 1972 etc.). Sheep and goats commonly harbour virus in the pharyngeal area, especially in tonsillar tissue, for as long as 5 months (Burrows 1966b, 1968) or 12 months (McVicar and Sutmoller 1968) after infection. In contrast, all attempts to demonstrate the carrier state in the pig (Burrows 1966b, 1968a, Sutmoller 1970) or guinea pig (Burrows 1968b) have been unsuccessful.

In one study there was no correlation between the sex and age of cattle and their carrier status (Hedger 1968).

Several factors may therefore contribute to the carrier state but our knowledge of the determinants of susceptibility or resistance is still rudimentary in this, as in other areas.

The recovery of virus in pharyngeal fluid is often intermittent and the titre may fluctuate quite widely. In 28 weekly samples from

10 carrier animals, the frequency of viral recovery varied between 1/28 and 21/28, moreover, on occasions virus could be recovered from only 1 of 2 samples taken from the same animal within a 5 minute period (Van Bekkum et al 1959). However, the frequency of recovery and the amount of virus generally decreases with time after infection. Ten steers had a mean infectivity of $10^{2.4}$p.f.u. per ml of pharyngeal fluid (range $10^{1.7}$ - $10^{3.0}$) 3 weeks after infection with type A119 but 24 weeks later virus was detectable in only 5 animals at a mean level of $10^{1.0}$p.f.u. per ml (range $10^{0.3}$ - $10^{2.0}$) (Burrows 1966a). Straver et al (1970) repeatedly sampled 66 cattle in 8 carrier herds after a type C epizootic; 38 animals were positive at least once, more than half of these became negative within 4 months of infection and the last animal became negative 24 months after the first sampling.

The development of clinical disease is not a prerequisite of the carrier state. A number of authors have reported on susceptible animals which did not develop lesions after infection but which did, nevertheless, show a chronic asymptomatic infection of the pharynx (Van Bekkum 1959, Sutmoller 1970, McVicar and Sutmoller 1969). Such animals usually develop circulating antibodies. Several workers have recorded an association between high serum antibody titre and the carrier state (Sutmoller and Gaggero 1965, Burrows 1966a, Van Bekkum 1966). Hedger (1970) was of the opinion that the presence of high levels of serum antibody in some members of the population after an outbreak of FMD was usually associated with the presence of carrier virus in the group and that, conversely, carrier virus was unlikely to be recovered from a population in which significant antibody levels could not be demonstrated. However, the same author (Hedger 1968) was able to show that while antibodies were found in the sera of 46 out of 50 carriers following a type SAT_3 epizootic, antibody could not be demonstrated in the sera of the 4 remaining carriers.

Cattle vaccinated using inactivated FMD vaccines, with high levels of humoral antibody and fully immune to the development of clinical disease, can acquire persistent subclinical infection as readily as non vaccinated animals (Van Bekkum et al 1959 and 1966, Burrows 1966a, Sutmoller et al 1967a and b and 1968, Hedger 1969, McVicar and Sutmoller 1969, Kaaden et al 1970 and Pustiglione Netto et al 1972). These authors are in agreement on the lack of correlation between a) pre-existing antibody levels and the development of the carrier state and b) the presence or absence of clinical lesions and the development of the carrier state. In the light of these findings it is not surprising that the asymptomatic carrier state can also follow the application of attenuated strain FMD vaccines (Burrows 1966a and b, Auge de Mello et al 1966 and 1970).

Claims have been made that pharyngeal virus was recovered more consistently, for longer periods and at a higher titre from cattle which were protected by inactivated vaccine and remained clinically immune on exposure to FMD than from non vaccinated cattle which developed clinical disease after exposure (Sutmoller et al 1968, McVicar and Sutmoller 1969, Sutmoller 1970). Passively immunised animals appeared to occupy an intermediate position (Sutmoller et al 1968). These authors postulated that the persistent infection which follows the exposure of vaccinated cattle to FMD may represent a more stable virus - host relationship than that which follows when susceptible cattle are exposed to the disease.

Burrows (1966a) investigated the specific localisation of virus in the carrier state by titration of suspensions of mucosae and epithelia taken from the pharyngeal and associated areas of convalescent carriers at post mortem. The dorsal surface of the soft palate and the walls of the pharynx were most often infected to highest titre in cattle following infection with wild strains while the tonsillar region figured more

prominently when attenuated strains were studied. Virus was recovered less frequently from the ventral surface of the soft palate and the glosso-epiglottic space and only occasionally from the tonsillar sinuses, tonsils, tongue, trachea and oesophagus. No virus was detected in the nasal turbinates. Van Bekkum et al (1966) also concluded that the cells lining the pharynx appeared to be the main sites of viral production in carrier cattle. Henderson (1966/67) recovered virus most consistently from the tonsillar region. Mohanty and Cottral (1971) studied cultures from the tissues of carrier cattle by fluorescent antibody techniques and infectivity assay and reported that the pharyngeal and oesophagal mucosae were important sites of persistence. The exact nature of the cells which support the carrier state is unknown.

Although the pharyngeal fluid of carrier cattle may contain quite large amounts of virus and infected droplets can be expelled by steers during coughing (Sutmoller et al 1967a), the significance of the carrier state in the maintenance and spread of FMD remains problematical. Many attempts have been made to demonstrate the experimental transmission of infection from carrier cattle to normal cattle but to date all have failed (Van Bekkum 1959, Burrows 1966[b], Brooksby 1967, Sutmoller et al 1967a and 1970, Straver 1970). The application of stress has also failed to influence the excretion of virus by carriers or its acquisition by recipient animals. Carrier and recipient cattle have been stressed by the injection of steroid hormones (Sutmoller and McVicar 1972), the simulation of prolonged trekking (Hedger, personal communication) and food and water deprivation (Garland unpublished observations). Recipient pigs have been stressed by the abrasion of their feet or by injection with embryonated ascaris eggs (Sutmoller and McVicar 1972).

Limited evidence does exist for the infection of normal animals by carriers in the field. Van Bekkum et al (1959) reported a rise in anti-

body in the absence of clinical disease in 1 of 30 calves born in a carrier herd 4 months after a type C outbreak. Hedger (1968) demonstrated type SAT_3 pharyngeal virus and serum antibody in one calf born some months after the last known outbreak of FMD in an enzootic area of Botswana. Sutmoller and McVicar (1972) recorded the development of low levels of serum antibody to type A in some animals among a group of pigs following contact with carrier cattle. No signs of FMD were observed and the animals were fully susceptible to the subsequent inoculation of type A virus.

A somewhat different situation exists when certain attenuated strains of FMD are employed. Virus was readily recovered from normal cattle in contact with animals vaccinated with type C (Resende) attenuated virus although serum antibody did not develop in the recipients. Such transmission was extremely rare when type A (Cruzeiro) attenuated virus was used (Auge de Mello et al 1966, 1970).

The almost total failure of transmission experiments between carriers and susceptible cattle has not been satisfactorily explained. However as discussed below (Section 5.1.4.) contributory factors may include: the presence of neutralising antibody in bovine saliva (Hyslop 1965a, Burrows 1966a, Van Bekkum 1966, Sutmoller et al 1967a, Kaaden and Matthaeus 1970); the periodicity of viral excretion in the carrier state (Van Bekkum et al 1959, Burrows 1966a, Sutmoller 1970 etc.); the low level of virus normally encountered in pharyngeal fluid (Burrows 1966a, Sutmoller and Cottral 1967, Sutmoller 1970 etc.); the production of interferon in the oropharynx and the fact that the main volume of upper alimentary and respiratory tract secretion is destined to be swallowed, disinfected and reabsorbed in the gut rather than be excreted from the mouth and nose.

Workers at Pirbright were the first to show that antigenic variation could be induced in FMDV by the serial passage of virus in the presence of immune bovine serum in tissue culture (Hyslop 1965b) and by serial passage in partially immune cattle (Hyslop and Fagg 1965, Fagg and Hyslop 1966). Burrows (1966b) extended these observations and showed, using serological techniques, that chronic residence of FMDV in the bovine pharynx could be associated with changes in the antigenic nature of the virus. Fellowes and Sutmoller (1970) and Sutmoller (1970) also demonstrated serological differences between the original virus and some subsequent carrier isolates from cattle and described additional differences between them in such characteristics as growth at various temperatures, resistance to heat, acid and ultraviolet energy and stabilisation by metallic salts. Strains of carrier virus have been isolated with reduced virulence for cattle (Burrows 1966b, Sutmoller et al 1967d, Straver et al 1970) and for pigs (Straver et al 1970). Such strains may recover full virulence for cattle after a single bovine passage (Sutmoller et al 1967d). Hedger used complement fixation tests to compare the antigenic characteristics of original virus and later carrier isolates from outbreaks of FMD in Africa. He found little variation after a type SAT_1 epidemic (Hedger 1970) but detected marked differences after a type SAT_3 outbreak (Hedger 1968). In Germany, Kaaden and co-workers (1970) also compared original and progeny virus strains and concluded that certain carrier isolates of type O virus behaved like modified strains of FMDV since they showed decreased virulence for mice and cattle, full virulence for pigs, slow development of cytopathic effect and reduced plaque size in tissue culture and an increased capacity for the stimulation of interferon. Straver et al (1970) made similar comparisons following an outbreak of type C in the Netherlands and found that some carrier isolates were of reduced pathogenicity for cattle and pigs, produced smaller plaques in some cell systems and showed an increased sensitivity to inactivation by pH and heat. Since the original

outbreak virus was composed of a heterogeneous population, some part of which exhibited the characteristics of subsequent carrier isolates, the authors suggested that selection was in operation during the course of the carrier state.

Other mechanisms of viral variation may operate during chronic residence of FMD in the bovine pharynx. The phenomenon of genetic recombination has been demonstrated experimentally in vitro between FMDV strains of distinct subtype (Pringle 1965, 1968, 1969, Pringle and Slade 1966, 1968) and the rate of mutation of some strains of the virus to thermal resistance can be as high as 10^{-4} (Pringle 1964). Recombination between types of FMDV has not been reported and in this context a number of workers have shown that reinfection of convalescent carrier cattle with virus of a different type results in the displacement of the first virus type in the pharynx in favour of the second type (Fellowes and Sutmoller 1970, Sutmoller 1970, Cottral and Gailiunas 1971 and unpublished work at Pirbright). Similarly, in superinfection experiments a second subtype of FMDV can readily supercede the original subtype in the bovine pharynx (Cottral and Gailiunas 1971).

Although pharyngeal infection is apparently restricted to one virus type or subtype at any one time, Sutmoller and Gaggero (1965) recovered type C and type A virus in one sampling from different cattle on the same farm and different types of FMDV have also been recovered from individual buffalo within a herd (Hedger 1971, Hedger et al 1969, 1973). Fellowes and Sutmoller (1970) presented data which suggests that recombination may occur between types of FMDV during mixed infection. They examined carrier virus derived from cattle convalescent to type O and reinfected with type A in cross neutralisation tests using antiserum prepared against the original and later isolates of type O and A virus. The results showed that at least 90% of the infectivity of some later

type A isolates could be neutralised by antiserum to both virus types. Simple mixed infection was considered unlikely since only type A virus could be detected by complement fixation and growth of the isolate in type O or type A antiserum failed to reveal the presence of more than one component.

The carrier state is a common sequel to acute FMD in cattle and considerable evidence exists to incriminate the carrier as a source of infection for other animals. Nevertheless, direct proof of such transmission is lacking and it is clear that spread of virus between carriers and susceptible animals occurs only rarely and is likely to involve special predisposing factors, such as antigenic variation. We know that FMDV can undergo such alteration during the course of the carrier state but we remain ignorant of the mechanisms involved in effective transmission. Rare though the event may be, however, the consequences can be extremely grave and the existence of an infected reservoir of such dangerous potential is clearly undesirable.

5.1.4. Host Defences.

Host defences against FMD include both natural, non specific defences and acquired or induced immunity. This section of the review deals only with those local and systemic host defence mechanisms which are known to oppose infection with FMDV and a later section (5.2.) is concerned with the broader aspects of local immunity including vaccination.

Non specific host defence mechanisms against FMD include innate immunity, physical barriers, pH and dilution effects, natural antibodies, interferon and interference between viruses. Acquired or induced defences following infection or vaccination include interferon, inflammation, cell mediated immunity and specific neutralising antibody which

may be found in internal and external secretions.

Many viral agents are species specific and such basic, innate resistance depends to a considerable extent on the presence or absence of either cellular receptor sites or intracellular growth mechanisms appropriate to particular virus. Both species and tissue susceptibility have been conclusively linked with the presence of receptors for some picornaviruses, particularly the enteroviruses polio and coxsackie, and susceptibility does not depend on the availability of intracellular pathways since cells which are resistant to intact virus can often be infected with RNA which has been uncoated (Holland 1961, 1962, 1964[a] Holland and Hoyer 1962). Alternatively particular cell types may resist infection, despite the presence of intracellular virus, because they lack the necessary biosynthetic pathway. Resistence of this type may occur in macrophages (Mims 1964) exposed to some arboviruses (Goodman and Koprowski 1962), to murine hepatitis virus (Bang and Warwick 1960) and to yellow fever virus (Sabin 1952).

The determinants of natural resistance or susceptibility are multiple and complex (see reviews by Smorodintsev 1960, Fenner 1968). The correlation between specificity <u>in vivo</u> and <u>in vitro</u> is far from absolute (Chaproniere and Andrews 1957) and depends on the dynamic interrelationship between virus and host. Resistant cells propagated <u>in vitro</u> may acquire receptors and develop susceptibility during culture, perhaps by the unmasking of preexisting sites or the derepression of synthetic mechanisms normally latent in the intact animal (Holland 1964[b]). Virus blind-passaged in a resistant host may adapt to grow well in that system (Sabin and Schlesinger 1945, Fenner and Cairns 1959 etc.).

Ontogeny of resistance is a feature of several virus diseases. The subject has been reviewed by Siegal (1952) and Burnet (1960) and the

effect has been experimentally investigated with polio (Sabin 1962), coxsackie (Kunin 1964) and FMD virus. At a clinical level transmissible gastroenteritis and pseudorabies viruses can cause mortality of nearly 100% in piglets of up to 10 days of age but produce only mild transient illness in adults. In contrast, yellow fever of man and vesicular stomatitis virus of cattle are examples of diseases which produce a much more severe syndrome in the adult than in the young (Beveridge 1967). The effects may be related to the development of immunological competence (Overman 1954, Overman and Kilham 1953), possibly to a better developed interferon system in the adult (Heineberg 1964) or to the establishment of other defences such as the reticulo-endothelial barrier (Chany et al 1966).

The inheritance of resistance to viruses has been studied, principally in the mouse, commencing with the work of Webster (1937) using St Louis Encephalitis and later by Sabin (1952) with yellow fever, Bang and Warwick (1960) with Mouse Hepatitis and Goodman and Koprowski (1962) with various arboviruses. These and other workers have shown that certain aspects of resistence are genetically controlled. Despite considerable efforts, little is known about the mechanisms which underlie the phenomenon of age resistance.

FMDV exhibits a well defined species specificity. For example, it will not replicate or produce disease in the horse. Age resistance is also a factor since the immature calf or piglet is usually much more severely ill than the adult and may well die. The determinants of susceptibility have not been investigated in domestic animals but several factors have been carefully examined in the mouse. Skinner (1951, 1953) pioneered the use of suckling mice in FMD research and later work supports his original observation that mice of less than 7 days of age were susceptible to the virus while increasing age was parallelled by increas-

ing resistance (Uhlmann and Traub 1958, Campbell 1960 etc.). Subak Sharpe made a particular study of age (1961a and c) and strain (1961b) resistance in this system and concluded that the difference in susceptibility between certain strains of mouse was determined by a single gene with incomplete dominance (1961a and c) but could also be due to an interaction between polygenic systems involving several loci in the mouse (controlling the complex character of susceptibility) and in the virus (determining the infectivity) (1961b).

Intact mucosa and epithelium can protect some sites against infection while mechanical damage to this barrier may predispose to disease. The experiments of Cottral et al (1965) in which the dorsum of the normal bovine tongue was resistant to infection while the mildly traumatised dorsum was susceptible have already been mentioned as has the work of Platt (1961, 1963a and b, 1966), Skinner and Knight (1964) and Jones (1968) who showed that mechanical injury to external situations was often associated with the development and exacerbation of lesions in the guinea pig. Infection of superficial sites via abrasions of the mouth or feet due for example to poor quality fodder containing sharp materials (e.g. old spiky hay, thorns and thistles) is frequently quoted as a portal of entry. Reports have also been made of the attempts to infect swine by the feeding of virus when infection occurred more often after the inclusion of bone fragments in the feed (Cox et al 1964, Stockman 1927, Andrewes et al 1937).

The FMD virion is stable over a relatively limited pH range of about 7.0 - 8.0 and pH values outside this range are associated with loss of viral infectivity which occurs very rapidly at acid values of 6.5 or less and more slowly at alkaline values of 8.0 to 10.0 (Bachrach et al 1957). The alkaline pH of bovine saliva has been cited as an antiviral factor (Donaldson 1972) as has the pH of bovine urine which may reach acid values

down to pH 5.3. The acid shift is particularly noticeable in bovine urine during the clinical phase of FMD when inappetance, fever, cachexia, and tissue breakdown combine to cause a metabolic acidosis (5th Progress Report, FMD Research Committee, Ministry of Agriculture and Fisheries, 1937, Cottral and Gailiunas 1969).

Mucosae and epithelia at the natural orifices of the body are kept moist by the continual secretion of physiological fluids. Thus occular, nasal, oral, pharyngeal, tracheal, bronchial, oesophageal and other surfaces are bathed in a moving film of secretions which overlies the outer aspect of superficial cells. Such secretions may play a double role in pathogenesis since on one hand they function to wash away particulate matter and to lubricate and protect surfaces against mechanical damage but on the other hand they offer a viscous medium which may serve to trap pathogens and bring them into intimate contact with susceptible cells. In the ruminant, especially in the bovine and most particularly in lactating cattle, salivation exhibits a number of unique features. The most remarkable feature is the volume of saliva secreted which may attain 190 litres per day (Bailey 1961). The secretion of such large amounts of saliva is likely to exert an antiviral effect simply by the dilution and washing away of pathogens in the oral and pharyngeal cavities.

The topic of natural antibody has been reviewed by Boyden (1966) and Michael (1969). Natural antibodies to a number of viruses occur fairly commonly in bovine serum (Allen et al 1958, Klein 1958) and even foetal calf serum may contain antibodies to the viruses of infectious bovine rhinotracheitis, bovine parainfluenza type 3 and bovine virus diarrhoea (Kniazeff et al 1967). Patty (1970) tested the serum of 20 normal steers and reported a high incidence of inhibition of FMDV types O, A and C. Ron et al (1972) examined 19 foetal calf sera for non

specific neutralisation against FMDV types O, A, SAT_1 and $Asia_1$ and found activity in all sera against one or more virus types. Immunoglobulins were detected in 6 sera but no correlation was found between neutralising activity and the presence of immunoglobulin. Non specific neutralisation of FMDV has also been shown in normal porcine serum (Bogel 1966, 1967, Bogel et al 1967). The antiviral effect of some normal sera against FMD is thus well established but the exact nature of the effect, be it inhibition or neutralisation, and the agent, be it immunoglobulin or another serum component, remains unknown. It is reasonable to assume that such a factor constitutes an additional defence mechanism. Natural, non specific antibodies have not been reported in other secretions.

Interference between viruses has long been recognised as a defensive factor and some of the first experiments in this area concerned the ability of vaccinia virus to protect guinea pigs against infection with FMDV (Gildmeister and Helm 1932). The subject of interference and interferon is more properly considered with induced host defences and it is necessary now only to mention that FMD is susceptible to heterologous interference which might contribute to protection in the special circumstances obtaining when an animal is concurrently infected with another virus. Interference of this type has been cited to explain the lack of clinical disease which may follow the exposure of known bovine carriers of enteroviruses or infectious bovine rhinotracheitis virus to virulent FMDV (Graves et al 1970, 1971b, Sutmoller 1970). Preexisting infection might also induce interferon to inhibit a superinfecting virus. Proof of these hypotheses is currently lacking.

Acquired or induced defences against FMD are of two types:

1. those which are brought into play as an immediate but relatively short lived counter to infection, restricting the extent of lesions and

promoting recovery from primary, acute infection and

2. those which are brought to bear more slowly to engender a longer lasting resistance to infection. The first type embraces those factors commonly included in the acute inflammatory response such as interferon production, local tissue hypoxia and acidity, cellular infiltration and fever. The second type includes cell mediated and antibody related immunity. This division is convenient but somewhat arbitrary, since factors such as antibody may function in both areas.

Interference phenomena have interested virologists since the 1930s and Heule (1950) and Schlesinger (1959) have reviewed the early investigations in this field. Despite intensive efforts the underlying mechanisms remained unknown and interference remained largely a descriptive term until a new era opened with the discovery of the first interferon by Issacs and Lindermann in 1957. An avalanche of research followed resulting in much information on the induction, nature and mode of action of the interferons, as recently reviewed by, among others, Allison (1971), Baron (1963, 1970a and b), Colby and Morgan (1971), DeClereq and Merigan (1970), Doshi and Shah (1972), Finter (1970), Glasgow (1970[a], 1971), Lockhart (1967), Vilcek (1969) and Wagner et al (1968).

Present knowledge may be briefly summarised as follows: interferons (IF) are a family of proteins of cellular origin capable of initiating a non specific intracellular inhibition of virus replication. They may be differentiated according to their source, spectrum of activity and physio-chemical properties. They are of a predominantly protein nature, possibly incorporating a carbohydrate moiety, and show a relatively high resistance to acid, alkali and heat. Interferons are a heterogeneous species differing in molecular weight (approximately 25,000 - 100,000 daltons), electric charge and stability to heat and acid, these differ-

ences perhaps being a reflection of the inducing agent and the cells of origin.

A wide variety of infectious and non infectious agents, their extracts and synthetic polynucleotides are capable of inducing the formation of IF and most inducers are characterised by their stable, helical, polyanionic structure. Many more substances will act as inducers *in vivo* than *in vitro*. Interferons are produced in most cell types including epithelial cells, fibroblasts, leucocytes, lymphocytes and macrophages although there is evidence to suggest that, *in vivo*, cells of the lymphoreticular series are chiefly involved. The precise mechanism of production is unclear but is thought to involve the derepression of a DNA interferon cystron leading to the production of an interferon messenger RNA which directs the synthesis of IF. Two mechanisms are postulated to explain the early appearance of IF after induction, one being rapid *de-novo* synthesis and the other being release of preformed IF. There have been attempts to link these mechanisms with the known existence of two broad classes of IF:

a) the stable, low molecular weight type produced in response to most viruses and statolon being synthesised following derepression and

b) the more labile, high molecular weight type produced in response to bacterial endotoxins and other non viral inducers being associated with the release of preformed IF. In any event, IF is detectable very soon after infection and is generally found in greatest amount around the time of clinical disease in infected animals.

Interferons exhibit a wide spectrum of non specific activity against most RNA and DNA containing viruses (the adenoviruses being relatively insensitive) and against other intracellular organisms. They act locally to protect the cell in which they were induced and are also released to protect adjacent or distant cells. The effect is largely

species specific in that IF produced in one host will protect others of the same or closely related species but will act at a much reduced level, if at all, in other species. Interferons have no direct action on viruses but act intracellularly by inhibition of the translation of viral messenger RNA to block the synthesis of new virus. Although generally stable in the laboratory at $+4^{o}C$, they are short lived in the animal. A half life of 7 - 11 minutes has been reported for IF in serum following intravenous injection in various species and a similar rapid clearance follows systemic induction. Claims have been made for the efficacy of endogenous and exogenous IF in the prevention and treatment of many viral infections of man and animals but it is usually difficult to disentangle the role of IF from that of other host defences.

Concentration of research on the interferons has tended to overshadow the fact that not all interference phenomena are IF mediated. Indeed, direct competitive interference between viruses is well recognised. The phenomena include heterologous interference (between unrelated viruses) and homologous interference (between slosely related viruses). In circumstances of high virus multiplicity, a special type of homologous interference may occur between virions of a single virus type and strain which has been called Autointerference. The issue may be further complicated by the fact that certain agents such as the rous inhibitory factor and herpes viruses can interfere directly with homologous virus (Rubin 1960, Roizman 1965) and can also induce IF (Bader 1962, Aurelian and Roizman 1965) so that special care is required to determine the mechanisms at work in any particular situation.

Means of direct competitive interference include the destruction of cytoplasmic membrane receptors (Crowell 1966) and competition for intracellular sites of synthesis as in mixed enterovirus infection (Cords and Holland 1964). Roizman (1965) showed that infection of non permis-

sive cells with a conditional lethal mutant of herpesvirus resulted in the formation of non-functional subunits coded for by the defective virus which interfered with the replication of a virulent mutant simultaneously introduced. The transmissable interfering component of vesicular stomatitis virus which gives rise to autointerference was first described in 1959 by Cooper and Bellett and has since been widely investigated.. Several workers have shown that the effect is mediated by a defective, incomplete form of the virus which interferes with the replication of the complete virion, possibly by introducing a coding error in the order of protein synthesis (Huang and Wagner 1966, Crick et al 1966, 1969 etc.).

It seems likely that both direct and interferon mediated interference figure among the host defences against FMD. The early work of Gildmeister and Helm (1932) with vaccinia and of Michelsen (1946) and Schafer (1946) with strains of FMD in guinea pigs showed that the virus was susceptible to both homologous and heterologous interference. The virus has been shown to induce IF in a number of tissue cultures of bovine origin (Dinter 1960, Dinter and Phililson 1962, Sellers 1963, Sellers et al 1968b and c). FMDV is also susceptible in vivo and in vitro to the action of a number of interferons (or interferon like substances) produced in response to viral agents other than FMD and to several non viral inducers. The viruses of Newcastle disease (Giacometti et al 1968, Gizatullin and Gumerov 1969) and Sendai (Sellers et al 1972) do not replicate in the guinea pig but will interfere with the replication of FMDV and a similar effect has been observed in calves treated with Semliki forest or Sendai virus (Sellers and Mowat unpublished results, Sellers et al 1972). Non viral inducers such as yeast RNA (Thely et al 1963), phytohaemagglutinin (Richmond 1969), Freund's complete adjuvant (Gorhe 1967) and synthetic anionic polymers (Sellers et al 1972, Richmond 1971) are also known to induce IF inhibitory to FMD.

Several workers have reported that avirulent strains of virus, naturally occurring or artificially produced, often induce higher levels of IF than more virulent strains (see Isaacs 1963). This phenomenon is by no means universal but in FMD some modified strains did induce more IF than their virulent counterparts in several in vitro systems (Sellers 1963, 1964, Sellers and Mowat 1968, Sellers et al 1968[a], 1968b) and the use of this trait has been suggested as a genetic marker of attenuation (Sellers et al 1968c).

The basic mechanism of IF action against FMD has been studied in tissue culture by Ahl (1970, 1971). He showed that strains of FMD could be classified according to an increase or lack of increase in their If sensitivity at infra or supra optimal growth temperatures and that IF sensitivity was potentiated by the removal from cells of divalent cations. Two independent anti-viral factors were distinguished in IF from cattle or tissue culture on the basis of physico-chemical and biological properties. Factor 1 was relatively acid sensitive and reduced the number of infective centres in a suspension of infected cells. Factor 2 was relatively unstable at 56°C and reduced the yield of virus per infective centre.

There are few reports of the occurrence and action of IF in natural infections of domestic stock with FMD. Cunha et al (1958) and Cottral and Gailiunas (1971) have described simultaneous inoculation of cattle with two or more types of FMD when, with a single exception, one type assumed sominance, possibly as a result of interference and/or IF production. Kubin (1961) described interference between the viruses of coital vesicular exanthema and FMD in cattle. Sellers and Mowat (1968) and Sellers et al (1968c) have shown that autointerference can occur within strains of modified, avirulent FMD since diluted preparations gave rise to lesions when inoculated into the bovine tongue whereas undiluted

virus gave rise to no lesions or lesions of reduced extent and severity. The same authors reported that modified FMDV could interfere with the development of tongue lesions in cattle when inoculated with virulent virus. They were also able to show that a mixture of virulent FMDV and IF showed less viral multiplication and cytopathegenicity than virulent virus alone in tissue culture, but were unable to demonstrate a similar effect when the mixtures were inoculated into the dermis of the bovine tongue. This failure may well have stemmed from technical problems concerning the retention of the inoculum in the lingual tissue and from differences between the two systems in the number and type of cells involved.

While it is probable that autointerference and IF act to limit the spread and promote the early regression of oral and other lesions in FMD, no direct evidence is currently available to this effect.

Local tissue hypoxia, hyperosmolarity and acidity have been accorded a non specific protective function in viral infections (Lwoff 1959, Bang and Luttrel 1961, Baron 1963 and 1967). These factors often occur together since inflammatory foci are associated with oedema and fibrin deposition which tends to impede the inward diffusion of oxygen, the outward diffusion of carbon dioxide and the transport of other acid metabolites. In addition, leucocyte infiltration tends to increase the accumulation of acid products and lowered oxygen tension is reflected in a shift towards anaerobic glycolysis with increased production of lactic acid. None of these effects has been specifically investigated in FMD but pH values of 5.5 - 6.5 have been recorded in inflammatory sites (Frunder 1953) and such values are likely to have a profound inactivating effect on a virus of extreme acid sensitivity. No studies have been made of the pH values of secretions from animals suffering from FMD, other than those on milk (Burrows et al 1971).

Cellular infiltration occurs in acute viral vesicular foci (Platt 1961, 1966, 1967) and involves lymphocytes and macrophages. Their function is undefined in FMD but presumably includes the traditional role of phagocytosis followed by intracellular breakdown (Mims 1964, Silverstein 1970, Smorodintsev 1960) and their more recently discovered role as producers of IF (Glasgow 1970) and processors of antigen as a first step in the priming of cells which are to play a part in antibody or cell mediated immunity (Craddock et al 1971, Mackaness 1970, 1971, 1972, Playfair 1971, Inanue and Cerottini 1970). There is no evidence to suggest that FMDV either grows in macrophages or attains distant target organs as a passenger in macrophages by the means employed in other viral diseases (see reviews by Rowley 1962, Mims 1964, Gresser and Lang 1966, Allison 1971). FMD viraemia, like that of other picornoviruses (Fenner 1968) is not cell associated (Burrows et al unpublished data).

Pyrexia is a normal component of the host response to FMD typically occurring during the peak of the clinical syndrome and for one or two days before and after it. As in many other diseases, the teleological significance of fever in FMD is difficult to assess (Fenner 1968, Allison 1971, Mims 1972). Elevation of body temperature may be associated with decreased virus growth as shown experimentally in animals with the viruses of coxsackie type B1, myxoma, Semliki forest, polio, Newcastle disease (Bennet and Nicastri 1960, Baron 1963) and canine herpes (Carmichael and Barnes 1969). Lwoff (1959) recorded that strains of polio virus of low virulence for monkeys grew poorly in tissue culture at febrile temperatures while mice infected intracerebrally with virulent strains survived longer if maintained 2 - 3°C above their normal body temperature. The same author (Lwoff 1969) reviewed the extensive studies of the effect of temperature on polio virus and proposed the unifying concept that fever resulted from increased metabolism due to viral

infection together with the release of pyrogenic substances from leucocytes mobilised as part of the inflammatory response. Temperatures above those normally found in the host act against the virus in at least two ways. Firstly they may depress viral multiplication by the inhibition of viral replicase and secondly they may increase lysosomal lesions so that enzymes, including ribonucleases, are released to destroy viral RNA. The correlation between optimal growth temperature and severity of disease is by no means absolute for all viruses but Barr (1969) has shown that strains of FMDV adapted to grow at 25 and 30°C were generally of reduced virulence for cattle, while strains adapted to grow at 40°C were often of high virulence. Hypothermia may contribute to host resistance against FMD by the mechanisms discussed but there is no direct evidence to support this view. The fact that the febrile response corresponds with the period of maximum symptoms does not alone enable us to ascribe a protective function and as fever usually occurs late in the incubation period of FMD following contact infection, it possibly occurs as a response to and a reflection of antigen-antibody complex formation or to generalised cell mediated immunity (Mims 1972).

The notion that antibody was an important factor in protection against disease gained general acceptance in the period 1880 - 1900 and passive immunisation against FMD was used prophylactically at the turn of the century.(Kitta 1892). Resistance to reinfection with FMD is thought to be largely attributable to the action of specific neutralising antibody. Four lines of evidence adduce its protective function, firstly, the correlation between the presence of neutralising antibody and resistance to challenge, secondly the correlation between the absence of neutralising antibody and susceptibility to challenge, thirdly the ability of passively transferred antibody to protect against challenge and fourthly, the specificity of antibody for a given viral antigen.

Tiselius and Kabat (1939) separated serum by electrophoresis and showed that antibodies were principally confined to the gamma globulin fraction. Brooksby (1949) and Bradish et al (1954a and b) applied this technique to the quantitative investigation of serum from normal and FMD infected cattle, showing that β globulin level rose 7 days after tongue inoculation and regained the normal level during the following week while the γ globulin rose 3 to 4 weeks after infection and remained high for several weeks.

FMDV has at least four associated antigens; the complete virion (140s), the 'empty' capsid (75s), the capsid subunit (12s) and the virus infection associated antigen (V.I.A., 4.5s) (Bachrach 1968, Brown 1972). All components react in complement fixation and agar gel precipitation tests but only 140s and 75s antigens give rise to neutralising antibody and immunity. The 140s and 12s components elicit antibodies of different electrophoretic mobility. Antibodies to the intact particle are type specific whereas antibodies to the subunit show a considerable degree of cross reaction between types in precipitin and complement fixation tests (Brown and Crick 1958). Antibody to the three larger antigens occurs in animals after disease or vaccination but antibodies to the V.I.A. component are found only in convalescent animals (Cowan and Graves 1966).

The serum antibody response after infection or immunisation with FMDV is typical for foreign protein. Early antibodies are detectable in cattle within 3 - 5 days of tongue infection, reach a peak at 7 - 14 days and gradually decline below detectable levels within 30 days (Brown et al 1964, Cowan 1966). They are macroglobulins of the IgM class (by analogy with human immunoglobulins) having a sedimentation coefficient of 19s, a fast γ1 or β2 globulin electrophoretic mobility (Brown 1960) and are sensitive to reductive cleavage with mercaptoethanol (Brown et

et al 1964). Early antibody will neutralise and precipitate FMDV, both homologous and heterologous (Brown and Graves 1959, Cowan 1966) but does not fix complement to any appreciable extent (Cowan 1966).

Later in infection, at 10 - 14 days, a second antibody type appears (Brown and Graves 1959) reaching a peak at about 28 days and persisting for months and years after infection (Cunliffe 1964, Cowan 1966, Graves et al 1972). Late antibody belongs to the IgG class having a sedimentation coefficient of 7s, both fast and slow γ globulin electrophoretic mobility (Brown 1960, Cowan 1966) and being resistant to the action of mercaptoethanol (Brown et al 1964). The 7s antibody shows neutralising, precipitating (Brown and Crick 1958) and complement fixing (Cowan 1966) activity of a type specific nature.

Skinner (1953) reported that bovine serum taken 7 days after infection with FMDV would neutralise both homotypic and heterotypic types of virus whereas 21 day serum was type specific in its neutralising activity. This effect was further confirmed and extended to include precipitin reactions between subtypes by early but not by late antibody (Brown and Graves 1959, Brown 1960). A mixed population of antibody types is also found in the IgG class since neutralising antibody persists for at least 4½ years (Cunliffe 1964, Graves et al 1972) whereas complement fixing antibody can no longer be detected after 90 days (Cunliffe 1964) or declines more rapidly and persists at a much lower level than neutralising antibody for up to 9 months after infection (Cowan 1966).

The antibody response of cattle to inactivated vaccines has also been studied and closely resembles that described following infection (McKercher and Giordano 1967, Chappuis et al 1971). A similar, non specific neutralising component is found mainly in the IgM fraction (Peleg and Modan 1972, Kalmar and Peleg 1972) but antibody to V.I.A.

is absent (Cowan and Graves 1966).

The extensive studies on the serum antibody response of the guinea pig to FMD are not reviewed here. In general they resemble those quoted for cattle although recent work has demonstrated that 19s guinea pig antibody was capable of differentiating antigenic variations within a strain of FMDV which were not detectable using 7s guinea pig or 19s and 7s cattle antibody (Wagner and Cowan 1971). Analogies should therefore be drawn with caution.

Passive immunisation has shown the protective nature of serum antibody in FMD. Antibody is secreted in the colostrum of convalescent or immunised cows (Schneider 1955, Stone and De lay 1959) and calves which suckle an immune dam rapidly acquire serum antibody (Graves 1963, Van Bekkum 1966) which will interfere with vaccination for 3 - 4 months (Wisniewski and Jankowska 1971, 1972) and protect against homologous challenge for at least 6 weeks (Srubar 1966). Passive immunisation using whole blood, convalescent plasma or immune and hyperimmune serum was extensively used in Europe prior to the advent of FMDV vaccines and was relied upon to give 10 - 14 days protection in the field (Galloway 1954). Brooksby (1949) demonstrated that protection could be passively transferred between adult cattle by purified and concentrated convalescent serum antibody and suggested that the dose of serum and the degree of protection were quantitatively related. Similar experiments were reported by Sutmoller et al (1968) who also pointed out that protection did not include freedom from pharyngeal infection. Many authors have reported that the protective efficacy of FMD vaccines can be estimated from the neutralising antibody titre of the serum (Martin and Chapman 1961, Van Bekkum et al 1963, 1969, Graves et al 1972 etc.) although individual variations and discrepancies do occur following vaccination (Borgen et al 1962, Graves et al 1968, Sutmoller and McVicar 1972) and

passive immunisation (Sutmoller et al 1968).

Preliminary studies on FMD antibodies in external secretions are fully discussed in section 5.2..

Cell Mediated Immunity (CMI) which resides in specifically sensitised, thymus-derived lymphocytes and may act via macrophages, is transferrable by cells (as opposed to serum) and is responsible for delayed hypersensitivity and homograft rejection phenomena. It has recently been ascribed significance in immunity to a number of viral conditions, particularly those caused by pox, herpes, leuko and some myxo and paramyxo viruses as reviewed by Allison (1971), Allison and Burns (1972) and Mims (1972). Evidence for the importance of CMI derives from clinical and experimental observations. Hypogammaglobulinaemic subjects recover normally from a number of viral infections (Good et al 1962). Persons with congenital abnormalities of CMI are prone to severe, recurrent and often fatal attacks by viruses which produce a much milder syndrome in the normal host, for example, thymic dysplasia is often associated with unusually severe pox virus infection despite a normal antibody response (Rossen 1968). Immunosuppressive techniques often exacerbate viral infections (Allison 1967) while immunity can often be restored by the transfer of sensitised lymphocytes as Blanden (1971) conclusively showed with ectromelia infection in mice.

Suppression of CMI by the use of thymectomy or anti-lymphocyte serum does not appear to potentiate infections due to certain enteroviruses or arboviruses. The use of agents which suppress antibody, such as cyclophosphamide, does potentiate them and normal immunity can be restored by the transfer of antibody (Murphy and Glasgow 1968, Zisman and Allison 1973, Zisman et al 1971, Zlotnick et al 1970). Some authorities have suggested that CMI may be important in generalised diseases and perhaps

plays no part in superficial infections (Mims 1972). Nevertheless use of the Macrophage Migration Inhibition techniques showed the presence of antigen sensitive lymphocytes in bronchial washings following immunisation with inactivated influenza virus (Waldman and Henney 1971, Waldman et al 1972) or attenuated respiratory syncytial virus (Chanock 1970) so that CMI is at least a possibility in these regions. Cell Mediated Immunity has not been investigated in FMD but it is reasonable to assume, from the data given above on antibody and the fact that the disease is caused by enterovirus, that it may not be of paramount importance.

Convalescent cattle may show a total clinical immunity to the same type of FMDV for 3 - 4 months after infection, resisting even challenge by tongue inoculation. Partial susceptibility may then ensue since localised lesions can develop at the inoculation site on the tongue but generalisation is resisted (Trautwein 1929, Galloway 1954, Davies et al 1963 etc.). Cunliffe (1964) investigated the duration of immunity in steers to homologous challenge following type O infection. At 335 - 363 days post infection 7 animals were challenged by tongue inoculation and, although primary vesicles developed at several inoculation sites in all animals, fever and secondary lesions were absent and disease did not spread to convalescent animals in contact. At 1708 days post infection 2 of 3 animals similarly challenged developed generalised disease but the third steer remained free of detectable lesions.

Cottral and Gailiunas (1971) confirmed an early observation by Trautwein (1927) when they showed that sequential infection of cattle with up to 7 types of FMDV resulted in a cumulative effect towards resistance but complete heterotypic resistance was not encountered until at least four different types had been applied. Cross neutralisation test reactions on serum antibody correlated well with challenge results. These observations are of mainly academic interest, however, since

infection with one type of FMD lends no resistance against heterologous challenge and repeated exposure to multiple types is rare, even in areas where 2 or 3 types of virus are endemic.

5.2. Local Immunity

References to protective substances in secretions are scattered through the early literature such as the finding of agglutinins to typhus in human faeces (Staubli 1903) and the demonstration of poliovirus neutralisation by human nasal washings (Amos and Taylor 1917) but the existence of a specialised, independent immune system operating at the external surfaces of the host seems to have been conceived by Besredka (1919, 1927). His studies of infections of the alimentary tract with enterobacteriaceae and the skin with B. anthracis led him to postulate the existence of such a system and to suggest that it might be manipulated in favour of host immunity.

Much original and subsequent work concerned infections due to bacteria, helminths and protozoa. These extensive studies have been reviewed by Pierce (1959), Tomasi and Bienenstock (1968) and Duncan (1971) and will only be alluded to in those areas which have pertinence for virology.

The initial observations of viral neutralising activity in secretions was greatly expanded in the period 1940 - 1954 by the work of two research groups under the leadership of Francis and Fazekas de St Groth who carried out systematic studies on local immunity in mice and men following infection or vaccination with influenza virus. Francis (1940) showed that convalescent human nasal washings could neutralise the virus and soon afterwards this effect was attributed to the presence of specific antibody (mucoantibody) in the secretions of the respiratory tract

(Francis and Brightman 1941, Francis 1943, Francis et al 1943). Intranasal exposure of mice to influenza was followed by the simultaneous appearance of antibody in bronchial mucus and in serum (Fazekas de St Groth and Donnelly 1950a, b and c). Similar results followed subcutaneous vaccination in man using live or inactivated virus, moreover antibody titres in secretion and serum showed parallel fluctuations (Francis et al 1943). Resistance to reinfection was thought to be primarily mediated by local antibody while antibody in serum was relatively ineffective. The evidence was circumstantial, being based on the fact that protection could be more closely correlated with the titre of antibody in respiratory secretion than with the titre in serum. Higher antibody levels were found in secretions after intranasal instillation than after parenteral inoculation of virus (Fazekas de St Groth and Donnelly 1950a). However, after passive immunisation a proportion of serum antibody could attain the secretions (Fazekas de St Groth and Graham 1954) and this proportion could be greatly increased by the intranasal application of heterologous virus, meta-periodate ion or certain astringents (Fazekas de St Groth et al 1951b). This latter phenomenon, so called 'pathotopic potentiation', was ascribed to mild inflammatory change with increased capillary permeability allowing the escape of serum antibody into secretions (Fazekas de St Groth 1951a).

In 1959 Heremans and co-workers discovered the IgA class of antibody. IgA differs from the other immunoglobulins (IgM, IgG, IgE and IgD) in immunological and physicochemical properties and also in distribution since it is a minor globulin type of serum but forms the major immunoglobulin (Ig) of human external secretions (Hanson 1961, Chodirker and Tomasi 1963). IgA has since been studied increasingly and the origin, structure, properties and function of the class have been reviewed by Tomasi and Bienenstock (1968), Heremans (1968, 1970), Tomasi (1970, 1972), Brandtzaeg (1973) and Vaerman (1973). IgA exists in two forms, the 7s

monomer with a molecular weight of approximately 160,000 daltons being found mainly in serum and the 11s dimer, comprising two monomers conjugated with an epithelial glycoprotein (the secretory piece or component) with a molecular weight of about 390,000 being found mainly in external secretions. The globulin is chiefly synthesised by plasma cells situated in the stroma of mucosae and glandular structures, close to the external surfaces of the body and, in man, constitutes the predominant Ig in milk, colostrum, saliva, tears, nasal fluid, bronchial fluid, gastrointestinal secretions, bile and urine. The exact function of the secretory piece is unknown but it does confer additional resistance against proteolytic enzymes and may serve as a specialised surface receptor holding IgA molecules at epithelial surfaces. In man, IgA predominates in external fluids but IgM, IgG and to a lesser extent IgD and IgE may also be found.

Bovine immunoglobulins analogous in physicochemical and antigenic characteristics to human IgG, IgM and IgA have been identified in serum and secretions and several recent reviews have been devoted to them (Butler 1969, 1973, Sullivan et al 1969, Aalund 1972, Duncan et al 1972, Porter 1973). IgG exhibits a particularly well defined heterogeneity in cattle with two prominent subclasses, IgG_1 and IgG_2 (Milstein and Feinstein 1968). IgG_2 is mainly confined to serum while IgG_1, with the lower isoelectric point, comprises from one half to two thirds of the serum IgG and nearly all the IgG in external secretions (Sullivan et al 1969, Duncan et al 1972). IgA is a minor globulin of bovine serum existing mainly as a dimer, unlike the monomeric form encountered in human serum (Mach and Pahud 1971, Butler 1971), and it is the main globulin of most external fluids, where it is found complexed with secretory piece (Vaerman 1970). IgA forms a relatively small part of the globulin in milk and colostrum where IgG_1 predominates, in contrast to the dominance of IgA in these secretions in man (Murphy et al 1964, Aalund 1968 etc.). Complete immunological cross reactivity has been demonstrated between

the heavy chains of human and bovine immunoglobulins (Murphy et al 1965) and free bovine or human secretory piece will combine equally readily with IgA from either species (Mach 1970, Mach and Pahud 1971).

Most bovine secretions have been investigated for immunoglobulin type and content and although quantative values differ widely between authors, probably as a result of different methods of collection and assay, the general pattern is fairly consistent. IgA has been identified in all secretions and is the principal globulin type in tears (Mach and Pahud 1971, Butler et al 1972, Pedersen and Nansen 1972), nasal fluid and saliva (Mach and Pahud 1971, Duncan et al 1972) and intestinal secretions (Porter and Noakes 1970, Mach and Pahud 1971). IgG_1 is commonly found in secretions and, in addition to its predominance in milk, colostrum and serum, it occurs in approximately equal amounts with IgA in small intestinal and vaginal mucus, spermatic fluid, bile and urine (Mach and Pahud 1971, Duncan et al 1972). Traces of IgG_2 are found in most secretions as the minor component although vaginal mucus, spermatic fluid, bile and colostrum contain appreciable amounts (Mach and Pahud 1971, Duncan et al 1972). IgM attains its highest levels in serum (Butler 1973) but also occurs in tears (Pedersen and Nansen 1972) and nasal fluid (Duncan et al 1972) in levels intermediate to those of IgA and IgG_1 as well as being a minor globulin of saliva, bile, milk and colostrum. Albumin has the highest concentration of any serum protein but only low levels have been recorded in tears, nasal fluid, saliva, milk and vaginal mucus (Butler et al 1972, Duncan et al 1972). Having the highest molecular weight of the serum proteins, the molecule is least likely to attain the secretions by diffusion and the serum:secretion ratio for albumin has been used as an index of serum protein transudation (Duncan 1971).

Immunoglobulins occur in secretions as a result of simple transud-

ation or selective transfer from serum, of local synthesis and release from superficial sites or by a combination of these. The overspill of serum protein has been studied in man using passively transferred, radioactively labelled globulin of various classes and, in general, such studies indicate that very little IgA is derived from the blood by transudation and only up to half the IgG content of secretions is derived in this way (Tomasi et al 1965, Butler et al 1967, Rossen 1971). Studies with IgA have not been made in cattle but similar experiments with IgG_1 and IgG_2 indicated some selectivity in the transport of IgG_1 in the gut, respiratory system (Curtain 1971), and eye (Sullivan et al 1969, Pedersen and Nansen 1972) and a highly developed system of selective transport in the mammary gland (Dixon et al 1961, Murphy et al 1964). Sullivan et al (1969) sampled the serum and secretions of a calf before suckling and showed them to be devoid of globulin. IgG_1 was detected in serum, tears and saliva 24 hours after the ingestion of colostrum suggesting the serum origin of secretory globulin. In contrast Provost (1970) was unable to demonstrate rinderpest antibody in the nasal secretion of calves born of immune dams although colostrum-derived serum antibody was plentiful. However, unusual membrane permeability is a feature of the neonatal calf and additional confirmation of these effects is required. The vast majority of IgG_1 in all secretions other than milk and colostrum is thought to be locally synthesised (Curtain et al 1971, Mach and Pahud 1971).

Local synthesis has been investigated in two ways. Firstly: immunohistochemical examination of human tissues using antisera specific for each class if Ig has usually shown that the majority of plasma cells in the lamina propria of the gut, the nasal and bronchial submucosa and the interstitial spaces of the salivary gland contain IgA while only a minority contain IgG or IgM (Brandtzaeg 1968, Tourville et al 1969a etc.). A similar situation obtains in the bovine gut (Vaerman 1970, Yurchak et al

1971, Porter et al 1972, 1973). Secondly: the synthesis of specific Ig types has been studied in vitro using explanted tissues nourished with media containing radioactive amino acids. In man, radioactively labelled IgA was produced by gastro intestinal tissue and by parotid and mammary gland. A little labelled IgG_1 was sometimes detected but no labelling of IgM occurred (Tomasi et al 1965, Rossen et al 1971 etc.). In cattle, IgA was the principal immunoglobulin synthesised by the ileum, duodenum, colon, lungs, nasal mucosa, oral pharynx and the parotid and lachrymal glands (Porter and Noakes 1970, Butler et al 1971, Mach and Pahud 1971, Hurliman and Darling 1971). Butler and colleagues (1973) confirmed these findings and, in extending them, drew attention to the fact that tissues in close anatomical proximity may synthesise different types and amounts of globulin. For example, they scored the production of IgA, IgG_1, IgG_2 and IgM on autoradiographic intensity as 2, 2, 2 and 1 in upper nasal mucosa and as 3, 1, 1 and 1 in lower nasal mucosa.

Quantitative studies on bovine secretory globulins have demonstrated wide fluctuations in values between animals, between samples in a 12 weekly series, before and after calving and even between quarters of the mammary gland (Butler et al 1972a, Duncan et al 1972). Breed has been considered as a source of differences (Butler et al 1972a). Disease can also alter membrane permeability and, in consequence, the distribution of Ig. Thus in mastitis IgG_2, present only in trace amounts in normal milk, may achieve levels equal to those of the principal globulin type, IgG_1 (Butler et al 1972a). Similarly, albumin may attain the secretions during inflammatory processes (Butler et al 1970, Duncan et al 1972).

The existence of a more or less distinct immunological system in external secretions has therefore been adduced from a number of interrelated observations: 1. the distribution of Ig types varies widely

and, in most instances, independently in serum and secretions. 2. IgA accounts for 60 - 100% of the Ig content of most external secretions but only 10 - 20% of the serum Ig. 3. IgA exists as different molecular species in serum (7s) and in secretions (11s). 4. Except in the case of lacteal secretion, plasma Ig contributes little to the protein content of external fluids. 5. The plasma cell population of superficial situations chiefly secretes IgA. 6. There is often a dissociation between the levels and class of antibody in serum and secretions following infection or immunisation. 7. Resistance to certain infections may be better correlated with secretory rather than humoral antibody.

Elucidation of the secretory immune system gave fresh impetus to investigations of pathogenesis and protection. Local immunity might be expected to exert its greatest influence against those diseases which are initiated in or which confine their effects to superficial sites in the host. It is not surprising therefore that much attention has been paid to its role in viral conditions of the respiratory and alimentary tracts. Work in the human has centred on polio (reviewed by Ogra and Karzon 1971) and influenza (reviewed by Mills et al 1970 and Rossen et al 1971), but many diseases have been investigated including those caused by parainfluenza virus (Artenstein et al 1964, Smith et al 1966, 1967, Tremonti et al 1968), rhinovirus (Perkins et al 1969a and b, Cate et al 1966, Douglas et al 1967, Butler et al 1970), respiratory syncytial virus (Anderson et al 1962, Kim et al 1969, Scott et al 1970), adenovirus (Artenstein et al 1964, Bellantini et al 1969, Chanock 1970), echovirus (Artenstein et al 1964, Ogra 1970), coxsackievirus (Rossen et al 1965, Bellantini et al 1965), measles (Bellantini et al 1969), rabies (Sirisinha and Charaputana 1970), rubella (Ogra 1971, Craddock et al 1973). and herpes (Centifanto et al 1973) viruses.

In the veterinary field, aspects of local immunity have been studied

in respect of influenza in horses (Rouse 1971, Rouse and Ditchfield 1970a, b and c, Fontaine 1972) and in ferrets (Potter et al 1972, Shore et al 1972), parainfluenza in calves (Bogel and Liebelt 1963, 1964, Gutenkunst et al 1969, Gates et al 1970, Morein 1970, 1972, Provost 1970, Marshall and Frank 1971, Frank and Marshall 1971, McKercher et al 1972, 1973, Bernhardt and Bengelsdorff 1973), infectious bovine rhinotracheitis in cattle (Provost 1970, McKercher and Crenshaw 1971, Todd et al 1971, 1972), rinderpest and mucosal disease in cattle (Provost 1970, Provost and Borredon 1972) and FMD in cattle (Hyslop 1965a, Scott 1966, Kaaden and Matthaeus 1970, Hedger 1970, Sutmoller 1970, Burrows et al 1971, Figueroa et al 1973) and in pigs (Wittman 1972).

Studies divide into those concerning infection and those concerning immunisation while further subdivisions are possible according to the use of inactivated or attenuated vaccines and the use of parenteral or topical routes. Secretory antibody usually appears in external secretions, as it does in serum, following infection with all the viruses listed above. Exceptions are known e.g. 7 children exposed to parainfluenza or respiratory syncytial virus showed a rise in nasal antibody but an equal number showed a fall and in 3 the level remained unchanged (Kim et al 1969). Nasal secretion, saliva and tears have been most commonly examined and specific neutralising activity has been shown to reside principally in the IgA fraction after infection of man with influenza, coxsackie (Butler et al 1970), parainfluenza (Smith et al 1967), rhino (Rossen et al 1966) and polio (Ogra et al 1968) viruses. Sputum (Mann et al 1968, Waldman et al 1970a and b, 1972b) and broncho-alveolar lavage fluid (Waldman et al 1973) showed a similar pattern after exposure to influenza virus. Gastrointestinal secretions also develop neutralising antibody of the IgA class after polio (Ogra and Karzon 1969a and b) and coxsackie virus infection (Ogra 1970). In studies with measles (Bellatini et al 1969) and polio (Ogra et al 1968) an IgG_1 component has

also been detected. Less classification of globulins has been undertaken in animals but cattle infected with parainfluenza (Morein 1970, 1972) or FMD (Kaaden and Matthaeus 1970) develop specific neutralising antibody in nasal and salivary secretions which is almost exclusively IgA. Provost (1970) however, found nasal antibody of both IgA and IgM classes in calves following rinderpest infection.

Little is known of the temporal development of the secretory antibody response. Antibody may be measurable within a few days of infection with polio (Ogra and Karzon 1971) but several workers have shown that, despite an immediate and lasting rise in the IgA concentration of nasal secretions, neutralising antibody may only develop 10 - 14 days after exposure to respiratory viruses (Alford et al 1967, Rossen et al 1971). Wide variations are also reported in the duration of the response. Values of 16 days (Rossen et al 1965) have been reported in human nasal secretion after infection with coxsackie type A_{21} virus, 3 weeks (Alford et al 1967) or up to 6 months (Kasel et al 1969, Waldman et al 1969) after influenza, up to 2 months after rhinovirus (Perkins et al 1969) and 2 years or 15 years after polio (Ogra and Karzan 1968, 1971). These variations probably stem from different methods of collection and assay together with the difficulty of precluding reexposure to these agents.

Few observations have been reported for cattle. Morein (1970) detected neutralising activity in the nasal secretion of calves naturally infected with parainfluenza type 3 virus. Neutralisation was demonstrated shortly after infection, persisted for at least 2 months and was contained in the IgA fraction. Marshall and Frank (1971) also detected neutralising activity in nasal secretions and serum from 2 calves at maximal levels within 6 days of infection with an aerosol containing $10^{8.5}$ $T.C.I.D._{50}$ of parainfluenza type 3 virus. Serum and secretory antibody levels were followed over a 17 week period. In one calf neither

class of antibody declined while in the other calf serum antibody remained high but nasal antibody showed a gradual fall. The secretory response was dependent upon dose, since a third calf exposed to $10^{3.5}$ T.C.I.D.$_{50}$ showed only a very slight, transient rise in nasal antibody and also on route of administration, since much less secretory antibody was produced when the intramuscular route was used. Repeated aerosol exposure also gave rise to increased humoral and secretory antibody.

Hyslop (1965a) showed that the oral saliva of normal steers possessed only slight, non specific antiviral activity whereas saliva collected 4 weeks after infection with FMD contained specific neutralising activity. Sutmoller et al (1967, 1970)and Figueroa et al (1973) also found that type specific neutralising activity developed in the secretions of convalescent cattle, sheep and goats. The reports provide few details but the activity was said to reach a peak 14 - 21 days post exposure to FMDV with parallel titres in pharyngeal, saliva and nasal secretions and uniformly higher levels in the latter. The effect was confirmed for pharyngeal fluid by Kaaden et al (1970) and Kaaden and Matthaeus (1970) went on to show by immunoelectrophoresis, dissociation and ultracentrifugation studies that the neutralising activity of 4 - 6 week convalescent bovine saliva was associated with a protein analogous to human secretory IgA.

Several workers have examined the relationship between antibody levels in secretions and recovery from or resistance to infection. Good correlation has been demonstrated in studies on rhinovirus (Cate et al 1966), parainfluenza virus (Smith et al 1966), influenza virus (Waldman et al 1969) and polio virus (Ogra and Karzon 1969a). Immunity was measured by an assessment of clinical disease and/or by the quantitation of viral multiplication and excretion after exposure and could be more closely related to the levels of secretory rather than serum antibody.

It must be emphasised that the data admit of possible correlation rather than proof. In other infections, such as those due adeno (Scott et al 1969), herpes (Centifanto et al 1970), rubella (Cradock-Watson et al 1973) and respiratory syncytial virus (Mills et al 1970) little or no correlation has been found between immunity and secretory antibody. Furthermore, while some persons suffering from selective IgA deficiency also suffer recurrent but usually mild infections, particularly of the respiratory tract (South et al 1965, Bellantini et al 1969), others may remain healthy (Tomasi and Bienenstock 1968, Heremans 1970).

Studies with attenuated viral vaccines produced results which closely resemble those found following natural infection, provided that the agent was applied topically, i.e. by aerosol or intranasal instillation. Parenteral vaccination usually stimulates a satisfactory humoral response but often elicits a very poor secretory response. Results of this type were obtained following vaccination with modified live virus vaccines against measles (Bellantini et al 1969) and influenza (Fazekas de St Groth 1950) viruses in man. Similar results were observed in cattle using attenuated viral vaccines of parainfluenza type 3 (Bogel and Liebelt 1963, 1964, Gutenkunst et al 1969, Gates et al 1970, Morein 1970, 1972, Frank and Marshall 1971, Marshall and Frank 1971, McKercher et al 1972, and Todd 1971), infectious bovine rhinotrachetis and bovine virus diarrhoea (Todd et al 1971, 1972). The reciprocal situation in which vaccination by the respiratory route gives rise to both secretory and serum antibody whereas parenteral vaccination produces only serum antibody, was also observed in many of the cattle experiments. Yet another response has been reported in man following infection with parainfluenza type 1 (Smith et al 1967) and influenza (Mann et al 1968) viruses when antibody developed in secretions but not in serum.

An interesting feature of the secretory immune system was noted by

Ogra and Karzon (1969b) who showed that the IgA response to poliovirus could be strictly localised. Implantation of modified poliovirus in different areas of the surgically isolated gut resulted in antibody formation only in the area of infection and in the serum but not in other regions of the intestine or pharynx. Coproantibody was exclusively IgA while serum antibody was of the IgM and IgG classes. Conversley, Douglas et al (1967) found rhinovirus only in the nasopharynx during infection but antibody levels rose in serum, saliva and tears as well as in nasal secretions. Duncan et al (1972b) recorded a similar finding in cattle following infection by intravaginal or intramuscular infection with v. foetus when serum, vaginal and nasal antibody achieved high levels but lachrymal or salivary levels were very low and variable.

Attempts to stimulate secretory immunity using inactivated viral vaccines have underlined the critical effect of dose rate and route as mentioned above and have generally produced less consistent results than attenuated virus (see reviews by Mills et al 1970 and Todd 1973). Human trials with dead influenza vaccines have compared the intranasal and subcutaneous or intramuscular routes. All three gave comparable serum antibody titres but appreciable levels of nasal or sputum antibody tended to develop more often, to higher titre and to persist longer after intranasal administration (Smith et al 1966, Waldman et al 1968, 1969, 1973,, Alford 1969, Fulk et al 1969, Downie 1970, 1973). Failure to produce nasal antibody was often associated with failure to protect against challenge. In one study similar low levels of antibody were detected in saliva after live intranasal or dead subcutaneous influenza vaccination (Mann et al 1968) but in most reports live attenuated preparations almost always elicited a greater secretory response. Virus serotype may influence the response since Kasel et al (1969) recorded that influenza A stimulated much more secretory antibody than influenza B when inactivated preparations were given intranasally.

Killed rhinovirus vaccine stimulated both nasal and serum antibody formation after intranasal administration (Perkins et al 1969a). Fulk and colleagues (quoted by Mills 1970) showed that inactivated parainfluenza type 3 virus stimulated nasal antibody in some persons after instillation in the nose. Chanock et al (1967) reported that parenteral immunisation with inactivated parainfluenza type 1 virus induced detectable levels of nasal antibody in only 12% of those vaccinated, as opposed to 83% of men infected intranasally with the virus. Similar results were quoted by Smith et al (1966). Ogra and co-workers (1968, 1969b) compared the effects of poliovirus infection and inactivated vaccination. Infection gave rise to antibody in serum and nasal secretions lasting for at least a year while intranasal vaccination produced only nasal antibody persisting for 2 - 3 months. Parenteral immunisation gave rise to serum antibody but no nasal or duodenal antibody was detected.

Subcutaneous administration of formalin inactivated parainfluenza type 3 virus in cattle failed to elicit nasal antibody after a single injection but did evoke such antibody after a second injection given 2 - 3 weeks later.(Morein 1970, 1972). Using the same virus, comparisons have also been made between dead vaccine administered as an aerosol and live vaccine given as an aerosol or parenterally. Serum antibody developed following attenuated vaccine regardless of the route of administration but nasal antibody was much more consistent and longer lasting after intranasal vaccination. The low titre of nasal antibody detected in some calves after live parenteral vaccination could be correlated with the titre concurrently present in serum. Low serum antibody levels developed after a single aerosol exposure to inactivated vaccine but nasal antibody was not detected. Calves reexposed in a similar manner 7 weeks later showed marked increases in serum antibody and developed low levels of nasal antibody (Frank and Marshall 1971, Marshall and Frank 1971).

In preliminary experiments FMD antibodies have not been detected in nasal secretion or saliva after a single application of inactivated antigen by the intranasal route in pigs (Wittman 1972) or cattle (Figueroa et al 1973). Hyslop (1965a) found low levels of antiviral activity, probably due to antibody, in the saliva of steers following subcutaneous immunisation with inactivated vaccine but Figueroa et al (1973) were unable to confirm this finding in calves.

Recent investigations of secretory immunity have concentrated on the IgA system almost to the exclusion of other factors. However, other mechanisms are at work. Interferon has been found in external secretions during the course of several viral infections of man (Cate et al 1969) and animals (Todd 1971, 1972, Rosenquist 1973) and Cell Mediated Immunity has also been demonstrated in the secretions of the respiratory tract (Henney and Waldman 1970, et al 1972, Ruben 1973, Wetherbee 1973). These factors have both been associated with recovery from primary viral infection and it is possible that other mechanisms, as yet unidentified, may also contribute to immunity at the external surfaces of the host.

6. MATERIALS AND METHODS

6.1. Tissue Culture

6.1.1. Primary Cultures: Calf Thyroid (CTY) (Snowdon 1966) and Calf Kidney (CK) (Sellers et al 1968a) cells were prepared by trypsinisation of minced thyroid gland or kidney cortex freshly obtained from local abattoirs. CTY cells were grown in equal parts of Eagle's medium and Hank's saline - yeast extract (0.01%) - lactalbumin hydrolysate (0.5%) medium (LYH) with 10% bovine serum and antibiotics. CK cells were grown in LYH medium with 10% ox serum and antibiotics. Cells were re-suspended at a rate of 5.0×10^5 viable cells per ml and 1.0 or 2.0 ml volumes were dispensed into rubber stoppered 153 mm x 16 mm glass tubes. In the case of CK cells, Roux flasks were also seeded with 100 ml of cell suspension. Monolayers became confluent after 5 - 7 days incubation in sloped, static racks at 37°C.

CTY cultures were used for the assay of FMDV in samples from cattle. CK tube cultures were used for the assay of interferon and CK Roux cultures for the production of standard IF preparations.

6.1.2. Continuous Cultures: Baby Hamster Kidney, strain 21, clone 13 cells (BHK) originated by Macpherson and Stoker (1962) were grown in Eagle's medium with 10% tryptose phosphate broth, 10% bovine serum and antibiotics. Cultures were seeded in Roux flasks (100 ml), 20 oz. medical flats (80 ml) or 6 cm glass or plastic petri dishes (5 ml) at a concentration of 5.0×10^5 viable cells per ml and incubated at 37°C until confluent 24 - 48 hours later. Petri dishes were held in gassed incubators supplied with air containing 5% carbon dioxide.

The IB-RS-2 cell line, a continuous pig kidney cell culture

originated by De Castro (1964), was grown in LYH medium with 10% bovine serum and antibiotics as described for BHK cells.

BHK and IB-RS-2 cultures were used in the production and assay of stock and concentrated FMD viruses. IB-RS-2 cultures were also used as an assay system in studies on the kinetics of virus inactivation.

6.1.3. Media: All media were supplied by the Tissue Culture Production Department of this Institute which also provided CTY cell suspension and BHK monolayers. Media contained Phenol Red (0.001%) as a pH indicator and antibiotics were routinely incorporated to a final concentration of: penicillin, neomycin and polymixin B each 100 units/ml, mycostatin 50 units/ml and streptomycin 400 µg/ml.

Cell monolayers were washed with 0.01% phosphate buffered saline free of calcium and magnesium ions prior to harvesting by means of a versene (0.01%) - trypsin (0.02%) solution.

6.2. Animals, (Breed, Management and Infection)

Mice, guinea pigs and rabbits were obtained from the breeding colony of this Institute. Cattle and pigs were supplied by a dealer.

6.2.1. Mice: suckling mice of the albino 'P' (Parkes) strain were kept as randomised litters of 10 with one or two lactating females in tins with sawdust litter and woodwool bedding. Pelleted diet was fed from hoppers and water was provided ad libitum. Mice were used in virus neutralisation studies.

6.2.2. Guinea pigs: groups of adult guinea pigs of the albino Hartley Dunkin strain weighing at least 500 g were kept in cages and fed on

pelleted diet with cabbage and grass in season and water ad libitum. They were used as a source of anti bovine globulin serum.

6.2.3. Rabbits: Adult rabbits of the New Zealand White or Dutch Half Lop breed were kept singly in cages on pelleted diet with water ad libitum, hay and green food in season. Rabbits were employed in the production of anti bovine globulin serum.

6.2.4. Pigs: Large White crossbred pigs weighing 20 - 30 Kg were housed in disease secure isolation units and fed on a proprietary balanced meal with water freely available. Pigs were infected with FMDV by inoculation to provide challenge virus for cattle.

6.2.5. Cattle: Steers of the Devon breed, 12 - 18 months old and weighing approximately 400 Kg, were supplied from the south west of England. The breed has been used at this Institute as the standard bovine animal for FMD research and the management and accommodation has been described by Henderson (1952) and Barr (1965). Up to 3 animals were housed in each loose box (3.65 m x 3.35 m x 3.05 m) in disease secure isolation units from which air was extracted through high efficiency filters. The daily ration included 4 lb mixed bran, rolled oats and concentrated cake together with 2 lb of concentrates fed in the morning and 8 lb of long hay fed in the afternoon. Alternatively 6 lb of lucerne cobs were given twice daily. Water was available ad libitum. Boxes were cleaned out daily.

6.2.6. Infection of animals with foot and mouth disease virus:
a) Infection of donor pigs: pigs were infected by the intradermal inoculation of stock FMDV into the bulbs of the heels of both fore feet. Eight sites were used, two on each claw, and animals received a total of approximately $10^{5.0}$ p.f.u. of virus as measured in IB-RS-2 cells.

Pigs usually developed primary lesions within 24 hours of inoculation and generalised disease ensued within 72 hours.

b) Infection of cattle: Cattle were infected by direct or indirect contact with FMDV excreted by donor pigs. Exposure was mainly confined to the period 48 - 58 hours after inoculation when donors were developing generalised disease and viral excretion is known to be at a high level. Direct contact was achieved by introducing 2 donor pigs into a loose box containing 2 or 3 cattle and housing the animals together for 2 hours. Indirect contact was achieved by holding groups of 3 steers for one hour in a loose box immediately after it had been vacated by 6 infected pigs. The amount of virus in the box was replenished by returning the donor pigs to it for one hour before the infection of the next group of cattle. In one experiment only (Section 7.5.2.) cattle were infected by the intradermal inoculation of FMDV into the dorsum of the tongue.

c) Infection of mice: suckling mice were inoculated with 0.03 ml of virus dilution by the intraperitoneal route (Skinner 1951).

6.3. Viruses

6.3.1. Foot and Mouth Disease Virus

6.3.1.1. Identity and History of FMD strains

Type	Subtype	Strain	Origin	Passage History at A.V.R.I.*		
O	1	Swiss 1/66	Swiss field sample 1966	C_1	P_6	C_1
O	1	BFS 1860	British field sample 1967	C_2		
A	12	119	British field sample 1932	C_{27}	BHK_1	C_1
C	-	Noville	Swiss field sample 1966	C_3		

* Passage history: passage in cattle (C), pigs (P) or baby hamster kidney cells (BHK).

The identity of all stock viruses and of a random sample of virus isolates from cattle was checked in complement fixation tests performed by the World Reference Laboratory at this Institute.

6.3.1.2. Production of FMDV

a) Production in animals: Stock virus was produced in cattle by multiple intradermal inoculation of the tongue. Vesicular epithelium was harvested 24 - 48 hours later. After mincing and grinding with sand in a pestle and mortar the epithelium was suspended in equal parts 0.04% phosphate buffer/Hartley's digest broth and filtered. An equal volume of sterile glycerol was added to the filtrate to give a final dilution of epithelium of 1:20 and the suspension was stored at -20°C. This stock was used for the infection of donor pigs and for tissue culture passage.

b) Production in tissue culture (Mowat and Chapman 1962, De Castro 1964): glycerinated seitz filtrates of bovine tongue vesicle epithelium were used to initiate tissue culture passage series in BHK or IB-RS-2 cells. Confluent monolayers in Roux flasks or 20 oz medical flats were washed with phosphate buffered saline (PBS, Dulbecco and Vogt 1954) at 37°C and 1.0 ml of a 1:100 dilution of stock virus was inoculated. After 30 minutes adsorption at 37°C the cultures were again washed with buffer and 60 - 100 ml of serum free maintenance medium was added. Infected monolayers were incubated at 37° C until 90 - 100% cytopathic effect (CPE) was observed at which time the culture field was harvested, centrifuged at 3000 rpm for 10 minutes and the supernatant collected. Viruses were adapted for growth in monolayer by up to 15 serial passages. Conditions of maximum virus yield were determined in growth curve experiments using fully adapted virus at multiplicities of 0.1 - 0.01 and virus produced under optimal conditions was filtered, glycerinated and

stored at -20°C. This virus was used in mouse neutralisation tests and for the infection of monolayers in the production of concentrated virus.

6.3.1.3. Concentration of FMDV

a) Salt precipitation (Brown and Cartwright 1963). Tissue culture virus harvests were clarified at 3000 rpm for 10 minutes and then mixed with an equal volume of saturated amonium sulphate at pH 7.4. After at least 1 hour at 4°C, the precipitate was collected by spinning at 2500 rpm for 30 minutes in a refrigerated centrifuge and resuspended in a reduced volume of PBS to give a physical concentration factor of x50 to x100. On occasions the suspension was further clarified by centrifugation at 10,000 rpm for 30 minutes.

b) Single phase polymer concentration (Fayet 1969, Wagner et al 1970). The method of concentration was essentially as described for salt precipitation except that ammonium sulphate was replaced by an aqueous solution of autoclaved polyethylene glycol (Molecular weight 6,000) which was added to the clarified virus harvest to a final concentration (w/v) of 6%. Precipitation was allowed to proceed for a minimum of 2 hours and on occasion a second precipitation was carried out.

c) Ultracentrifugation (Brown and Cartwright 1963). Virus concentrated by salt or polymer precipitation was clarified by spinning at 10,000 rpm for 30 minutes and then further purified and concentrated by ultracentrifugation at 25,000 rpm for 3½ hours at 4°C. The pellet was allowed to soften overnight at 4°C under a small volume of PBS before being resuspended in a reduced amount of buffer.

Concentrated virus was stored in 1.0 ml volumes in bijoux bottles at -70°C and served as antigen in immunodiffusion and immunoelectro-

phoresis experiments.

6.3.1.4. Assay of Virus Infectivity.

a) Estimation of 50% End Points in Tissue Culture. Confluent CTY monolayers were washed with PBS at 37°C and 0.2 ml volumes of tenfold dilutions of virus in PBS were inoculated using 2 or 4 tubes per dilution. After 30 minutes at 37°C, 2.0 ml of serum free maintenance medium was added and the tubes were incubated at 37°C on a roller apparatus. Cultures were examined for CPE by low power light microscopy at 24 and 48 hours post inoculation and 50% end points were calculated by the method of Reed and Muench (1938).

This method was used to estimate the viral infectivity of samples from cattle and titres were expressed as log ID_{50} per ml of nasal secretion, oral saliva, pharyngeal fluid, urine and serum per g of faeces or per sample in the case of tears or preputial swabs.

b) Plaque Assay (Dulbecco 1952, Sellers and Stewart 1960). Confluent BHK or IB-RS-2 monolayers in petri dishes were infected by the method described for CTY tubes using 2 or 3 plates per dilution. After 60 minutes adsorption the infected monolayers were overlaid with 5.0 ml of Eagle's (BHK) or LYH (IB-RS-2) medium containing 1.0% (w/v) nutrient agar (Difco Bacto) and antibiotics. After 48 hours incubation at 37°C the monolayers were stained with a 1:10,000 dilution of neutral red in PBS or with methylene blue solution and the plaques counted. Results were expressed as plaque forming units (p.f.u.) per ml of inoculum.

The method was used in titrations of stock virus infectivity (BHK) and in studies on the kinetics of virus inactivation by various antiviral factors (IB-RS-2).

c) Estimation of 50% End Points in Mice. This method was used in virus neutralisation studies and is detailed under section 6.6.2.

6.3.1.5. Storage of Virus.

Glycerinated stocks of vesicular epithelium or tissue culture virus were stored at -20°C. Concentrated virus stocks were held at -70°C. Samples from cattle were stored at 4°C for up to 48 hours after collection or at -70°C for longer periods until assayed for infectivity.

6.3.2. Vesicular Stomatitis Virus.

The Indiana C strain of vesicular stomatitis virus (VSV) was used at the 15th BHK passage level as challenge virus in interferon assays and as an inducer in the production of reference stocks of IF.(see section 6.5.). Bottles containing VSV were wrapped in silver foil to protect the light sensitive virus. Tissue culture virus was stored at -20°C in 50% sterile glycerol.

6.4. Foot and Mouth Disease Vaccine

Inactivated FMD vaccine was kindly supplied by Burroughs Wellcome and Company. The vaccine was prepared from virus of type C (strain Noville) by the standard production method whereby virus is grown in BHK cell suspension cultures, filtered, inactivated with acetylethyleneimine and saponin is incorporated as an adjuvant. The vaccine contained more than six 50% Cattle Protective Doses per 2.0 ml dose and was administered by subcutaneous injection.

6.5. Interferon

6.5.1. Interferon Assay

Samples from cattle were assayed for interferon (IF) by measuring their capacity to inhibit CPE in CK tube cultures (Sellers and Fitzpatrick 1962, Sellers et al 1968b and c).

Each 4.0 ml volume of bovine nasal secretion or dialysed pharyngeal fluid was acidified to pH 2.0 by the addition of approximately 0.3 ml of N/1 hydrochloric acid (HCl) in order to inactivate any FMDV in the sample. After 24 hours at 4°C the pH was readjusted by the addition of an equivalent amount of N/1 sodium hydroxide (NaOH) and final adjustments were made to bring the pH of the sample within the range 7.4 - 7.6 by the addition of N/10 HCl or NaOH.

A five fold dilution series of each sample was prepared in LYH medium with antibiotics to cover the range 1:1 to 1:625 and 0.5 ml was inoculated into confluent CK cultures containing fresh growth medium with 2% heat inactivated bovine serum previously tested to show the absence of IF. Three tubes were inoculated for each dilution. After 24 hours rotation at 37°C, 0.2 ml of LYH containing approximately 100 CK $TCID_{50}$ of vesicular stomatitis virus (VSV) was added and the cultures were again rolled at 37°C.

Controls included: a) A titration of challenge virus, b) a challenge virus control in which the sample was replaced by 0.5 ml of growth medium, c) a control on sample toxicity in which the undiluted sample was included and the virus was replaced by 0.2 ml of medium, d) a control on the efficiency and reproductibility of the system in which a standard preparation of CK interferon replaced the test samples.

The cultures were examined microscopically and when control tubes

showed maximum CPE (usually at 24 hours) the degree of CPE was estimated in all tubes and the IF titre calculated as the final dilution of the sample that protected 50% of the cells. The calculation was made according to the method of Reed and Muench (1938) and the titre was expressed as log units of IF in 0.5 ml of original sample.

6.5.2. Production of Standard Interferon Preparation.

A standard preparation of IF was produced from a 48 hour harvest of CK monolayers in Roux flasks infected with VSV by the method described for FMDV (see Section 6.3.1.2.). The infected tissue culture fluid was centrifuged at 3,000 rpm for 10 minutes and the supernatant acidified to pH 2.0 by the addition of N/1 HCl. After 24 hours at 4°C, the pH of the harvest was corrected to pH 7.6 by the addition of N/1 NaOH. When the preparation had been satisfactorily tested for the presence of IF and the absence of VSV, it was stored at 4°C.

6.5.3. Characterisation of Interferon.

The standard IF preparation and some samples from cattle were partially characterised by the demonstration of a) lack of direct effect on FMDV, b) marked loss of activity in non bovine assay systems, c) approximately equal effect on various types of FMDV and on VSV showing the non specific nature of the inhibition.

6.6. Antibodies

6.6.1. Convalescent Bovine Serum.

6.6.1.1. Preparation of Convalescent Bovine Serum.

Four steers developed fully generalised disease after infection with FMDV type O, strain BFS 1860 by intradermolingual inoculation. One month post infection the jugular vein was catheterised and 5.0 - 6.0 litres of blood were taken from each animal. Serum was obtained from clotted blood in the usual way and filtered through pads of a sterilising grade. The filtered serum was dialysed against running tap water for 48 hours to precipitate the euglobulin fraction and the remaining serum proteins were precipitated by the addition of an equal volume of 66% saturated ammonium sulphate solution. The precipitates were collected by centrifugation at 2,500 rpm for 30 minutes at 4°C and resuspended in a reduced volume of normal saline. The supernatant was reconcentrated and the two precipitates combined in a volume which was about 50 times less than that of the original serum. The concentrate was dialysed against repeated changes of normal saline until sulphate ions were no longer detectable in the dialysate (Campbell et al 1963). The protein concentration was followed throughout the procedure by direct spectrophotometry at 280 nm and 72 - 75% was retained in the final preparation. The mouse neutralisation index of the original material averaged 6.8 and of the concentrate 7.8 per ml. Concentrated serum was stored at 4°C and used within 4 weeks of preparation.

Concentrated antibody preparations were pooled and their globulin type was investigated by immunoelectrophoresis, the patterns being developed with antiserum against bovine immunoglobulin and against concentrated homologous FMDV. Activity against FMD resided principally in the IgG_1 and IgG_2 molecules with some activity in the IgM fraction.

6.6.1.2. Passive Immunisation.

Concentrated convalescent bovine serum to FMDV type O, strain BFS 1860 was administered to 3 steers by intravenous injection. Each

received 200 ml of concentrate administered via a flutter valve. Blood samples and secretion samples were taken before immunisation, at 2 hourly intervals for 8 hours after immunisation, and then daily for 21 days.

6.6.2. Measurement of Antibody to FMDV.

The neutralising activity of antibody to FMDV in sera and secretions was measured by means of neutralisation tests in mice using a constant dilution of serum or secretion and varying dilutions of virus (Skinner 1953, et al 1952).

Samples were routinely tested at the following dilution: serum 1:10, tears approximately 1:2, nasal secretion 1:1, dialysed saliva and pharyngeal fluid 1:1 or after concentration by a factor of 10 to 100. On occasions, these secretions were also examined at other dilutions and additional tests were carried out on lower respiratory and alimentary tract secretions at a dilution of 1:10 and on faecal suspension (1:10 or x50) and urine (1:1 or x100).

On the day of testing samples were diluted in PBS and heated at 56°C for 30 minutes. They were then distributed in 0.25 ml volumes in bijoux bottles and an equal amount of the appropriate tenfold dilution of virus in PBS was added to each. Viral titrations diluted in an equal volume of PBS were also included and the specificity of the neutralisation was controlled by testing samples against both homologous and heterologous types of FMDV. The reproductibility of the test was monitored by the inclusion of a standard homologous bovine antiserum. When all the reagents had been added the caps were replaced, the bottles thoroughly shaken and the neutralisation reaction allowed to proceed for 60 minutes at 37°C in a water bath.

Residual viral infectivity was assayed in mice. Each sample was inoculated intraperitoneally in 5 - 7 day old mice using 5 or 10 mice per dilution. Litters were examined daily for 7 days and the number of mice paralysed or dead was recorded. Mouse 50% infective doses were calculated by the method of Reed and Muench (1938) and the neutralisation index (NI) was given by the difference in infectivity between the virus control and the test sample. Results were expressed as Mouse Neutralisation Indices per 0.03 ml of sample at the dilution tested.

Periodically the identity of virus in tissue suspensions from infected mice was checked by complement fixation. Within tests the standard error of the NI was 0.184 and the coefficient of variation was 3.640% (20 observations). Between tests the values were 0.228 and 6.582% (20 observations).

6.6.3. Preparation of Bovine Immunoglobulins.

The immunoglobulins IgM, IgG_1, IgG_2 and secretory IgA were isolated and purified from bovine serum, colostrum and saliva by a combination of techniques as described by Sullivan et al 1969, Porter and Noakes 1970, Mach and Pahud 1971, Porter 1971, Butler 1971, et al 1972a, b and c, Duncan et al 1972.

Colostrum was centrifuged at 2,500 rpm for 60 minutes and the fat layer removed with a spatula. Caseins were removed by lowering the pH to 4.6 with 0.1 N HCl, incubating at 37°C for 30 minutes and spinning out the precipitate at 2,500 rpm for 30 minutes. The pH of the supernatant was readjusted to 7.4 by the addition of 0.1 N NaOH.

Serum and colostral whey were dialysed against running tap water for 6 hours and against 3 daily changes of distilled water at 4°C to

precipitate the euglobulins. After dialysis the precipitate was collected by centrifugation as above and resuspended in PBS. The water soluble proteins in the supernatant were precipitated by the addition of 33% saturated ammonium sulphate solution (pH 7.4) at room temperature with continuous mixing. After centrifugation the precipitate was resuspended in a reduced volume of PBS and dialysed against the same buffer until sulphate ions were no longer detectable in the dialysate.

Saliva was clarified by low speed centrifugation and the supernatant concentrated by positive pressure dialysis at 4°C.

IgM was isolated from the euglobulin fraction of serum by collecting the leading edge of the first peak to emerge after exclusion chromatography on sephadex gels. The peak was concentrated and twice recycled on the same column. Contaminating α_2 macroglobulin was separated by pevikon block electrophoresis and the resulting highly purified IgM was collected and concentrated.

IgG_1 was isolated from colostrum. After defatting, decaseination and salt precipitation as described, the resuspended precipitate was dialysed against 2 changes of 0.1 M Tris - 0.05 M Saline buffer at 4°C. Once equilibriated the solution was fractionated by anion exchange chromatography using gradient elution. The second and largest peak was concentrated and applied to a G 200 sephadex column from which 3 peaks emerged. The third peak was collected, concentrated and twice recycled on G 200 gels to yield highly purified IgG_1.

IgG_2 was isolated from serum. After dialysis, precipitation and equilibration as described for IgG_1 the protein mixture was spearated by anion exchange chromatography on DEAE A50 cellulose. The first peak was collected, concentrated and recycled on sephadex G 200 to yield

highly purified IgG_2.

Secretory IgA was obtained from concentrated saliva. The concentrate was equilibrated with the starting buffer for anion exchange and chromatographed on a DEAE A50 cellulose cloumn. Secretory IgA was obtained with a minor IgG_1 component and the two were separated by running the concentrated material through a sephadex G200 column which allowed the collection of highly purified secretory IgA.

The identity and purity of separated immunoglobulins was assessed by their pattern of elution from chromatography columns, their electrophoretic mobility and precipitation pattern in immunoelectrophoresis using (i) polyvalent antisera directed against whole serum or secretions and (ii) monospecific antisera directed against particular globulin classes. Absolute purity of immunoglobulins is difficult to achieve even when ultracentrifugation or iso electric focussing is included in the method. The difficulty stems from a) the marked heterogeneity of globulin classes, particularly of bovine IgG and b) the existence of commom antigenic determinants in different globulin classes. However, contaminating protein reactivity in antisera raised using these Ig preparations could be removed or greatly reduced by reciprocal absorption with the appropriate protein as described in sections 6.6.4. and 6.6.5.6..

6.6.4. Production of Antiserum to Bovine Immunoglobulins.

Antisera to whole bovine serum, tears, nasal secretion, concentrated saliva and purified and concentrated samples of IgM, IgG_1 and secretory IgA were produced in rabbits. A one ml solution containing 1 to 5 mg of appropriate protein was emulsified in an equal volume of Freund's complete adjuvant and two 0.5 ml injections were given at separate intramuscular sites each month for at least three months. Prior to bleeding,

1 - 5 mg of the required protein was injected intravenously and serum was prepared from blood collected by cardiac puncture 10 days later. Some rabbits were rendered tolerant to IgG prior to immunisation with IgM and secretory IgA by the method of Spiegelberg and Weigle (1968). Antiserum to bovine IgG_2 was produced in guinea pigs by the method of Binaghi et al (1967).

Many antisera showed unwanted cross reactivity in respect of precipitin formation in gels. Contaminating antibodies were removed or minimised by repeated reciprocal absorption with the appropriate heterologous immunoglobulin to produce monospecific antisera. Thus antisera to IgM, IgG_2, and secretory IgA was absorbed with IgG_1 to remove anti light-chain reactivity and antisera to IgG_1 was absorbed with IgG_2 (Mach and Pahud 1971, Downie 1970). The method of absorption is given in section 6.6.5.6. and the specificity of antisera is shown in Plates 5 - 8.

6.6.5. Methods for the Isolation and Characterisation of Antibody.

6.6.5.1. Gel filtration (Exclusion chromatography).

Sephadex G150 or G200 (Pharmacia, Uppsala, Sweden) was swollen in boiling water for 6 hours and the resultant gel was equilibrated in 5 daily changes of 0.1M Tris - 0.2M NaCl buffer containing 0.02% sodium azide at pH 7.6. Fines were carefully decanted at each buffer change and the gel was poured in 100 cm x 2.5 cm glass columns. Final equilibration was achieved by passing a further 1,000 ml of buffer through the column after which the flow characteristics and void volume of the column were determined by observing the elution of 3 ml of 0.1% blue dextran (molecular weight 2.0×10^6) in Tris - Saline buffer. Serum or secretion samples containing 200 - 300 mg of protein were applied to the bottom

of the column in amounts of up to 3% of the bed volume and eluted in an ascending flow of Tris - saline buffer under a constant hydrostatic head adjusted to give a flow rate of 30 ml per hour at room temperature.

Column effluent passed through a device which allowed automatic scanning and recording of ultraviolet light absorption at a wavelength of 280 nm. (Isco U - V Absorptiometer Model 4A, Flow Cell Type 4, Recorder Type BD7, Shandon Southern Ltd, Camberley, Surrey). Fractions of 2, 5 or 10 ml were separated by means of a volumetric siphon and collected automatically (The Central Fraction Collector, Scientific Supplies Ltd., London).

6.6.5.2. Anion Exchange Chromatography.

The method employed was essentially that of Binaghi et al (1967). DEAE sephadex type A - 50 (Pharmacia, Uppsala, Sweden) was equilibrated in 3 daily changes of 0.1M Tris - 0.05M NaCl buffer containing 0.02% azide at pH 8.3 and the gel poured in 45 cm x 2.5 cm glass columns. Samples of serum or secretion were dialysed to equilibrium with the starting buffer and volumes of up to 3.0 ml were applied to the top of the column. Protein was eluted sequentially from the gel by the action of a continuous gradient of increasing ionic strength rising from the starting buffer containing 0.1M Tris and 0.05M NaCl to the final buffer containing 0.1M Tris and 0.22M NaCl. All buffers were at pH 8.3 and contained 0.02% sodium azide. The gradient was produced using a reservoir made up of three cylinders connected in series via tubes at the base of each cylinder. The third reservoir which led to the column was stirred mechanically and contained 270 ml of starting buffer while the first and second reservoirs each contained 270 ml of final buffer. Buffer was allowed to run under gravity at an initial flow rate of 25 ml per hour at room temperature and fractions were monitored, recorded and collected

automatically as described for gel filtration in section 6.6.5.1..

6.6.5.3. Immunodiffusion.

Double diffusion in agar gel was performed according to the method of Oughterlony (1958) with minor modifications. Ion agar number 2 (Oxoid) was dissolved by steaming in veronal buffer ionic strength 0.1 containing 0.1% sodium azide at pH 8.3.. Ten ml amounts of 1.5% (w/v) agar were dispensed in 6 cm plastic petri dishes. Wells of 3 mm diameter set 3 mm apart were cut in the agar to a present pattern by means of a special punch and filled with 10 µl of the appropriate antigen or antibody solution. Diffusion at room temperatures in a humified chamber was followed by the appearance of precipitin lines within 3 days.

6.6.5.4. Immunoelectrophoresis.

Micro immunoelectrophoresis was carried out according to Scheidegger (1955) with minor modifications using Shandon equipment. Glass slides, 25 mm x 75 mm, held on a levelling table were coated with a 2 mm deep layer of 1.0% Ionagar No. 2 (Oxoid) dissolved by steaming in barbitone acetate buffer, ionic strength 0.05, pH 8.6.. Wells and troughs were cut in the agar to a preset pattern by means of a special tool. Wells were filled with 5 - 10 µl of test sample and the slides were arranged above the two chambers of an electrophoresis tank each of which contained 500 ml of barbitone acetate buffer, 0.1M, pH 8.6. Filter paper wicks joined the ends of the slide to the buffer solution in each compartment. A constant potential difference of 6 volts/cm was applied to the slides for 2 hours.

After electrophoresis the separated proteins were precipitated with anti globulin serum or concentrated FMDV which was allowed to diffuse

from troughs running parallel to the direction of migration of the test sample. Diffusion was at room temperature in a humidified chamber. Slides were examined daily for 5 days and the results were recorded by means of drawing or photography.

6.6.5.5. Preparative electrophoresis.

The method of Aalund (1968) was followed. Pevikon C-870 (polyvinyl acetate copolymer, Feinbiochemica, Heidelberg, West Germany) was cast in a block measuring 22 cm x 25 cm x 1 cm in 0.125M barbital acetate buffer pH 8.6. Samples were prepared as a slurry in Pevikon and applied to a slot 1 cm x 23 cm running the width of the block at a point 5 cm from its cathodal side. Electrophoresis was carried out at room temperature for 18 hours using a constant current of 45 milliamps in the equipment described in section 6.6.5.4. Subsequently the block was sectioned at 1 cm intervals across its width and the protein eluted in PBS.

6.6.5.6. Absorption of antibody.

Immunoglobulins were removed from samples by the addition of 2 volumes of polyvalent or class specific antiglobulin serum followed by incubation at $4^{\circ}C$ for 16 hours. Precipitates were removed by centrifugation at 25,000 rpm for 30 minutes. The degree of absorption was tested by immunodiffusion and immunoelectrophoresis and if necessary the process was repeated using small increments of antiglobulin serum until the specific precipitin could no longer be detected (Downie 1970).

6.6.5.7. Dissociation of antibody.

Dissociation experiments were performed by mixing equal volumes of immunoglobulin solution and 0.2M 2 - mercaptoethanol in PBS at pH 7.4.

Reduction of disulphide bonds was allowed to proceed for 18 hours at 4°C and the material was then alkylated by the addition of an equal amount of 0.2M iodoacetamide in PBS at the same pH (Fleischman et al 1962). Toxic residues were removed by overnight dialysis against PBS at 4°C. Control preparations were treated in an identical manner except that PBS was added in place of mercaptoethanol and iodoacetamide. The effect of dissociation was examined by neutralisation tests in mice.

6.6.6. Methods of antibody concentration.

Antibody in volumes of more than 1.0 L was generally concentrated by salt or polymer precipitation as described under sections 6.3.1.3. and 4.6.3. Smaller volumes, particularly column chromatography fractions, were concentrated by means of negative pressure dialysis (Sartorius membranfilter type SM 132, Gottingen, West Germany), positive pressure dialysis in stirred cells (Amicon, membrane types XM 50 and CM 50, High Wycombe, Bucks), centrifugal dialysis (Amicon, Cone type CF 50 A) or polyacrylamide gel absorption ('Lyphogel', Gelman, Lancing, Sussex).

6.6.7. Dissociation of FMDV antigen - antibody complexes.

A modification of the method of fluorocarbon dissociation described by Brown and Cartwright (1960) was applied to recover viral infectivity from neutral mixtures of FMDV virus and antibody. One volume of trichloro trifluoroethane ('Arklone P', ICI Ltd., Runcorn, Cheshire) was mixed with one volume of antigen-antibody complex and the mixture blended together for 60 seconds at 16,000 rpm. The resultant emulsion was centrifuged at 3,000 rpm for 5 minutes and the upper phase containing the virus was aspirated. The procedure was repeated on four occasions and bovine plasma albumin was added to the final product to a concentration of 0.1%.

6.6.8. Kinetics of antibody neutralisation of FMDV.

Tissue culture adapted strains of FMDV containing approximately $10^{6.5}$ pfu per ml were mixed with an equal volume (2.0 ml) of bovine serum (1:10), nasal secretion (1:1) or dialysed saliva (1:1 or x10). Biological fluids had been derived at various times from normal and convalescent cattle and were heated at 56°C for 1 hour before testing. Mixtures were incubated at 37°C for up to 4 hours and 0.2 ml samples removed at 20 minute intervals were immediately diluted tenfold in PBS containing 0.1% bovine plasma albumin (BPA). Loss of viral infectivity due to causes other than neutralisation were measured by the inclusion of a virus titration diluted in PBS containing 0.1% BPA. Residual infectivity was measured by the plaque technique in IB-RS-2 monolayers using 3 cultures per dilution and results were expressed as the percentage of the original virus surviving per unit time.

6.7. Bovine Secretions

6.7.1. Methods of collection and processing.

6.7.1.1. Ante mortem collection.

a) Lachrymal secretion: tears were collected on cotton swabs held in the pocket formed by the everted lower eye lid using 10 swabs for each eye. On average 4.6 ml of secretion was obtained (range 3.2 - 8.5 ml) and after washing in 5.0 ml of PBS, the fluid was collected by centrifugation at 2,500 rpm for ten minutes.

b) Nasal secretion: Absorbent cotton tampons were held in the nares by means of a muslin mask as shown in plate 1. After 15 - 20 minutes tampons were removed and about 15 ml of secretion was harvested by placing the

tampon in the barrel of a disposable 20 ml syringe and squeezing the fluid out in a special press incorporating a disposable bacteriological filter, designed and constructed for this purpose at this Institute (Plate 2).

c) Oral saliva: mixed saliva was collected from the floor of the mouth and the region between the teeth and the cheek by suction using a 20 ml disposable mucus extractor (Sterilin Ltd., Richmond, Surrey). Debris was removed by centrifugation at 3,000 rpm for 10 minutes and concentration was achieved by positive pressure dialysis.

d) Pharyngeal secretion: fluid was collected in 10 ml amounts by means of a cup probang passed through the mouth into the pharynx as described by Van Bekkum (1959) and Burrows (1966) or by a similar instrument modified by the attachment of a 'lid' to facilitate repeated passage through the fauces. Amounts of 100 ml were readily obtained by means of a special cup probang fitted with a metal tube and a suction device designed and constructed for the purpose at this Institute. The three types of instrument are shown in plates 3 and 4. Pharyngeal fluid was subsequently processed as described above for saliva.

e) Faeces: faeces collected from the rectum were prepared as a 1:10 suspension by mechanical shaking in PBS with double strength antibiotics and the supernatant was collected after spinning at 3,000 rpm for 20 minutes. Some samples were clarified by centrifugation at 10,000 rpm for 30 minutes and filtered prior to concentration by polymer precipitation as in section 6.3.1.3.

f) Urine: samples obtained during urination were centrifuged at low speed and some were concentrated by polymer precipitation as above.

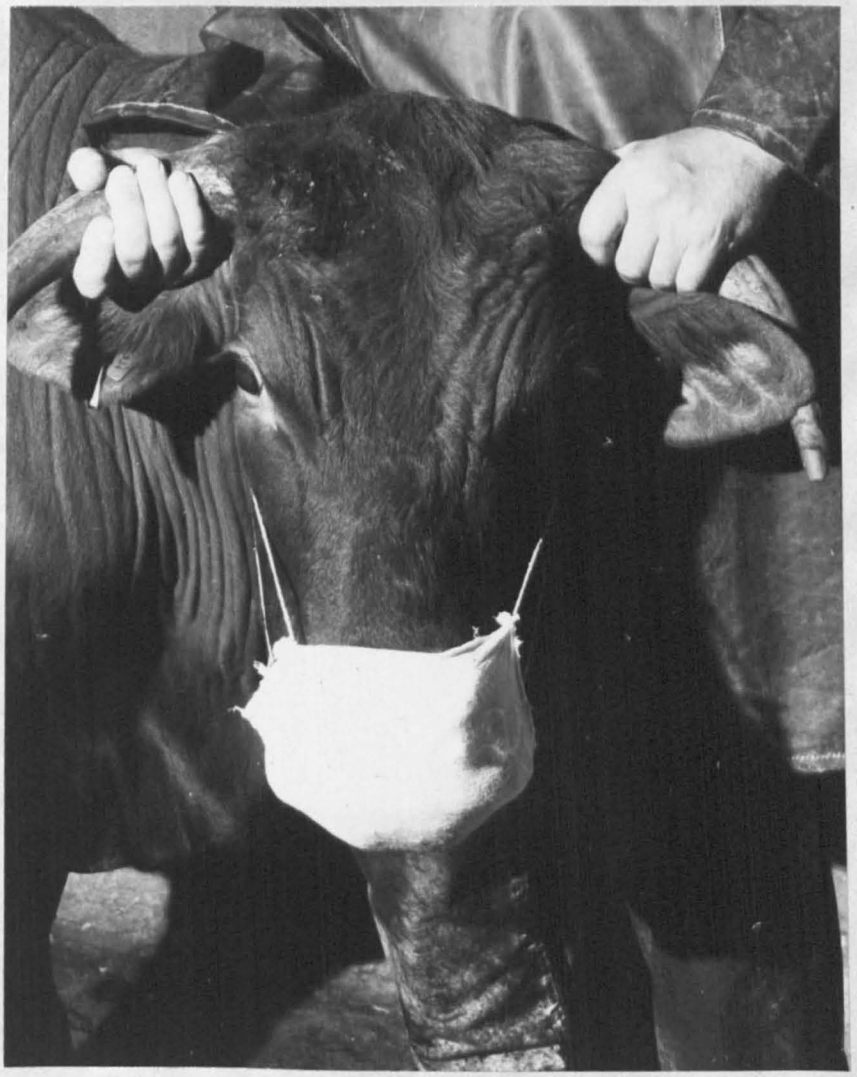


PLATE 1. Collection of bovine nasal secretion.

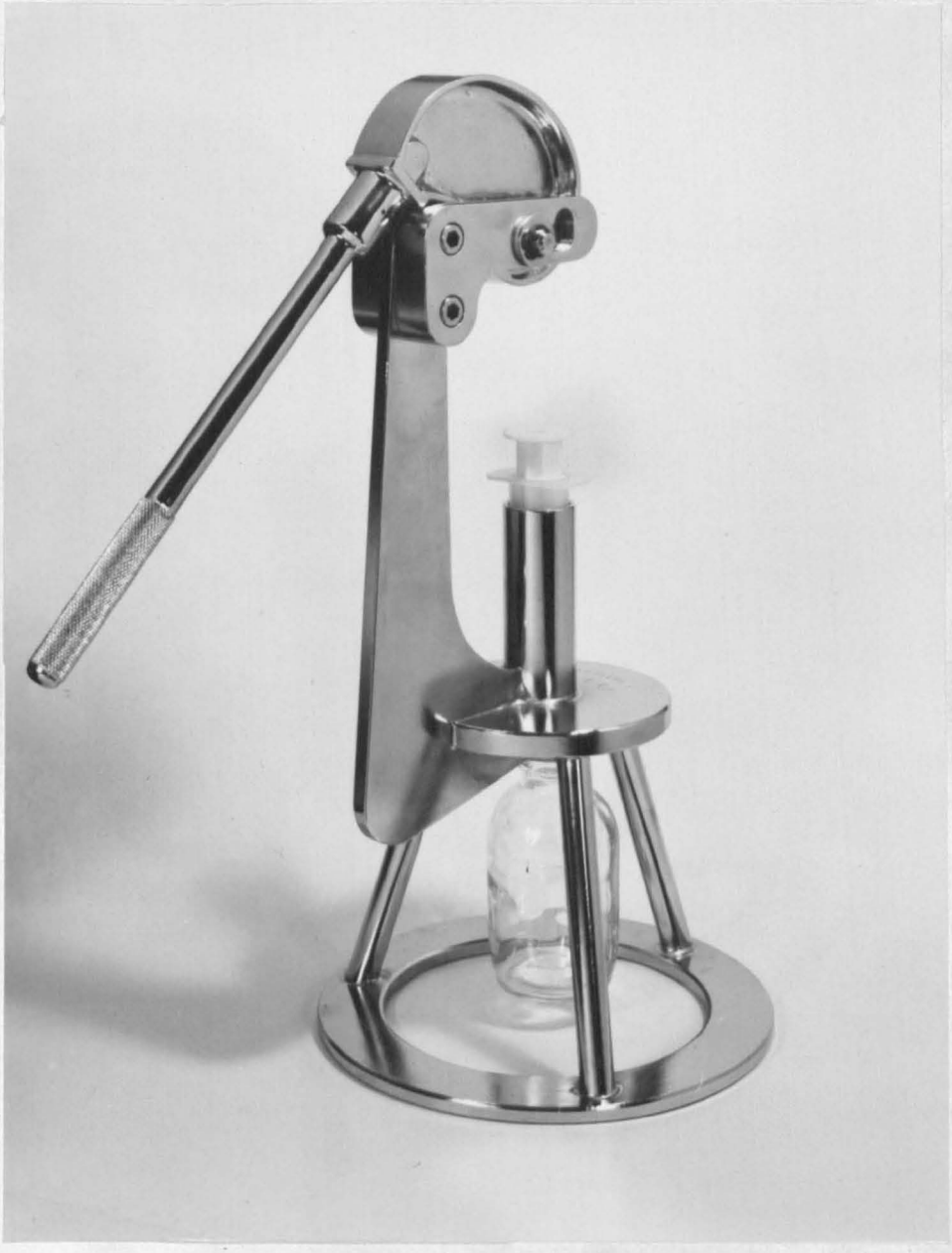
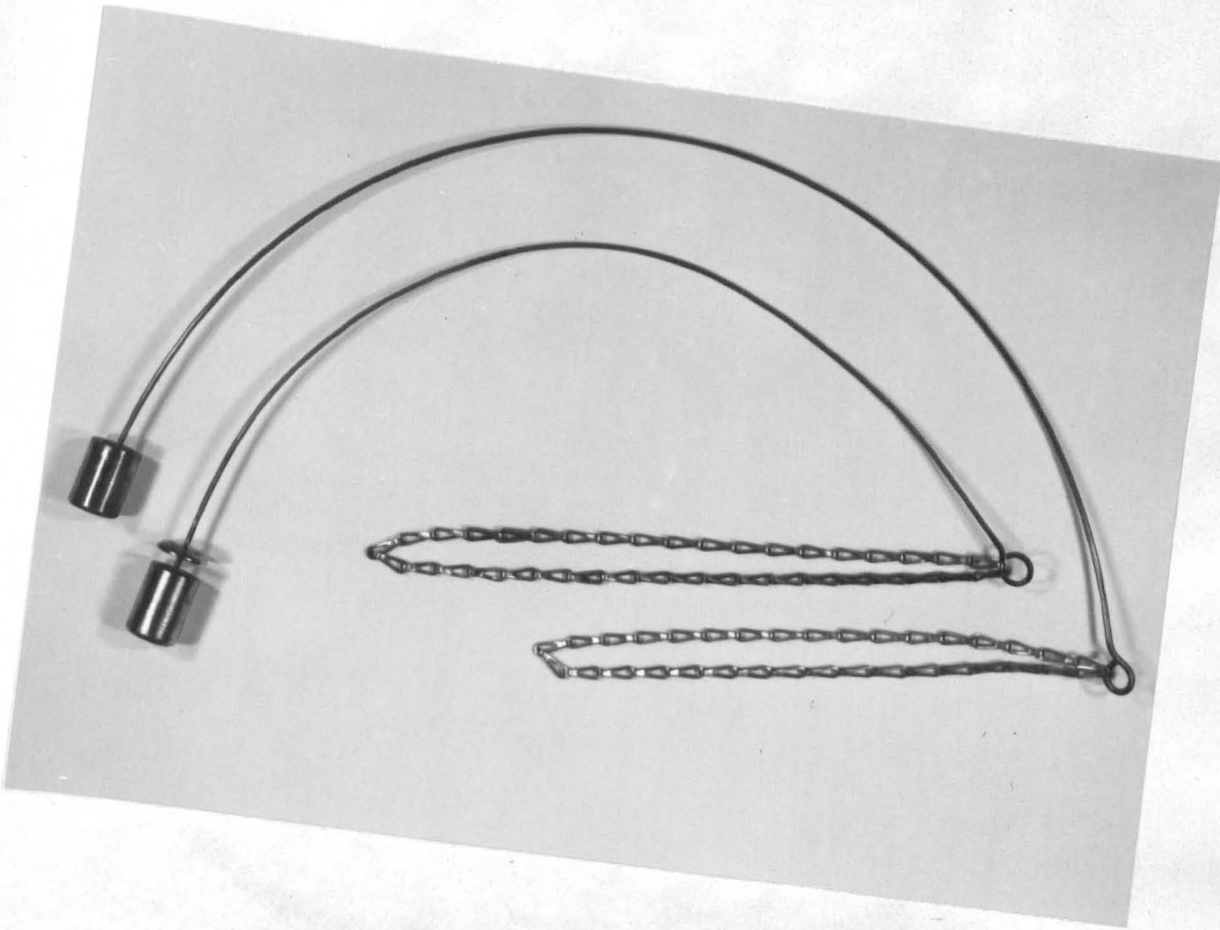


PLATE 2. Laboratory press for the recovery of nasal secretion from tampons.



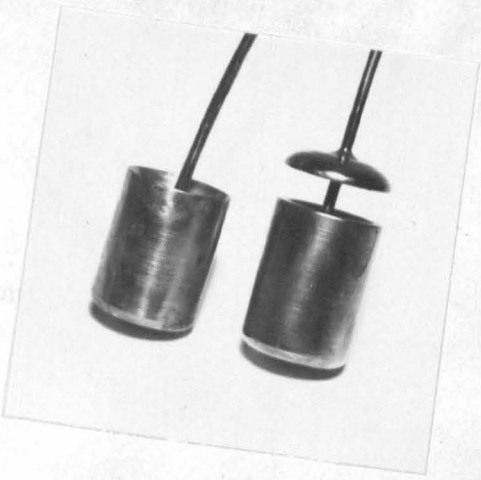


PLATE 3. Cup probangs. Original and modified versions. Overall length of wire 600 centimetres.

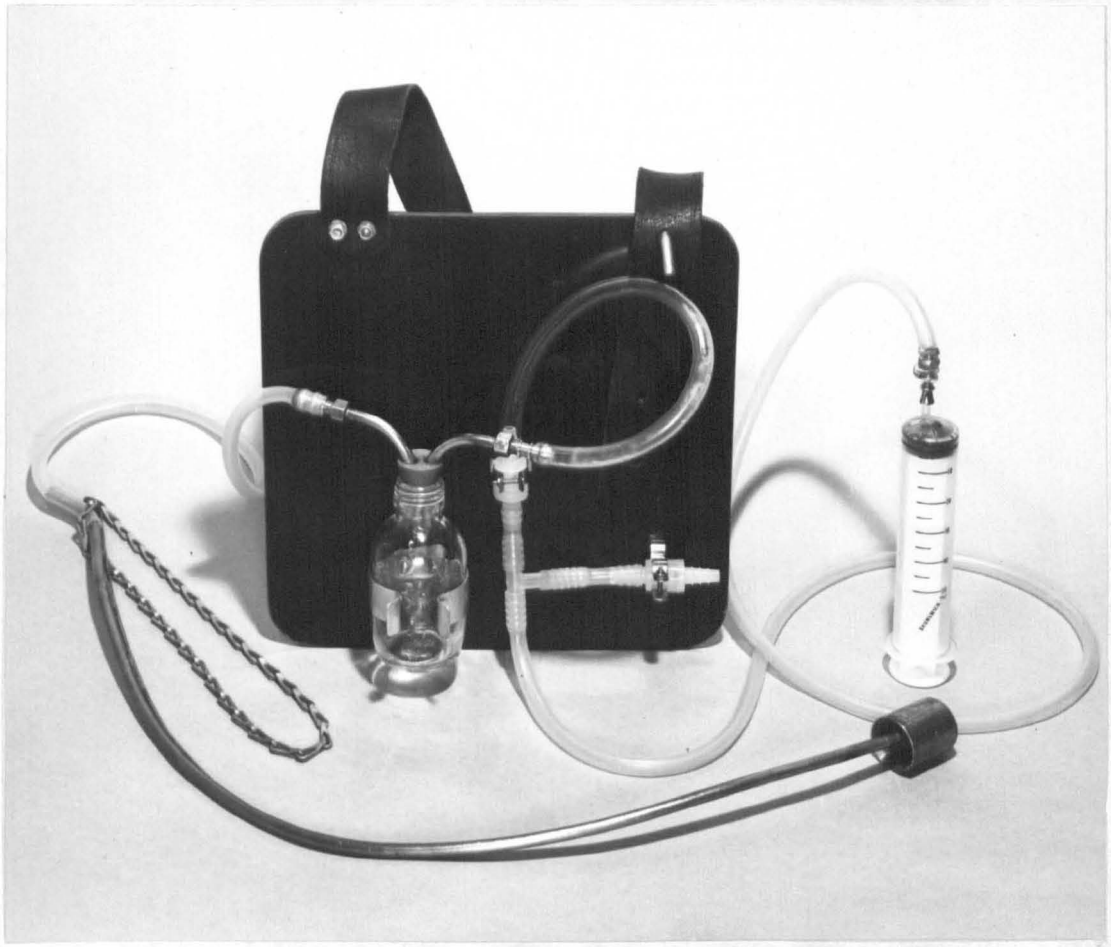


PLATE 4. Cup probang with suction device.

g) Serum: blood obtained by jugular venupuncture was allowed to clot and the serum separated after centrifugation.

6.7.1.2. Post Mortem Collection

Post mortem secretions were obtained from cattle which had been bled out after the lethal intravenous injection of barbiturate solution.

a) Respiratory secretions: mucus was obtained from the external surfaces of the trachea and major bronchi by gently running the blunt end of a scalpel handle over the mucosa and collecting the accumulated secretion under suction. Mucus was resuspended by vigorous pipetting in PBS at a dilution of 1:10.

Broncho-alveolar washings were taken by introducing 500 ml of PBS into the right apical lobe of the lung via the stem bronchus. The bronchus was clamped off, the lobe gently massaged and the available washings poured out of the air passages. Only about 60% of the buffer was recoverable. Washings were centrifuged at 3,000 rpm for 15 minutes to remove cellular material.

b) Alimentary secretions: the abomasum, small intestine, caecum and colon were incised, emptied and washed under gently running tap water. Mucus was collected and processed as described above for respiratory secretions.

Secretions were concentrated by the methods given under sections 6.3.1.3., 6.6.3 and 6.6.6.. Samples were stored at -20°C or at -70°C when infectivity was to be preserved.

6.7.2. Detection of Haemoglobin.

Secretion samples were examined for the presence of haemoglobin (as an indication of contamination with blood) by means of proprietary reagent strips ('Labstix', Ames Co., Stoke Poges, Bucks.).

6.7.3. Measurement of pH

The pH of secretions was measured by means of a portable pH meter with a combined glass microelectrode (Meter Type 30C, Electrode Type 9259/81, E.I.L. Ltd., Richmond, Surrey). The instrument was equipped with automatic temperature compensation and was accurate to within $\pm$ 0.1 of a pH unit with discrimination to the level of 0.05 of a pH unit.

The pH was measured either directly in vivo (tears), immediately after collection (saliva, pharyngeal fluid, urine, faeces, blood) or immediately after processing (nasal secretion, serum). Thirty seconds was the maximum time elapsing between the collection of samples and the measurement of pH in all cases except serum where much longer periods elapsed.

6.7.4. Investigation of antiviral factors in secretions.

6.7.4.1. Interferon and Antibody.

The methods employed have been described in sections 6.5. and 6.6 respectively.

6.7.4.2. pH and Other effects.

a) Range of pH stability of FMDV strains: buffers were prepared to cover the range 5.0 - 10.0 in steps of a quarter of a pH unit. A potassium phosphate - disodium phosphate buffer(Sorenson) was used for the range

5.0 - 8.0, a potassium phosphate - borax buffer for the range 8.25 - 9.0 and a glycine - sodium hydroxide buffer (Sorenson) for the range 9.25 - 10.0. Buffers were prepared in glass distilled water containing 0.1% bovine plasma albumin according to Documenta Geigy (1968) and pH was measured using a direct reading electronic meter. Buffer capacities were sufficient to hold the pH within 0.1 of a unit throughout the test.

Stock virus was diluted in buffer at various pH values to give 4.0 ml of test solution containing $10^{6.0}$ pfu per ml. Two series of each were prepared in capped bijoux bottles, one of which was held at room temperature while the other was held at 37°C in a water bath. Test solutions achieved 37°C within 3 minutes of partial immersion. After one hour samples were diluted a hundred fold in PBS containing 0.1% BPA and infectivity was measured by plaque assay in IB-RS-2 plates using 3 cultures per dilution. Results are derived from 3 experiments with each virus strain.

b) Kinetics of Inactivation of FMDV strains in secretions at physiological pH values: pools of secretions from normal or convalescent cattle were heated to 56°C for 30 minutes and the pH was then adjusted by the addition of N/10 HCl or NaOH to values of 8.5 (pharyngeal fluid or oral saliva), 8.0 (nasal secretion) or 7.5 (tears). FMDV was diluted in the secretions and in buffer solutions at corresponding pH values (prepared as in section 4.7.4.2.a) to give $10^{6.0}$ pfu/ml and 4.0 ml volumes were held in capped bijoux bottles at room temperature or at 37°C in a water bath. Mixtures were incubated for up to 4 hours and 0.2 ml samples withdrawn at 20 minute intervals were immediately diluted tenfold in PBS containing 0.1% BPA. Infectivity was measured by plaque assay in IB-RS-2 monolayers using 3 cultures per dilution and results were derived from 3 experiments with each virus strain.

6.8. Protein Estimation

Protein concentrations were determined by the method of Lowry et al (1951) or by direct measurement of optical density at 280 nm followed by comparison with a standard absorbance curve for bovine plasma albumin. Optical density was measured in silica cells of 1 cm pathlength using a Unicam Model SP500 Spectrophotometer or the Isco Model UA4 Absorbance Monitor described in section 6.6.5.1.

7. INVESTIGATIONS, RESULTS AND CONCLUSIONS

7.1. Investigations of secretions from the normal animal

Secretions derived from 61 cattle prior to any experimental proceedure involving FMDV and from 40 cattle following experimental procedures were measured to establish several normal parameters including : pH, total protein concentration, globulin type, the occurrence of interferon and the presence and amount of other non specific anti viral factors.

7.1.1. Normal pH values

The pH of secretions might have a bearing on FMDV infection and excretion either directly, by inactivation of the pH sensitive virus, or indirectly, by influencing other mechanisms such as neutralisation. A survey was conducted to establish the mean and range of pH in secretions from normal cattle.

7.1.1.1. Diurnal variations

The secretions of 3 cattle were sampled at four hourly intervals throughout a 24 hour period. The animals were fed once, immediately after the first set of samples had been taken. The results are summarised in Table 1. Inactivation of virus by pH would be most likely to occur at the alkaline values obtained for pharyngeal fluid, oral saliva and nasal secretion and less likely to occur at the pH of the other secretions.

7.1.1.2. Daily variations

Table 2 shows the mean pH values of secretions derived from 6 cattle sampled daily for seven days.

7.1.1.3. Seasonal variations

Table 3 shows the mean pH values of bovine secretions measured at monthly intervals throughout one year. The figures are derived from 3 steers convalescent to FMDV and were used when it had been established that FMD infection produced no effect on the pH values of these secretions. No seasonal variation was discerned since the values obtained fell at random within the range found for weekly variation in pH values. The housing and diet of these animals remained constant throughout the period so that they were not exposed to the type of seasonal variation encountered in the field.

7.1.1.4. Effect on diet

Comparisons were made between two groups of 3 steers convalescent to FMDV, one fed on a lucerne cob diet and the other fed on a diet of hay and concentrates. The results are shown in Table 4. Weekly measurements of secretion pH over a five week period revealed no significant differences between the groups for all secretions excepting urine for which the group fed on hay and concentrates gave a consistently lower value. The acid pH would tend to inactivate FMDV.

7.1.1.5. Collected pH values

Table 5 and Fig. 3a show the mean value and range of pH of normal bovine secretions. Values were derived from 120 observations for each secretion taken from various animals over a three year period. All measurements were made at the animal as expeditiously as possible.

Table 1. Mean pH values of bovine secretions measured at four hourly intervals for one day.

Time of day (hours)	L/S	Ph/F	O/S	N/S	F	U	B
0800 *	7.43	8.23	8.31	7.72	7.23	6.74	7.46
1200	7.16	8.70	8.46	7.86	6.52	-	7.54
1600	7.40	8.35	8.37	7.82	6.84	7.52**	7.51
2000	7.42	8.40	8.55	8.00	7.03	-	7.48
2400	7.08	8.52	8.56	7.93	6.78	7.63**	7.55
0400	7.33	8.50	8.42	7.86	6.92	-	7.51
0800	7.51	8.36	8.50	7.88	7.11	6.82	7.60
$\bar{x}$	7.33	8.43	8.45	7.86	6.91	7.17	7.52
S.E.	0.058	0.056	0.034	0.031	0.088	0.231	0.017

* Sampling of the first animal commenced at the times specified

** Mean of only two samples

L/S Lachrymal Secretion

Ph/F Pharyngeal Fluid

O/S Oral Saliva

N/S Nasal Secretion

F Faeces

U Urine

B Blood

Table 2. Mean pH values of bovine secretions measured at daily intervals for one week.

	L/S	Ph/F	O/S	N/S	F	U	B
Monday	7.42	8.35	8.39	7.90	6.58	8.26	7.60
Tuesday	7.08	8.62	8.45	7.65	7.11	6.48	7.65
Wednesday	7.25	8.71	8.36	7.86	7.06	7.35	7.53
Thursday	7.36	8.73	8.32	8.22	7.20	7.62	7.48
Friday	7.27	8.33	8.57	8.01	7.04	6.46	7.61
Saturday	7.44	8.26	8.55	8.05	6.82	6.53	7.58
Sunday	7.58	8.34	8.46	7.92	6.85	7.05	7.68
$\bar{x}$	7.34	8.47	8.44	7.94	6.95	7.10	7.59
S.E.	0.060	0.075	0.034	0.066	0.080	0.258	0.244

Key as for Table 1.

Table 3. Mean pH values of bovine secretions measured at monthly intervals throughout the year.

	L/S	Ph/F	O/S	N/S	F	U	B
January	7.43	8.61	8.56	7.83	7.10	6.73	7.51
February	7.60	8.32	8.40	7.56	7.25	7.26	7.64
March	7.05	8.41	8.63	7.91	6.63	6.93	7.64
April	7.60	8.48	8.52	8.21	6.95	7.50	7.50
May	7.13	8.73	8.55	8.05	6.73	7.63	7.71
June	7.42	8.31	8.47	8.12	7.20	7.28	7.52
July	7.25	8.65	8.38	7.95	7.14	8.00	7.63
August	7.33	8.69	8.42	7.73	6.84	6.41	7.44
September	7.36	8.54	8.55	8.24	7.00	6.56	7.56
October	7.27	8.75	8.32	8.02	6.93	6.84	7.42
November	7.54	8.55	8.47	7.77	6.53	7.62	7.70
December	7.40	8.63	8.52	7.92	6.68	7.93	7.54
$\bar{x}$	7.36	8.55	8.48	7.94	6.91	7.22	7.56
S.E.	0.048	0.042	0.024	0.057	0.067	0.529	0.095

Key as for Table 1.

Table 4. Mean pH values of bovine secretions from steers fed on different diets.

Week	L/S		Ph/F		O/S	
	A	B	A	B	A	B
1	7.43	7.37	8.61	8.42	8.56	8.34
2	7.34	7.45	8.23	8.36	8.32	8.40
3	7.05	7.31	8.66	8.45	8.61	8.45
4	7.28	7.06	8.37	8.56	8.33	8.51
5	7.60	7.42	8.41	8.42	8.63	8.38
$\bar{x}$	7.34	7.32	8.45	8.44	8.49	8.41
S.E.	0.090	0.069	0.078	0.031	0.067	0.028

	N/S		F		U	
	A	B	A	B	A	B
1	7.83	7.92	7.10	7.15	6.73	6.48
2	8.21	7.85	6.83	6.90	7.26	6.50
3	8.00	7.89	7.10	6.53	7.68	6.49
4	7.81	8.06	6.74	7.03	8.08	6.52
5	7.56	7.94	7.25	7.11	7.26	6.70
$\bar{x}$	7.88	7.93	7.00	6.94	7.40	6.53
S.E.	0.107	0.034	0.094	0.111	0.226	0.040

A Lucerne Cob Diet B Hay and Concentrate Diet

Key otherwise as for Table 1.

The figures agree closely with the results of daily, weekly and monthly measurements (Tables 1, 2, 3 and 4), and show that remarkably little variation, less than 1 pH unit, occurs in the pH of these secretions with the exception of urine. The results were used to select pH values which were employed in further tests on the kinetics of inactivation of FMDV by antiviral factors in secretions.

Table 5. Collected pH values of bovine secretions measured at the animal.

Sample	pH Value	
	Mean	Range
Tears	7.32	7.01 - 7.60
Pharyngeal Fluid	8.52	8.23 - 8.75
Oral Saliva	8.48	8.30 - 8.58
Nasal Secretion	8.01	7.67 - 8.40
Faeces	7.01	6.52 - 7.20
Urine	6.76	6.41 - 8.08
Blood	7.56	7.40 - 7.72

7.1.2. Normal protein values.

Proteins occurring naturally in secretions might influence FMDV infection and secretion by acting as a stabilising agent for viral infectivity or, in contrast, by exerting anti viral effects via mechanisms such as neutralisation. For this reason and because normal values were required for use in tests on the antiviral activity of secretions, a survey of total protein levels was conducted in normal cattle. Sampling was as

described in the corresponding section 7.1.1.1. - 7.1.1.5.

7.1.2.1. Diurnal variation

Table 6. Mean protein values of bovine secretions measured at four hourly intervals for one day (mg/100 ml).

Time of Day (hours)	L/S	Ph/F	O/S	N/S	U	Se
0800*	690	127	146	380	24.8	6840
1200	517	150	273	416	-	6720
1600	560	155	187	592	18.2**	6420
2000	679	270	190	463	-	6680
2400	749	306	432	458	37.0**	6710
0400	693	310	360	615	-	6850
0800	762	264	251	504	10.3	6840
$\bar{X}$	664	226	262	489	22.3	6722
S.E.	34.84	29.86	38.73	32.93	5.69	5.71

Se Serum Key otherwise as in Table 1.

* Sampling of the first animal commenced at the time specified

** Mean of only two samples.

The results in Table 6 show that total protein levels vary widely between secretions. Urine had the lowest levels, tears and nasal secretions had similar levels as did oral saliva and pharyngeal fluid while serum contained levels which were at least 12 times greater than the highest concentration found in external secretions.

7.1.2.2. Daily variations

The results were similar to those obtained during sampling throughout a 24 hour period as shown in Table 7.

Table 7. Mean protein values of bovine secretions measured at daily intervals for one week (mg/100 ml).

	T	Ph/F	O/S	N/S	U	Se
Monday	417	115	92	470	12.5	6490
Tuesday	563	167	117	545	13.2	6510
Wednesday	489	208	183	490	37.5	6830
Thursday	563	124	212	305	29.0	6810
Friday	605	463	309	580	60.0	6530
Saturday	587	202	620	557	10.2	6770
Sunday	521	651	306	720	35.6	6560
$\bar{x}$	535	274	262	523	28.3	6640
S.E.	24.59	76.62	67.55	47.58	6.81	5.76

Key as for Tables 1 and 6.

7.1.2.3. Seasonal variation

No seasonal variation was discerned in the protein content of secretions since values fluctuated at random throughout the period as shown in Table 8 below.

7.1.2.4. Effect of diet

The diets had no influence on the protein content of the secretions during the period of investigation as is shown in Table 9.

Table 8. Mean protein values of bovine secretions measured at monthly intervals for one year (mg/100 ml).

	L/S	Ph/F	O/S	N/S	U	Se
January	408	145	102	708	9.7	6570
February	516	290	196	825	3.2	6640
March	673	168	208	621	41.6	6690
April	605	192	314	900	35.0	6730
May	375	305	266	801	25.6	6650
June	791	673	435	672	27.0	6820
July	804	204	197	442	63.7	6770
August	563	337	250	750	45.0	6510
September	600	562	386	675	11.6	6770
October	726	457	251	638	18.0	6550
November	787	518	520	800	35.2	6610
December	583	213	418	681	14.6	6720
$\bar{x}$	619	338	295	709	27.5	6669
S.E.	143.35	50.25	35.14	34.34	5.03	2.80

Key as for Tables 1 and 6.

Table 9. Mean protein values of bovine secretions from steers fed on different diets (mg/100 ml).

Week	T		Ph/F		O/S	
	A	B	A	B	A	B
1	407	516	210	193	237	145
2	533	401	163	205	165	286
3	345	563	180	175	179	162
4	417	390	227	185	147	205
5	390	461	158	170	163	104
$\bar{x}$	418	466	187	185	178	180
S.E.	31.19	33.11	13.40	6.27	15.55	30.97

	N/S		U		Se	
	A	B	A	B	A	B
1	670	695	11.7	20.5	6780	6810
2	728	862	22.1	17.6	6510	6770
3	470	545	32.6	22.8	6810	6530
4	490	580	9.4	15.0	6620	6490
5	557	720	25.2	30.7	6850	6780
$\bar{x}$	583	680	20.2	21.2	6710	6670
S.E.	50.34	56.20	4.30	2.68	6.42	6.83

A Lucerne Cob Diet B Hay and Concentrate Diet

Key as for Table 6.

7.1.2.5. Collected protein values for bovine secretions (mg/100 ml) and comparisons with values from the literature

Table 10.

			Values from the literature.		Reference
	Mean	Range	Mean	Range	
Tears	540	390 - 804		824 - 896	A
			896		B
			639	350 - 1210	C
Nasal Secretion	596	305 - 900	931	660 - 1360	D
			224	179 - 244	A
			201	144 - 239	E
			735	329 - 1569	F
Pharyngeal Fluid	242	115 - 673			
Saliva	235	102 - 620		130 - 144	B
Urine	23	3.2 - 60			
Serum	6682	6420 - 6850	7600		G
				6120 - 6150	B

Reference			Breed
A	Duncan et al	(1972)	Holstein - Friesian
B	Butler et al	(1972)	Holstein - Friesian
C	Pedersen and Nansen	(1972)	Danish Red
D	Rouse and Angulo	(1970)	Unspecified
E	Mach and Pahud	(1971)	Simmental
F	McKercher et al	(1973)	Unspecified
G	Altman and Dittmer	(1961)	Various.

There is general agreement between the results of this new survey and the values from the literature. Specific discrepancies may reflect differences of age, sex, breed, management and techniques (see section 7.1.2.6.).

7.1.2.6. Effect of collection method on the protein content of nasal secretion.

The levels of total protein in nasal secretion reported by Duncan et al (1972) and Mach and Pahud (1971) were below those found by Rouse and Angelo (1970), McKercher et al (1973) or in the current survey. Since the lower levels were found in fluid collected as washings and the higher values in fluid collected on tampons, the method apparently influenced the results. To test this possibility and to investigate the possible exudation of serum protein caused by the tampon method, nasal secretions were obtained from steers on three successive days. On Day 1 secretion was collected in a rubber tipped pipette as it accumulated in the nares, on Day 2 secretion was collected by washing with PBS and on Day 3 by the use of tampons. The results in Table 11 show that a) less protein was recovered in washings, b) comparable levels were recovered by the slow collection of fluid as secreted or by the tampon method and c) the use of tampons did not increase the extravasation of serum protein.

Table 11.

Animal	Collection Method		
	Washing	Pipette	Tampon
HR8	237*	463	506
HR9	190	512	489
HR10	188	373	462
HR11	304	685	613
$\bar{X}$	229.7	508	517
S.E.	27.21	65.57	33.09

* Protein concentration in mg/100 ml of nasal secretion

7.1.3. Normal globulin classes in bovine secretions.

A number of recent reports have concerned the identification of globulin classes in bovine secretions. However, no data is available for the Devon breed and no single author has reported on the range of secretions investigated in this thesis. Moreover, little information is available on the globulin type in secretions following exposure to FMDV and none on possible alterations in the type of antibody found at different times after exposure. A survey was therefore conducted to establish the type of antibody present in normal bovine secretions.

Samples taken from 15 steers immediately before infection and from one normal animal at post mortem were examined by a number of techniques including gel filtration, anion exchange chromatography, immunoelectrophoresis and immunodiffusion.

Protein elution profiles are shown for secretions following gel filtration (Fig. 1) and anion exchange chromatography (Fig. 2). The secretions were from one steer but the profiles are representative of all the samples examined. Secretions and selected concentrated fractions from column chromatography were examined by immunoelectrophoresis (Plates 5 - 9) and immunodiffusion (Plate 10) using antisera directed against whole secretions or against single globulin classes.

Serum showed the characteristic triple peaks after gel filtration. IgM, IgG subclasses and albumin accounted for the main precipitating antibody reactions in the first, second and third peaks respectively. IgA was also detected in serum in concentrated fractions from the trough between the first and second peaks. Following anion exchange chromatography, serum proteins separated into a fall-through peak, containing principally IgG_2, and a second broad peak made up of at least two minor peaks containing, in order of elution, IgG_1, IgA, IgM and albumin. The findings were in accordance with those of other workers and indicated that the techniques of separation and identification were functioning adequately.

Lachrymal secretion gave two distinct peaks on gel filtration, the first and major peak contained IgA with lesser amounts (as measured by the intensity of the precipitin reaction) of IgM. IgG_1 was detected in the trough and the smaller second peak contained albumin. No IgG_2 could be detected. Anion exchange results showed a small fall-through peak followed by a trough with a second distinct peak, composed largely of secretory IgA, running into a third ill-defined peak containing secretory IgA, IgM and Albumin. Mach and Pahud (1971) reported similar results but were also able to detect low levels of IgG_2 in some lachrymal secretions from Friesian cattle.

Nasal secretion separated into two peaks on sephadex G200; the first sharply defined peak contained chiefly IgA with traces of IgM while the trough and second peak were rich in IgG_1 with traces of albumin occurring at the tail of the pattern. Results of anion exchange chromatography resembled those obtained for tears.

The elution patterns and precipitin reactions obtained with concentrated oral saliva and pharyngeal fluid were indistinguishable. The first, well defined peak on gel filtration contained secretory IgA while IgG_1 was detected in the trough and in the second diffuse peak. The absence of IgG_2 was confirmed on anion exchange chromatography and the single broad peak contained principally secretory IgA with some IgG_1. The low levels of IgG_2 and IgM detected in saliva by Mach and Pahud (1971) and Duncan et al (1972) were not confirmed.

Intestinal mucus and bronchial washings were subjected only to precipitin tests. Reactions were particularly strong with antiserum to secretory IgA and less well defined with antiserum to IgG_1. No reactions were elicited in tests with antiserum to IgG_2 and IgM.

It is to be noted that no attempt was made to distinguish the minor protein components of serum and secretions reported by other workers and mentioned in the literature review of this ~~this~~ thesis. The classes were named by analogy with human immunoglobulins according to a) the elution pattern in chromatography, b) the relative position of precipitin lines in immunodiffusion against specific antisera. The results are shown in Table 12. In summary, the predominant globulin class of external secretions in steers of the Devon breed was IgA. IgG_1 was also commonly detected in smaller amounts while IgM was a minor component of certain secretions. The secretory globulins of the breed are essentially similar to those reported for other breeds of cattle.

Fig 1. Gel Filtration Protein Elution Profiles For Bovine Secretions.

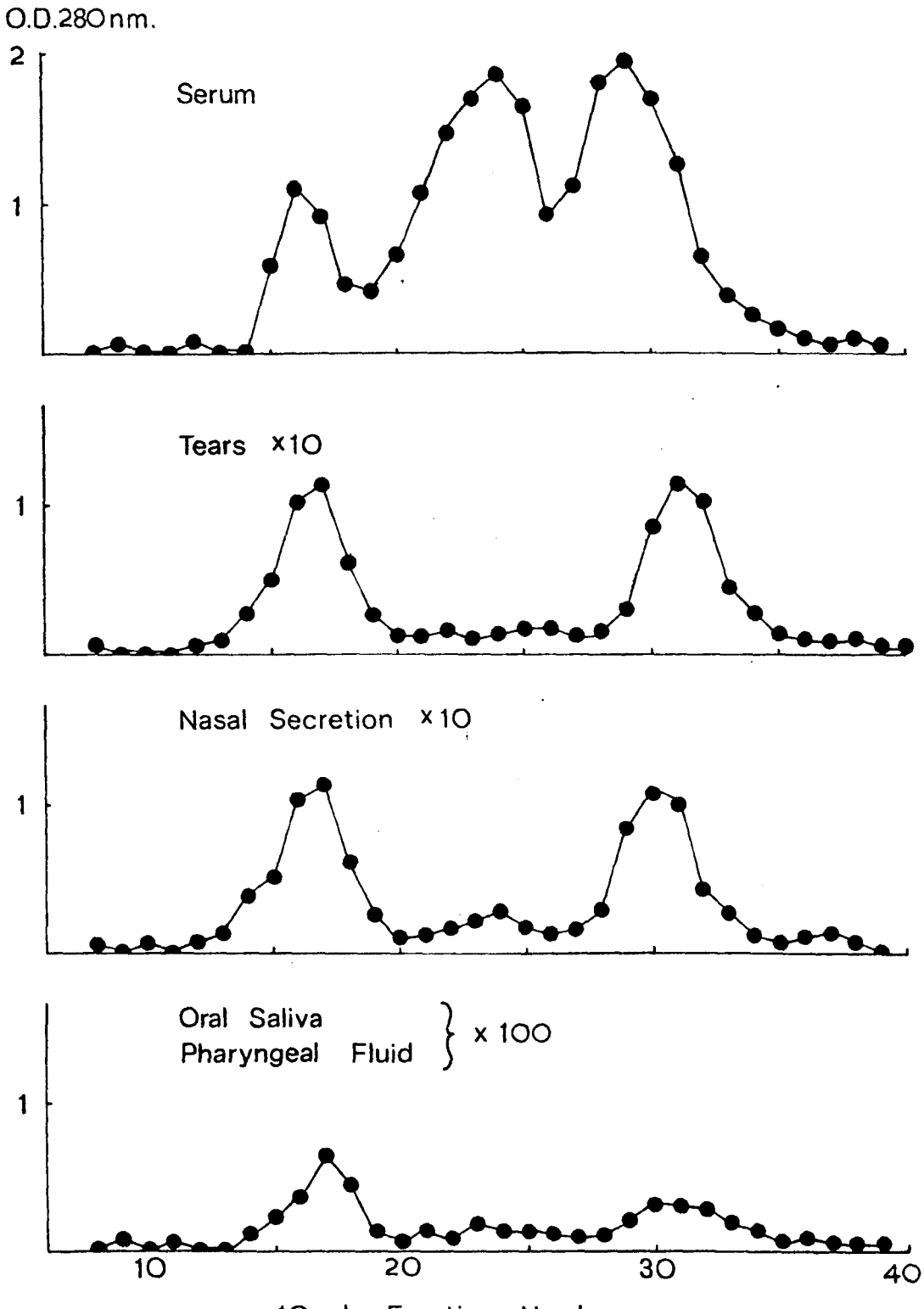
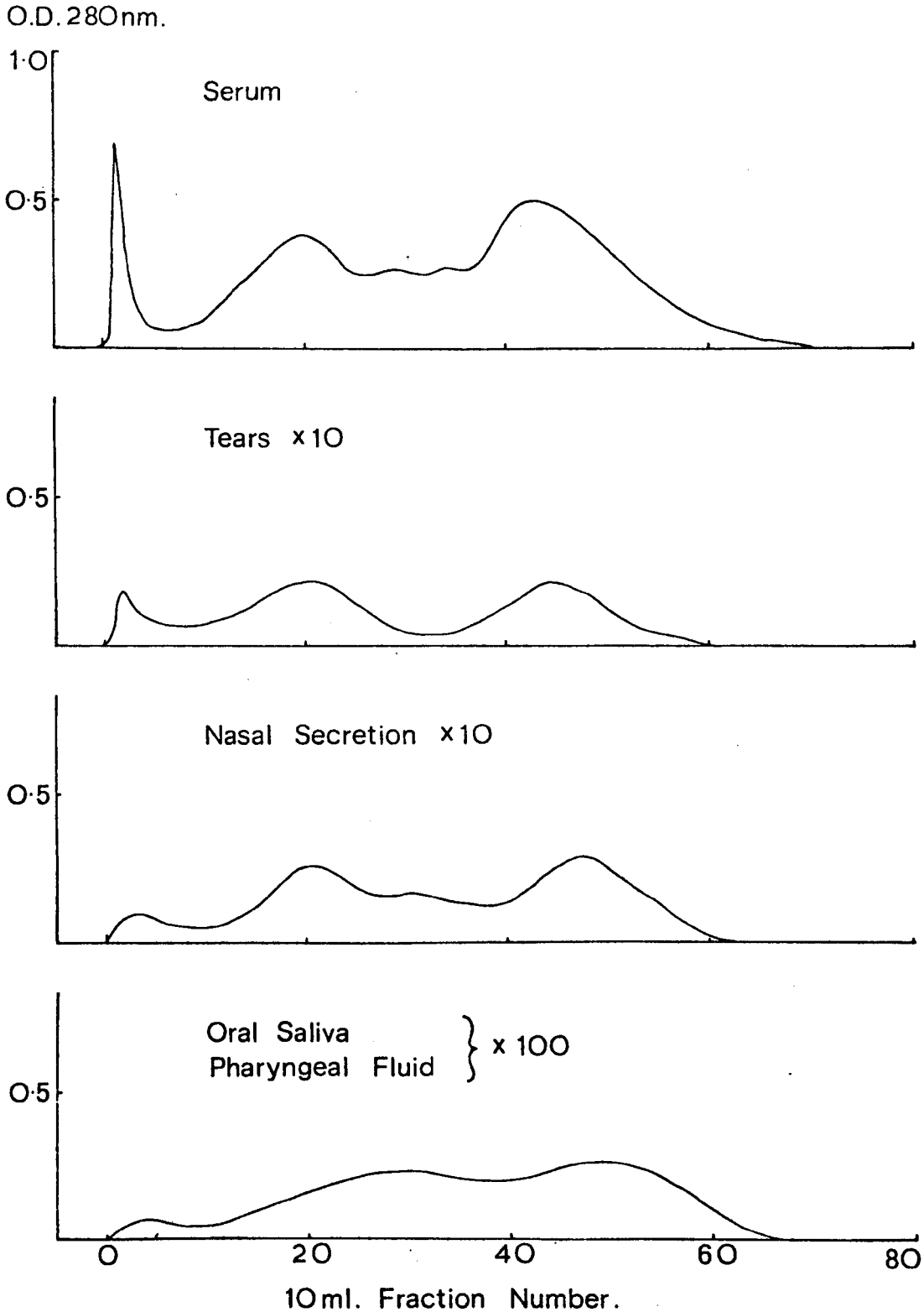


Fig 2. Anion Exchange Chromatography Protein Elution Profiles For Bovine Secretions.



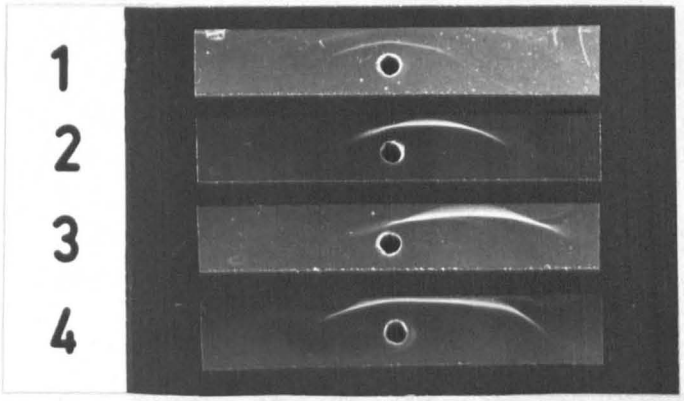


PLATE 5. Immunoelectrophoretic patterns for bovine immunoglobulins.

Wells contained: 1, 3 and 4 - bovine serum.
2 - bovine colostrum.

Troughs contained:
1. Antiserum to bovine IgM
2. Antiserum to bovine IgA
3. Antiserum to bovine IgG_1
4. Antiserum to bovine IgG_2

Cathode on the right.

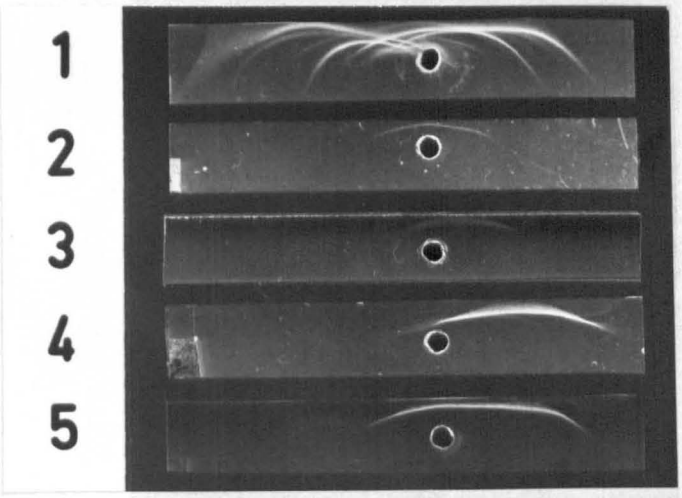


PLATE 6. Immunoelectrophoretic patterns for bovine serum.

Wells contained bovine serum.

Troughs contained:
1. Antiserum to whole bovine serum.
2. Antiserum to bovine IgM.
3. Antiserum to bovine IgA.
4. Antiserum to bovine IgG_1.
5. Antiserum to bovine IgG_2.

Cathode on the right.

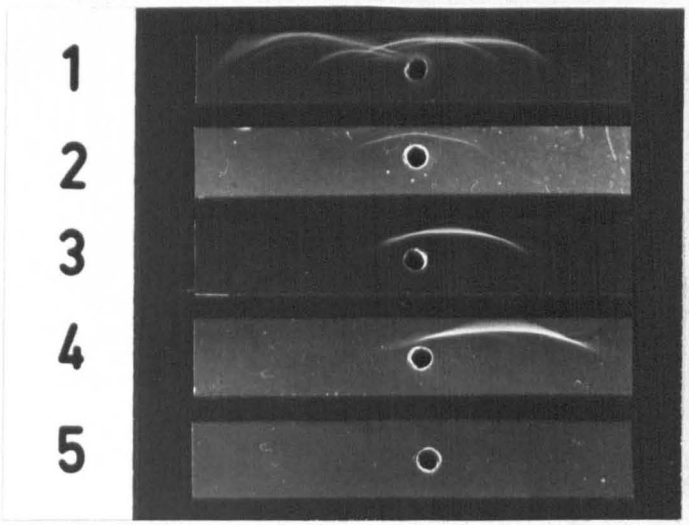


PLATE 7. Immunoelectrophoretic patterns for bovine lachrymal secretion.

Wells contained bovine lachrymal secretion x10.

Troughs contained:
1. Antiserum to whole bovine lachrymal secretion.
2. Antiserum to bovine IgM.
3. Antiserum to bovine IgA.
4. Antiserum to bovine IgG_1
5. Antiserum to bovine IgG_2.

Cathode on the right.

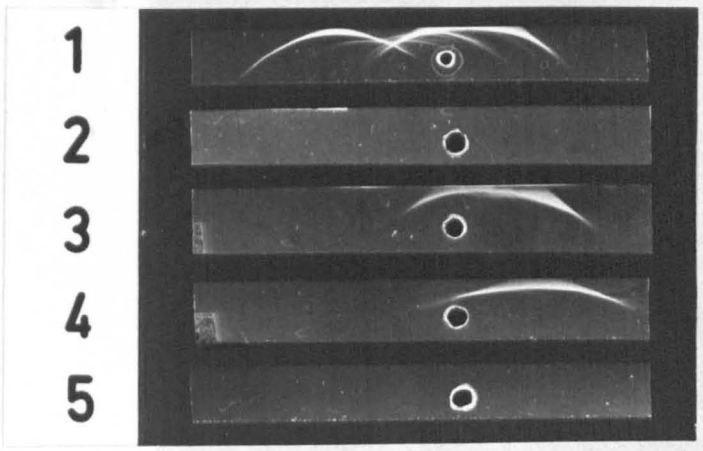


PLATE 8. Immunoelectrophoretic patterns of bovine nasal secretions.

Wells contained bovine nasal secretion.

Troughs contained:
1. Antiserum to whole bovine nasal secretion.
2. Antiserum to bovine IgM.
3. Antiserum to bovine IgA.
4. Antiserum to bovine IgG_1.
5. Antiserum to bovine IgG_2.

Cathode on the right.

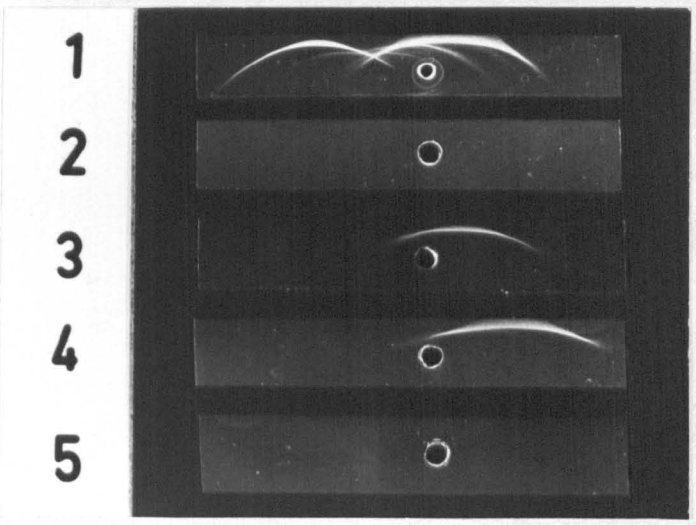


PLATE 9. Immunoelectrophoretic patterns for bovine oral saliva/pharyngeal fluid.

Wells contained oral saliva/pharyngeal fluid x100.

Troughs contained:
1. Antiserum to whole bovine oral saliva/pharyngeal fluid.
2. Antiserum to bovine IgM.
3. Antiserum to bovine IgA.
4. Antiserum to bovine IgG_1.
5. Antiserum to bovine IgG_2.

Cathode on the right.

Table 12. Distribution of globulin classes in normal bovine secretions.

Secretion	IgM	IgA	IgG_1	IgG_2
Lachrymal	++	+++	+	-
Nasal	+	+++	++	-
Salivary (x20)	-	+++	+	-
Pharyngeal (x20)	-	+++	+	-
Bronchial	-	+++	+	-
Intestinal	-	+++	+	-
Serum	+++	+	++++	+++

Precipitin Reactions: - None, + Faint, ++ Distinct, +++ Strong, ++++ Very Strong.

A subjective assessment was made of the maximum intensity of the precipitin reaction which occurred during 5 days of observation.

7.1.4. Antiviral factors in normal secretions.

7.1.4.1. Interferons.

No interferon was detected in serum or in lachrymal, nasal, salivary or pharyngeal secretion from each of 16 normal cattle or in intestinal or lower respiratory secretions from one steer.

7.1.4.2. pH and other effects.

a) The range of pH stability of FMDV strains:

Since pH could play a part in the inactivation of FMDV in secretions and since the effect might be compounded with that of other anti-

viral factors, preliminary studies were carried out to establish the range of pH stability of the virus strains in experiments uncomplicated by factors other than hydrogen ion concentration. The survival of type O,(Swiss 1/66), A and C FMDV for 60 minutes at 37°C in buffers containing 0.1% BPA and covering the pH range of 5 - 10 is shown in Fig. 3b.

Under the conditions of the test optimal survival of viral infectivity occurred over the pH ranges 7.0 - 8.25 (type A), 7.0 - 8.0 (type O) and 7.5 - 8.5 (type C). The pH values of pharyngeal fluid, oral saliva and nasal secretion may exceed these ranges of pH stability while those of faeces and urine may fall below them. In these circumstances, the pH of the secretion might itself serve in a protective capacity. However, virus inactivation proceeded only slowly through further increments of 0.25 - 0.5 pH units on either side of the range of optimal stability and traces of viruses persisted below pH 6.5 or above 9.0 for more than 60 minutes. The intrinsic antiviral effect of pH in secretions is likely to be of minor importance.

b) The kinetics of inactivation of FMDV in secretions at physiological pH values.-

Preliminary studies indicated that the inactivation of virus at 37°C proceeded more rapidly in certain secretions than in buffers at the same pH. Experiments were therefore conducted to examine the kinetics of virus inactivation in normal tears, nasal fluid, oral saliva and pharyngeal fluid. Virus was suspended in secretion or buffer at the appropriate mean physiological pH value and the inactivation of virus was measured over a four hour period at 37°C. Results are shown in Fig. 4. FMDV in tears or nasal secretion lost titre at a rate which was equal to or slightly less than the rate in the corresponding buffer. The slight sparing effect may have been due to the extra protein in the secretions. However, viruses were inactivated much more rapidly in oral

Fig 3 A. Mean And Range Of pH Of Normal Bovine Secretions .

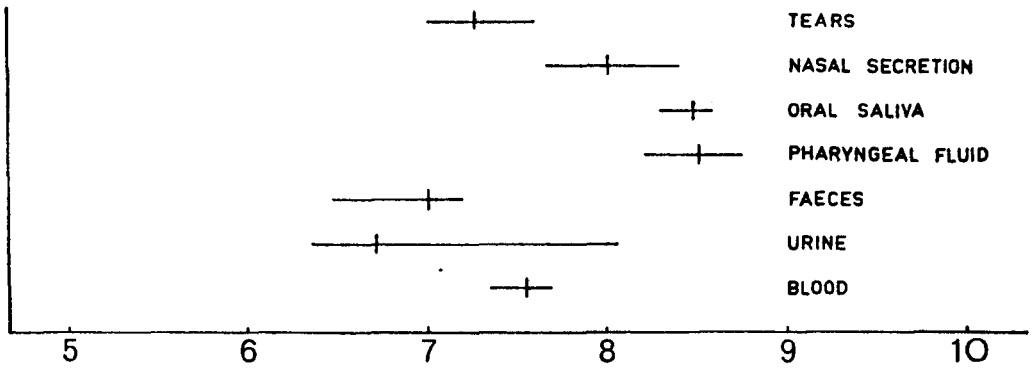
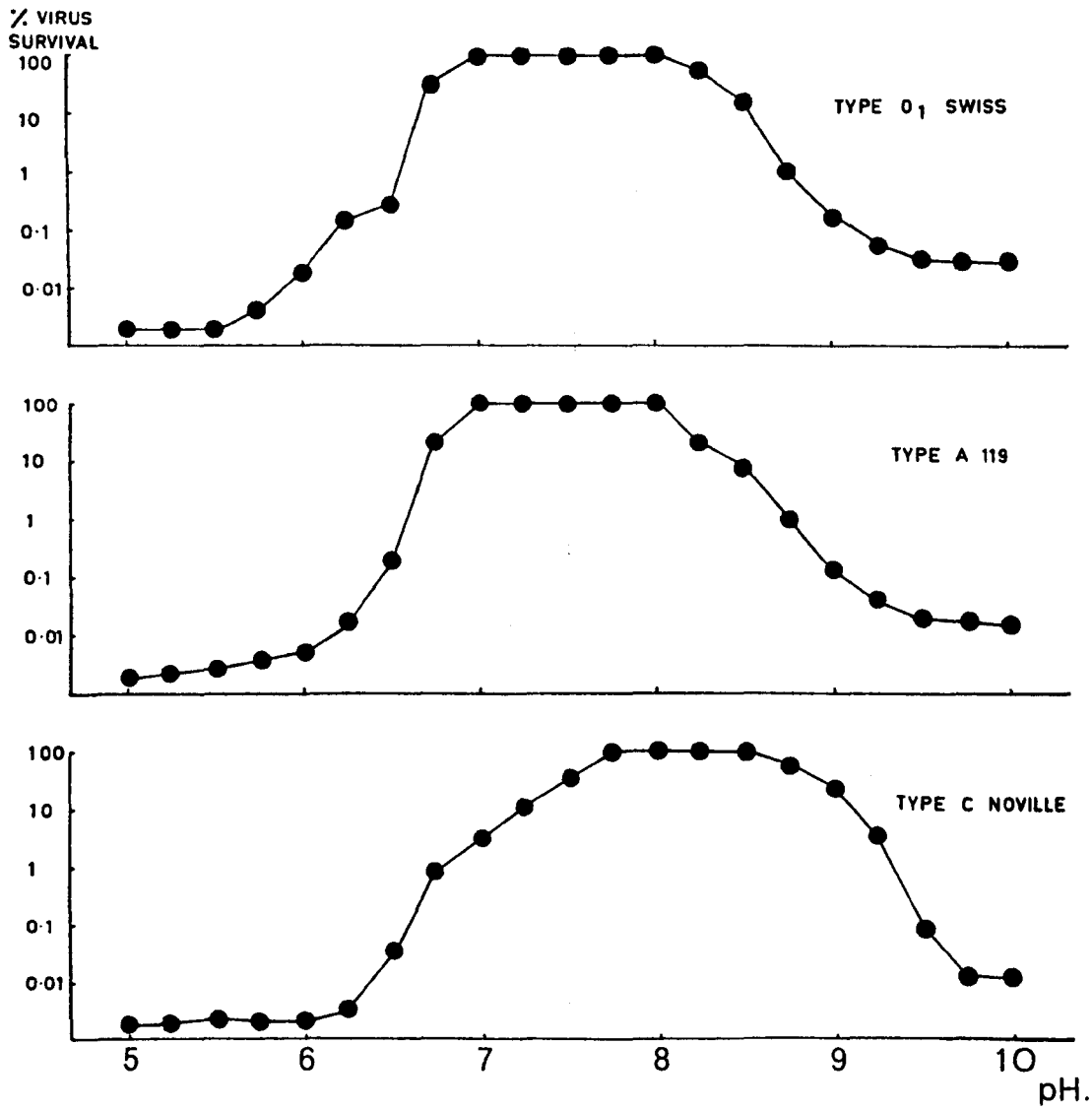


Fig 3 B. Range Of pH stability Of FMDV Strains At 37°C .



saliva and pharyngeal fluid than in buffer solution at the same pH. A difference of 2 - 3 $\log_{10}$ p.f.u. was apparent for all strains of virus with marginally greater inactivation occurring with types O and A than with type C.

Normal bovine saliva and pharyngeal fluid therefore contained two non specific antiviral factors: an adverse pH effect and an additional unidentified component.

c) Investigations of the additional, non specific antiviral factor in saliva and pharyngeal fluid.

Partial characterisation of the additional antiviral factor was carried out using a pool of normal bovine saliva and type O (Swiss 1/66) FMDV to investigate the effect of concentration, pH, heat and dialysis.. The standard assay method was employed using virus at $10^{6.0}$ p.f.u./ml, sampling after 4 hours incubation at 37°C and assaying by the plaque technique in IB-RS-2 monolayers.

(i) Effect of concentration.

Virus was diluted in varying proportions of saliva or glycine - sodium hydroxide buffer at pH 8.5 and the mixtures were held at 37°C for 4 hours. The virus titre decreased as the proportion of saliva increased (Table 13) showing the concentration dependancy of the inactivation.

(ii) Effect of pH

Saliva was heated to 56°C for 30 minutes and aliquots were adjusted to pH values of 7.5, 8.0 and 9.0 by the addition of NaOH or HCl. Virus was suspended in saliva or buffer at these pH values at $10^{6.0}$ p.f.u./ml

and the mixtures were held at 37°C for 4 hours. Although the pH values altered during incubation (Table 14) the results showed virus inactivation by saliva to be independent of pH values over the range 7.5 - 8.5.

Table 13. Effect of the concentration of saliva on the inactivation of FMDV.

Diluent Ratio Buffer/Saliva	Virus Titre $\log_{10}$ p.f.u./ml
100/0	4.52*
75/25	3.84
50/50	3.25
25/75	2.70
0/100	2.35

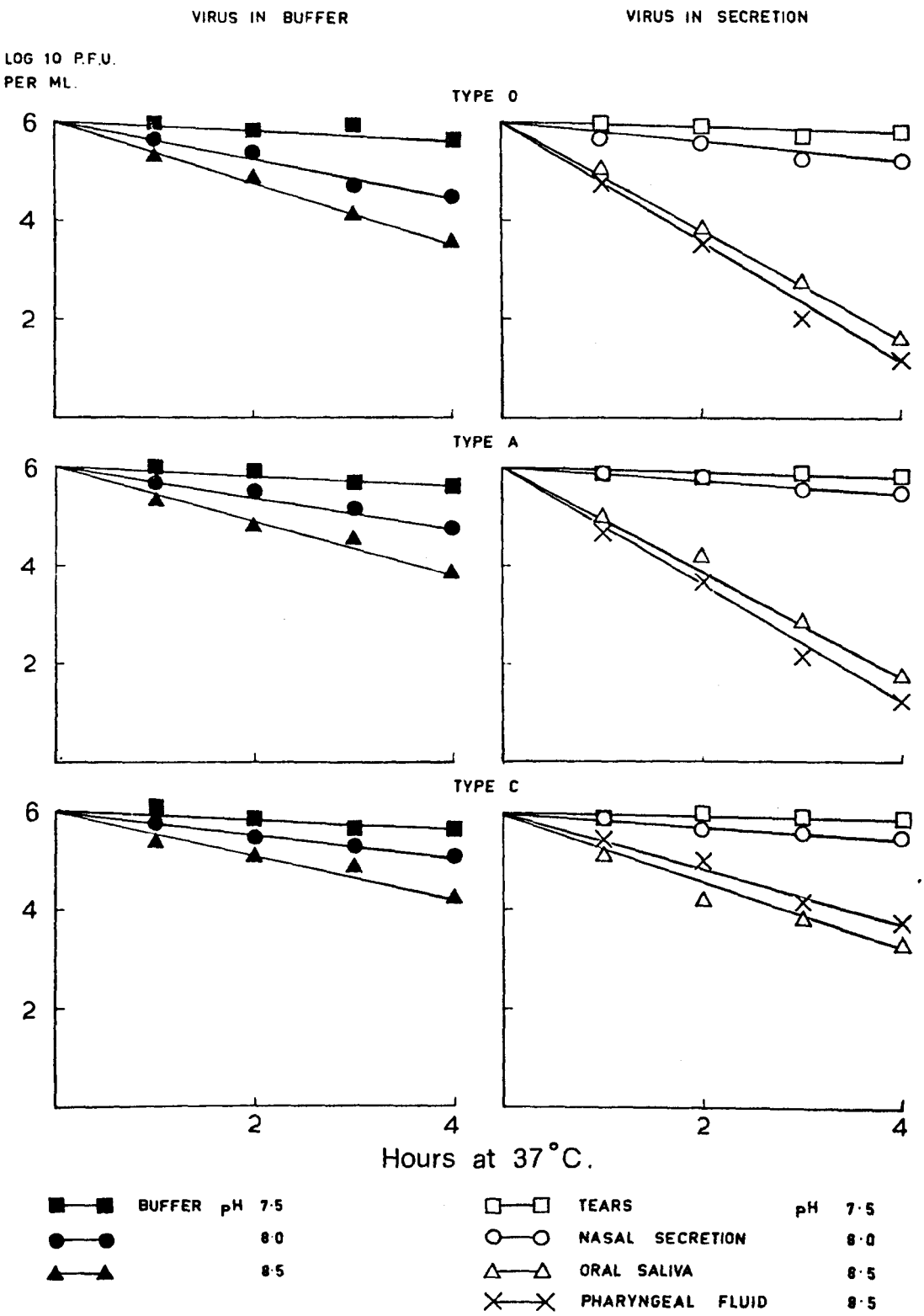
* Virus titre after 4 hours at pH 8.5 and 37°C.
Initial titre $10^{6.0}$ p.f.u./ml. Mean of 3 experiments.

Table 14. Effect of pH on the inactivation of FMDV by saliva.

Diluent	pH		Virus Titre
	Initial	Final	$\log_{10}$ p.f.u./ml
Buffer	7.50	7.55*	5.82**
	8.00	8.10	5.57
	8.50	8.53	5.14
	9.00	9.15	4.63
Saliva	7.50	7.70	2.50
	8.00	8.22	2.65
	8.50	8.65	2.58
	9.0	9.02	2.14

* Maximum pH deviation in 3 experiments. ** Virus titre after 4 hours at 37°C. Initial titre $10^{6.0}$ p.f.u./ml. Mean of 3 experiments.

Fig4. Kinetics Of Inactivation Of FMDV In Buffers And Bovine Secretions At Physiological pH Values.



(iii) Effect of heat

Saliva was heated at 37°C, 65°C or 70°C and samples were withdrawn at ten minute intervals for up to 1 hour. The pH was adjusted to 8.5 by the addition of HCl (1.0 or 0.1N), virus was suspended in the saliva samples and the mixtures incubated at 37°C.

The antiviral factor was not affected by heating at 65°C for 60 minutes but was partially destroyed after 10 minutes and completely destroyed after 20 minutes at 70°C (Table 15).

Table 15. Effect of heat on the antiviral factor in saliva

Diluent.	Incubation Temp. (°C)	Time of incubation (mins.) 0	10	20	30	40	50	60
Saliva	37	3.40*	-	-	-	-	-	3.31
	65	3.58	-	-	-	-	-	3.50
	70	3.45	1.76	0.98	1.0	-	-	-
PBS	-	1.20						

* Reduction in titre, ($\log_{10}$ p.f.u./ml) occurring when virus at $10^{6.0}$ p.f.u./ml was incubated for 4 hours at 37°C and pH 8.5 in PBS or in saliva which had been heated at 37, 65, 70°C for various times. Mean of 3 experiments.

(iv) Effect of Dialysis.

Saliva was dialysed against PBS at pH 7.5 or 8.5 overnight at 4°C with constant stirring using a saliva : PBS ratio of 1 : 50. The inactivation of virus was studied in dialysed and non dialysed saliva and in buffer at the same pH. Dialysis at pH 8.5 resulted in the

removal of the additional antiviral factor while dialysis at pH 7.5 resulted in the removal of both the pH effect and the additional antiviral agent (Table 16).

Table 16. Effect of dialysis on the antiviral factors in saliva.

Diluent	pH	Virus titre	pH	Virus titre
PBS	7.5	6.01*	8.5	5.23
Saliva		2.70		2.58
Dialysed saliva		5.83		5.16

* $\log_{10}$ p.f.u./ml after 4 hours at 37°C. Mean of 3 experiments.

7.1.5. Conclusions.

The first section of experimental results was largely concerned with the establishment of normal values for bovine secretions. The mean and range of pH and protein content was determined for tears, nasal secretions, oral saliva, pharyngeal fluid, faeces, urine and serum. Values varied between animals and between samples from the same animal but no diurnal, daily and seasonal pattern was apparent. Two different diets were investigated for their effect on the pH and protein content of secretions. An effect was discernible only in the case of urine which was of a consistently lower pH in cattle fed on a diet of hay and concentrates than in cattle fed on lucerne cobs.

The major immunoglobulin classes were identified in a number of secretions. In common with the other breeds of cattle so far investigated, IgA was found in all secretions together with an IgG_1 component while IgM was also identified in lachrymal secretion.

The range of pH stability of the FMDV strains was determined in buffer solutions. The pH values for type C Noville (pH 7.5 - 8.5) were greater than for type O Swiss (pH 7.0 - 8.0) or type A 119 (pH 7.0 - 8.25).

The antiviral effect of normal secretions was studied in experiments which incorporated the baseline data established for pH and protein content. No interferon was detected in normal secretions. Comparisons between the normal pH values for secretions and the range of pH stability of the viruses revealed that hydrogen ion concentration alone could exert an antiviral effect in some instances. However, although the effect was dependent on the virus strain, it was likely to be of only minor significance in protection because of the slow rate of viral inactivation. The rate at which FMDV strains lost infectivity (log/hour) in buffers at 37°C was :

pH	type O	type A	type C
7.5	0.100	0.125	0.100
8.0	0.375	0.325	0.200
8.5	0.625	0.575	0.375

Virus in tears at pH 7.5 or nasal secretion at pH 8.0 and 37°C lost infectivity at rates very similar to those occurring in buffer.

Apart from the pH effect an additional, non specific, antiviral factor was discovered in normal bovine saliva or pharyngeal fluid. The factor was most active against types O and A and less so against type C. Inactivation rates for virus at pH 8.5 and 37°C were as follows:

	type O	type A	type C
Saliva	1.075	1.185	0.700
Pharyngeal Fluid	1.250	1.075	0.750

The factor was concentration dependent, active over the pH range of at least 7.5 - 8.5, unaffected by heating at 60°C for 1 hour, partially destroyed after 10 minutes and totally destroyed after 20 minutes at 70°C and able to be removed by dialysis. The protective capacity of this factor was also likely to be small since viral inactivation proceeded only slowly under its influence.

7.2. Secretions from convalescent animals

The role of secretions in pathogenesis was investigated in steers experimentally infected with FMDV types O, A and C. The experiments involved short term studies (made between Day 0 and Day 56 post exposure to infection) and long term studies (made between 2 months and 5½ years after infection). Factors investigated were: the acquisition and excretion of virus, the development of clinical disease, the induction of interferon, the antibody response (neutralising capacity, kinetics of neutralisation and type of immunoglobulin), the pH and protein content of secretions, the carrier state and the interrelationships between these factors.

A standard method of direct infection was used whereby groups of 2 - 3 steers were housed in one box with two infected donor pigs. After 2 hours of contact the steers were moved into separate, clean boxes and the pigs were slaughtered.

7.2.1. Short term observations.

7.2.1.1. The acquisition and secretion of FMDV

In order to elucidate the comparative importance of various routes of infection and excretion of FMDV, a study was made of virus excretion

following infection by natural routes. Cattle were sampled immediately prior to infection on Day 0, at approximately 24 hour intervals for up to 12 days and then at 2 - 3 day intervals for up to 56 days post exposure. Samples were assayed for infectivity on CTY monolayers in tubes within 4 hours of collection or after longer periods of storage at -70°C and the identity of a random sample of cultures showing CPE was checked by complement fixation.

The frequency of recovery and the amount of virus in secretions and excretions is given in Table 17 (type A, 119), Table 18 (type O, Swiss 1/66), Table 19 (type O, BFS 1860), Table 20 and Figure 5 (type C, Noville). The development of clinical disease is shown in Tables 22 and 23.

The method of exposure was akin to that occurring in the field when the diseased animal infects a stall mate. Exposure resulted in the development of generalised disease between 2 and 4 days post infection but the degree of challenge in each case was unknown. However, comparison of the amounts of virus recovered on Day 1 suggests that the steers exposed to type O, Swiss 1/66 (Table 18) experienced a far greater challenge than any of the other groups (Tables 17, 19 and 20). Alternatively the Swiss O strain might be a more invasive agent.

Samples may have been contaminated during collection and those containing minimal amounts of virus at the peak of the syndrome are particularly suspect in this respect. Nevertheless the pattern of excretion is clear (Fig. 5). Virus was generally detected earliest and to highest titre in pharyngeal fluid. Oral saliva and nasal secretion were often positive at the same time as pharyngeal fluid but almost always to a lower level. Lachrymal and preputial swabs also contained appreciable amounts of virus before the onset of clinical disease. Urine and faeces

contained little if any virus during this period. Viraemia ensued within 1 - 3 days of exposure and persisted for 3 - 6 days.

Once the disease was apparent, high levels of FMDV could be detected in all the secretions with the exception of urine, from which sporadic recoveries were made, and faeces which contained the least amount of virus. One steer (JT71, Table 18) infected with type O_1 Swiss 1/66 showed unusually high levels of virus in urine. Maximum excretion of virus occurred during the period 1 - 3 days before and after the development of vesicles in the nose and mouth with peak levels in saliva and pharyngeal fluid at the time of their rupture. Of the 4 virus strains examined the O_1 Swiss 1/66 strain was excreted earliest in greatest amounts, Excretion of the other strains followed a similar pattern but at a later time (C Noville) or a lower level (O_1 BFS 1860, A 119). Table 21 gives the titre of virus in secretions and excretions as measured in this study together with values from the literature.

Fluorocarbon was employed to dissociate possible antigen - antibody complexes in secretions during the acute phase of FMD. In some instances an increased amount of virus was recovered following treatment (details in section 7.2.1.6.)

Clinical signs developed in a characteristic manner (Tables 22 and 23). The febrile response varied, some animals showed little deviation of temperature while others were pyrexic for up to 4 days. Neither the extent and severity of lesions nor the degree of viral excretion could be correlated with the temperature response. It is noteworthy that one steer in each group infected with types O and C remained free of tongue lesions despite the development of extensive vesicles elsewhere.in the mouth. The frequent involvement of the prepuce is also of interest as a feature which has not been commonly recorded. No conclusions could

Table 17. Excretion of FMDV type A (119) from steers.

Animal No.	Sample	Days post exposure										
		0	1	2	3	4	5	6	7	8	9	10
					+							
HX20	N/S	0.0	Tr	3.0	5.0	5.3	4.0	1.5	0.0	Tr	0.0	0.0
	O/S	0.0	1.0	4.8	6.5	7.0	6.5	4.3	2.8	0.0	0.0	0.0
	Ph/F	0.0	1.8	4.5	7.3	6.8	5.0	5.0	3.5	1.3	1.0	2.0
	F	0.0	0.0	Tr	1.3	2.0	0.0	1.5	0.0	0.0	0.0	0.0
	U	0.0	0.0	-	2.0	2.3	0.0	1.8	0.0	0.0	0.0	0.0
	P/S	0.0	Tr	2.8	4.6	5.3	2.1	0.0	1.2	0.0	0.0	0.0
	Se	0.0	0.0	1.5	6.0	6.3	5.5	1.8	0.0	0.0	0.0	0.0
						+						
HX21	N/S	0.0	Tr	0.0	2.8	6.0	5.0	4.8	3.3	0.0	0.0	0.0
	O/S	0.0	0.0	1.3	4.5	6.8	6.5	5.3	4.0	1.3	0.0	0.0
	Ph/F	0.0	1.0	2.5	3.8	6.5	6.5	6.0	3.5	2.8	1.3	2.0
	F	0.0	0.0	0.0	0.0	2.0	0.0	0.0	0.0	0.0	0.0	0.0
	U	0.0	-	0.0	Tr	1.8	2.5	0.0	-	Tr	0.0	0.0
	P/S	0.0	1.3	1.6	2.6	2.3	3.8	4.1	1.3	0.0	0.0	0.0
	Se	0.0	0.0	0.0	3.5	6.5	5.8	4.3	1.3	0.0	0.0	0.0

Four CTY tubes per dilution.

Key		Sample	Lowest dil. tested	Results expressed as
L/S	:	Lachrymal Swab	1:2 (approx.)	CTY ID_{50}/sample
N/S	:	Nasal secretion	1:1	CTY ID_{50}/ml
O/S	:	Oral Saliva	1:1	CTY ID_{50}/ml
Ph/F	:	Pharyngeal Fluid	1:1	CTY ID_{50}/ml
F	:	Faeces	1:10	CTY ID_{50}/g
U	:	Urine	1:1	CTY ID_{50}/ml
P/S	:	Preputial Swab	1:5 (approx.)	CTY ID_{50}/ml
Se	:	Serum	1:1	CTY ID_{50}/ml

\+ : Earliest detection of clinical lesions
\- : No sample
Tr : Trace of virus (one or two tubes showing CPE at the lowest dilution tested.
0.0 : No virus detected at the lowest dilution tested.

Table 18. Excretion of FMDV type O (Swiss 1/66) from steers.

Animal No.	Sample	Days post exposure										
		0	1	2	3	4	5	6	7	8	9	10
JT71	L/S	0.0	2.8	4.8+	5.3	6.3	2.8	2.8	1.8	0.0	0.0	0.0
	N/S	0.0	4.5	6.8	6.3	4.3	4.3	3.3	0.0	0.0	0.0	0.0
	O/S	0.0	5.3	6.8	6.8	5.0	4.8	4.3	0.0	0.0	0.0	0.0
	Ph/F	0.0	6.3	5.8	7.3	4.5	5.3	3.3	2.8	0.0	2.3	3.0
	F	0.0	0.0	2.3	2.3	0.0	2.5	1.8	0.0	0.0	0.0	0.0
	U	0.0	0.0	3.8	4.3	5.5	4.8	4.0	0.0	0.0	0.0	0.0
	P/S	0.0	2.8	5.6	5.8	4.1	4.6	4.8	0.0	0.0	0.0	0.0
	Se	0.0	4.3	5.8	5.3	4.5	0.0	0.0	0.0	0.0	0.0	0.0
JT72	L/S	0.0	Tr	2.3	4.6+	6.1	5.3	3.6	1.8	0.0	0.0	0.0
	N/S	0.0	2.8	4.8	6.3	5.0	4.5	3.3	0.0	0.0	0.0	0.0
	O/S	0.0	5.8	7.3	6.8	6.5	5.3	4.8	Tr	0.0	0.0	0.0
	Ph/F	0.0	6.8	7.8	6.8	5.3	3.0	4.8	2.3	1.8	0.0	3.3
	F	0.0	0.0	2.3	Tr	0.0	0.0	2.3	0.0	0.0	0.0	0.0
	U	0.0	0.0	2.3	3.8	1.3	0.0	2.0	0.0	0.0	0.0	0.0
	P/S	0.0	1.8	3.6	4.8	2.8	4.3	4.8	0.0	0.0	0.0	0.0
	Se	0.0	1.5	5.3	4.8	4.8	2.0	0.0	0.0	0.0	0.0	0.0
JT73	L/S	0.0	5.8	3.8+	4.8	4.3	4.1	5.3	1.6	0.0	0.0	0.0
	N/S	0.0	2.8	5.8	7.3	5.8	3.8	3.0	0.0	0.0	0.0	0.0
	O/S	0.0	4.3	8.3	7.8	5.3	3.8	3.0	2.8	0.0	0.0	0.0
	Ph/F	0.0	4.8	6.3	5.8	4.8	4.5	4.8	2.8	2.3	1.3	2.8
	F	0.0	0.0	3.0	0.0	1.8	0.0	2.3	0.0	0.0	0.0	0.0
	U	0.0	0.0	2.8	3.8	1.8	0.0	2.3	0.0	0.0	0.0	0.0
	P/S	0.0	Tr	4.6	5.8	4.8	4.3	4.8	0.0	0.0	0.0	0.0
	Se	0.0	4.3	6.3	6.8	3.5	0.0	0.0	0.0	0.0	0.0	0.0

Four CTY tubes used per dilution. Key as for Table 17.

Table 19. Excretion of FMDV type O (BFS 1860) from steers.

Animal No.	Sample	Days post exposure										
		0	1	2	3	4	5	6	7	8	9	10
HR8	N/S	0.0	0.0	1.5	4.0	5.5+	5.0	4.5	2.5	0.0	Tr	0.0
	O/S	0.0	Tr	2.0	4.5	5.5	6.0	5.5	3.0	1.0	0.0	0.0
	Ph/F	0.0	0.0	4.5	4.0	6.0	4.5	4.5	5.0	1.5	1.0	1.5
	F	0.0	0.0	0.0	0.0	Tr	0.0	1.5	0.0	0.0	0.0	0.0
	U	0.0	-	0.0	0.0	1.0	1.5	1.5	0.0	-	0.0	0.0
	P/S	0.0	0.0	Tr	1.5	3.5	4.0	4.0	2.5	0.0	0.0	0.0
	Se	0.0	0.0	0.0	1.2	5.2	5.2	2.2	0.0	0.0	0.0	0.0
HR9	N/S	0.0	Tr	0.0	1.5	5.0+	5.0	4.5	3.0	1.0	0.0	0.0
	O/S	0.0	1.5	2.0	3.5	5.5	6.0	5.5	1.5	0.0	0.0	0.0
	Ph/F	0.0	2.0	5.5	4.0	4.5	4.5	3.5	2.5	1.5	1.0	1.5
	F	0.0	0.0	0.0	Tr	1.5	Tr	0.0	1.5	0.0	0.0	0.0
	U	0.0	0.0	-	1.0	2.0	0.0	2.5	0.0	0.0	0.0	0.0
	P/S	0.0	0.0	Tr	2.5	4.0	4.5	4.0	1.0	0.0	0.0	0.0
	Se	0.0	0.0	Tr	1.2	4.2	5.2	1.5	0.0	0.0	0.0	0.0
HR11	N/S	0.0	2.0	4.0+	5.0	3.5	2.0	0.0	0.0	0.0	0.0	0.0
	O/S	0.0	3.5	5.8	5.5	6.0	4.0	2.3	0.0	0.0	0.0	0.0
	Ph/F	0.0	6.5	7.0	5.0	4.5	3.5	2.5	2.0	0.0	1.0	1.0
	F	0.0	0.0	1.5	0.0	Tr	0.0	0.0	0.0	0.0	0.0	0.0
	U	0.0	-	1.0	1.0	0.0	-	1.0	0.0	0.0	0.0	0.0
	P/S	0.0	1.5	3.5	3.5	2.0	0.0	1.0	0.0	0.0	0.0	0.0
	Se	0.0	3.0	3.0	4.5	2.0	0.0	0.0	0.0	0.0	0.0	0.0

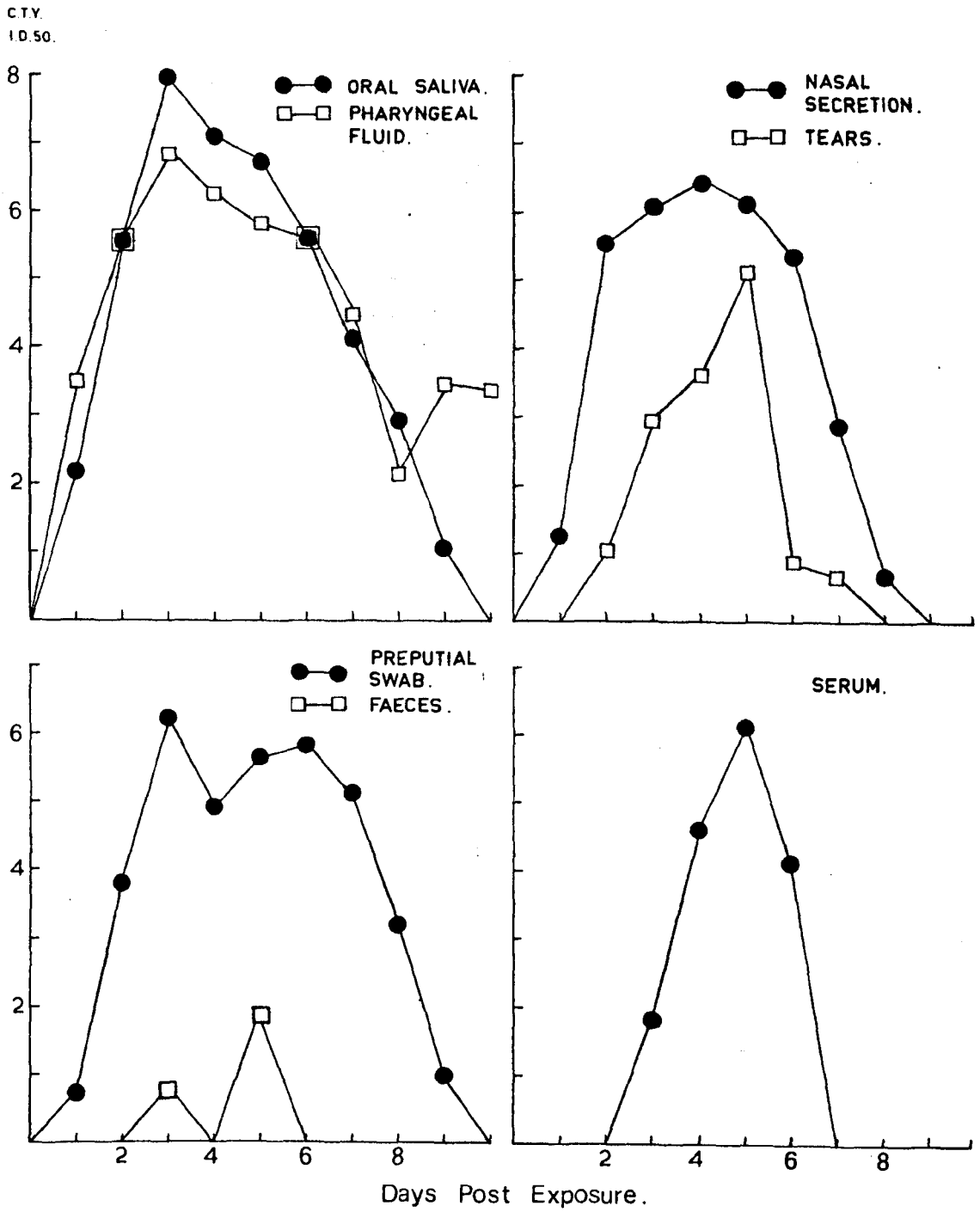
Two CTY tubes used per dilution. Key as for Table 17.

Table 20. Excretion of FMDV type C (Noville) from steers.

Animal No.	Sample	Days post exposure										
		0	1	2	3	4	5	6	7	8	9	10
JS57	L/S	0.0	0.0	1.8	3.8+	3.8	5.3	Tr	0.0	0.0	0.0	0.0
	N/S	0.0	1.5	6.3	8.3	6.3	5.8	3.5	3.0	1.3	0.0	0.0
	O/S	0.0	3.8	5.3	8.3	7.0	6.3	4.0	3.3	2.5	Tr	0.0
	Ph/F	0.0	3.8	6.8	7.8	6.3	5.3	5.8	4.3	2.0	2.2	3.2
	F	0.0	0.0	0.0	2.3	0.0	Tr	0.0	0.0	0.0	0.0	0.0
	U	0.0	-	1.3	2.8	0.0	-	0.0	Tr	0.0	0.0	0.0
	P/S	0.0	1.8	3.3	7.3	4.8	5.8	5.8	2.3	0.0	0.0	0.0
	Se	0.0	0.0	4.8	6.3	7.8	1.3	0.0	0.0	0.0	0.0	0.0
JS59	L/S	0.0	0.0	1.8	4.1	4.1+	5.1	2.8	2.3	0.0	0.0	0.0
	N/S	0.0	1.3	5.3	4.8	6.3	6.8	4.5	2.3	0.0	0.0	0.0
	O/S	0.0	2.3	7.8	8.8	7.3	6.3	5.8	4.8	2.8	Tr	0.0
	Ph/F	0.0	3.3	5.0	7.3	8.3	5.3	4.8	3.8	2.8	2.2	2.2
	F	0.0	0.0	0.0	2.0	3.3	0.0	0.0	0.0	0.0	0.0	0.0
	U	0.0	0.0	-	3.3	1.5	0.0	1.5	0.0	0.0	0.0	0.0
	P/S	0.0	0.0	3.8	7.3	5.8	7.0	3.8	2.8	1.8	0.0	0.0
	Se	0.0	0.0	0.0	4.2	6.2	5.2	0.0	0.0	0.0	0.0	0.0
JS63	L/S	0.0	0.0	0.0	0.0	2.8+	6.1	1.8	0.0	0.0	0.0	0.0
	N/S	0.0	1.3	4.8	5.3	6.8	6.3	6.8	2.3	0.0	0.0	0.0
	O/S	0.0	1.8	4.3	7.0	6.8	6.8	6.8	4.3	3.0	1.3	Tr
	Ph/F	0.0	3.3	5.3	6.3	5.3	7.3	6.3	5.8	1.8	2.2	2.8
	F	0.0	0.0	0.0	1.3	0.0	2.3	0.0	0.0	0.0	0.0	0.0
	U	0.0	0.0	2.3	-	2.5	0.0	-	-	0.0	0.0	0.0
	P/S	0.0	0.0	3.5	3.5	3.5	5.1	4.3	3.8	0.0	0.0	0.0
	Se	0.0	0.0	0.0	2.8	5.3	5.3	0.0	0.0	0.0	0.0	0.0

Four CTY tubes used per dilution. Key as for Table 17

Fig 5. Mean Virus Content Of Samples From Three Steers Infected With FMDV Type C Noville By Contact.



LESIONS FIRST DETECTED ON DAY 3 (ONE STEER) AND DAY 4 (TWO STEERS) FOLLOWING EXPOSURE.

Table 21. Titre of FMDV in some secretions and excretions of cattle .

Secretion/ Excretion	Volume or Weight	Virus Strain	Max Virus Titre	Total Virus content	Ref.
Blood/	30 l	A119	$10^{5.8}$/ml	$10^{10.3}$	A
Serum		O Canefa-9	$10^{5.6}$/ml	$10^{10.1}$	B
		Various	$10^{6.0}$/ml	$10^{10.5}$	C
		O, BFS 1860	$10^{5.2}$/ml	$10^{9.7}$	E
		O, Swiss 1/66	$10^{6.8}$/ml	$10^{11.3}$	E
		A119	$10^{6.5}$/ml	$10^{11.0}$	E
		C Noville	$10^{7.8}$/ml	$10^{12.3}$	E
Lachrymal	Unknown	O Canefa-2	$10^{7.0}$/s		F
Secretion		O, Swiss 1/66	$10^{6.3}$/s		E
		C Noville	$10^{6.1}$/s		E
Nasal	Unknown	Various	$10^{7.7}$/g		C
Secretion		O, BFS 1860	$10^{5.5}$/ml		E
		O, Swiss 1/66	$10^{7.3}$/ml		E
		A119	$10^{6.0}$/ml		E
		C Noville	$10^{8.3}$/ml		E
Oral	98 - 190 l	Various	$10^{8.0}$/ml	$10^{13.3}$	C
Saliva	per day	O Israel/63	$10^{8.5}$/ml	$10^{13.8}$	G
		O, BFS 1860	$10^{6.7}$/ml	$10^{12.0}$	D
		O, BFS 1860	$10^{6.0}$/ml	$10^{11.3}$	E
		O, Swiss 1/66	$10^{7.8}$/ml	$10^{13.1}$	E
		A119	$10^{7.0}$/ml	$10^{12.3}$	E
		C Noville	$10^{8.8}$/ml	$10^{14.1}$	E

Secretion/ Excretion	Volume or Weight	Virus Strain	Max Virus Titre	Total Virus content	Ref.
Pharyngeal	probably as	O, BFS 1860	$10^{7.4}$/ml	$10^{12.7}$	D
Fluid	saliva	O, BFS 1860	$10^{7.0}$/ml	$10^{12.3}$	E
	98 - 190 l	O, Swiss 1/66	$10^{7.8}$/ml	$10^{13.1}$	E
	per day	A119	$10^{7.3}$/ml	$10^{12.6}$	E
		C Noville	$10^{8.3}$/ml	$10^{13.6}$	E
Faeces	14 - 45 kg	O Canefa-2	$10^{4.1}$/g	$10^{8.7}$	F
	per day	O BFS 1860	$10^{2.0}$/g	$10^{6.7}$	D
		O, Swiss 1/66	$10^{3.0}$/g	$10^{7.7}$	E
		A119	$10^{2.0}$/g	$10^{6.7}$	E
		C Noville	$10^{3.3}$/g	$10^{8.0}$	E
Urine	8.8 - 22 l	A119	$10^{4.9}$/ml	$10^{9.2}$	H
	per day	O M 11	$10^{4.6}$/ml	$10^{8.9}$	H
		O, BFS 1860	$10^{2.5}$/ml	$10^{6.8}$	E
		O, Swiss 1/66	$10^{5.5}$/ml	$10^{9.8}$	E
		A119	$10^{2.5}$/ml	$10^{6.8}$	E
		C Noville	$10^{3.3}$/ml	$10^{7.6}$	E

Physiological data from Sellers (1971) and Dukes (1970)

A. Cottral and Bachrach 1968. Titre in mouse ID_{50}/ml or g. Infected by tongue inoculation. Report gives details of types and strains of FMDV not listed in the table.
B. Cottral 1969. Titre in mouse ID_{50}/ml or g. Infected by tongue inoculation.
C. Scott et al 1966. Titre in Calf Kidney p.f.u./ml or g. Infected by tongue inoculation. Results obtained with 7 types of FMDV but report gives only collected figures.
D. Sellers et al 1969. Titre in IB-RS-2 p.f.u./ml or g. Infected by indirect contact.
E. This Thesis. Titre in CTY ID_{50} per ml or g. or sample (s). Infected by direct contact.
F. Sutmoller and McVicar 1973. Titre in Calf Kidney p.f.u./ml, g or s. Infected by intraconjunctival installation.
G. Hyslop 1965a. Remarks as for A..
H. Cottral et al 1968. Remarks as for A..

Table 22. Development of clinical disease in cattle exposed to FMDV.

Virus / Animal No.	Days post exposure 2	3	4	5	6	7
A119						
HX20	103.8*	103.8 UL T.G. 4F	101.4 LL HP	103.4 RN	103.2	102.4
HX21	101.0	101.3	104.2 2F	103.4 UL LL T. HP. P 4F	104.6	103.4
C Noville						
JS57	101.4	105.2 RN. LN. M UL. LL. T 4F	104.3	104.0	101.6	101.0
JS59	100.8	101.4	105.0 RE. RN.LN UL. LL. T 4F P	103.6 LE HP	103.4	101.2
JS63	101.2	101.4	104.0 UL. LL.HP 1F	106.0 LN P 4F	104.2	101.8

* Rectal temperature °F.

Lesion Sites:

RE, LE: Right and left eye (conjunctivitis)
M: Muzzle
RN, LN: Right and left nostril
UL, LL: Upper and lower lip
1F - 4F: one to 4 feet
G: Gum
DP: Dental Pad
HP: Hard Palate
T: Tongue
P: Prepuce

Table 23. Development of clinical disease in cattle exposed to FMDV.

Virus	Days post exposure					
Animal No.	1	2	3	4	5	6
0, BFS 1860						
HR8	101.4*	101.5	102.6	103.8 RN LN UL P 4F	103.8	102.0
HR9	100.8	101.4	102.8	104.2 UL G 2F	101.8 LL T. P. 4F	101.4
HR11	101.5	103.8 RN	104.0 LE LN M DP T 4F	102.4 HP	101.4	101.6
0, Swiss 1/66						
JT71	101.6	104.6 RN LN UL LL T 3F	103.8 P 4F	103.0	102.5	101.5
JT72	100.8	102.8	103.6 RN M UL LL G 2F	103.2 LN DP 4F	101.5	101.4
JT73	101.4	102.8 RN UL LL T P 4F	103.0	101.5	100.8	101.4

be drawn about the origin of virus in urine. However, virus was rarely found in urine outside the viraemic phase or in the absence of preputial virus, either or both of which might act as a source of urinary FMDV.

Internal secretions were also examined for virus at post mortem. Six steers were slaughtered at 7 day intervals between 7 and 42 days after exposure to type O, Swiss 1/66. All animals developed fully generalised disease. Prior to slaughter the usual samples were taken while after death 42 samples were taken of mucosae, tissues and associated lymph nodes throughout the alimentary and respiratory systems. Table 24 includes only samples from which virus was recovered. No lesions were found in the lower respiratory or alimentary tracts, nor was virus detected in these regions. From 14 days post exposure, virus persisted only in pharyngeal fluid and in oropharyngeal tissues.

Table 24. Recovery of FMDV in ante and post mortem samples from steers convalescent to type O, Swiss 1/66.

Animal Number	HR10	HR8	HR9	HR11	HR6	HR7
Days post exposure	7	14	21	28	35	42
Oral Saliva	Tr	-	-	-	-	-
Pharyngeal Fluid	1.5*	2.3	1.5	1.2	-	2.3
Tongue Epithelium	2.5	-	-	-	-	-
DSSP	2.5	1.0	1.3	1.5	2.2	2.5
Pharyngeal Mucosa	1.5	-	Tr	-	1.5	-
Tonsil	3.0	1.0	1.0	-	-	-
RPLN	1.5	-	-	-	-	-
Oesophageal Mucosa	1.5	-	-	-	-	-

DSSP: Dorsal surface of the soft palate
RPLN: Retropharyngeal lymph node
* Titre in CTY ID_{50} per ml or g. 5 tubes per dilution used.
Tr.: trace of virus at lowest dilution examined. -: No virus detected.

7.2.1.2. pH values during infection.

Inflammation may result in the alteration of normal pH values and since FMDV produces an acute inflammatory response and the virus is pH labile, the hydrogen ion content of secretions might serve in a defensive capacity during infection. Fourteen daily measurements were made of the pH values of tears, nasal secretion, oral saliva, pharyngeal fluid, faeces, urine and blood from two groups of 3 cattle before, during and after clinical disease following exposure to FMDV types O, Swiss 1/66 and C Noville.

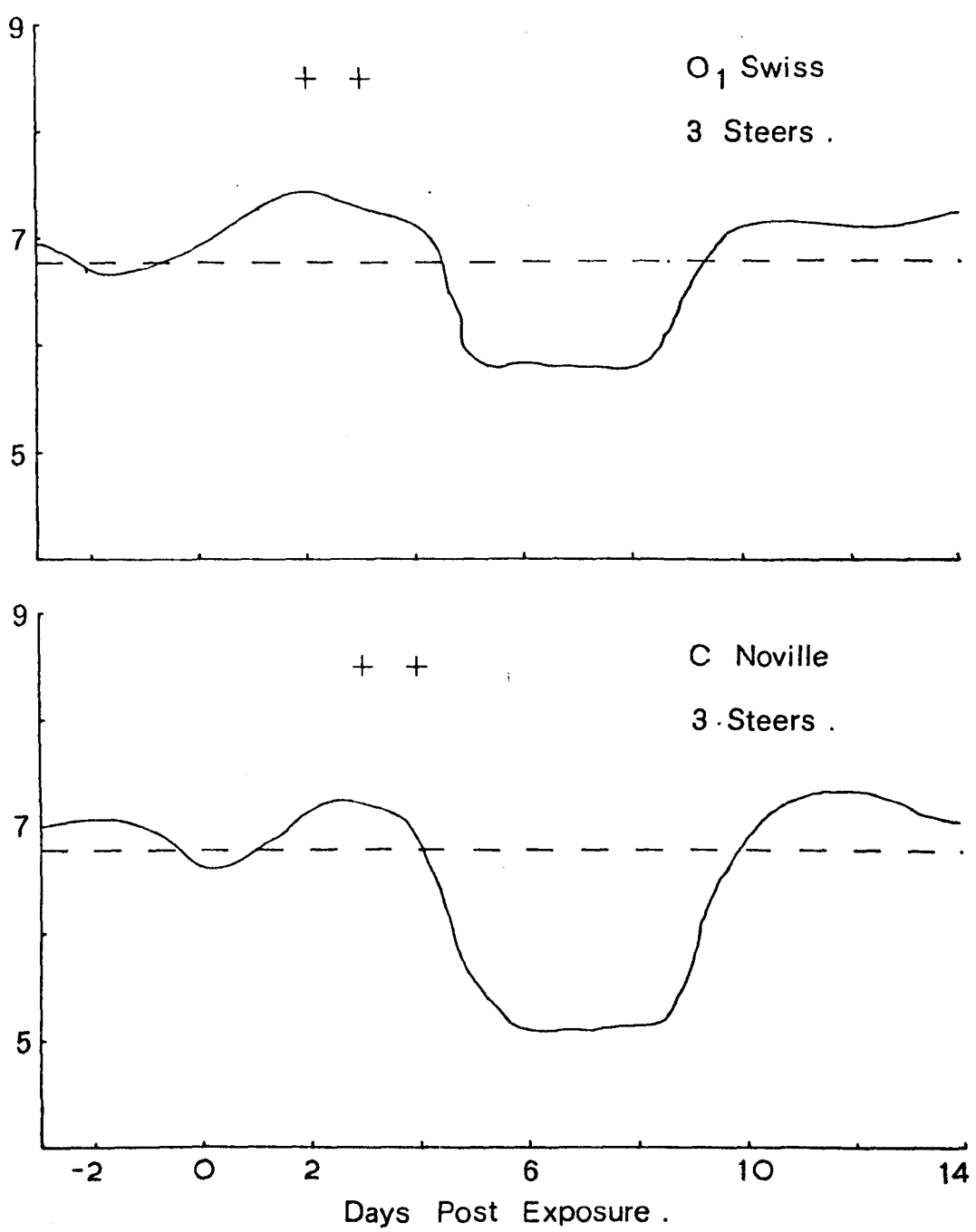
Urine alone showed a consistent deviation from the normal range of pH with a tendency to become more acid as disease became generalised (Fig. 6). The shift was more pronounced after type C infection and the lowest value recorded was pH 5.2. Low pH persisted for 2 - 4 days at levels known to be inimical to the survival of FMDV infectivity (c.f. Fig. 3). Type C virus was recovered once from urine during the acid shift (Table 20) but type O was recovered on several occasions (Table 18). Both viruses were recovered from preputial swabs taken during this period.

7.2.1.3. Protein levels during infection.

The survival of FMDV may be influenced by the amount of protein in secretions. At least two effects may be envisaged, a non specific protective effect or a specific antiviral effect due to neutralising antibody.

Total protein levels were measured by the Lowry method in secretions derived from cattle throughout infection as described in section 7.2.1.2.. Tears, nasal secretion, oral saliva and pharyngeal fluid showed a marginal increase in protein content lasting only 1 - 2 days at the peak of

Fig 6 . Mean pH Of Bovine Urine During The Course Of FMD .



+ Earliest detection of lesions .

---- Mean pH value of normal bovine urine .

clinical disease (Table 25). In many animals the serous, nasal and lachrymal secretions became mucoid or muco-purulent at this time while oral saliva and pharyngeal fluid tended to become more viscid. These changes might explain the increased protein levels which are found.

7.2.1.4. Interferon levels during infection.

IF occurs in secretions during the course of a number of viral diseases and in some instances it is known to exert an antiviral effect. The IF response of cattle to infection with FMDV was therefore investigated. Fourteen daily sample sets were taken from 4 groups of cattle before, during and after clinical disease caused by FMDV types O, Swiss 1/66, O, BFS 1860, A119 and C Noville. After overnight acidification at pH 2.0 to destroy FMDV, samples were brought to a neutral pH and assayed for IF by measuring their capacity to inhibit CPE in Calf Kidney tube cultures challenged with Vesicular Stomatitis Virus. Results are shown in tables 26, 27, 28 and 29 and Fig. 7. In general IF was detected only during the development of clinical lesions. In some instances the appearance of IF apparently preceeded the development of vesicles by 24 hours but it is possible that undetected lesions were present in such areas as the nasopharynx at the earlier time.

The maximum time of detection of IF in any single animal or secretion was 4 days but IF was more commonly present for only 2 or 3 days. Greatest amounts of IF were found in tears, nasal secretion and serum, smaller amounts occurred in oral saliva and pharyngeal fluid while none was detected in faecal suspension or urine. The lower levels in oral and pharyngeal secretion may merely reflect their dilution with saliva which forms the major constituent of these fluids. Types A119 and C Noville induced more IF than type O, BFS 1860 and type O, Swiss 1/66 induced least, a fact which correlates to some extent with the amounts

of virus recovered from secretions (c.f. Tables 17 - 20).

Table 25. Mean protein content of secretions during FMD.

Virus	Sample	Days post exposure						
Animal No.		0	1	2	3	4	5	6
				+	+			
O, Swiss 1/66	L/S	406*	510	906	950	800	566	450
	N/S	567	483	975	1060	647	472	485
JT 71, 72, 73	O/S	164	155	710	302	210	154	368
	Ph/F	298	360	961	274	354	400	210
	U	45	16	10	24	4	60	45
	Se	6430	6570	6610	6580	6490	6610	6520
					+	+		
C Noville	L/S	520	413	613	852	987	964	565
	N/S	612	704	621	900	1010	1040	600
JS 57, 59, 63	O/S	196	257	316	588	740	216	230
	Ph/F	309	264	338	682	840	506	310
	U	22	53A	12A	10A	43	40A	6A
	Se	6550	6610	6420	6560	6610	6530	6490

*: Protein content mg/100 ml.

+: Earliest detection of clinical lesions

Values are the mean of 3 samples from individual steers on each day.

A: Mean of only 2 samples

Sample abbreviations as in Table 17.

Table 26. Interferon in bovine secretions during infection with FMDV type O (Swiss 1/66).

Animal No.	Sample	Days post exposure						
		0	1	2	3	4	5	6
JT71	L/S	-	-	0.5 +	1.3	-	-	-
	N/S	-	-	0.8	1.8	-	-	-
	O/S	-	-	0.5	-	-	-	-
	Ph/F	-	-	0.8	0.8	-	-	-
	Se	-	-	1.0	1.0	-	-	-
JT72	L/S	-	-	-	0.8 +	1.3	-	-
	N/S	-	-	0.8	0.8	2.1	-	-
	O/S	-	-	-	0.5	0.5	-	-
	Ph/F	-	-	-	0.8	0.5	-	-
	Se	-	-	-	1.3	0.8	-	-
JT73	L/S	-	-	0.3 +	1.5	-	-	-
	N/S	-	-	0.5	2.0	0.8	-	-
	O/S	-	-	0.3	0.5	-	-	-
	Ph/F	-	-	0.5	0.5	-	-	-
	Se	-	-	1.0	1.3	-	-	-

IF titre expressed as log units per 0.5 ml of secretion.

+: Earliest detection of clinical lesions

-: No IF detected

Otherwise Key as for Table 17.

Table 27. Interferon in bovine secretions during infection with FMDV type O (BFS 1860).

Animal No.	Sample	Days post exposure 0	1	2	3	4	5	6	7
HR8	N/S	-	-	-	-	1.2+	2.3	0.5	-
	O/S	-	-	-	-	0.5	1.3	0.3	-
	Ph/F	-	-	-	0.5	1.0	1.0	-	-
	Se	-	-	-	-	0.5	2.0	1.3	-
HR9	N/S	-	-	-	-	1.5+	2.0	-	-
	O/S	-	-	-	0.3	0.8	0.5	-	-
	Ph/F	-	-	-	0.5	1.2	0.8	-	-
	Se	-	-	-	-	1.0	0.5	-	-
HR11	N/S	-	-	2.0+	2.3	-	-	-	-
	O/S	-	-	0.8	1.0	-	-	-	-
	Ph/F	-	-	1.0	0.3	-	-	-	-
	Se	-	0.3	0.8	1.2	-	-	-	-

Key as for Table 17.

Table 28. Interferon in bovine secretions during infection with FMDV type A (119).

Animal No.	Sample	Days post exposure							
		0	1	2	3	4	5	6	7
HX20	N/S	-	-	-	1.5 +	2.6	-	-	-
	O/S	-	-	-	0.8	1.5	0.3	-	-
	Ph/F	-	-	0.3	1.0	1.2	-	-	-
	Se	-	-	-	1.5	1.5	0.3	-	-
HX21	N/S	-	-	-	-	2.6 +	1.8	0.5	-
	O/S	-	-	-	-	1.2	1.0	-	-
	Ph/F	-	-	-	-	0.8	1.2	-	-
	Se	-	-	-	-	1.0	1.2	-	-

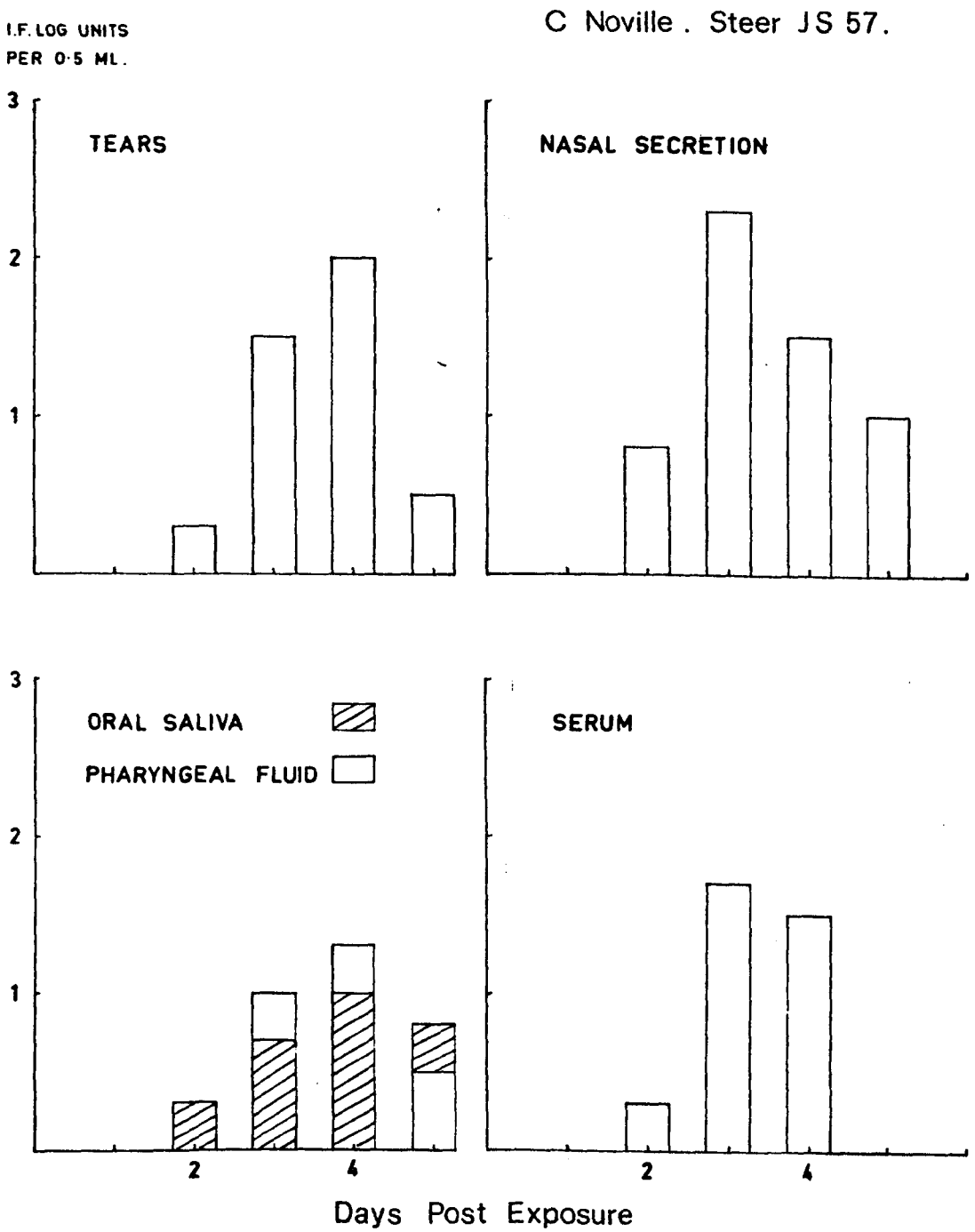
Key as for Table 17.

Table 29. IF in bovine secretions during infection with FMDV type C (Noville).

Animal No.	Sample	Days post exposure							
		0	1	2	3	4	5	6	7
JS57	L/S	-	-	0.3	1.5 +	2.0	0.5	-	-
	N/S	-	-	0.8	2.3	1.5	1.0	-	-
	O/S	-	-	-	1.0	1.3	0.5	-	-
	Ph/F	-	-	0.3	0.7	1.0	0.8	-	-
	Se	-	-	0.3	1.7	1.5	-	-	-
JS59	L/S	-	-	-	1.3	1.0 +	1.5	-	-
	N/S	-	-	0.5	0.8	2.2	1.8	-	-
	O/S	-	-	-	1.0	1.5	0.8	0.3	-
	Ph/F	-	-	0.5	1.2	1.5	0.3	-	-
	Se	-	-	-	0.5	2.0	0.8	-	-
JS63	L/S	-	-	-	-	- +	1.5	-	-
	N/S	-	-	-	-	1.2	2.3	0.5	-
	O/S	-	-	-	0.3	0.8	1.2	0.3	-
	Ph/F	-	-	-	0.5	1.0	0.5	1.2	-
	Se	-	-	-	-	2.3	1.8	-	-

Key as for Table 17.

Fig 7. Interferon In Bovine Secretions During The Course Of FMD.



7.2.1.5. Neutralising antibody in infection.

a). Development of neutralising antibody.

Antibody has been found in secretions after a number of viral infections and in some instances an important protective role has been assigned to it. Preliminary reports indicated that secretory antibody was produced after infection with FMD but detailed information was lacking. A close study was made of the humoral and secretory neutralising antibody response in groups of steers which developed generalised disease after exposure to FMDV types O_1 (Swiss 1/66) and (BFS 1860), A (119) and C (Noville). Sets of secretion samples were obtained for the first 10 days and then at weekly intervals for up to 6 weeks post infection.

Saliva and pharyngeal fluid were dialysed overnight against PBS at pH 7.5 to remove the adverse alkaline pH effect and the additional antiviral factor. Tears, nasal secretion, saliva and pharyngeal fluid were tested undiluted, serum and post mortem mucus at a dilution of 1:10 and urine and faecal suspension after concentration by a factor of 10 - 100 times. All secretions were heated at 56°C for 30 minutes prior to measurement of the homologous neutralisation index in suckling mice and the specificity of neutralisation was detected by testing the heterologous neutralising capacity of selected samples.

The neutralising antibody response was similar for the 4 strains of FMDV examined. Initial development during the first 10 days after infection is shown in Tables 30 to 33 and the subsequent pattern during the first six weeks is shown in Tables 34 to 37. The antibody response to type C Noville is also depicted in Fig. 8. Neutralising antibody was detectable in secretion within 3 - 6 days (mean 5 days) of exposure. Antibody attained a peak 21 - 28 days after exposure and persisted at

declining levels for at least 6 weeks. Long term studies are presented in section 7.2.2. Levels in external secretions were almost always well below those found concurrently in serum and antibody was often detected in serum one day earlier than in secretions, even when humoral antibody was sought at a dilution of 1:10. The development of antibody was more closely related to time after exposure virus than to time after development of lesions and in one animal (HR9, Table 31) antibody was detected at the same time as lesions.

The amount of antibody was greatest in serum. Of the external secretions, nasal contained most antibody - slightly more than lachrymal, while antibody was found at a lower level and in approximately equal amounts in oral saliva and pharyngeal fluid. Very little antibody could be detected in urine or faecal suspension collected 28 days after infection, even when concentrated up to 100 times. Heterologous neutralising activity was confined to the early part of the response and was not detectable 3 weeks after exposure to virus. Representative cross neutralisation results are given in Table 38.

The development and decline of antibody in mucus samples obtained at post mortem from the lower respiratory and alimentary tracts (Table 39) resembled that of ante mortem secretions. Antibody occurred in appreciable amounts in mucus and although the levels were always below those found in serum they were sufficient to raise doubts about the apparent lack of antibody in faecal suspension.

The influence of infection on the additional non specific antiviral factor in oral saliva and pharyngeal fluid was also investigated. Sequential undialysed samples were brought to pH 7.5 by the addition of acid and examined in heterologous neutralisation tests. The factor remained constant throughout the course of disease and convalescence.

Table 30. Neutralising antibody in bovine secretions following infection with FMDV type O_1 (Swiss 1/66). Day 0 - 10.

Animal No.	Sample	Days post exposure 0	1	2	3	4	5	6	7	8	9	10
				+								
JT71	L/S	0.2*	0.0	0.4	0.0	0.0	1.0	1.6	1.6	1.4	1.8	2.2
	N/S	0.0	0.2	0.0	0.0	0.4	1.8	2.4	1.8	2.0	2.0	2.4
	O/S(D)	0.4	0.0	0.2	0.0	0.2	0.8	1.0	2.0	1.8	2.0	2.0
	Ph/F(D)	0.2	0.2	0.0	0.4	0.4	1.0	1.4	1.8	1.6	1.8	2.0
	Se	0.0	0.4	0.2	0.6	1.2	2.6	2.2	2.8	2.8	3.0	3.8
					+							
JT72	L/S	0.0	0.0	0.0	0.2	0.2	0.8	1.0	1.8	2.0	1.6	1.8
	N/S	0.4	0.0	0.2	0.0	0.0	1.2	2.4	2.6	2.0	2.4	2.8
	O/S(D)	0.2	0.0	0.0	0.4	0.0	0.6	1.2	1.8	1.6	2.0	2.2
	Ph/F(D)	0.4	0.2	0.0	0.0	0.2	0.0	1.0	2.0	1.8	1.8	2.4
	Se	0.2	0.0	0.4	0.2	0.0	2.0	2.8	3.4	2.8	3.0	3.2
				+								
JT73	L/S	0.2	0.0	0.2	0.2	0.0	0.4	1.4	1.8	1.6	1.6	2.0
	N/S	0.0	0.2	0.0	0.0	0.2	1.2	2.4	2.6	2.4	2.6	3.0
	O/S(D)	0.4	0.2	0.4	0.0	0.4	0.2	0.8	2.2	1.8	2.0	1.8
	Ph/F(D)	0.0	0.4	0.0	0.2	0.0	0.4	1.0	2.2	1.4	1.8	2.4
	Se	0.0	0.2	0.0	0.2	1.0	2.0	2.4	2.8	2.6	3.0	2.8

* : Mouse neutralisation index per 0.03 ml of sample

(D) : Dialysed

0.0 : No antibody detected at the lowest dilution tested

Key otherwise as Table 17

Note 1. Antibody could not be detected in urine or faeces even after concentration. See text.

Table 31. Neutralising antibody in bovine secretions following infection with FMDV type O_1 (BFS 1860). Day 0 - 10

Animal No.	Sample	Days post exposure 0	1	2	3	4	5	6	7	8	9	10
HR8	N/S	0.0	0.2	0.2	0.0	0.4+	0.8	1.0	1.6	2.0	2.0	2.4
	O/S(D)	0.4	0.0	0.2	0.0	0.0	0.4	0.6	1.2	1.6	1.6	1.8
	Ph/F(D)	0.1	0.2	0.0	0.2	0.4	0.4	0.8	0.8	1.2	1.8	1.6
	Se	0.0	0.2	0.0	0.4	0.4	0.8	1.4	2.4	2.8	2.6	3.2
HR9	N/S	0.0	0.0	0.0	0.2	0.8+	0.4	1.2	1.6	1.8	2.0	2.6
	O/S(D)	0.2	0.2	0.0	0.2	0.2	0.0	0.6	1.0	1.2	1.4	1.6
	Ph/F(D)	0.0	0.0	0.4	0.2	0.0	0.2	0.6	1.0	1.0	1.6	1.8
	Se	0.2	0.4	0.4	0.0	1.0	1.2	2.0	1.8	2.4	3.0	3.6
HR11	N/S	0.0	0.2	0.0+	0.8	1.0	1.0	1.4	1.8	1.6	2.0	2.6
	O/S(D)	0.2	0.2	0.4	0.4	0.6	0.6	1.0	1.6	2.0	1.8	2.0
	Ph/F(D)	0.0	0.2	0.0	0.0	0.6	0.8	0.8	1.3	1.8	1.6	1.8
	Se	0.2	0.0	0.4	0.4	1.2	1.8	2.4	2.4	2.8	3.0	2.8

Key as for previous tables.

Table 32. Neutralising antibody in bovine secretions following infection with FMDV type A (119). Day 0 - 10.

Animal No.	Sample	Days post exposure 0	1	2	3	4	5	6	7	8	9	10
					+							
HX20	N/S	0.2	0.0	0.2	0.4	0.2	0.6	0.8	1.6	1.2	1.4	1.6
	O/S(D)	0.0	0.4	0.2	0.0	0.2	0.4	0.6	1.0	0.8	1.0	1.2
	Ph/F(D)	0.4	0.0	0.0	0.0	0.4	0.2	0.8	1.2	1.0	0.8	1.2
	Se	0.4	0.0	0.2	0.2	0.2	1.4	2.0	2.6	2.8	3.2	3.0
						+						
HX21	N/S	0.2	0.2	0.4	0.0	0.6	1.0	1.2	1.8	1.8	1.6	2.0
	O/S(D)	0.2	0.2	0.0	0.0	0.4	0.8	1.0	1.4	1.0	1.2	1.4
	Ph/F(D)	0.0	0.0	0.2	0.2	0.0	0.6	1.2	1.2	1.2	1.4	1.6
	Se	0.4	0.2	0.2	0.2	0.2	1.4	2.2	2.0	2.2	3.0	2.8

Key as for previous tables.

Table 33. Neutralising antibody in bovine secretions following infection with FMDV type C (Noville). Day 0 - 10.

Animal No.	Sample	Days post exposure 0	1	2	3	4	5	6	7	8	9	10
					+							
JS57	L/S	0.2	0.2	0.0	0.0	0.2	0.8	1.0	1.0	1.6	1.4	1.6
	N/S	0.2	0.0	0.4	0.2	0.2	1.0	2.4	2.4	2.4	2.6	2.4
	O/S(D)	0.4	0.2	0.0	0.2	0.4	0.6	0.8	1.0	1.2	1.0	1.6
	Ph/F(D)	0.0	0.0	0.4	0.0	0.0	0.8	1.0	1.2	1.2	1.4	1.6
	Se	0.0	0.4	0.0	0.6	0.4	1.8	2.2	2.8	2.8	3.2	3.0
						+						
JS59	L/S	0.0	0.2	0.4	0.0	0.4	0.6	0.6	1.2	1.8	2.0	2.2
	N/S	0.4	0.2	0.2	0.2	0.2	0.6	0.6	2.0	2.4	2.4	2.6
	O/S(D)	0.2	0.0	0.0	0.4	1.0	1.2	0.8	1.2	1.4	1.6	2.2
	Ph/F(D)	0.2	0.2	0.2	0.0	0.4	1.6	1.0	1.2	1.2	1.0	2.8
	Se	0.0	0.4	0.2	0.0	0.6	1.8	2.4	2.4	2.6	2.4	2.8
						+						
JS63	L/S	0.4	0.2	0.0	0.0	0.4	0.8	1.0	1.0	1.0	1.4	1.8
	N/S	0.4	0.2	0.4	0.2	0.4	0.8	1.2	1.4	2.2	1.8	2.4
	O/S(D)	0.4	0.0	0.0	0.2	0.6	1.2	1.0	1.0	1.2	1.4	1.6
	Ph/F(D)	0.0	0.0	0.2	0.4	0.0	1.4	0.8	1.0	1.2	1.6	1.4
	Se	0.2	0.4	0.2	0.4	0.0	1.0	1.6	2.6	2.8	2.6	3.0

Key as for previous tables.

Table 34. Neutralising antibody in the secretions of cattle following infection with FMDV type O_1 (Swiss 1/66). Week 0 - 6.

Animal No.	Sample	Days post exposure 0	7	14	21	28	35	42
JT71	L/S	0.2	1.6	2.0	2.6	2.6	1.8	0.8
	N/S	0.0	1.8	2.4	3.0	3.6	2.4	2.6
	O/S(D)	0.4	2.0	1.8	2.4	2.2	2.0	1.0
	Ph/F(D)	0.2	1.8	1.6	2.0	2.0	2.0	1.2
	Se	0.0	2.8	3.8	4.6	4.4	4.6	3.8
JT72	L/S	0.0	1.8	1.8	2.4	2.8	1.4	1.2
	N/S	0.4	2.6	2.6	3.0	2.6	1.8	2.2
	O/S(D)	0.2	1.8	2.0	2.4	2.0	1.0	1.2
	Ph/F(D)	0.4	2.0	2.4	2.6	2.2	1.4	1.0
	Se	0.2	3.4	4.0	5.0	5.2	4.8	4.4
JT73	L/S	0.2	1.8	1.8	2.0	2.0	1.4	1.0
	N/S	0.0	2.6	-	2.8	2.4	1.8	1.4
	O/S(D)	0.4	2.2	2.0	2.2	2.0	2.2	1.0
	Ph/F(D)	0.0	2.2	1.6	1.8	2.0	1.6	0.8
	Se	0.0	2.8	3.0	4.2	3.4	3.4	3.6

Key as for previous tables

Table 35. Neutralising antibody in the secretions of cattle following infection with FMDV type O_1 (BFS 1860). Week 0 - 6.

Animal No.	Sample	Days post exposure 0	7	14	21	28	35	42
HR10	N/S	0.0	1.5					
	O/S(D)	0.2	1.0					
	Ph/F(D)	0.0	1.4					
	Se	0.4	1.8					
HR8	N/S	0.0	1.6	3.3				
	O/S(D)	0.4	1.2	2.4				
	Ph/F(D)	0.2	0.8	2.3				
	Se	0.0	2.4	4.1				
HR9	N/S	0.0	1.6	2.6	3.4			
	O/S(D)	0.2	1.0	1.8	1.9			
	Ph/F(D)	0.0	1.0	1.2	2.2			
	Se	0.2	1.8	3.8	4.5			
HR11	N/S	0.0	1.8	2.8	2.7	3.3		
	O/S(D)	0.2	1.6	1.5	1.2	1.9		
	Ph/F(D)	0.0	1.3	1.7	-	2.4		
	Se	0.2	2.4	3.6	3.7	4.4		
HR6	N/S	-	-	4.1	3.7	3.3	2.8	
	O/S(D)	-	-	2.0	2.2	0.9	1.7	
	Ph/F(D)	-	-	1.9	1.8	1.8	1.2	
	Se	-	-	4.8	4.5	4.3	3.8	
HR7	N/S	-	-	3.4	3.3	3.1	2.6	1.7
	O/S(D)	-	-	1.6	2.2	1.1	0.9	1.0
	Ph/F(D)	-	-	2.1	1.8	1.7	1.4	0.9
	Se	-	-	3.0	4.5	4.3	3.4	3.0

Table 36. Neutralising antibody in the secretions of cattle following infection with FMDV type A (119). Week 0 - 6.

Animal No.	Sample	Days post exposure 0	7	14	21	28	35	42
HX20	N/S	0.2	1.6	2.2	3.0	3.2	2.8	2.0
	O/S(D)	0.0	1.0	1.4	2.2	2.0	1.4	0.4
	Ph/F(D)	0.4	1.2	1.8	1.8	2.2	1.6	0.6
	Se	0.4	2.6	3.8	4.6	3.4	3.0	3.0
HX21	N/S	0.2	1.8	2.0	2.6	3.0	2.6	1.8
	O/S(D)	0.2	1.4	1.6	1.8	2.0	1.6	0.6
	Ph/F(D)	0.0	1.2	1.8	2.2	1.4	1.0	0.4
	Se	0.4	2.0	4.2	4.6	4.2	3.6	3.2

Key as for previous tables.

Table 37. Neutralising antibody in the secretions of cattle following infection with FMDV type C (Noville). Week 0 - 6.

Animal No.	Sample	Days post exposure 0	7	14	21	28	35	42
JS57	L/S	0.2	1.0	3.8	4.2	4.0	2.2	1.8
	N/S	0.2	2.4	3.4	4.6	4.2	3.8	3.0
	O/S(D)	0.4	1.0	2.4	2.8	2.2	0.8	1.2
	Ph/F(D)	0.0	1.2	2.6	3.0	1.8	1.0	1.0
	Se	0.0	2.8	5.6	5.6	5.2	4.8	4.2
JS59	L/S	0.0	1.2	4.0	4.8	4.4	4.0	1.8
	N/S	0.4	2.0	4.0	4.8	4.4	4.0	2.8
	O/S(D)	0.2	1.2	1.8	2.6	2.6	1.8	1.2
	Ph/F(D)	0.2	1.2	2.0	3.0	2.4	1.2	0.8
	Se	0.0	2.4	5.2	5.6	5.4	4.6	4.8
JS63	L/S	0.4	1.0	3.4	3.6	3.8	3.0	1.2
	N/S	0.4	1.4	3.8	4.6	4.2	3.6	3.2
	O/S(D)	0.4	1.0	2.4	2.8	2.0	0.8	0.8
	Ph/F(D)	0.0	1.0	1.6	2.2	1.8	1.2	0.4
	Se	0.2	2.6	4.2	5.6	5.6	4.8	4.0

Key as for previous tables.

Fig 8. Mean Neutralising Antibody Response In Bovine Secretions Following Infection With FMDV Type C Noville.

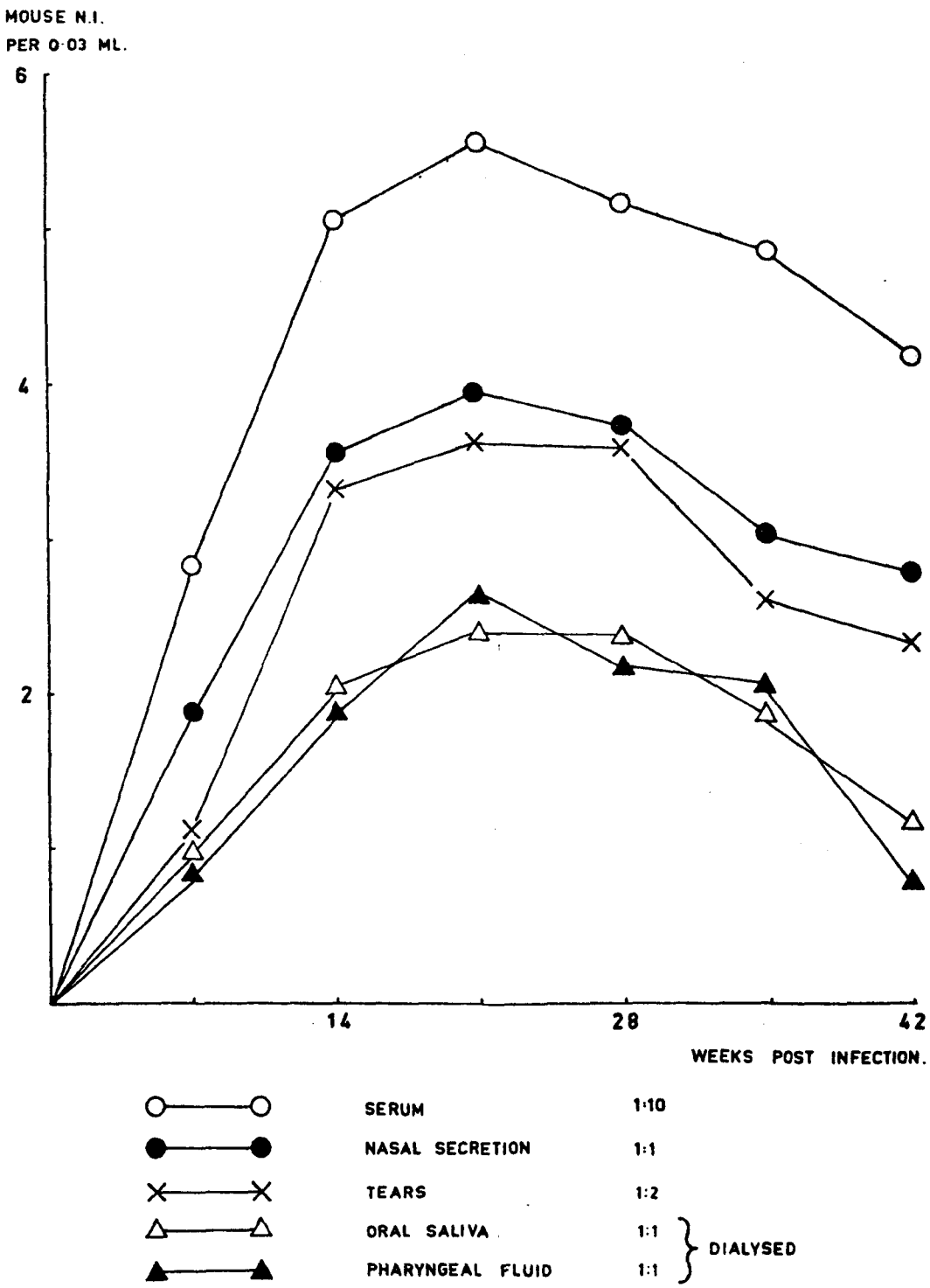


Table 38. Homologous and heterologous neutralisation by bovine secretion following infection with FMDV type C (Noville).

Sample	Days post exposure			
	0	7	14	21
L/S	0.2/0.0	1.0/0.4	3.6/2.4	4.2/0.2
N/S	0.2/0.2	2.0/1.2	3.4/1.6	4.4/0.4
O/S(D)	0.4/0.2	1.0/0.4	2.4/0.8	2.6/0.4
Ph/F(D)	0.0/0.2	1.2/0.6	2.2/1.0	2.8/0.2
Se	0.2/0.0	2.4/1.6	4.8/1.2	5.6/1.0

Results expressed as the Neutralisation Index (NI) per 0.03 ml of undiluted sample or, in the case of serum, of sample at 1:10 dilution.

Numerator : Homologous N.I. against C Noville

Denomenator Heterologous N.I. against O Swiss 1/66.

Table 39. Neutralising antibody in the secretion of cattle following infection with FMDV type O_1 (BFS 1860). Post mortem samples.

Animal Number	HR10	HR8	HR9	HR11	HR6	HR7
Days post exposure	7	14	21	28	35	42
Tracheal Mucus	0.5*	1.2	1.5	1.7	0.8	0.8
Bronchial Mucus	0.8	1.5	1.0	1.4	1.1	0.5
Broncho-alveolar Washings	1.0	2.4	2.0	2.2	1.2	1.0
Abomasal Mucus	0.5	0.8	1.2	1.0	0.8	0.8
Small Intestinal Mucus	0.8	1.2	1.8	1.4	0.5	0.8
Caecal Mucus	0.5	1.0	1.2	1.2	0.8	0.6
Colonic Mucus	0.8	0.8	1.0	1.4	0.8	0.8
Serum	1.8	4.1	4.5	4.4	3.8	3.0

* Mouse Neutralisation Index per 0.03 ml of sample

All samples apart from broncho-alveolar washings tested at a dilution of 1 : 10.

b) Immunoglobulin type.

Convalescent sera, secretions and fractions separated from them by column chromatography were investigated with regard to immunoglobulin type by means of neutralisation tests, reductive cleavage procedures, absorption with specific antisera and immunodiffusion or immunoelectrophoresis tests using antiserum specific for bovine globulin classes and concentrated homologous FMDV as diagnostic reagents. Since results were essentially similar for all the viruses investigated, only those obtained with type C will be reported.

The chromatographic separation of proteins by gel filtration from the secretions of a steer 21 days convalescent is shown in Fig. 9. The elution pattern resembles that of secretions from normal animals (cf Fig. 1) and of other samples taken between 7 and 42 days post infection. Similar remarks apply to the results of anion exchange chromatography. FMD infection does not therefore cause changes in the proteins if secretions which are detectable by the chromatographic methods employed although it may cause a transient rise in total secretory protein at the height of clinical disease (section 7.2.1.3.).

Figure 9 illustrates the distribution of neutralising activity in the isolated fractions and compafison of the elution profiles for protein and neutralisation gave an indication of the immunoglobulin types involved. Closer identification was provided by the results of immunodiffusion (Plate 10), immunoelectrophoresis (Plates 5 - 9), reductive cleavage (Table 40) and absorption (Table 41) tests. The composite data showed that the specific neutralising activity of convalescent bovine secretions was associated with protein molecules possessing the characteristics of immunoglobulins. In serum the neutralising activity corresponded with the first and second peaks eluted in gel filtration. As reported by

other workers, the neutralising activity during the first 14 days was linked mainly with the IgM class of antibody, being highly susceptible to disulphide bond reduction, showing typical electrophoretic mobility and being largely removed by absorption with antiserum to IgM. A gradual change over to IgG (both IgG_1 and IgG_2 subclasses) occurred during the period 14 - 21 days post infection with neutralisation becoming increasingly resistant to mercaptoethanol, showing characteristic gamma electrophoretic mobility and reacting with antiserum to IgG. Long term neutralising capacity resided almost entirely in the IgG class.

In tears, nasal secretions, oral saliva and pharyngeal fluid, early antibody was of the IgA class with some activity in IgM molecules (particularly in lachrymal secretion). IgA was mainly eluted from secretions in the area between the first and second peaks of the gel filtration profile for serum and showed a sensitivity to mercaptoethanol which was intermediate to that of IgM and IgG. Again the molecules displayed a characteristic electrophoretic mobility and gave specific precipitin reactions with antiserum to IgA. Neutralising capacity persisted in the IgA class of antibody and was also detected in the IgG_1 subclass from 10 days after exposure to infection, particularly in oral saliva and pharyngeal fluid.

c). Kinetics of neutralisation.

The rate at which virus was neutralised by antibody in serum or secretions was examined using samples obtained 21 - 28 days after exposure to FMD. Saliva and pharyngeal fluid were dialysed prior to testing. Samples were incubated at $37^{o}C$ and pH 7.5 with homologous virus at $10^{6.0}$ p.f.u. per ml and aliquots withdrawn at 20 minute intervals were assayed for residual virus in tissue culture monolayers. The results were similar for the 4 virus strains although neutralisation appeared to proceed

slightly faster and to a greater degree with the C Noville system than with the other strains. The type C virus may have elicited antibody of higher avidity or, alternatively, the virus itself may be more susceptible to neutralisation. However the quantitative aspects of the experiments were not sufficiently well defined to allow any conclusions to be drawn. Representative results are shown in Figure 10. In 20 minutes incubation 90 - 99% of virus was neutralised by homologous antibody. A fraction of virus escaped neutralisation and persisted for at least 4 hours at 37°C. When similar experiments were carried out in nasal secretion at pH 8.0 or in undialysed oral saliva and pharyngeal fluid at pH 8.5 the residual, non neutralised fraction was usually totally inactivated after 4 hours at 37°C, probably by means of pH and thermal inactivation in these alkaline secretions together with the action of the additional non specific antiviral factor.

7.2.1.6. Dissociation of antibody-antigen complexes.

Antigen-antibody complexes might be expected to form in many secretions during the acute phase of FMD especially at the time when viral excretion is decreasing while antibody levels are increasing. Fluorocarbon treatment has been successfully employed in the reactivation of FMDV from neutralised complexes (Brown and Cartwright 1960, Sutmoller and Cottral 1967c etc.) and the technique was applied to the secretions of 3 steers collected during the first 10 days after exposure to type C virus.

Attempts to reactivate virus did not produce clear cut results (Table 42). In some samples an increase in viral titre followed dissociation but in others the titre was unaltered or reduced after treatment. Furthermore, the results varied independently of the virus titre in untreated samples. Despite the unpredictable nature of the results three features were apparent. Firstly, fluorocarbon treatment consistently

failed to increase the titre of virus in samples taken during the first three days of exposure although large amounts were shed in the secretions at that time (see Table 17). Secondly, reactivation was principally achieved in samples taken between 5 and 7 days after exposure at the time when antibody was developing. Thirdly, in only one instance (Steer JS63, Serum, Day 7) did dissociation result in the recovery of virus from a sample which had been negative prior to treatment. The first and second observations indicated that neutralising antibody did play a part in limiting the excretion of infective virus. The third observation suggested that virus is recoverable from complexes for a rather short time and that the disappearance of virus from all secretions (except pharyngeal fluid) is mediated to some extent by antibody but also by other antiviral agencies.

7.2.2. Long term observations.

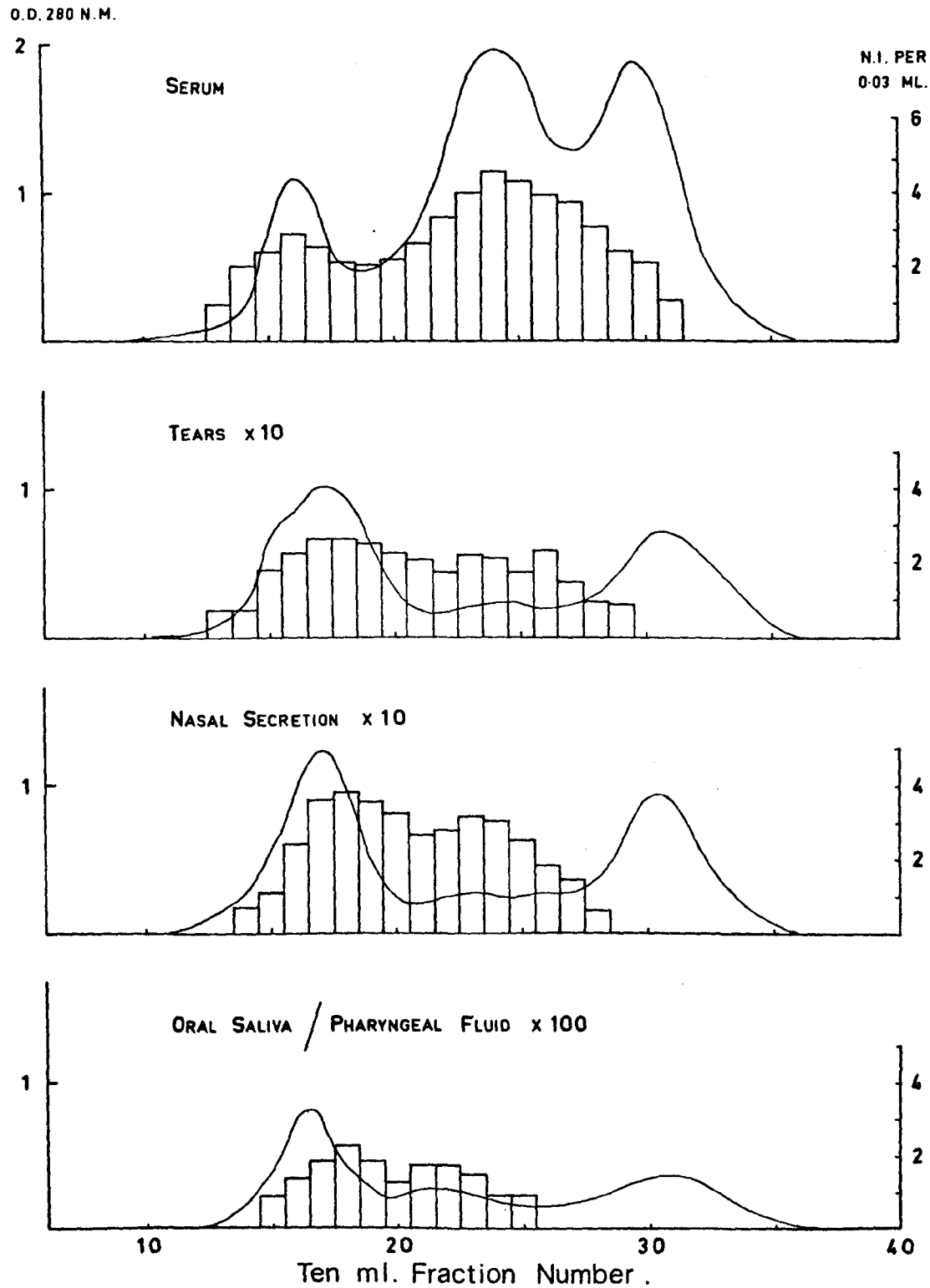
Two groups of steers were followed for extended periods of time after infection with FMD to determine the persistence of antibody and virus in secretions. The first group was sampled at approximately monthly intervals during 11 months of convalescence after infection with type O (BFS 1860) and the second group at irregular intervals during the period 4 - 5½ years after infection with type A (119). Samples were treated with fluorocarbon in an attempt to increase virus recovery by the dissociation of possible antibody-antigen complexes.

No virus was recovered in six samplings from the 8 steers infected with type A virus. These animals had carried virus for as long as 105 weeks after infection (Brooksby 1967). One month after infection of steers with type O, the virus could not be detected in tears, nasal secretion, oral saliva, faeces, urine or serum from 3 animals but was recovered from pharyngeal fluid for up to 4 months post infection (Table 43).

Fluorocarbon treatment resulted in little if any increase in viral recovery.

Serum neutralising antibody persisted at high levels in both groups and showed only a gradual decline 5½ years post infection. Nasal and lachrymal antibody also persisted at low levels. Antibody in oral saliva and pharyngeal fluid declined below detectable levels 2 - 3 months after infection with type 0 but was still demonstrable in concentrated secretions (x10 - 100) for as long as 11 months after type 0 and 5½ years after type A infection (Tables 44 and 45). It is of interest that levels of antibody and virus declined in pharyngeal fluid at about the same time (see Tables 43 and 44).

Fig 9. Gel Filtration Profiles For Protein Content And Neutralising Activity Of Convalescent Bovine Secretions.



SECRETIONS SAMPLED 21 DAYS POST INFECTION WITH FMDV TYPE C NOVILLE.

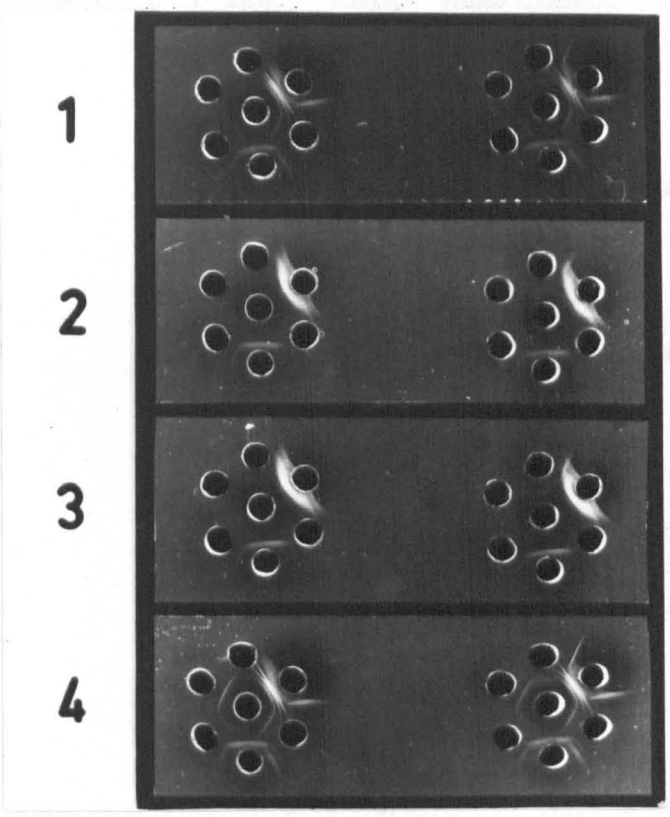


PLATE 10. Immunodiffusion patterns for normal and convalescent bovine secretions.

Details overleaf.

Key for PLATE 10.

Central wells
1. Lachrymal secretion.x10.
2. Nasal secretion.
3. Oral saliva/pharyngeal fluid x100.
4. Serum.

Left hand pattern - samples taken before infection.
Right hand pattern - samples taken 21 days after infection.

Peripheral wells
A. Homologous concentrated FMDV.
B. Antiserum to 1, 2, 3 or 4.
C. Antiserum to IgM.
D. Antiserum to IgA.
E. Antiserum to IgG_1.
F. Antiserum to IgG_2.

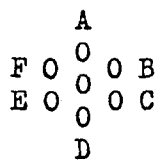


Table 40. Effect of reductive cleavage on the neutralising capacity of whole and fractionated convalescent bovine secretions. (FMDV type C Noville)

Sample	Days post exposure			
	7	14	21	42
Lachrymal Sec.	1.0/0.2*	3.6/1.8	4.0/2.8	1.0/0.8
Nasal Sec.	1.6/1.0	3.6/2.8	4.4/3.2	2.8/2.0
Oral Saliva	0.8/0.8	2.0/1.6	2.4/1.0	0.8/0.8
Pharyngeal Fl.	1.0/0.6	2.2/1.8	2.8/1.0	0.8/0.6
Serum	2.2/0.6	4.6/1.4	5.4/5.0	4.2/4.4
IgM	-/-	3.0/0.2	-/-	-/-
IgA	-/-	-/-	3.6/2.4	-/-
IgG_1	-/-	-/-	4.8/4.2	-/-
IgG_2	-/-	-/-	5.0/4.6	-/-

Results expressed as the neutralisation index per 0.03 ml of sample.

Samples tested at a dilution of 1:3 apart from serum which was tested at 1:30.

-/- : Not tested

* Numerator : Neutralisation Index of non reduced sample

Denomenator Neutralisation Index of reduced sample.

Table 41. Effect of immunoglobulin absorption on the neutralising capacity of whole and fractionated convalescent bovine secretions. (FMDV type C Noville).

Sample	Absorbed with:-	Days post exposure			
		7	14	21	42
Lachrymal Secretion	anti L/S	96*	98	100	98
(L/S)	anti IgM	58	45	32	10
	anti IgA	69	75	70	75
	anti IgG_1	10	15	18	20
	anti IgG_2	0	2	0	4
Nasal Secretion	anti N/S	100	94	90	98
(N/S)	anti IgM	11	12	15	2
	anti IgA	74	76	60	79
	anti IgG_1	22	18	25	30
	anti IgG_2	5	2	3	5
Oral Saliva	anti O/S	96	98	97	96
(O/S)	anti IgM	2	3	5	0
	anti IgA	80	75	68	77
	anti IgG_1	12	15	20	26
	anti IgG_2	6	3	0	4
Pharyngeal Fluid	anti Ph/F	98	97	100	100
(Ph/F)	anti IgM	4	0	2	0
	anti IgA	76	70	70	65
	anti IgG_1	39	25	22	31
	anti IgG_2	2	4	8	3

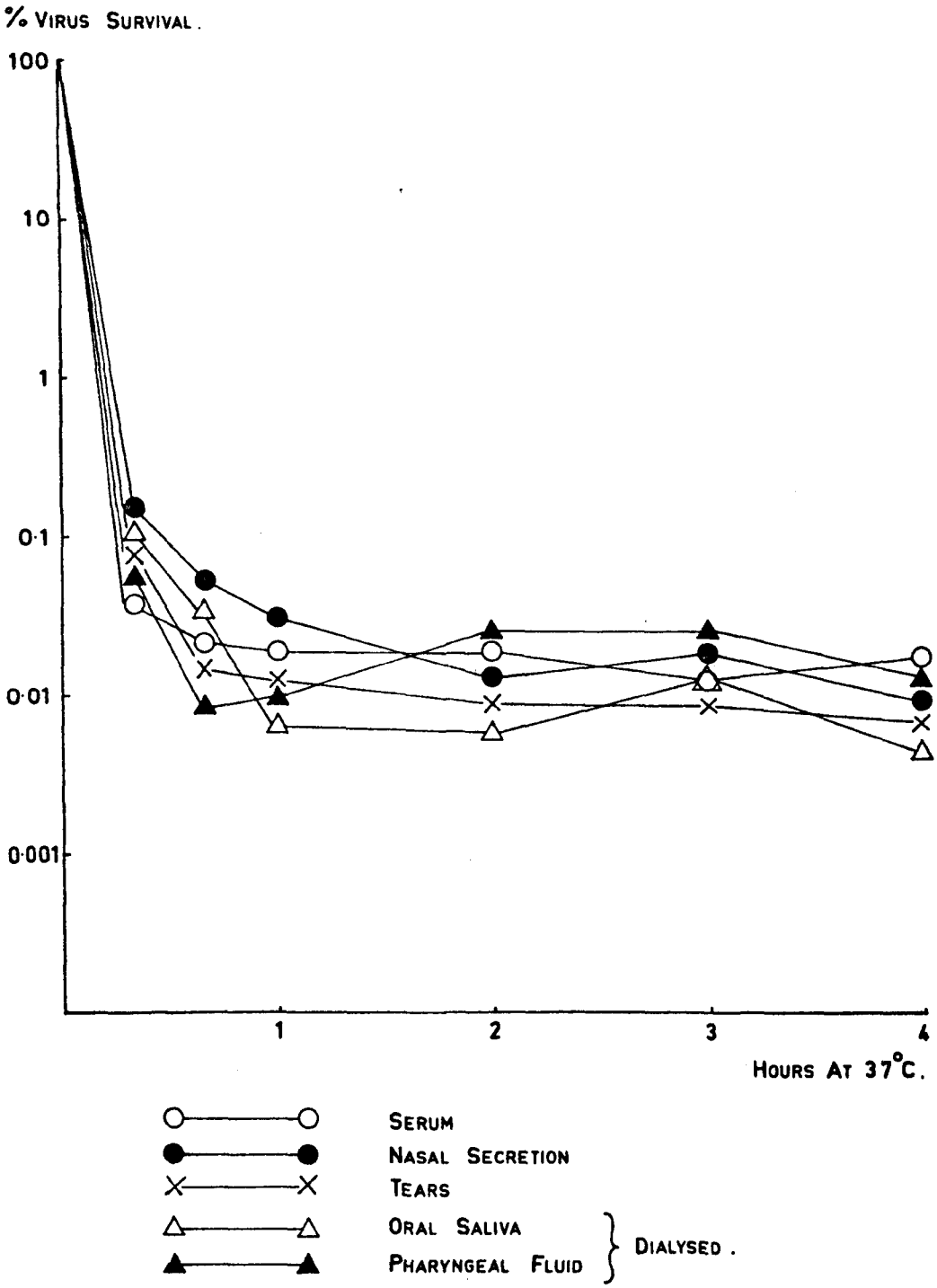
continued overleaf.

Sample	Absorbed with:-	Days post exposure			
		7	14	21	42
Serum (Se)	anti Se	100*	100	98	100
	anti IgM	82	70	21	4
	anti IgA	5	2	10	13
	anti IgG_1	75	26	87	95
	anti IgG_2	11	15	73	86
IgM	anti IgM	-	97	-	-
	anti IgA	-	10	-	-
	anti IgG_1	-	3	-	-
	anti IgG_2	-	5	-	-
IgA	anti IgM	-	-	15	-
	anti IgA	-	-	89	-
	anti IgG_1	-	-	4	-
	anti IgG_2	-	-	2	-
IgG_1	anti IgM	-	-	6	-
	anti IgA	-	-	2	-
	anti IgG_1	-	-	100	-
	anti IgG_2	-	-	21	-
IgG_2	anti IgM	-	-	5	-
	anti IgA	-	-	5	-
	anti IgG_1	-	-	35	-
	anti IgG_2	-	-	98	-

* Reduction in the Neutralisation Index of sample after absorption with specific antiserum expressed as a percentage of the original unadsorbed value.

- : not tested.

Fig 10. Kinetics Of Neutralisation Of FMDV By Convalescent Bovine Secretions.



Secretions sampled 21 days post infection with FMDV type C.

Table 42. Effect of fluorocarbon dissociation on the titre of virus in secretions from cattle infected with FMDV. Type C Noville. Day 3 - 10.

Animal No.	Sample	Days post exposure 3	4	5	6	7	8	9	10
JS57	L/S	+ -	-	0.4*	-	-	-	-	-
	N/S	-	-	1.0	1.2	-	0.8	-	-
	O/S	-	-	-	1.5	0.4	-	-	-
	Ph/F	-	-	-	-	1.2	-	0.8	-
	Se	-	0.8	1.0	-	-	-	-	-
JS59	L/S	-	+ -	1.0	-	0.8	-	-	-
	N/S	-	-	-	1.5	0.4	-	-	-
	O/S	-	-	1.8	1.8	-	1.0	-	-
	Ph/F	-	-	0.6	1.4	0.8	-	-	0.6
	Se	-	1.0	1.4	0.4	-	-	-	-
JS63	L/S	-	+ -	1.0	0.4	-	-	-	-
	N/S	-	-	-	1.5	0.6	-	-	-
	O/S	-	0.4	-	1.8	-	0.6	0.8	-
	Ph/F	-	-	1.3	1.5	-	0.4	-	-
	Se	-	1.2	0.8	-	0.8	-	-	-

\+ : Earliest detection of lesions.
* : Increase in virus titre (C.T.Y. ID_{50} per ml) after treatment with fluorocarbon.
\- : No increase in virus titre.
Note: Several samples lost titre after treatment with fluorocarbon.
Key otherwise as for previous tables.

Table 43. Persistence of virus in pharyngeal fluid samples following infection with FMDV type O_1 (BFS 1860).

Month post Infection **	Animal Number JE36	JE37	JE38
1	0.7*	2.0	1.2
2	1.2	1.5	1.0
3	1.5	0.0	1.5
4	1.3	0.0	0.7
5	Tr	0.0	0.0
6 - 11	0.0	0.0	0.0

* CTY ID_{50} per ml of sample

** Animals sampled at approximately monthly intervals

0.0 = No virus detected in undiluted sample

Tr = One or two tubes showing CPE at 10^{-0} dilution.

Table 44. Neutralising antibody in bovine secretions following infection with type O_1 (BFS 1860). Month 1 - 11.

Animal No.	Sample	Months post exposure** 1	2	3	4	5	6	7	8	9	10	11
JE36	L/S	-	-	-	-	-	1.2	1.0	1.4	1.0	1.2	0.8
	N/S	3.6	2.0	1.2	1.4	-	1.6	1.8	2.2	1.6	1.4	1.2
	O/S(D) 1:1	1.2	0.4	0.2	NT	-	NT	NT	NT	NT	NT	NT
	O/S(D) x10	1.8	1.4	1.4	1.0	-	0.8	1.0	1.2	0.8	1.0	1.0
	Ph/F(D) 1:1	0.8	0.6	0.0	NT	-	NT	NT	NT	NT	NT	NT
	Ph/F(D) x10	1.6	1.6	1.0	1.0	-	1.0	1.2	1.4	1.2	0.8	1.0
	Se	4.6	3.8	3.4	3.6	-	3.8	3.6	4.0	3.6	3.0	3.2
JE37	L/S	-	-	-	-	-	1.0	1.4	1.2	1.2	1.0	0.6
	N/S	3.0	2.8	2.0	2.2	-	1.4	1.0	1.6	1.2	1.0	1.0
	O/S(D) 1:1	0.8	0.2	0.4	NT	-	NT	NT	NT	NT	NT	NT
	O/S(D) x10	2.0	1.0	1.4	1.6	-	1.0	1.4	1.0	1.2	1.4	0.8
	Ph/F(D) 1:1	1.6	0.0	0.4	NT	-	NT	NT	NT	NT	NT	NT
	Ph/F(D) x10	2.2	1.2	1.6	1.2	-	1.6	1.2	0.8	1.4	1.0	1.0
	Se	4.9	3.2	3.6	3.6	-	4.0	3.8	3.6	3.2	3.0	2.8
JE38	L/S	-	-	-	-	-	1.0	1.2	1.4	0.8	1.0	1.0
	N/S	3.2	2.8	1.4	1.0	-	1.2	1.4	1.6	1.4	1.0	1.0
	O/S(D) 1:1	1.4	0.6	0.0	NT	-	NT	NT	NT	NT	NT	NT
	O/S(D) x10	2.0	1.2	1.4	1.6	-	1.0	1.4	0.8	1.2	1.4	0.8
	Ph/F(D) 1:1	1.2	0.2	-	NT	-	NT	-	NT	-	-	NT
	Ph/F(D) x10	1.8	1.0	-	0.8	-	1.4	-	1.0	-	-	1.0
	Se	3.8	3.8	3.0	4.2	-	3.8	3.8	4.0	3.6	3.8	3.4

** Animals sampled at approximately monthly intervals.
Dialysed saliva and pharyngeal fluid tested undiluted or after x10 conc.
NT: not tested
Key otherwise as for Table 30 etc..
Note: Pharyngeal fluid samples from steer JE38 are incomplete because the animal often regurgitated persistently during sampling.

Table 45. Neutralising antibody levels in bovine secretions following infection with FMDV type A (119). 4 - 5½ years.

Sample and Dilution		Months post infection	
		49	66
Lachrymal Secretion	1:1	1.0*	0.8
		(0.6 - 1.5)	(0.4 - 1.2)
Nasal Secretion	1:1	1.4	1.2
		(0.6 - 2.0)	(0.6 - 1.8)
Oral Saliva	x50	1.0	1.0
		(0.4 - 1.8)	(0.4 - 1.6)
Pharyngeal Fluid	x50	1.2	0.8
		(0.4 - 1.8)	(0.4 - 1.4)
Serum	1:10	2.8	2.6
		(2.4 - 3.2)	(2.3 - 3.2)

* Geometric mean (and range) of Neutralisation Indices per 0.03 ml of secretion tested at the specified dilution.

Eight steers in the group.

7.2.3. Conclusions.

Cattle exposed to simulated natural infection with 4 strains of FMD by direct contact with donor pigs developed generalised disease, lesions being first detected between 2 - 4 days after exposure. The method of infection entailed a number of intrinsic variables, e.g. the known difference in aerosol excretion between strains of FMDV (Donaldson et al 1970), the probable differences in excretion by individual donor pigs and the relative positions of donors and recipients in the loose box during exposure. These variables may explain in part the different amounts of virus recovered from various groups 24 hours after exposure (considerably more was recovered from recipients of FMDV type O_1 Swiss) and the different rate at which disease developed within groups (lesions were detected in one steer, HR11, on Day 2 and in two other steers, HR8 and HR9, on Day 4 after exposure to FMDV type O BFS 1860). Important determinants of susceptibility may reside in individual recipients. The fact that steer HR11 succumbed 48 hours earlier than box mates may have been due to a particularly high degree of susceptibility rather than to the acquisition of a larger dose of challenge virus. However differences between individual animals in respect of the amount of virus recovered and the rate at which lesions developed could not be related to the pH value, the total protein content or the level of natural antiviral factors in secretions at the time of infection.

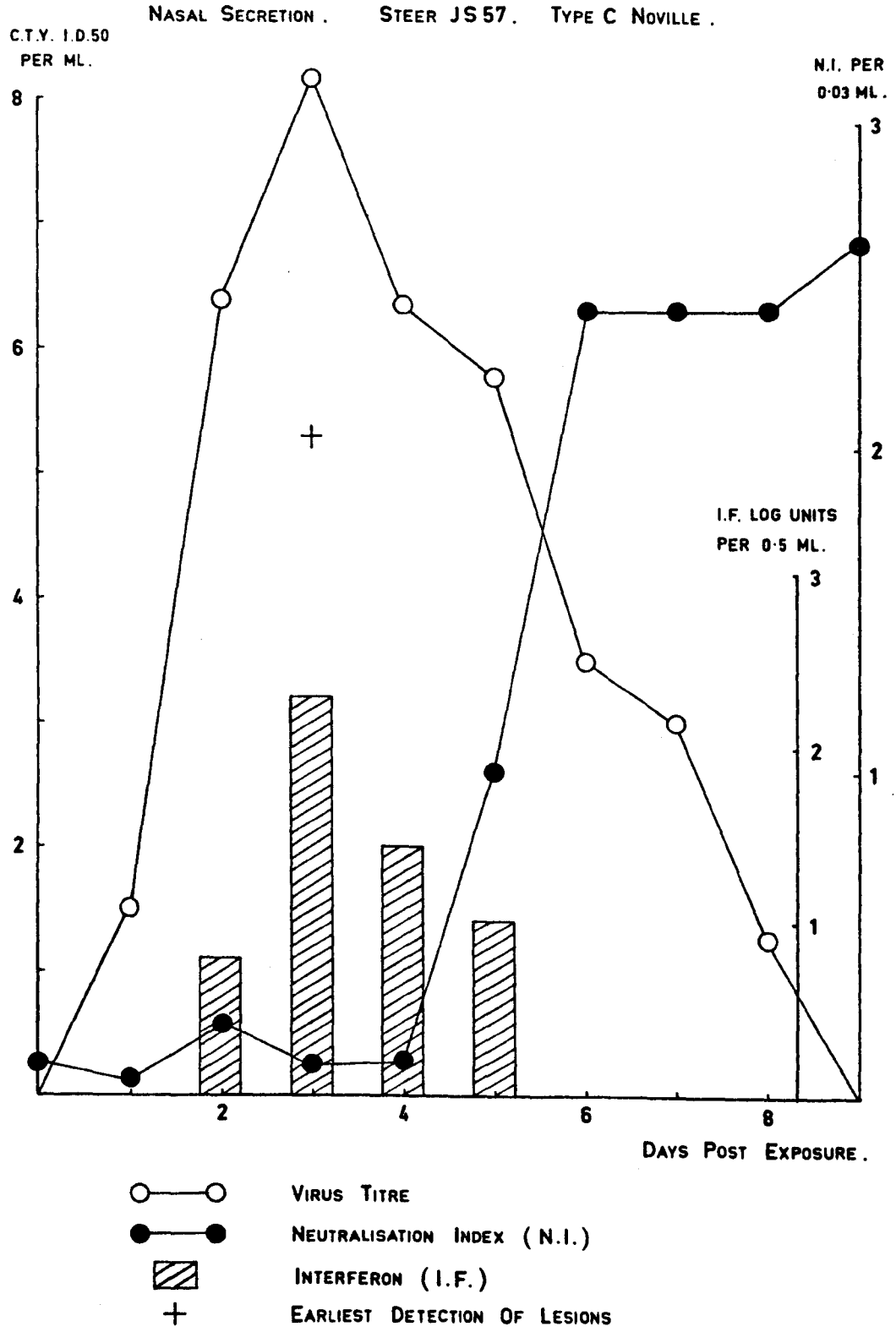
Interferon was present in the secretions of all animals between 2 - 4 days after exposure to virus and was usually detected for 2 or 3 days (maximum 4 days). Most IF was induced in response to types A and C, less to type O viruses and in all secretions the presence of IF showed a good correlation with the time of development of lesions and a poor correlation with time after exposure to infection.

Neutralising antibody was detected in secretions 3 - 6 days (mean 5 days) after exposure to virus, its development being more closely related to the time after exposure to virus than with the time of development of lesions. Antibody levels were similar for all 4 strains of virus although slightly more was produced in response to type C infection. Peak levels were reached between 21 and 28 days post infection and the levels in external secretions were always lower than those found in serum. Antibody was found in order of decreasing concentration in nasal secretion, tears, saliva and pharyngeal fluid. Mucus from the lower respiratory tract and alimentary tract at post mortem also possessed neutralising capacity. No antibody was detected in urine or faecal suspension despite concentration of the samples.

Infection produced a transient rise in the total protein content of secretions coinciding with the height of clinical disease but very little alteration of pH was observed, except in urine which became more acid. The distribution of protein in secretions was unaltered as assessed by column chromatography but specific neutralising antibody developed in the immunoglobulin fractions. The neutralising capacity of various secretions was associated with different immunoglobulins; in serum initially with IgM and later with IgG_1 and IgG_2, in tears initially with IgM and IgA and later with IgA and IgG_1 and in oral saliva and pharyngeal fluid with IgA and later with IgA and IgG_1.

The temporal relationship between virus excretion, clinical disease, IF induction and antibody production was examined for each secretion. Figure 11, for example, shows a graphical representation of this relationship for bovine nasal secretion following infection with type C virus. In general IF was detected at the height of the syndrome and antibody was detected 1 - 3 days later. Viral excretion declined from its peak as IF was induced and decreased markedly in the face of rising antibody

Fig 11 . Relationships Between Virus Excretion , Interferon And Antibody In Bovine Secretions During Infection With FMDV .



levels. Evidence for a casual relationship between rising antibody and declining virus was provided by the fact that fluorocarbon treatment resulted in an increased viral recovery from some samples (Table 42), particularly those taken during the period immediately after the peak of clinical disease and viral excretion. This effect was by no means universal and for the most part the data admits of association rather than proof.

From the tenth day after infection virus was detectable only in pharyngeal fluid samples. Neutralising antibody was detectable for at least 5½ years after infection in serum (at high levels), in nasal secretion and tears (at lower levels) and in oral saliva and pharyngeal fluid (in low levels detectable only after concentration procedures had been applied).

7.3. Secretions from Passively Immunised Animals

Studies in convalescent animals (section 7.2.) showed that specific neutralising antibody appeared in bovine secretions after exposure to FMDV. The antibody could have originated by overspill from serum, by local production in peripheral situations or by a combination of these. Passive immunisation was employed to investigate the possibility of antibody attaining the secretions by simple transudation or by selective transfer from serum. Secretions from 3 steers were sampled before immunisation, at 2 hourly intervals for 8 hours and then daily for 21 days after the intravenous application of concentrated bovine antiserum to FMDV type O BFS 1860. The total protein content and the neutralising capacity of samples was measured but in the light of the negative findings in respect of pH in convalescent animals, the hydrogen ion content was not studied.

After immunisation one of the three steers (JC67) showed clear clinical signs of immediate hypersensitivity with tachypnoea, shivering, defecation, urination, lachrymation and salivation, swelling of the eyelids and submaxillary oedema.

7.3.1. Protein levels following passive immunisation.

No changes attributable to immunisation were detected in the total protein content of serum or secretions sampled during the first 72 hours after the passive transfer of concentrated antiserum.

7.3.2. Neutralising antibody following passive immunisation.

Humoral antibody declined slowly after immunisation showing a half life of 18 days (range 16 - 21 days). Antibody was detected in secretions within 2 hours of passive immunisation and was measurable in nasal secretion for 3 - 4 days and in oral saliva and pharyngeal fluid concentrated by a factor of 10 for 2 - 3 days after immunisation. The highest levels of secretory antibody were found in samples taken 2 hours after immunisation from the steer which showed anaphylaxis but by 4 hours the levels were similar in all animals. Individual results are shown in Table 46 and mean results are displayed in Figure 12. Neutralising antibody in secretions was principally of the IgG_1 class.

7.3.3. Conclusions.

At face value the findings support the concept that serum may act as a source of secretory antibody in the bovine species, but the results must be qualified in at least two respects. a) One animal showed striking signs of immediate hypersensitivity which were reflected in high levels of antibody (but not in total protein levels) in early secretion

samples. It is possible that subclinical vascular lesions occurred in the other steers with increased capillary permeability allowing an abnormal escape of antibody into the secretions. b) The finding that the neutralising activity of secretions was associated chiefly with immunoglobulin of the IgG_1 class must be set against the fact that the immune serum had been derived from cattle 28 days convalescent to FMD when the neutralising capacity is known to reside chiefly in that class. The absence of antibody of the IgM or IgA classes was not therefore surprising.

Despite these shortcomings the experiment proved that serum antibody could attain the external secretions in certain situations. The idea of the overspill mechanism was supported by the fact that antibody was detectable in secretions when the serum had a neutralisation index of around 2.5 but could not be demonstrated (at least in saliva) when serum antibody levels fell below that threshold. The results also indicated a degree of selectivity in the transfer since the antiserum contained both IgG_1 and IgG_2 molecules whereas IgG_1 predominated in the secretions after immunisation.

7.4. Secretions from Actively Immunised Animals

The serum neutralising antibody response to potent, inactivated FMD vaccine is well established but very little is known about secretory antibody following vaccination. Antibody levels were therefore measured in serum and secretions collected from cattle vaccinated once, twice or three times with conventional inactivated type C Noville vaccine given by the subcutaneous route. Total protein and interferon levels were also measured. One group of 3 steers was used and samples of serum, lachrymal and nasal secretion, oral saliva and pharyngeal fluid were taken before each vaccination, daily for 10 days and at weekly or monthly

Table 46. Neutralising antibody levels in the secretions of cattle following passive immunisation. (Antiserum to FMDV type O BFS 1860).

Animal No.	Sample	Time post immunisation								
		Hours					Days			
		0	2	4	6	8	1	2	3	4
JC70+	N/S	0.2*	2.0	1.2	0.8	1.0	0.8	0.6	0.8	0.4
	O/S	0.0	1.0	1.0	0.8	0.8	0.6	0.4	0.6	0.2
	Ph/F	0.2	1.2	0.8	1.0	0.8	0.4	0.2	0.4	0.0
	Se	0.4	2.6	2.6	2.4	2.6	2.4	2.0	2.0	1.8
JC71	N/S	0.0	1.0	1.0	1.2	1.0	1.0	0.8	0.4	0.2
	O/S	0.2	0.6	0.8	0.8	1.0	0.8	0.4	0.6	0.0
	Ph/F	0.2	0.8	0.8	1.0	0.8	0.6	0.8	0.0	0.2
	Se	0.2	2.8	2.4	2.8	2.6	2.6	2.2	1.8	1.8
JC72	N/S	0.4	1.2	1.2	0.8	1.0	1.0	0.8	0.6	0.2
	O/S	0.0	0.8	0.8	1.0	0.8	1.0	0.8	0.6	0.2
	Ph/F	0.0	0.6	0.8	0.6	0.8	0.6	0.6	0.4	0.2
	Se	0.2	3.0	2.8	3.2	2.6	2.4	2.0	1.8	1.6

N/S Nasal Secretion 1:1

O/S Oral Saliva Dialysed x10

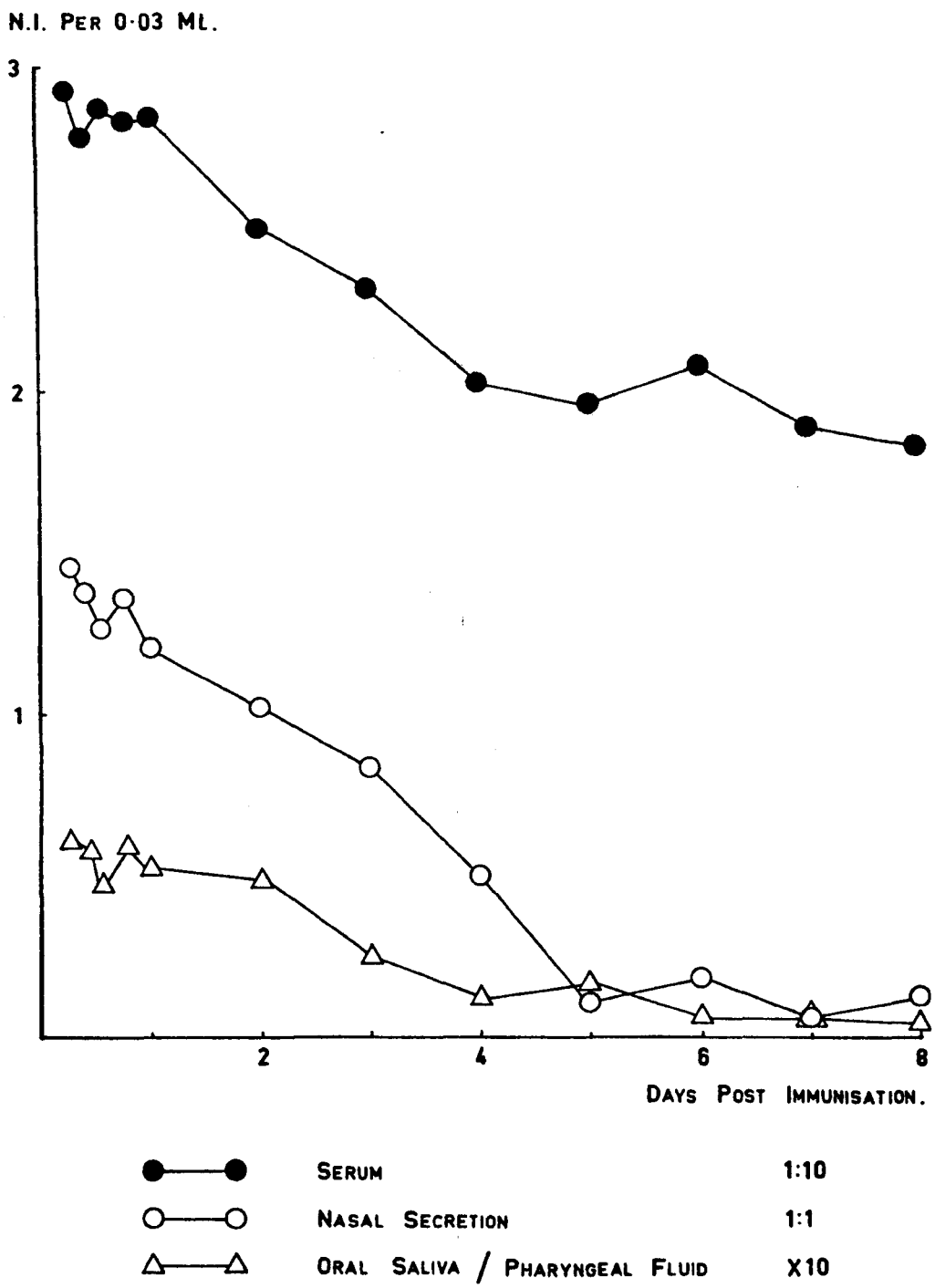
Ph/F Pharyngeal Fluid Dialysed x10

Se Serum 1:10

* Homologous Neutralisation Index per 0.03 ml

\+ Animal showed clinical signs of anaphylaxis. See Text.

Fig 12. Neutralising Antibody Response In Bovine Secretions Following Passive Immunisation (Mean Of Three Steers).



intervals thereafter.

7.4.1. Total Protein and Interferon levels.

No changes attributable to vaccination were detected in the total protein content of serum or secretions. Interferon was not detected in samples taken during the first 72 hours after vaccination or revaccination.

7.4.2. Neutralising Antibody Response.

Primary vaccination engendered a normal humoral response with antibody being first detected after 5 days and reaching a peak at about 21 days. Antibody was found in tears and nasal secretion at low levels but not in dialysed oral saliva or pharyngeal fluid even when these secretions were concentrated as much as a hundredfold. Serum antibody levels had declined to about 2.0 (Neutralisation Index per 0.03 ml at 1:10 dilution) and nasal and lachrymal antibody had fallen to baseline levels when the animals were revaccinated 35 days after the initial immunisation.

After secondary vaccination a typical anamnestic response ensued in serum and antibody also became evident in external secretions. Serum antibody levels were invariably greater than those found in secretions and more antibody was found in fluids from the eyes and nose than in those from the mouth or pharynx. Serum antibody showed a gradual decline of about 2 logs (Neutralisation Index) over 12 months of sampling but 2.5 - 3.0 logs of neutralising activity remained. Antibody was detectable in tears for 6 - 8 weeks, in nasal secretion for 8 - 12 weeks and in oral saliva or pharyngeal fluid for 4 - 5 weeks after revaccination. In salivary secretions antibody was initially detectable in unconcentrated

dialysed fluid but later it was necessary to concentrate by 50 - 100 fold.

Approximately one year after secondary immunisation the steers were vaccinated for a third time. Serum antibody rose rapidly to levels as high as those seen in convalescent animals (cf Table 37 and Fig. 8) and high levels of activity were also demonstrated in all the secretions in the order: nasal secretion, tears, oral saliva and pharyngeal fluid. Sampling continued for only 14 days after tertiary vaccination at which time antibody levels were still rising in each location. The pattern of neutralising antibody response followed repeated vaccination is shown in Fig. 13..

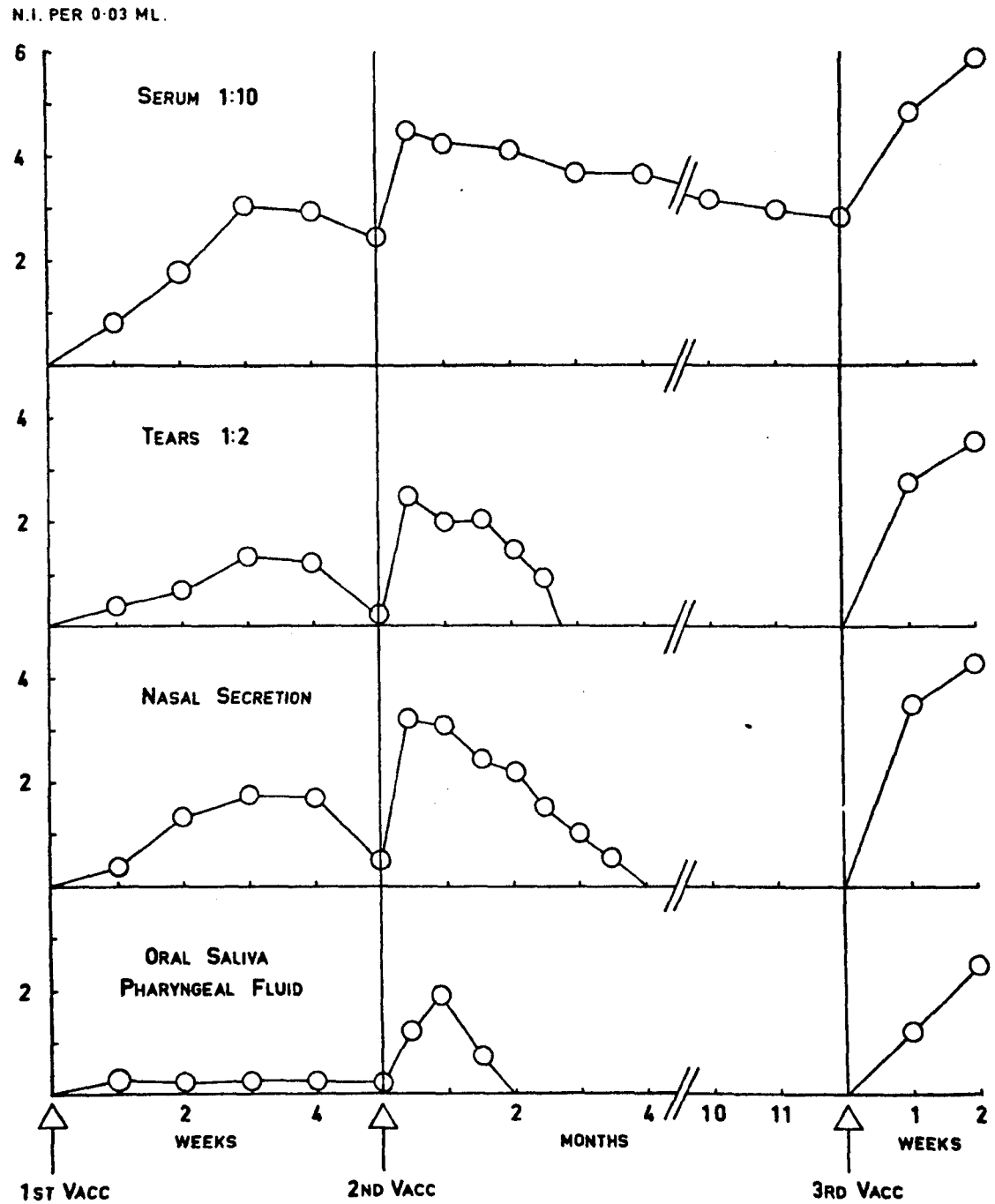
7.4.3. Immunoglobulin type.

Pooled samples collected at weekly intervals after each vaccination were examined by gel diffusion, anion exchange chromatography, immunodiffusion, immunoelectrophoresis and adsorption procedures to determine the immunoglobulin type responsible for neutralisation.

The early IgM response in serum was replaced by IgG and antibody of the IgG_1 and IgG_2 classes was responsible for most of the humoral neutralising activity from 28 days after primary vaccination to 14 days after tertiary vaccination. In the secretions of animals once or twice vaccinated neutralisation was principally associated with antibody of the IgG_1 class (with traces of IgM in early samples, particularly of tears). However, in samples derived from steers 14 days after the third vaccination neutralisation was associated not only with the IgG fraction but also with the IgA class (Table 47).

7.4.4. Conclusions.

Fig 13. Neutralising Antibody Response In Bovine Secretions Following Repeated Vaccination.



TYPE C NOVILLE INACTIVATED VACCINE. MEAN RESULTS FROM THREE STEERS.

Table 47. Effect of Immunoglobulin absorption on the neutralising capacity of secretions from vaccinated cattle.

Sample (and dilution)	Absorbed with*	Days post vaccination: 1° Vacc		2° Vacc		3° Vacc	
		14	21	7	28	7	14
Serum (1:10)	IgM*	92**	18	6	3	0	3
	IgA	3	9	2	5	13	15
	IgG_1	10	97	88	96	100	100
	IgG_2	12	85	100	92	95	96
Lachrymal	IgM	49	15	5	16	12	18
Secretion	IgA	12	73	86	80	19	43
(1:2)	IgG_1	2	87	97	100	97	98
	IgG_2	4	14	10	13	18	10
Nasal	IgM	10	8	2	6	4	4
Secretion	IgA	5	6	3	4	2	49
(1:1)	IgG_1	65	100	96	100	96	100
	IgG_2	13	16	17	10	13	15
Oral	IgM	-	-	5	2	0	3
Saliva	IgA	-	-	3	5	10	36
(x10 dialysed)	IgG_1	-	-	94	90	100	96
	IgG_2	-	-	15	13	19	12
Pharyngeal	IgM	-	-	0	3	5	5
Fluid	IgA	-	-	5	8	15	32
(x10 dialysed)	IgG_1	-	-	87	100	91	100
	IgG_2	-	-	13	16	15	14

*: Sample absorbed with antiserum against the specified immunoglobulin class.

**: Reduction in the Neutralisation Index of sample after absorption with specific ahtiserum expressed as a % of the original unabsorbed value.

-: not tested.

The amount of antibody detectable in the external secretions of cattle immunised by the subcutaneous application of inactivated FMD vaccine was dependant upon the number of times that the animals had been vaccinated. After a single vaccination neutralising antibody was detected for a brief period in small amounts in tears and nasal secretions but not in oral saliva or pharyngeal fluid even after these fluids had been concentrated. Serum antibody was plentiful. When animals were revaccinated 35 days later antibody became apparent in all the external secretions. Secretory antibody levels were always below those found concurrently in serum and greater amounts were detected in tears and nasal secretion than in oral sadiva and pharyngeal fluid. High levels of serum antibody persisted for at least 12 months after secondary vaccination. Secretory antibody, however, declined more rapidly and was longest detected in nasal secretion (12 weeks). After one or two doses of vaccine antibody in all secretions was principally of the IgG_1 class.

A third vaccination, 12 months after the second, resulted in increased antibody in all samples to levels above those elicited by one or two doses and comparable with the amounts seen in convalescent animals (cf Figs. 8 and 13). An interesting alteration occurred in the distribution of neutralising activity in secretions following the third vaccination since an IgA component was demonstrated in addition to IgG_1.

Vaccination did not give rise to detectable IF in serum or secretion nor did it produce changes in the total protein content or the distribution of protein classes which were measurable by the technique employed.

The fact that antibody could be recovered from secretions after subcutaneous vaccination with killed virus confirmed the possible involvement of the secretory immune system in the protection of cattle

vaccinated by this route.

7.5. Secretions from Immunised and Challenged Animals

Recovered animals are highly immune to a second challenge by homologous FMDV. Furthermore, complete clinical protection against FMD is readily conferred upon cattle by subcutaneous vaccination with conventional inactivated vaccines. The immunity does not extend to the pharyngeal region however and vaccinated animals frequently become asymptomatic carriers of virus after challenge. Studies already described showed that neutralising antibody could be detected in bovine secretions during convalescence (Section 7.2.) and after passive (Section 7.3.) or active (Section 7.4.) immunisation. The final series of experiments was conducted to investigate the role of secretory antibody in the protection of cattle against FMD. Local immunity was studied after a) vaccination or b) infection. Immunity was assessed according to the degree of protection afforded against clinical disease, the amount of viral shedding in secretions and the development of the carrier state.

7.5.1. Animals challenged after vaccination.

Eight groups of 3 steers were vaccinated and revaccinated by the subcutaneous route using an inactivated type C Noville vaccine applied at varying intervals before exposure to challenge with homologous FMDV. Table 48 shows the schedule of vaccination. Samples of pharyngeal fluid, tears, nasal secretion and serum were collected for antibody assay before vaccination and at weekly intervals thereafter until the time of challenge. Oral saliva was not sampled since previous work had shown (Sections 7.2, 7.3. and 7.4) that antibody levels in saliva were indistinguishable from those in pharyngeal fluid.

Animals were exposed to infection by holding 3 steers for one hour in a loose box immediately after it had been vacated by 6 donor pigs infected with FMDV 48 hours previously. The amount of virus in the box was replenished by reintroducing the donor pigs for one hour before the infection of the next group of cattle. Recipient groups were returned to their original uninfected box and the donors were slaughtered after the final exposure period.

Steers were examined and sampled daily for ten days and then at irregular intervals for up to 42 days after exposure. Oral saliva, faeces and preputial swabs were taken in addition to those secretions sampled before exposure and all were examined for the presence and amount of virus.

7.5.1.1. Antibody levels at the time of exposure.

The vaccination schedule resulted in widely different levels of neutralising antibody in secretion at the time of exposure. Group mean neutralisation indices varied between 0.1 - 3.6 for tears, 0.3 - 4.3 for nasal secretion, 0.0 - 2.6 for pharyngeal fluid and 0.0 - 5.4 for serum (Table 49). The response to vaccination resembled that already described (Section 7.4.) except for group 2 in which antibody could not be detected 7 days after primary vaccination.

7.5.1.2. Clinical protection

Groups 1 and 2 succumbed with fully generalised disease. There was a total absence of clinical signs in groups 3 - 8 and no significant temperature response was detected in these animals.

7.5.1.3. Excretion of virus.

Table 48. Schedule of vaccination prior to homologous challenge with FMDV type C Noville.

Group	Status	Days vaccinated before exposure		
		First Vacc.	Second Vacc.	Third Vacc.
1	Vacc. x1	0	-	-
2		7	-	-
3		14	-	-
4		21	-	-
5	Vacc. x2	28	0	-
6		35	7	-
7		42	14	-
8	Vacc. x3	414	379	14

Three steers were included in each group.

Table 49. Group mean Neutralising antibody levels at the time of exposure to FMDV type C Noville.

Group	Status	Secretion			
		L/S	N/S	Ph/F	Se
1	Vacc. x1	0.1*	0.3	0.0	0.1
2		0.3	0.5	0.0	0.2
3		1.8	1.6	0.2	2.2
4		1.1	1.9	0.0	2.8
5	Vacc. x2	1.1	1.7	0.3	2.8
6		1.9	3.6	1.2	3.3
7		2.9	3.9	2.4	5.1
8	Vacc. x3	3.6	4.3	2.6	5.4

* Homologous mouse neutralisation index per 0.03 ml. Geometric mean of results derived from 3 animals.

L/S : Lachrymal secretion 1:2

N/S : Nasal secretion 1:1

Ph/F : Pharyngeal Fluid Dialysed. x10 conc.

Se : Serum 1:10

The frequency of recovery of virus in samples taken daily from each group between Days 1 and 11 after exposure is given in Table 50. Viral excretion in animals vaccinated once on the day of exposure or seven days previously closely resembled that seen in non vaccinated animals (cf Table 20). However, excretion was almost completely inhibited in all situations excepting pharyngeal fluid in cattle vaccinated one or more times at least 14 days before challenge. The sporadic recovery of virus from sites other than the pharynx and the small amount of virus involved (equal or less than 1.5 CTY ID_{50} per sample) were commensurate with contamination during sampling rather than with true infection.

Pharyngeal fluid from group 3 contained virus as often and at the same level as samples from groups 1 and 2. However, viral recovery from this secretion was somewhat less frequent in groups 4 - 7 and strikingly less in group 8. Pharyngeal virus was confined to a single animal in this froup and recoveries were made on only 3 occasions during the first 10 days of sampling and 3 out of 10 subsequent samples taken at intervals during the period 10 - 42 days post exposure.

7.5.1.4. Detection of Interferon.

Interferon was not detected in serum or secretion samples taken during the first 48 hours after exposure to virus.

7.5.1.5. Recovery of virus at post mortem.

The marked reduction in the amount of virus recoverable in pharyngeal fluid samples from thrice vaccinated steers in group 8 was investigated further when the animals were slaughtered 42 days after challenge. Tissue from the dorsal and ventral surfaces of the soft palate, pharyngeal

Table 50. Recovery of virus from vaccinated cattle during the first ten days after exposure to FMDV type C Noville.

Group	Sample						
	L/S	N/S	O/S	Ph/F	P	F	Se
1	15*	25	27	26	20	4	10
2	18	21	25	24	21	9	9
3	3	0	2	29	3	0	0
4	0	0	1	19	1	0	0
5	0	2	0	16	0	1	0
6	0	0	0	17	0	0	0
7	1	0	2	19	0	0	0
8	0	0	0	3	0	0	0

* Number of times that virus was recovered from 30 samples (3 cattle sampled daily for 10 days). Infectivity assay performed in CTY tube cultures.

L/S	Lachrymal Secretion	1:2	P	Preputial Swab	1:5
N/S	Nasal Secretion	1:1	F	Faeces	1:10
O/S	Oral Saliva	1:1	Se	Serum	1:1
Ph/F	Pharyngeal Fluid	1:1			

wall and tonsil were obtained at post mortem, processed and examined for FMDV by inoculation onto CTY monolayer cultures. Although no virus could be demonstrated in fluid samples taken immediately prior to slaughter, type C virus was recovered from the pharngeal tissues of all 3 animals. Virus was recovered in amounts of 1.0 - 1.7 CTY ID_{50} per gram of tissue, and only in samples from the dorsal surface of the soft palate.

7.5.2. Animals challenged after infection.

The experiment described in section 7.5.2. was performed conjointly with Mr. J.A. Mann of this Institute.

Eight steers were challenged with homologous virus approximately $5\frac{1}{2}$ years after infection with FMDV type A119. The animals had been asymptomatic carriers of virus for as long as 105 weeks during the initial convalescence (see Section 7.2.2.) but no virus was detected in 6 samples taken during the period 4 - $5\frac{1}{2}$ years post infection. The steers were housed in pairs and challenge was effected by the intradermal inoculation of $10^{5.0}$ Bovine Tongue ID_{50} over 10 sites on the lingual dorsum of one animal in each pair. Two normal steers were treated in the same way to act as challenge controls. Secretions were sampled before reexposure to infection and for as long as 28 days after as previously described (Section 7.5.1.).

7.5.2.1. Antibody levels at the time of exposure.

Table 45 gives the mean and range of neutralisation indices in the secretions of the convalescent steers at the time of reexposure to virus, 66 months after primary infection. The antibody levels and immunoglobulin types have been discussed in section 7.2.2..

7.5.2.2. Clinical protection.

Clinical disease in convalescent steers was confined to the tongue inoculation sites, some of which showed mild, non progressive lesions of an erosive nature along the track of the needle reminiscent of the effect often noted after the intradermolingual inoculation of attenuated strains of FMDV. Control animals succumbed with generalised disease.

7.5.2.3. Excretion of virus.

Virus was not detected in tears, nasal secretion, oral saliva, pharyngeal fluid, preputial swabs, faeces or serum sampled during the first 10 days after reexposure or in pharyngeal fluid obtained on 6 occasions during the period 10 - 28 days after challenge. Virus was readily detectable in the secretion of control animals where excretion conformed to the pattern previously established (Section 7.2.1.1. and Table 17).

7.5.2.4. Recovery of virus at post mortem.

Convalescent steers were slaughtered 26 - 28 days after reexposure to FMDV and tissue samples from the dorsal and ventral surface of the soft palate, the wall of the pharynx and the tonsils were processed and examined for infective virus. Virus was not detected, even after fluorocarbon treatment had been applied to the samples.

7.5.2.5. Antibody response after reexposure.

Although both control animals developed generalised disease the absence of detectable virus in the secretions of animals after reexposure called into question the adequacy of the challenge procedure, particularly

for the non inoculated member of each convalescent pair. Proof of adequate exposure was sought by examining the neutralising antibody response to challenge. Table 51 shows the differences in the neutralisation index of serum and secretions measured immediately prior to re-exposure and again 10 days later. Two interesting features are apparent. All 4 inoculated and 2 non inoculated steers showed a rise in serum antibody but no such increase occurred in the other 2 non inoculated steers infected by contact (FE54 & FE75). However, in one of these (FE54) there was evidence of a rise in the neutralising capacity of external secretions in the absence of a humoral response.

The results showed that 7 of 8 steers were exposed to antigen in sufficient concentration to stimulate an increase in neutralising antibody while one animal exposed by contact infection may not have received an adequate challenge. The results also indicate a possible dissociation between the local and humoral responses in the production of neutralising antibody following challenge by contact.

7.5.3. Conclusions.

Various schedules of single, double and triple vaccination gave rise, as planned, to a wide range of antibody level at the time of exposure to infection. At one extreme steers vaccinated once on the day of exposure or 7 days previously were without detectable antibody while at the other, steers vaccinated for the third time 14 days before exposure exhibited antibody levels comparable with those of convalescent animals. As in previous experiments serum antibody was detectable earlier and attained higher levels than secretory antibody and all levels increased with repeated vaccination.

In convalescent animals serum antibody showed higher levels than

Table 51. The neutralising antibody response in serum and secretions after reexposure of steers to FMDV type A119.

Box	Animal No.	Method of Infection*	Sample Se	L/S	N/S	Ph/F
1	FE51	Tongue	2.0**	1.0	1.4	0.8
	FE53	Contact	1.2	1.0	1.2	0.8
2	FE56	Tongue	1.9	0.8	1.4	1.0
	FE75	Contact	0.0	0.2	0.0	0.2
3	FE50	Tongue	2.0	1.2	1.6	1.2
	FE54	Contact	0.2	0.8	1.2	0.8
4	FE74	Tongue	1.6	1.0	1.6	1.2
	FE55	Contact	2.2	1.4	1.8	1.4

* Steers were infected by intradermal inoculation of the tongue or by direct contact with the inoculated animal.

** Rise in homologous neutralisation Index of the sample between Day 0 and Day 10 post challenge (N.I. per 0.03 ml).

Se Serum 1:10

L/S Lachrymal Secretion 1:2

N/S Nasal Secretion 1:1

Ph/F Pharyngeal Fluid Dialysed 1:1.

secretory antibody. However, 1 of 8 convalescent steers reexposed to homologous challenge proved to be a notable exception in that antibody levels rose in secretions but not in serum.

Animals which had been vaccinated one or more times at least 14 days before exposure were immune to the development of clinical lesions, resisting challenge which produced severe generalised FMD within 3 days of exposure in steers vaccinated on the day of exposure or 7 days previously. The susceptibility of the latter group was unexpected since earlier work in this field had shown that cattle vaccinated as early as 2 - 3 days before exposure could be protected against clinical disease (Graves et al 1968, Garland, Burrows and Greig unpublished).

Convalescent animals showed only mild local lesions following homologous challenge $5\frac{1}{2}$ years after primary infection.

Viral excretion in vaccinated but non immune animals was essentially similar to that already established in normal cattle. Vaccination procedures which produced clinical protection were also associated with the virtual elimination of viral shedding in all secretions other than pharyngeal fluid. The sporadic recovery of virus from sites other than the pharynx and the small amounts involved (1.5 CTY ID_{50}) were commensurate with contamination during sampling rather than with true infection and excretion.

One or two doses of vaccine failed to prevent the acquisition of the carrier state although there were indications of a slight reduction in the frequency of viral recovery after a second vaccination. However, a highly significant reduction was observed in the recovery of carrier virus from animals vaccinated three times. In sampling during the period 0 - 42 days post exposure pharyngeal virus was detected in only 1 of 3

steers in this group and on only 6 of 20 occasions. The next lowest frequency, found in animals twice vaccinated at exposure, gave corresponding figures of 3/3 and 15/20. Nevertheless all 3 steers were found to be harbouring virus at post mortem. Three doses of vaccine therefore failed to prevent the acquisition of the carrier state but did succeed in producing a dramatic reduction in the excretion of virus in pharyngeal fluid.

Virus was not detected in the secretions of convalescent steers after reexposure to homologous challenge. The animals did not become carriers and no virus could be recovered from pharyngeal predilection sites at post mortem. Immunity engendered by previous infection may therefore be sufficient to prevent the acquisition of the carrier state.

Neutralising antibody in secretions after one or two vaccinations was principally of the IgG_1 class and was associated with resistance to infection in all superficial sites except the pharynx. In the secretions of convalescent or thrice vaccinated animals neutralising antibody was associated with IgG_1 and also with IgA. Increased pharyngeal immunity as shown by reduced viral excretion in thrice vaccinated animals or by the ability of convalescent animals to resist pharyngeal infection was therefore associated with the presence of IgA antibody in secretions at the time of exposure.

DISCUSSION

In natural infection of cattle with FMD the initial encounter between virus particle and susceptible cell may occur at one of several portals including the conjunctiva, nasal passages, oral cavity, pharynx and lower respiratory tract. A common feature of these sites is the presence of a film of secretion which bathes the outer mucosal and epithelial surfaces. These secretions may play an important part in the defence of the animal against invading pathogens including viruses as indicated by early work on mucoantibody and coproantibody in influenza and poliomelitis. More recently the discovery of the interferons and the partial elucidation of the secretory antibody system have given fresh impetus to the study of local immunity. Since primary sites of FMD infection are in superficial locations and since preliminary reports have shown the presence of antibody in bovine secretions after infection with the virus, close study of local immunity seemed appropriate.

This study began with an investigation of the physiological properties of normal bovine secretions to determine their influence on the process of infection with FMDV. Techniques and equipment were devised for the collection of large amounts of secretion. The total protein content, pH range, Ig class and antiviral activity of tears, nasal secretion, oral saliva and pharyngeal fluid were examined in samples from 101 normal steers.

The total protein content of the secretions of Red Devon cattle fell within those quoted for other breeds (Table 10). Levels in sequential samples varied less than the widely divergent values reported by Butler et al (1972a) or Duncan et al (1972a). Their work differs in respect of breed, sex and physiological status since it concerned lactat-

ing cattle of the Holstein-Friesian breed. The variability in dairy cattle would however be unlikely to influence susceptibility to FMD to any marked extent.

The type and distribution of immunoglobulins in the secretions of Red Devon steers (Table 12) was similar to that described for other breeds of cattle. IgA predominated, IgG_1 was also commonly found and IgM occurred in certain secretions, notably in tears. The origin of local Ig in cattle has not been fully elucidated. In man various sources are accepted e.g. salivary Ig may be derived from a) the major and minor salivary glands, b) serum via transudation, c) crevicular fluid and d) local production by plasma cells (Dayton et al 1969). Mach and Pahud (1971) have shown the synthesis of secretory IgA by bovine parotid and submaxillary glands in vitro and paramucosal cells containing IgA have been demonstrated in the lower gut, lungs, nasal mucosa, oral pharynx, parotid and lachrymal glands (Porter and Noakes 1970, Butler et al 1971, Mach and Pahud 1971, Hurliman and Darling 1971). Passive immunisation in cattle using radioactively labelled IgG_1 and IgG_2 have shown some selectivity in the transport of IgG_1 into alimentary, respiratory and occular fluids (Curtain 1971, Sullivan et al 1969, Pedersen and Nansen 1972). Evidence from passive immunisation studies must be interpreted with caution, since the results depend not only on the constituents of the transferred serum but also on the integrity of the recipient vascular system. Nevertheless the results obtained in this thesis (Section 7.3.) lend support to the view that some secretory antibody can be accounted for by overspill from serum. The results of active immunisation and infection studies discussed below indicate that secretory Ig can also be derived from other sources.

The pH of secretions was measured in the animal or as soon as possible after collection and normal values established (Table 5). The pH may

have altered during collection. For example, carbon dioxide may have escaped from blood while it was expressed from the syringe or from nasal secretions during recovery from tampons. However, the figures for blood compare reasonably well with the theoretical values derived using the Henderson - Hasselbach equation (Altman and Dittmer 1961). Moreover the pH value of nasal secretion collected by means of a pasteur pipette as it accumulated in the nares differed little from that of fluid expressed from tampons, Differences between values quoted in this thesis and those obtaining in the animal are therefore likely to be small.

The effect of hydrogen ion concentration on FMDV is well known (Bachrach et al 1957, Mussgay 1959). Several authors have commented on the antiviral activity of alkaline pH in oro-pharyngeal secretions (Van Bekkum et al 1966, Hedger 1968, Burrows 1966, 1972) but there have been no detailed studies of pH inactivation in natural bovine secretions. The limits of pH stability of the 4 virus strains were first investigated in buffers (Fig. 3b). Comparison of the range of pH stability with the pH value of secretions (table 5 and Fig. 3a) showed that nasal secretion and more especially oral saliva and pharyngeal fluid were sufficiently alkaline to cause damage to capsid protein while urine could be sufficiently acid to disrupt the virion exposing the nucleic acid to unfavourable pH and to the action of ubiquitous nucleases.

Inactivation of virus in normal tears and nasal fluid at 37°C and under physiological conditions of pH and protein content proceeded at a slow rate similar to inactivation in buffer and commensurate with thermal inactivation. In oral saliva and pharyngeal fluid virus was inactivated at a rate greater than that attributable to pH alone (Fig. 4). The additional antiviral factor was effective against 3 types of FMDV, concentration dependant, active over the pH range 7.5 - 8.5, unaffected by heating at 60°C for 1 hour, partially destroyed after 10 minutes and

totally destroyed after 20 minutes at 70°C and removable by dialysis.

Concurrent work at this Institute on the aerosol stability of FMDV type O_1 BFS 1860 has shown the virus to be more unstable when suspended in bovine salivary fluid than when suspended in cell culture fluid, milk, faeces or nasal secretion (Donaldson 1972, 1973, Barlow and Donaldson 1973). Inactivation was caused by an undefined organic molecule which was dialysable and sensitive to heating at 70°C but not at 60°C. It is probable that the factor causing inactivation of type O_1 BFS 1860 in aerosols of bovine salivary fluid is the same factor causing inactivation of types O_1 BFS 1860, O_1 Swiss 1/66, A119 and C Noville in oral saliva and pharyngeal fluid in this study.

No amylase or protease has been reported in bovine saliva and the secretion is apparently devoid of peroxidase and lysosyme which exist in the saliva of other species (Bailey and Balch 1961a and b, Hyslop 1965a, Kay 1966, Phillipson 1970). Nevertheless the additional factor might be a dialysable enzyme or an enzyme-coenzyme system and although the addition of some inhibitors (mercuric chloride, sodium azide and iodoacetamide) or a chelating agent (ethylene-diaminetetra acetic acid) had no effect on its capacity to inactivate FMDV in aerosols (Barlow and Donaldson 1973) or in suspensions (Garland unpublished data) the results do not preclude this possibility.

Secretions were obtained from 101 individual steers, many sampled repeatedly, and the additional antiviral factor was detected in all samples of oral saliva and pharyngeal fluid tested. Other workers have stated that normal bovine saliva possessed no antiviral activity (Hyslop 1965a, Figueroa et al 1973) or made no mention of such activity (Kaaden and Matheaus 1970). These reports are difficult to reconcile with the invariable occurrence of the factor in the present study but might be

explained by the use of different breeds of cattle, assay of activity in different systems and the focus of attention on antibody rather than on other factors.

The fact that type C Noville was more resistant to alkaline pH and to the additional antiviral factor than were types O_1 Swiss or A119 (Section 7.1.3. and Fig. 3b) illustrates the important differences which may exist between types and strains of FMDV in this respect.

The maximum rate of viral inactivation due to the combined agencies of temperature, alkaline pH and the additional non specific factor in salivary secretion was 1.25 log units per hour. This value was determined from in vitro kinetic studies and may well be lower than the rate in vivo since it makes no allowance for the continuous admixture of fresh secretion which occurs in the animal. The flow rate of parotid saliva varies from 2.0 ml/minute in the resting steer to 30 - 50 ml/minute during feeding and the lactating cow may secrete 98 - 190 litres of mixed saliva per day, the volume depending to some extent on the diet (Bailey 1961, Bailey and Balch 1961a and b). The importance of the natural non specific, antiviral factors must be considered in relation to the rate of infection of cells with FMDV. Experiments in tissue culture gave a half time of penetration of cells by FMDV of 30 seconds at 37°C (Thorne 1962) and current work in organ culture on the infection of tissue from the pharyngeal region of the calf has shown that cells may become infected with FMDV during an exposure time of 5 seconds (Garland and Hamblin, unpublished data). Even allowing for the prodigious secretion of saliva in the cow with its diluting and washing effect and the fresh addition of viral inhibitors, it is probable that the natural antiviral factors in bovine secretions are of limited protective value since viral inactivation proceeds only slowly under their influence. Indeed, the incidence of disease, the rate at which lesions

developed and the severity of the syndrome in normal cattle showed no correlation with the presence or amount of non specific antiviral factors in secretions. It should be noted, however, that a severe challenge was employed and it is possible that the non specific factors might assume a more significant role in animals exposed to minimal amounts of virus.

Cattle vary in their susceptibility to FMDV. Variations in severity of clinical disease is usually apparent in any group of infected animals and titration of a viral suspension by a standard method of intradermolingual inoculation will give different results in different individuals (Henderson 1949). Variations in natural secretory antiviral factors may contribute to the differing susceptibility but since the secretions of all the cattle examined contained similar antiviral activity this possibility could not be tested.

A related topic concerns the transmission of FMDV between carrier and susceptible cattle. Transmission experiments have had little success although epidemiological evidence strongly suggests that such spread does occur. The event is rare and probably requires a combination of special circumstances, one of which might be a deficiency of non specific antiviral factors. An animal with such a deficiency could play the part of the super receiver or super excretor of virus as envisaged by Schulman and Kilbourne (1963) and others in the spread of influenza.

An extensive survey was carried out to investigate the occurrence of variations and abnormalities in the pH value, protein content, Ig class and antiviral activity of bovine secretions (Sections 7.1.1. - 7.1.5.). Samples taken from cattle at 4 hourly intervals over a 24 hour period, at daily intervals over a week or at monthly intervals over a

year showed only minor variations. Samples from 101 individual animals all contained the additional antiviral factor. Interferon in any measurable amount was consistently absent. Secretions bathing the predilection sites showed very little fluctuation. Two different diets caused changes only in the pH of urine. On the other hand rare deficiency states are known, e.g. Nansen (1972) has recently reported on the existence of specific dyscriases of IgG in Red Danish cattle and other immunological abnormalities are likely to emerge. It is possible that abnormalities of secretory antiviral factors may be involved in both the varying susceptibility of cattle to FMDV and in the sporadic transmission of FMDV from the carrier animal but the present studies show that such abnormalities are uncommon.

The occurrence, nature and significance of induced antiviral factors in secretions was next examined during the course of infection of steers with FMDV. Viral excretion, clinical symptoms, interferon induction, secretory antibody production and the relationship between them were studied in cattle infected by exposure to virus excreted from donor pigs. The method was chosen because it combined a natural route of infection with a high degree of efficiency. It does however have the inherent disadvantage of not allowing precise measurement of the challenge dose.

All normal steers exposed to FMDV developed generalised disease, lesions being first detected within 2 - 4 days of exposure (Tables 22 and 23). No correlation could be drawn between the rate at which lesions developed, the severity of the syndrome and the amount of viral excretion on the one hand and the amount of natural antiviral activity in secretions at exposure on the other. The different rates at which individual animals became clinically ill may have been related to inequalities in the challenge dose and/or to differences in one or more of the many determinants of host susceptibility.

Pathogenesis of disease is dependant upon a multifactorial complex with four principal interrelated components; the virus, the environment, the host and the chance which combines them. Each component comprises a number of elements, many poorly understood. Viral factors include those of type, subtype, strain, history, resistance to inactivation, virulence, dose and method of presentation. Environmental factors include those of season, metereology and husbandry. Host factors include species, breed, sex, age, nutritional plane, physiological condition, intercurrent infection, disease history, vaccinal status and the presence and development of specific and non specific host defences.(the latter have already been discussed in Section 5.1.4.). In the present study an attempt has been made to investigate some important factors in the pathogenesis of FMD but the existence and potential of many other determinants of resistance of susceptibility must also be borne in mind.

A cautious interpretation of viral infection and excretion was necessary. The method of challenge was likely to result in the deposition of virus in all superficial sites so that the early recovery of virus from an area might be due to residual contamination rather than true infection and multiplication. Similarly, as infection developed, virus shed from active sites of multiplication might well contaminate other regions in the same animal or in box mates. With the onset of viraemia infectivity would become widely disseminated in tissues and possibly in secretions and thus the origin and significance of virus recovered in samples at that time would be in doubt. These considerations apply to all the challenged animals but steers exposed to type O_1 Swiss illustrate the effect of overwhelming infection particularly well since 24 hours after exposure virus was recovered from all sites other than faeces and urine (Table 18). Challenge with the other strains appears to have been less severe (Tables 17, 19 and 20) and in one animal (Steer HR8, Table 19) the secretions were virtually devoid of

virus at the first sampling, one day after exposure. Despite these difficulties it is possible to discern a pattern of infection based on the relative amounts of virus in concurrent samples, the time at which virus appeared and the development and distribution of lesions in each situation.

The results (Tables 17 - 20 and Fig. 5) confirm the primary involvement of the pharynx since virus was recovered most consistently in secretions from this area, often earlier and almost always to higher titre than in samples from other regions. Several other workers have shown that FMDV was recoverable from pharyngeal fluid for several days before other secretions became infected and before the development of lesions following the exposure of cattle to aerosols (Burrows 1968a, et al 1971, Sellers et al 1968, 1969), intramammary instillation (Burrows et al 1971) or intravenous inoculation (Mann and Garland, unpublished data). Examination of upper respiratory and alimentary tract tissues from cattle slaughtered during the prodromal period confirmed the involvement of the pharynx (Burrows 1972, Garland unpublished data). The suggestion that nasal mucosa might be a site of primary infection (Korn 1957) has received less attention but in one report samples from this area were much less frequently infected than pharyngeal tissues in the early stages of the disease (Burrows 1972). Sutmoller and McVicar (1973) showed that primary infection of the bovine conjunctiva could occur and that virus spread to pharyngeal fluid within minutes or hours of conjunctival instillation. In these studies samples were taken from only some of the natural secretions in each case whereas in the current work comprehensive samples were obtained.

Appreciable amounts of virus were recovered in samples from the eyes, nostrils and mouth as well as from the pharynx during the prodromal period showing that early foci might also develop in these situations.

One novel finding was the frequent presence of virus in preputial swabs at the first sampling 24 hours post exposure. The rising titre of successive samples and the subsequent development of lesions indicates a true infection of the prepuce (e.g. Steer HX21, Tables 17 and 22) which must therefore be considered as another focus of primary infection in the steer. Since steers spend much of their time in a recumbent position it is possible that low grade trauma caused by the pressure of the ventral abdomen on the floor of the box might predispose to infection of the prepuce in the same way that mild mechanical damage is known to exacerbate tongue lesions in cattle or pad lesions in the guinea pig (see literature review Section 5.1.2.).

Many aspects of the excretion of FMDV have been investigated. Most workers, however, have been concerned with a limited number of secretions, many have used artificial methods of infection and employed assay systems of low sensitivity for FMDV. The present study provides a detailed account of viral excretion for 4 strains of FMDV in all the secretions of steers following simulated natural infection as measured in CTY tissue culture, the most sensitive assay system currently available.(Snowdon 1966, Donaldson et al 1970). In general the results accord well with previous reports. Useful comparisons may be made between the present findings and those of other workers who have approached the topic from different standpoints. Two examples follow.

Scott et al (1966) followed the excretion of virus in Hereford steers infected with FMDV by intramuscular or intradermolingual inoculation and were able to recover virus in CK monolayers from nasal secretion for up to 6 days and from saliva for up to 9 days after the detection of lesions. Their work differs from the present study not only in methods of infection and assay but also in emphasis since it was concerned more with the limits of viral persistence in lesion material (vesicle epithelium from

the muzzle, mouth and feet) than with the overall pattern of excretion. The method of infection has an obvious influence on the rate of development of disease, the distribution of lesions and the shedding of virus during the incubation period but comparison of samples common to both reports indicates that it has little bearing on the excretion of virus after the appearance of lesions.

Viral excretion has also been studied in cattle following indirect contact infection at this Institute (Burrows 1968[a], 1972, et al 1971, Sellers et al 1968[b], 1969). Animals were housed in the same compound as infected donors but at some distance from them and in different boxes. Infection therefore differed from that in the current experiments in that cattle were usually exposed to lower doses of virus presented principally by the airborne route. The emphasis of this work was on the detection of early sites of viral multiplication and on excretion during the prodromal period. Sampling was normally discontinued at the onset of clinical lesions. Disease was characterised by a longer incubation period (5 - 10 days) than that normally found after infection by direct contact (2 - 4 days in this study) and by the recovery of virus from various sites for several days before the appearance of disease. In particular pharyngeal fluid contained virus for as long as 9 days (range 0 - 9 days) before the development of lesions.

In this thesis the topic of viral excretion was approached from another related standpoint with two main aims in view. Firstly, to provide a comprehensive survey of excretion in the external secretions of steers throughout the course of the disease and secondly, to furnish a yardstick with which to measure the efficiency of antiviral substances in the secretions. The methods used and the results obtained in pursuing these ends occupy a somewhat intermediate position between the two types of approach discussed above and may be used to relate these and similar

observations.

Virus was found in all the natural secretions and excretions in increasing amounts during the prodromal period and excretion reached a peak immediately before, during and after the formation and rupture of vesicles. Viraemia occurred at about the same time and lasted for 3 - 5 days. Urine and faeces contained the least amounts of virus and recovery from these excretions was intermittent. Virus persisted in the pharyngeal fluid of all animals for more than 10 days and the longest periods for which virus was recovered from other secretions were: tears 7 days, nasal secretion 9 days, oral saliva at least 10 days (9 days in most animals), faeces and urine 7 days, preputial swabs 8 days, and serum 7 days.

The airborne excretion of FMDV was not measured but there can be no doubt that infected secretions provide a source of virus for aerosols. Expired air could be contaminated during passage through the mouth, nose and pharynx, particularly when lesions are present in these areas. Aerosols might be created during mastication, sneezing, snorting, bellowing, coughing, lowing and the smacking movements of the tongue often seen during the clinical phase of FMD. In the case of pigs, at least, the virus appeared to originate from expired air rather than from other sources of viral excretion (Sellers et al 1970, 1973). However, other sources of virus are available such as milk, urine and faeces which may be voided with splashing and aerosol formation. Data is available on the airborne dissemination of all the viruses used in this thesis except strain A119. The maximum amount of virus in secretion was recovered from cattle at the time when lesions first appeared which also corresponds with the peak of aerosol excretion (Sellers and Parker 1969, Donaldson et al 1970).

The total output of virus in any secretion or excretion may be related to its volume and values may be calculated to show the maximum theoretical content in each case (Sellers 1971 and Table 21). These figures emphasise the potential hazards but provide only a rough guide to actual excretion since they make no allowance for other effects of the disease such as increased salivation, reduced faecal output or low urinary pH.

The persistence of virus in pharyngeal fluid for extended periods of time after exposure provided further confirmation of the carrier state in FMD. The possibility of virus survival in other areas of the convalescent animal has received less attention but assay of 42 representative tissues from all areas of the respiratory and alimentary tract of 6 steers at post mortem revealed the presence of virus only in samples from the pharyngeal region (Table 24).

There have been no reports of IF production in domestic animals after natural infection with FMDV. IF has been detected in cattle after the inoculation of the viruses of Newcastle disease (NDV), vaccinia and parainfluenza type 3 (PI-3) (Rosenquist and Loan 1969a, Ahl and Straub 1972) and infectious pustular vulvovaginitis (IPV) (Ahl and Straub 1972). The viruses differ greatly in their capacity to stimulate IF with NDV (a non replicating agent in cattle) being a very potent inducer and PI-3 (a natural bovine pathogen) being a poor inducer (Trueblood and Manjara 1972). The peak IF response in cattle may occur 12 hours after inoculation (NDV), at 2 days (IPV) or 5 days (IBR) after infection and IF may be detected for 2 days (NDV), 7 - 14 days (IBR) or 9 days (IPV) after inoculation. The amount may vary widely between locations in the animal, thus nasal and vaginal secretion contained 6 or 10 times more IF than did serum after infection with IBR or IPV viruses respectively and IF was detected for much longer periods in the external secretions

(Ahl and Straub 1972).

IF was detected in the current study following clinical infection but not after vaccination or vaccination and subsequent exposure of immune animals. The appearance of IF was strictly confined to the period of maximum viral multiplication which in turn coincided with the full expression of clinical disease (Tables 26 - 29). IF was commonly detected for only 2 or 3 days (maximum 4 days). Nasal secretion contained most IF followed by serum and tears, considerably less was found in oral saliva and pharyngeal fluid and none in urine and faecal suspension. The low levels seen in salivary fluids may merely reflect the dilution of IF in these secretions and the same factor may be involved in the apparent absence of IF in pharyngeal fluid during the carrier state. On the other hand, chronic residence of virus in the pharynx might be associated with the hyporeactive state which may develop in cells under repeated viral stimulation (Ho and Kono 1965, DeClerq and Merigan 1970) resulting in the production of less IF. This possibility is supported to some extent by the work of Phillipson and Dinter (1963) who were able to demonstrate IF in persistently infected CK cultures during the first 10 days but not during the 20th to 40th day of chronic infection with FMDV.

Since the initial report by Hyslop (1965a) several workers have mentioned the presence of specific neutralising antibody or antiviral substances in the secretions of cattle convalescent to FMD (see Section 5.2.). These reports have been of a preliminary nature and apart from the weekly sampling of nasal fluid and saliva in calves for one month following infection (Figueroa et al 1973) and the association of the neutralising capacity of concentrated, 6 week convalescent, bovine saliva with an immunoglobulin analogous to human secretory IgA (Kaaden and Matthaeus 1970) few details are available of the way in which antibody

develops and persists in secretions or the class of Ig involved.

Daily sampling of all secretions for 10 days after exposure revealed that antibody was detectable within 3 - 5 days of exposure in all infected animals (Section 7.2.1., Tables 30 - 33 and Fig. 8). Peak levels were reached within 21 - 28 days (Tables 34 - 37) and antibody persisted for at least $5\frac{1}{2}$ years after infection (Tables 44 &45). Serum antibody levels were above those found in the external secretions in all but one animal (Section 7.5.2.5., Table 51) and were usually demonstrable at a dilution of 1:10. Nasal secretion and tears contained the most secretory antibody, normally demonstrable in unconcentrated fluid at the height of the response, while oral saliva and pharyngeal fluid contained least and neutralising antibody was often detectable only after concentration of the fluid by as much as a hundred fold. Blood sampling therefore continues as the method of choice for diagnostic and epidemiological purposes.

Detailed observations on the development of lesions, the excretion of virus, the induction of IF and the production of antibody in acute FMD provided a clear picture of the temporal and quantitative relationship between these factors (Fig. 11). The appearance of IF showed a good correlation with the time of development of lesions whereas the appearance of antibody was better correlated with the time elapsing after exposure to virus. In general virus declined in secretions after the appearance of IF and the development of antibody was attended by a marked decrease in the amount of recoverable virus. These observations are in accordance with the hypothesis that IF, which is induced early in the course of the disease, may serve to limit the multiplication of virus in primary foci and to localise the spread of lesions while antibody, which appears slightly later, acts to neutralise virus, promote resolution, rid the host of infection (except in the pharynx) and provide lasting

immunity against infection.

The observations show that such interrelationships could exist and some supporting evidence for the role of antibody is provided by the results of dissociation procedures (Table 42). However, it must be emphasised that the correspondence of factors in time means that a significant interaction is possible but does not in itself prove any teleological effect. The difficulty in separating the defensive roles of secretory and humoral factors has already been discussed (Section 5.2.) and the possible involvement of cell mediated immunity and other protective mechanisms as yet unrecognised should be borne in mind.

FMD has been controlled in many countries by means of prophylactic vaccination. With few exceptions animals are immunised by means of inactivated antigen with adjuvant given by the subcutaneous route. Having studied secretory antibody following infection, the investigation turned to the question of what part, if any, was played by such antibody in the immunity of animals which have been vaccinated according to current practice.

Neutralising antibody was detected in serum but, apart from a transient, low level response in lachrymal and nasal secretion, very little antibody was detected in secretions following a single dose of vaccine. Levels rose in serum and secretions after a second and third application of vaccine (Section 5.3., Fig. 13) but secretory antibody was always found in lower amounts than humoral antibody.

Parenteral application of inactivated viral vaccines has generally given rise to low levels of antibody in secretions, despite the successful stimulation of serum antibody. The current results are in general accordance with those seen in man following subcutaneous vaccination with

dead influenza viruses (Waldman et al 1968, 1969, 1972b, 1973, Mann et al 1968, Kasel et al 1969, Alford 1969 and Downie 1970, 1973) and parainfluenza virus type 1 (Smith et al 1966, Chanock et al 1967) or intramuscular vaccination with rhinovirus (Perkins et al 1969). Experiments in cattle using parainfluenza type 3 virus gave similar results (Morein 1970, 1972) and Figueroa et al (1973) have recently reported on the failure to detect secretory antibody in 6 calves sampled weekly for one month after a single subcutaneous dose of inactivated FMDV vaccine. These findings are at odds with those of Hyslop (1965a) who reported the presence of antibody in unconcentrated saliva samples taken from 5/6 steers 4 weeks after primary subcutaneous vaccination.

In the current studies repeated application of inactivated vaccine by the subcutaneous route gave rise to increasing levels of secretory antibody. The variable appearance and low level of secretory antibody evoked by subcutaneous vaccination with inactivated influenza viruses has been noted by several workers as has the tendency for repeated doses to produce greater amounts of antibody in secretions (Waldman 1968, 1969 and Downie 1973). Duncan and Thomson (1970) recorded that the first of several sequential doses of killed Pasturella haemolytica vaccine gave rise to serum antibody in calves whereas nasal antibody was detected only after the fourth dose. Morein (1972) using an inactivated PI-3 vaccine in cattle found humoral but not nasal antibody after a single subcutaneous injection but secretory antibody was detected after a second dose of vaccine given 2 - 3 weeks later.

The discovery that subcutaneous vaccination in man often failed to confer clinical immunity led to the pursuit of other methods of vaccination including the use of attenuated strains and the aerosol route, both of which produced more consistent results in terms of secretory antibody levels, inhibition of viral excretion and protection against clinical

disease (see Literature Review Section 5. 2.). Mowat (personal communication) was not able to immunise cattle by exposing them to an aerosol of inactivated FMDV vaccine. Nevertheless the fact that the immunity conferred by vaccination does not extend to the pharyngeal region provides an incentive for further investigation of alternative methods of vaccination.

Neutralising antibody in the serum and secretions of steers following infection and active or passive immunisation were characterised by means of column chromatography (Fig. 1 and 2), immunodiffusion (Plate 10), immunoelectrophoresis (Plates 5 - 9), reductive cleavage (Table 40) and specific absorption of immunoglobulins (Tables 41 and 47). Distinct differences were observed between the Ig classes in secretions from convalescent animals (principally IgA with some IgG_1), passively immunised animals (IgG_1) and actively immunised animals (IgG_1 after the first and second doses of vaccine with an IgA component appearing after the third dose). Comparisons between serum and secretions in respect of the time of development of antibody, the levels attained and the Ig classes involved suggest that at least two mechanisms may be involved in the production of local antibody. These are discussed under A and B below.

(A). Since antibody levels in serum were always greater than those in secretions the outward diffusion of Ig from serum to secretions was feasible. Evidence for the spillover mechanism was provided by the detection of antibody in secretions after passive immunisation (evidence which must be viewed with reservations - see Section 7.3.) and after active immunisation, together with the fact that antibody was not found in secretions until the level in serum exceeded a neutralisation index of around 2.5. The observation that serum contained neutralising antibody of the IgM, IgG_1 and IgG_2 classes whereas the activity of secretions was associated principally with IgG_1 suggested the existence of selective

rather than simple diffusion.

(B). Neutralising activity associated with IgA was detected in the secretions of animals following infection or after a third subcutaneous application of inactivated vaccine. According to the results of absorption procedures, serum contained less neutralising IgA than did secretions so that the molecule is unlikely to have been of humoral origin. IgA may well have been produced locally and such a source is known to exist in the bovine (Butler et al 1971). The production of antibody in this system may depend on the penetration of antigen in sufficient mass to prime the immunocompetent cells of the paramucosal tissues, an event which is readily envisaged in the diseased animal. Possible explanation of the observations in vaccinated animals might be that either: a) after repeated vaccination sufficient antigen eventually reaches the paramucosal tissues perhaps in macrophages, and stimulates the production and release of detectable IgA or b) lymphocytes primed for the production of IgA elsewhere in the body finally settle out in peripheral situations and there secrete IgA.

The current studies provide indications of the origin of Ig classes in secretions after FMDV infection or vaccination. It would be of interest to determine the amount of each Ig class in different situations by a technique such as radial immunodiffusion (Mancini et al 1965).

In the final section of the work an attempt was made to assess the importance of local antibody in the immunity engendered by vaccination or infection. Without the availability of naturally occurring dysgammaglobulinaemic animals such as assessment is problematical because of the extreme difficulty in disentangling the role of secretory antibody from that of serum antibody and other host defences.

The experimental approach was to create groups of steers with widely different levels of secretory antibody at the time of challenge and to examine the relationships between antibody level, clinical protection, acquisition and excretion of virus (in sites other than the pharynx) and the carrier state after exposure to FMDV. The schedule of vaccination (Table 48) did evoke a wide range of neutralising antibody levels at the time of challenge (Table 49) but antibody was not detected in the serum of the group vaccinated once, 7 days before exposure. The reason for this unexpected failure is unknown but it may be that the vaccine lost petency before being used in these animals. Previous experiments (Graves et al 1968, Garland, Burrows and Greig unpublished data) had shown that cattle could be protected within 3 - 4 days of vaccination with inactivated antigen applied parenterally so that the capacity of one or more doses of baccine to engender clinical protection when applied at least 14 days before challenge in these studies was as expected.

There was no gradation in the recovery of virus from samples other than pharyngeal fluid since the frequency (Table 50) and amount of virus either resembled that seen in fully susceptible cattle (groups vaccinated on Day 0 and Day 7 before exposure) or was almost totally eliminated by immunisation (all other groups). If a threshold of immunity exists for each site, other than the pharynx, it had already been exceeded by the time that animals had been vaccinated for 14 days. The observations confirm and extend the observations of Sellers et al (1969a) who compared viral excretions in steers 19 days vaccinated at the time of exposure and bulls unvaccinated at exposure when a similar reduction was demonstrated in the recovery of virus from immunised cattle. In future experiments it might be possible to create animals of intermediate immunity by vaccinating closer to the time of exposure.

When thrice vaccinated animals were exposed to type C virus a strik-

ing decrease followed in the recovery of infectivity from all samples including pharyngeal fluid and virus was detected in this secretion in only 1 of 3 animals. Virus was, however, recovered from the tissues of all three steers at post mortem (Section 7.5.1.4.). When 6 convalescent steers were reexposed to homologous type A119 virus $5\frac{1}{2}$ years after the initial infection no virus was recovered during ante mortem or post mortem sampling (Section 7.5.2.). It is of interest that IgA antibody was present in the secretions of both groups of steers which exhibited a modification of the carrier state.

This thesis has been concerned with the study of natural and induced factors inhibitory to foot and mouth disease virus in bovine secretions. The results indicate that in the normal course of events natural secretory factors are of minor significance in the early phase of the disease and that secretory abnormalities are uncommon. Induced factors do appear to have a bearing on the course of infection since interferon and neutralising antibody were invariably detected in secretions as infected animals began to recover and virus excretion diminished. Secretory antibody may be particularly important in highly immune animals. Thus the marked reduction in viral excretion from animals exposed to virus after repeated vaccination and the total absence of excretion from even long term convalescent cattle after reexposure to virus showed a close correlation with the presence of neutralising antibody of the IgA class in secretions.

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10. BIBLIOGRAPHY

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