VIRUS PARTICLES, ANTIGENS AND ANTIBODIES IN SHEEP INFECTED WITH ORF, WITH SPECIAL REFERENCE TO DIAGNOSIS

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VIRUS PARTICLES, ANTIGENS AND ANTIBODIES IN SHEEP INFECTED WITH ORF, WITH SPECIAL REFERENCE TO DIAGNOSIS

by

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SUMMARY

The roles of various laboratory aids for confirming a diagnosis of orf were assessed. The most sensitive technique was the detection of heat-stable complement-fixing antigen. The next best was visualisation of negatively stained virus particles by electron microscopy and the third useful technique was the detection of heat-labile precipitating antigens by immuno-diffusion. The virus particles and the antigens were different entities with differing molecular sizes.

Cell culture techniques yielded promising results but the nature and specificity of the cell destruction are unknown. Fluorescent antibody techniques and haemagglutination tests were unreliable.

The optimal samples were scabs collected from lesions that were one to two weeks old.

Precipitating antibodies which were gamma globulins appeared in the sera of all convalescent lambs and most of the vaccinated lambs within four weeks of exposure but they were transient disappearing in eight to sixteen weeks. Similarly complement-fixing antibodies which were Ig^M globulins appeared in the sera of all convalescent and all vaccinated lambs within four weeks of exposure. They persisted longer than the precipitating antibodies.

The sera and colostrums of some ewes at parturition contained both precipitating and complement-fixing antibodies which were transferred passively to the lambs.

INTRODUCTION

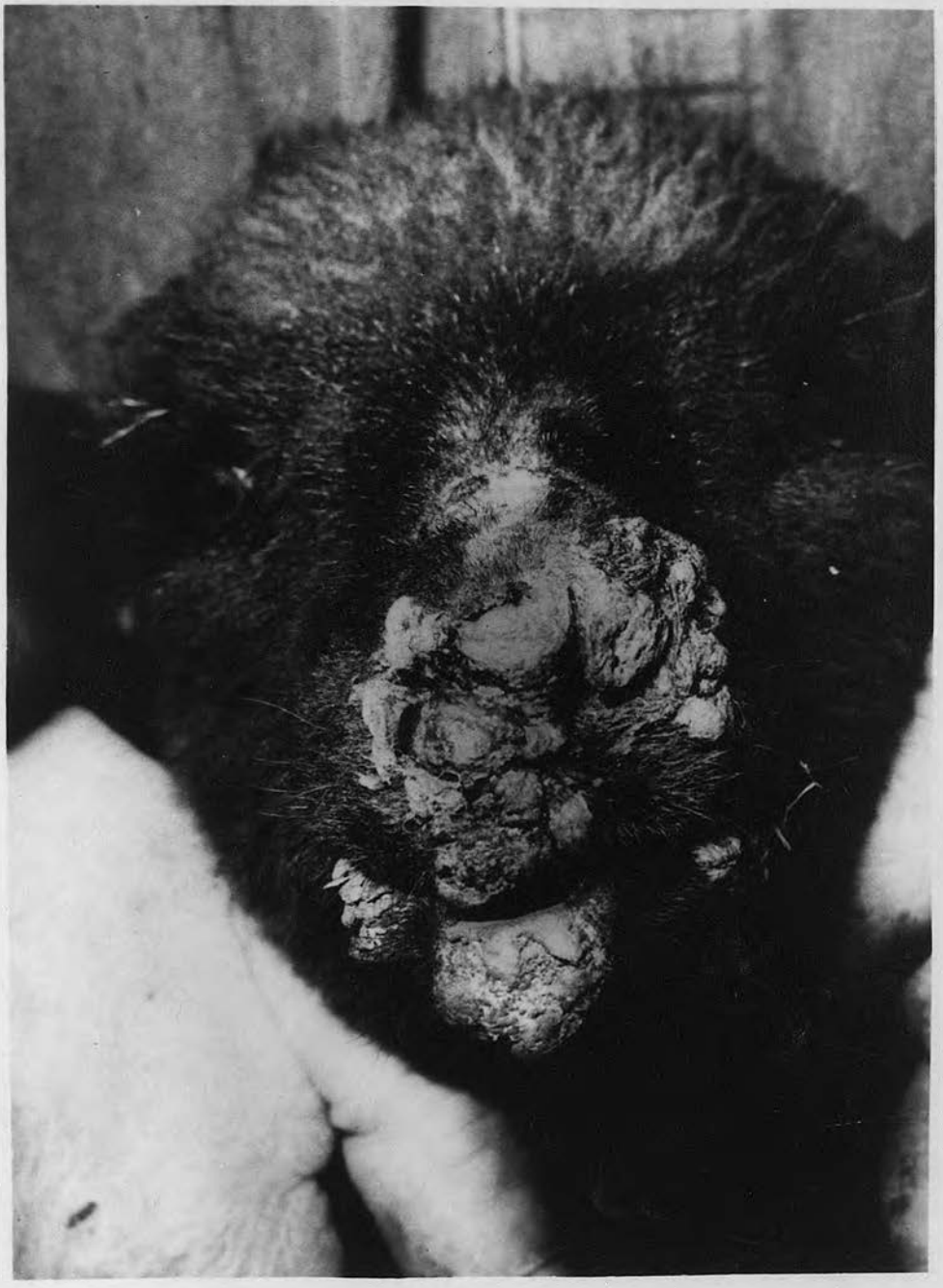
Orf is a benign pox-like disease of sheep and goats that occurs wherever sheep are raised. It affects primarily the lips of young animals producing characteristic proliferative lesions (Fig. 1). Occasionally lesions develop on the feet, particularly around the coronets and in the interdigital clefts. Ewes suckling infected lambs often develop lesions on the teats. Systemic invasion is rare.

Diseases of a similar nature have long been known. Youatt (1837) drew attention to an epidemic described by Paulet that occurred among sheep in France in 1745. Its contagious nature was recognised as early as 1787 by Steeb. To-day the disease is recognised in the national flocks and herds of most of the major sheep and goat raising countries.

Diagnosis in the United Kingdom is based usually on the clinical findings and is relatively easy because the lesions are so characteristic and

are /

Fig. 1 Typical orf.



are unlike the lesions of any other sheep disease known in the United Kingdom except, perhaps, strawberry foot-rot. Overseas, however, there are problems in differential diagnosis. Bluetongue, "peste des petits ruminants" and labial streptothricosis all simulate orf at some stage of their clinical evolution. Our objective therefore, was to evaluate the potential role of laboratory aids in confirming a diagnosis of orf in the hope that proven simple techniques could be defined that would permit rapid differentiation of orf from other diseases.

REVIEW OF THE LITERATURE

THE DISEASE

SYNONYMS

Orf has many synonyms. Purists would restrict the term "orf" to the disease form characterised by lesions on the feet and geographically restricted to the border counties of Scotland and England. Nisbet (1954), for example, emphasised at length that orf was primarily a condition affecting the leg and contagious pustular dermatitis, the commonest syndrome with a world-wide distribution, primarily affected the lips and nostrils. Colloquially, however, the term "orf" embraces both syndromes. Virologists prefer the term "orf" because of its antiquity. It is derived from the Old English word "hreof" meaning scabby (Nagington, Tee and Smith, 1965).

The common technical synonyms now current are "contagious pustular dermatitis" and "contagious ecthyma". The former is favoured by British workers and the latter by American and Continental workers. The international tag used in the FAO-WHO-OIE Animal Health Yearbooks is "Ecthyma contagiosum". Other technical labels are "malignant aphtha" (Walley, 1888), "pustular fever" (Walley, 1888), "carbuncle of the coronary band" (Walley, 1890), "contagious dermatitis" (Hoare /

(Hoare, 1913), "lip and leg ulceration" (Hoare, 1913), "Crusta labialis" (Hoare, 1913), "ulcerative stomatitis" (Hoare, 1913), "contagious pustular stomatitis" (Aynaud, 1923), "contagious aphtha" (Glover, 1928) and "infectious labial dermatitis" (Seddon and Belschner, 1929).

The term "ulcerative dermatosis" (Tunnicliff, 1949) is commonly applied to the syndrome prevalent in the United States of America characterised by granulating ulcers of the skin of the lips, feet and genital organs (Trueblood, 1966).

Other colloquial terms used in the United Kingdom include "black muzzle" (Youatt, 1837), "hair and hoof" (Hoare, 1913), "mouth and foot" (Hoare, 1913), "outburst" (Glover, 1933) and "crack" (James, 1968). In Australia "scabby mouth" is used (Seddon and Belschner, 1929) and in the United States of America "sore mouth" (Boughton and Hardy, 1934).

EPIDEMIOLOGY

Locus: Orf has a world-wide distribution (Selbie, 1944). The current prevalence in the 25 major sheep and goat raising countries is shown in Table 1.

Natural host range: The natural hosts of orf virus are /

TABLE 1

PREVALENCE OF ORF IN COUNTRIES WITH MORE THAN 10^7 SHEEP AND GOATS*

Low	Moderate	High	Unrecorded
Bulgaria	Afghanistan	Australia	Brazil
Morocco	Argentine	India	China
Nigeria	New Zealand	Iran	Ethiopia
Rumania	Pakistan	South Africa	Mexico
U.S.A.	Peru	United Kingdom	Mongolia
Yugoslavia	Spain	Uruguay	
	Turkey		
	U.S.S.R.		

* Abstracted from the FAO-WHO-OIE Animal Health Yearbook 1968 (Anon., 1969)

are sheep and goats. In addition, man is susceptible and acquires the disease through contact with affected animals (Oppermann and Stumpke, 1937; Gray, 1949; Hart, Hayston and Keast, 1949). The disease has also been detected in wild thar (Hemitragus emlaicus) (Kater and Hansen, 1962), chamois (Rupicapra rupicapra) (Grausgruber, 1964) and, more recently, in alpacas (Lama pacos) (Moro, 1965).

Pathogenesis: Within two to three days of infection traumatised skin is erythemic and swollen (Aynaud,1923; Glover, 1928). The swelling arises from vacuolation of the cytoplasm of the superficial nucleated cells of the epidermis. The dermis is mildly inflamed. The vacuolar degeneration of the epidermis spreads to the deeper layers and there is increased mitotic activity in the Malpighian layer which more than doubles its thickness. The rete pegs are elongated. Three to four days after infection a few granular intracytoplasmic inclusions appear in the swollen cells but they are transient and are seldom observed after the sixth day (Jubb and Kennedy, 1963). The dermis is heavily infiltrated with neutrophils. The older cells on the surface necrose and small multilocular pustules form from the liquefaction of infiltrating neutrophils. Eight /

Eight to thirteen days after infection the pustules rupture and the exudate coagulates into a scab. Beneath the scab there is intensive proliferative activity which results in the formation of verrucose masses. The scab is at first moist but in eleven to twenty days it dries and finally drops off in about four weeks.

Transmission: Orf is very contagious (Berry, 1901). Once the disease gains entry to a flock it spreads easily (Aynaud), 1923) and records exist claiming that fifty per cent of a flock were infected in two days and one hundred per cent in five days (Gardiner, Craig and Nairn, 1967). The virus is hardy and may lie dormant on grazing areas for very long periods (Boughton and Hardy, 1935).

Prevalence: Sheep and goats of any age are susceptible but the disease is more frequently and perhaps more readily transmitted and is more severe in animals under one year of age (Berry, 1901). Newly weaned lambs are particularly prone to attack (Glover, 1933). Outbreaks in sucking lambs are not uncommon and the disease spreads to the teats of the nursing ewes (Glover, 1930). The disease therefore appears to have a seasonal prevalence but, in fact, the /

the prevalence is related to sheep husbandry practices rather than the seasons.

CLINICAL SIGNS

Incubation period: The natural incubation period in sheep is not known but it is tacitly assumed to be a few days. In man it is five days (Andrewes, 1967). The incubation period of the experimental lesion in sheep and goats is one to three days (Aynaud, 1923; Glover, 1928; Boughton and Hardy, 1934).

Pathography: The virus has a propensity to produce several superficially dissimilar syndromes, the commonest of which is facial orf.

Facial orf: The first sign usually noticed by shepherds is the appearance of scabs adherent to the lips and nostrils. They are derived from lesions that progress through the classical pox stages of papules, macules, vesicles and pustules. The pustules rupture about the eighth or ninth day and the exudate forms the scab, moist and yellow at first but later dry and dark. The scabs fall off three to five weeks after they are formed leaving a white scar of new epithelium. Individual scabs range in size from /

from 0.5 to 1.0 cm; Glover (1928) likened them to a filbert nut. Frequently they coalesce. The scabs are firmly adherent and their removal exposes a verrucose mass which bleeds rapidly. Occasionally, the disease spreads to other parts of the body such as the coronet, under surface of the tail, the thighs and axillae (Glover, 1928). We have ourselves observed extension to tail stumps, castration wounds, ear wounds, circumorbita and the interdigital clefts.

Oral orf: The problem in the flocks of the Veterinary Field Station, University of Edinburgh, was the regular appearance of the disease in sucking lambs and frequently the first lesions were associated with tooth eruption and affected the oral mucosa producing whitish verrucose plaques. Labial lesions followed. The disease was benign and the course was typical. Darbyshire (1961), however, observed a syndrome in older lambs which was characterised by multiple ulcerative or pustular eruptions in the buccal cavity, cheeks, tongue and lips together with extension to the gastro-intestinal tract. The mortality rate was high and ranged in different outbreaks from seven to seventy-eight per cent.

Teat /

Teat orf: A sequel to orf in sucking lambs is the development of orf lesions on the teats of the lactating ewes. The course is typical but mastitis frequently complicates the outcome.

Ulcerative dermatosis: The term "ulcerative dermatosis" was proposed by Tunnicliff in 1949 to cover the diseases previously described as lip and leg ulceration, posthitis, balanoposthitis and ulcerative vulvitis. The syndrome is characterised by epidermal and subcutaneous tissue destruction resulting in raw, granulating ulcers of the skin of the lips, legs, feet and genital organs. The ulcers vary in depth and are covered with a scab. Removal of the scab reveals pus on a raw bleeding floor. The course ranges from one to two months. Mortality is low.

Border orf: Nisbet (1954) used the term "border orf" to describe the syndrome that is restricted geographically to the border counties of Scotland and England. It is a dermatitis primarily affecting the legs. The lesion is usually solitary and most commonly occurs in the regionof the coronet or fetlock but may be found anywhere up to the knee or hock. Rarely there is extension to other parts such /

such as the skin around the eyes, base of the ear, the lips and nostrils. A scab varying in size from 2 to 4 cm covers a shallow ulcer filled with thin greyish pus, the result of secondary bacterial infection which invariably occurs. The raw surface bleeds freely. The scabs drop off in five to eight weeks.

Human orf: Human infections are usually restricted to the hands and arms. There is seldom any constitutional disturbance except after biopsy (James, 1968). The clinical lesions vary from multiple vesicles to the classical indurated cherry-red nodule. The course is about six weeks (Hart et al., 1949). Differential diagnosis from milkers' nodule infection is based on knowledge of the animal source, (Nagington et al., 1965).

Complications: Orf rarely kills. It can cause considerable loss of condition when the lesions interfere with the prehension of food or sucking (Aynaud, 1923; Manley, 1934; Selbie, 1944; Hart et al., 1949). Occasionally superimposed bacterial infections (Aynaud, 1923; Hart et al., 1949), or blow fly strikes (Boughton and Hardy, 1934; Hart et al. 1949; Yepez and Ronderos Posse, 1962) decimate affected lambs. Mastitis often follows infection of the /

the teats of lactating ewes with loss of the affected gland and sometimes death if the secondary mastitis becomes gangrenous. Glover (1930) reported that the virus itself induced mastitis when injected into the udder through the teat canal.

IMMUNITY

The literature on orf is full of conflicting statements on the nature, degree and duration of the immunity engendered in sheep by the virus of orf. In addition, the existence of varying antigenic strains of the virus has both been mooted and questioned.

Naturally - acquired active immunity: Jacotot (1926) found that sheep recovered from naturally - acquired disease resisted experimental re-infection for at least two and a half years. Yepez and Ronderos Posse (1962) in Colombia and Hardy (1964) in the U.S.A. believed that the resistance following recovery from the natural disease was lifelong provided the same strain of virus was involved.

Schmidt (1962) challenged sheep that had recovered from the natural disease by applying a suspension of orf-infected scabs to scarified upper lips and found that /

that the sheep were immune from thirteen days to five months after the onset of the natural disease. However, when the challenge site was changed from the lips to the inner thighs or chest, eighty-eight per centof the sheep were judged susceptible two months after the onset of the natural disease.

Naturally - acquired passive immunity: The colostrum of immune ewes did not contain any perceptible protective substances. Lambs born of immune ewes were therefore not protected passively (Hardy, 1964; Richter and Jansen, 1968).

Artificially - acquired active immunity using inactivated virus: Aynaud (1921) was the first to claim success in protecting sheep against orf artificially. He dried infected scabs over sulphuric acid, inactivated the virus with chloroform and emulsified the treated scabs in glycerin. The emulsion was applied to the scarified skin of the inner thigh. The immunity so conferred lasted at least nine months. Altara (1925) affirmed that the immunity was not always of long duration and disappeared in from five to eight months.

Pegreffi (1930) used formalin - inactivated virus /

virus as a vaccine. Olah and Elek (1953) improved the efficiency of formalin - inactivated vaccine by adding two per cent aluminium hydroxide gel as an adjuvant. The vaccine was administered subcutaneously in doses of 3 to 5 ml. Immunity developed twenty-one days after vaccination and persisted for at least two months.

Richter and Jansen (1968) compared the efficacies of an unheated vaccine containing live virus and a vaccine which was held at 60°C for forty-five minutes. Both types of vaccine were applied to the scarified skin in the axilla region. The unheated vaccine induced a "good" immunity but suffered from the disadvantage that the reactions at the vaccination sites were severe in some animals. The heated vaccine gave very light local reactions and evoked a "reasonable" immunity. Newborn lambs, the progeny of immune ewes, also developed a "reasonable" immunity after vaccination in the skin of the auricle with the heated vaccine.

Artificially - acquired active immunity using live virus: Moussu (1923) succeeded in immunising sheep using infected scabs suspended in blood from infected animals /

animals and emulsified in glycerin. Glover (1928) followed Aynaud's technique but omitted the chloroform and this type of vaccine is still produced commercially in the United Kingdom. A similar vaccine has long been in use in the United States of America (Boughton and Hardy, 1934).

Ishii, Kawakami and Fukuhara (1953) inoculated a suspension of infected scabs into the lips of susceptible sheep and goats and found that the animals resisted re-infection for at least three months.

Glover (1928) injected relatively large doses of virus subcutaneously and intravenously. Clinical disease was not a sequel but the inoculations, nevertheless, appeared to confer a considerable degree of immunity.

Abdussalam (1957) infected rabbits experimentally and detected a partial resistance to re-infection in recovered rabbits. The partial resistance, however, disappeared in about fifty days.

Resistance developed within ten days of applying live virus to the scarified skin of the inner thigh and it persisted for at least six months, the longest period tested, (Olah and Elek, 1953). Nisbet (1954) considered that the immunity was solid for about three months /

months after which the degree of resistance varied. MacDonald and Bell (1961) concluded that immunity to orf was very poor. Johnston (1964) found that the immunity disappeared slowly until at twelve weeks after vaccination it was no longer detectable. Nevertheless Nisbet (1954) concluded that sufficient protection was probably conferred against the natural disease for up to six months. On the other hand, Hardy (1964) believed that the immunity after live vaccination was lifelong.

Trueblood, Chow and Griner (1963) reviewed the equivocal reports on immunity to orf and concluded that they could be explained by the existence of different antigenic strains of the virus, by differences in susceptibility of sheep, and by unawareness of previous exposure of sheep to orf. An alternative explanation of the discrepant claims is differing interpretations of challenge reactions. Some workers, like Olah and Elek (1953), Nisbet (1954), MacDonald and Bell (1961) and Johnston (1964) interpreted any reaction at the challenge site as lack of resistance. Others like Hart et al. (1949) recorded resistance in sheep for up to sixteen months after /

after vaccination because the lesions at the challenge site healed in ten to thirteen days. Hardy (1964) used the failure to develop the natural disease as his criterion.

Artificially - acquired passive immunity: Olah and Elek (1953) failed to influence the course of the natural disease by injecting affected sheep sub-cutaneously with 10 to 15 ml. of serum from convalescent sheep. Moreover, hyperimmune serum even in doses of 50 ml. failed to protect sheep infected simultaneously by scarification.

Type of immunity: From the first Aynaud (1921) recognised that the immunity in orf was local. Later he (1923) confirmed that the immunity was cellular and not humoral. Support for his hypothesis came from the observation that lambs received no protection from immune ewes and that there were no protective substances secreted in the milk (Boughton and Hardy, 1934). Johnston (1965) challenged sheep on various sites including the vaccination site and found that resistance to re-infection persisted longer at the vaccination site than at other sites.

AETIOLOGY

HISTORY

For many years the cause of orf was believed to be a bacterium, Fusiformis necrophorus, because of the regularity with which it was recovered from the lesions (Hoare, 1913). It was not until 1921 that a viral aetiology was mooted by Aynaud. He stated that the disease was caused by a specific virus which survived in dried scabs from which fresh animals were infected long after the resolution of the original outbreak. Aynaud's findings were confirmed by Lanfranchi (1925), Glover (1928) and Manley (1934). In 1954 Nisbet observed small cocci under the light microscope and pox-like particles in metal-shadowed preparations examined by electron microscopy. In 1961 Nagington and Whittle observed the criss-cross pattern in negative stained preparations that was to prove characteristic of orf virus.

STRUCTURE

The morphology, structure and characteristics of orf virus are now well defined (Nisbet, 1954; Abdussalam and Cosslett, 1957; Nagington and Whittle, 1961; Kujumgiev and Todorov, 1961; Nagington and Horne /

Horne, 1962; Reczko, 1962; Knocke, 1962; Nagington, Newton and Horne, 1964; and Schulze and Schmidt, 1964).

The morphology of orf virus is of special interest (Nagington, 1964). Virus particles observed in the electron microscope are smaller than other pox viruses; 260 x 160 nanometers (Nagington and Whittle, 1961), 208 x 143 nanometers (Kujumgiev and Todorov, 1961), 240 x 170 nanometers (Knocke,1962). Estimates of the diameter from ultrafiltration through collodion membranes range from 100 - 272 nanometers (Franco and Vrtiak, 1957) to 200 - 250 nanometers (Ishii et al., 1953).

Orf virus has a cylindrical shape instead of the brick shape typical of pox viruses (Nagington and Horne, 1962). The most striking morphological feature is a criss-cross pattern formed by tubular threads 8 - 10 nanometers wide. These were especially prominent when sodium phosphotungstate negative staining was used (Nagington and Horne, 1962). The thread appeared to be a component of the thick outer wall of the virus particle and probably has an important mechanical role in protecting the viral nucleic acid (Nagington, Newton and Horne, 1964).

Using /

Using cytoplasmic extracts of cells infected with orf virus, Nagington and Horne (1962) showed that there were two kinds of virus particles; the apparently complete form consisting of a nucleoid with surrounding envelope and the incomplete form consisting of envelope material without a nucleoid component.

PROPERTIES

Composition: The internal component of orf virus consists of desoxyribonucleic acid with a particle weight of 2.85×10^{-6} grams (Nagington, Newton and Horne, 1964). Evidence of the presence of lipids is equivocal; some strains are resistant to treatment with ether (Liess, 1962) others are sensitive (Glover, 1928; Plowright and Ferris, 1959; Trueblood and Chow, 1963). English and German strains are partially inactivated by chloroform (Glover, 1928; Liess, 1962).

Haemagglutinins: Some of the pox viruses possess specific haemagglutinins (Andrewes, 1967). There have, therefore, been several attempts to detect orf-specific haemagglutinins. Olah and Elek (1953) tried differing sources of virus and different types of erythrocytes. The results were not reliable. Erythrocytes from hens, rabbits and sheep /

sheep were never agglutinated. On occasion, tests with erythrocytes from frogs, guinea pigs and horses gave irregular and unpredictable reactions. The most promising system was one using hamster erythrocytes which were sometimes agglutinated with 1:500 dilutions of virus. Nevertheless the results were not reproducible and the reactions, when they occurred, were not reversible.

Kujumgiev (1954) sometimes demonstrated agglutination of guinea pig erythrocytes but only with high concentrations of virus. Erythrocytes from chickens, horses, pigs, rabbits and sheep were not agglutinated. Abdussalam (1958) used both extracts of orf-infected skin and suspensions of elementary bodies but neither agglutinated calf, fowl, goat, guinea pig, horse, mouse, pigeon, rabbit and sheep erythrocytes nor human Rh-positive erythrocytes sensitised with incomplete anti-D Rh serum nor bovine erythrocytes sensitised with Paul-Bunnel antibody.

Sawhney (1966b), on the other hand, passaged two strains of orf virus on the chorio-allantoic membranes of fowl embryos and claimed that the passaged virus agglutinated erythrocytes from guinea pigs, hens, mice and men but not from horses, rabbits and sheep.

Haemagglutination /

Haemagglutination titres of low passaged virus were modest but the titres of high passaged virus were significantly greater.

Olah and Elek (1953) succeeded in inhibiting haemagglutination only once using convalescent serum. Sawhney (1966b), however, readily confirmed the specificities of his haemagglutination reactions by inhibition tests with hyperimmune anti-orf sera prepared in fowls, rabbits and sheep. In addition, he claimed that anti-vaccinia serum inhibited the reaction. Sawhney's findings have yet to be confirmed by other workers.

Resistance: Aynaud (1923) discovered and Glover (1928) confirmed that orf virus possessed great powers of resistance to desiccation, dried scabs remaining fully virulent for at least twelve months. Later, virulent virus was recovered from dried scabs stored at room temperature for fifteen years (Hart et al., 1949) and at 45°F for twenty-two years (Livingston and Hardy, 1960). Orf virus also survived lyophilisation (Olah and Elek, 1953).

The virus was not inactivated by glycerine or liquid paraffin (Glover, 1928). Merthiolate at a concentration /

concentration of 0.01 per cent and formalin at a concentration of 0.4 per cent inactivated the virus but 0.5 per cent carbolic acid was harmless even after forty-five days contact at 4°C (Olah and Elek, 1953). Purification of viral suspension s by successive treatments with fluorocarbon resulted in little or no loss of infectivity (Trueblood and Chow, 1963).

ANTIGENICITY

Types: Blanc, Melanidi and Caminopetros (1922) demonstrated by cross-immunisation in lambs that vaccinia virus, sheep pox virus and what was described as an eruptive disease of goats of viral aetiology did not confer immunity against each other. Lambs, previously vaccinated with the goat virus reacted to challenge with either sheep pox or vaccinia virus. A sheep pox vaccinated lamb reacted when challenged with the goat virus and a vaccinia-vaccinated lamb also reacted when challenged with the goat virus. Jacotot (1926) reported similar findings; goats and sheep previously vaccinated with vaccinia virus reacted when challenged with orf virus and vice versa.

Similarly MacDonald (1951) and Abdussalam (1958) could /

could not demonstrate any cross-reaction between contagious pustular dermatitis and vaccinia in complement fixation tests. On the other hand, Webster and Miles (1957) and Webster (1958) claimed to have detected a serological relationship between contagious pustular dermatitis, ectromelia and vaccinia virus. In the complement fixation test, cross fixation with dermatitis antigen was only obtained with concentrated vaccinia and ectromelia sera but fixation occurred with the antigen diluted up to 1:256. The antigen titre in the homologous antiserum was 1:4096. Contagious pustular dermatitis, vaccinia and ectromelia also showed a precipitating antigen but no line of precipitation was obtained between dermatitis serum and fowl pox virus. Cross-neutralisation in sheep and rabbits with dermatitis virus and vaccinia and ectromelia antisera did not reveal any significant neutralising activity but cross-neutralisation on mice showed that dermatitis antisera significantly neutralised ectromelia virus. Vaccinia virus was not neutralised by dermatitis antisera when virus antiserum mixtures were inoculated on to the chorioallantoic membrane of the developing fowl embryo.

Sawhney /

Sawhney (1966b) adapted a strain of orf virus to grow on the chorioallantoic membranes of developing fowl embryos. Orf virus produced haemagglutinins that were specifically inhibited by anti-orf serum at dilutions as high as 1:256 and by vaccinia antiserum up to 1:16. The authors suggested therefore that both viruses were immunologically related.

Schmidt (1967e) demonstrated a relationship between orf and vaccinia virus, and between orf and sheep pox by the use of the partial complement-fixation test. By immuno-diffusion, one line of identity was given by orf and vaccinia virus. He suggested that the antigen shared by orf and vaccinia virus was probably a group specific antigen.

Hudson (1931) scarified and applied a 1:1,000 suspension of orf scabs to the skins of two sheep previously vaccinated with sheep pox virus and induced typical orf lesions. A weaned lamb inoculated with orf virus developed fever and pustules when injected with sheep pox virus. Sheep pox virus was recovered from the pustules. Hudson (1931) concluded that no cross-immunity existed between orf and the strain of sheep pox virus that existed in East Africa. On the other hand, Sharma and Bhatia (1958) showed by virus neutralisation tests carried out on orf and goat pox susceptible kids, that /

that goat pox sera neutralised orf virus whereas orf antisera failed to neutralise goat pox virus. They also challenged with orf virus four kids that had been vaccinated five months earlier with goat pox vaccine. A control group of four kids that had suffered from a natural attack of orf were challenged with virulent goat pox virus. The kids with previous orf experience and challenged with virulent goat pox virus developed local and thermal reactions while the kids previously vaccinated against goat pox and challenged with orf virus were unaffected. In other words, goat pox virus protected animals against goat pox and orf but orf virus did not protect against goat pox. Mundu and Mohan (1961) confirmed the ability of goat pox virus to confer resistance against orf virus but, nevertheless, they considered the two viruses to be distinct. The numbers of animals used in these cross-resistance trials are too small to allow definite conclusions.

More recently Sharma (1966) detected by cross-complement fixation a cross-reaction between sheep pox virus and ecthyma virus, and also between goat pox virus and ecthyma virus, and <u>vice versa</u>. In gel precipitation plates using sheep pox antigen and ecthyma hyperimmune sera, one line was observed. Goat pox antigen did not give /

give any line against the ecthyma sera. Finally no cross precipitation was observed between ecthyma antigen and goat pox or sheep pox sera in agar gels. In cross-protection tests no relationship was found between any of the pox viruses of goats and sheep and orf and vice versa.

Liebermann (1966) using contagious pustular dermatitis and bovine papular stomatitis sera labelled with fluorescein isothiocyanate, found specific immuno-fluorescence in the cytoplasm of calf kidney and calf testis cell monolayers infected with dermatitis virus, bovine papular stomatitis, milkers' nodules and pseudo-cow pox viruses. He concluded that all four viruses were antigenically closely related. No fluorescence was seen in the cytoplasm of cell monolayers infected with vaccinia virus or with the Neethling strain of lumpy skin disease virus. Papadopoulos, Dawson, Huck and Stuart (1968) confirmed that orf virus and pseudocowpox virus shared a common sub-group specific soluble precipitating antigen. No cross-reaction was observed with cowpox or vaccinia viruses.

Strains: Glover (1928) examined different sheep strains of orf virus and showed by cross-protection tests that they were immunologically identical. Similarly Olah and Elek (1953) used viruses from three different localities and /

and found that they induced resistance against each other. They concluded that there was no evidence of plurality of strains. On the other hand, MacDonald and Bell (1961) failed to get satisfactory cross-protection results in sheep given a cell cultured-virus and a standard commercial virus vaccine. Hardy (1964) likewise reported resistance to homologous strains but not, in one animal, to a heterologous strain. More recently Sawhney (1966c) claimed to have detected the existence of at least two antigenic forms of the virus by cross-protection and virus-serum neutralisation tests.

Nisbet (1954) examined the relationship between contagious pustular dermatitis virus and Border orf virus. By complement fixation tests he demonstrated that the viruses were serologically similar; both antisera fixed complement up to a dilution of 1:64 against contagious pustular dermatitis and Border orf.

Similarly Sharma (1966) demonstrated that contagious pustular dermatitis and contagious ecthyma were the same. He used cross-protection, immuno-diffusion and complement fixation tests.

Trueblood (1966) found by cross-neutralisation tests in cell cultures inoculated with contagious ecthyma /

ecthyma and ulcerative dermatosis viruses that they were readily neutralised by the heterologous antiserum.

Both viruses also shared a common precipitating antigen. Earlier Trueblood, Chow and Griner (1963) had concluded from a critical appraisal of the literature that the possibility that there was a multiplicity of strains had not been adequately investigated.

CULTIVATION

Experimental hosts: Early attempts to adapt orf virus to rabbits failed (Aymaud, 1921; Blanc et al., 1922; Lanfranchi, 1925) but later trials using massive doses were claimed to have been successful (Selbie, 1944; Kujumgiev, 1954; Abdussalam, 1957). Cattle were susceptible to experimental inoculation by scarification (Lanfranchi, 1925; Ishii et al., 1953; Papadopoulos et al., 1968). Cats (Lanfranchi, 1925), chickens Lanfranchi, 1925), dogs (Blanc et al., 1922; Lanfranchi, 1925; Ishii et al., 1953; Abdussalam,1957) guinea pigs (Aynaud, 1921; Blanc et al., 1922; Lanfranchi, 1925; Ishii et al., 1953; Abdussalam, 1957), horses (Blanc et al., 1922), pigs (Ishii et al., 1953), and mice (Abdussalam, 1957) were refractory. There is one report of experimental infection of a monkey (Macacus rhesus) with orf virus of goat origin (Blanc et al., 1922).

Fowl /

Fowl embryos: Many investigators have tried unsuccessfully to grow and adapt the virus of orf to the chorioallantoic membranes of developing fowl embryos (Hart et al., 1949; Ishii et al., 1953; Kujumgiev, 1954; Greig, 1956; MacDonald and Bell, 1961; Valadao, 1961). Abdussalam (1951), however, observed, forty-eight hours after inoculation, opaque pin-point lesions having a maximal diameter of 0.25 mm on chorio-allantoic membranes of embryos previously incubated for twelve days. The lesions regressed after ninety-six hours. Histological examination revealed that the lesions at first consisted of proliferating ectodermal and endodermal cells. Later, the lesions consisted of necrotic cells together with inflammation of the mesoderm. The virus was passaged serially on the chorio-allantoic membranes of fowl embryos but the lesions were produced only in the first four passages. Virus harvested from the fourth passage was infective for lambs but harvests of the sixth and ninth passages were non-infective.

Sawhney (1966a) also succeeded in adapting orf virus to fowl embryos. There was no apparent intensification of the reaction over fifteen passages but the pock-forming titre rose to 5×10^6 pock-forming units /

units per ml. Lambs scarified with virus from the tenth egg passage reacted but the reaction was much milder than that produced by the original virus. The difference was attributed to attenuation.

Cell cultures: Greig (1957) was the first to succeed in propagating orf virus in cell culture monolayers. He used primary monolayers grown from embryonic sheep skin. Cytopathic effects commenced three to four days after inoculation and by the seventh day destruction of the monolayers was usually complete. Three strains of the virus behaved similarly and all were shown to be viable by sheep inoculation after ten serial passages in cell cultures.

Plowright, Witcomb and Ferris (1959) grew the virus in monolayer cultures derived from lamb and calf testis and ovine bovine and caprine embryonic kidneys. Absorption of virus to testis cells was more complete than to kidney cells. Moreover, the virus was disseminated more rapidly and completely and the peak viral titres were higher in cultures of testis cells. Virus demonstrable in the cell culture fluid was consistently less than that in attached cells.

Kujumgiev and Todorov (1961) cultivated the virus from a suspension of dried infected scabs in monolayers derived /

derived from kidneys of newborn rabbits. After four consecutive passages the virus induced a well pronounced cytopathic effect which was manifested twenty-four hours after inoculation, and reached its maximum forty-eight hours later. The highest titre obtained was $5 \times 10^{-3.5}$ TCD_{50} per ml.

MacDonald and Bell (1961) extended the cell culture spectrum of orf virus by growing the virus in monolayers of human amnion cells, human embryonic kidney cells, human embryonic liver cells and sheep embryo kidney cells. Schimmelpfennig and Liess (1962) also grew human and ovine strains of the virus in primary cell cultures of calf testes. Trueblood and Chow (1963) adapted the virus to monolayer cultures of bovine embryonic kidney cells and found after ten passages that there was no reduction in pathogenicity for sheep. Ramyar (1963) cultivated and passaged orf virus in monkey kidney cells, and when fifth-passaged virus was injected around the lips of a goat and a lamb, the animals developed lesions at these points. Trueblood (1966) grew the virus in fowl embryo fibroblasts.

Schmidt (1967b) cultivated the Wellcome Institute strain of orf virus in calf kidney cell cultures. After /

After fifteen passages the virus was completely absorbed within sixteen hours of inoculation and the first cell changes were produced within eighteen hours. New virus first appeared after twenty-four hours reaching a maximum titre of $10^{4.85}$ ID_{50} per ml. at seventy-two hours. In contrast to the original material the virus was highly infective for sheep. The calf kidney cell cultured virus usually lacked a serologically active component which, however, was formed in the infected animal.

CLASSIFICATION

Walley (1888) and later Aynaud (1923) recognised that orf was closely allied to the poxes because of its mode of evolution. Abdussalam and Cosslett (1957) on morphological grounds assigned the virus of orf to the quadriform groups of Ruska and Kausche (1943) which contained the viruses of molluscum contagiosum, ectromelia, rabbit myxoma, canary pox, vaccinia, pig pox, fowl pox and varicella. In 1964 Huck classified orf virus along with the poxes of horse, sheep, goats, pigs and camels and bovine papular stomatitis virus in a sub-group of the pox viruses called the ungulate poxes. Peters, Muller and Buttner (1964) proposed the descriptive /

descriptive term "paravaccinia" for the sub-group which they limited to orf, bovine papular stomatitis and milkers' nodule viruses. Nagington et al. (1965) pleaded for the term "orf sub-group" instead of "paravaccinia sub-group" and they added the viruses of chamois contagious ecthyma and bovine X virus. Their plea was heeded and Melnick and McCombs (1966) classified orf virus in Subgroup II (Orf-like viruses) of the Pox virus group.

DIAGNOSIS

Most outbreaks of orf are diagnosed from an assessment of the history of the outbreak and the clinical signs. Confirmation of the field diagnosis is seldom attempted. Death is rare and consequently pathological and histo-pathological examinations play little part in confirming a diagnosis. The usefulness of examining specimens collected by biopsy is limited because the significant histo-pathological changes all occur within a few days of the onset of infection before scab formation has commenced (Aynaud, 1923; Glover, 1928; Nisbet, 1954; Wheeler and Cawley, 1956).

ISOLATION AND IDENTIFICATION OF THE VIRUS

Biological tests: The commonest method used to confirm a provisional diagnosis is the biological test. Suspensions of scabs from suspect cases are applied to the scarified skin of the groin or axilla of sheep or goats. If the reactions are restricted to the scarified sites and progress through the typical stages of macules, papules, vesicles, pustules and scabs, then the diagnosis of orf is considered confirmed! Even as late as 1951 it was stated that sheep scarification was the only certain means of diagnosis. (Lloyd, MacDonald and Glover, 1951).

Hart /

Hart et al. (1949) introduced an important modification by applying the suspect suspension to the scarified skins of known-susceptible and known-immune sheep. They, therefore, had some measure of the specificity of any reactions that developed. The reactions in susceptible animals were first obvious three days after scarification and were manifested by erythema and swelling and, on occasion, by pustules. In eight to thirteen days the pustules coalesced and ruptured. A thick scab covered the lesion which healed in twenty-seven to twenty-nine days. On the other hand, reactions in immune animals were very mild and the lesions healed in seven to fifteen days.

Ishii et al. (1953) used cattle and rabbits in addition to sheep and goats and claimed good results. Scarification of dogs, guinea pigs, horses and pigs failed.

Typical reactions were induced with suspensions of lesions collected after the macule stage (Jacotot, 1926). The highest yields of virus were obtained from scabs collected from lesions five to seven days old (Olah and Elek, 1953).

Isolation /

Isolation of virus in cell cultures: Isolation of orf virus in cell cultures is not yet a routine procedure largely because of the frequency with which non-specific cytopathic changes occur within twenty-four hours of inoculation (Plowright et al., 1959). MacDonald and Bell (1961) believed that these changes were specific and their early occurrence was due to a heavy inoculum of virus. They described the sequence of changes in lamb embryo kidney cells as being an early rounding and swelling of the cells followed by clumping. On the other hand, Plowright et al. (1959) considered the specific changes to be the formation of large granular cytoplasmic inclusions consisting of an eosinophilic paranuclear "matrix area" and a more basophilic outer zone. Kujumgiev and Todorov (1961) also observed the formation of cytoplasmic inclusions which displayed several stages of development and maturation beginning with eosinophilic granular accumulations and ending with a well-defined compact body with a diameter of two to eight micrometers and situated paranuclearly within a light halo. Schimmelpfennig and Liess (1962) likewise detected specific intracytoplasmic inclusions in infected cell cultures. Most infected cells were basophilic and their /

their cytoplasms contained ribonucleic proteins and glycogen which could be demonstrated histochemically. The intracytoplasmic inclusions in the basophilic cells were often Feulgen-positive. Some cells early in course of infection were acidophilic and contained intracytoplasmic inclusion bodies that were Feulgen-negative.

Another problem in isolating orf virus from scabs is the heavy load of bacteria in the scabs (Johnston, 1964). Once the virus is isolated the yield is increased if the pH of the maintenance medium is kept at pH7 or above (Johnston, 1964).

The ideal cell culture system for isolating orf virus has not been defined. Plowright et al. (1959) and Sawhney (1966d) favoured lamb testis cells, Kujumgiev and Todorov (1961) used rabbit kidney cells and Schimmelpfennig and Liess (1962) used calf testis cells. Pig kidney cells were not as good as lamb and calf testis cells for isolating most strains of the virus except the Iranian strain (Sawhney, 1966d). MacDonald and Bell (1961) believed that human ammion cells were best for isolating orf virus from human lesions.

Sawhney (1966d) isolated several strains of orf virus in lamb and calf testis cells. The cellular change induced by the strains were all similar but strains virulent for sheep were less cytopathogenic in cell cultures than avirulent strains.

Recently /

Recently Nagington (1968) described the factors affecting the growth of paravaccinia viruses in cell culture systems. The dose was important, the degree of cytopathic change being proportional to the number of virus particles present. Similarly the average time taken to produce recognisable cytopathic changes was proportional to the number of virus particles in the inoculum. However, continued growth was not related to the amount of virus in the inoculum. Cytotoxicity was ruled out because diluted virus did not give better growth. Different batches of cells differed in their ability to support viral multiplication and, consequently, only the first four or five subcultures of the most susceptible batches of cells were useful. Bovine serum in the media delayed the onset of cytopathic changes and Nagington (1968) attributed the delay to antibody or to other non-specific inhibitors.

Orf virus isolated from sheep clinical specimens has a clear-cut specificity for growth in sheep testis cells only (Nagington, 1968). Eight isolations were successful out of eight clinical specimens cultured. All paravaccinia viruses studied produced plaques of rounded cells. Orf virus of sheep origin tended to give /

give "open plaques" with rounded cells which readily detached from the glass leaving a clear central area surrounded by cells in the process of rounding.

Light microscopy: In 1937 Gordon drew attention to the work of Dr. John Buist, an Edinburgh physician, who detected by ordinary light microscopy the elementary bodies of smallpox virus in stained preparations of vesicular fluid. The elementary bodies were re-discovered in 1906 by Paschen who suggested that their demonstration was of diagnostic value. The application of staining methods for the diagnosis of animal poxes was, however, delayed until the 1950s. Nisbet (1954) found Paschen's staining technique to be the most satisfactory method, but, nevertheless, consistent results were not easily obtained. He first used smears prepared from the fluid contents of vesicles from lambs experimentally infected with orf. The virus particles stained a deep pink colour and appeared as tiny cocci about 300 nanometers in diameter. Later he prepared smears from washed centrifuged suspensions of five day old orf lesions which when stained by Paschen's method showed numerous virus particles. Abdussalam and Cosslett /

Cosslett (1957) confirmed the utility of Paschen's method. Nevertheless, the technique as a diagnostic aid was limited because the best time for collecting samples was the fourth day after inoculation by scarification (Abdussalam and Cosslett, 1957). After the sixth day non-specific particulate matter confused the recognition of the virus particles.

Electron microscopy: Electron microscopy was used as a diagnostic aid as early as 1948 during an outbreak of smallpox in New York (Van Rooyen and Scott, 1948). Nevertheless it is only comparatively recently that advances in technique have enabled diagnosticians to use the electron microscope routinely. The key technique was negative staining which revealed intimate structural details sufficient to allow differentiation between viruses (Cruickshank, Bedson and Watson, 1966).

Orf viruses were first seen with the electron microscopy in preparations of elementary bodies (Nisbet, 1954). The specimens were shadow-cast with gold palladium alloy and the viruses were seen as coccobacillary structures. Occasional coccal forms were also observed but no details of the internal structure were discernible. Nisbet's findings were confirmed later by Abdussalam and Cosslett (1957).

It /

It was not until 1961 that Nagington and Whittle saw and described the characteristic criss-cross pattern and oval shape of orf virus, in preparations that were negatively stained. Since then, the size, shape and structure of orf virus has been found to be characteristic of its group, the paravaccinia virus group.

Another member of the group is milkers' nodule virus and Nagington, Tee and Smith (1966) found that electron microscopy was a quicker and more reliable method of confirming a provisional diagnosis of milkers' nodule than attempted isolation in cell cultures which is rendered difficult by the presence of bacterial and fungal contaminants in the specimens to be examined. Johnston and Shorey (1967) used the electron microscope to differentiate between pseudo-cowpox and ulcerative mammillitis infections in Australian cattle and they used a preparation of powdered orf vaccine as their reference control.

ISOLATION AND IDENTIFICATION OF ANTIGENS

Precipitating Antigens: The first technique used to detect specific orf antigens was precipitation in tubes /

tubes but the results were not satisfactory when the antigenic source was dried scabs (Manley, 1934). Later, Webster (1958) detected a soluble precipitin which appeared to consist of two fractions. The specificity of the antigen, however, was queried because the fractions were not identified by absorption methods and therefore they could be similar haptens of different molecular sizes. Webster (1958) claimed to have demonstrated an immunological relationship between orf, vaccinia and ectromelia by cross-precipitation and he considered that one of the soluble precipitins corresponded to zone III of vaccinia and ectromelia. Abdussalam (1958) also demonstrated a precipitation reaction in tubes when using orf-immune sera prepared in rabbits and various extracts from elementary bodies.

The development of the Ouchterlony technique whereby the reagents were diffused through agar gels greatly simplified the detection of precipitating antigens and antibodies (Ouchterlony, 1948). The technique introduced two advantages over precipitation in tubes; firstly, no clarification of the antigen was necessary, and, secondly, a more immediate comparison could be made between the material under test /

test and a positive control. Specific precipitation "line or lines" formed by the interaction of suspect antigen and known antiserum bend and fuse with the corresponding precipitation "lines" formed between the positive control antigen and antiserum. The technique was used by Dumbell and Nizamuddin (1959) in the diagnosis of smallpox. They used extracts from selected scabs as antigens and the line of precipitation, appeared in two hours and linked up with the control lines within four to five hours. They also tested suspect antisera but concluded that the main use of the test was for detecting antigens.

The role of immune diffusion as a diagnostic aid for orf was tentatively explored by Johnston (1962). He prepared his anti-Orf serum in rabbits using multiple inocula of saline suspensions of orf-infected scabs harvested from sheep. The test antigens were similar saline suspensions of orf scabs. When the reagents were diffused against each other through agar gel several lines of precipitation were formed. Johnston (1962) postulated that some of the lines were due to the presence of antibodies to sheep proteins in the test sera. He, therefore, absorbed out the sheep protein antibodies by incubating the sera with sheep white /

white blood cells overnight at 37°C. Diffusion of the absorbed sera against orf scab suspensions yielded only one line of precipitation.

Johnston (1962) also attempted to eliminate unwanted reactions by using orf virus purified on a phosphate column for the production of anti-orf sera in rabbits. The antiserum to the purified virus always produced one line of precipitation and sometimes two when diffused against suspensions of orf scabs. Later, Johnston (1963) adapted orf virus of human origin to rabbit kidney cell cultures grown and maintained in culture media supplemented only with rabbit sera. When the cultured virus was injected into rabbits for the production of antiserum the inoculum contained no foreign proteins other than orf virus. The rabbits were later bled for serum which was diffused against a suspension of orf scabs and produced a line of precipitation. Unfortunately, however, an unexpected line of precipitation also appeared between the virus suspension and normal rabbit serum. Johnston (1963) found that the unexpected reaction was caused by antigens of staphy-:lococcal bacteria in the scab suspension precipitating with /

with antibodies to staphylococcal bacteria in the rabbit sera; the rabbit colony had earlier experienced an epidemic of "snuffles". There was no evidence of a reaction between orf antigen and orf antibody. Negative results were also obtained with antisera prepared in rabbits inoculated with orf virus passaged fifty times in cell cultures (Johnston, 1964). Thereafter Johnston (1965) tried anti-Orf serum produced in sheep; twenty-seven out of thirty-two scabs positive by biological tests were also positive when diffused against sheep anti-orf serum.

Trueblood (1966) used immuno-diffusion tests to study the relationship between the viruses of ulcerative dermatosis and orf. He concluded that the two viruses were related but orf virus contained an antigen component not found in ulcerative dermatosis virus.

Sharma (1966) showed by immuno-diffusion that Indian strains of orf virus isolated from sheep and goats had two precipitating antigens which were identical. Neither was reactive with anti-sheep pox or anti-goat pox sera. On the other hand, suspensions of sheep pox lesions, but not suspensions of goat pox lesions, produced one line of precipitate when diffused against anti-orf serum.

Using /

Using a hyperimmune anti-orf serum prepared in rabbits by injecting "reasonably purified" orf virus Schmidt (1967d) detected seven precipitation lines, four of which Schmidt considered to be specific orf antigen-antibody reactions. The precipitating antigens were inactivated by repeated freezing and thawing as well as by storage for several months at a few degrees below or above 0°C. On the other hand, Papadopoulos et al. (1968), found that their preparation of pseudocowpox precipitating antigen was stable at low storage temperatures; no alteration in activity was detected after three months at 4°C, after six months at -20°C nor after twenty cycles of alternate freezing and thawing. Both research groups reported that the precipitating antigens were heat labile. Reactivity was unchanged after twenty-four hours at 37°C but within ten days at 37°C a two-fold decrease in titre occurred (Papadopoulos et al., 1968). Reactivity was completely destroyed within ten minutes at 60°C (Papadopoulos et al., 1968) and within five minutes at 100°C (Schmidt, 1967d). Orf complement fixing antigens, in contrast, were heat stable and survived exposure to 90°C for one hour (Schmidt, 1967d). Orf precipitating antigens also differed from orf complement /

complement fixing antigens in their lability to acid treatment which actually enhanced the titre of the complement fixing antigen (Schmidt, 1967d). Sonication did not affect orf precipitating antigens and centrifugation at 64,000 G for thirty minutes did not sediment them (Papadopoulos et al., 1968). Both Schmidt (1967d) and Papadopoulos et al., (1968) concluded that the precipitating antigens were "soluble" antigens.

Like Johnston (1963; 1964), Schmidt (1967d) was unable to detect precipitating antigens in cell culture systems infected with orf virus. Papadopoulos et al. (1968), however, were successful using calf testis cells but they had to concentrate the culture fluids fifty times. The antigen was not detected until the sixth day after inoculation when fifty per cent of the cells were showing cytopathic changes. Maximal titres of antigen were reached nine to ten days after inoculation when ninety to one hundred per cent of the cells were damaged.

Rabbit anti-pseudocowpox serum reacted with the orf precipitating antigen preparation to give one line of precipitation which fused exactly with the line between the anti-pseudocowpox serum and pseudocowpox antigen /

antigen (Papadopoulos et al., 1968). The same rabbit anti-pseudocowpox serum did not react when diffused against antigens prepared from cowpox, vaccinia and bovine herpes mammillitis viruses (Papadopoulos et al., 1968). The results of the cross-reaction tests confirmed the hypothesis based on morphological and structural similarities that orf and pseudocowpox viruses were members of a sub-group of the pox viruses and were in agreement with the hypothesis that the soluble antigens of the pox viruses were sub-group specific (Woodroofe and Fenner, 1962).

Complement fixing antigens: Glover (1933) developed a complement fixation test using scabs as the source of antigen and hyperimmune anti-orf serum produced in rabbits. He found that scabs from natural and experimental cases nearly always fixed complement. Since then various groups of research workers have used complement fixation to detect orf-specific antigens. Only one worker, Manley (1934), concluded that the complement fixation test was not satisfactory as an aid in the diagnosis of orf.

The various reported techniques differ in detail but not in the essentials. Most workers used orf-infected scabs as the source of antigen. Glover (1933) extracted /

extracted the antigens by treating the scabs with ether for seven days at 37°C and he never had trouble with anti-complementary activity. Other workers used saline extracts of the scabs; Webster (1958), in addition, fortified the saline with calcium and magnesium ions. Sheep proteins and contaminating bacteria present in scab extracts did not have any adverse effect on the reaction (Schmidt, 1967c). The most fundamental variant in technique was that proposed by Schmidt (1967c) who preferred the partial complement fixation reaction to the direct complement fixation test. By using the partial C4 fixing reaction he demonstrated an antigenic relationship between orf and vaccinia virus on the one hand and, on the other, between orf and sheep pox viruses.

MacDonald and Bell (1961) used infected cell culture fluids as their source of antigen but they gave no details of the properties of their antigen. Ether-extracted antigen was heat-labile being inactivated within one hour at 65°C (Glover, 1933) whereas saline extracts of infected scabs were heat-stable. Webster (1958) claimed that antigen titres were unaffected by heating at 70°C and 90°C for one hour. On the other hand, Schmidt (1967a) while he preferred /

preferred to heat the antigen extract for thirty minutes at 70°C to destroy anti-complementary factors, found that heating for longer than thirty minutes at 70°C lowered the antigen titre. Abdussalam (1958), nevertheless, detected activity in boiled antigen extracts.

The nature of the orf complement-fixing antigen is ill-defined. Live infectious virus was not essential for fixation (Abdussalam, 1958). In fact, the particle size of the antigen was smaller than that of the intact virus and it was not sedimented when subjected to centrifugation for one hour at ±40,000 g (Webster, 1958).

Antigens detected with fluorescent antibody: In recent years the fluorescent antibody technique has been in vogue in diagnostic laboratories; it is, for example, the preferred method in confirming a diagnosis of rabies (Beauregard, Boulanger and Webster, 1965). Murray (1963) used the indirect fluorescent antibody technique described by Weller and Coons (1954) as an aid in the diagnosis of smallpox. Volkova, Caliev and Prasolova (1967) were the first to apply the technique to detect orf virus particles; impression smears /

smears of natural and experimental orf lesions were first treated with sheep anti-orf serum and then with anti-sheep serum labelled with fluorescein isothio-:cyanate. They claimed that the virus particles were readily recognised because of the specific bright green fluorescence. They seemed to have ignored non-specific fluorescence due to the presence of sheep proteins in the smears.

Recently Benda, Noskov, Danes, Serbezov and Goldin (1968) detected vaccinia and smallpox virus particles and their aggregates by using a direct fluorescent antibody technique. The fluorescent spots were about one third larger than virus particles. The best results were obtained when smears were prepared from vesicular fluids.

DETECTION OF ANTIBODIES

Neutralising antibodies: In his studies on the nature of the causal agent of orf Aynaud (1923) examined the protective power of the serum of recovered sheep both in vivo and in vitro. The virus was unaffected and Aynaud concluded that resistance to orf was local and not humoral in origin. Glover (1933) titrated mixtures of virus and sheep "hyperimmune" anti-orf serum on scarified sheep skin and /

and claimed to have neutralised a 1:100 dilution of virus from scabs that were autolysed. Manley (1934) likewise detected some neutralising activity in serum from recovered cases.

Sharma and Bhatia (1958) carried out titrations of serum-virus mixtures intradermally in goat kids and detected cross-neutralisation of orf virus by anti-goat pox serum but not by anti-goat dermatitis serum. Webster (1958) also used intradermal inoculations in sheep to examine the reaction between orf virus and anti-orf, anti-vaccinia and anti-ectromelia sera. Anti-orf serum lowered the virus titre one hundred-fold but anti-vaccinia and anti-ectromelia had no effect.

Trueblood, Chow and Griner (1963) incubated orf virus with sheep anti-orf serum and reported that infectivity for sheep was not lost. They summarised their own and other's findings and agreed with Aynaud that orf infection of sheep did not induce protective antibody.

Abdussalam (1958) titrated orf virus and anti-serum mixtures intradermally in rabbits. Convalescent sheep serum and convalescent human serum inhibited virus activity but convalescent rabbit did not.

Webster /

Webster (1958) also used rabbits as indicator hosts but he failed to find any significant drop in the titres of virus mixed with anti-orf, anti-vaccinia and anti-ectromelia sera. He claimed, however, to have detected substantial neutralisation of ectromelia virus in mice by anti-orf serum; he recorded a titre fall of 1.8 logs in one trial and 1.0 logs in a second trial.

The technical difficulties and inconvenience of titrations on the skin of sheep or rabbits were largely overcome by using cell cultures as indicators of free virus. Plowright et al. (1959) titrated serum-virus mixtures in sheep embryonic kidney cells; both convalescent sheep sera and rabbit hyperimmune anti-orf sera caused a variable degree of neutralisa-:tion which was hardly significant. Similarly MacDonald and Bell (1961) failed to detect unequi-:vocally evidence of neutralising activity in sheep and human convalescent serum in their cell culture system. Trueblood (1966) ran neutralisation tests in cell cultures using sera from hyperimmunised sheep and hyperimmunised rabbits. The sheep sera failed to neutralise the virus whereas the rabbit sera did. On the other hand, Naginton and Whittle (1961) used the more sensitive plaque reduction technique /

technique and claimed to have detected neutralising antibodies in the sera of convalescing human patients and sheep.

Sawhney (1966c) detected more than one antigenic form of orf virus by neutralisation tests in lamb testis cell monolayers using the virus dilution-serum constant technique. He reported significant neutralisation of an English strain of the virus by anti-sera prepared against a Bulgarian strain and vice versa. Rumanian and Slovakian strains were not neutralised by anti-sera to the English and Bulgarian strains nor were anti-sera against the Rumanian and Slovakian strains reactive against the English and Bulgarian strains.

Complement-fixing antibodies: Glover (1933) reported that orf virus induced the development of complement-fixing antibodies in the sera of convalescent and hyperimmunised sheep. The antibodies were difficult to demonstrate, the key to the problem being the use of a suitable antigen.

Sera collected from sheep three weeks after recovery from experimental infection fixed complement but the antibody titres were very low and they fell so rapidly /

rapidly that sera collected nine weeks after infection contained no detectable antibodies. Glover (1933) concluded that attempted differentiation of susceptible and immune sheep by examining their sera for complement fixing antibodies was hardly possible.

Rottgardt, Aramburu and Pirazzi (1949) detected complement-fixing antibodies in the sera of sheep bled eight and thirty-one days after experimental infection. Olah and Elek (1953) reported the presence of complement-fixing antibodies in the sera of twenty-six lambs eleven days after vaccination that persisted in nineteen out of the twenty-six lambs for five months. Kujumgiev (1954), similarly, found antibodies in the sera of lambs from one to five months after vaccination.

Nisbet (1954) titrated the complement-fixing antibodies in the sera of a lamb bled at different times after experimental infection and detected an increase in titre with time. The highest titre, however, was only 1:12. Moreover, the sera from five other lambs bled at intervals up to one month after infection had non-specific levels of antibody. In contrast, MacDonald and Bell (1961) reported antibody titres of 1:64 in the sera of sheep three weeks /

weeks after infection. They used an antigen derived from orf-infected sheep embryonic kidney cultures.

Blakemore, Abdussalam and Goldsmith (1948) identified infections in two human patients as orf by detecting complement-fixing antibodies in the convalescent sera. The reactions were very weak being similar to those reported by Glover (1933) for sera from convalescent sheep. MacDonald (1951) also confirmed a human infection by detecting complement-fixing antibody and he reported that serum taken three to four weeks after the first appearance of the lesion gave complete fixation up to a dilution of 1:32 and seventy-five per cent fixation at the 1:40 dilution.

In contrast to the low levels of complement-fixing antibodies found in convalescent sera, the sera of hyperimmunised sheep readily fixed complement (Glover, 1933; Blakemore et al., 1948; Schmidt, 1958). Similarly the sera of hyperimmunised rabbits contained high levels of complement-fixing antibodies, the titres being around 1:64 (Blakemore et al. 1948; Nisbet, 1954).

The complement-fixing activity of anti-orf sera was /

was restricted to the fractions containing globulins; the albumin fractions did not fix complement (Olah and Elek, 1953).

Schmidt (1958) preferred the partial complement fixation test to the direct test. He detected antibodies in both convalescent and hyperimmune sera but because the antibody titres were low it was essential to use high grade antigens.

Nisbet (1954) measured the complement-fixing antibody titres of sera that had been absorbed with homologous and heterologous antigens and clearly demonstrated that the disease then known as "border orf" was immunologically identical with contagious pustular dermatitis. Sharma (1966) used cross-complement fixation tests to detect a slight cross-reaction between orf and sheep pox and between orf and goat pox.

Precipitating antibodies: The sera of sheep convalescent from orf and hyperimmunised against orf contain precipitating antibodies, but little is known about their nature or development. Abdussalam (1958) and Webster (1958) detected them by a tube precipita-:tion test. More recent studies, however, are based on /

on immuno-diffusion techniques in agar gels (Johnston, 1965; Sharma, 1966; Trueblood, 1966; Papadopoulos et al. 1968).

Webster (1958) reported the presence of two precipitating antibodies in the sera of hyperimmune sheep and his findings were confirmed by Trueblood (1966) and Sharma (1966). Johnston (1965) immunised sheep with cell cultured-orf virus in Freund's adjuvant. Sera collected six weeks later contained only non-specific precipitating antibodies. The serum of one animal bled after nine weeks appeared to give a specific reaction when diffused against orf antigen. When the same animal was bled after twelve weeks the specific antibody was well defined. An attempt to repeat the procedure failed (Johnston, 1966).

Papadopoulos et al. (1968) used a pseudocowpox antigen to detect a precipitating antibody in the serum of a sheep experimentally infected with orf. The pre-infection serum was free of antibody. They also followed the development of the precipitating antibody in a cow infected with both pseudocowpox and orf viruses. The antibody reached its maximum titre /

titre two weeks after the primary inoculation and fell gradually but was still detectable four months later. A second virus inoculation produced a very rapid serological response. The titres fell rapidly within a month to the level which existed prior to the second inoculation.

DIFFERENTIAL DIAGNOSIS

BLUETONGUE

Excellent and comprehensive reviews of the literature on bluetongue have been published in recent years by Howell (1963) and Neitz (1966).

Definition: Bluetongue is an insect-borne virus disease of ruminants characterised in sheep by fever, excoriations of the mucous membranes of the mouth and nasal passages, coronitis and laminitis.

Epidemiology: Locus: Bluetongue has been classified as one of the emerging diseases of animals (Howell, 1963). For many years it was confined to Africa but in the past twenty years it has spread out of Africa into southern Europe, the Middle East, Pakistan, the Far East and North America.

Natural host range: Overt disease is commonest in sheep but there is a wide variation in innate resistance. African and Asian breeds possess a high innate resistance, Merinos a moderate degree of innate resistance and English mutton breeds have little or no resistance (Neitz, 1948). Goats are susceptible and clinical signs have been observed in goats affected in virgin epidemics (Hardy and Price, 1952). Similarly, cattle /

cattle are susceptible but are rarely observed sick in Africa. In virgin epidemics elsewhere, however, overt disease in cattle is not uncommon (Howell, 1963).

Transmission: In nature bluetongue is transmitted by biting insects particularly night-flying Culicoides (Du Toit, 1944). Transmission by direct contact between sick and healthy sheep does not occur but the virus has been isolated from foetuses of reacting ewes (Schulz and DeLay, 1955).

Prevalence: Because the virus is insect-borne the disease has a well-defined seasonal prevalence, the incidence rising with the onset of the rainy season and falling with the arrival of frosts or the dry season. The mechanism for the maintenance of the virus over the inter-epidemic periods is obscure (Neitz, 1966).

Diagnosis: The provisional diagnosis is based on the history of the outbreak, the clinical signs and autopsy findings. The confirmatory diagnosis is based on the isolation of the virus and its serological identification.

History: Pertinent factors are the locus and season. Exotic sheep imported into an area where the disease is endemic will often inadvertently monitor the presence of the virus.

Clinical /

Clinical signs: The natural incubation period lasts about one week. The onset of illness is manifested by fever which persists for six to twelve days. Within twenty-four hours of the onset of illness salivation and nasal discharge indicate hyperaemia of the buccal and nasal mucosae. Oedema and sometimes purplish discolouration of the lips and tongue follow. The epithelium thickens and is shed leaving exposed haemorrhagic craters. The nasal discharge thickens and dries in crusts around the nostrils. As the fever regresses coronitis and laminitis become evident and the affected animals are disinclined to move. Progressive emaciation precedes death for several days. Surviving animals recover slowly and patchily shed their fleece. The mortality rate among indigenous sheep in an endemic area seldom exceeds ten per cent but among exotic sheep it approaches ninety per cent.

Autopsy findings: Dead sheep are usually emaciated. Grey necrotic debris coats the buccal and nasal mucosæ, crusty eruptions cover the skin, hyperaemia and haemorrhages streak the feet, and red gelatinous fluid infiltrates the intramuscular connective tissues. Sometimes the muscles are grey and mottled, but at other times, they are studded with small haemorrhages.

Virus /

Virus isolation: The most sensitive technique for isolating bluetongue virus is sub-inoculation of non-coagulated blood or a suspension of spleen from an early clinical case into known susceptible sheep. A less sensitive but rapid and cheaper technique is the inoculation of blood intravascularly into fowl embryos previously incubated for ten to thirteen days (Goldsmith and Barzilai, 1968).

Virus identification: Isolated virus is identified by complement fixation and neutralisation tests. There are sixteen serotypes of bluetongue virus (Howell, 1966).

Diagnostic confusions with orf: On numerous occasions bluetongue has been mis-diagnosed as orf (Gambles, 1949; Fry, 1958). Less commonly outbreaks of orf have been suspected as being bluetongue. Recent examples include outbreaks of fatal mucosal infections of sheep in southern England reported by Darbyshire in 1961 and an outbreak of scabby mouth complicated by trauma caused by the shrub, Templetonia retusa, in Western Australia described by Gardiner et al. (1967).

PESTE /

PESTE DES PETITS RUMINANTS

There is no adequate review of the literature on "peste des petits ruminants". A brief anonymous contribution in the second edition of the "Handbook of Animal Diseases in the Tropics" published by the British Veterinary Association in 1968 draws attention to the disease. The best description is found in the paper by Mornet, Orue, Gilbert, Thiery and Sow Mamadou (1956) who demonstrated the viral aetiology of the condition and concluded that it was a strain of rinderpest virus naturally adapted to goats and sheep.

Definition: "Peste des petits ruminants" is a viral contagion of goats and sheep characterised in its acute phase by a rinderpest-like syndrome and in its chronic phase by the development of prominent labial scabs.

Epidemiology: Locus: The disease appears to be restricted to West Africa (Anon., 1968).

Natural host range: Most clinical cases are observed in goats. Sheep, however, are also susceptible (Mornet et al., 1956). Cattle are refractory to natural exposure but they can be infected if the virus is adminstered parenterally (Gargadennec and Lalanne, 1942).

Transmission: /

Transmission: "Peste des petits ruminants" is a contagion and transmission occurs after contact between sick and healthy goats. Cattle are not infected by direct contact with sick goats (Mornet et al. 1956). Nevertheless unknown vectors are suspected to play a role in the spread to new foci (Mornet et al., 1956).

Prevalence: Outbreaks are sporadic. In Nigeria a similar if not identical disease known locally as "Kata" appears to have a seasonal prevalence, the incidence rising sharply with the onset of the rains. (Whitney, Scott and Hill, 1967).

Diagnosis: Most cases are diagnosed from the clinical signs and autopsy findings. Confirmation depends upon isolation of the virus and its serological identification.

Clinical signs: The acute phase of the disease mimics rinderpest. The inoculation period varies from two to six days and the course of the acute phase lasts about five days. There is a prodromal fever and then erosions appear on the epidermoid mucous membranes. Diarrhoea develops two or three days later. Most affected goats die at this stage but those that survive develop a labial dermatitis.

Autopsy /

Autopsy findings: Death occurs in the acute phase. The prominent lesions are erosive stomatitis and haemorrhagic gastro-enteritis together with secondary broncho-pneumonia.

Virus isolation: Mornet et al. (1956) and Whitney et al. (1967) readily transmitted the disease to susceptible goats by injecting them with blood or suspensions of spleen from febrile goats. On the other hand, Johnson and Ritchie (1968) failed to isolate the virus from blood in primary cell cultures although they readily isolated the virus from mucosal surfaces and the associated drainage lymph nodes.

Virus identification: Isolated virus is identified either by cross-protection tests in goats or cattle (Mornet et al., 1966), or by the characteris-:tic cytopathic effect on cell cultures (Gilbert and Monnier, 1962; Johnson and Ritchie, 1968) or by serum-virus neutralisation tests.

Diagnostic confusion with orf: The labial scabs that develop in surviving goats are reminiscent of orf (Mornet, et al., 1956; Whitney et al., 1967).

LABIAL STREPTOTHRICOSIS

Streptothricosis was first described as a disease of cattle by Van Saceghem in 1915 in the Belgian Congo. Infection of sheep was first described by Bekker in 1928 in South Africa. Extensive reviews of the literature have been published by Austwick (1958) and Roberts (1967).

Definition: Labial streptothricosis is a chronic exudative dermatitis affecting the lips of sheep and goats characterised by scab formation and caused by the bacterium, Dermatophilus congolensis.

Epidemiology: Locus: Streptothricosis in varying forms in various species has been reported from most parts of the world. One form is strawberry foot-rot which appears to be restricted to sheep in the United Kingdom, particularly north-eastern Scotland (Selbie, 1946; Harriss, 1948). Another is a dermatitis confined to the hairy parts of the skin, scrotum and legs of sheep which has been reported from East Africa (Hudson, 1937) and Scotland (Nisbet and Bannatyne, 1955). Labial streptothricosis was reported first by Memery in 1960 from West Africa.

Natural host range: The causal organism has a wide host range and commonly affects cattle, goats, horses, sheep and wild ruminants.

Transmission: /

Transmission: The dominant mode of transmission is unknown. Vectors, particularly ticks, have been incriminated. Cutaneous abrasions from thorns and spiny plants are an important predisposing cause of overt lesions. Extension of existing lesions is facilitated by water.

Prevalence: Outbreaks appear to be linked to high rainfall, high humidity and high environmental temperature.

Diagnosis: The diagnosis is often based solely on clinical signs and it is readily confirmed by the examination of smears of the under surfaces of the scabs or by isolating the organism.

Clinical signs: The dominant feature is the skin lesions. The dermis is inflamed and exudate separates the epidermis from the dermal matrix. New epidermis forms under the exudate but likewise it is separated from the dermis by exudate. The process continues to form a thick scab composed of alternate layers of cornifying epidermis and exudate (Roberts, 1967).

Bacterial isolation: Dermatophilus congolensis is a filamentous bacterium and it is recognised in smears by /

by the typical branched filaments which divide transversely and longitudinally into thick bundles of coccoid forms. On culture it forms greyish white adherent colonies on solid media that may be rough or smooth. The mycelium and spores are Gram positive (Austwick, 1958).

Diagnostic confusion with orf: Labial streptothricosis in goats in West Africa was first diagnosed as orf and doubts only arose with the extension of the lesions and their prolonged course (Memery, 1960). Similarly strawberry foot-rot was first considered to be viral in origin (Selbie, 1946).

MATERIALS AND METHODS

VIRUSES

Orf virus: The strain of orf virus used, was isolated from a naturally affected Suffolk lamb at Easter Bush, Roslin, Midlothian, in the Spring of 1964. It was passaged experimentally in susceptible lambs one to six times and maintained between passages as infected scab at -18°C.

Bovine papular stomatitis virus: The strain of bovine papular stomatitis virus was isolated from a bovine tongue lesion seen at Gorgie Slaughterhouse in 1969. It was passaged twice in susceptible calves.

EXPERIMENTAL ANIMALS

Sheep: Blackface, Cheviot, Greyface and Suffolk sheep were used. Most were one to two weeks old. A few were six months old. Some were ewes. Sighting experiments had shown that similar animals were susceptible to orf and, in fact, all proved to be susceptible.

Cattle: Young Ayrshire calves known to be susceptible to bovine papular stomatitis were utilised to propagate the virus and to produce immune serum.

Rabbits: /

Rabbits: Young rabbits of the New Zealand White breed were kept individually in isolation and used for the production of anti-sera.

BIOLOGICAL TESTS

SOURCE OF SAMPLES

Scabs collected from lambs and sheep infected with orf both naturally and experimentally were screened for live virus by biological tests (Fig. 2). The scabs were stored at -18°C until required, the longest storage period being five years.

PROCESSING OF SCABS

The scabs were weighed and a twenty per cent (w/v) suspension prepared in phosphate buffer saline in a mortar and pestle. The suspension was centrifuged at 3,500 r.p.m. for ten minutes and the resulting supernatant fluid was collected and treated with antibiotics to control bacterial contaminants; 1,000 units of penicillin, 1,000 micrograms of streptomycin and 50 micrograms of fungizone respectively per ml. The suspension was held overnight at 4°C before being tested in animals.

TECHNIQUE

The skins of the inner thighs of young susceptible lambs were scarified and 0.1 ml of the scab suspension

was /

was dropped on to the scarified area and evenly distributed with a sterile swab. The animals were examined at least weekly for the development of typical orf lesions on the scarified area.

Fig. 2 - Collection of scab sample.

CELL CULTURE STUDIES

SOURCE OF VIRUS

Orf scabs were harvested from lesions produced experimentally on the scarified skins of susceptible lambs. The scabs were weighed and ten or twenty per cent suspensions (w/v) were prepared in phosphate buffer saline in a mortar and pestle. The suspensions were centrifuged at 3,500 r.p.m. for ten minutes and the supernatant fluids were collected and treated with antibiotics; 1,000 units of penicillin, 1,000 micrograms of streptomycin and 50 micrograms of fungizone respectively per ml. The suspensions were kept at 4°C overnight to allow the antibiotics to act on the bacteria commonly contaminating the scabs.

PRODUCTION OF CELL CULTURES

Primary cultures: Lamb and calf testis cells: Testes were surgically removed with aseptic precautions from young lambs and calves. The parenchyma was exposed, detached from the Tunica albuginea and rinsed several times in lactalbumin hydrolysate. Then it was minced with scissors. The fragments were rinsed with lactalbumin hydrolysate until free of blood and tryprinised in twenty volumes of 0.05 per cent trypsin solution /

solution at 35°C. A single cycle of ninety minutes sufficed for lamb testis cells and a single cycle of two hours sufficed for calf testis cells. The trypsinised suspensions were filtered through gauze and centrifuged at 800 r.p.m. for ten minutes. The sedimented cells were re-suspended in Earle's growth medium containing ten per cent bovine serum and seeded into flasks or tubes. Usually good monolayers were formed within seventy-two hours. The cells, thereafter, were sustained in medium containing two per cent foetal bovine serum.

Lamb and calf kidney cells: Kidneys were removed with aseptic precautions from freshly killed lambs and calves. The perirenal fat and the renal capsules were removed and discarded. The cortex was minced with scissors and the fragments were rinsed in Hank's solution. Thereafter the fragments were subjected to as many as eight seven-minute cycles of trypsinisation at 35°C in 0.25 per cent trypsin. Finally the cells were sedimented, re-suspended in Earle's growth medium fortified with ten per cent tryptose phosphate broth and seeded into flasks or tubes. Monolayers formed within seventy-two hours and were sustained in a maintenance medium containing two per cent foetal bovine serum.

Foetal /

Foetal lamb skin cells: Strips of skin were removed aseptically from lamb foetuses in slaughtered ewes. They were chopped into millimetre fragments and rinsed in lactalbumin hydrolysate until all the blood was washed out. The fragments suspension was diluted in twenty volumes of 0.5 per cent trypsin solution and agitated for one hour at 35°C. Fresh trypsin solution replaced the old and the cycle was repeated three or four times. The cell suspension was filtered and lightly centrifuged for ten minutes. The sedimented cells were re-suspended in Earle's growth medium fortified with tryptose broth and containing ten per cent bovine serum and seeded into flasks or tubes. Monolayers developed in six to seven days and were maintained in medium containing tryptose broth and two per cent bovine serum.

Sheep thyroid cells: Thyroids were removed from slaughtered young adult sheep. The fat and capsule were removed and the tissue was chopped into small fragments with scissors, rinsed in lactalbumin hydrolysate to remove all blood and diluted in twenty volumes of 0.5 per cent trypsin solution. Three or four cycles of trypsinisation were usually necessary and cells were harvested at the end of each cycle.

The /

The cell suspensions were pooled, filtered, centrifuged and re-suspended in Earle's growth medium containing ten per cent bovine serum. Monolayers developed in flasks and tubes six to seven days after seeding and thereafter were sustained by a maintenance medium containing two per cent bovine serum.

Continuous cell lines: The following continuous cell lines were used; bovine kidney cells grown and maintained in Earle's media containing ten and two per cent bovine serum respectively; rabbit kidney cells grown and maintained in Eagle's media containing ten and two per cent bovine serum respectively; Hep-2 cells grown and maintained in Eagle's media containing five and two per cent bovine serum respectively; human ammion cells grown and maintained in 199 medium containing ten and two per cent bovine serum respectively.

INOCULUM

The viral suspensions were inoculated into cell cultures either without further dilution or, on occasion, after dilution in phosphate buffer saline or Hank's balanced salt solution using ten-fold steps down to 10^{-6}. Three suspensions were further centrifuged /

centrifuged at 15,000 r.p.m. for forty-five minutes and both the supernatant fluids and the pellets were tested, the pellets being re-suspended in phosphate buffer saline up to the original volume. Finally, three viral suspensions were processed through a chromatographic column containing Sephadex G-200 gel [1] and the fractions known to contain infectious virus were used.

One crude scab suspension was subjected to ultrasonication at 20,000 cycles for two minutes before being centrifuged at 3,500 r.p.m. for ten minutes to remove the larger particles. The supernatant fluid was re-centrifuged at 32,000 r.p.m. for thirty minutes and both the supernatant fluid and the re-suspended pellet were examined.

TECHNIQUE

Some tissue culture tubes containing coverslips were seeded with cells and a confluent monolayer was allowed to form before inoculation with virus. Others were inoculated with virus immediately after the cells were seeded. Orf-scab suspensions were inoculated /

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1 Pharmacia Fine Chemicals AB, Uppsala, Sweden.

inoculated into tubes in volumes varying from 0.1 to 0.3 ml and were left to be adsorbed at temperatures varying from 4°C to 37°C for periods of time ranging from thirty minutes to three hours. At the end of the adsorption period the inocula were decanted and the monolayers were washed three times with warm phosphate buffer saline, Earle's medium or Hank's solution. Then the appropriate maintenance media were added. The cells were observed for non-specific changes at the end of the adsorption period and every twenty-four hours. Coverslip preparations were usually stained with the Giemsa six hours after inoculation. Some were stained with haemotoxylin and eosin and others were stained with acridine orange. Direct fluorescent antibody techniques were also tried.

ELECTRON MICROSCOPY

SOURCE OF SAMPLES

Orf-infected samples: Experimental orf was produced by scarifying the skins of the inner thighs of susceptible lambs and applying, on the scarified area 0.1 ml of a twenty per cent orf-scab suspension (w/v). The scabs formed at the scarification site were stripped off with forceps and stored at -18°C in screw-capped containers until they were processed. Several scab harvests were made from each animal on different days.

Samples from clinically-diagnosed cases of orf: Scabs were harvested from orf-like lesions on the nostrils, lips, tail, tongue and ears of lambs and teats of ewes.

Samples from clinically-diagnosed cases of bovine papular stomatitis: Lesions were collected by biopsy from the tongue, dental pad and scrotum of affected cattle.

MATERIALS

Electron microscope: The electron microscope used was the AEI-EM-6B [1].

Stain: Phosphotungstic acid was used for negative staining /

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[1] Associated Electrical Industries Ltd., Harlow, Essex.

staining and was prepared by dissolving 1.5 grams of phosphotungstic acid [1] in 100 ml of de-ionised distilled water. The pH was adjusted to 7.2 by slowly adding drops of a solution of 1-N potassium hydroxide.

Grids; "Athene" electron microscope specimen grids [2], new 200 type were used. They had a diameter of 3.05 mm.

Grid boxes: Specimen grid boxes 4828B [3] were used to keep the already scanned grids.

Forceps: Ordinary watchmaker forceps were used to manipulate the grids.

Photographic plates: The special lantern contrast plates, 3¼" x 3¼" [4], were used to record by photography all the positive samples found.

Dissecting needles: Because hundreds of specimens had to be processed and because it was essential to avoid cross-infection, sterile disposable hypodermic needles [5] were used instead of dissecting needles.

Collodion /

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1 George T. Gurr Ltd., London.

2 Smethurst High-Light Ltd., Lancs., England.

3 LKB-Produkter AB, Stockholm, Sweden.

4 Ilford Limited, England.

5 Gillette Industries Ltd., Isleworth, Middlesex.

Collodion film: The collodion film was prepared by mixing 2 grams of low viscosity nitrocellulose, 50 ml of amyl acetate and 25 ml of ether. The mixture was kept in a screw-cap bottle.

Carbon coating: The AEI vacuum coating unit was used to carbon coat the collodion membranes on the grids. First a sheet of copper mesh was placed at the bottom of a petri dish[1] and covered with distilled water up to the lip of the dish. The grids were immersed one by one and located on the copper mesh without overlap. They were coated with collodion in batches of approximately fifty. A drop of the collodion suspension was dropped on the surface of the distilled water where it formed a pellicle or membrane which was then removed with forceps. The process was repeated three or four times until a good pellicle was obtained. The object was to clean the water surface of dust particles and so allow better formation of a collodion membrane. The distilled water was carefully siphoned off thus allowing the collodion membrane to settle on the grids sitting on the copper mesh. The petri dishes were then placed in a calcium chloride desiccator. When dried, the grids were transferred to the vacuum coating unit[2] and coated with carbon.

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1 Griffin & George Ltd., Wembley, Middlesex.

2 Associated Electrical Industries Ltd., Harlow, Essex.

METHOD

Caps from the plastic containers of disposable hypodermic needles were placed on Whatman filter paper and used as staining wells. The needle was removed from its container, attached to a syringe containing the negative stain and two drops of the stain were delivered into the cap well. The same needle was used to dissect the scab to be examined and a portion about 2 square mm in size was transferred to and stirred in the stain for approximately thirty seconds. Disintegration occurred and released the contents of the scab. The scab debris was removed and discarded into a container filled with chloros solution.

A carbon-coated collodion grid was held between fine forceps and layered on top of the stain for thirty seconds, the coated surface facing downwards. The grid, then, was lifted off and held with the coated surface upwards for another thirty seconds, and the excess stain blotted up gently with filter paper. The grids were left to dry overnight in a desiccator containing calcium chloride before being screened in the electron microscope.

Good intact fields were searched at an initial magnification of 1,500 and then scanned for the presence /

presence of virus at a magnification of 10,000. Once the virus particles were located the magnification was increased for final identification and photography. The minimum screening time was ten minutes, although some samples were screened for as long as twenty-five minutes. The time spent to find orf-virus particles was always recorded.

VIRUS PARTICLE LYSIS

Reagents: Scabs from lambs experimentally scarified seven days earlier with orf virus were harvested and scanned with the electron microscope. Scabs rich in particles were used in the lytic experiments with sheep anti-orf serum and serum from colostrum-deprived lambs. Gel chromatographic fractions of sheep anti-orf serum were also used.

Method: Aliquots of the orf scabs, approximately 2 sq. mm in size, were added to 0.4 ml volumes of serum and incubated for one week at 37°C. The scabs were then removed and scanned with the electron microscope after negative staining.

DETECTION OF HAEMAGGLUTININS

PROCESSING OF SAMPLES

Orf scabs were weighed and ten to twenty per cent suspensions (w/v) were prepared in Kolmer's saline. The suspensions were centrifuged at 3,500 r.p.m. for ten minutes and the supernatant fluids were tested for haemagglutinins without further treatment, after treatment with kaolin for twenty minutes at 4°C, after re-centrifugation at 14,000 r.p.m. for forty-five minutes when both the supernatant fluid and the pellet re-suspended in Kolmer's saline to half the original volume were examined, after sonication at 20,000 cycles for two minutes, and after fractionation through Sephadex G-200.

RED BLOOD CELLS

Avian, mammalian and primate red blood cells were suspended in Alsever's solution or were heparin-:ised. They were washed three times in Alsever's solution and stored as packed cells at 4°C until required. The concentrations used in the test were 0.5 and 1.0 per cent.

TECHNIQUE /

TECHNIQUE

Two-fold dilutions of the suspect haemagglutinin preparations were made in 0.2 ml blanks of Kolmer's saline in perspex agglutination plates up to a dilution of 1:512. Then 0.1 ml of Kolmer's saline and 0.2 ml of the red blood cell suspension were added to each well. A red blood cell control was always included in each test. The plates were incubated at 37°C for one hour and then allowed to stand overnight at 4°C. The end-point was the highest dilution causing agglutination.

DETECTION OF PRECIPITATING ANTIGENS

SOURCE OF SAMPLES

Virus-infected samples: Lambs were scarified on the inner side of the thigh and infected by applying on the scarified area 0.1 ml of a twenty per cent scab suspension (w/v) previously shown to contain orf virus particles by electron microscopy. The scabs that developed at the scarification sites were harvested and stored individually at -18°C until they were processed. Several scab harvests were made from each animal at varying times after scarification until the lesions were healed. The objective was to assess the development of precipitating antigens in the scabs and to determine the optimal time for detecting the antigens.

Virus-free samples: One lamb was scarified on the inner aspects of both thighs and a heavy growth of Staphylococcus aureus was rubbed into the wounds. The scabs that developed over the lesions were harvested.

Three lambs were scarified on the inner thighs and a 1:12 ointment of mercuric iodide in vaseline was applied to the scarified area. Severe inflammatory reactions resulted and scab formation followed. The scabs were harvested.

PROCESSING /

PROCESSING OF SAMPLES

The scabs were weighed and twenty per cent (w/v) suspensions were prepared in Dulbecco "A" phosphate buffer saline, using mortars and pestles. The suspensions were centrifuged at 3,500 r.p.m. for ten minutes. The tissue debris were discarded but the supernatant fluids were retained and tested for precipitating antigens.

PRODUCTION OF ANTI-SERA

Specific anti-sera reactive against orf virus were produced experimentally in sheep and rabbits. Anti-sera reactive against bovine papular stomatitis virus were produced experimentally in calves.

Sheep anti-orf sera: Three Greyface, one cross-bred Dorset, and two Cheviot lambs were used. The Greyfaces were immune through earlier vaccination but the cross-bred Dorset and the Cheviots were known to be susceptible. The lambs were hyperimmunised with different materials and, therefore, the procedure for each is given separately.

Greyface 7: A ten per cent suspension of a scab shown by electron microscopy to contain numerous orf virus /

virus particles was prepared in phosphate buffer saline and subjected to two centrifugation cycles at 3,500 r.p.m. for five and ten minutes respectively. The supernatant fluid was collected and treated with antibiotics. Two ml of the suspension were slowly incorporated into 2 ml of incomplete Freund's adjuvant[1] and emulsified by vigorous shaking. Two ml of the emulsion were injected intravenously into Greyface lamb 7. The lamb was bled for serum at irregular intervals from the seventh day after inoculation. Seven months after the adjuvant injection the animal was challenged by applying a suspension of virus-infected scab to scarified sites on both inner thighs. Blood for serum was collected ten days later.

Greyface 10: A ten per cent suspension of a scab shown by electron microscopy to contain numerous orf virus particles was prepared in phosphate buffer saline and subjected to two centrifugation cycles at 3,500 r.p.m. for five and ten minutes respectively. Two ml of the supernatant fluid was re-centrifuged at 14,000 r.p.m. for forty-five minutes at 4°C. The supernatant fluid was discarded, and the pelleted virus re-suspended in /

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1 Difco Laboratories, Detroit, Michigan, U.S.A.

in 2 ml of phosphate buffer saline. Two ml of the re-suspended virus were emulsified with 2 ml of incomplete Freund's adjuvant and 2 ml of the emulsion were injected intravenously into Greyface lamb 10. The lamb was bled from the seventh day after injection. It was challenged eight weeks later by the same route and again in fifteen days by injecting 2 ml of re-suspended orf virus pellet in phosphate buffer saline without adjuvant. Thereafter the lamb was bled for serum at irregular intervals. Seven months after the first adjuvant injection the animal was re-challenged by applying a suspension of virus-infected scab to scarified sites on both inner thighs. Blood for serum was collected ten days later.

Cross-bred Dorset 64: A twenty per cent suspension of orf scabs was fractionated through Sephadex G-200 and the fractions shown to contain orf virus were used for immunisation. The skin of the inner thigh was scarified and 0.3 ml of the infective fractions were applied to the scarified area. Six weeks later 5 ml of the infective fractions were mixed with 2.5 ml of the fluorocarbon, Arcton 113[1]. The mixture was shaken for ten minutes in a Griffin flask shaker[2], and then centrifuged /

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[1] Imperial Chemical Industries Ltd., Runcorn, Cheshire.

[2] Griffin & George Ltd., Wembley, Middlesex.

centrifuged in the cold at 3,500 r.p.m. for ten minutes. The clear liquid phase on top contained the virus and was harvested and emulsified with an equal volume of incomplete Freund's adjuvant. Two ml of the virus-adjuvant emulsion were injected intravenously into Greyface lamb 64. Seven days later fresh virus-adjuvant emulsion was prepared and 2 ml were injected intravenously and 2 ml intramuscularly. Third and fourth intravenous injections of 2 ml. of freshly prepared emulsion were given at weekly intervals. The lamb was bled for serum between injections and at irregular intervals after the last injection. Seven months later the animal was challenged by applying 0.3 ml of infective Sephadex G-200 fraction to scarified skin on both inner thighs. It was bled for serum eleven days after challenge.

Greyface 4: A ten per cent suspension of orf scab was prepared in phosphate buffer saline and subjected to two centrifugation cycles at 3,500 r.p.m. for five and ten minutes respectively. The supernatant fluid was re-centrifuged in the cold at 14,000 r.p.m. for forty-five minutes. The pellet was re-suspended in phosphate buffer saline and 2.5 ml of the re-suspended pellet /

pellet were thoroughly mixed with 2.5 ml of complete Freund's adjuvant[1] to form a thick emulsion. Doses of 2.5 ml of the emulsion were injected intramuscularly at two sites in Greyface lamb 4. One month later the supernatant fluid from a centrifuged ten per cent orf scab suspension was mixed with an equal volume of incomplete Freund's adjuvant and 3 ml of the emulsion were injected intravenously. The lamb was bled between and after the inoculations. Six months later the lamb was re-challenged by scarification with orf-virus on the inner side of both thighs and bled two weeks later.

Cheviot 54: Sephadex G-200 fractions containing orf-precipitating antigen (Peak 2) were pooled, concentrated ten times at 4°C with polyethylene glycol[2] and dialysed at 4°C for twenty-four hours against phosphate buffer saline. The dialysed antigen was emulsified with an equal volume of incomplete Freund's adjuvant and 2 ml of the emulsion were injected intravenously into Cheviot lamb 54. The skin of the inner left thigh was scarified but no agent was applied to the site. The animal was bled for serum at weekly intervals.

Cheviot /

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[1] Difco Laboratories, Detroit, Michigan, U.S.A.

[2] Carbowax PEG-20M, Union Carbide Ltd., London.

Cheviot 76: Sephadex G-200 fractions containing orf-precipitating antigen (Peak 3) were pooled and concentrated ten times with polyethylene glycol and dialysed at 4°C for twenty-four hours against phosphate buffer saline. The dialysed fractions were thoroughly mixed with an equal volume of incomplete Freund's adjuvant and 2 ml of the emulsion were injected intravenously into the susceptible Cheviot lamb 76. The skin of the inner left thigh was scarified but no agent was applied to the site. The animal was bled for serum at weekly intervals.

Rabbit anti-orf sera: Eight rabbits were used. The different immunisation regimes are recorded separately.

Rabbits 01 and 35: A twenty per cent suspension of orf scabs was prepared in phosphate buffer saline and centrifuged at 3,500 r.p.m. for five minutes. The supernatant fluids were re-centrifuged three times at 3,500 r.p.m. for five, fifteen and thirty minutes respectively. The final supernatant fluid was treated with antibiotics, diluted in an equal volume of phosphate buffer saline and then emulsified with an equal volume of incomplete Freund's adjuvant. One ml of the emulsion was injected intravenously and 0.2 ml were injected subcutaneously at five sites in both rabbits.

Rabbits /

Rabbits 02, 21 and 22: Sephadex G-200 fractions containing infectious orf virus were thoroughly mixed with an equal volume of incomplete Freund's adjuvant. Each rabbit was injected intravenously with 1 ml of the emulsion and subcutaneously with 1 ml in 0.2 ml amounts at five different sites. The animals were rested for two months and then re-injected intravenously with 1 ml of a similar but freshly prepared emulsion. The three rabbits were bled to death ten days later and their sera pooled.

Rabbits 03 and 04: A twenty per cent suspension of orf scabs was prepared and lightly centrifuged four times. One ml of the final supernatant fluid was injected intravenously into each rabbit.

Rabbit 011: Precipitation lines that formed in the orf immuno-diffusion system were cut out, blended with phosphate buffer saline and centrifuged at 3,500 r.p.m. for ten minutes. The supernatant fluid was harvested and emulsified with an equal volume of incomplete Freund's adjuvant. One ml of the emulsion was injected subcutaneously at five different sites. Three further injections were given at weekly intervals using similarly prepared inocula.

Bovine /

Bovine anti-bovine papular stomatitis sera: Two susceptible calves were infected experimentally with bovine papular stomatitis at scarified sites. The scabs were harvested and pooled and a ten per cent suspension of the pooled scabs was prepared in phosphate buffer saline. The suspension was centrifuged at 3,500 r.p.m. for ten minutes and the supernatant fluid was then emulsified in an equal volume of incomplete Freund's adjuvant. One of the calves previously infected experimentally was inoculated intravenously with 3 ml of the virus-adjuvant emulsion. It was bled for serum four weeks later.

PREPARATION OF AGAR GELS

One per cent agar gels were prepared by autoclaving one gram of Oxoid Ionagar No.2 [1] in 100 ml of Dulbecco 'A' phosphate buffer saline for ten minutes at 10 lbs per square inch. Thiomersal for bacteriostatic purposes was added to give a concentration of 0.04 per cent.

Macro-system: Plastic petri dishes, 5 cm in diameter, were filled with 5.0 ml of the one per cent agar and were /

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[1] Oxoid Ltd., London.

were stored at 4°C until required. Wells for the reagents were punched out of the agar using a standard pattern that was hexagonal in outline with the wells at the angles and in the centre. The wells were 6 mm in diameter and were 4 mm apart. Each held 0.1 ml of reagent.

Micro-system: Plastic trays[1] each holding six glass microscope slides were flooded with molten agar which solidified to give a depth of 1 to 2 mm. The agar-coated trays were stored at 4°C until required. Wells for the reagents were punched out using a hexagonal pattern similar to that used in the macro-system except that the wells were only 3 mm in diameter and were only 2 mm apart. Each well held 0.01 ml of reagent.

METHOD

The reagents were a suspension of known-positive antigen, hyper-immune serun and suspensions, and sometimes dilutions of the suspect antigens. Pasteur pipettes were used to distribute the reagents in the wells. Diffusion proceeded at room temperature (18-22°C). The plates and slides were examined for visible lines of precipitation after twelve, twenty-four and forty-eight hours.

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[1] LKB - Produkter AB, Stockholm, Sweden.

A positive result was only recorded if the precipitation line between the suspect antigen and the hyperimmune serum fused exactly with the line between the known-positive antigen and the hyperimmune serum.

Some reactions were photographed directly. Other reactions were stained either with amido black or thiazine red after the plates were washed overnight in saline.

PROPERTIES OF THE PRECIPITATING ANTIGEN

Effect of alternate freezing and thawing: Orf suspensions with known precipitating activity were frozen at -70°C in a mixture of solid carbon dioxide and alcohol and immediately thawed in cold running water. Ten cycles of freezing and thawing were done, sampling and testing for precipitating activity at every alternate cycle.

Effect of temperature: Test tubes containing dilutions of orf-scab suspensions were held in a water-bath at 40, 50, 56, 60 and 70°C. Tubes were removed at various times, quickly cooled under cold running water and /

and the contents were titrated for precipitating activity using two-fold dilution steps in phosphate buffer saline. The half-life periods at the various temperatures were derived from the slopes of the regression lines calculated by the method of least squares (Snedecor, 1965) using the formula

$$t_{\frac{1}{2}} = \frac{0.3}{b}$$

where b is the slope of the regression line relating the precipitating titres in logarithms to time.

Sedimentation: Orf scab suspensions were centrifuged in the cold at 15,000 r.p.m. for forty-five minutes (26,168 r.c.f.) in a M.S.E. "Highspeed 17" centrifuge[1]. The supernatant fluids were tested for precipitating activity. The pellet was re-suspended in phosphate buffer saline to approximately one half of the original volume and then examined for precipitating activity.

Filtration: Orf scab suspensions were centrifuged through Hemmings asbestos pad filters[2] at 4,000 r.p.m. for fifteen minutes. The filtrates were tested for precipitating activity. Materials adsorbed by the filter pads were extracted by soaking the pads in volumes /

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[1] Measuring and Scientific Equipment Ltd., London.

[2] Carbon-Ford Sales Ltd., Lancashire.

volumes of phosphate buffer saline equivalent to one half of the original volumes. The extracts were tested for precipitating activity.

DETECTION OF COMPLEMENT-FIXING ANTIGENS

PROCESSING OF SAMPLES

Virus-infected and non-infected scabs examined by electron microscopy and for precipitating antigens were also tested for complement-fixing antigens. They were weighed and a one per cent suspension (w/v) was prepared in barbitone buffer[1] in a mortar and pestle. The suspension was centrifuged at 3,500 r.p.m. for ten minutes. The supernatant fluid was the base-line dilution of the antigen, the sediment and lipid scum being discarded.

PRODUCTION OF ANTI-SERA

The sheep anti-orf sera prepared for the immuno-diffusion tests were used to detect complement-fixing antigens.

COMPLEMENT

Freeze-dried commercial guinea pig complement[2] was reconstituted according to the manufacturer's instructions and titrated for haemolytic activity. The highest dilution causing one hundred per cent haemolysis in the presence of two minimal haemolytic units /

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[1] Oxoid Ltd., London.

[2] Burroughs Wellcome Ltd., Beckenham, Kent.

units of haemolysin was considered to be one unit of complement. Four units were used in the complement fixation tests.

HAEMOLYTIC SYSTEM

Sheep red blood cells were obtained by bleeding from the jugular vein into Alsever's solution. The cells were centrifuged and re-suspended in fresh Alsever's solution three times before being finally packed and stored at 4°C until required.

The source of haemolytic serum was commercially-prepared rabbit anti-sheep red blood cell serum[1]. Checker-board titrations were used to determine the highest dilution of haemolytic serum causing fifty per cent lysis of a two per cent suspension of sheep red blood cells in the presence of a slight excess of complement. The end-point was designated the minimal haemolytic unit and two units were used in the complement fixation tests.

METHOD /

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[1] Burroughs Wellcome Ltd., Beckenham, Kent.

METHOD

The direct complement fixation procedure was used and was carried out in WHO perspex agglutination plates[1]. Two-fold dilutions of the one per cent orf scab suspension were made in blanks containing 0.1 ml barbitone buffer up to a dilution of 1:6,400. Sheep anti-orf sera were diluted 1:5 in barbitone buffer and inactivated for forty minutes in a water-bath at 56°C. Then 0.1 ml of the inactivated diluted sera and four units of complement in 0.1 ml were added to each antigen dilution. Fixation proceeded at 35°C for forty-five minutes.

Controls set up with each test were scab dilutions mixed with complement, anti-serum and complement, non-infected sheep scab suspension in anti-serum and complement, known virus-infected orf scab suspension in anti-serum and complement, known virus-infected orf scab suspension in normal sheep serum and complement, and a check of the activity of the complement using 4, 2, 1, 0.5 and 0.25 units of complement.

Fifteen minutes before the end of the fixation period the haemolytic system was assembled by mixing equal volumes of two per cent sheep red blood cells and haemolysin diluted to contain two minimal haemolytic doses per 0.1 ml and held at room temperature.

Each /

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[1] Prestware Ltd., London.

Each well received 0.1 ml of the haemolytic system and thereafter the plates were incubated with shaking every ten minutes for a further forty minutes at 35°C. The mixtures were allowed to settle at room temperature for two hours before being assessed. The end-point was taken as the highest dilution of the antigen suspension fixing fifty per cent of the four units of complement in the presence of anti-orf serum (Fig. 3).

PROPERTIES OF THE COMPLEMENT-FIXING ANTIGEN

Effect of alternate freezing and thawing: One per cent suspensions of orf-infected scabs in barbitone buffer known to fix complement were frozen at -70°C in a mixture of solid carbon dioxide and alcohol and immediately thawed under cold running water. Ten cycles of freezing and thawing were carried out, sampling and testing for complement-fixing activity at every second cycle.

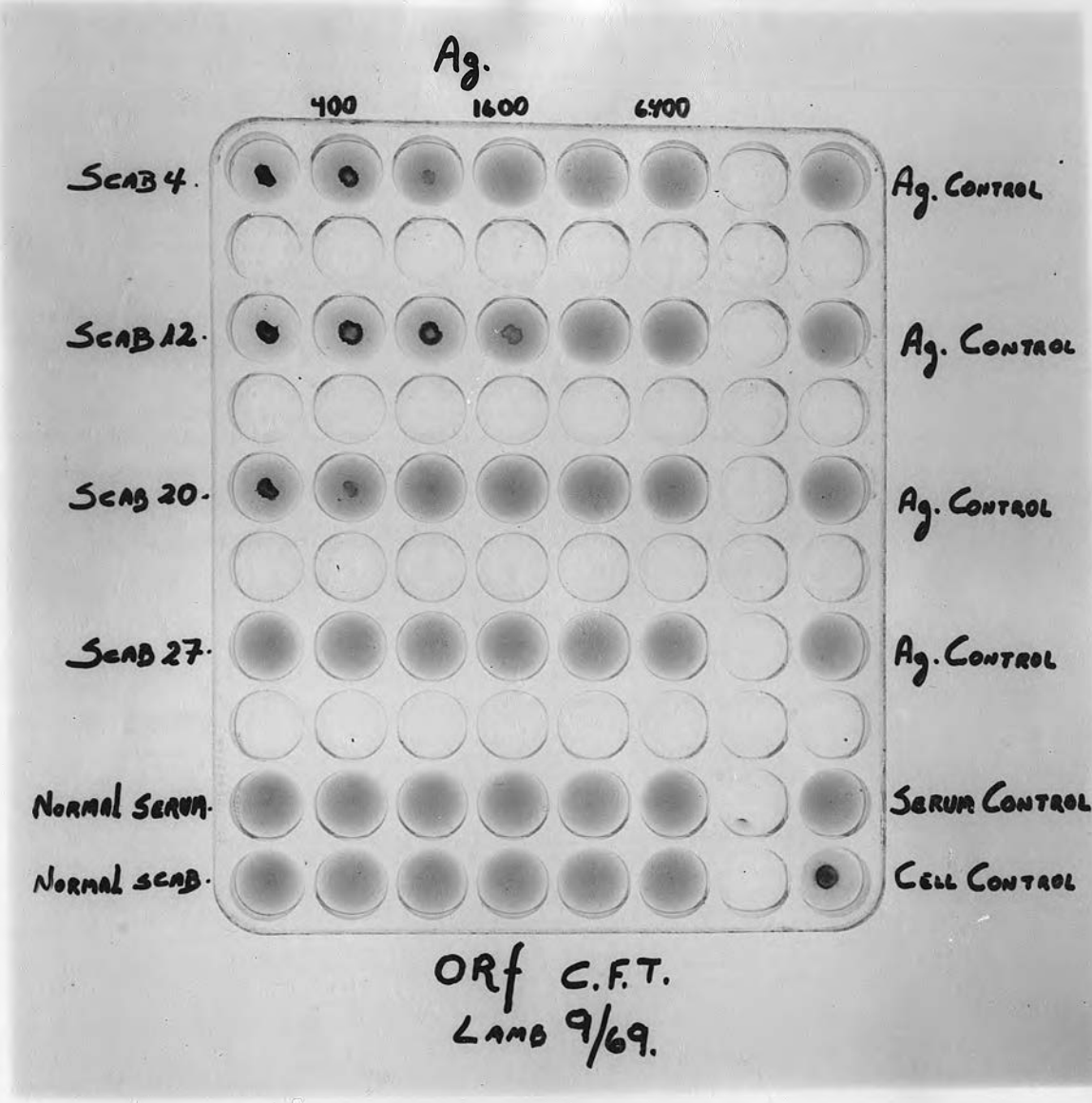
Effect of temperature: Test-tubes containing one per cent suspensions of orf-infected scabs were held in a water-bath at 56, 60 and 70°C for varying periods. When tubes were removed, the contents were cooled under cold running water and then titrated for complement-fixing activity.

Sedimentation: /

Sedimentation: One per cent suspensions of orf-infected scabs were centrifuged in the cold at 15,000 r.p.m. for forty-five minutes (26,168 r.c.f.) in a M.S.E. "Highspeed 17" centrifuge. The supernatant fluids were tested for complement-fixing activity. The pellets were re-suspended in a volume of barbitone buffer equivalent to the original volume and then tested for complement-fixing activity.

Filtration: One per cent suspensions of orf-infected scabs were centrifuged through Hemmings asbestos pad filters at 4,000 r.p.m. for fifteen minutes. The filtrates were tested for complement-fixing activity.

Fig. 3 - A typical complement-fixation test for the detection of orf-specific antigen.



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DETECTION OF ANTIGENS BY FLUORESCENT ANTIBODIES

PROCESSING OF SAMPLES

Fresh orf scabs harvested in the first two weeks after scarification were used for immuno-fluorescent studies.

Impression smears: The technique described by Benda et al. (1968) was followed. Impression smears of the under-surface of the scabs were made on clean microscope slides. They were allowed to dry and then used as such or after fixation in acetone for twenty minutes.

Re-suspended pellets: A twenty per cent scab suspension in phosphate buffer saline was prepared and centrifuged in the cold at 3,000 r.p.m. for thirty minutes. The supernatant fluid was carefully removed and re-centrifuged at 15,000 r.p.m. for one hour. The pellet was re-suspended in a few drops of phosphate buffer saline and smears were prepared on clean microscope slides. The smears were allowed to dry and used as such or were fixed in acetone for twenty minutes.

Sephadex G-200 fractionated virus: Orf virus suspensions were fractionated through Sephadex G-200 and the virus-containing fractions were examined by the

fluorescent /

fluorescent antibody technique. Smears were prepared on clean microscope slides and allowed to dry and were used as such or were fixed in acetone for twenty minutes.

Kaolin-treated scabs: One volume of fresh orf-scabs was ground in two volumes of phosphate buffer saline and centrifuged at 3,000 r.p.m. for ten minutes. An equal volume of twenty-five per cent kaolin solution was added to the supernatant fluid and the mixture was agitated for twenty minutes at 4°C. After centrifugation smears of the supernatant fluid were prepared and were dried or fixed in acetone for twenty minutes.

Fluorocarbon-treated scabs: One volume of fresh orf-scabs was ground in two volumes of phosphate buffer saline and then centrifuged at 3,000 r.p.m. for ten minutes. Half a volume of Arcton[1] was added to the supernatant fluid and the mixture was agitated for ten minutes and then centrifuged at 3,500 r.p.m. for ten minutes. The clear liquid phase was carefully removed and smears were prepared on clean microscope slides and allowed to dry. The smears were used as such or after fixation in acetone for twenty minutes.

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[1] Imperial Chemical Industries Ltd., Runcorn, Cheshire.

PRODUCTION OF CONJUGATED SERA

Sheep hyperimmune anti-orf sera were fractionated by column chromatography using Sephadex G-200. The fractions containing orf-precipitating activity were identified in the 7S peak and utilised as the source of specific globulins. Pools of the 7S globulins were dialysed at 4°C against phosphate buffer saline and the protein content adjusted to two per cent either by further concentration against polyethylene glycol and dialysis or by the addition of phosphate buffer saline. Fluorescein isothiocyanate[1] that had been dried in a desiccator containing calcium chloride was used to label the globulins. The final concentration was twenty-five micrograms of fluorescein per milligram of globulin and the conjugation was carried out at a final protein concentration of ten milligrams per ml at pH 9.0 by diluting the globulin solution to twice the original volume in a 0.15 molar sodium chloride solution and a volume of carbonate-bicarbonate buffer equal to one tenth of the final volume. The globulin-fluorescein mixture was gently agitated for eighteen hours at 4°C. Then the conjugate was dialysed in the cold through Visking tubing No. 24/32[2] against phosphate buffer /

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[1] George T. Gurr Ltd., London.

[2] Scientific Instrument Centre Ltd., London.

buffer saline until the dye ceased to come out. Several changes of phosphate buffer saline were required. The conjugate was further purified by passing it through a chromatography column containing Sephadex G-25 coarse to remove unbound fluorescein. Two separate yellow bands were observed to migrate down the column; the first fast moving band was collected in different fractions and concentrated in the cold against polyethylene glycol to approximately the original volume. The concentrate constituted the fluorescent anti-orf serum.

TECHNIQUE

The direct fluorescent antibody technique was used. Smears were flooded with two drops of the conjugate either undiluted, diluted 1:2 or 1:4 in phosphate buffer and were incubated in a humid chamber at 35°C for thirty minutes. Then excess conjugate was tipped off and the smears were washed three times for ten minutes with phosphate buffer saline. After drying the smears were examined under ultra-violet light or blue light using the Niken model S microscope[1].

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[1] Nippon Kogaku K.K., Tokyo, Japan.

GEL CHROMATOGRAPHY FRACTIONATION OF ORF SCABS

APPARATUS

An LKB gel chromatography[1] unit was used The gel was a cross-linked dextran, Sephadex G-200[2], which had a particle diameter of 40 to 120 micrometers and the columns were either 2.5 cm in diameter and 100 cm long (25/100), or, more often, 2.5 cm in diameter and 45 cm long (25/45). Fractions were collected in an LKB Radi-Rac which had a time-control. The flow was monitored by a Uvicord[1] ultra-violet light spectrophotometer and recorded.

TECHNIQUE

Sephadex G-200 was allowed to swell in an excess of de-ionised water for twenty-four hours with occasional stirring. The supernatant fluid was decanted and discarded. Dulbecco "A" phosphate buffer saline at pH 7.3 was added. The mixture was stirred and then allowed to settle. Again the supernatant fluid was decanted and discarded. The process was repeated several times until the Sephadex gel was equilibrated in the buffer. The swollen thick slurry was de-gassed by autoclaving for ten minutes at a pressure of 10 lbs per square inch.

The /

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[1] LKB - Produkter AB, Stockholm, Sweden.

[2] Pharmacia Fine Chemicals Inc., Uppsala, Sweden.

The columns were fixed in a plumb vertical position, one quarter filled with phosphate buffer saline and topped with the gel. Two volumes of buffer were then passed through the gravity-packed gel. Blue dextran 2000[2] was passed through the column to determine the void volume. Orf scab suspensions were centrifuged and the supernatant fluids were injected into the bottom of the chromatography column in volumes that varied from 5 to 7 ml. A continuous upward flow of buffer ensured continuous elution. Homogeneous fractions were pooled, concentrated with polyethylene glycol at 4°C and dialysed against phosphate buffer saline for twenty-four hours at 4°C.

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2 Pharmacia Fine Chemicals Inc., Uppsala, Sweden.

DETECTION OF PRECIPITATING ANTIBODIES

SOURCE OF SERA

Convalescent sera: Suffolk and Cheviot home-bred orf-susceptible lambs were observed every week for the development of natural orf. As soon as the animals showed the first signs of the disease they were bled and the bleeding was repeated at one, two, four, eight, twelve and sixteen weeks.

Passively-transferred antibodies: Six ewes were bled as soon as possible after lambing and pre-suckling colostrum obtained. Their newly born lambs were bled before they sucked colostrum and twice within twenty-four hours after the ingestion of colostrum. The colostrum samples were centrifuged at 3,500 r.p.m. for fifteen minutes to separate the butter-fat. The casein-containing whey was saved and tested for precipitating antibodies. The sera, likewise, were tested for antibodies.

Post-vaccinal sera: Ellon lambs: A flock of twenty-five Greyface lambs six months of age were bought from a farm where the disease had been absent for the last six years. The lambs were bled and vaccinated by scarification on the inner side of the thigh with 0.1 ml of a twenty per cent orf scab suspension containing antibiotics /

antibiotics. The lambs were bled four, eight, twelve and sixteen weeks after the primary vaccination and their sera were assayed for precipitating antibodies.

Home-bred lambs: Home-bred Suffolk and Cheviot lambs were bled within seven days of birth and were then vaccinated by scarification. The lambs were observed every week and bled every four weeks for twenty weeks and their sera were assayed for precipitating antibodies.

Post-challenge sera: Groups of vaccinated Greyface lambs were bled and challenged by scarifying the inner thighs and applying a suspension of orf scabs four, eight, twelve and sixteen weeks after vaccination. They were bled when challenged and again one and two weeks later. The sera was assayed for precipitating antibodies.

Control sera: Sheep anti-orf sera: The sera of sheep hyperimmunised against orf were used as the positive control sera.

Orf-negative sera: Sera were collected from colostrum-deprived lambs and from lambs known to be free of infection and were used as negative control sera.

PROCESSING /

PROCESSING OF SERA

Lambs were bled from the jugular vein using disposable syringes. The blood was allowed to clot at room temperature for at least three hours. The samples were shaken to free the clots and then centrifuged at 3,500 r.p.m. for ten minutes. The supernatant serum was harvested and stored at -20°C until required.

PRODUCTION OF ANTIGENS

Orf scabs known to be rich in virus particles were weighed and twenty per cent suspensions (w/v were prepared in phosphate buffer saline. The supernatant fluids separated by light centrifugation were used as the known positive antigens. Similarly known negative antigens were prepared from non-infected sheep scabs.

TECHNIQUE

The macro-immuno-diffusion system in petri-dishes was always used to test unknown sera. The central well was filled with the known positive control serum, the top and lower right wells contained the known positive /

positive antigen suspension and the lower left well contained the known negative antigen suspension. The unknown serum was distributed into the upper left and right wells and the bottom well. The plates were held at room temperature and examined for lines of precipitation twenty-four and forty-eight hours later.

DETECTION OF COMPLEMENT-FIXING ANTIBODIES

PROCESSING OF SAMPLES

Sera from lambs convalescent from orf, from lambs vaccinated and challenged with orf and from non-infected lambs tested for precipitating antibodies were also examined for complement-fixing antibodies. In addition samples of colostrum were tested after the butter-fat had been removed and after the casein in the whey had been precipitated out by the slow addition of 1 N hydrochloric acid until the pH was 4.6.

The sera were diluted 1:4 with barbitone buffer and inactivated in a water-bath for forty minutes at 56°C. The pH of the whey was adjusted to 7.0 by adding drops of one molar sodium hydroxide and then the whey was diluted 1:4 with barbitone buffer. The diluted whey was also inactivated at 56°C for forty minutes.

PRODUCTION OF ANTIGENS

Orf scab suspensions known to fix complement in the presence of sheep hyperimmune anti-orf sera were used at a constant dilution of 1:100. Similarly known negative sheep scab suspensions were used as negative control antigens.

TECHNIQUE /

TECHNIQUE

The direct complement fixation procedure was used and was carried out in perspex agglutination plates. Two-fold dilutions of inactivated sera or whey were made in blanks containing 0.1 ml barbitone buffer up to a dilution of 1:256. Known positive antigen diluted 1:100 in barbitone buffer was added using volumes of 0.1 ml together with four units of guinea pig complement in 0.1 ml. Fixation was allowed to proceed at 35°C for forty-five minutes.

Controls incorporated in each test were serum dilutions mixed with complement, positive antigen and complement, negative serum with positive antigen and complement, known positive serum with known positive antigen and complement, and a check of the activity of the complement using four, two, one, 0.5 and 0.25 units.

The haemolytic system was sensitised for fifteen minutes at room temperature and then 0.1 ml calculated to contain one minimal haemolytic dose was added to each well. The plates were incubated for a further forty minutes at 35°C being shaken every ten minutes. The test was read after a two hour settling period at room temperature, the end-point being taken as the highest dilution of serum fixing fifty per cent of the complement.

DETECTION OF NEUTRALISING ANTIBODIES

PROCESSING OF SAMPLES

Sera from sheep hyperimmunised against orf and known to contain precipitating and complement-fixing antibodies were used. Negative sera were collected from colostrum-deprived lambs. All sera were diluted 1:5 with phosphate buffer saline and inactivated by heating to 56°C for thirty minutes. They were then filtered through asbestos pads and some were added to Eagle's or Earle's growth media to give a final concentration of twenty per cent.

PRODUCTION OF VIRUS

Ten per cent suspensions of orf scabs known to be rich in virus particles were prepared in phosphate buffer saline and centrifuged. The supernatant fluids were harvested and treated with antibiotics.

TECHNIQUES

The objective was to neutralise the so-called "cytotoxic effect" of orf virus on cell cultures. Two techniques were tried. First, a series of two-fold and, sometimes, ten-fold dilutions of orf scab suspensions /

suspensions were mixed with equal volumes of 1:5 sheep anti-orf serum and similar series were mixed with equal volumes of 1:5 known negative sheep serum. The serum-virus mixtures were held at room temperature for two hours before 0.2 ml volumes were inoculated into cell monolayer culture tubes using four tubes per dilution. After two hours adsorption the inocula were decanted and replaced with 1.0 ml of Eagle's maintenance medium containing two per cent calf serum. The cultures were incubated for twenty-four hours at 35°C and then assessed for cell damage.

Secondly, cell monolayers were infected with ten-fold dilutions of orf scab suspensions. After thirty minutes adsorption the inocula were discarded and the monolayers were washed three times with phosphate buffer saline. One ml of culture medium containing twenty per cent sheep anti-orf serum was added to each of four tubes inoculated with the same concentration of virus. Simultaneously four equivalent tubes received one ml of culture medium containing twenty per cent known negative sheep serum and four other tubes received one ml of culture medium containing two per cent calf serum. The cultures were incubated for twenty-four hours at 35°C and then assessed for cell damage.

GEL CHROMATOGRAPHY FRACTIONATION OF SERUM

Sheep anti-orf serum, rabbit anti-orf serum and rabbit anti-whole sheep serum were fractionated through Sephadex G-200 gels packed in a 25/45 chromatography column. The apparatus used was the LKB gel chromato-:graphy unit and the flow was monitored spectrophoto-:metrically. The volume of serum fractionated varied from 3 to 8 ml and 0.02 per cent sodium azide was incorporated in the buffer solution.

IMMUNO-ELECTROPHORESIS

PROCESSING OF SAMPLES

Rabbit anti-whole sheep serum serum: One ml of adult sheep serum was mixed with an equal volume of incomplete Freund's adjuvant and one drop of Tween 80. One ml of the adjuvant emulsion was injected intravenously and simultaneously 0.2 ml were injected subcutaneously into five sites in a rabbit. Five and six weeks later the rabbit was given identical courses of injections. One week after the last course the rabbit was bled for serum and challenged by the intravenous injection of one ml adult sheep serum. After a further week one ml of /

of the Sephadex G-200 fraction containing the 19S globulins was injected intravenously. The rabbit was bled out for serum five days after the last injection.

Sheep sera and colostrums: Six ewes were bled at parturition and colostrum samples were taken. Their new-born lambs were bled for serum before and after taking colostrum.

The butter-fats in the colostrum samples were removed by centrifugation of the samples at 3,500 r.p.m. for fifteen minutes and discarded. The casein-containing whey was examined without further treatment.

TECHNIQUE

Immuno-electrophoresis was carried out in the LKB Immunophor Standard Set[1]. Two grams of Ionagar No. 2[2] were dissolved in 98 ml of distilled water by autoclaving at 10 lbs per square inch for ten minutes. Two volumes of barbitone buffer at pH 8.6 were added to one volume of distilled water, heated to 60°C and mixed with the two per cent agar at 60°C. Thiomersal was added as a bacteriostatic to give a final concentration of 0.04 per cent. The buffer-agar mixture /

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1 LKB - Produkter AB, Stockholm, Sweden.

2 Oxoid Ltd., London.

mixture was poured over six microscope slides previously coated thinly with 0.1 per cent agar. The treated slides were stored in a humid chamber at 4°C for up to five days until required.

Wells were excavated in the agar overlays and were filled with 0.01 ml of the serum or colostrum samples. A voltage of 200V was applied for two hours to separate the antigens electrophoretically. Then a trough was cut between the wells and filled with 0.1 ml of rabbit anti-whole sheep serum serum. Diffusion proceeded for twenty hours at room temperature (18-20°C).

The nomenclature used by Jonas (1968) was adopted to describe and differentiate the immunoglobulins.

RESULTS

BIOLOGICAL TESTS

Suspensions of orf scabs collected from primary lesions on forty-two experimentally-infected lambs during the first twenty-nine days after scarification with virus produced, without exception, typical lesions of experimental orf when scarified on the inner sides of the thighs of susceptible lambs (Table 2; Fig. 4). The durations of the experimentally-produced orf lesions were closely followed in twenty-four susceptible lambs. They ranged from three to nine weeks with a median of five weeks in all but one lamb in which the lesion persisted for thirteen weeks (Table 3; Fig. 5). The overall median was 5.5 weeks and the overall mean 5.9 weeks.

TABLE 2

BIOLOGICAL TEST IN SUSCEPTIBLE LAMBS

Age of scabs (days)	Number examined	Number positive
10	7	7
11	1	1
12	1	1
13	1	1
14	12	12
16	2	2
17	6	6
18	1	1
21	4	4
22	2	2
24	2	2
25	1	1
26	1	1
29	1	1

Fig. 4 - Distribution by age of orf scabs positive by biological tests.

Biological Test in Susceptible Lambs

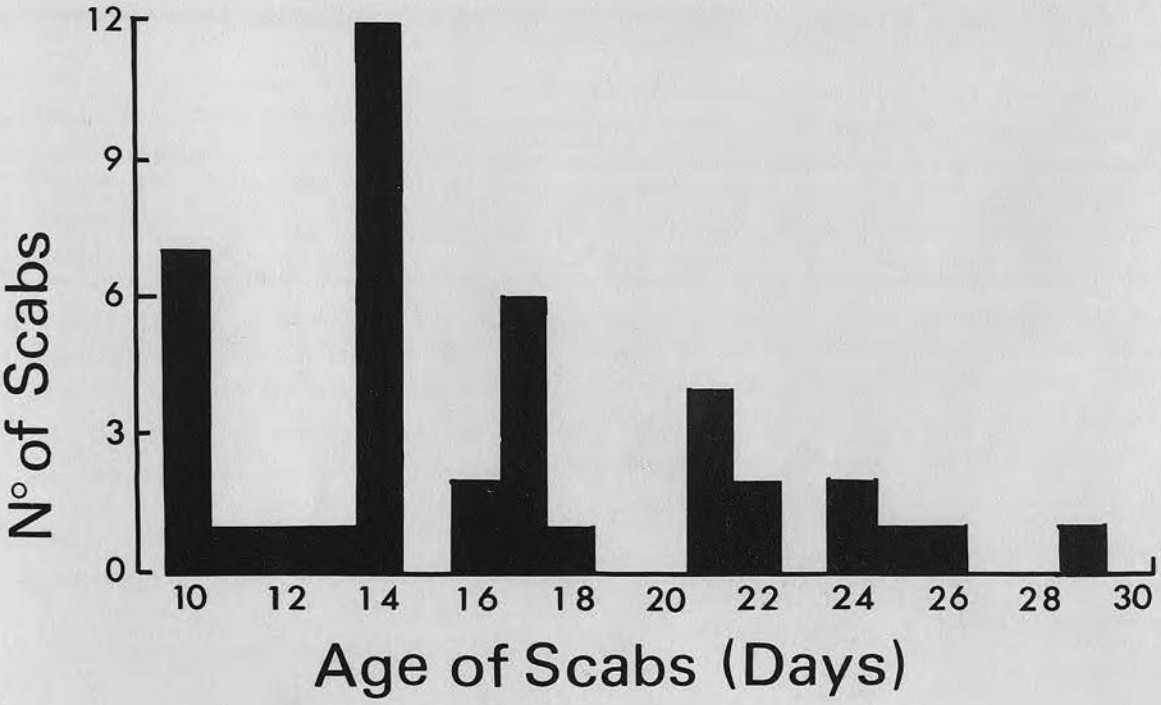


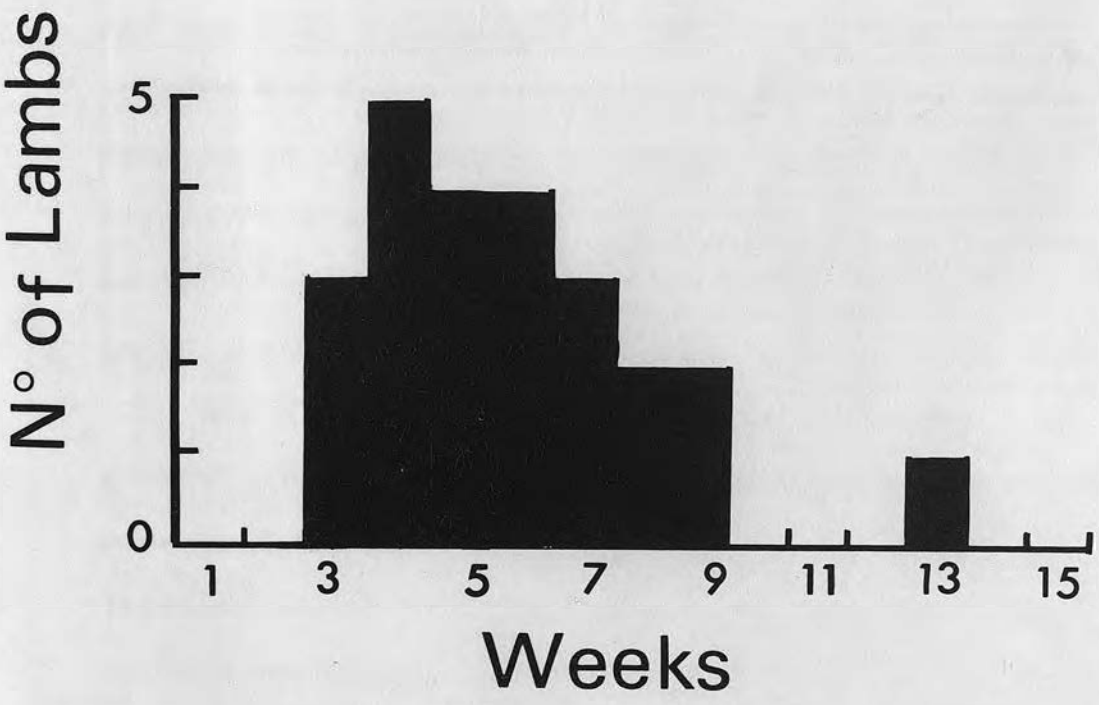
TABLE 3

DURATION OF THE PRIMARY REACTIONS IN SUSCEPTIBLE LAMBS

Duration of Lesions (weeks)	Number of Lambs
1	0
2	0
3	3
4	5
5	4
6	4
7	3
8	2
9	2
10	0
11	0
12	0
13	1

Fig. 5 - Distribution of the durations of primary orf reactions in susceptible lambs.

Duration of the Primary Reaction in Susceptible Lambs



CELL CULTURAL STUDIES

A total of thirty-six scabs collected from orf lesions that were five to twenty-eight days old and were known, by electron microscopy, to contain virus particles were processed and the suspensions were inoculated into ten types of cell cultures. All induced cytopathic changes within twenty-four hours and complete destruction of the cell sheets within seventy-two hours whereas suspensions of two virus-free scabs were innocuous (Figs. 6 and 7). The titres of eight scabs in Hep 2 cells ranged from $10^{-4.5}$ to $10^{-5.5}CPD_{50}$ per gram and the titres of three scabs in RK_{13} cells were all $10^{-4.5}CPD_{50}$ per gram (Table 4).

The cytopathic effect was manifested by a generalised refractile rounding of cells; the nuclei were pyknotic and the cytoplasms were contracted. Inoculation of fresh monolayers with affected cell culture medium or with frozen and thawed preparations of whole affected cultures did not cause cytopathic changes. Inoculation of cell suspensions, however, depressed the plating efficiency of the cells in three serial passages but not in four.

Cytopathogenesis /

Cytopathogenesis was closely linked to the virus particles. The activity, for example, was sedimented with the virus particles when scab suspensions were subjected to high-speed centrifugation, the supernatant fluids being harmless. Similarly the activity was found to occur in the Sephadex G-200 gel chromatography fractions corresponding to the first peak and ascending side of the second peak. In other words, it overlapped the fractions containing the virus particles. The cytopathogenic activity was not dialysable and it was unaffected by absorption with kaolin, a technique known to remove toxic lipoproteins. Partial neutralisation of the activity was achieved by prior incubation with sheep anti-orf serum.

The cytopathic effect was only demonstrable on the first serial passage of virus on established monolayers. On subsequent passages no cytopathic effects were observed though a depression of plating efficiency did occur.

Fig. 6 - Cell monolayer twenty-four hours after inoculation with a suspension of virus-free scab.

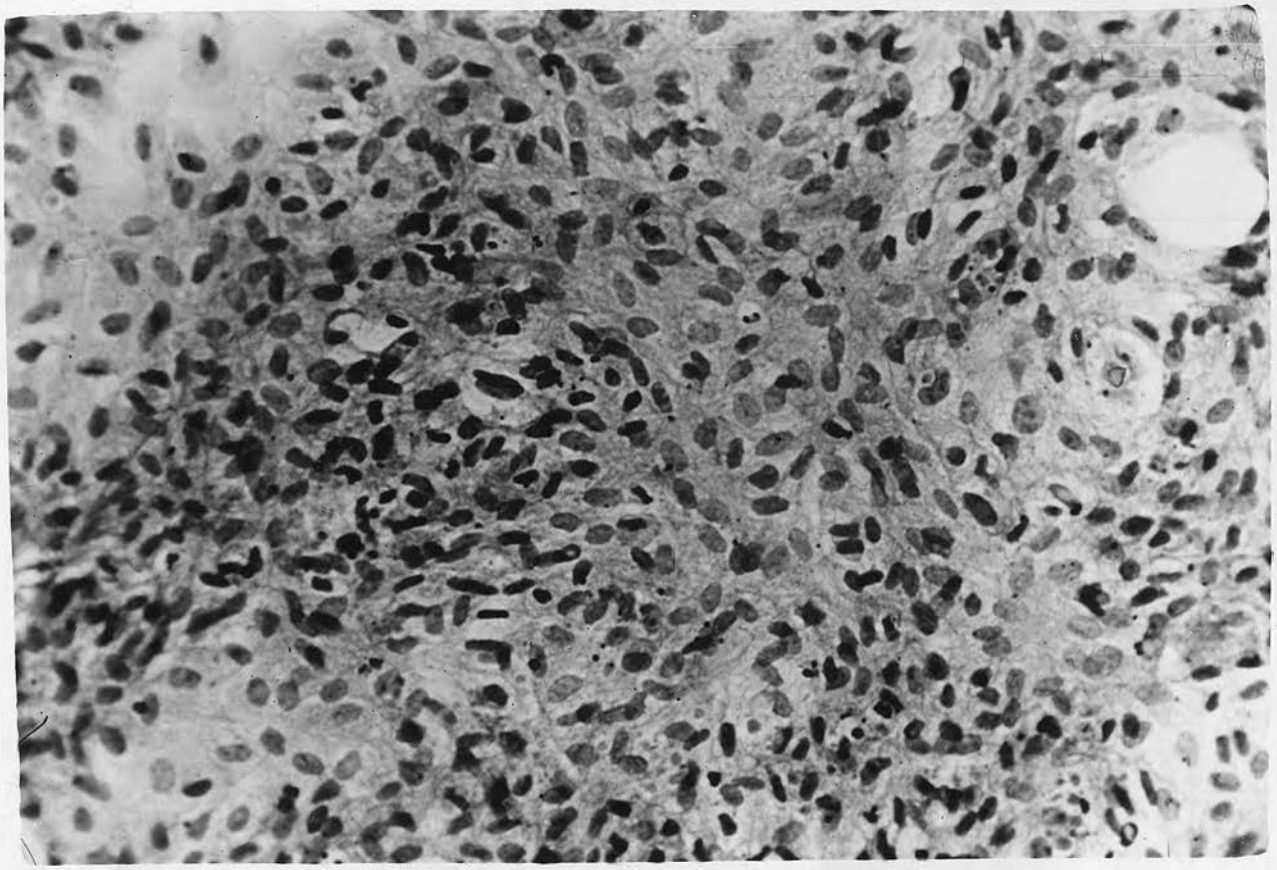


Fig. 7 - Cell monolayer twenty-four hours after inoculation with a suspension of orf virus infected scab.

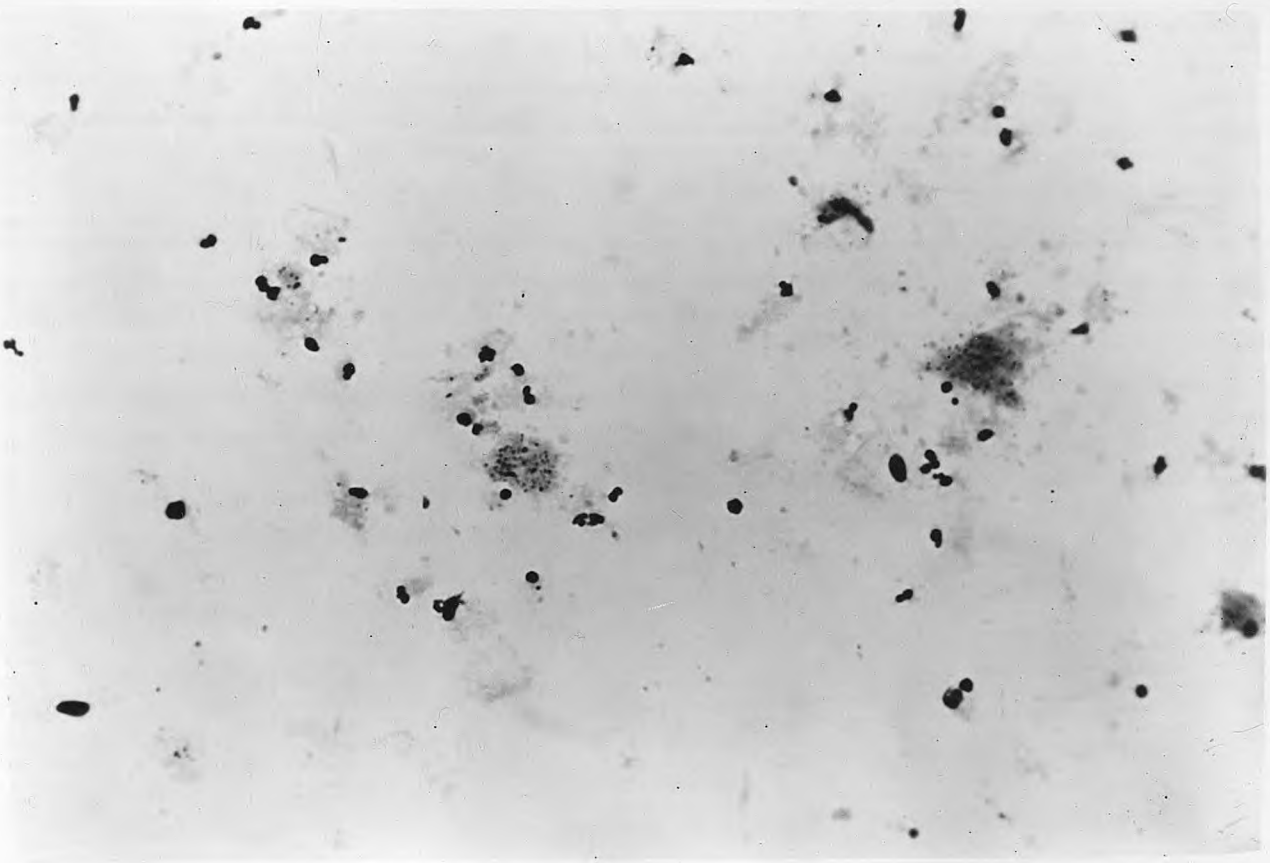


TABLE 4

Titres of Orf Scab* Suspensions in Cell Cultures

Days after scarification	Hep 2 cells	RK_{13} cells
5		4.5
7	5.5	
8		4.5
11	4.5	
12	4.5	
15	5.5	
16		4.5
18	4.5	
21	4.5	
22	4.5	
24	4.5	

* Expressed as the logarithms of the reciprocals of the CPD_{50}

ELECTRON MICROSCOPY

EXPERIMENTALLY-PRODUCED ORF SCABS

A total of four hundred and twenty-four orf scabs produced experimentally in sheep were examined with the electron microscope after direct negative staining. The technique proved to be quick, simple and reliable. Orf viruses were identified when present by their characteristic morphology, shape and size. They were readily differentiated from tissue debris and stain aggregates. Virus particles were seen in three hundred and eighty-two specimens (90.1 per cent) (Fig. 8).

Scabs harvested four to ten days after scarification with virus contained many virus particles (Fig. 9). Thereafter the number of particles observed decreased slowly up to day fourteen. From days fifteen to nineteen particles were always detected but their numbers were fewer. After day nineteen some negative specimens were encountered. In ten specimens only one virus particle was observed (Fig. 10). They were all scabs collected within one week of resolution of the experimental lesion.

The scanning time for scabs collected in the first /

first fourteen days was measured in seconds because of the great numbers of particles present. On the other hand, the scanning time for scabs collected twenty days or more after scarification was often measured in minutes before typical particles were found. Specimens without recognisable virus particles were scanned for ten minutes before being assessed as negative.

Types of particles observed: Characteristic particles of orf virus had the so-called criss-cross pattern or woven appearance and were oval in shape. They were observed in all the positive specimens (Fig. 11).

A second type of virus particle characterised by the absence of the criss-cross structure was also observed but not in all specimens. They had a double membrane and an oval internal electron-dense nucleoid (Fig. 12). The size and shape was the same as that of typical orf viruses.

Virus particles were readily identified at all magnifications above 1,500. At a magnification of 1,500 they were seen as small oval rings demarcated by black margins (Fig. 13). No structure was observed. At a magnification of 3,000 the shape of the viruses was characteristic and the criss-cross pattern was vaguely seen (Fig. 14).

Scabs /

SCABS FROM NATURAL CASES OF ORF

Scabs from lambs: A total of twenty-three scabs were harvested from the lips, feet and noses of lambs suffering from orf and diagnosed as such clinically. Orf virus particles were seen in fifteen (65 per cent). The numbers of particles seen varied but were usually fewer than those seen in experimentally-produced scabs.

Scabs from ewes: A total of eighteen scabs were collected from the teats of ewes showing orf-like lesions. Orf virus particles were only seen in four (22.2 per cent). The number of virus particles present was always small in comparison with the positive experimentally produced orf scabs and in one specimen only one virus particle was detected.

SCABS FROM SUSPECT CASES

A total of fifteen scabs were collected from lambs showing lesions that were considered clinically to be suggestive of orf. Five (33.3 per cent) contained orf virus particles.

LESIONS FROM NATURAL CASES OF BOVINE PAPULAR STOMATITIS

Eight samples were scanned and virus particles were observed in five (63 per cent) (Fig. 15).

VIRUS /

Fig. 8 - Distribution by age of orf scabs positive by electron microscopy.

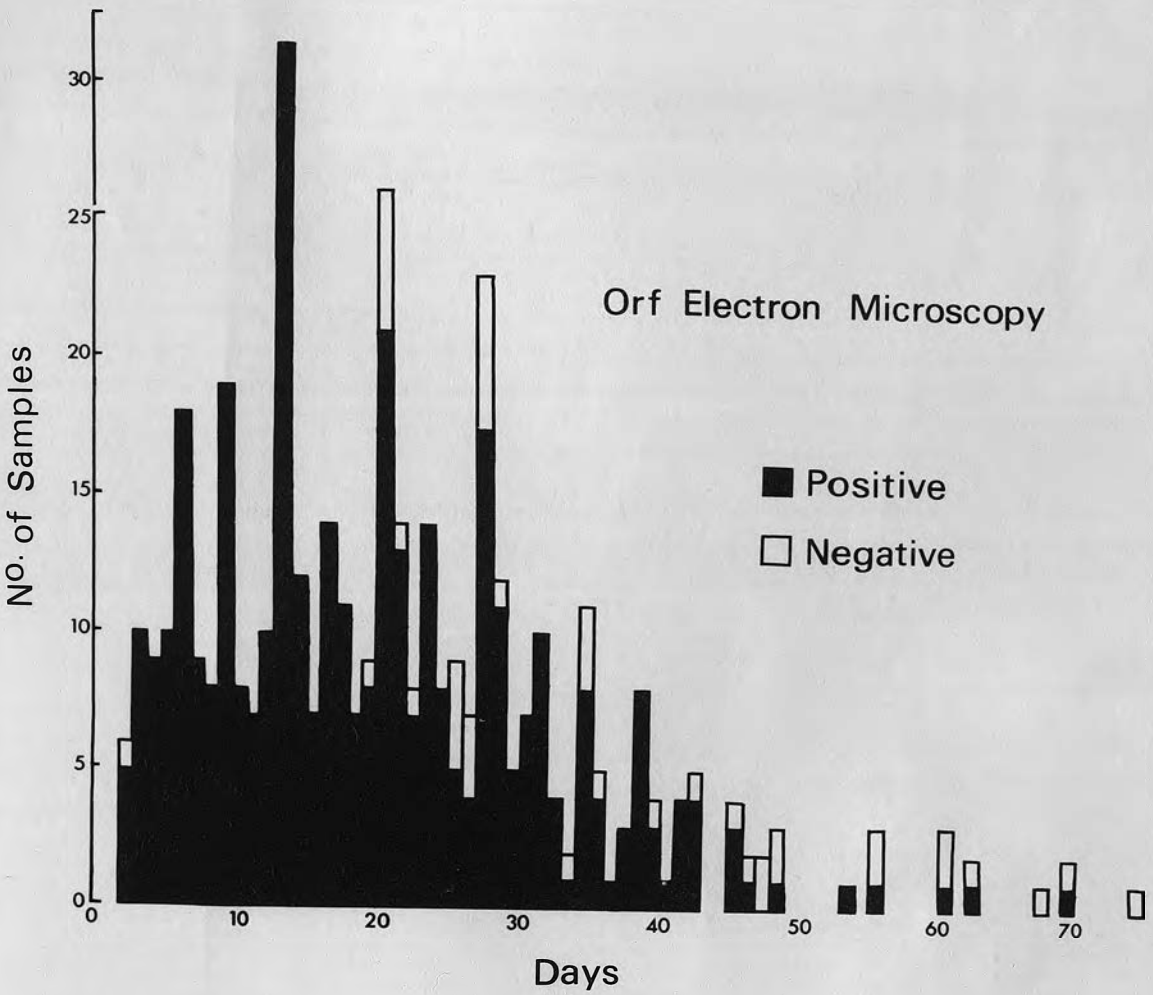

Orf Electron Microscopy
Positive
Negative
No. of Samples
Days
0
5
10
15
20
25
30
0
10
20
30
40
50
60
70

Fig. 9 - Orf virus particles in a seven day scab.

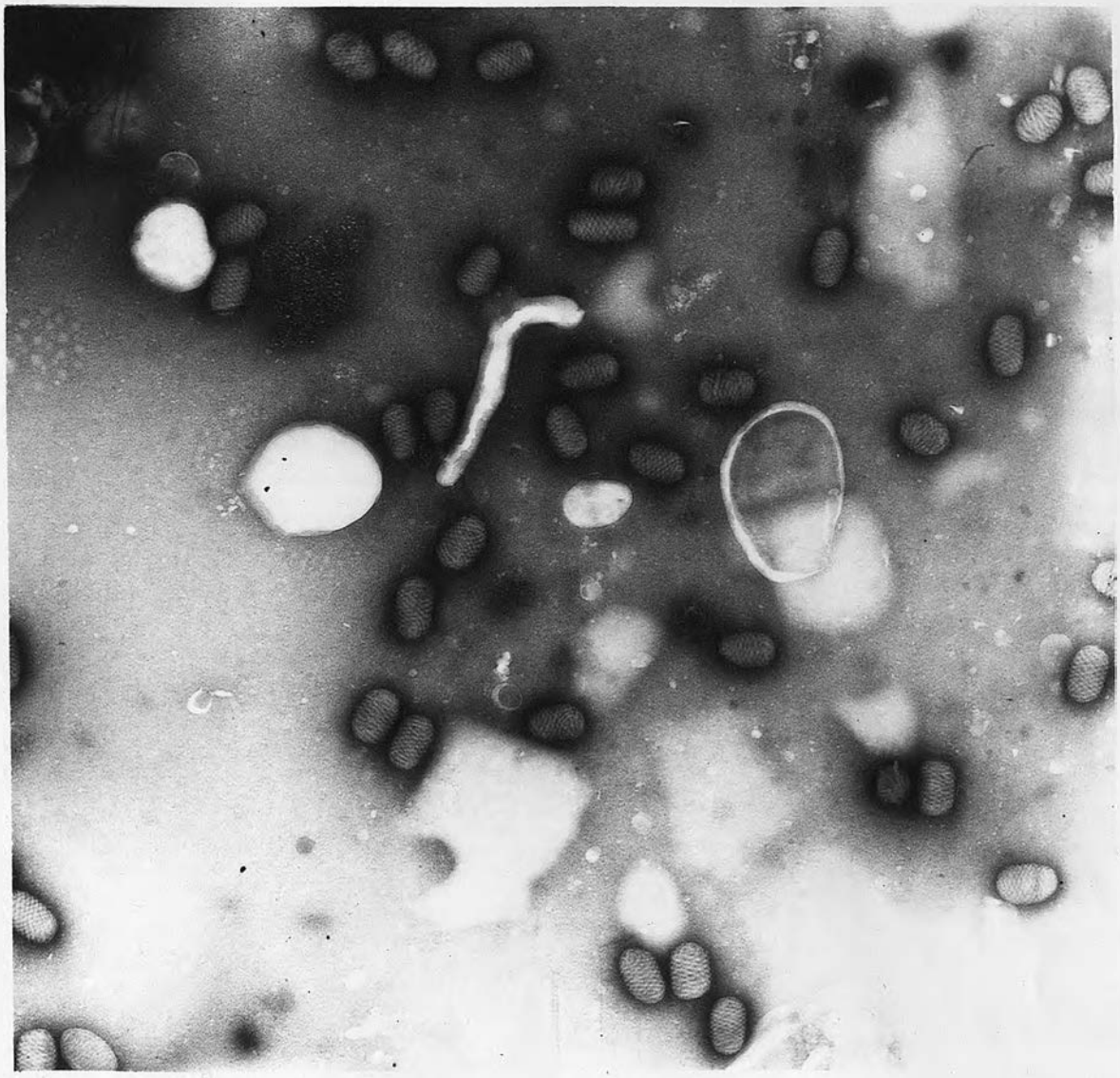


Fig. 10 - Single orf virus particle.

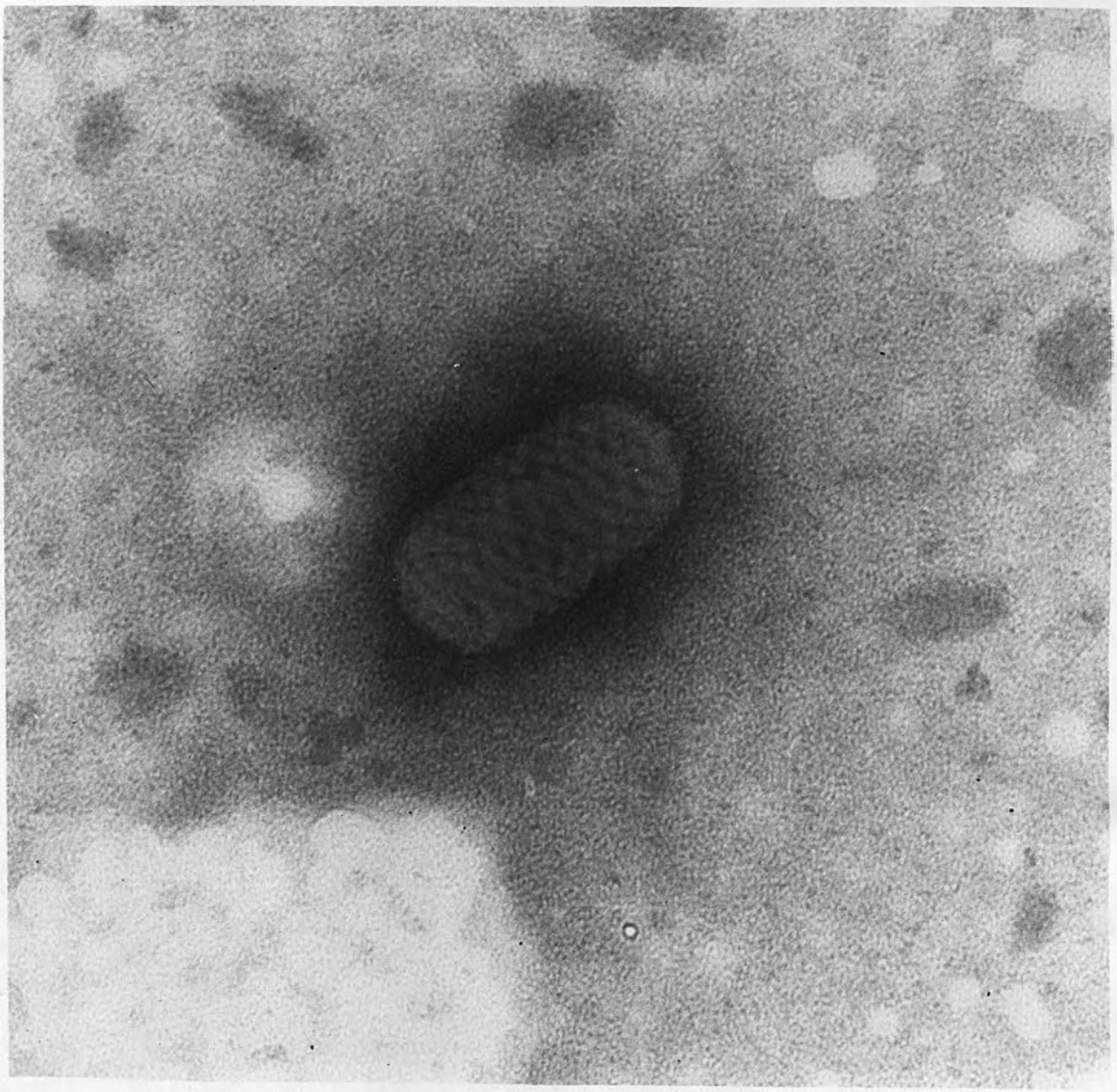


Fig. 11 - Characteristic orf virus structure.

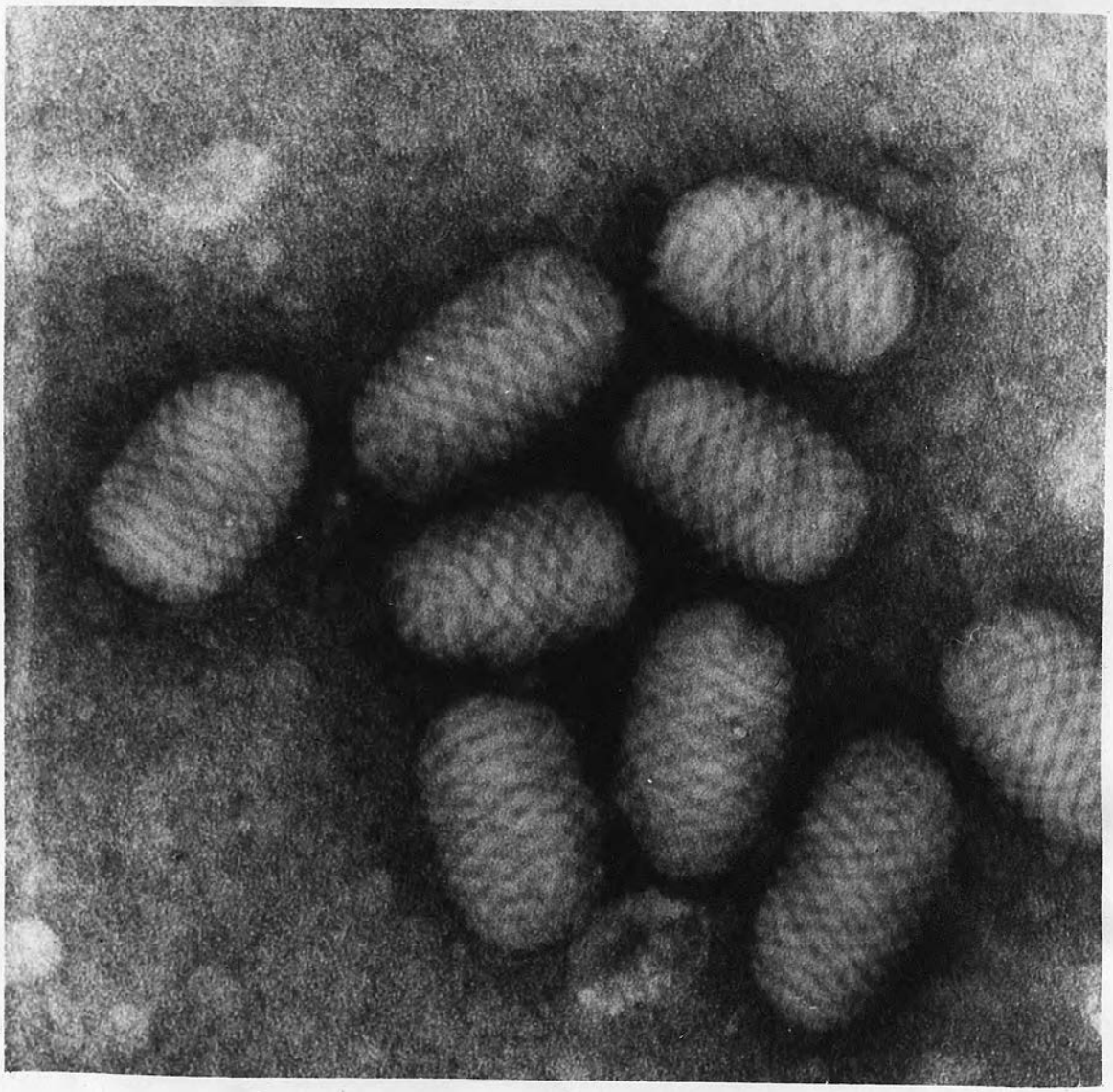


Fig. 12 - Second type of orf virus particles.

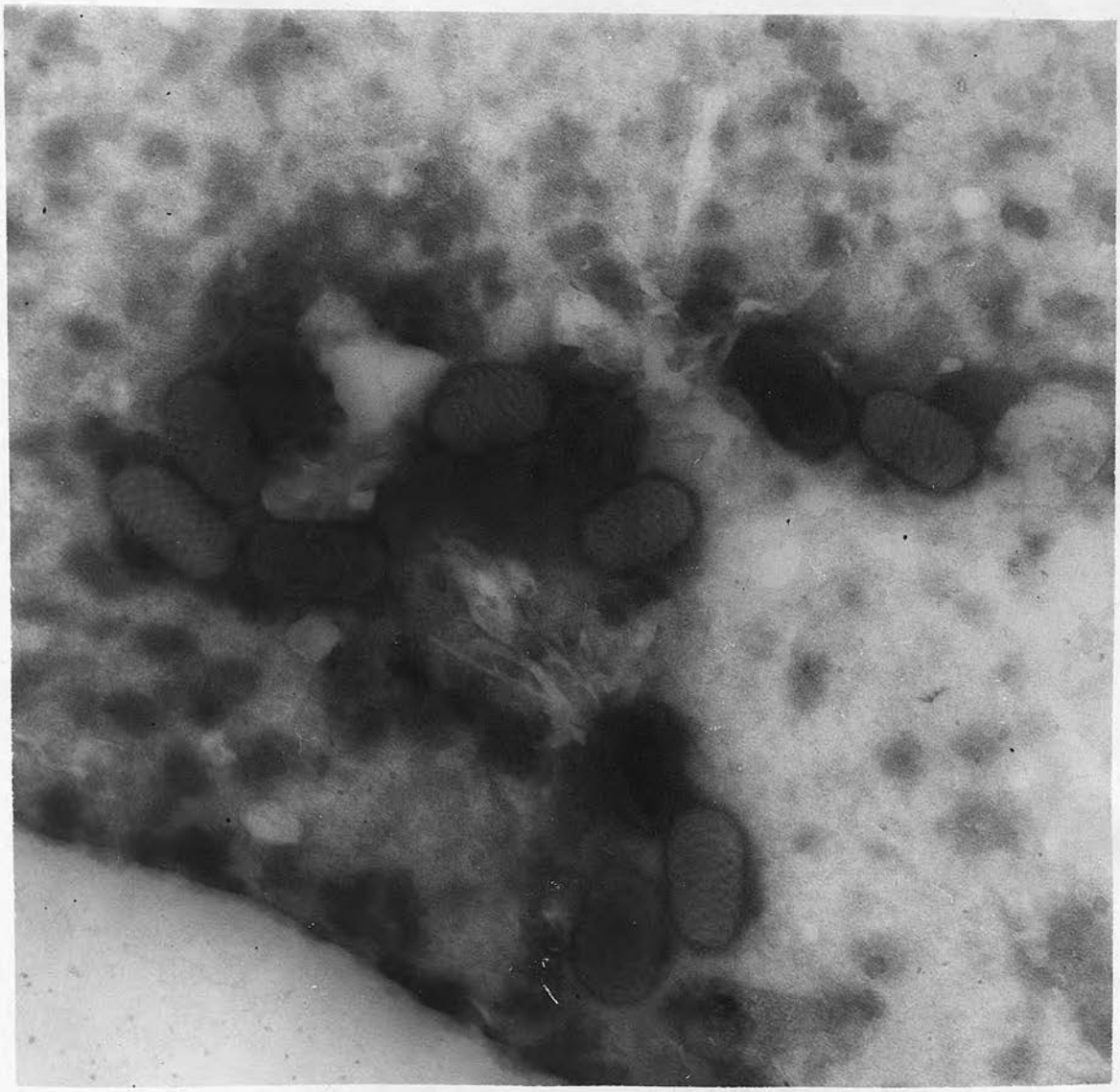


Fig. 13 - Orf virus particles X 1,500.

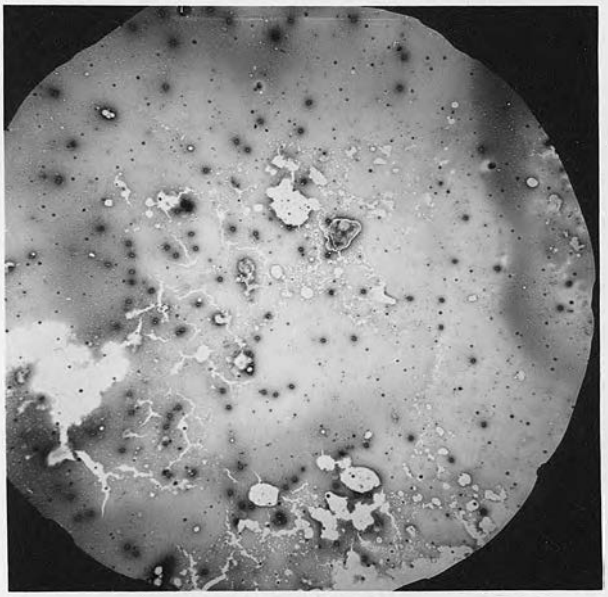


Fig. 14 - Orf virus particles X 3,000.

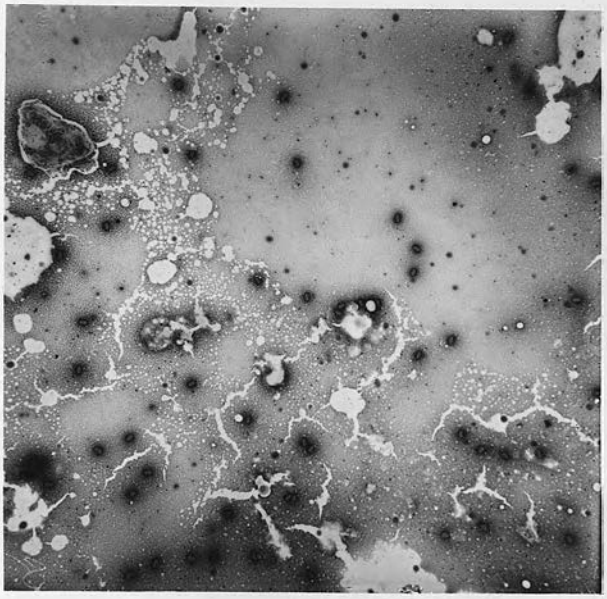
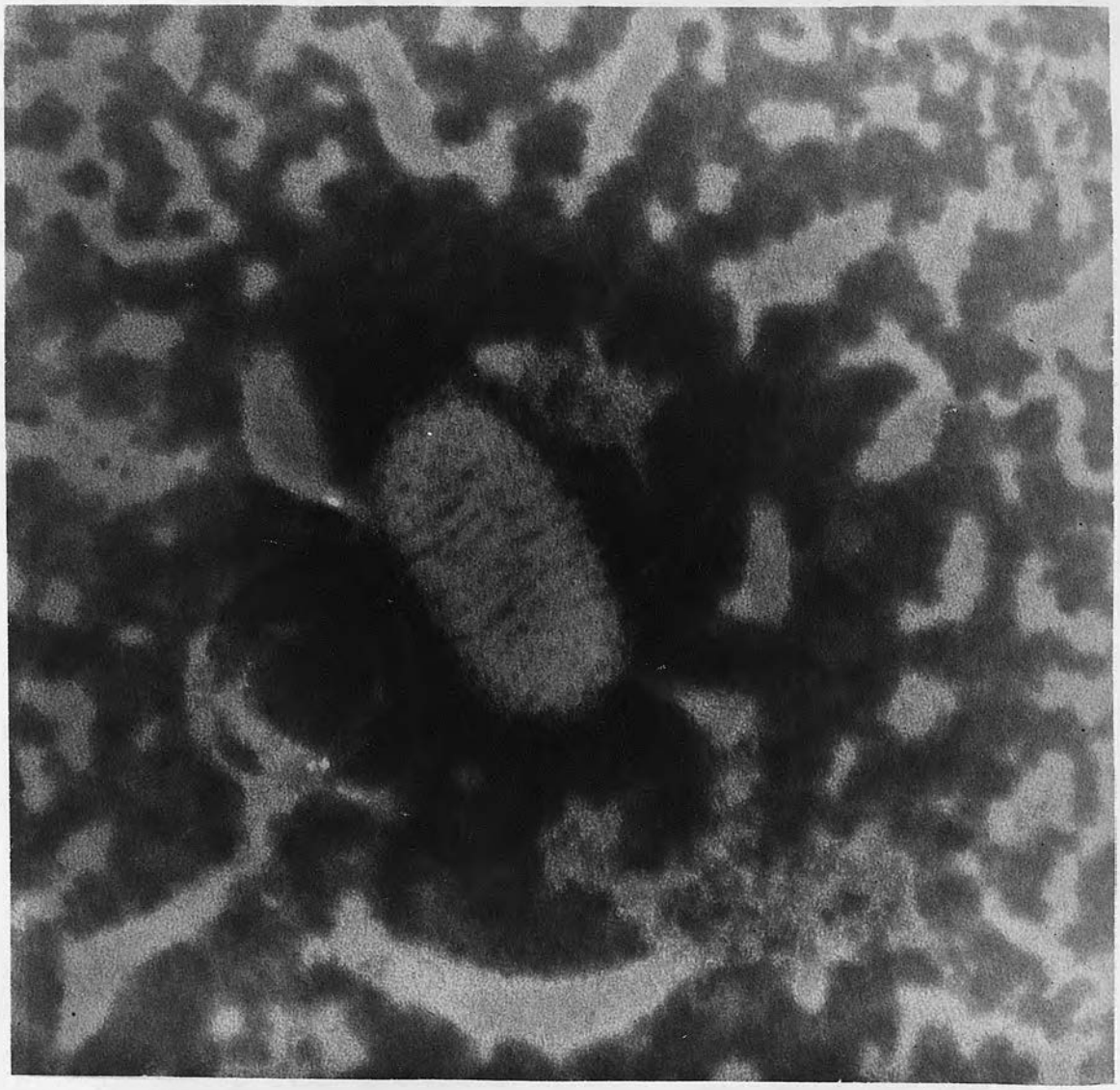


Fig. 15 - Bovine papular stomatitis virus.



VIRUS PARTICLE LYSIS

The appearance and amount of orf virus particles from scabs that had been incubated for a week in orf-negative colostrum-deprived lamb serum did not deviate from the appearance and amount of virus found in the control aliquot of the same scab (Fig. 16). On the other hand, scabs that had been kept for a week in neat sheep anti-orf serum, in 19S or 7S fractions contained considerably fewer virus particles and the particles themselves were ill-defined and deviated from the characteristic orf virus appearance because the criss-cross patterns were hardly discernible. (Fig. 17).

The specimens prepared from scabs kept in the albumin fraction of the hyperimmune sheep serum were not satisfactory because the albumin precipitated. Nevertheless, many virus particles were observed, and they did not deviate from the normal appearance of orf virus. On the other hand, the specimens prepared from scabs that had been incubated in a fraction of the fourth peak of the fractionated hyperimmune serum contained very few virus particles, all of which were ill-defined and without the characteristic criss-cross pattern. Comparatively, the preparations from the fourth peak deviated most from the normal.

Fig. 16 - Orf virus particles incubated for one week at 37°C in colostrum-deprived lamb serum.

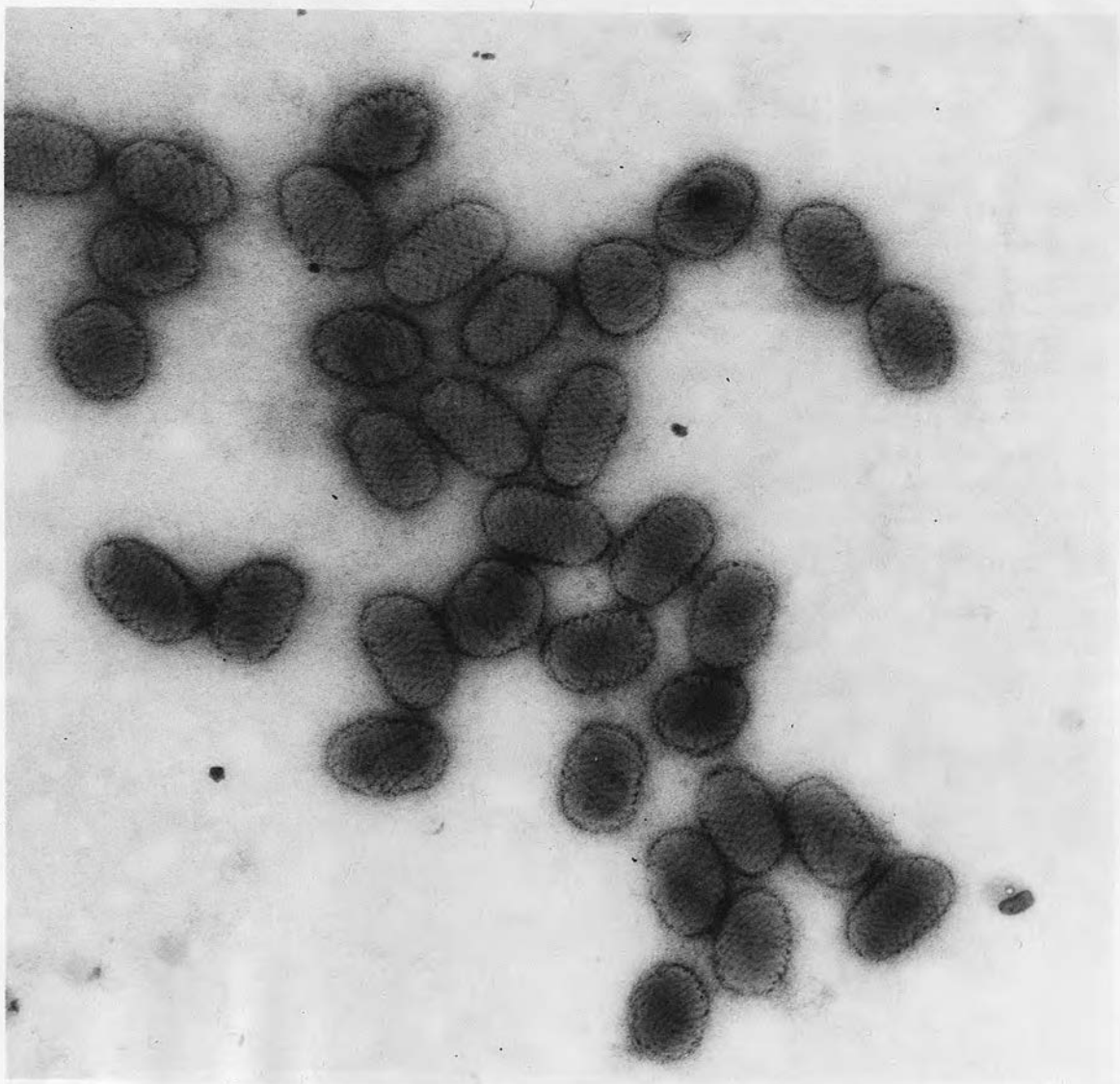
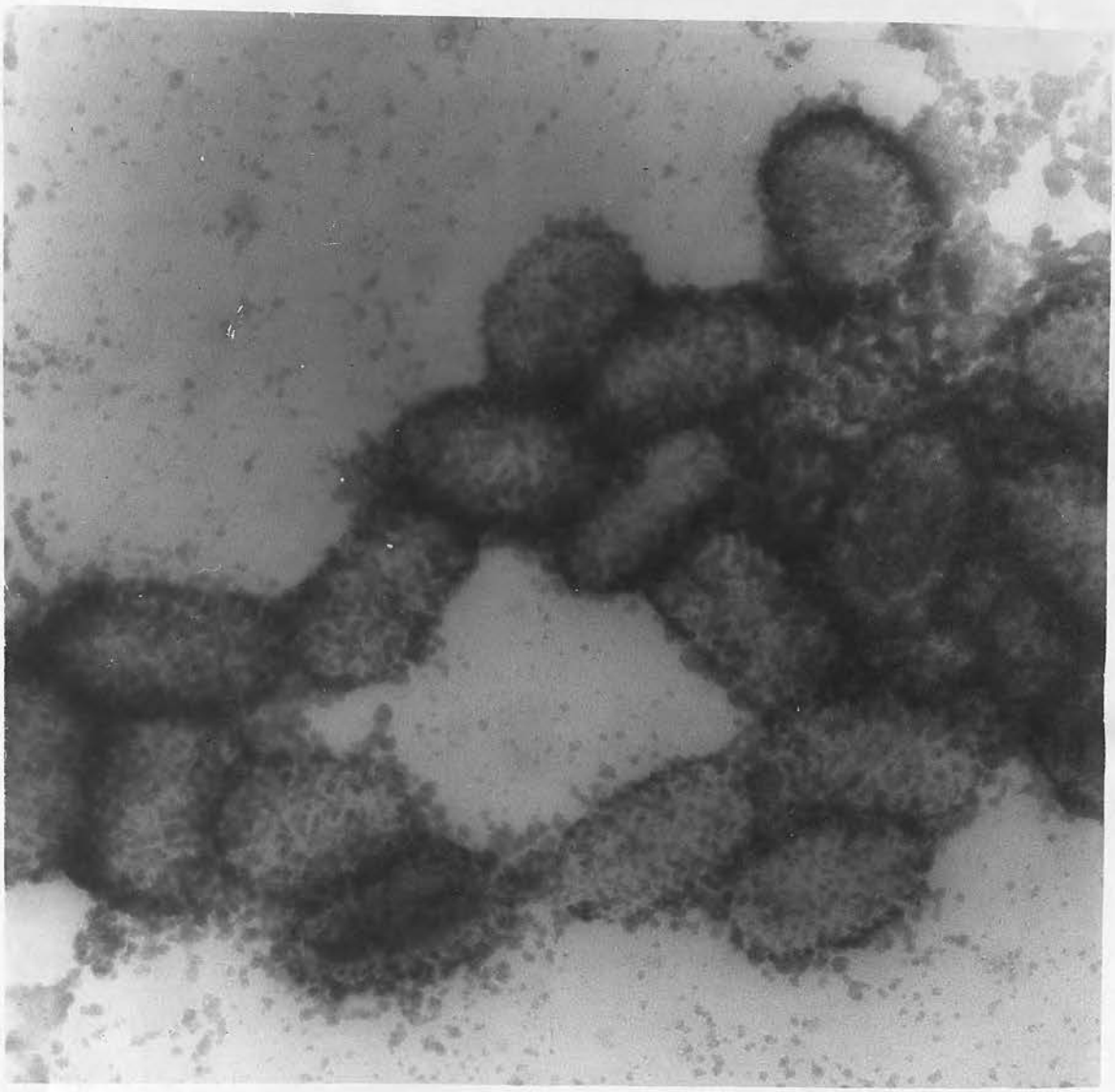


Fig. 17 - Orf virus particles incubated for one week at 37°C in sheep anti-orf serum.



DETECTION OF ANTIGENS BY FLUORESCENT ANTIBODIES

Impression smears: Fluorescing aggregates and granules were always observed in the smears prepared from fresh orf-positive specimens and nullified interpretation.

Re-suspended pellets: Fluorescing aggregates and granules were readily detected. The sizes were very heterogenous and comparably larger than the virus particles.

Sephadex G-200 fractionated virus: Smears prepared from infectious fractions always fluoresced in the form of spots and small aggregates. Some of the isolated spots resembled orf virus in appearance; they were oval in shape but they were larger than the virus particles.

Kaolin-treated scabs: Very few fluorescent spots were observed and smears of control scabs similarly treated contained similar fluorescent spots.

Fluorocarbon-treated scabs: Very few fluorescent granules of varying size were seen. Similar granules were observed in smears of orf-negative scabs.

HAEMAGGLUTINATION

Orf scab suspensions: A total of twenty-three known-positive orf scabs harvested between ten and twenty-nine days after experimental scarification were assayed for haemagglutinating activity against baboon, calf, fowl, guinea pig, human type O, mouse, pig, rabbit and sheep erythrocytes. No haemagglutinating activity was ever found. Treatment of the orf suspensions by ultra-:sonication, ultracentrifugation or kaolin did not help.

Sephadex G-200 fractions: Fractions corresponding to the lower descending side of the third or albumin peak obtained after Sephadex G-200 fractionation of a pool of ten day old scabs agglutinated guinea pig, human type O, fowl, mouse and rabbit erythrocytes. There was no activity against baboon, calf, pig or sheep erythrocytes (Table 5). The haemagglutination titres ranged from 1:2 against mouse erythrocytes to 1:16 against rabbit erythrocytes. The scab pool before fractionation did not exhibit haemagglutinating activity. The specificity of the reactions was not confirmed because anti-orf serum failed to inhibit them.

TABLE 5

NON-SPECIFIC HAEMAGGLUTINATION BY FRACTIONS OF ORF SCAB SUSPENSIONS

Erythrocytes	Haemagglutination titre
Baboon	Nil
Calf	Nil
Fowl	1:4
Guinea pig	1:8
Human type O	1:8
Mouse	1:4
Pig	Nil
Rabbit	1:16
Sheep	Nil

PRECIPITATING ANTIGENS

EXPERIMENTALLY-PRODUCED ORF SCABS

Macro-system: A total of three hundred and sixty experimentally-produced orf scabs were tested for the presence of precipitating antigens by the macro-system. The age of the scabs ranged from three to seventy-five days post-scarification. Antigens were detected in preparations from two hundred and twenty-five scabs (62.5 per cent) (Fig. 18).

Optimal reactions were usually obtained after overnight incubation of the plates at room temperature. The earliest observable reactions occurred after five hours at room temperature if the suspensions were derived from young scabs.

Orf-precipitating antigens were detected as early as three days after experimental exposure when the lesions were pustular; five out of six scabs were positive. Scabs harvested from day four up to day nineteen after scarification were always positive. Beyond day nineteen, a considerable number of scabs suspensions failed to give any reaction. Nevertheless some suspensions of eight and ten week-old scabs were positive. In general, suspensions of scabs contained no precipitating antigens if harvested one week or less /

less from resolution of the lesions (Table 6). If the course of the experimental lesion was unusually protracted because of the development of secondary reactions around the original site precipitating antigens were detected up until two weeks before complete resolution (Table 7).

Two precipitating antigens were detected but the ability to detect both antigens depended upon the source of anti-orf serum. Sheep immunised once either by scarification with live virus or by intravenous inoculation of live virus yielded sera that reacted with only one antigen (Fig. 19). Immune sheep that were challenged with live virus and were bled one week after challenge yielded sera that reacted with both antigens (Fig. 20). The single antigen detected by all the sheep anti-orf sera had a slower mobility than the second antigen detected by sheep hyperimmune anti-orf sera and the reaction occurred nearer to the antigen-containing well.

No lines of precipitation developed between known virus-free scab suspensions and sheep anti-orf sera. Similarly suspensions of the scabs produced by infection with Staphylococcus aureus and suspensions of colonies of Staphylococcus aureus were non-reactive.

Antigen /

Antigen titres: The titres of two scabs harvested at one week were 1:5 and 1:20 (Table 8; Fig. 21). One scab aged ten days had a titre of 1:40. Most scabs harvested at two weeks had titres of 1:20. Scabs harvested at three weeks had titres ranging from <1:5 to 1:20. The titres of scabs harvested later were low. In other words, precipitating antigens were at their peak between the first and second weeks.

Micro-system: Suspensions of eighty-four experimentally produced orf scabs were tested for the presence of precipitating antigens by the micro-system. The age of the scabs ranged from ten to forty-three days after experimental scarification. Antigens were detected in twenty-six (31 per cent) (Fig. 22).

The micro-system proved to be less sensitive than the macro-system. When the same suspensions were tested by both systems, eight positive in the macro-system were negative in the micro-system (Table 9). Moreover, one hundred per cent of the suspensions prepared from scabs harvested between the tenth and eighteenth day after scarification were positive in the macro-system whereas only sixty-five per cent were found positive by the micro-system.

SCABS /

SCABS FROM NATURAL CASES

Scabs from lambs: A total of thirty-three orf scabs collected from animals suffering from the natural disease were tested by the macro-system and eleven were found to be positive (33 per cent). The antigens were identical with those found in experimentally-produced orf scabs.

Scabs from ewes: Although twenty-five scabs from lesions on the teats of lactating ewes were examined by the macro-system only one (4 per cent) contained precipitating antigen. On the other hand, suspensions of four [redacted] teat scabs contained precipitating antibodies reactive when diffused against known positive orf antigen.

SCABS FROM SUSPECT CASES

Suspensions were prepared from scabs collected from thirty-two cases of suspected orf and were tested by the macro-system. Only two (6 per cent) contained precipitating antigens identical with those found in suspensions of experimentally-produced orf scabs.

PROPERTIES OF THE PRECIPITATING ANTIGEN

Freezing and thawing: Five suspensions of orf scabs were /

were subjected to ten cycles of freezing and thawing. The precipitating antigen titres before and after treatment were identical (Table 10).

Heat inactivation: The precipitating antigen titre was unaffected by exposure at 40°C for thirty-six hours. Heat inactivation at higher temperatures proceeded linearly like a first-order reaction except at 70°C when it was curvilinear. The half-life at 50°C was fifty minutes. (Fig. 23). At 56°C it was 11.5 minutes and no activity was detected after forty-five minutes exposure (Fig. 24). At 60°C the half-life was 2.2 minutes and no activity was detected after ten minutes exposure (Fig. 25). At 70°C the half-life was twenty seconds and no activity was detected after sixty seconds exposure (Fig. 26).

Sedimentation: Four orf scab suspensions were tested before and after high speed centrifugation. Precipitating antigen activity was restricted to the supernatant fluids. None of the re-suspended pellets were reactive.

Filtration: The fast moving antigen was found in the filtrate. Most of the slow moving antigen was recovered in the filtrate but some of it was trapped within the asbestos pads.

Fig. 18 - Distribution by age of orf scabs tested for precipitating antigens by the macro-system.

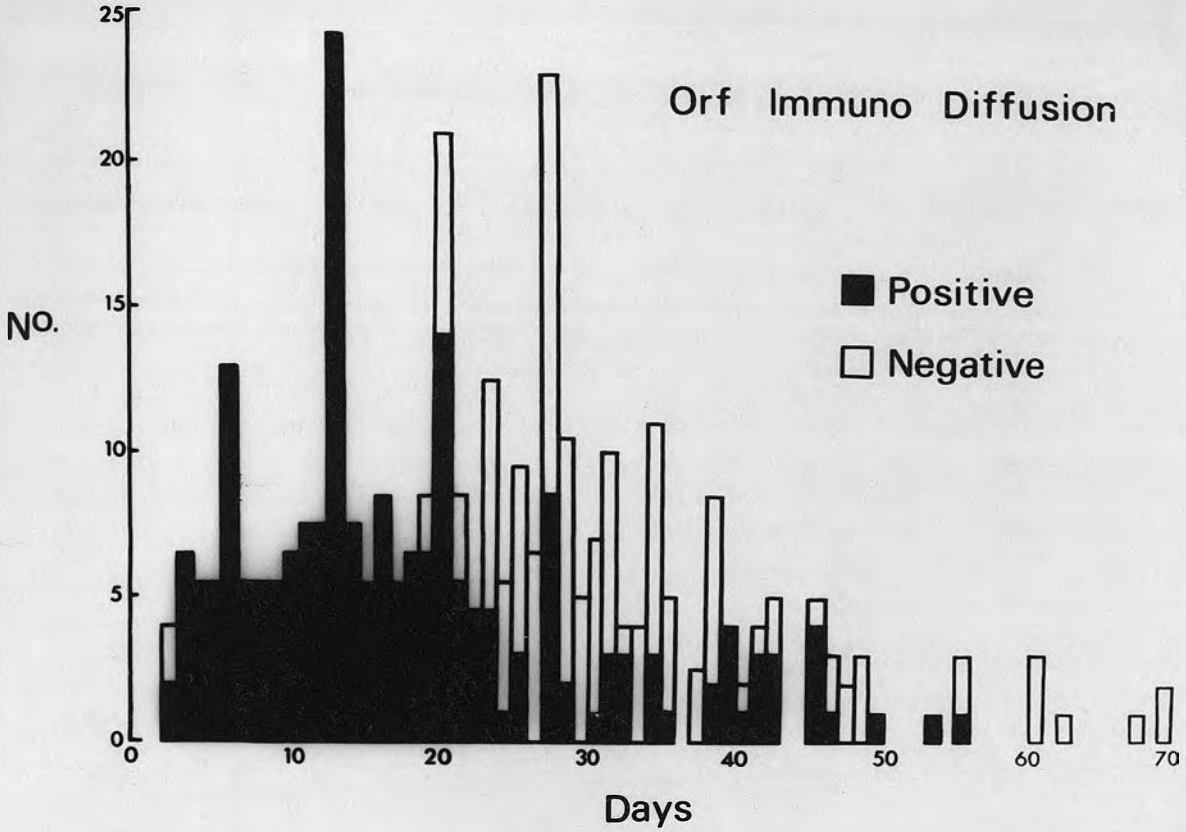

Orf Immuno Diffusion
Positive
Negative
NO.
25
20
15
10
5
0
0
10
20
30
40
50
60
70
Days

TABLE 6

ORF PRECIPITATING ANTIGEN IN SCABS HARVESTED FROM LAMBS IN WHICH THE LESION HAD A MEDIAN DURATION.

Lamb	Weeks post vaccination (infection)				
	1	2	3	4	5
1	+	+	+	-	H
3	+	+	-	H	
5	+	+	+	-	H
7	+	+	+	-	H
9	+	+	-	-	H
11	+	+	+	-	H
27	+	+	-	H	
C17	+	+	+	-	H
C29	+	+	+	.	H
C35	+	+	+	.	H
C45	+	.	+	-	H
S55	+	+	+	-	H

. Not done + Positive - Negative H Healed

TABLE 7

ORF PRECIPITATING ANTIGEN IN SCABS HARVESTED FROM LAMBS IN WHICH THE LESION HAD AN ABNORMALLY LONG DURATION

Lamb	Weeks post vaccination (infection)										
	1	2	3	4	5	6	7	8	9	10	11
13	+	+	+	-	-	+	+	.	.	-	H
23	+	+	+	+	+	+	-	-	-	H	
35	+	+	+	+	+	+	-	+	-	-	Died
C19	+	+	.	+	-	.	H				
43	+	+	+	-	-	H					
C41	.	+	.	-	-	H					
S57	+	+	.	-	.	H					

. Not done + Positive - Negative H Healed

Fig. 19 - Immuno-diffusion plate illustrating a single precipitation line. The top and lower right wells contained sheep anti-orf serum and the lower left well contained sheep normal serum. The central well contained the known positive antigen and the upper left, upper right and bottom wells contained the unknown suspension which proved positive.

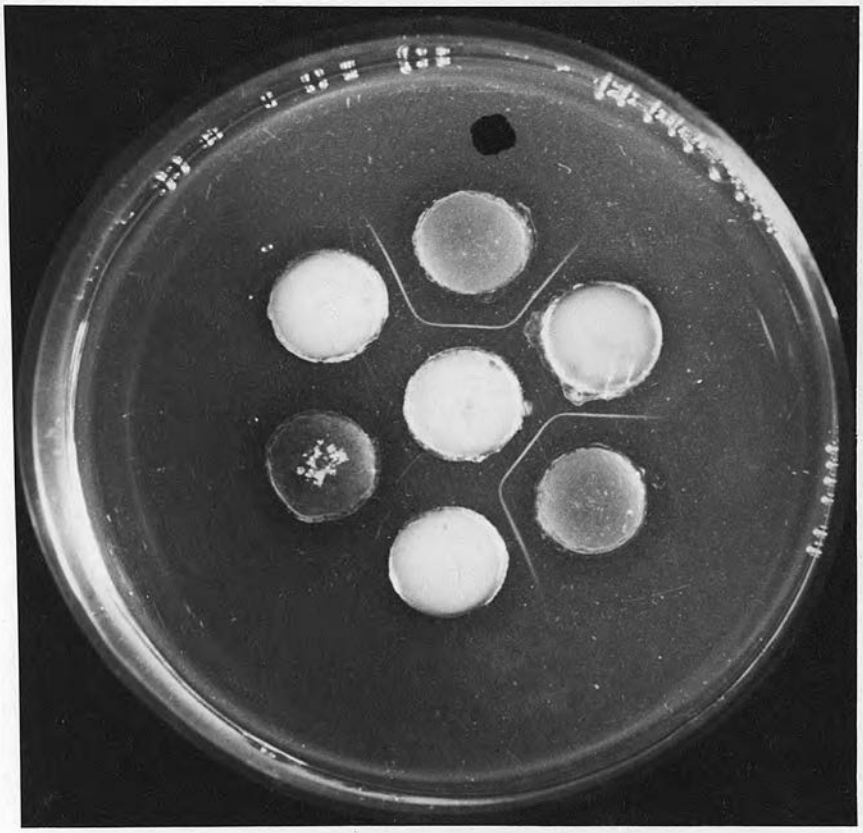


Fig. 20 - Immuno-diffusion plate illustrating two precipitation lines. The top and lower right wells contained sheep hyperimmune anti-orf serum and the lower left well contained sheep norman serum. The central well contained the known positive antigen and the upper left, upper right and bottom wells contained the unknown suspension which proved positive.

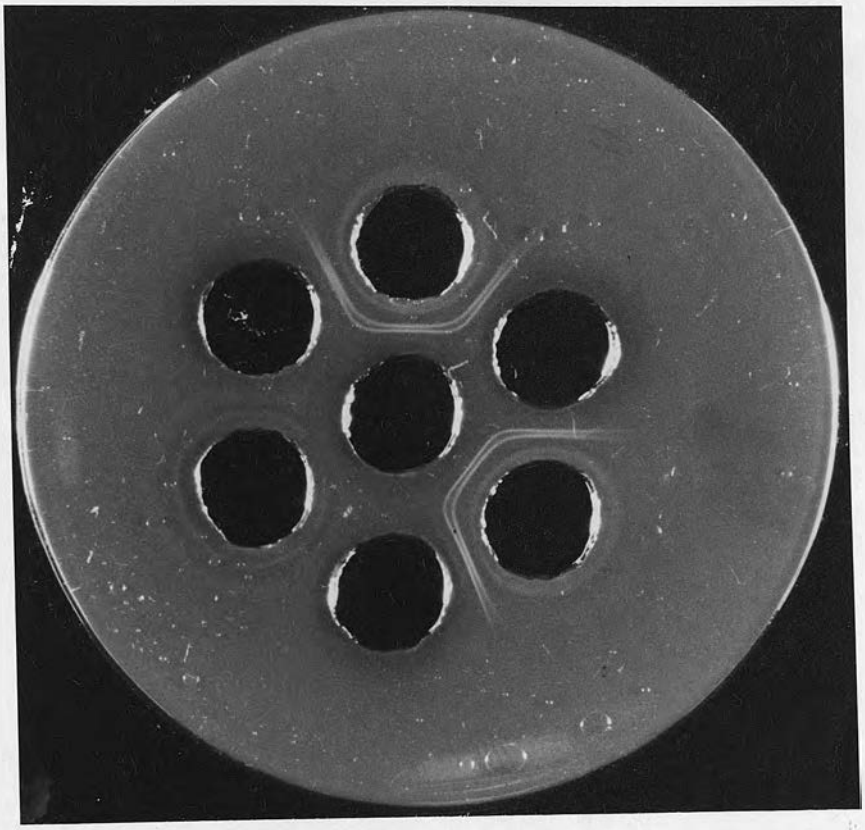


TABLE 8

PRECIPITATING ANTIGEN TITRES* IN ORF SCABS

Age of Scabs (Weeks)				
1	2	3	4	5
5	10	10	5	5
20	20	20	< 5	< 5
	20	5		
	20	20		
	20			
	5			
	10			

* Expressed as the reciprocal of the highest dilution giving precipitation.

Fig. 21 - Median titres of precipitating antigens in scabs harvested weekly after experimental scarification.

Median titres of Precipitating Antigens

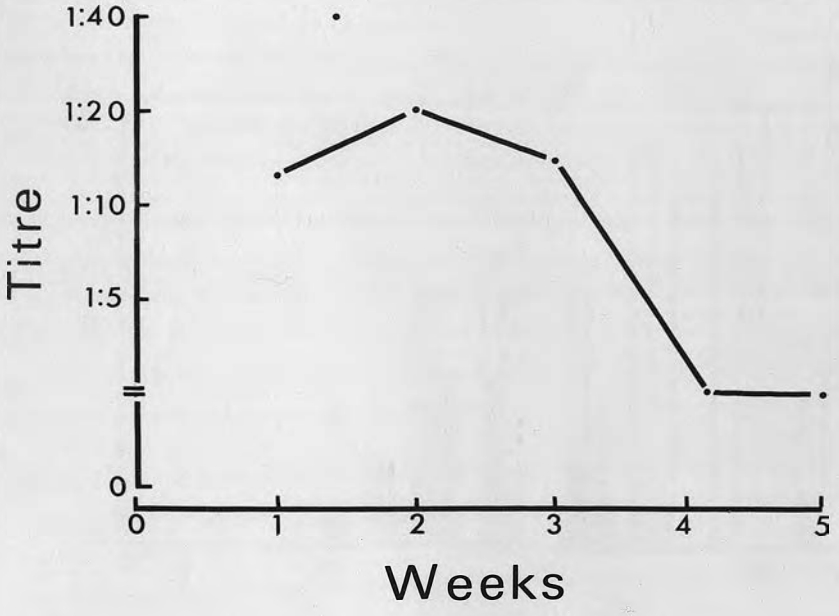


Fig. 22 - Distribution by age of orf scabs tested for antigens by the micro-system.

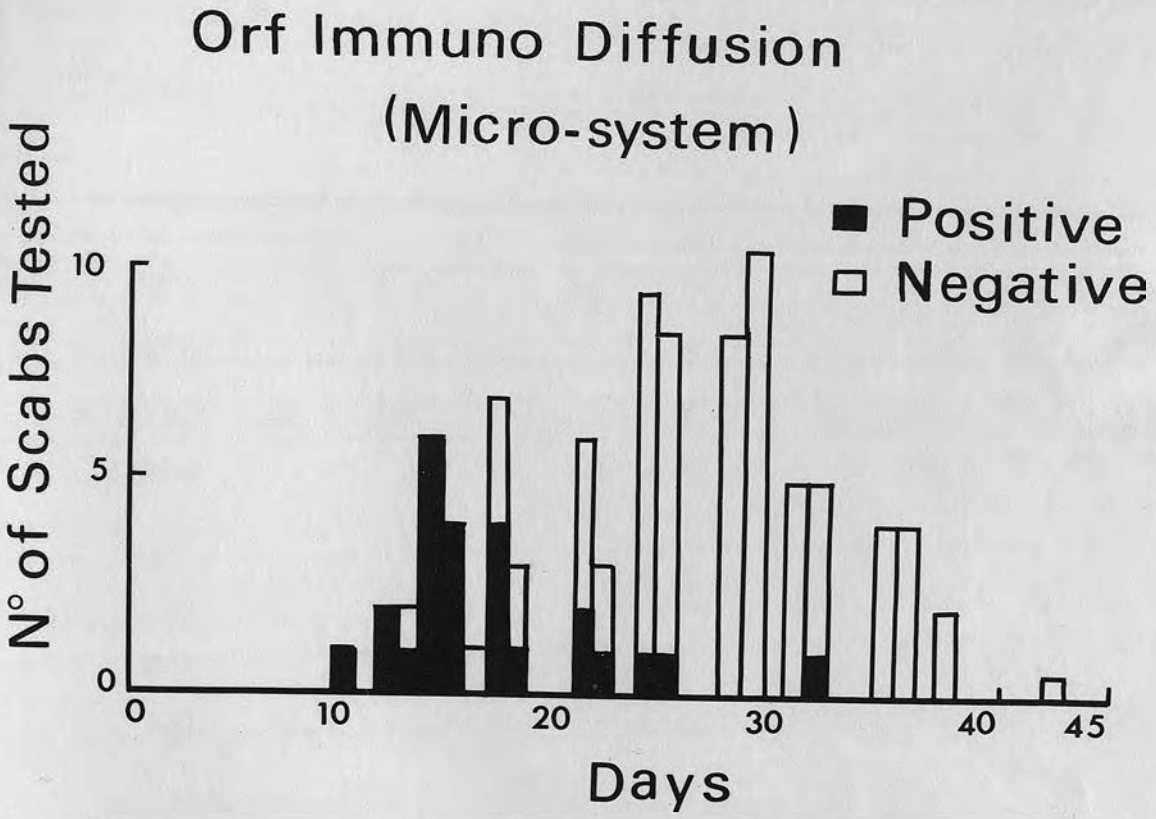

Orf Immuno Diffusion
(Micro-system)
Positive
Negative
N° of Scabs Tested
10
5
0
0
10
20
30
40
45
Days

TABLE 9

ORF SCABS TESTED BY BOTH IMMUNO-DIFFUSION TECHNIQUES

Scab	Age (days)	Macro-system	Micro-system
D75	17	+	-
D76	17	+	-
47	21	+	-
D79	21	+	-
28	24	+	-
47	24	+	-
D79	28	+	-
28	31	+	-

TABLE 10

EFFECT OF FREEZING AND THAWING ON PRECIPITATING ANTIGENS

Age of Scab (Days)	Titre of untreated suspension	Titre of treated suspension
12	1:5	1:5
13	1:10	1:10
16	1:10	1:10
19	1:5	1:5
21	1.5	1:5

Fig. 23 - Heat inactivation of precipitating antigen at 50°C.

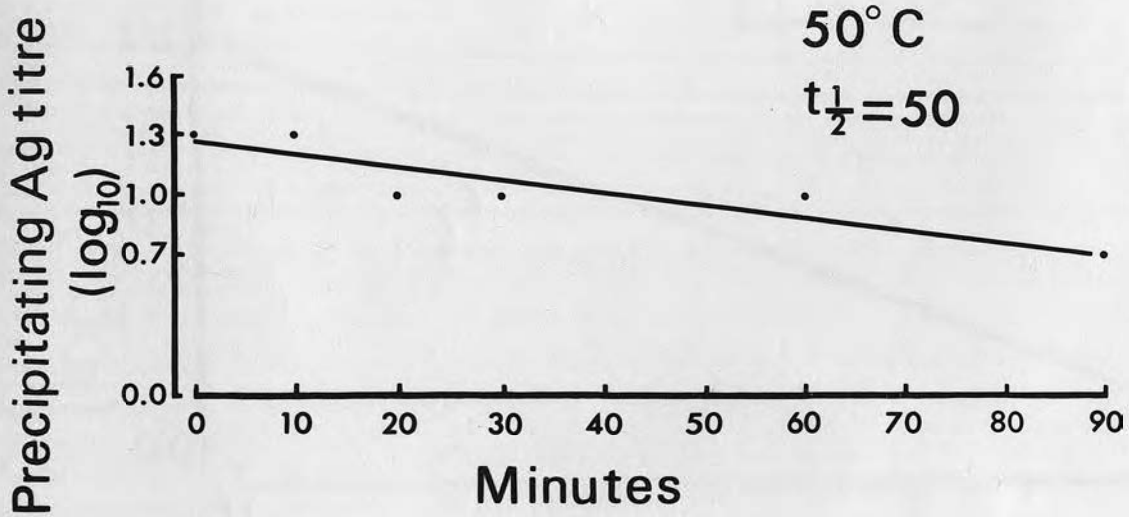

50° C
$t_{\frac{1}{2}}$=50
Precipitating Ag titre
(log_{10})
1.6
1.3
1.0
0.7
0.0
0
10
20
30
40
50
60
70
80
90
Minutes

Fig. 24 - Heat inactivation of precipitating antigen at 56°C.

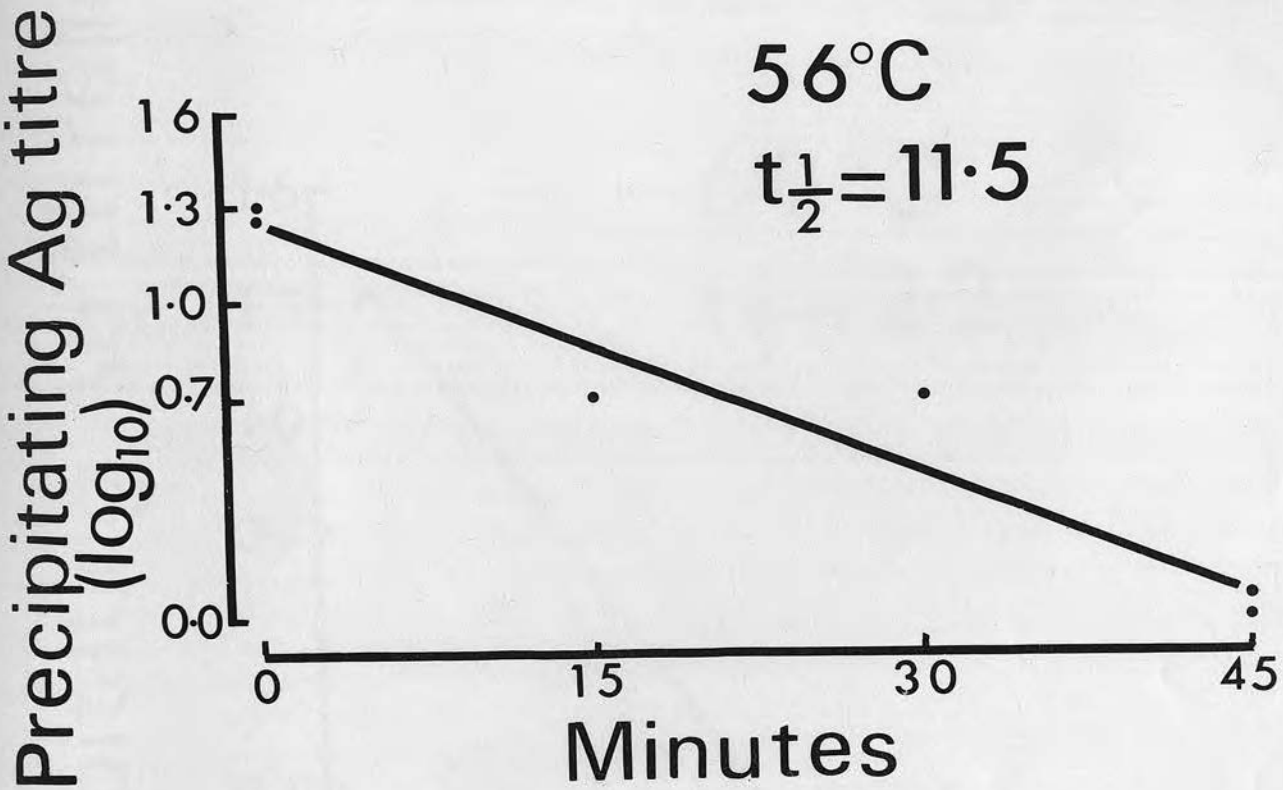
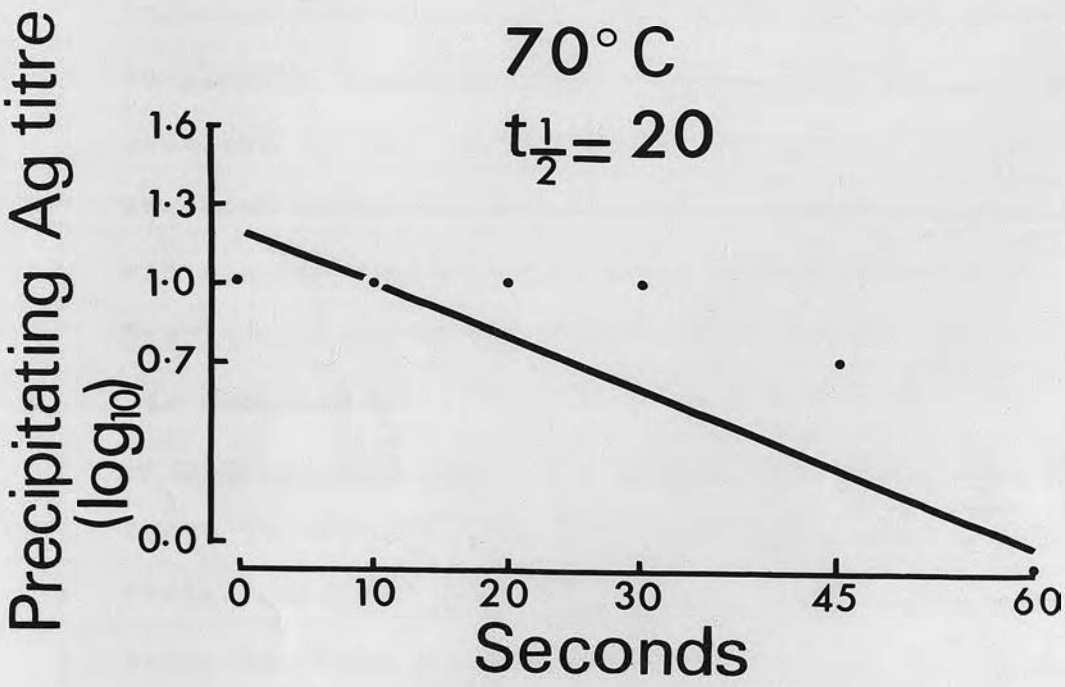

56°C
$t_{\frac{1}{2}}$=11·5
Precipitating Ag titre
(log_{10})
1·6
1·3
1·0
0·7
0·0
0
15
30
45
Minutes

Fig. 25 - Heat inactivation of precipitating antigen at 60°C.

Precipitating Ag titre(log10)
60°C
t½=2·2
1·6
1·3
1·0
0·7
0·0
0
5
10
15
Minutes

Fig. 26 - Heat inactivation of precipitating antigen at 70°C.


70° C
$t_{\frac{1}{2}} = 20$
Precipitating Ag titre
$(\log_{10})$
1·6
1·3
1·0
0·7
0·0
0
10
20
30
45
60
Seconds

COMPLEMENT-FIXING ANTIGENS

EXPERIMENTALLY-PRODUCED ORF SCABS

Quantal response: A total of two hundred and sixty-three orf scabs harvested from sixty-eight lambs infected experimentally were tested by the direct complement fixation test. Specific antigens were detected in two hundred and forty-seven (94 per cent), the base dilution being 1:100. Non-infective scabs and suspensions prepared from pure cultures of Staphylococcus aureus isolated from orf scabs did not fix complement.

Sequential samples: The ages of the scabs ranged from three to seventy days after scarification. Complement fixing antigens were detected in scabs harvested as early as three days after scarification and thereafter throughout the course of the experimental infection (Fig. 27).

Antigen titres: Scabs from experimental lesions on twenty-four lambs were harvested at weekly intervals and their complement-fixing antigen contents were titrated. The lambs fell into two groups, one of eighteen lambs in which the experimental lesions persisted for less than the expected median period of five weeks and a second group of six lambs in which the experimental /

experimental lesions persisted beyond the expected median period. Antigen titres of the scabs harvested from the first group of lambs reached their peak one week after scarification and declined after the second week (Table 11; Appendix Figs. 1-13; Fig. 28). The highest titre was 3.6 i.e. a fifty per cent end-point dilution of 1:4,200. The antigen titres of the second group of lambs reached their peak two weeks after scarification and thereafter declined very slowly and significant titres were still present eight and nine weeks after scarification (Table 12; Appendix figs. 14-19; Fig. 29). The highest titre in the second group of lambs was 3.5 i.e. a fifty per cent end-point dilution of 1:3,200.

Efficacy of extraction technique: Complement-fixing antigens were prepared from eight scabs by the ether-extraction technique described by Glover (1933) and Nisbet (1954). Eight antigens were also prepared from the same scabs by our technique of barbitone buffer extractions. The titres were compared (Table 13). Six were similar and the other two scabs had higher titres when extracted in barbitone buffer than when extracted with ether.

SCABS /

SCABS FROM NATURAL CASES

Scabs from lambs: Scabs from sixteen cases of clinically diagnosed orf in lambs naturally infected were assayed for the presence of complement-fixing activity. Antigens were detected in twelve scabs (75 per cent). The titres ranged from 2.0 to 2.6 (Table 14).

Scabs from ewes: Scabs from seventeen cases of clinically diagnosed teat orf in lactating ewes naturally infected were examined for complement-fixing antigens and five (29 per cent) proved positive. The complement-fixing antigen titres ranged from 2.2 to 2.9 (Table 15).

PROPERTIES OF THE COMPLEMENT-FIXING ANTIGEN

Freezing and thawing: Two orf scabs harvested fourteen days after experimental infection were subjected to ten cycles of alternate freezing and thawing. The titres remained unaltered (Table 16).

Heat inactivation: The orf complement-fixing antigen proved to be remarkably heat stable; even after fifteen hours exposure to 56, 60 and 70°C preparations possessed high levels of complement-fixing activity (Table 17).

Sedimentation: The complement-fixing activity of orf scab suspensions, and of fractions corresponding to the /

the top of the first peak obtained after Sephadex G-200 fractionation was not sedimented by centrifugation at 26,168 r.c.f. per minute for forty-five minutes. The titres obtained with the supernatant fluids were identical with the titres obtained before centrifugation. The re-suspended pellets did not fix complement (Table 18).

Filtration: Eight one per cent suspensions of orf-infected scabs were filtered through asbestos pads. The filtrates were devoid of complement-fixing activity (Table 19).

Fig. 27 - Distribution by age of orf scabs tested for complement-fixing antigens.

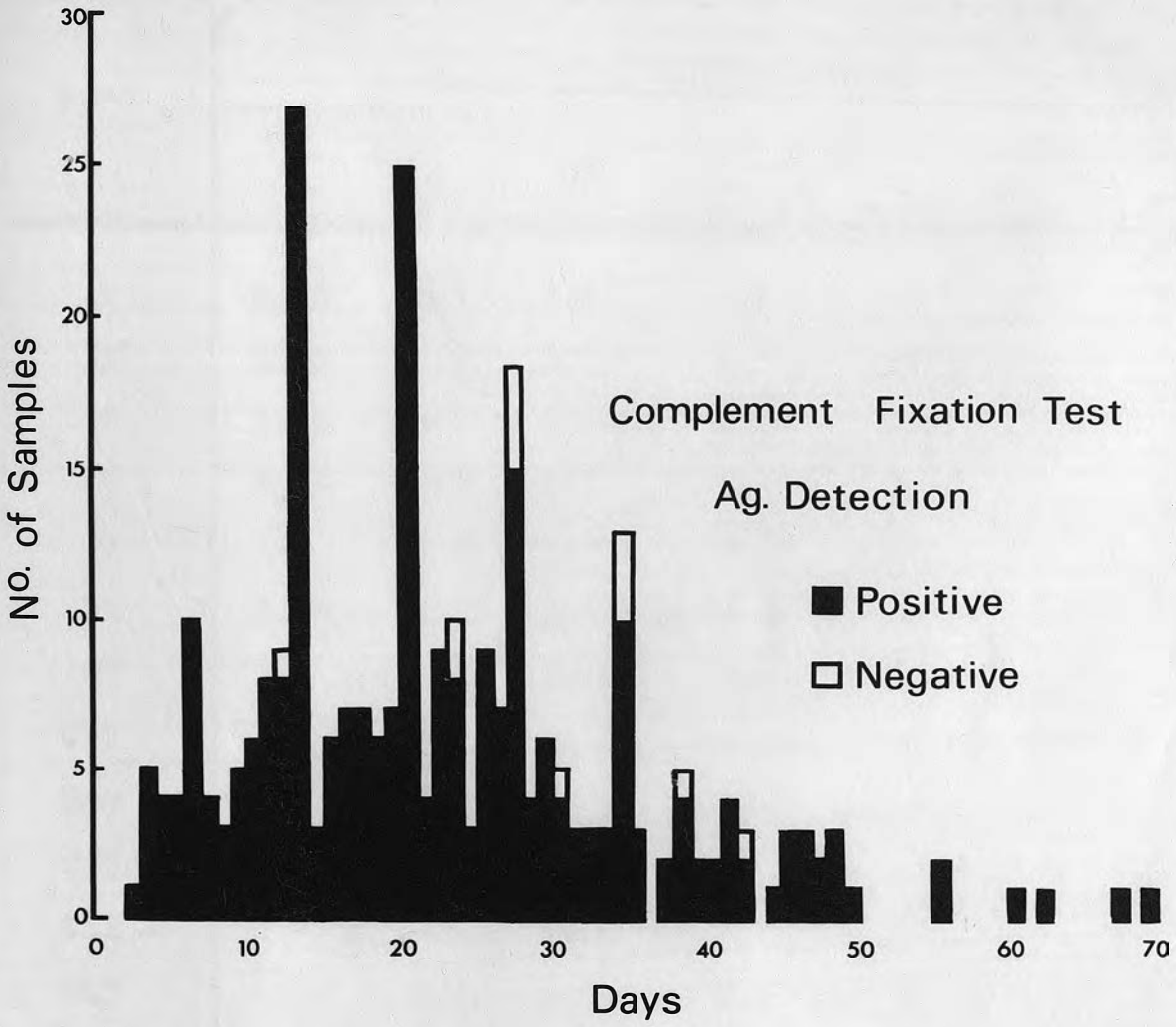

30
25
20
15
10
5
0
No. of Samples
Complement Fixation Test
Ag. Detection
Positive
Negative
0
10
20
30
40
50
60
70
Days

TABLE 11

COMPLEMENT-FIXING ANTIGEN TITRES* OF ORF SCABS PERSISTING FOR LESS THAN FIVE WEEKS

Lamb	Weeks after scarification				
	1	2	3	4	5
1	2.78	3.3	3.08	2.6	H
3	2.45	3.2	2.3	2.3	H
5	3.63	3.0	2.82	2.7	H
7	3.08	3.08	2.9	2.60	H
9	3.0	3.12	2.9	2.42	H
11	2.90	3.03	2.78	2.6	H
27	3.2	3.03	2.6	2.3	H
C17	.	2.9	2.78	2.0	H
C19	3.3	3.08	.	2.48	< 2.0
C35	2.6	2.9	2.6	H	
C41	.	2.3	2.6	< 2.0	H
C43	2.48	2.3	2.6	< 2.0	H
C45	.	3.08	2.18	< 2.0	H
C53	.	2.9	2.6	< 2.0	H
C57	.	2.6	2.3	2.3	H
C69	.	3.08	2.3	< 2.0	H
S55	3.2	2.82	2.68	< 2.0	H
S57	3.2	2.3	.	2.0	H
Medians	3.04	3.02	2.60	2.30	< 2.0

* Expressed as the logarithm of the reciprocal of the 50 per cent end-point dilution.

H lesion healed.

Fig. 28 - Median complement-fixing antigen titres of orf scabs persisting for less than five weeks.

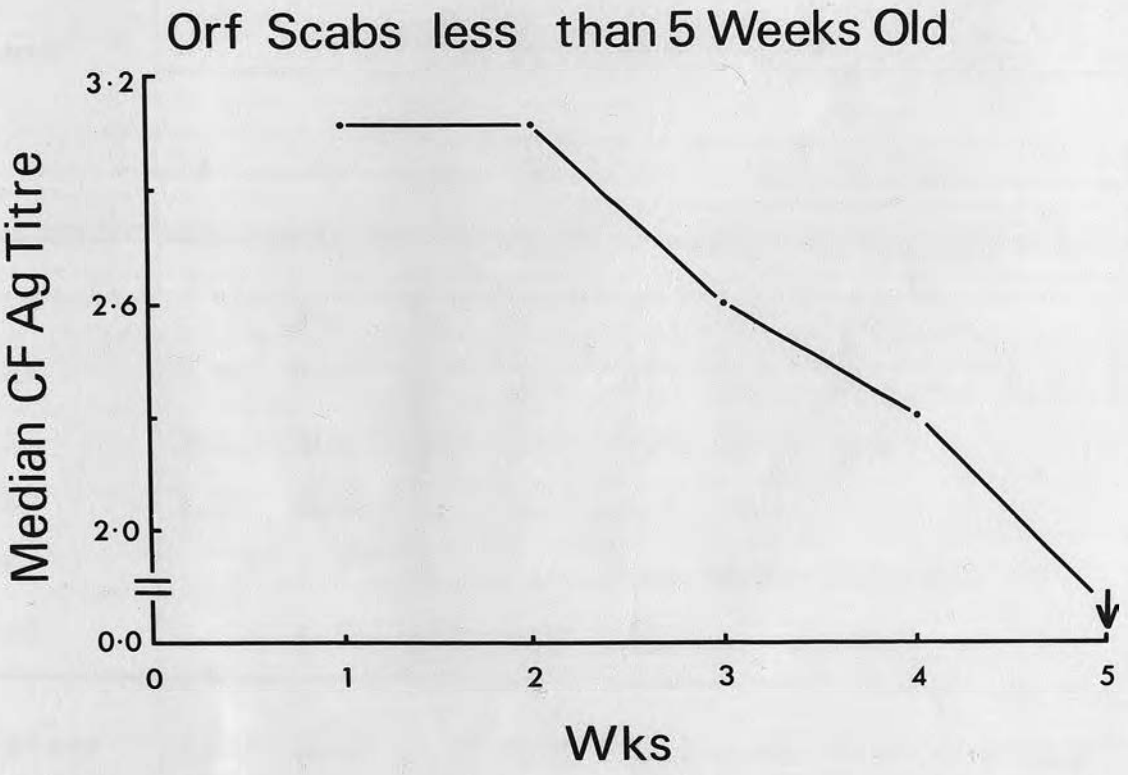

Orf Scabs less than 5 Weeks Old
Median CF Ag Titre
3·2
2·6
2·0
0·0
0
1
2
3
4
5
Wks

TABLE 12

COMPLEMENT-FIXING ANTIGEN TITRES* OF ORF SCABS PERSISTING FOR MORE THAN FIVE WEEKS

Lamb	Weeks after scarification								
	1	2	3	4	5	6	7	8	9
13	.	3,1	2.7	2.6	2.6	2.6	2.0	.	.
23	2.9	3.4	2.9	2.9	2.7	2.8	2.8	2.6	2.5
31	3.1	3.5	3.5	3.2	2.9	2.8	2.3		
43	3.2	2.6	.	2.6	2.3	H			
S65	.	3.2	.	.	2.3	2.3	2.3	2.3	.
S68	.	3.5	3.1	2.9	2.2	.	H		
Medians	3.10	3.30	3.00	2.90	2.45	2.70	2.30	2.45	2.52

* Expressed as the logarithm of the reciprocal of the 50 per cent end-point dilution.

. = not done.

H = lesion healed.

Fig. 29 - Median complement-fixing antigen titres of orf scabs persisting for more than five weeks.

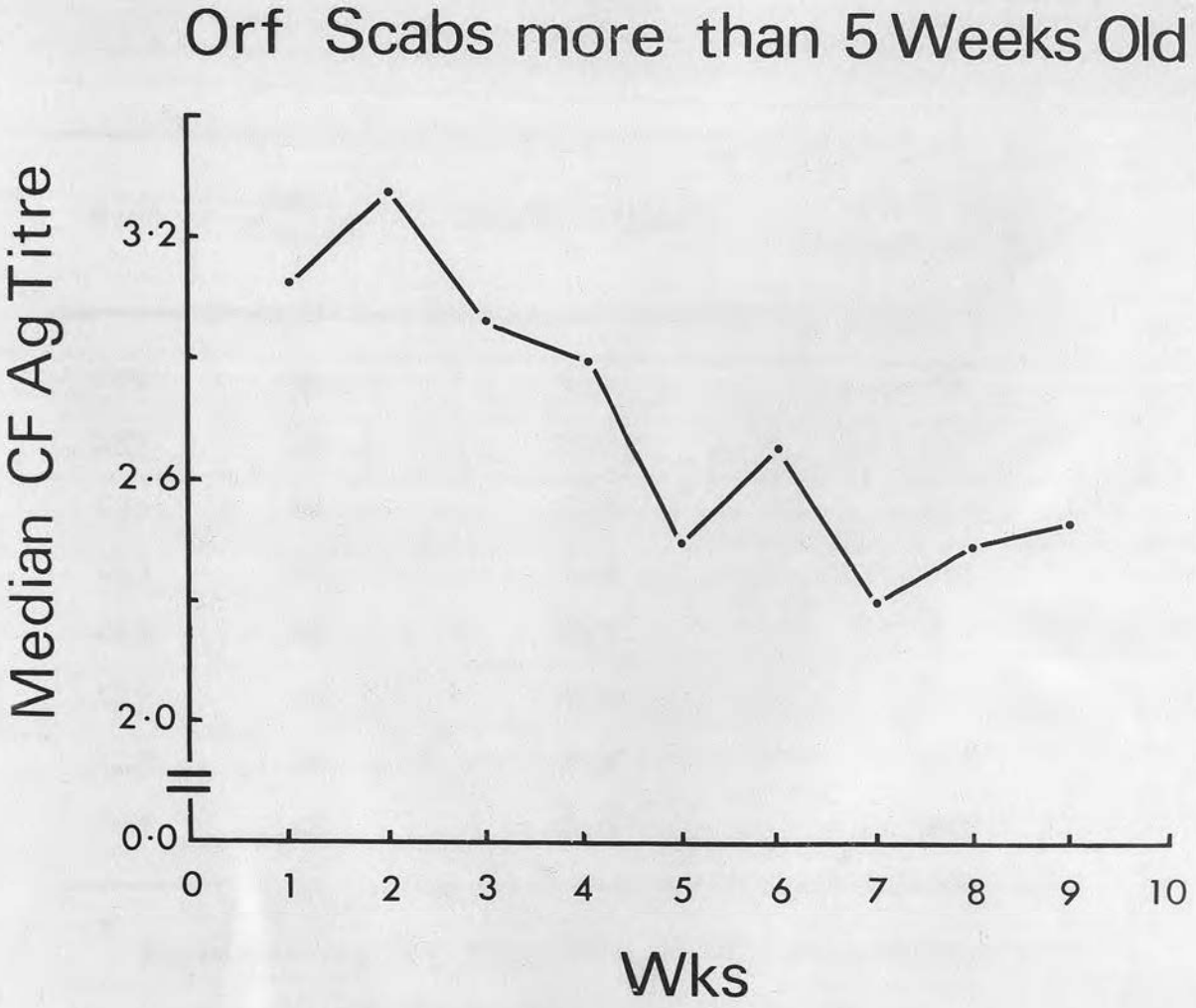

Orf Scabs more than 5 Weeks Old
Median CF Ag Titre
3·2
2·6
2·0
0·0
0
1
2
3
4
5
6
7
8
9
10
Wks

TABLE 13

COMPLEMENT-FIXING ANTIGEN TITRES* OF ORF SCABS EXTRACTED WITH ETHER AND BARBITONE BUFFER

Scab	Age (days)	Ether-extract	Barbitone buffer extract
C29	14	2.5	2.5
S57	14	2.0	2.6
C35	21	2.6	2.6
C43	21	2.3	2.3
C45	21	2.5	2.5
C17	28	2.6	2.6
C19	28	2.3	2.3
S65	28	2.6	2.9

* Expressed as the logarithm of the reciprocals of the 50 per cent end-point dilutions.

TABLE 14

COMPLEMENT-FIXING ANTIGEN TITRES* OF CLINICALLY DIAGNOSED ORF SCABS

Date	Scab	Titre
21.2.67	40	2.6
21.2.67	G1	2.3
14.10.67	Wd	< 2.0
7.2.68	13	< 2.0
19.2.68	H.968	2.4
27.3.68	5	< 2.0
20.3.68	29	< 2.0
26.3.68	C9	2.0
17.4.68	C17	2.0
30.7.68	C51	2.6
27.12.68	24	2.3
17.1.69	24	2.3
10.4.69	32	2.3
1.5.69	35	2.3
3.6.69	C39	2.3
10.6.69	G3	2.3

* Expressed as the logarithm of the reciprocal of the 50 per cent end-point dilutions.

TABLE 15

COMPLEMENT-FIXING ANTIGEN TITRES* OF TEAT ORF SCABS

Date	Scab	Titre
20.2.69	U11	< 2.0
6.3.69	U11	< 2.0
13.3.69	U11	< 2.0
20.3.69	U11	< 2.0
20.2.69	U56	< 2.0
6.3.69	U56	2.6
13.3.69	U56	2.9
20.3.69	U56	< 2.0
13.3.69	T87	2.6
24.3.69	10	2.2
27.3.69	W4	< 2.0
20.3.69	W1	< 2.0
20.3.69	W50	2.3
13.3.68	U12	< 2.0
27.3.68	S36	< 2.0
3.3.68	T85	< 2.0
20.2.69	T90	< 2.0

* Expressed as the logarithm of the reciprocal of the 50 per cent end-point dilution.

TABLE 16

TITRES* OF COMPLEMENT-FIXING ANTIGENS AFTER FREEZING AND THAWING

Cycles of freezing and thawing	Scab Suspension	
	C.29	S.57
0	2.5	2.6
2	2.6	2.6
4	2.6	2.6
6	2.6	2.6
8	2.6	2.6
10	2.6	2.6

* Expressed as the logarithm of the reciprocals of the 50 per cent end-point dilutions.

TABLE 17

COMPLEMENT-FIXING ANTIGEN TITRES* OF ONE PER CENT SCAB SUSPENSIONS AFTER HEAT TREATMENT

Exposure time (hours)	56°C	60°C	70°C
0	3.3	3.0	3.0
1	2.8	2.6	
2	2.8		
3	2.8	2.6	2.6
4	2.7		
15	2.6	2.6	2.3

* Expressed as the logarithm of the reciprocals of the 50 per cent end-point dilutions.

TABLE 18

COMPLEMENT-FIXING ANTIGEN TITRES* AFTER SEDIMENTATION

Scab	Before Sedimentation	Supernatant Fluid	Resuspended pellet
7	2.9	2.9	< 2.0
10-14	3.2	3.2	< 2.0
18	2.9	2.9	< 2.0
SP1	3.7	3.5	< 2.0

* Expressed as the logarithm of the reciprocals of the 50 per cent end-point dilutions.

TABLE 19

EFFECT OF FILTRATION THROUGH HEMMINGS ASBESTOS PADS ON THE COMPLEMENT-FIXATION ANTIGEN TITRES*

Scab	Before filtration	After filtration
C.29	2.5	< 2.0
S.57	2.6	< 2.0
C.35	2.6	< 2.0
C.43	2.3	< 2.0
C.45	2.5	< 2.0
C.17	2.6	< 2.0
C.19	2.3	< 2.0
S.65	2.9	< 2.0

* Expressed as the logarithm of the reciprocals of the 50 per cent end-point dilutions.

ANTIGEN FRACTIONATION

Three orf scab suspensions were fractionated in a K25/45 column packed with Sephadex G-200. All had the same profile with four distinct peaks (Fig. 30). A fourth suspension was passed through Sephadex G-200 in a K25/100 column and yielded a profile with six distinct peaks (Fig. 31).

LOCUS OF INFECTIVITY

Infectious virus occurred only in the first or macro-molecular peak. Fractions corresponding to the ascending side of the first peak produced an inflammatory reaction on the scarified skin of a susceptible lamb but a typical orf lesion did not develop. Fractions corresponding to the top and higher half of the descending side of the first peak induced typical orf lesions on the scarified skins of three susceptible lambs.

Infectivity of the orf suspension subjected to fractionation through the K25/100 column was also restricted to the top and higher part of the descending side of the first peak.

LOCUS OF COMPLEMENT-FIXING ANTIGEN ACTIVITY

Most of the complement-fixing activity was located, like infectivity, in the fractions corresponding /

corresponding to the top and higher half of the descending side of the first peak (Fig. 32). Fixation of a low order was obtained with fractions corresponding to the ascending side and lower half of the descending side of the first peak.

Concentration of the fractions against polyethylene glycol extended the range of complement-fixing activity into the second peak and ascending side of the third peak (Fig. 33).

LOCUS OF PRECIPITATING ANTIGEN ACTIVITY

Precipitating antigen activity was restricted to the fractions corresponding to the second peak. Concentration of the fractions did not extend the range of precipitating activity.

LOCUS OF HAEMAGGLUTINATING ACTIVITY

Fractionation of two of the orf scabs suspensions yielded fractions that agglutinated erythrocytes from guinea pigs, hens and rabbits and human type O erythrocytes. Titres were low being around 1:8 and the haemagglutination was not inhibited by anti-orf sera. The fractions containing the haemagglutinins corresponded to the lower part of the descending side of the third peak.

Fig. 30 - Typical profile of an orf scab suspension fractionated through Sephadex G-200 in a K25/45 column.

Orf Scab Sephadex G200

Column K25/45

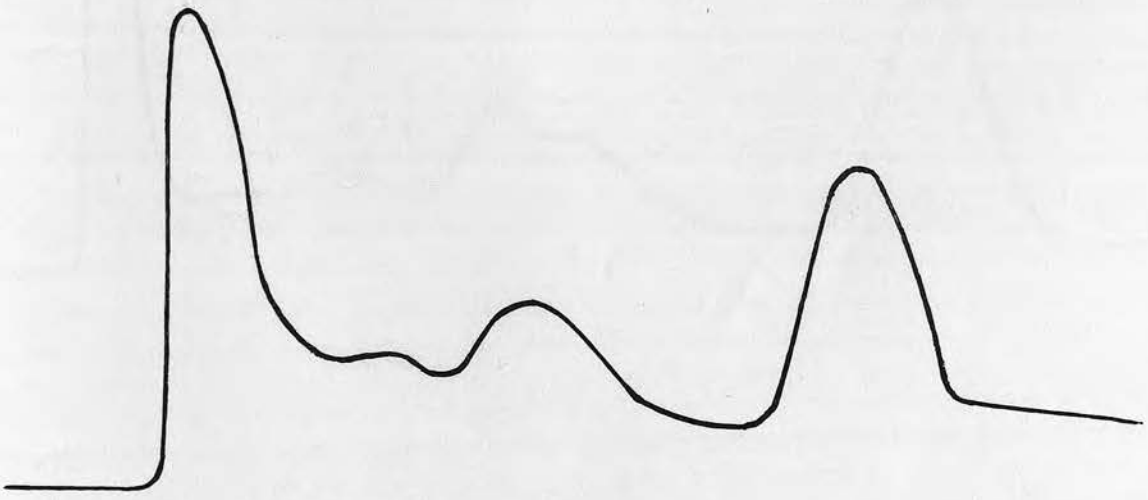


Fig. 31 - Profile of an orf scab suspension fractionated through Sephadex G-200 in a K25/100 column.

Orf Scab Sephadex G200

Column K25/100

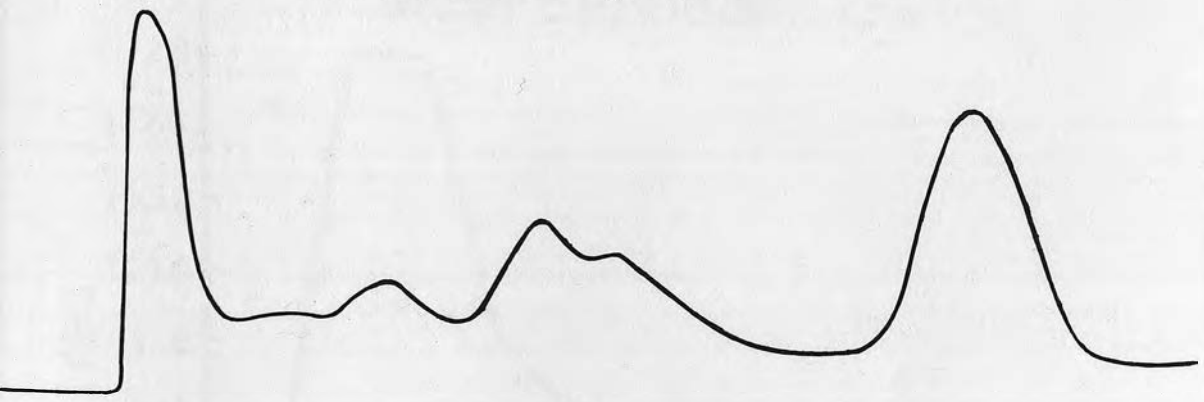


Fig. 32 - Location of complement-fixing antigens in orf scab suspension fractionated through Sephadex G-200.

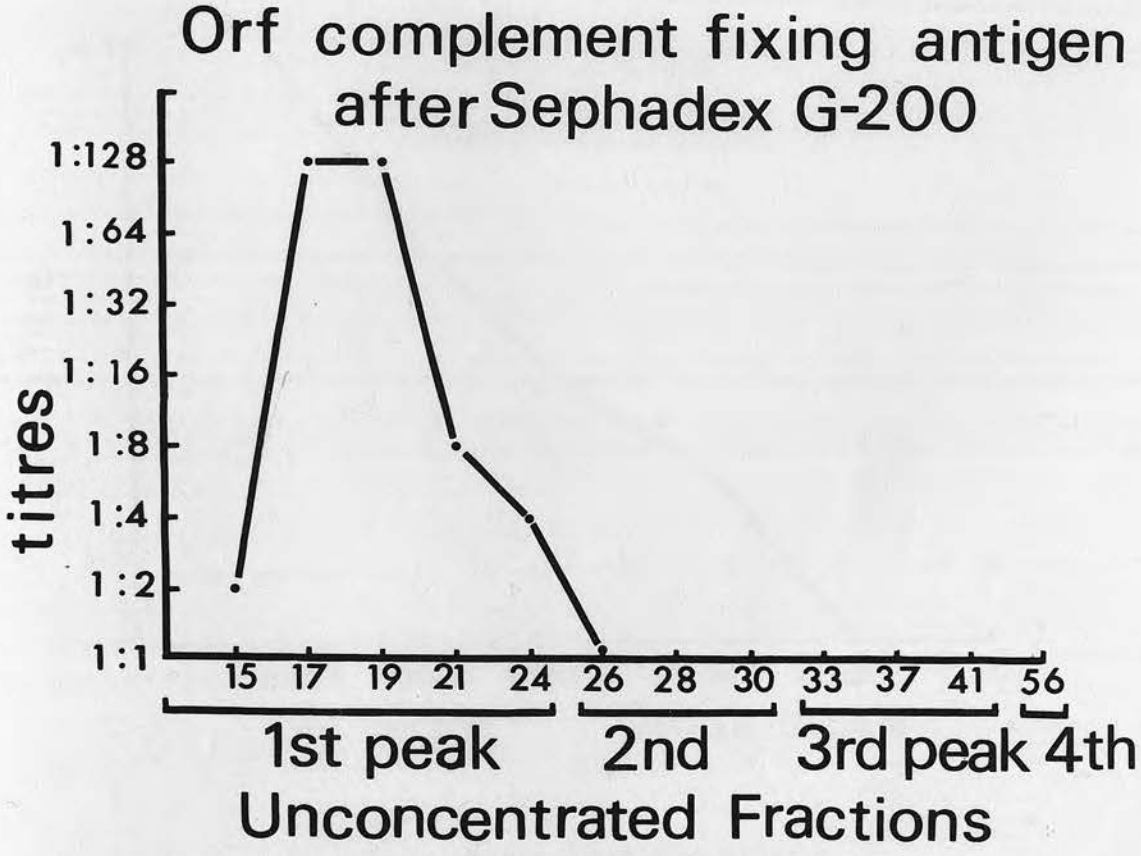
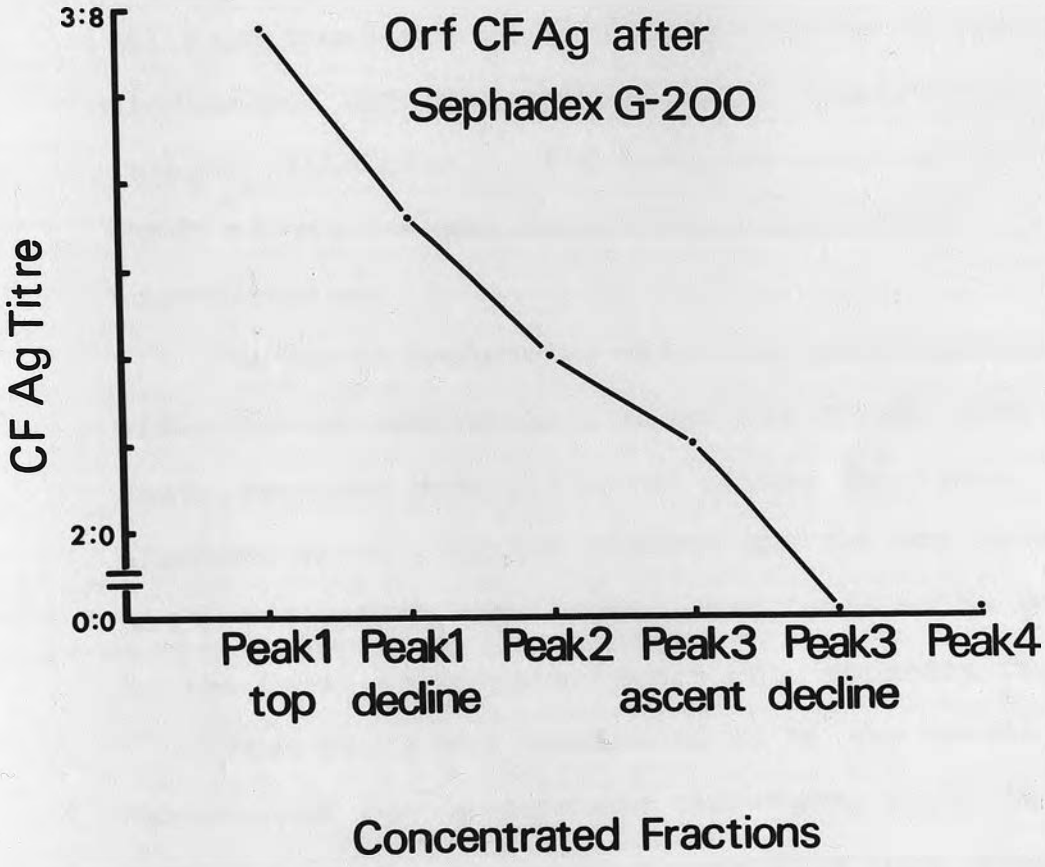

Orf complement fixing antigen
after Sephadex G-200
titres
1:128
1:64
1:32
1:16
1:8
1:4
1:2
1:1
15 17 19 21 24 26 28 30 33 37 41 56
1st peak
2nd
3rd peak
4th
Unconcentrated Fractions

Fig. 33 - Location of complement-fixing antigens in concentrated fractions of orf scab suspensions after passage through Sephadex G-200.


Orf CF Ag after
Sephadex G-200
CF Ag Titre
3:8
2:0
0:0
Peak1 top
Peak1 decline
Peak2
Peak3 ascent
Peak3 decline
Peak4
Concentrated Fractions

DIAGNOSTIC EFFICIENCY OF METHODS FOR DETECTING PARTICLES AND ANTIGENS

EXPERIMENTALLY-PRODUCED ORF SCABS

A total of two hundred and forty-five experimentally-produced orf scabs were assayed by electron microscopy, agar gel diffusion and complement-fixation antigen techniques. The scabs were harvested from three to sixty-eight days after experimental scarification.

The three techniques were equally sensitive and efficient in confirming a diagnosis of orf when the scabs examined were collected within the first nineteen days; all one hundred and two orf scabs collected within this period were found to be positive by the three techniques (Table 20; Appendix fig. 20).

Five weeks was considered to be the normal duration of the lesions and therefore, scabs harvested from day twenty up to day thirty-five were grouped. Within this period the complement-fixation test proved to be the most sensitive by detecting as positive one hundred and two out of one hundred and eleven orf scabs examined (92 per cent). Electron microscopy detected ninety-two positives (83 per cent) and the gel diffusion technique detected only forty-five positives (41 per cent).

Lesions /

Lesions that had an abnormally long duration of more than five weeks were included in a third group. Again, the complement fixation test was the most efficient method; thirty-two orf scabs collected between thirty-five and sixty-eight days were examined and thirty were positive (93 per cent) whereas electron microscopy gave nineteen positives (59 per cent) and gel diffusion detected twelve positives (38 per cent).

SCABS FROM NATURAL CASES

Scabs from lambs: Scabs from seven clinically diagnosed cases of orf were assayed for the presence of virus particles, precipitating and complement-fixing antigens. In only two cases did all three techniques give positive results. Three other scabs were shown to be positive by electron microscopy and by complement-fixation techniques. Two scabs were positive only by the complement-fixation test (Table 21).

Scabs from four other clinically diagnosed cases of orf were examined by immuno-diffusion and complement-fixation techniques. Two were judged negative and one was considered positive by both methods. The fourth scab contained complement-fixing antigen but not precipitating antigen.

Finally /

Finally a scab from one case was checked by electron microscopy and by complement fixation. Both techniques yielded a negative result.

Scabs from ewes: Thirteen scabs were harvested from the teats of nine ewes diagnosed clinically as suffering from naturally-acquired orf. The scabs were examined for the presence of virus particles by electron microscopy and for precipitating and complement-fixing antigens. Seven scabs were negative by all three techniques (Table 22). Two were positive by electron microscopy and complement fixation. One was positive by immuno-diffusion and by complement fixation. One was positive only by electron microscopy, one distorted particle only being detected. Finally two were positive only by complement fixation.

SCABS FROM SUSPECT CASES

When orf suspected scabs were processed by two of the three techniques, the following results were obtained. Of a total of twenty-two scabs examined by gel diffusion and by electron microscopy, seven were considered positive and ten were deemed negative by both techniques (Table 23). Five other scabs were positive by electron microscopy only.

TABLE 20

COMPARATIVE EFFICIENCIES OF ELECTRON MICROSCOPY, GEL DIFFUSION AND COMPLEMENT-FIXATION TESTS IN CONFIRMING A DIAGNOSIS OF ORF.

Age of scabs (days)	Electron microscopy (%)	Gel diffusion (%)	Complement-fixation (%)
1 - 19	100	100	100
20 - 35	83	41	92
36 - 68	59	38	93

TABLE 21

DIAGNOSTIC EFFICIENCY OF METHODS FOR CONFIRMING NATURAL CASES OF ORF IN LAMBS

Scab	Electron Microscopy	Gel Diffusion	Complement fixation titre *
C39	+	+	2.3
61	+	+	2.3
24	+	-	2.3
32	+	-	2.3
35	+	-	2.3
40	-	-	2.6
63	-	-	2.3
29	.	-	< 2.0
5	.	-	< 2.0
C9	.	-	2.0
C51	.	+	2.6
13	-	.	-

* Expressed as the logarithm of the reciprocal of the 50 per cent end-point dilution.

TABLE 22

DIAGNOSTIC EFFICIENCY OF METHODS FOR CONFIRMING NATURAL TEAT ORF

Date	Scab	Electron Microscopy	Gel diffusion	Complement fixation *
13.3.68	U12	-	-	-
13.3.68	T85	-	-	-
20.2.69	T90	-	-	-
20.2.69	U11	-	-	-
6.3.69	U11	-	-	-
20.2.69	U56	-	-	-
6.3.69	U56	-	-	2.6
13.3.69	U56	-	-	2.9
20.3.69	U56	-	-	-
20.3.69	W50	+	-	2.3
13.3.69	T87	+	-	2.6
24.3.69	10	-	+	2.2
20.3.69	W1	+	-	-

* Expressed as the logarithm of the reciprocal of the 50 per cent end-point dilution.

TABLE 23

EFFICIENCY OF METHODS FOR CONFIRMING A DIAGNOSIS ON SUSPECT CASES

Date	Scab	Electron Microscopy	Gel Diffusion
24.2.67	34	+	+
24.2.67	50	+	+
13.3.69	2	+	+
26.3.69	W	+	+
10.4.69	14	+	+
3.6.69	C23	+	+
10.6.69	30	+	+
21.2.68	5	-	-
21.2.68	26	-	-
13.3.68	C11	-	-
13.3.68	29	-	-
13.3.68	40	-	-
27.3.68	30	-	-
5.4.68	19	-	-
8.5.69	20	-	-
22.5.69	38	-	-
3.6.69	C11	-	-
13.3.68	1	+	-
13.3.68	15	+	-
20.3.68	24	+	-
26.4.69	20	+	-
1.5.69	43	+	-

PRODUCTION OF ANTISERA

SHEEP ANTI-ORF SERA

Anti-sera reactive against orf virus in cyto-toxicity-inhibiting tests, fluorescent antibody tests, immuno-diffusion tests and complement-fixation tests were produced successfully in five out of the six experimentally-inoculated lambs. None of the sera reacted when tested with antigens prepared from virus-free scabs, pure colonies of stock strains of Staphylococcus aureus or colonies of Staphylococcus aureus isolated from orf-infected scabs. The sera never reacted with orf-susceptible and orf+immune sheep sera or with each other. The antibodies were not absorbed out when the sera were incubated with sheep red blood cells, antigens from Staphylococcus aureus or virus-free scabs (Tables 24 and 25). Complement-fixing activity, however, was increased slightly by absorption with sheep red blood cells.

Greyface 7: Although Greyface 7 was an immune lamb from vaccination six months before and although it developed natural orf fifteen days after vaccination, its serum collected before hyperimmunisation did not contain detectable precipitating and complement-fixing antibodies.

Precipitating /

Precipitating antibodies: The serum collected one week after the intravenous injection of the orf scab suspension in incomplete Freund's adjuvant was reactive and the titre of the precipitating antibody was 1:16 (Table 26; Fig. 34). The titre was maintained at this level up to the fifth week but thereafter the titre fell to a barely detectable level at sixteen weeks.

Serum obtained five weeks after hyperimmunisation was fractionated through Sephadex G-200 and the precipitating activity was found to be restricted to the fractions corresponding to the second or 7S peak.

No precipitating antibody was detected when the animal was challenged seven months after the adjuvant injection. There was an anamnestic response (Table 27; Fig. 34). Moreover a second precipitating antibody developed and its titre ten and fifteen days after challenge was the same as the first antibody. The titres of both antibodies fell to 1:16 at thirty days after challenge. At seventy days after challenge only one antibody was detectable.

When sera containing two antibodies were tested alongside sera containing one antibody, the single antibody was found to be directed against a slow-moving antigen. The second antibody reacted with

a /

a fast-moving antigen which was never detected with serum containing only one antibody. Both antibodies occurred in the Sephadex G-200 fractions corresponding to the 7S peak.

Complement-fixing antibodies: The serum collected one week after the intravenous injection of the orf scab suspension in incomplete Freund's adjuvant contained no complement-fixing antibody but at two weeks the serum had a titre of 1:12 (Table 26; Fig. 35). Thereafter the titres fluctuated between 1:8 and 1:16.

Serum collected five weeks after hyperimmunisation was fractionated through Sephadex G-200 and the entire complement-fixing activity was found in the fractions corresponding to the descending side of the first or 19S peak.

The animal was challenged seven months after the adjuvant injections when the complement-fixing titre of the serum was 1:16. There was an anamnestic response and the titre rose to 1:32 within ten days and was maintained at this level for at least another twenty days after which it declined (Table 27; Fig. 35).

Greyface 10: Although Greyface 10 was an immune lamb from vaccination six months before and although it developed /

developed natural orf one month after vaccination, its serum collected before hyperimmunisation did not contain detectable precipitating antibodies.

Precipitating antibodies: The serum collected one week after the intravenous injection of the re-suspended orf virus pellet in incomplete Freund's adjuvant was reactive and the titre of the precipitating antibody was 1:2 (Table 28; Fig. 36). A week later the titre rose to 1:4 but samples obtained at four, five and seven weeks had titres of 1:2.

Serum obtained five weeks after hyperimmunisation was fractionated through Sephadex G-200 and the precipitating activity was found to be restricted to the fractions corresponding to the second or 7S peak.

The lamb was challenged for the first time eight weeks after hyperimmunisation when the precipitating antibody activity was 1:1. No precipitating antibodies were demonstrated in the sera obtained one and two weeks later. The lamb was challenged for a second time two weeks after the first challenge. No precipitating activity was detected in the serum obtained one week after the second challenge but a serum sample collected two weeks later was reactive and had a titre of 1:2 (Table 29; Fig. 36). Six weeks after the second challenge /

challenge another serum sample was obtained but no precipitating activity was detected. Twenty weeks after the second challenge the serum was still devoid of precipitating activity. The lamb was re-challenged by scarification. The serum sample obtained ten days later had a precipitating titre of 1:2. Despite three challenges, Greyface 10 produced only one antibody which was always directed against the slow-moving antigen.

Complement-fixing antibodies: The serum collected one week after the intravenous injection of the re-suspended orf virus pellet in incomplete Freund's adjuvant had a complement-fixing antibody titre of 1:4 (Table 28: Fig. 37). Thereafter, the titres fluctuated between 1:10 and 1:12. The lamb was challenged eight weeks after the adjuvant injection when the complement-fixing antibody titre was 1:8. There was an anamnestic response and the titre rose to 1:32 within one week (Table 29: Fig. 37). Two weeks after challenge the animal was challenged for a second time and the serum obtained a week later had a complement-fixing antibody titre of 1:32. Sera obtained six and seventeen weeks after the second challenge had titres of 1:12 and 1:16 respectively. The lamb was challenged for a third time, twenty weeks after the second challenge and the serum obtained ten days later had a titre of 1:32.

Greyface 4: Greyface lamb 4 was vaccinated against orf. Eight months later it was hyperimmunised but the serum collected at the time of hyperimmunisation did not contain detectable precipitating and complement-fixing antibodies.

Precipitating antibodies: The serum collected six weeks after the intramuscular injection of the suspended orf virus pellet in complete Freund's adjuvant was reactive and contained two antibodies, the titres of which were 1:16 (Table 30; Fig. 38). The titres were maintained at this level up to the seventeenth week, the longest period tested. Six months after hyperimmunisation the lamb was challenged by scarification and the serum obtained two weeks later again contained two antibodies both having a titre of 1:32.

By testing the serum alongside sera containing one antibody, the single antibody was found to be directed against a slow-moving antigen and the second antibody reacted with a fast-moving antigen which was never detected with serum containing only one antibody. Both antibodies occurred in the Sephadex G-200 fractions corresponding to the 7S peak.

Complement-fixing antibodies: The serum collected six weeks after the beginning of the hyperimmunisation regime /

regime contained complement-fixing antibodies. The titre was 1:12 (Table 30; Fig. 39). Eleven weeks later the complement-fixing titre was 1:20. Seven months after the intramuscular injection of the re-suspended orf virus pellet in complete Freund's adjuvant the lamb was challenged by scarification and the complement-fixing antibody titre of a serum sample obtained two weeks later was 1:16.

When the serum collected two weeks after challenge was fractionated through Sephadex G-200 complement-fixing activity was found in the fractions corresponding to the first or 19S peak and in the fractions corresponding to the ascending side of the second or 7S peak.

Cross-bred Dorset 64: The cross-bred Dorset lamb 64 was injected with Sephadex G-200 orf-infected fractions but no precipitating antibodies were detected in its serum before hyperimmunisation.

Precipitating antibodies: The serum collected five weeks after hyperimmunisation was reactive and the titre of the precipitating antibody was 1:16 (Table 31; Fig. 40). The titre was maintained at this level up to the eleventh week. The precipitating titre of the serum /

serum obtained five months after hyperimmunisation was 1:8 and thereafter fell until at the eighth month, when the animal was challenged, the precipitating antibody activity of the serum was 1:2. Eleven days after challenge, there was evidence of an anamnestic response (Table 32; Fig. 40). Moreover, a second precipitating antibody developed, and its titre was the same as the first antibody, namely, 1:32.

When the serum containing the two antibodies was tested alongside sera containing one antibody, the single antibody was found to be directed against a slow-moving antigen. The second antibody reacted with a fast-moving antigen which was never detected with serum containing only one antibody. Both antibodies occurred in the Sephadex G-200 fractions corresponding to the 7S peak.

Complement-fixing antibodies: The serum collected five weeks after the initial intravenous injection of infective fractions in incomplete Freund's adjuvant contained complement-fixing antibodies titrating to 1:16 (Table 31; Fig. 41). Four weeks later, the titre was found to be 1:64 and this titre was maintained until eight months after the first adjuvant injection. When the lamb was challenged by scarification, the serum obtained eleven days after challenge had /

had a complement-fixing antibody titre of 1:64 (Table 32; Fig. 41). The post-challenge serum was fractionated through Sephadex G-200 and the entire complement-fixing activity was restricted to the fractions corresponding to the descending side of the first or 19S peak.

Cheviot 54: Cheviot 54 was a susceptible lamb and serum collected before hyperimmunisation did not contain detectable complement-fixing antibodies or precipitating antibodies.

Precipitating antibodies: The serum collected one week after the intravenous injection of the precipitating fractions in incomplete Freund's adjuvant was reactive but only when assayed undiluted (Table 33; Fig. 42). By the second week the serum had a precipitating antibody titre of 1:2 and by the third week the titre had risen to 1:8. The titre was maintained at this level up to the sixth week, the longest period tested.

Complement-fixing antibodies: The sera collected one and two weeks after the intravenous injection of Sephadex G-200 fractions containing precipitating antigen in incomplete Freund's adjuvant contained no complement-fixing antibody but at three and four weeks the sera had titres of 1:4 (Table 33; Fig. 43). The sera obtained five and six weeks after hyperimmunisation failed to fix complement.

Cheviot 76: Cheviot 76 was a susceptible lamb and its serum collected before hyperimmunisation did not contain detectable precipitating antibodies. On the other hand, complement-fixing antibodies were present to a titre of 1:4.

Precipitating antibodies: None of the sera collected after injection contained precipitating antibodies.

Complement-fixing antibodies: The sera collected weekly up to five weeks after the intravenous injection of Sephadex G-200 fractions in Freund's adjuvant contained no complement-fixing antibodies.

RABBIT ANTI-ORF SERA

Production of specific anti-orf sera in rabbits with the antigens available was not satisfactory. Four rabbits died within twenty-four hours of inoculation and death was attributed to the procedure. The autopsies revealed pulmonary oedema with a few patches of pneumonia, hydrothorax, hydropericardium, and severe vascular congestion of the upper respiratory tract and along the folds of the intestinal mucosa.

Rabbits 02, 21 and 22: The serum pool derived from rabbits 02, 21 and 22 contained at least five precipitating /

precipitating antibodies which were reactive when diffused through agar against orf scab suspensions and at least four antibodies reactive against normal sheep serum (Fig. 44). The extra line of precipita-:tion in the rabbit serum-orf scab suspension reaction did not fuse to give a line of identity with the control reaction between the same orf scab suspension and sheep anti-orf serum.

Rabbit 011: Despite attempted purification of the antigen used to hyperimmunise rabbit oll two antibodies were formed. One reacted with orf scab antigen but the precipitation line was not identical with the control line formed between the same antigen and sheep anti-orf serum. The other antibody reacted with sheep serum only.

BOVINE ANTI-BOVINE PAPULAR STOMATITIS SERUM

The calf hyperimmunised against bovine papular stomatitis was earlier infected experimentally with the virus. Nevertheless its serum collected prior to hyperimmunisation did not contain detectable precipitating and complement-fixing antibodies.

Precipitating antibodies: Serum collected four weeks after the intravenous injection of bovine papular stomatitis /

stomatitis infected scab suspension in incomplete Freund's adjuvant was reactive when diffused against five orf scab suspensions known to contain orf-precipitating antigen. The titre of the serum, however, was only 1:1 (Table 34).

The reaction between bovine anti-bovine papular stomatitis serum and orf antigen was tested alongside orf systems giving two lines of precipitation and was found to fuse with the reaction associated with the slow-moving antigen.

Titration of orf antigens simultaneously against bovine anti-bovine papular stomatitis serum and sheep anti-orf serum yielded identical end-points of 1:20. Heat treatment of the antigen suspension for fifteen minutes at 56°C destroyed all reactivity with the bovine anti-bovine papular stomatitis sera whereas the titre against sheep anti-orf serum fell to 1:5.

Complement-fixing antibodies: The calf serum taken four weeks after hyperimmunisation did not fix complement in the presence of orf antigen suspension.

TABLE 24

PRECIPITATING ACTIVITY* OF SHEEP ANTI-ORF SERA BEFORE AND AFTER ABSORPTION

Serum	Sheep erythrocytes		Staphylococcus aureus		Virus-free scabs	
	Before	After	Before	After	Before	After
G7	1:16	1:16	1:16	1:16	1:16	1:16
G10	1:4	1:4	1:4	1:4	1:4	1:4
G4	1:16	1:16	1:16	1:16	1:16	1:16
D64	1:16	1:16	1:16	1:16	1:16	1:16
C54	1:4	1:4	1:4	1:4	1:4	1:4

* Expressed as the highest dilution giving precipitations.

TABLE 25

COMPLEMENT-FIXING ACTIVITY OF SHEEP ANTI-ORF SERA* BEFORE AND AFTER ABSORPTION

Serum	Sheep erythrocytes		Staphylococcus aureus		Virus-free scabs	
	Before	After	Before	After	Before	After
G7	1:16	1:32	1:16	1:16	1:16	1:16
G10	1:12	1:16	1:12	1:12	1:12	1:12
G4	1:20	1:32	1:20	1:20	1:20	1:20
D64	1:64	1:64	1:64	1:64	1:64	1:64

* Expressed as the 50 per cent end-point dilution

TABLE 26

ANTIBODY RESPONSE OF GREYFACE LAMB 7

AFTER HYPERIMMUNISATION

Weeks after hyperimmunisation	Precipitating antibodies	Complement-fixing antibodies
0	Nil	Nil
1	1:16	Nil
2	1:16	1:12
3	1:16	
4	1:16	1:8
5	1:16	1:8
8	1:4	
10	1:4	1:12
16	1:1	1:12
27	Nil	1:16
30	Nil	1:16

Fig. 34 - The precipitating antibody response of immune Greyface lamb 7 after hyperimmunisation and challenge.

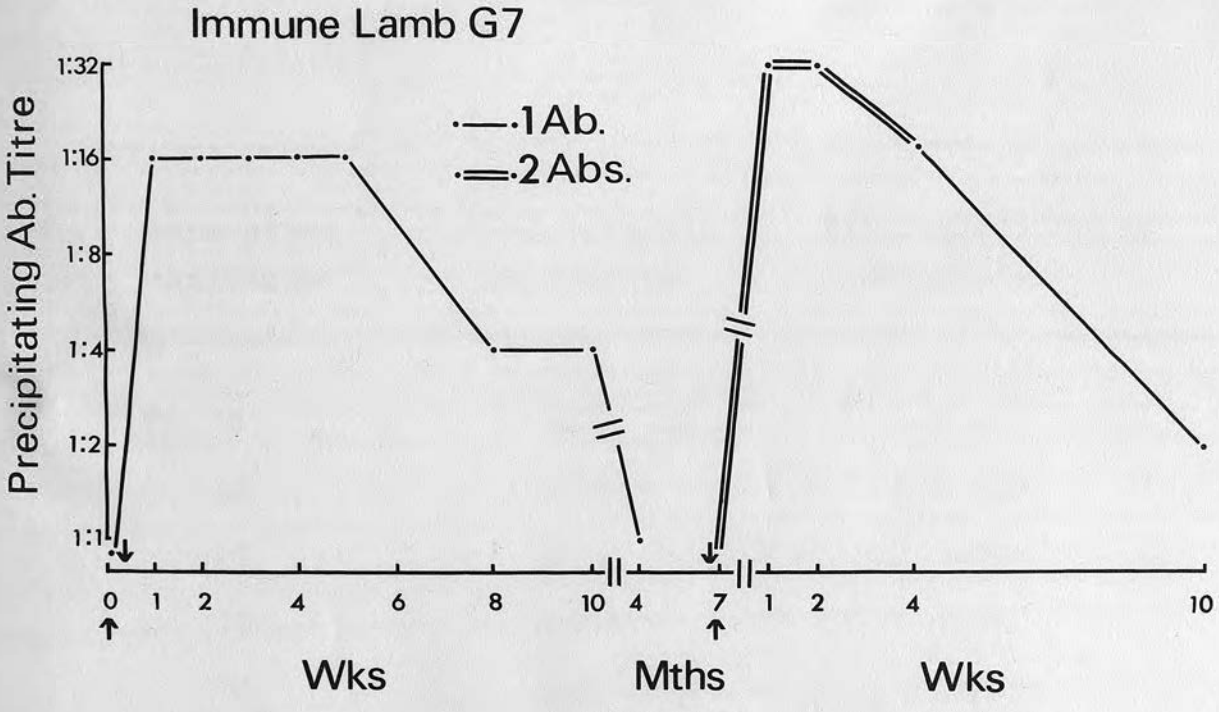

Immune Lamb G7
Precipitating Ab. Titre
1:32
1:16
1:8
1:4
1:2
1:1
1Ab.
2Abs.
0 1 2 4 6 8 10 4 7 1 2 4 10
Wks
Mths
Wks

TABLE 27

ANTIBODY RESPONSE OF HYPERIMMUNISED GREYFACE LAMB 7 AFTER CHALLENGE

Days after challenge	Precipitating antibodies	Complement-fixing antibodies
0	Nil	1:16
10	1:32	1:32
15	1:32	1:32
30	1:16	1:32
70	1:2	1:12

Fig. 35 - The complement-fixing antibody response of immune Greyface lamb 7 after hyperimmunisation and challenge.

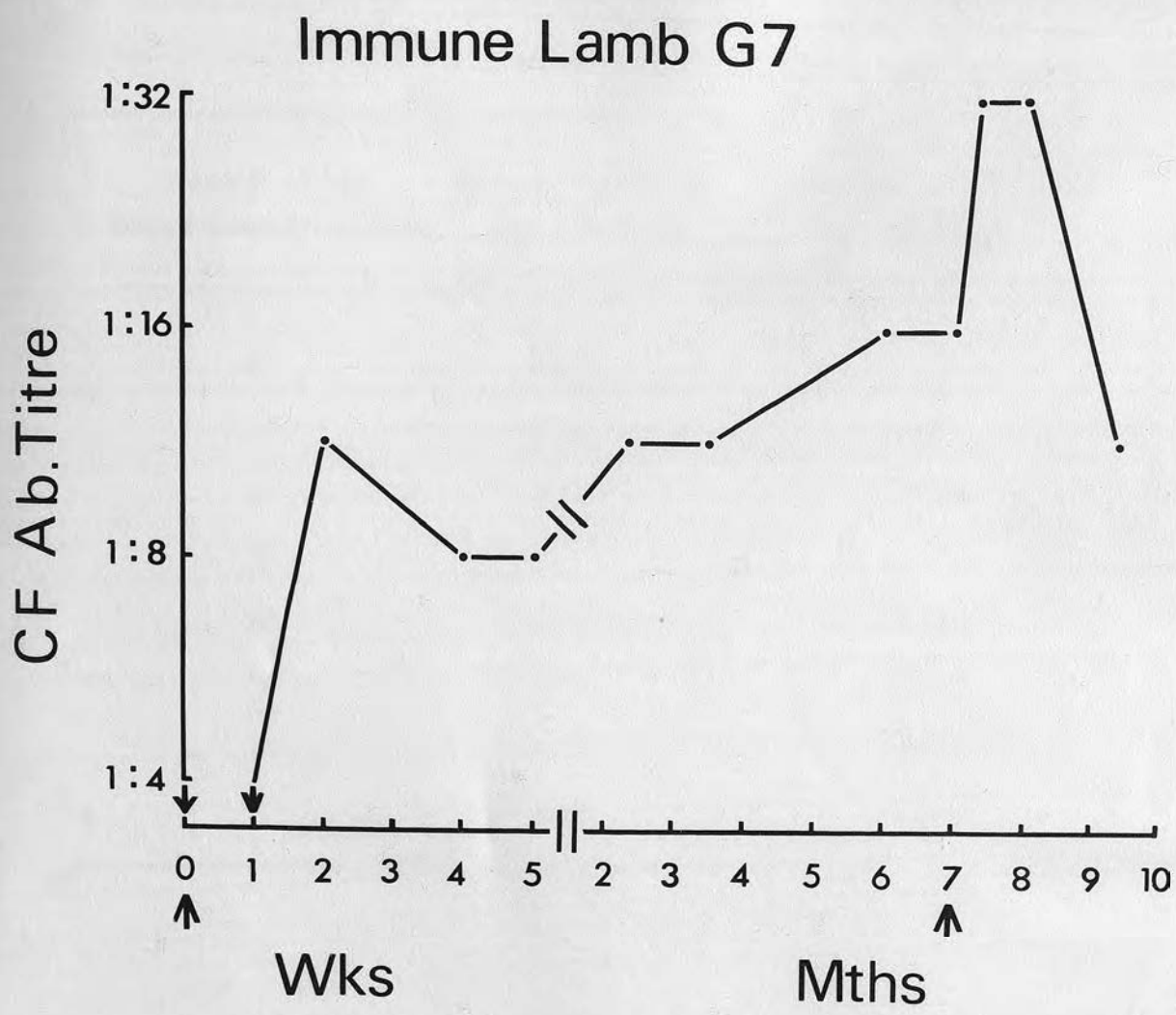

Immune Lamb G7
CF Ab.Titre
1:32
1:16
1:8
1:4
0
1
2
3
4
5
2
3
4
5
6
7
8
9
10
Wks
Mths

TABLE 28

ANTIBODY RESPONSE OF GREYFACE LAMB 10

AFTER HYPERIMMUNISATION

Weeks after hyperimmunisation	Precipitating antibodies	Complement-fixing antibodies
0	Nil	
1	1:2	1:4
2	1:4	1:12
3		
4	1:2	1:10
5	1:2	1:12
7	1:2	1:12
8	1:1	1:8

Fig. 36 - The precipitating antibody response of immune Greyface lamb 10 after hyperimmunisation and three challenges.

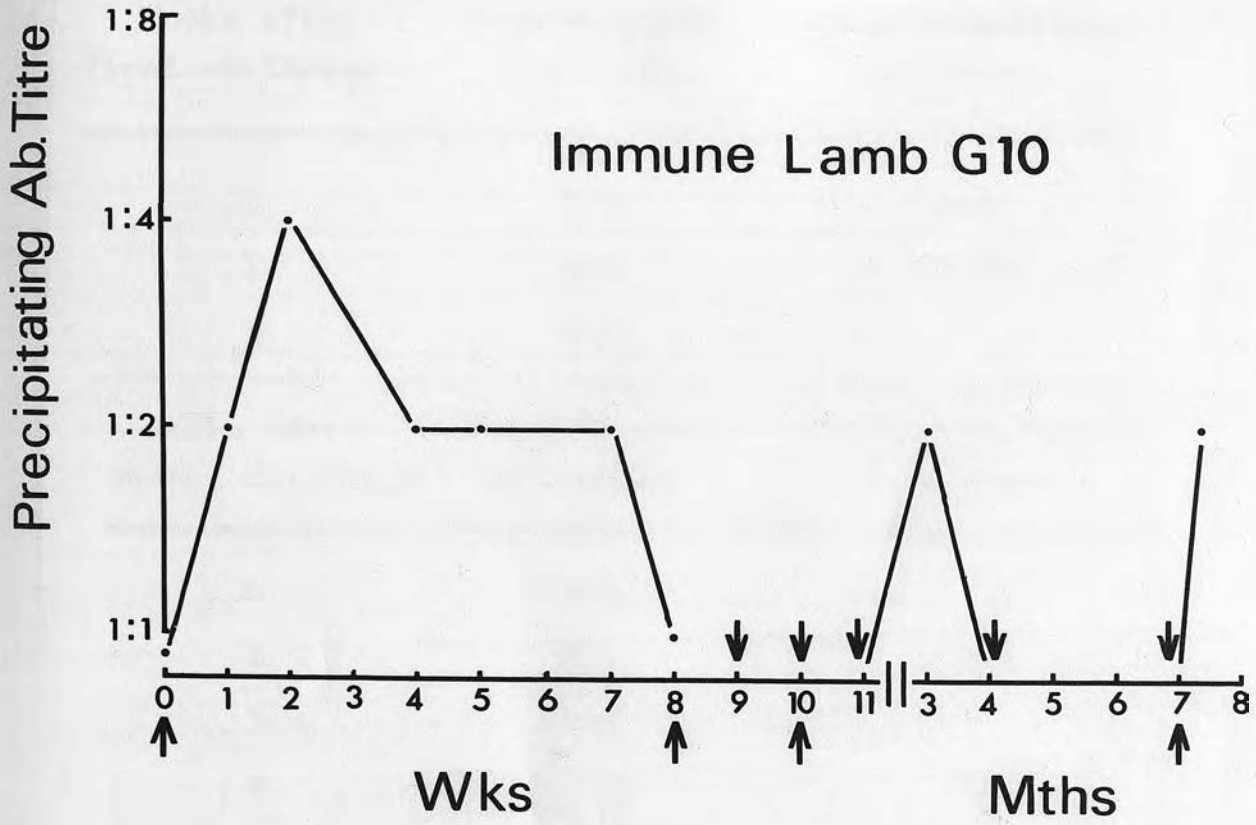

Immune Lamb G10
Precipitating Ab. Titre
1:8
1:4
1:2
1:1
0 1 2 3 4 5 6 7 8 9 10 11 3 4 5 6 7 8
Wks
Mths

TABLE 29

ANTIBODY RESPONSE OF HYPERIMMUNISED GREYFACE LAMB 10 AFTER CHALLENGE

Weeks after first challenge	Precipitating antibodies	Complement-fixing antibodies
0	1:1	1:8
1	Nil	1:32
2	Nil	

Weeks after second challenge	Precipitating antibodies	Complement-fixing antibodies
0	Nil	
1	Nil	1:32
3	1:2	
6	Nil	1:12
17		1:16
20	Nil	

Days after third challenge	Precipitating antibodies	Complement-fixing antibodies
0	0	
10	1:2	1:32

Fig. 37 - The complement-fixing antibody response of immune Greyface lamb 10 after hyperimmunisation and three challenges.

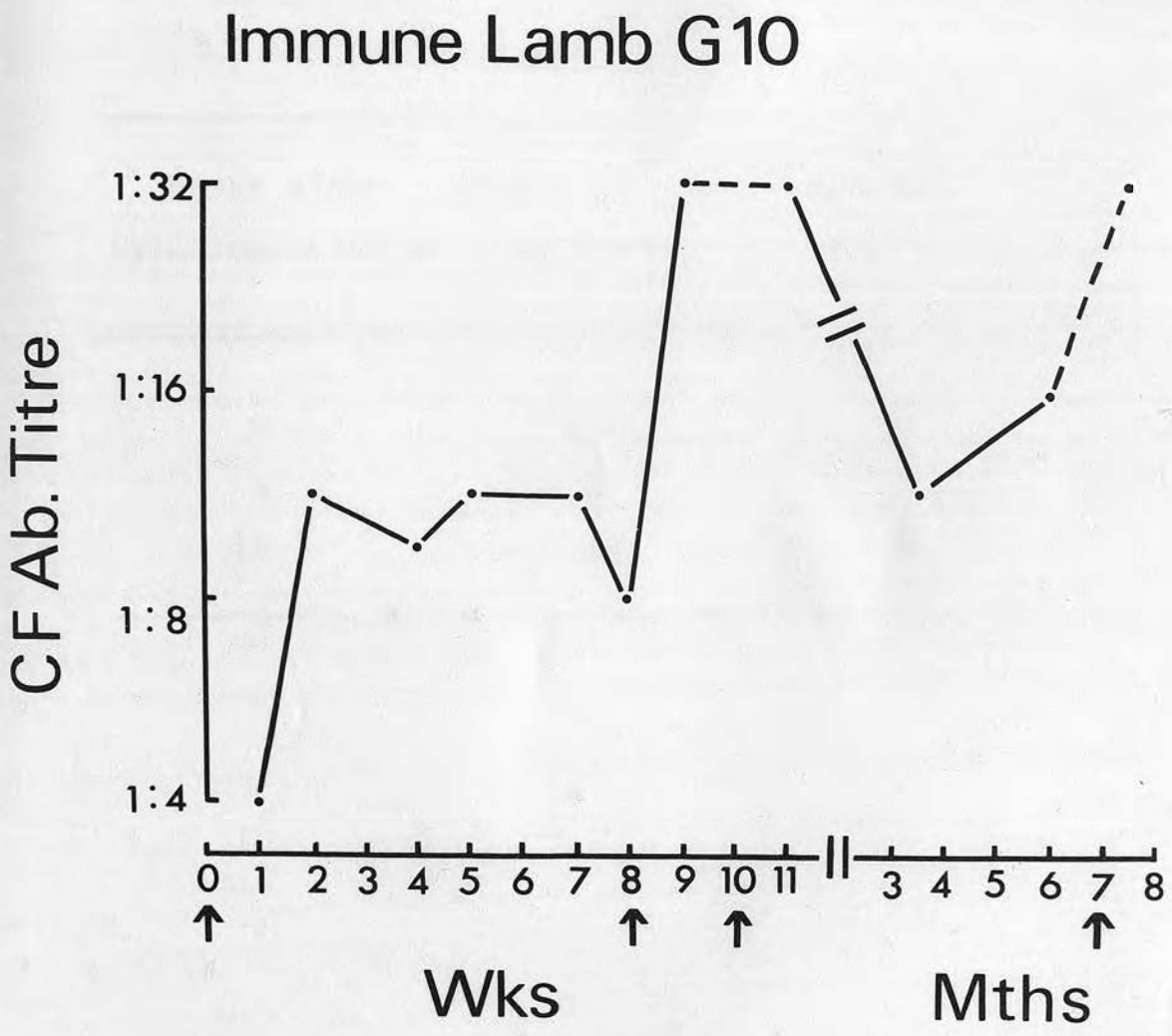

Immune Lamb G10
CF Ab. Titre
1:32
1:16
1:8
1:4
0 1 2 3 4 5 6 7 8 9 10 11
3 4 5 6 7 8
Wks
Mths

TABLE 30

ANTIBODY RESPONSE OF GREYFACE LAMB 4

AFTER HYPERIMMUNISATION

Weeks after hyperimmunisation	Precipitating antibodies	Complement-fixing antibodies
0	Nil	Nil
6	1:16	1:12
17	1:16	1:20

Fig. 38 - The precipitating antibody response of immune Greyface lamb 4 after hyperimmunisation and challenge.

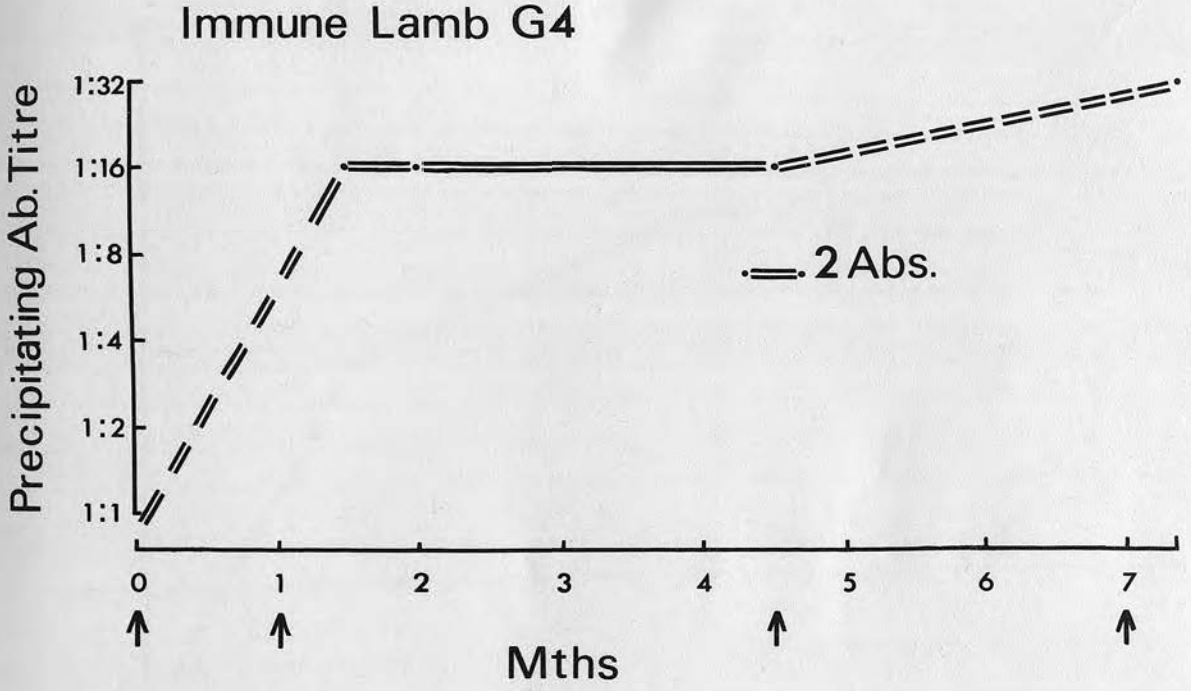

Immune Lamb G4
Precipitating Ab. Titre
1:32
1:16
1:8
1:4
1:2
1:1
2 Abs.
0
1
2
3
4
5
6
7
Mths

Fig. 39 - The complement-fixing antibody response of immune Greyface lamb 4 after hyper-immunisation and challenge.

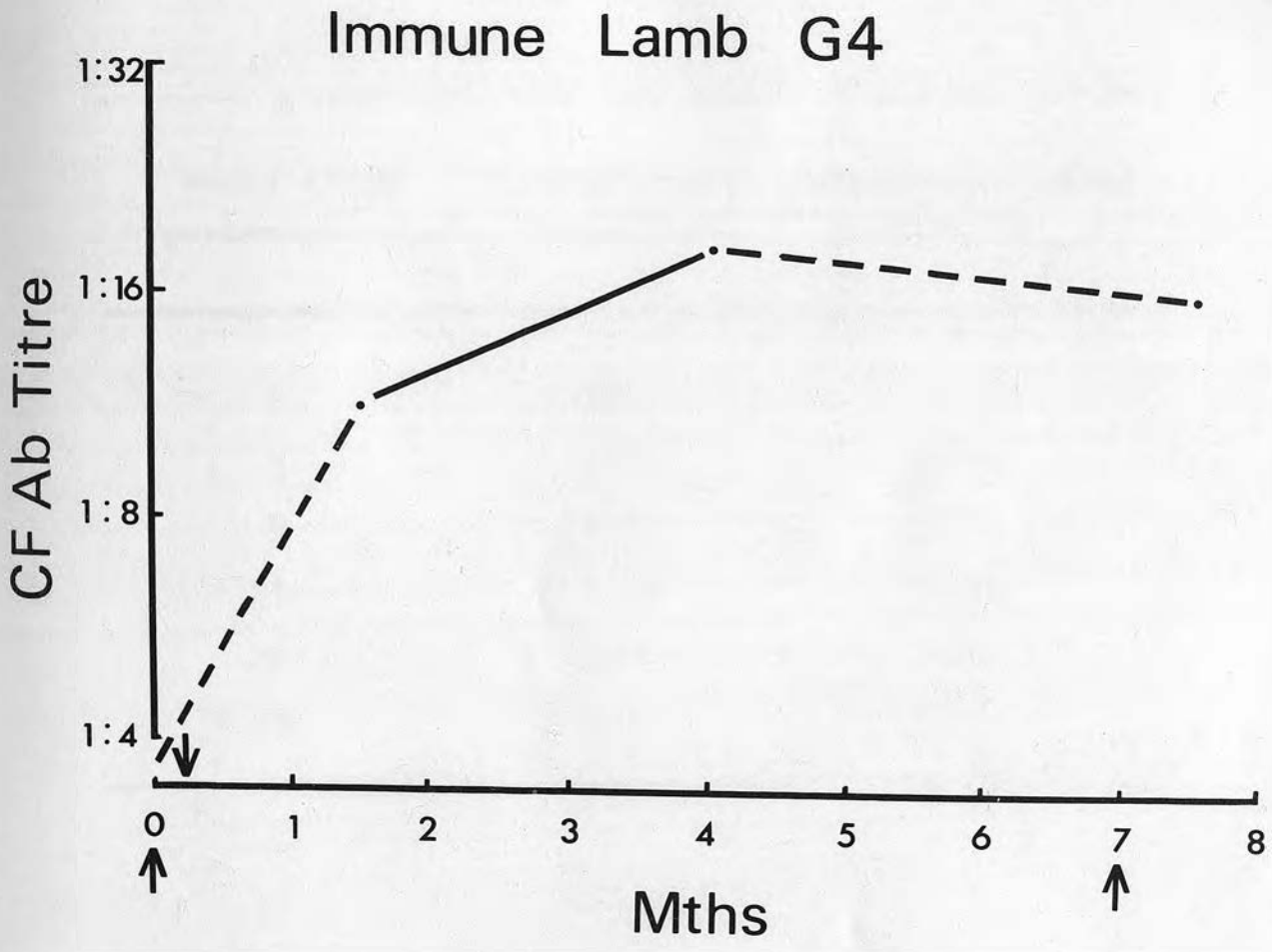

Immune Lamb G4
CF Ab Titre
1:32
1:16
1:8
1:4
0
1
2
3
4
5
6
7
8
Mths

TABLE 31

ANTIBODY RESPONSE OF CROSSBRED DORSET LAMB 64 AFTER HYPERIMMUNISATION

Weeks after hyperimmunisation	Precipitating antibodies	Complement-fixing antibodies
0	Nil	
5	1:16	1:16
9	1:16	1:64
11	1:16	1:64
20	1:8	1:64
35	1:2	1:64

Fig. 40 - The precipitating antibody response of crossbred Dorset lamb 64 after hyperimmunisation and challenge.

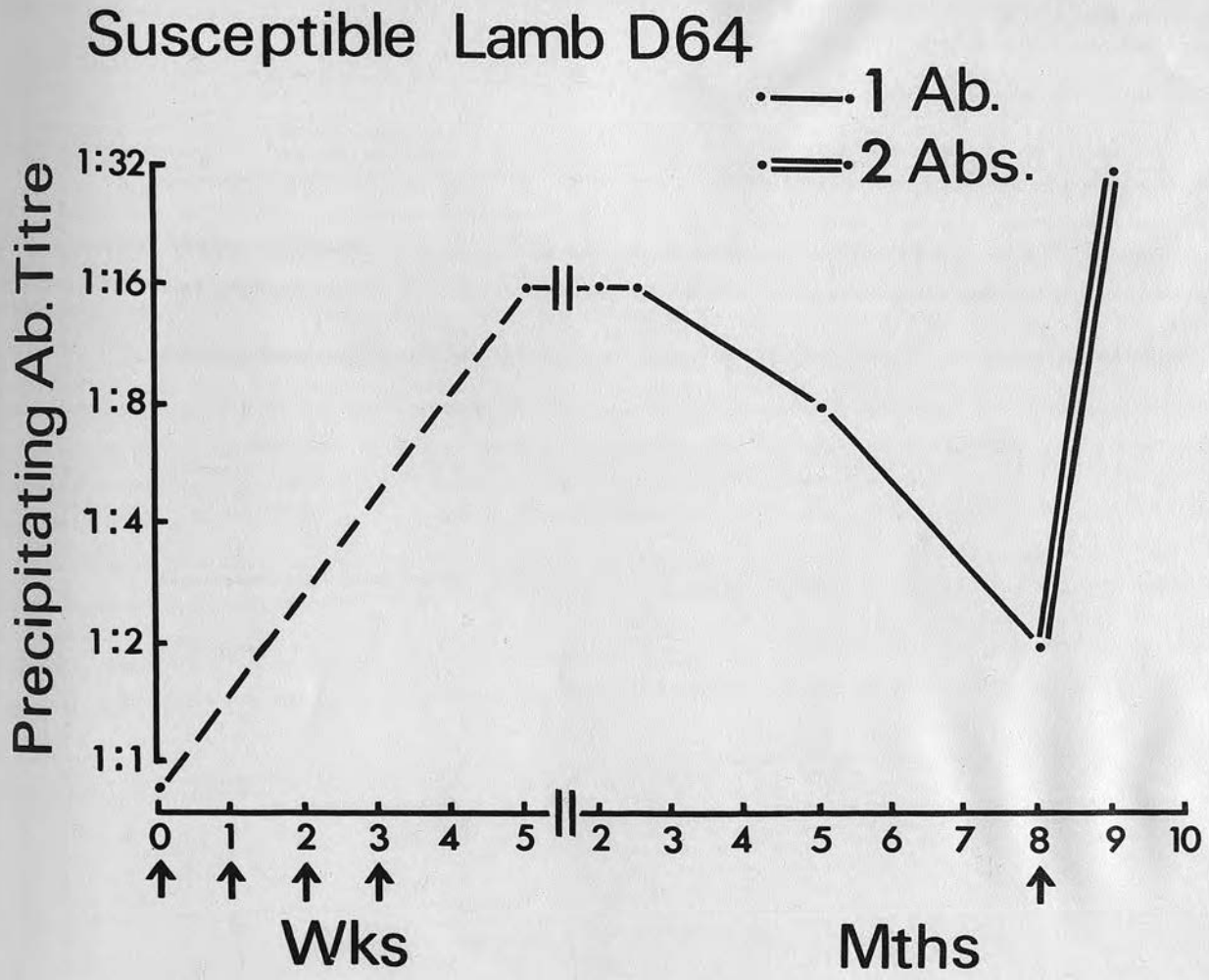

Susceptible Lamb D64
1 Ab.
2 Abs.
Precipitating Ab. Titre
1:32
1:16
1:8
1:4
1:2
1:1
0 1 2 3 4 5 2 3 4 5 6 7 8 9 10
Wks
Mths

TABLE 32

ANTIBODY RESPONSE OF HYPERIMMUNISED CROSSBRED DORSET LAMB 64 AFTER CHALLENGE

Days after challenge	Precipitating antibodies	Complement-fixing antibodies
0	1:2	1:64
11	1:32	1:64

Fig. 41 - The complement-fixing antibody response of crossbred Dorset lamb 64 after hyperimmunisation and challenge.

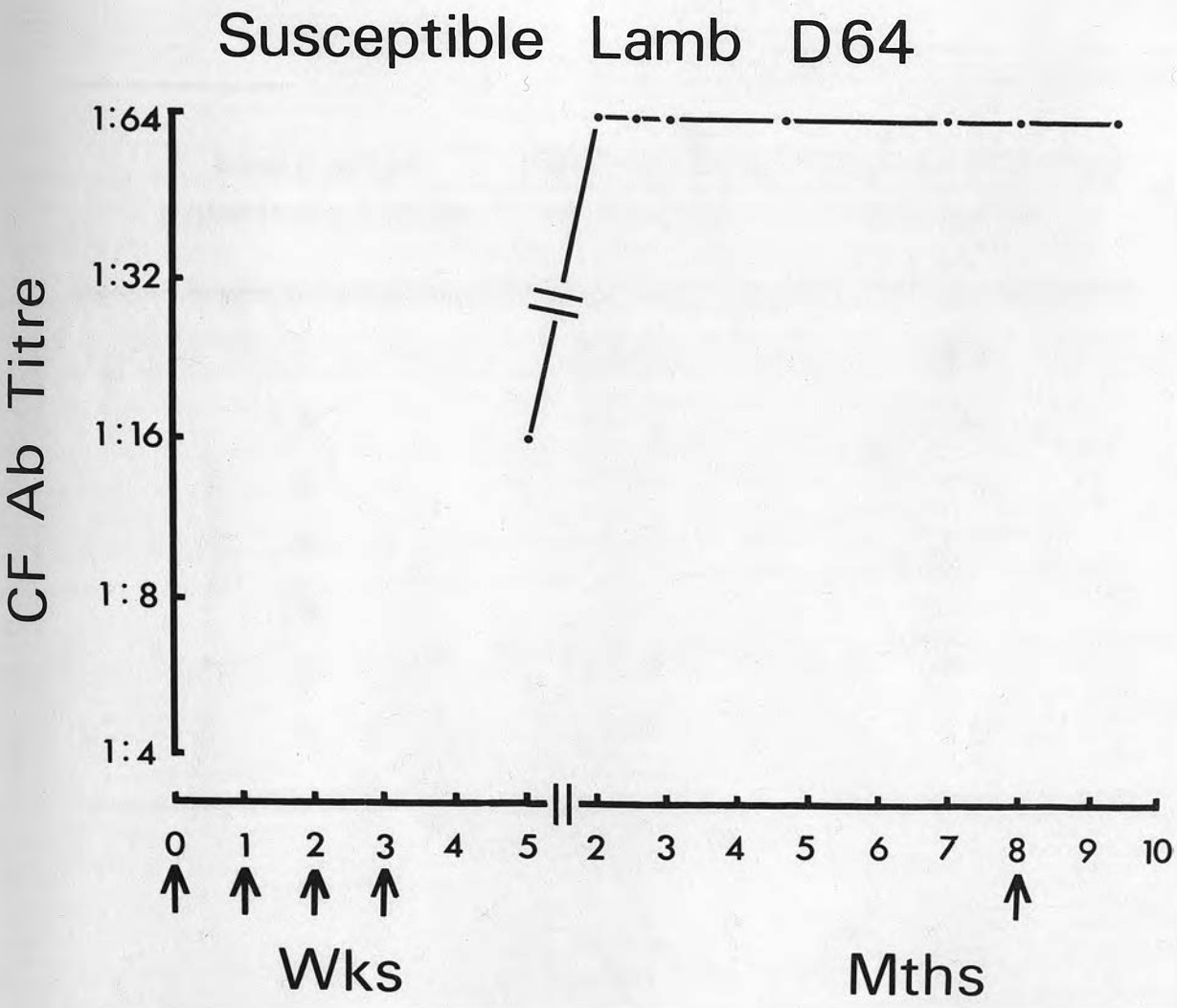

Susceptible Lamb D64
CF Ab Titre
1:64
1:32
1:16
1:8
1:4
0 1 2 3 4 5 2 3 4 5 6 7 8 9 10
Wks
Mths

TABLE 33

ANTIBODY RESPONSE OF CHEVIOT LAMB 54

AFTER HYPERIMMUNISATION

Weeks after hyperimmunisation	Precipitating antibodies	Complement-fixing antibodies
0	Nil	Nil
1	1:1	Nil
2	1:2	Nil
3	1:8	1:4
4	1:8	1:4
5	1:8	Nil
6	1:8	Nil

Fig. 42 - The precipitating antibody response of Cheviot lamb 54 after hyperimmunisation.

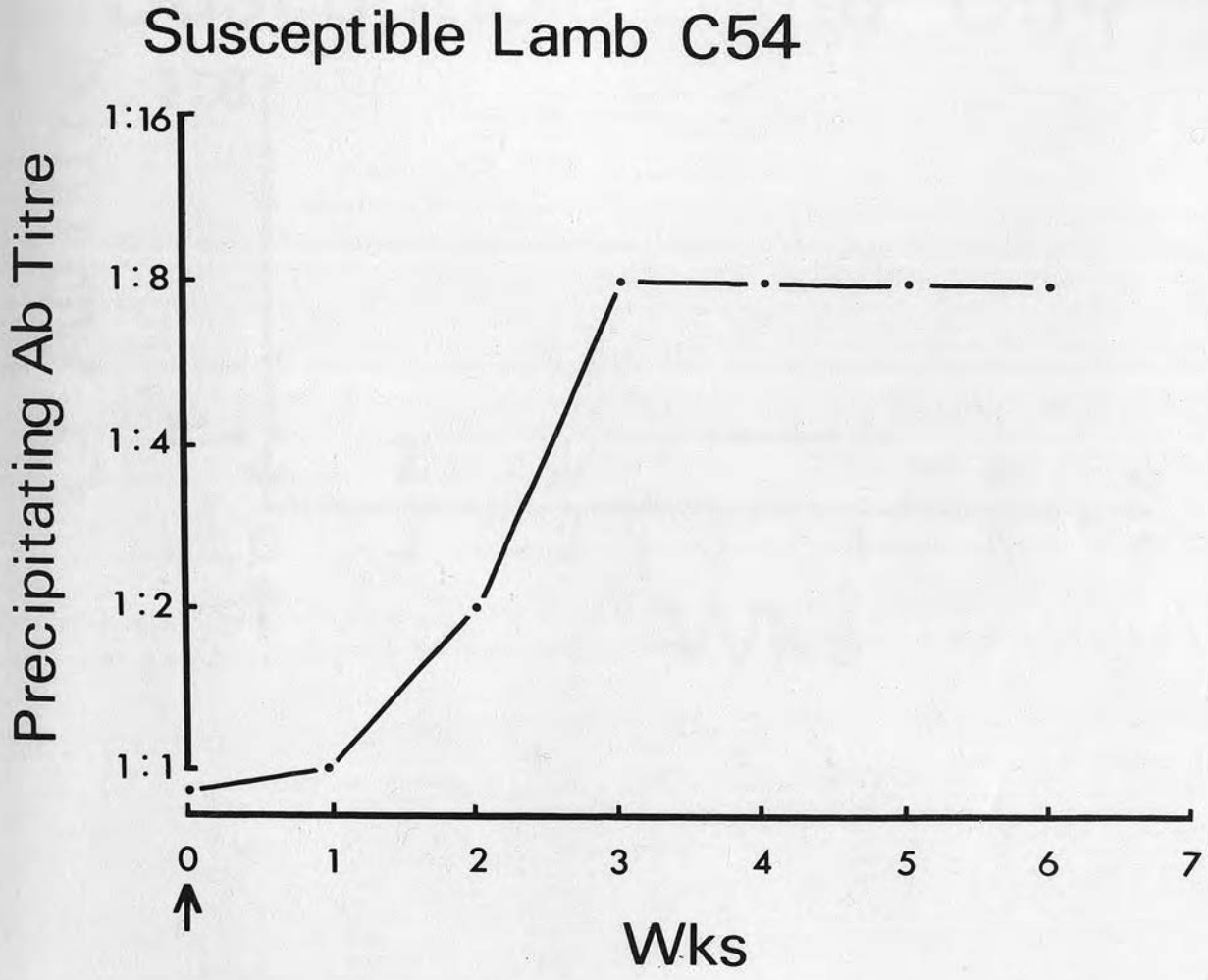

Susceptible Lamb C54
Precipitating Ab Titre
1:16
1:8
1:4
1:2
1:1
0
1
2
3
4
5
6
7
Wks

Fig. 43 - The complement-fixing antibody response of Cheviot lamb 54 after hyperimmunisation.

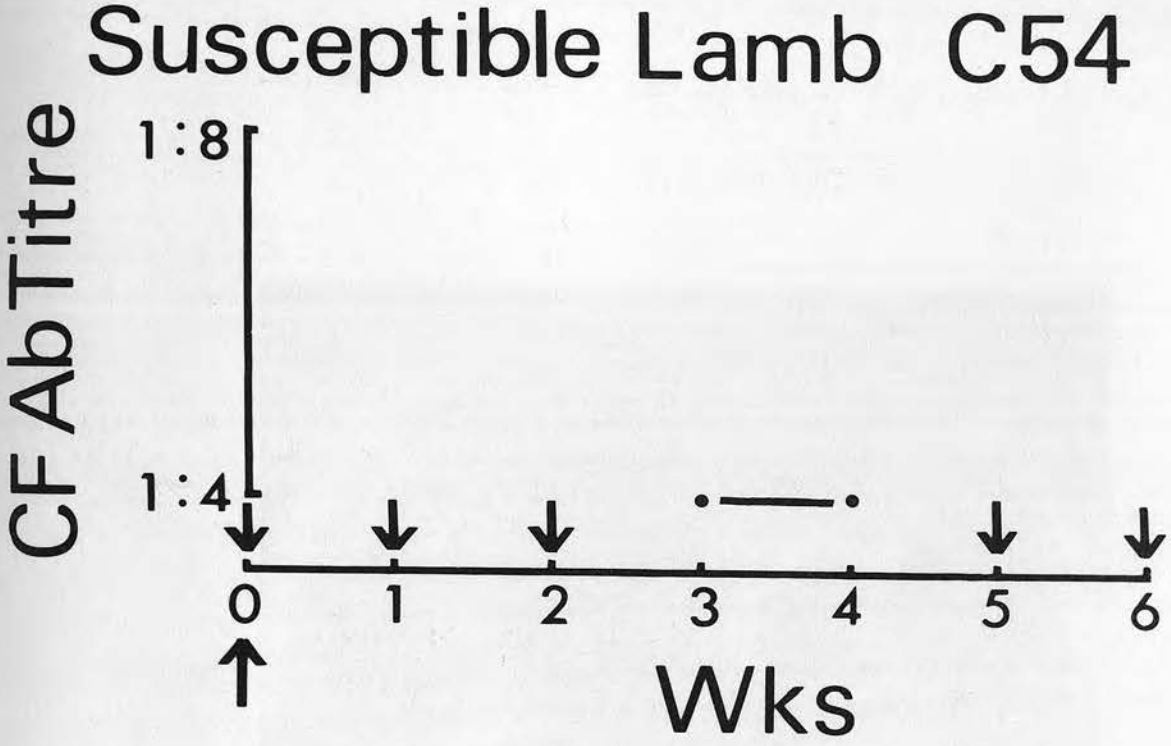

Susceptible Lamb C54
CF Ab Titre
1:8
1:4
0
1
2
3
4
5
6
Wks

Fig. 44 - Immuno-diffusion plate illustrating the reactions produced by pooled sera from rabbits 02, 21 and 22. The top and bottom wells contained sheep anti-orf serum. The lower left and upper right wells contained the rabbit serum pool. The upper left, central and lower right wells contained orf antigen.

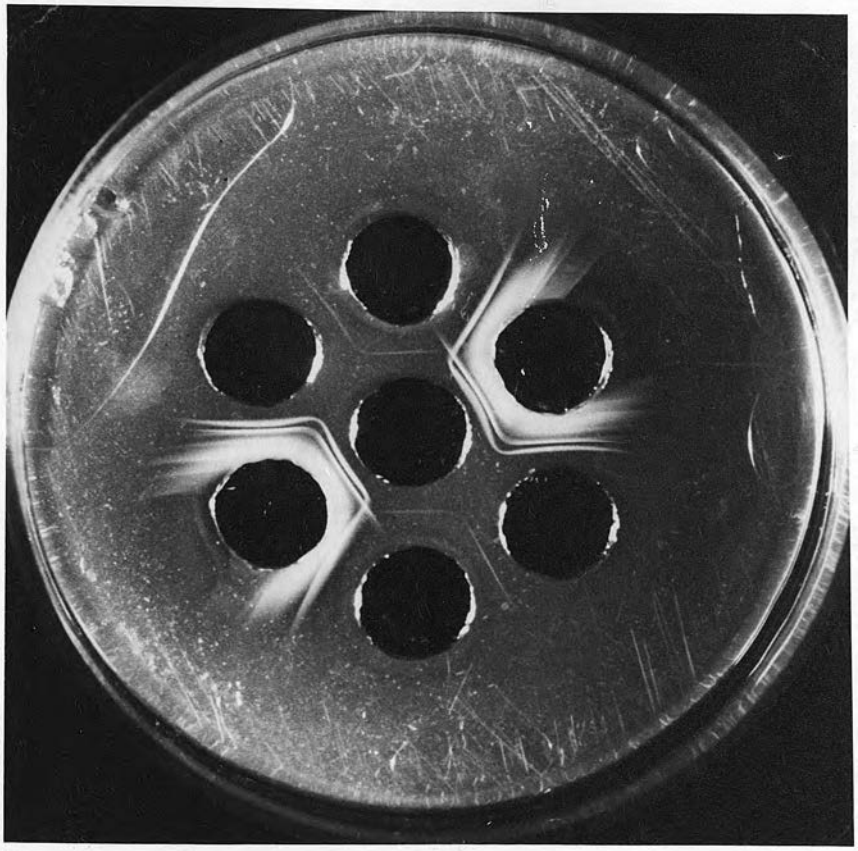


TABLE 34

PRECIPITATING ANTIBODY TITRES OF BOVINE ANTI-BOVINE PAPULAR STOMATITIS SERUM WHEN DIFFUSED AGAINST ORF PRECIPITATING ANTIGEN

Age of orf scabs (days)	Titre of bovine papular stomatitis antiserum
13	1:1
14	1:1
17	1:1
18	1:1
22	1:1

PRECIPITATING ANTIBODIES

The specificity of precipitation reactions induced by unknown sera were checked by fusion with simultaneous control reactions using known positive sheep anti-orf sera.

CONVALESCENT SERA

Seventeen home-bred lambs clinically examined weekly from birth were bled as soon as they were observed to be affected with orf and regularly thereafter. The sera were examined by the macro-system of immuno-diffusion.

Precipitating antibodies appeared one week after the onset of the disease in four out of the seventeen lambs (24 per cent). Two weeks after the onset of the disease nine of fifteen lambs tested had precipitating antibodies in their sera (60 per cent). By the fourth week sixteen animals were positive (Table 35; Fig. 45). Thereafter the percentage of lambs with detectable antibodies fell to eighty-seven at eight weeks, to fifty at twelve weeks and to nil at sixteen weeks. All positive convalescent sera produced one line of precipitation only.

The sera of one lamb, No. 16, was consistently negative. The lesions suspected as being caused by orf /

orf disappeared within one week, whereas the course of the lesions in the other lambs was typical of orf.

POST-VACCINAL SERA

Ellon lambs: A flock of twenty-five six month-old lambs allegedly susceptible to orf were purchased at Ellon in Aberdeenshire. They were bled for serum on arrival and then vaccinated. Every four weeks thereafter a group was bled for serum and challenged.

None of the twenty-five sera collected before vaccination contained precipitating antibodies (Table 36; Fig. 46). Five of the six lambs bled four weeks after vaccination had precipitating antibodies but none of the lambs bled eight, twelve and sixteen weeks was positive.

Home-bred lambs: Twelve home-bred lambs born of immune ewes were bled and vaccinated within one week of birth. They were re-bled for serum every week and then every four weeks until the twentieth week.

Five of ten sera collected from lambs within one week of birth and before vaccination had precipitating antibodies. The ages of the lambs with antibodies ranged from a few hours up to four days (Table 37). The ages of the lambs without antibodies ranged from three /

three to seven days. In other words, the younger lambs had antibodies and the older lambs did not.

One week after vaccination none of the lambs' sera contained antibody. Two weeks after vaccination one out of seven sera tested (15 per cent) was positive. Three and four weeks after vaccination two out of seven sera tested (30 per cent) and three out of ten sera tested (30 per cent) respectively were positive. Thereafter the percentage of positives rose to sixty-three at eight weeks and then fell to forty-two at twelve weeks and thirty-three at sixteen weeks (Table 38; Fig. 47). None of the six sera collected twenty weeks after vaccination contained detectable antibodies. All positive sera gave one line of precipitation only.

A comparison of the responses of convalescent lambs and vaccinated lambs revealed that at four weeks after the onset of the natural disease one hundred per cent of the affected lambs had precipitating antibodies whereas only thirty per cent of the vaccinated lambs had antibodies four weeks after vaccination (Fig. 48). At eight weeks after the onset of disease eighty-seven per cent had antibodies but at eight weeks after vaccination the percentage positive rose to sixty-three per cent. Thereafter the percentage with antibodies in /

in both groups declined and no antibodies were detected in the convalescent lambs sixteen weeks after the onset of the disease and in the vaccinated lambs twenty weeks after vaccination. In other words, lambs affected with natural orf develop antibodies faster than vaccinated lambs but the persistence of antibodies in convalescent lambs is shorter than in vaccinated lambs.

POST-CHALLENGE SERA

Convalescent lambs: Eight sets of home-bred twins were split at birth; one lamb was weaned and reared in isolation and the other was nursed by the ewe. The eight sucking lambs contracted orf naturally whereas the disease was never observed in the eight weaned lambs. The sucking lambs together with their weaned twins were challenged by applying virus to scarified skin seven to twenty weeks after the onset of the natural disease.

One sucking lamb, No. 20, had a single precipitating antibody in its serum when challenged. The sera of the other fifteen lambs were negative. One week later all the sucking convalescent lambs had antibodies, five having two antibodies and two of the weaned /

weaned disease-free lambs (25 per cent) had single antibodies (Table 39).

Vaccinated lambs: Groups of vaccinated Ellon lambs were challenged four, eight, twelve and sixteen weeks after vaccination. Five of the six lambs challenged four weeks after vaccination had single precipitating antibodies in their sera at the time of challenge. None of the animals challenged later had antibodies when challenged (Table 40). All the lambs possessed antibodies one and two weeks after challenge. The lambs challenged four, eight and sixteen weeks after vaccination possessed one antibody whereas three of the six lambs challenged twelve weeks after vaccination had two antibodies.

TABLE 35

PRECIPITATING ANTIBODIES IN THE SERA OF CONVALESCENT LAMBS

Lamb	Weeks after onset of Natural Orf			
	4	8	12	16
2	+	?	+	-
4	+	+	+	-
8	+	+	+	.
10	+	+	.	.
12	+	?	-	.
14	+	?	-	.
16	-	-	-	-
20	+	+	+	-
22	?	+	+	-
24	+	-	-	.
26	+	?	-	-
30	+	+	+	-
32	+	+	-	.
34	+	+	-	-
36	+	+	?	-
38	+	.	.	.
42	+	.	.	.

+ Positive ? Probably positive - Negative . Not done

Fig. 45 - Evolution of precipitating antibodies in the sera of lambs affected naturally with orf.

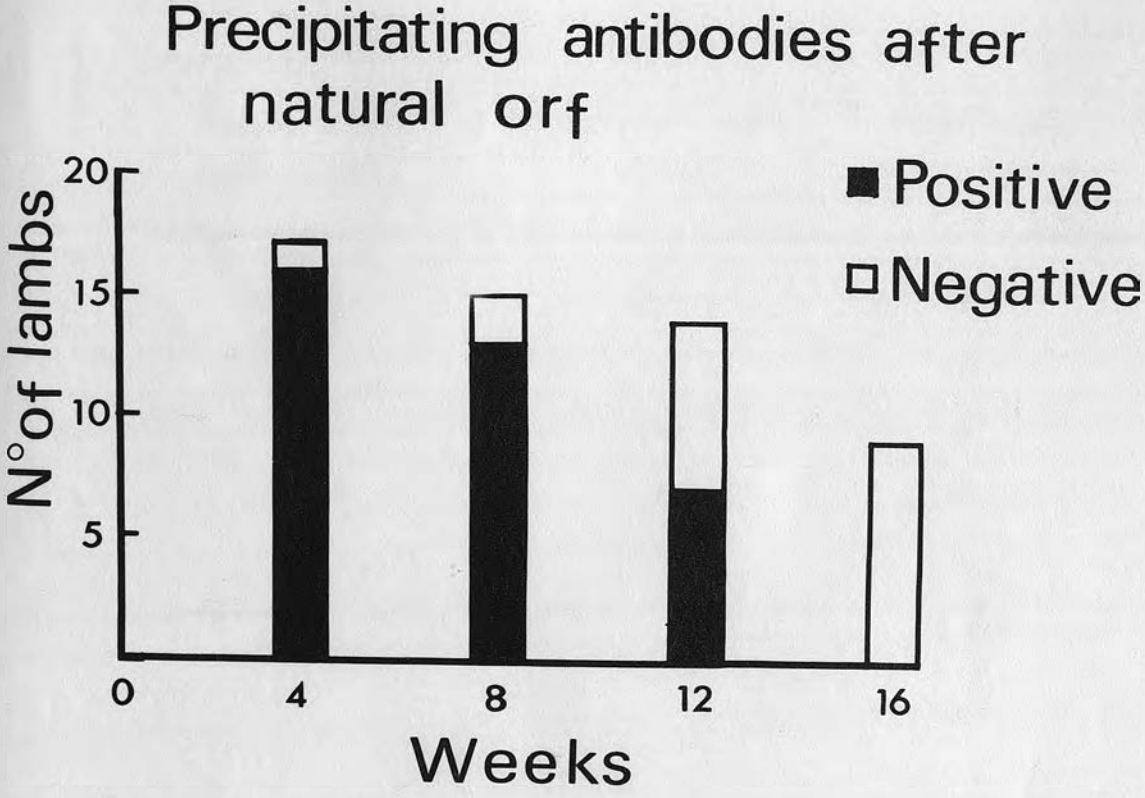

Precipitating antibodies after natural orf
N°of lambs
20
15
10
5
0
4
8
12
16
Weeks
Positive
Negative

TABLE 36

PRECIPITATING ANTIBODIES IN THE SERA OF VACCINATED EELON LAMBS

Weeks after vaccination	Number of sera tested	Number of sera positive
0	25	0
4	6	5
8	6	0
12	6	0
16	7	0

Fig. 46 - Evolution of precipitating antibodies in the sera of vaccinated Ellon lambs.

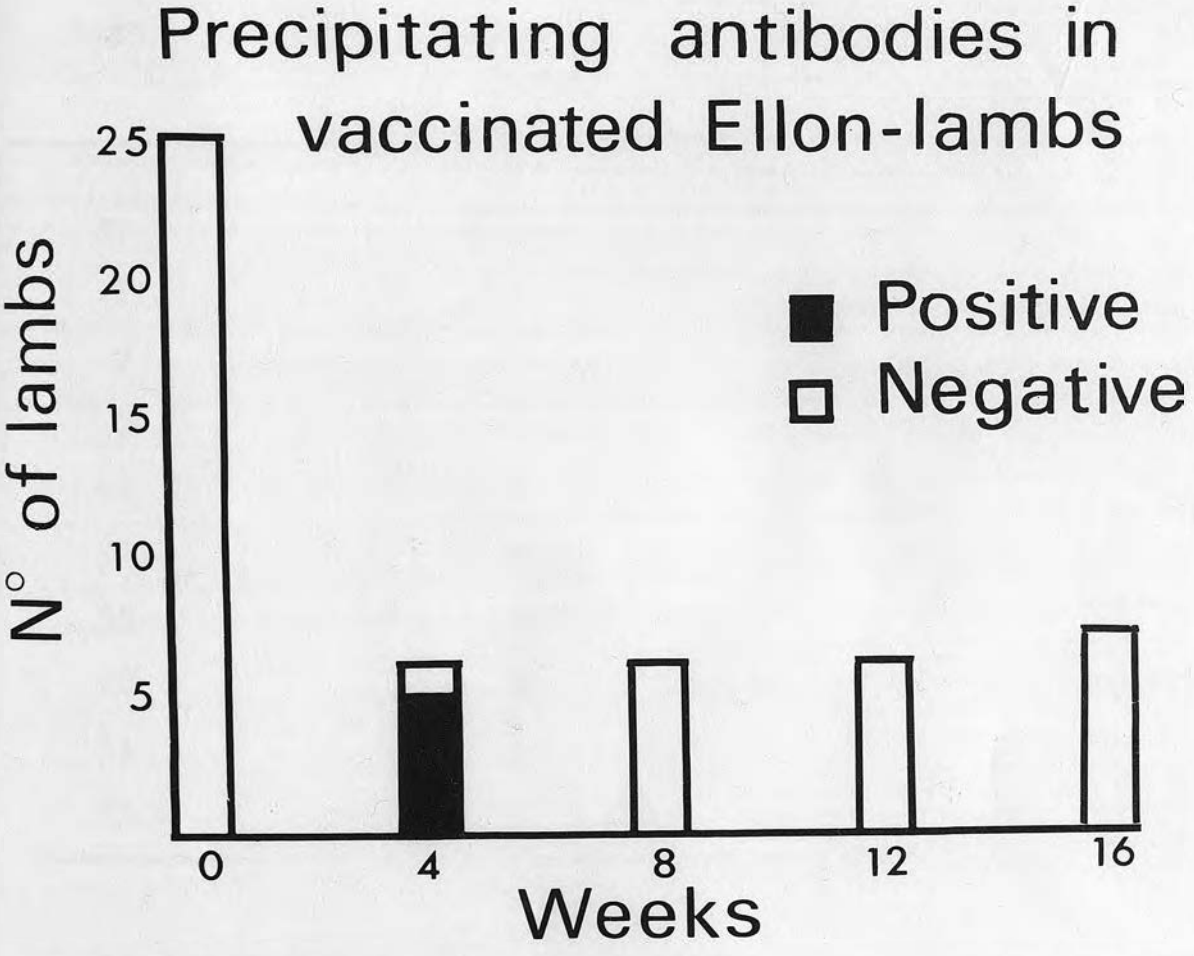

Precipitating antibodies in vaccinated Ellon-lambs
N° of lambs
25
20
15
10
5
Positive
Negative
0
4
8
12
16
Weeks

TABLE 37

AGES OF HOME-BRED LAMBS CHECKED FOR PRECIPITATING ANTIBODIES WITHIN ONE WEEK OF BIRTH

Lamb	Age (days)	Antibodies
13	< 1	+
7	1	+
9	1	?
5	3	-
43	3	-
31	4	+
35	4	?
27	5	-
11	6	-
23	7	-

+ Positive ? Probably positive - Negative

TABLE 38

PRECIPITATING ANTIBODIES IN THE SERA OF VACCINATED HOME-BRED LAMBS

Lamb	Weeks after vaccination					
	0	4	8	12	16	20
1	.	.	+	-	-	-
3	.	.	-	+	-	-
5	-	+	+	+	?	-
7	+	-	+	-	?	-
9	?	-	.	+	?	-
11	-	-	-	-	-	.
13	+	+	+	-	-	.
23	-	-	-	+	.	.
27	-	-	-	-	-	-
31	+	-	?	-	-	.
35	?	?	+	+	.	.
43	-	-	+	-	.	.

\+ Positive ? Probably positive - Negative

. Not done

Fig. 47 - Evolution of precipitating antibodies in the sera of home-bred lambs born of immune ewes.

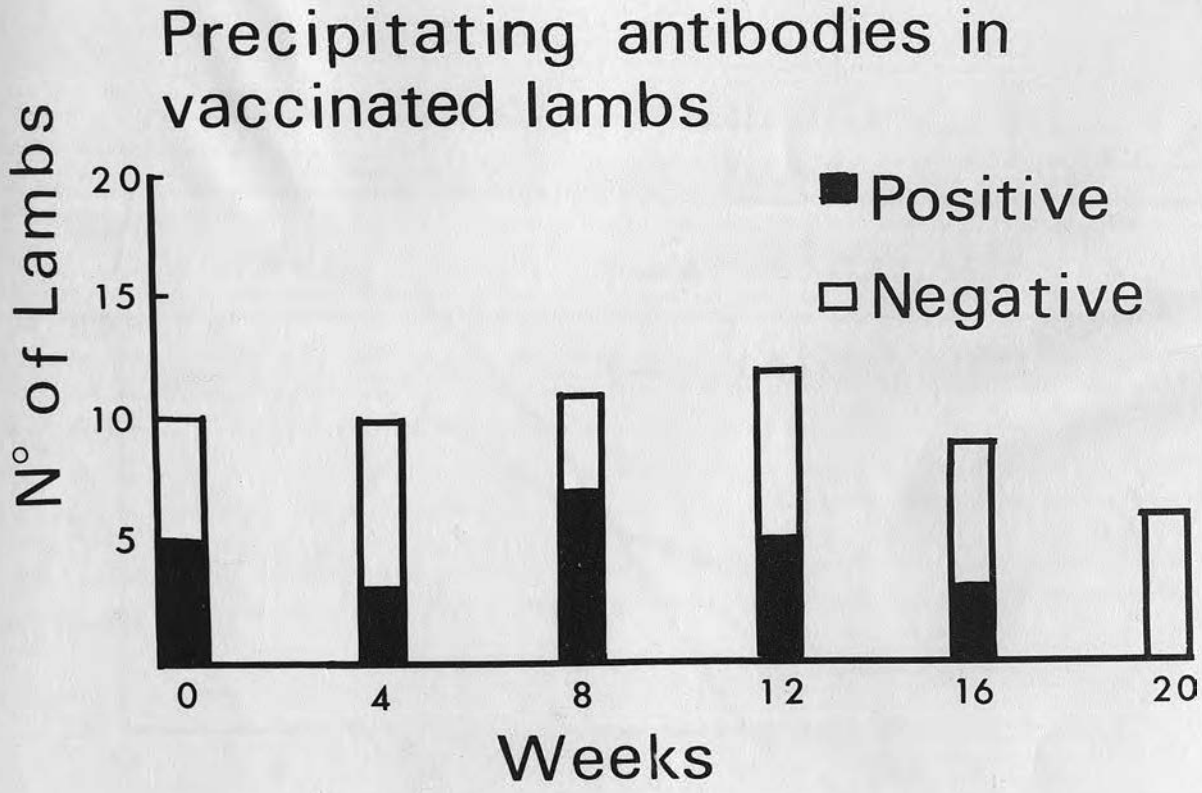

Precipitating antibodies in vaccinated lambs
N° of Lambs
20
15
10
5
Positive
Negative
0
4
8
12
16
20
Weeks

Fig. 48 - A comparison of the percentages of lambs with antibodies following natural infection and vaccination.

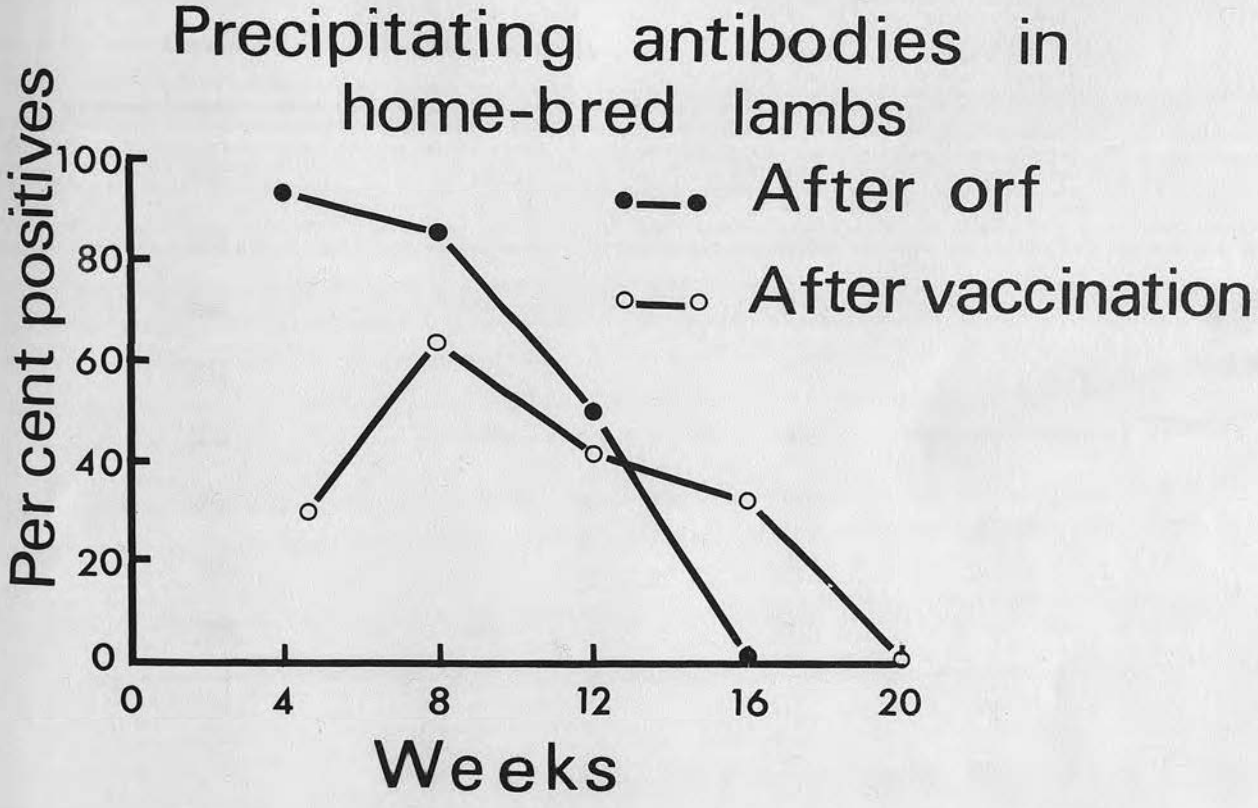

Precipitating antibodies in home-bred lambs
After orf
After vaccination
Per cent positives
100
80
60
40
20
0
0
4
8
12
16
20
Weeks

TABLE 39

PRECIPITATING ANTIBODIES IN THE SERA OF CONVALESCENT LAMBS AND THEIR NON-CONVALESCENT TWINS ONE WEEK AFTER CHALLENGE

Nursed convalescent lambs		Weaned disease-free lambs	
Lamb	Antibodies	Twin	Antibodies
16	+	15	-
18	+	17	-
20	++	19	+
22	++	21	-
26	++	25	-
34	+	33	-
36	++	37	-
38	++	39	+

++ Two precipitating antibodies

\+ One precipitating antibody

\- No antibody

TABLE 40

DETECTION OF PRECIPITINS IN THE SERA OF VACCINATED ELLON LAMBS AFTER CHALLENGE

Weeks after vaccination	Lamb	Weeks after challenge		
		0	1	2
4	1	?	+	+
	3	+	+	+
	5	+	+	+
	7	+	+	+
	9	-	+	+
	11	+	+	+
8	13	-	+	+
	15	-	+	.
	17	-	+	+
	19	-	+	+
	21	-	+	.
	23	-	+	+
12	25	-	+	.
	27	-	++	++
	29	-	+	.
	31	-	++	++
	33	-	++	++
	35	-	+	.

TABLE 40 (Contd.)

Weeks after vaccination	Lamb	Weeks after challenge		
		0	1	2
16	37	-	+	.
	39	-	+	.
	41	-	+	.
	43	-	+	+
	45	-	+	.
	47	-	+	.
	49	-	+	+

++ Two antibodies

\+ One antibody

? Probable antibody

\- No antibody

COMPLEMENT FIXING ANTIBODIES

CONVALESCENT SERA

Twelve home-bred lambs were bled as soon as orf lesions were seen to appear and thereafter weekly. The complement-fixing antibody content of the sera was estimated using the direct complement fixation technique.

The sera of lambs obtained when the lesions were first observed failed to fix complement at the dilutions tested, namely, 1:4 to 1:32 (Table 41; Fig. 49). Four weeks after the onset of the disease complement-fixing antibodies were demonstrated in eight out of eight sera tested, the titres ranging from 1:4 to 1:20, the median being 1:12. At eight weeks twelve out of twelve sera tested were positive with titres that ranged from 1:4 to 1:20, the median being 1:9. At week twelve ten sera were tested and activity was found in nine of the sera. The titres varied from less than 1:4 to 1:20, the median being 1:10. At sixteen weeks five sera were tested, and all were positive. The titres varied from 1:4 to 1:20 with a median of 1:8. Finally, at twenty weeks after orf was first observed the one serum available had a titre of 1:4.

POST /

POST-VACCINAL SERA

Ellon lambs: No complement fixing antibodies were demonstrated in the sera of twenty-five Ellon lambs bled before vaccination. Four weeks later, six out of six vaccinated lambs had complement-fixing antibodies in their sera, with titres that ranged between 1:8 and 1:24. The median was 1:14 (Table 42; Fig. 50). Eight weeks after vaccination six other lambs were bled; their titres ranged from 1:6 to 1:12, the median being 1:7. At twelve weeks after vaccination four out of six other lambs had complement fixing antibodies with titres varying from 1:4 to 1:8, the mediam being 1:4. Finally at sixteen weeks after vaccination, complement fixing antibodies were demonstrated in six out of seven lamb sera tested. The titres ranged from 1:8 to 1:32 with a median of 1:12.

Home-bred lambs: Eleven home-bred lambs were bled and vaccinated within one week of birth and again weekly for four consecutive weeks. Thereafter, the lambs were bled every four weeks up to week twenty-four.

Complement fixing activity was detected in six out of eight sera from new-born lambs. The titres ranged from 1:6 to 1:26 with a median of 1:4 (Table 43; Fig. 51). Four weeks after vaccination nine out of nine /

nine sera fixed complement, the titres ranging from 1:6 to 1:11 with a median of 1:8. The median titre was maintained around this level through to the sixteenth week. At twenty weeks four out of four sera tested were positive, the median titre being 1:15. At twenty-four weeks a decline was evident; only two out of the five sera tested contained low levels of antibodies.

POST-CHALLENGE SERA

Groups of six Ellon lambs were challenged four, eight, twelve and sixteen weeks after vaccination and the complement fixing activity of their sera was assayed before and one and two weeks after challenge.

Overall, challenge did not affect the complement-fixing antibody levels (Tables 44 and 45).

TABLE 41

COMPLEMENT-FIXING ANTIBODIES* AFTER NATURAL ORF

Lamb	Weeks after onset					
	0	4	8	12	16	20
2	< 4	4	4	6	6	.
4	< 4	.	8	20	20	4
8	.	.	8	6	.	.
10	.	.	10	.	.	.
14	.	12	12	12	.	.
16	.	12	6	6	4	.
20	.	20	16	12	12	.
24	.	12	20	20	.	.
26	.	.	8	< 4	.	.
34	.	10	10	8	8	.
36	.	16	14	16	.	.
38	.	16	4	.	.	.

* Expressed as the reciprocals of the 50 per cent end-point dilutions.

Fig. 49 - Median titres of complement-fixing antibodies in the sera of convalescent lambs.

Median titres of complement fixing antibodies in Convalescent lambs

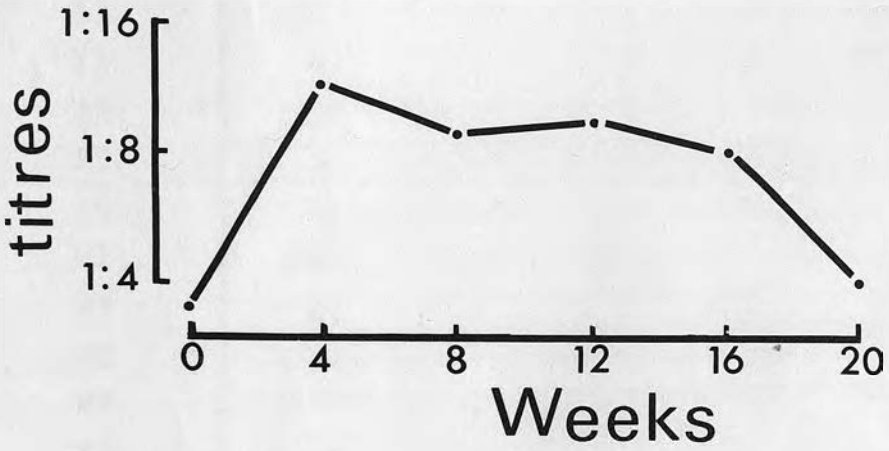


TABLE 42

COMPLEMENT-FIXING ANTIBODIES* IN VACCINATED ELLON LAMBS

Lamb	Weeks after Vaccination				
	0	4	8	12	16
1	< 4	12	.	.	.
3	< 4	24	.	.	.
5	< 4	8	.	.	.
7	< 4	22	.	.	.
11	< 4	16	.	.	.
13	< 4	.	8	.	.
15	< 4	.	6	.	.
17	< 4	.	8	.	.
19	< 4	.	12	.	.
21	< 4	.	6	.	.
23	< 4	.	6	.	.
25	< 4	.	.	< 4	.
27	< 4	.	.	4	.
29	< 4	.	.	< 4	.
31	< 4	.	.	8	.
33	< 4	.	.	4	.
35	< 4	.	.	8	.
37	< 4	.	.	.	12
39	< 4	.	.	.	8
41	< 4	.	.	.	32
43	.	.	.	.	12
45	< 4	.	.	.	< 4
47	< 4	.	.	.	12
49	< 4	.	.	.	8

* Expressed as the reciprocals of the 50 per cent end-point dilutions.

Fig. 50 - Median titres of complement-fixing antibodies in the sera of vaccinated Ellon lambs.

Median titres of complement fixing antibodies in vaccinated Ellon lambs

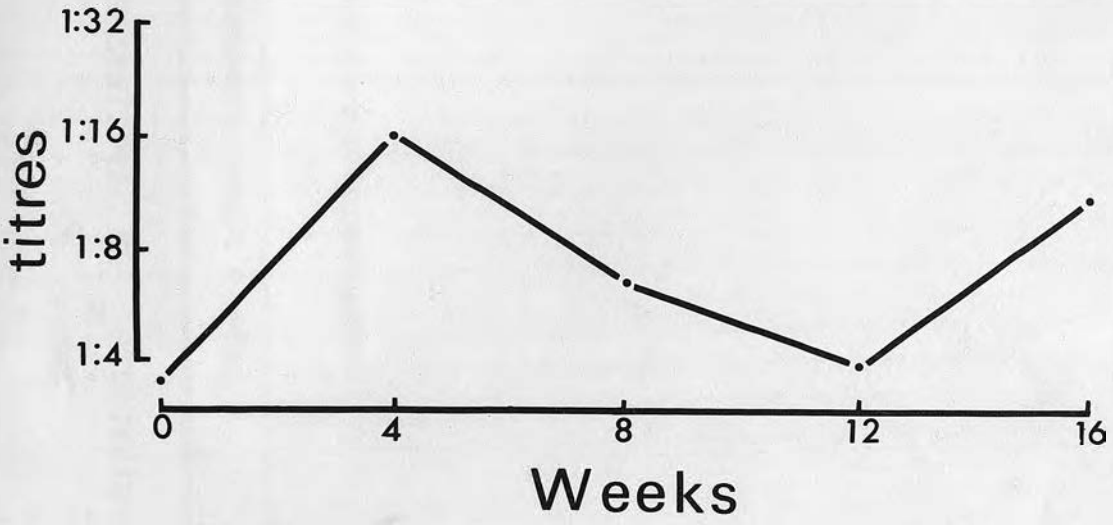


TABLE 43

COMPLEMENT-FIXING ANTIBODIES* IN VACCINATED HOME-BRED LAMBS

Lamb	Weeks after Vaccination						
	0	4	8	12	16	20	24
1	.	.	6	8	8	20	4
3	.	.	6	12	12	10	< 4
5	< 4	8	8	10	20	20	8
7	8	10	8	< 4	< 4	8	< 4
9	6	6	8	8	8	.	< 4
13	15	8	6	8	12	.	.
23	6	11	11	11	.	.	.
27	8	8	4	.	8	.	.
31	< 4	6	8	7	4	.	.
35	26	8	24	22	.	.	.
43	.	10	6	4	.	.	.
Median	4	8	8	8	8	15	< 4

* Expressed as the reciprocals of the 50 per cent end-point dilutions.

Fig. 51 - Median titres of complement-fixing antibodies in the sera of vaccinated home-bred lambs.

Median titres of complement fixing antibodies in vaccinated home-bred lambs

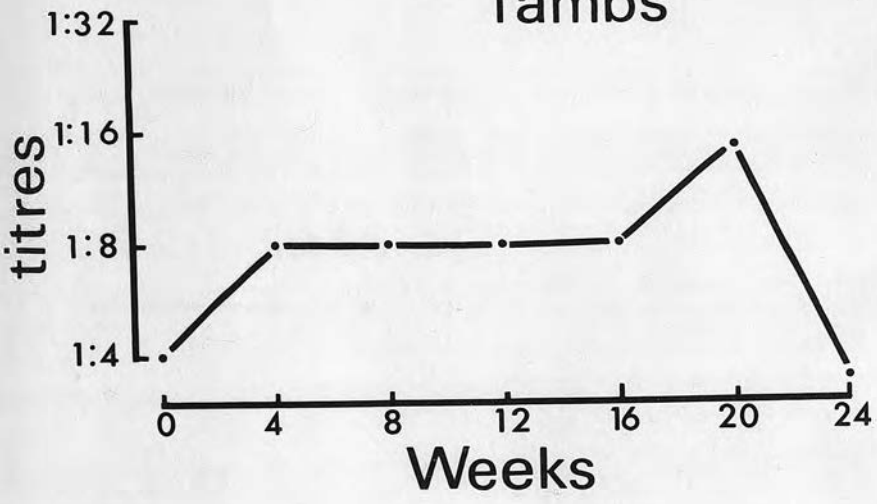


TABLE 44

COMPLEMENT FIXING ANTIBODIES* IN CHALLENGED ELLON LAMBS

Weeks after Vaccination	Lamb	Weeks after Challenge		
		0	1	2
4	1	12	< 4	6
	3	24	4	24
	5	8	8	8
	7	22	12	8
	9	8	10	10
	11	16	24	32
8	13	8	< 4	4
	15	6	4	.
	17	8	10	16
	19	12	12	12
	21	6	4	.
	23	6	4	4
12	25	< 4	< 4	4
	27	4	< 4	< 4
	29	< 4	10	8
	31	8	10	8
	33	4	< 4	< 4
	35	8	8	8

TABLE 44 (Contd.)

Weeks after Vaccination	Lamb	Weeks after Challenge		
		0	1	2
16	37	12	16	.
	39	8	12	.
	41	32	32	.
	43	12	10	8
	45	< 4	12	.
	47	12	16	.
	49	8	8	8

* Expressed as the reciprocals of the 50 per cent end-point dilutions.

TABLE 45

MEDIANS OF COMPLEMENT FIXING ANTIBODIES*

BEFORE AND AFTER CHALLENGE

Weeks between vaccination and challenge	Weeks after challenge		
	0	1	2
4	14	9	9
8	7	4	8
12	4	4	6
16	12	12	8
Overall	8	10	8

* Expressed as the reciprocals of the 50 per cent end-point dilution.

NEUTRALISING ANTIBODIES

SERUM-VIRUS MIXTURES

The titre of a seven day old orf scab in Hep 2 and AV_3 cell monolayers was $10^{-5.5}TCD_{50}$ per gram. Prior incubation with bovine normal serum or colostrum-deprived lamb serum did not alter the titre whereas prior incubation with sheep anti-orf serum lowered the titre one hundred-fold (Table 46).

POST-INFECTION EXPOSURE

The titre of the same seven day old orf scab in Hep 2 and AV_3 cell monolayers was not affected by the addition of bovine normal serum or colostrum-deprived lamb serum to the cell culture maintenance medium. On the other hand, the titre was lowered one hundred-fold when the cell culture maintenance medium contained twenty per cent anti-orf serum.

TABLE 46

ORF SCAB TITRES* IN CELL CULTURES AFTER INCUBATION WITH SERUM

Serum	Hep 2 cells	AV_3 Cells
None	5.5	5.5
Bovine normal	5.5	5.5
Colostrum-deprived lamb	5.5	5.5
Sheep anti-orf	3.5	3.5

* Expressed as the logarithms of the reciprocals of the TCD_{50} end-points.

PASSIVELY-TRANSFERRED ANTIBODIES

PRECIPITATING ANTIBODIES

Three out of six ewes bled for serum at parturition had precipitating antibodies detectable by diffusion against known-positive orf-scab suspensions (Table 47). Four of the six ewes also had precipitating antibodies in the colostrum. The precipitating antibody titres of the colostrumswere always higher than the corresponding serum titres.

No precipitating antibody activity was ever found in the sera of the offspring of the six ewes collected before colostrum had been taken. On the other hand, three of the four offspring of the ewes that had precipitating antibodies in their colostrums acquired precipitating antibodies in their sera after sucking. The offspring of the ewes without antibodies in their colostrums did not have antibodies after sucking. One lamb born of an immune ewe had precipitating antibodies six hours after sucking, one had antibodies eighteen hours after sucking and three had antibodies twenty-four hours after sucking.

COMPLEMENT-FIXING ANTIBODIES

All six ewes bled for serum at parturition had detectable /

detectable orf complement-fixing antibodies (Table 48). Five also had complement-fixing antibodies in their colostrums.

No complement-fixing activity was ever detected in the lambs born of the six ewes and bled for serum before they sucked. After sucking, five of the lambs were found to have acquired complement-fixing antibodies which were first detected in two lambs at six hours. The highest titre was 1:48 and the median titre was 1:9.

NATURE

Ewe sera at parturition: The immuno-electrophoretic patterns of the sera of six ewes at parturition were examined (Fig. 52). The immuno-globulins migrated towards the cathode and the albumin, alpha and beta globulins towards the anode. Three arcs of precipitation were observed in the immuno-globulin area of reaction. The fast and slow moving gamma-globulins were easily identified because of their position and characteristic gull-wing appearance. The IgM arc of precipitation was seen as a faint but well-defined arc that occurred also towards the cathode side between the fast moving gamma-globulin and the corresponding well.

Ewe /

Ewe colostrum at parturition: At least three arcs of precipitation were observed on the cathode side after ewe colostrum was electrophoretically separated (Fig. 52). They were situated in the area where immunoglobulins react.

Colostrum-deprived lamb sera: The sera from six colostrum-deprived lambs were examined by immuno-electrophoresis and none was found to contain immuno-globulins (Fig. 53).

Nursing lamb sera: Sera from lambs that had sucked colostrum contained immunoglobulins (Fig. 53). Fast and slow gamma-globulins were clearly demarcated in the eighteen and twenty-four hour samples but in the six hour samples the slow gamma-globulin was less distinct. The IgM arc was also more distinct in the later samples than in the six hour samples.

TABLE 47

PASSIVE TRANSFER OF PRECIPITATING ANTIBODIES* THROUGH THE COLOSTRUM

Ewe			Lamb				
No.	Serum	Colostrum	No.	Before sucking	After sucking (hours)		
					6	18	24
X52	1:2	1:4	C76	< 1:1	.	1:1	1:1
X54	1:2	1:8	104	< 1:1	.	< 1:1	< 1:1
X72	< 1:1	< 1:1	108	< 1:1	.	< 1:1	< 1:1
X77	< 1:1	1:8	C70	< 1:1	< 1:1	.	1:1
X86	1:2	1:4	S71	< 1:1	1:1	.	1:1
B10	< 1:1	< 1:1	C82	< 1:1	< 1:1	.	< 1:1

* Expressed as the highest dilution giving precipitation

A

B

C

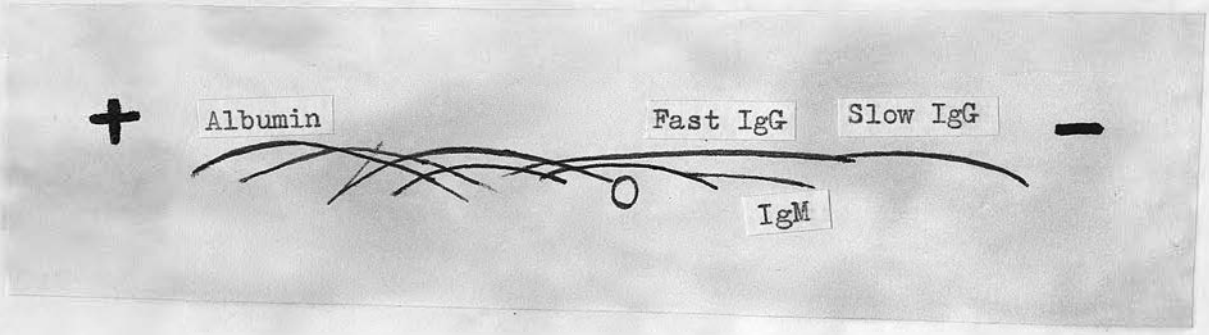
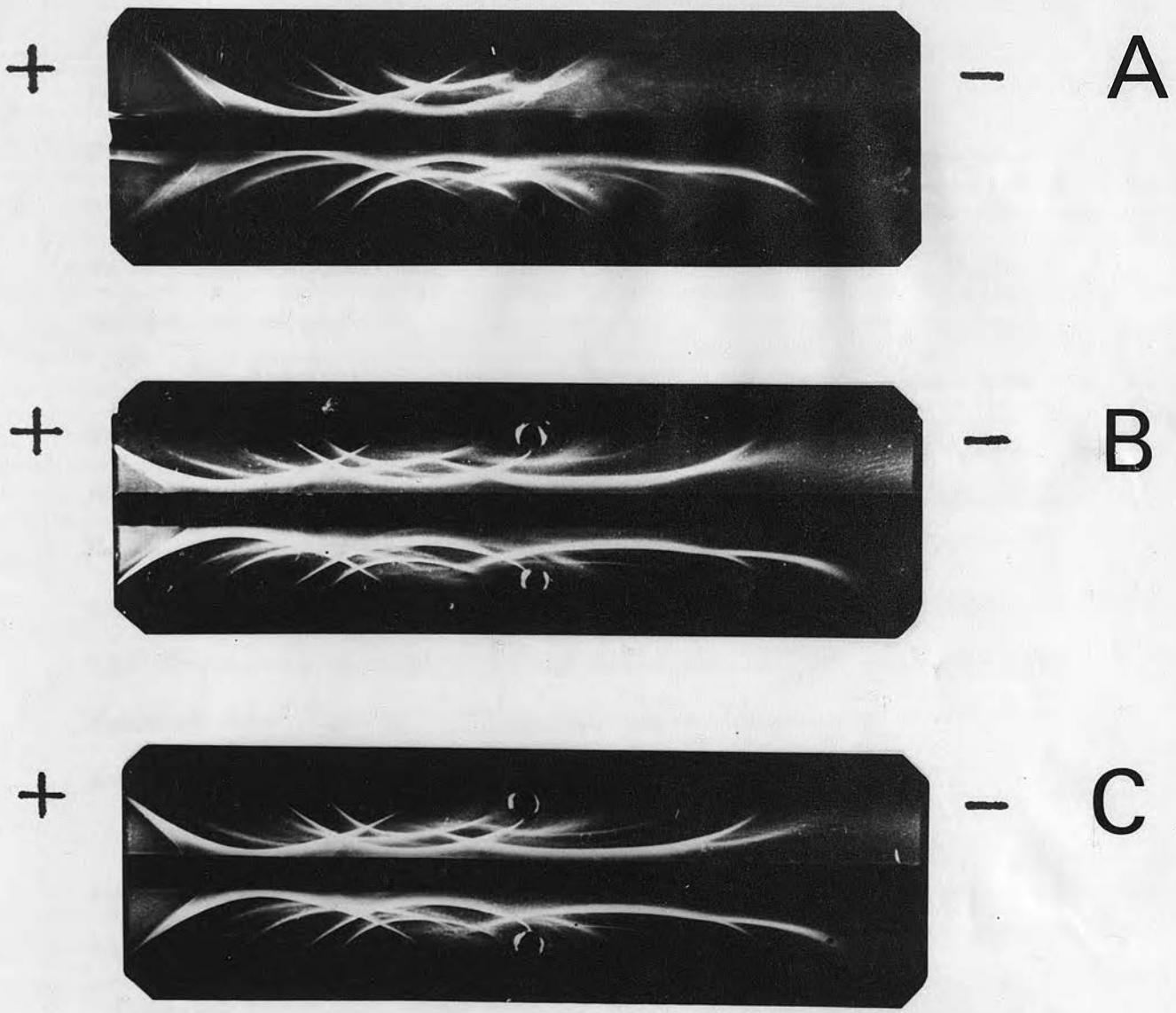


Fig. 52 - Immuno-electrophoretic patterns of ewe serum and colostrum. The colostrum filled the top well and the serum filled the bottom well. The trough contained rabbit anti-whole sheep serum serum.

+

−

Fig. 53 - Immuno-electrophoretic patterns of lamb sera before and after sucking colostrum. A was the serum collected before sucking. B was the serum collected six hours after sucking, and C was the serum collected twenty-four hours after sucking. All the bottom wells contained normal sheep serum and all the troughs contained rabbit anti-whole serum serum.


+
−
A
+
−
B
+
−
C

ANTIBODY FRACTIONATION

SHEEP SERA

Sera from sheep vaccinated against orf and hyper-immunised against orf were fractionated in a K25/45 column packed with Sephadex G-200. All had the same profile with four main peaks (Fig. 54). The first peak was the macroglobulin or 19S peak. The second peak was the 7S gamma-globulin peak, the third was the albumin peak, and the fourth contained proteins of low molecular size.

The fractions corresponding to the first peak were subjected to immuno-electrophoresis and were found to contain I_gM antibody and alpha 2 macroglobulin (Fig. 55). Most of the orf complement-fixing antibody activity was located in the first peak; a little occurred in the fractions from the ascending side of the second peak. None of the fractions from the first peak had precipitating antibody activity.

The second peak was always higher than the first but no overlap occurred. Immuno-electrophoresis of the fractions corresponding to the second peak revealed slow and fast moving gamma-globulins and an alpha 1 globulin (Fig. 56). Only the fractions from the lower ascending side of the second peak fixed complement /

complement and antibody titres were low being about 1:4. On the other hand, all the precipitating antibody activity was restricted in the fractions corresponding to the top of the second peak. The antibodies reacting with the slow and fast moving antigens were both 7S antibodies.

Immuno-electrophoresis of the fractions from the third peak failed to reveal an IgM arc of precipitation. Indistinct bands occurred from trailing of the slow and fast moving gamma-globulins originating in the second peak (Fig. 57). The fractions were always devoid of complement-fixing antibody and precipitating antibody activity.

No immuno-electrophoretic activity was detected when fractions from the fourth peak were examined. Similarly the fractions possessed neither complement-fixing nor precipitating antibodies, (Fig. 58).

RABBIT SERA

Rabbit anti-whole sheep serum: Sera from rabbits hyperimmunised with whole sheep serum had typical fractionation profiles with four peaks. The anti-sheep activity was assayed in all the fractions by immuno-diffusion against sheep serum and it was found to be restricted to the fractions corresponding to the top of the second or 7S peak.

Rabbit anti-orf serum: The serum pool from rabbits 02, 21 and 22 which were hyperimmunised with orf virus purified by gel chromatography had a typical profile when fractionated. All the precipitating antibody activity against both sheep serum and orf scab suspensions was found in the fractions corresponding to the top of the second or 7S peak.

Fig. 54 - Typical profile of sheep anti-orf serum fractionated through Sephadex G-200 in a K25/45 column.

Sheep Orf Serum
Sephadex G200

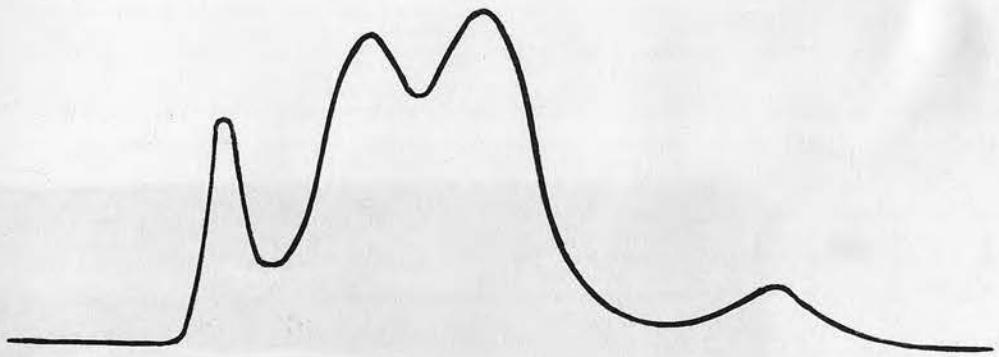


Fig. 55 - Immuno-electrophoresis of concentrated fractions of sheep anti-orf serum corresponding to the first or 19S peak. The top well contained the first peak fractions and the bottom well contained sheep serum. The trough contained rabbit anti-whole sheep serum serum.

+

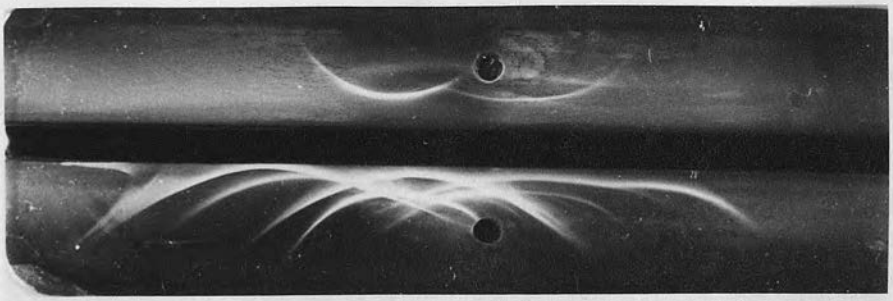


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Fig. 56 - Immuno-electrophoresis of concentrated fractions of sheep anti-orf serum corresponding to the second or 7S gamma-globulin peak. The top well contained the second peak fractions and the bottom well contained sheep serum. The trough contained rabbit anti-whole sheep serum serum.

+

−

Fig. 57 - Immuno-electrophoresis of concentrated fractions of sheep anti-orf serum corresponding to the third or albumin peak. The top well contained the third peak fractions and the bottom well contained sheep serum. The trough contained rabbit anti-whole sheep serum serum.

+

−

Fig. 58 - Immuno-electrophoresis of concentrated fractions of sheep anti-orf serum corresponding to the fourth peak. The top well contained the fourth peak fractions and the bottom well contained sheep serum. The trough contained rabbit anti-whole sheep serum serum.

+

−

DISCUSSION

DETECTION OF VIRUS

Orf virus was readily isolated and identified by applying suspensions of infected scabs to the scarified skin of susceptible lambs. On the other hand, proven isolation in cell cultures was fraught with difficulty.

The electron microscope proved to be a useful tool in confirming a diagnosis of orf or bovine papular stomatitis rapidly. Negative staining readily revealed the internal structure of the particles and allowed confident recognition. Differentiation between orf virus and bovine papular stomatitis virus, however, was impossible without knowledge of the origin of the samples.

Large numbers of virus particles were always observed in early scabs, whereas older scabs contained fewer and fewer particles until none was found in scabs collected within a few days of complete resolution of the lesion. The essential difference between early scabs and late scabs was believed to be linked to the development of antibody and the experiment designed to confirm this hypothesis succeeded; scabs rich in virus particles were incubated with sheep anti-orf serum and the numbers of intact virus particles were spectacularly reduced. These findings conflict with the /

the traditional concept expressed by Glover (1928) that the disease is spread by means of the crusts. We believe that by the time the scabs drop off there are few or no virus particles in the scabs.

The detection of orf particles in smears using fluorescent antibody techniques was impractical because old scabs showed too much non-specific fluorescence. Smears of early scabs contained many fluorescent spots but no detail was discernible.

PRECIPITATION TEST

The immuno-diffusion precipitation technique was successfully used to demonstrate precipitating antigens in orf lesions and precipitating antibodies in sheep sera. The test itself proved to be cheap and easy to perform. The key to success was, on the one hand, the quality of the anti-serum used, and, on the other hand, the preparation of known-positive antigens from orf lesions one to two weeks old. Unlike Mansi (1958) we found the macro-system to be more sensitive than the micro-system, a result we attributed to larger volumes of the reagents diffusing across proportionately shorter distances. Preparations of orf scabs yielded two precipitating antigens, labelled for convenience as the /

the slow and fast moving antigens. Both resisted freezing and thawing and both were heat-labile in contradistinction to the heat-stable complement-fixing antigen. Neither sedimented with the virus and they were readily separated from virus particles by fractionation through Sephadex G-200. Fractionation, however, failed to separate the two antigens both of which eluded in the fractions corresponding to the second chromatographic peak. In other words, they had similar molecular sizes.

The number of precipitating antibodies produced against orf virus varied. Susceptible lambs that were vaccinated or suffered from the natural disease or were hyperimmunised by injecting virus or precipitating antigen with incomplete Freund's adjuvant formed one precipitating antibody whereas challenged immune animals produced two antibodies. Both antibodies were eluded in the fractions corresponding to the 7S peak after Sephadex G-200 chromatography and by immuno-electrophoresis they were found to be gamma-globulins.

Bovine anti-bovine papular stomatitis serum reacted with orf scab suspensions to produce one line of precipitation. Our findings confirm those of Papadopoulos et al. (1968) who postulated the existence of a common sub-group specific precipitating antigen among /

among the paravaccinia viruses. The antibody was directed against the slow moving antigen which suggests that the slow antigen is the specific sub-group antigen.

Antigen development: Precipitating antigens were readily detected within four days of applying virus to scarified skin. Peak titres persisted between the first and second weeks and thereafter the amounts of precipitating antigen declined to barely detectable levels at three weeks and over. The decline coincided with the fall in the number of virus particles which, in turn, was correlated with the appearance of antibody in the sera. Regular recovery of precipitating antigens from lesions persisting for more than five weeks was attributed to secondary lesions which were observed to develop around the periphery of the original site.

Antibody development: The sera of colostrum-deprived lambs and lambs known never to have had orf did not contain precipitating antibodies reactive against suspensions of orf-infected scabs. The sera of lambs infected naturally and typically with orf all contained precipitating antibodies within four weeks of the onset of the disease. The antibodies were first /

first detected in twenty-four per cent of naturally-infected lambs within one week of the onset of the disease and they were last detected at twelve weeks. Precipitating antibodies induced by experimental infection were likewise transient first appearing in the sera of a minority of lambs two weeks after vaccination and persisting for less than twenty weeks. A few vaccinated lambs never developed detectable precipitating antibodies. Our findings in regard to the appearance of antibody are in agreement with those of Abdussalam (1958) and Papadopoulos et al. (1968) but they conflict in regard to the persistence of the antibody. Abdussalam (1958), for example, detected antibodies in the sera of one sheep recovered for five months. In addition we found a clear difference in the antibody behaviour of the home-bred and Ellon lambs; none of the Ellon lambs had detectable precipitating antibodies eight or more weeks after vaccination. We suggest that the difference reflects differences in innate resistance. The home-bred lambs were Suffolks and Cheviots and were vaccinated within the first week of life whereas the Ellon lambs were Greyfaces and were vaccinated when six months old. Further work is necessary to define whether the important factor is breed or age.

The /

The transient nature of the precipitating antibodies in lambs contrasts with the finding that three out of six ewes had detectable levels of precipitating antibodies in their sera at parturition and four of the six ewes had antibodies in their colostrums. The discrepancy merits re-examination because it suggests that either the ewes contracted orf within a few weeks of parturition or the stress of pregnancy and parturition activated a latent orf infection.

In the light of Halliday's work (1965a) on the transfer of antibodies from ewes to their lambs, the demonstration of the passive transfer of precipitating antibodies was not unexpected. We confirmed also his finding that sheep have higher titres of antibody in their colostrum relative to that in the maternal serum. One of our lambs born of an immune ewe did not acquire antibody. Subsequent enquiries revealed that the lamb was deprived of colostrum inadvertently for more than eight hours after birth. Recently Gay, Anderson, Fisher and McEwan (1965) reported that some calves were unable to absorb globulins from colostrum four to six hours after birth and it is therefore tempting to suggest that this is the explanation /

explanation for the failure of our lamb to absorb antibody. Halliday (1965b) found that many hill lambs that died at ages up to ten days had no gamma-globulin in their sera and he attributed the phenomenon to ewes deserting their lambs.

Anamnestic responses were always induced in actively immune sheep by challenge with orf virus. In fact the technique could be used to prove or disprove past exposure to the virus or vaccination in flocks; if immune all the sheep would have detectable antibodies one week after challenge whereas if they were susceptible none would have detectable antibody.

The production of hyperimmune anti-orf sera in rabbits was bedevilled by other antigen-antibody reactions and we abandoned the technique. Others also experienced difficulties when they hyperimmunised rabbits (Johnston, 1962; 1963; Scmidt, 1967d). The problem arises through the use of crude antigen suspensions and a likely solution would be to use refined antigen similar to that prepared by Papadopoulos et al. (1968) from concentrated and differentially centrifuged virus grown in cell cultures. Even so, they had to remove anti-calf factor antibodies by mixing the rabbit serum with normal calf serum.

In /

In contrast the production of hyperimmune anti-orf sera in sheep was easy. Unlike Trueblood (1966) who found that sheep did not produce suitable antibody titres and unlike Johnston (1965; 1966) who was only able to produce reliable antibody in one sheep we were able to produce high yields of precipitating antibodies in all but one of our sheep. Presumably differences in the immunisation schedules rather than differences in innate resistances account for the discrepancy because all our challenges of actively immune sheep stimulated the rapid appearance of antibody. The ideal regime of inoculations for hyperimmunisation is not known. We used too few animals and too many inoculation regimes to permit drawing any firm conclusions but we have the impression that multiple inoculations spread over a period of weeks yielded higher titres of antibody for a longer period than inoculations given simultaneously. Hyperimmune sheep behaved like sheep convalescent from the disease or vaccinated for the first time in that precipitating antibodies were transient.

COMPLEMENT-FIXATION TEST

The direct complement-fixation technique has been successfully used to detect complement-fixing antigens /

antigens in experimentally produced orf scabs and scabs from natural cases of the disease and for the detection of complement-fixing antibodies in the sera of sheep after hyperimmunisation, vaccination and challenge and in the sera of orf-convalescent animals. The main difficulty encountered in the technique was the standardisation of the reagents. The sheep sera were heated at 56°C for forty minutes to destroy anti-complementary activity because it had been previously found that some sera were slightly anti-complementary after inactivation for only thirty minutes. Absorption of sera with sheep red bloods increased the titres slightly presumably by removing non-specific inhibitors. Pilot experiments also showed that scab suspensions used at dilutions that ranged from 1:10 to 1:50 were strongly anti-complementary. Some 1:80 dilutions had slight anti-complementary activity and, therefore, we used as our base a dilution of 1:100.

Previous workers reported that ether extractions of orf scabs increased the quality of the antigen (Glover, 1933; Nisbet, 1954) and also suppressed the anti-complementary activity of the scabs. Our results conflict because we demonstrated that there was no advantage over barbitone buffer extraction which is easy and rapid.

Like /

Like the precipitating antigen, the complement-fixing antigen was distinct from the virus particles. Moreover the two antigens were distinct. The two striking differences were first the heat lability of the precipitating antigen and the heat stability of the complement-fixing antigen and, secondly, the precipitating antibodies were gamma-globulins whereas the complement-fixing antibodies were IgM globulins.

Antigen development: The detection of complement-fixing antigens was the most sensitive method of monitoring lesions; ninety-four per cent of the scabs harvested from experimental lesions contained detectable amounts of complement-fixing antigens. Moreover the titres of the complement-fixing antigens were always much greater than the titres of the precipitating antigens. The period during which the titres were highest was the same for both antigens, namely, between the first and second weeks. The decline of the complement-fixing antigen curve was slower than that of the precipitating antigen curve but it was also linked to the decline in numbers of virus particles.

Complement-fixing antigen titres of scabs harvested /

harvested from the minority of lambs in which the experimental lesion persisted abnormally long because of secondary lesion development around the original site were higher than the titres of scabs harvested from lambs in which the course of the experimental lesion was typical. A major factor in prolonging the high titres was undoubtedly the evolution of new virus in the secondary sites but, in addition, the high titres may reflect a lower innate resistance of the lambs permitting greater multiplication of the virus at the primary site and permitting also the development of secondary lesions. Further work is required to clarify the point but it may be possible to predict the development of secondary lesions by titrating the complement-fixing antigen in early scabs.

Antibody development: The sera of colostrum-deprived lambs and lambs known never to have had orf were always free of complement-fixing antibodies. Antibodies were readily detected in the sera of convalescent sheep four weeks after the onset of the disease and they persisted for at least twenty weeks, the longest period tested. Their evolution therefore differed significantly from that of the transient precipitating antibodies. Similarly complement-fixing antibodies persisted longer than precipitating antibodies in the sera of hyper-immunised sheep. However, complement-fixing

antibodies /

antibodies may also be transient because the sera of some vaccinated lambs collected twenty-four weeks after vaccination were negative. The number of animals screened was small and definite conclusions are therefore not possible.

Our data on the complement-fixing activity of the sera of pregnant ewes at the time of parturition, like our data on the precipitating antibodies in the same sera, conflict with the hypothesis that the complement-fixing antibodies are transient. The sera of the six ewes examined contained complement-fixing antibodies but only five of the ewes also had complement-fixing antibodies in their colostrums and the titres were lower than the serum titres. Nevertheless five lambs acquired complement-fixing antibodies after sucking.

All vaccinated lambs developed complement-fixing antibodies whereas a few vaccinated lambs did not develop precipitating antibodies. Challenge of convalescent or vaccinated lambs, however, did not evoke an anamnestic response of the complement-fixing antibodies whereas it did evoke anamnestic levels of precipitating antibodies. On the other hand varied regimes of hyperimmunisation produced complement-fixing antibodies /

antibodies in one to two weeks in lambs previously immune but containing no complement-fixing antibodies in their sera at the time of hyperimmunisation. Challenge of hyperimmune lambs appeared to induce a slight anamnestic response.

CONCLUSIONS

Sequential observations of orf scabs produced experimentally revealed that the virus particles, precipitating antigens and complement-fixing antigens all reached peak levels between the first and second weeks after scarification and before antibodies were detectable. The efficiencies of the methods for detecting particles and antigens varied and are illustrated by our success rates in confirming cases of the natural disease. We detected virus particles in sixty-five per cent of the scabs taken from animals which were unequivocally diagnosed as facial orf from the clinical signs, precipitating antigens in thirty-three per cent and complement-fixing antigen in seventy-five per cent. Similarly in scabs taken from ewes unequivocally diagnosed as teat orf from the clinical signs we detected virus particles in twenty-two per cent, precipitating /

precipitating antigens in four per cent and complement-fixing antigens in twenty-nine per cent. In scabs from suspect cases of orf virus particles were in thirty-three per cent and precipitating antigens in six per cent. We therefore concluded that the complement-fixation test was the most accurate technique for confirming a diagnosis of orf.

Our ability to confirm a diagnosis of teat orf was poor. There are two and, perhaps three, explanations. First, clinical diagnosis of teat orf is unreliable. Secondly, the lesions tended to be relatively old before they were observed and consequently the scabs were collected after the optimal period for detecting virus particles or antigens. Thirdly, some "typical" lesions may be hypersensitivity reactions activated by contact with virus in lesions of the lips and oral mucosa of the sucking lambs and therefore free or nearly free of replicating virus and its antigens. In support of the third explanation was our finding that sixteen per cent of the scabs from the teats of lactating ewes contained precipitating antibodies.

Antibodies to orf in convalescent or vaccinated lambs were transient yet they were detected in the sera of ewes at parturition. Their diagnostic significance therefore /

therefore is doubtful. Similarly we concluded that the diagnostic roles of virus isolation techniques in cell cultures, haemagglutination techniques and fluorescent antibody techniques were unreliable.

Isolations of the virus by applying suspensions of suspect scabs to the scarified skins of susceptible lambs was a sensitive method of confirming a diagnosis but the cost of the technique and the delay before a result is obtained render the method unsuitable for routine diagnostic purposes. Instead we recommend that scabs be first examined by electron microscopy after negative staining. If no particles are seen then the scab should be tested for complement-fixing antigens. If no electron microscope is available we suggest that scabs be first examined by the immuno-diffusion technique and, if the result is negative, by complement-fixation techniques.

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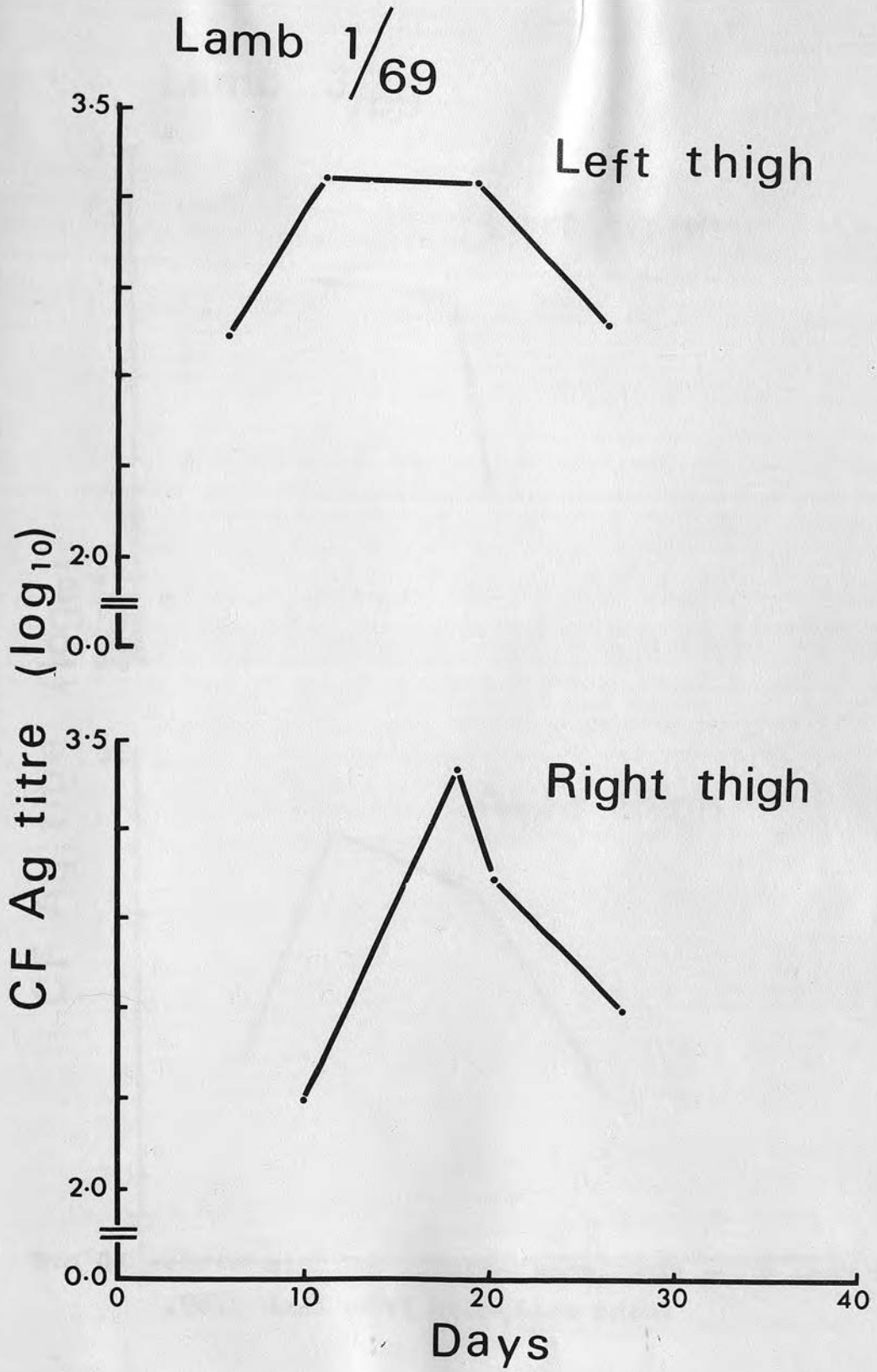
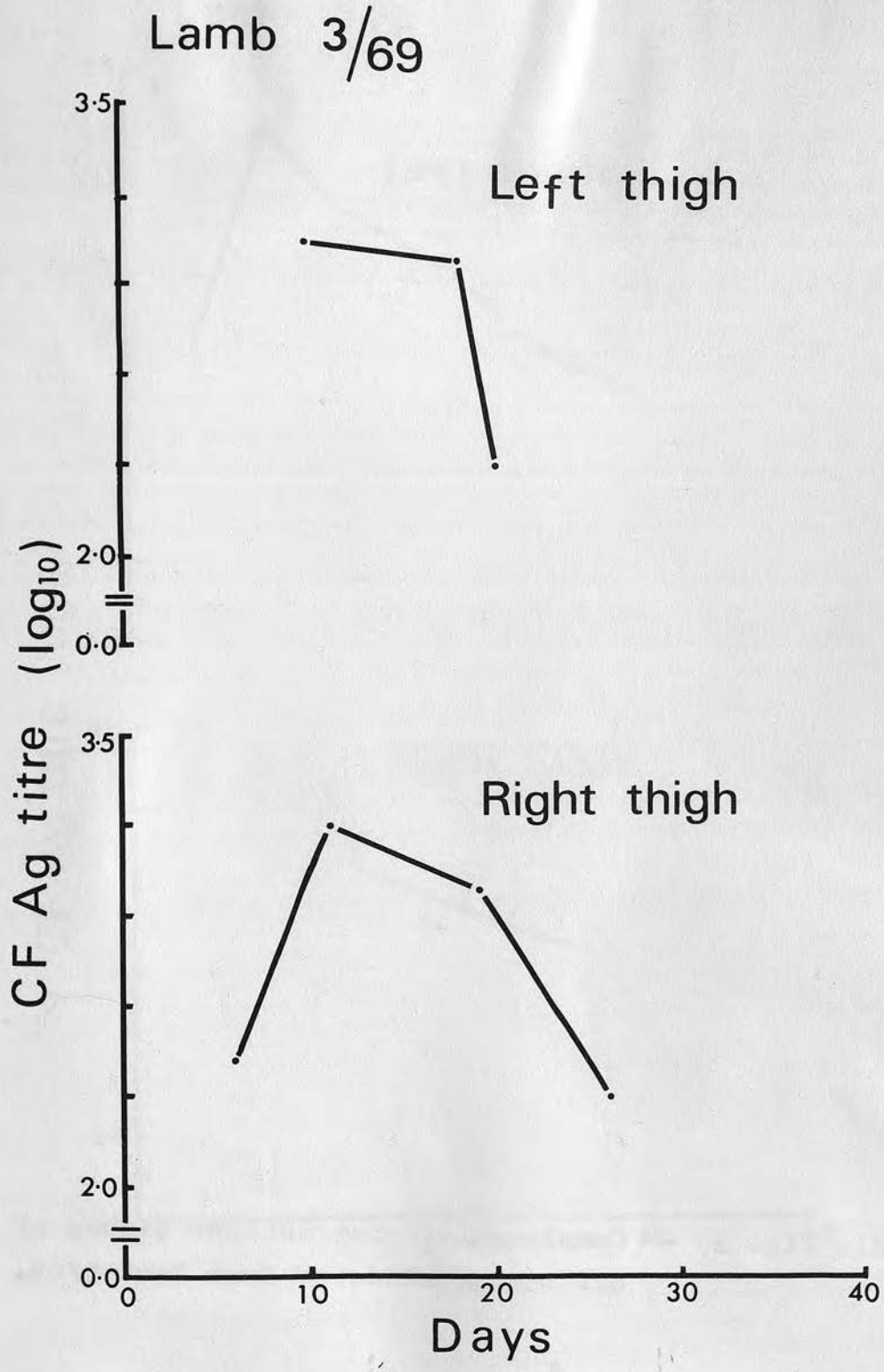
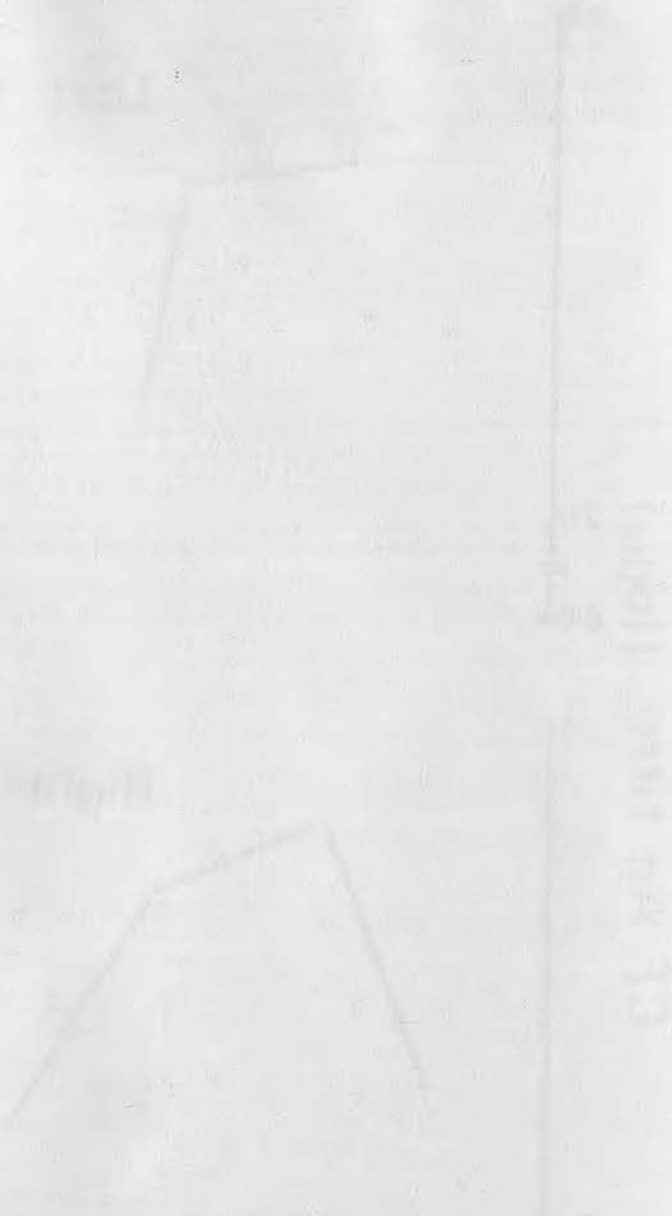
APPENDIX

Appendix fig. 1 - Complement-fixing antigen titres of orf scabs collected from lamb 1/69.

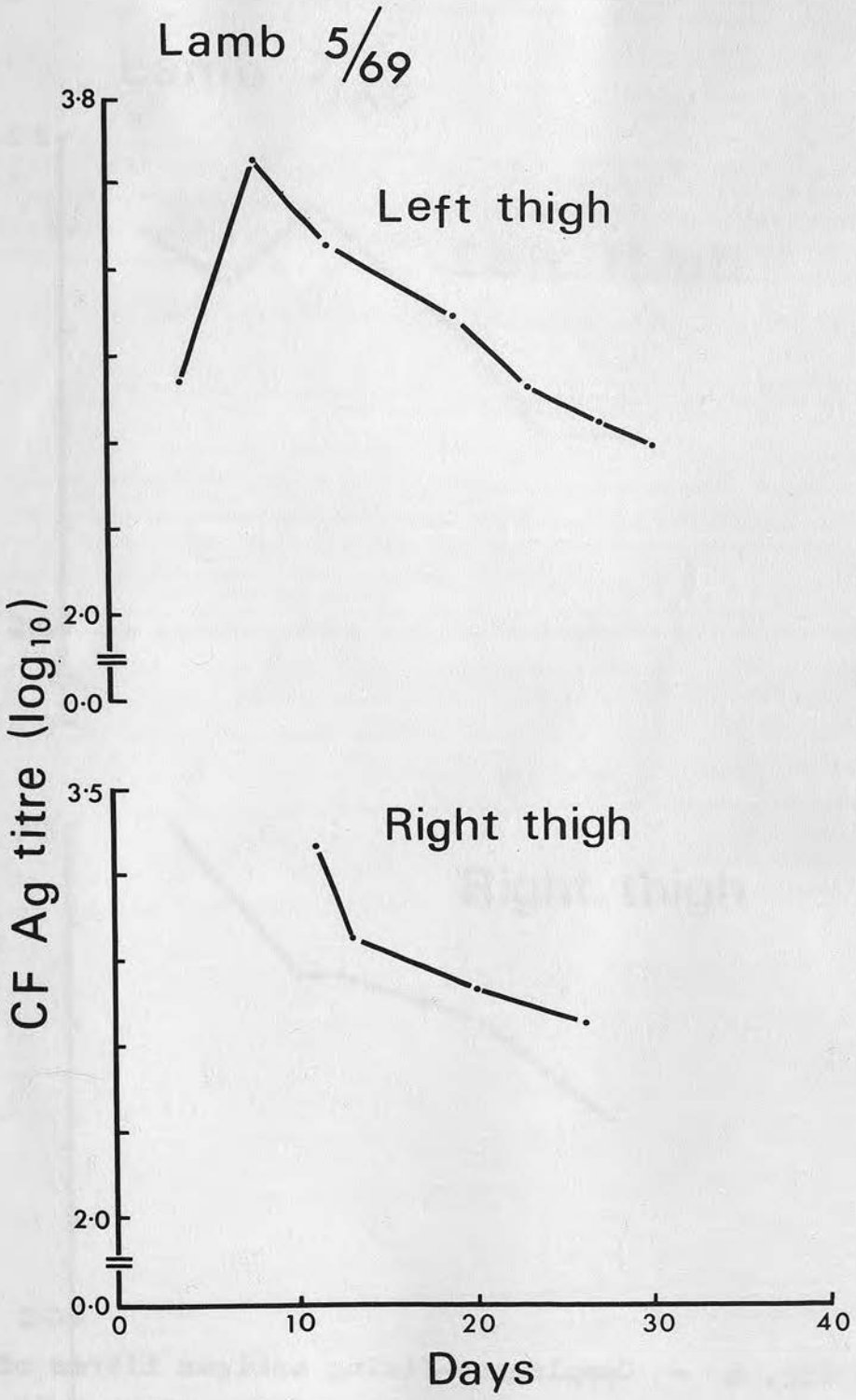

Lamb 1/69
Left thigh
Right thigh
CF Ag titre (log10)
3·5
2·0
0·0
0
10
20
30
40
Days

Appendix fig. 2 - Complement-fixing antigen titres of orf scabs collected from lamb 3/69.

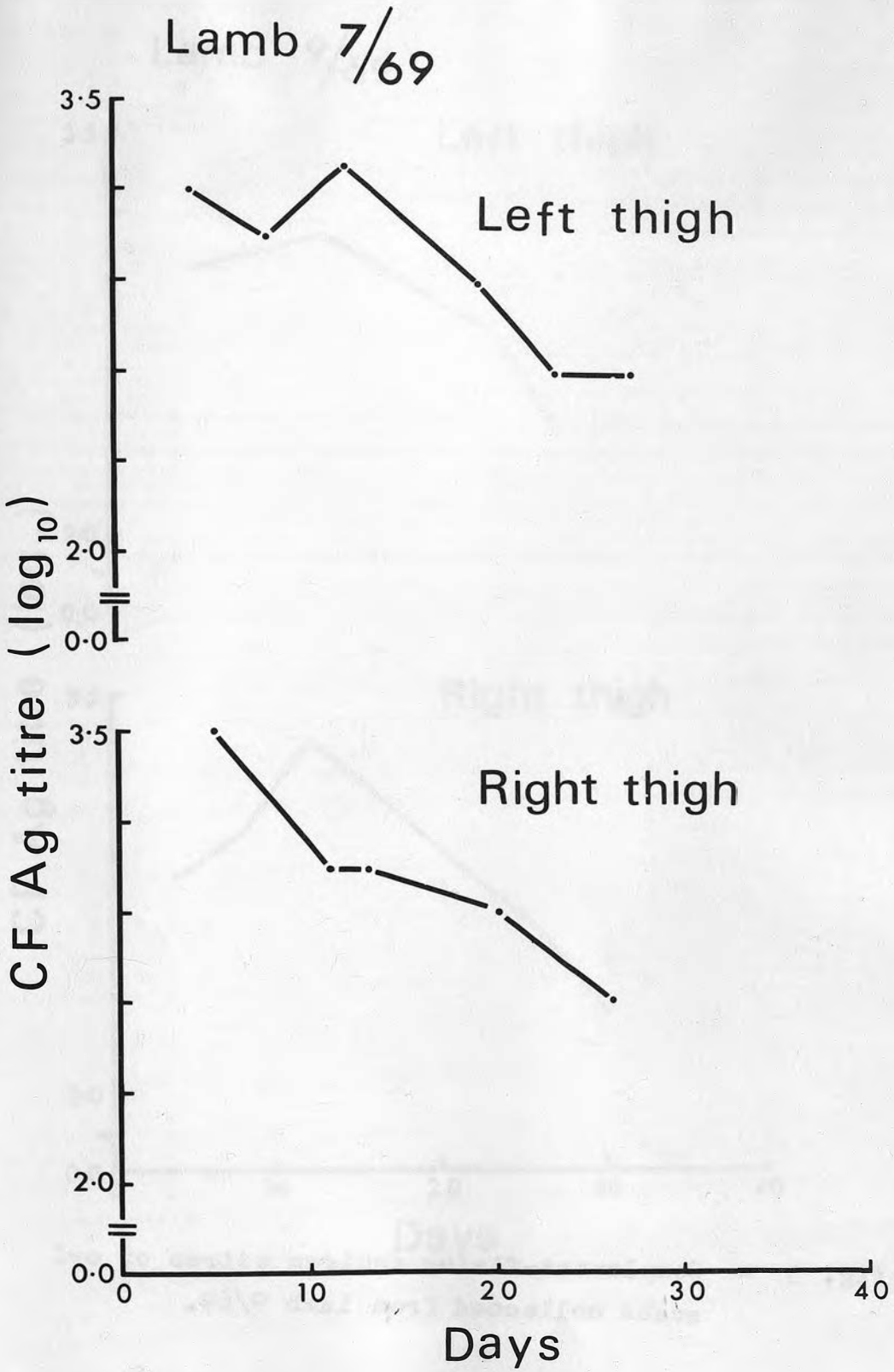

Lamb 3/69
Left thigh
Right thigh
CF Ag titre (log10)
3·5
2·0
0·0
0
10
20
30
40
Days

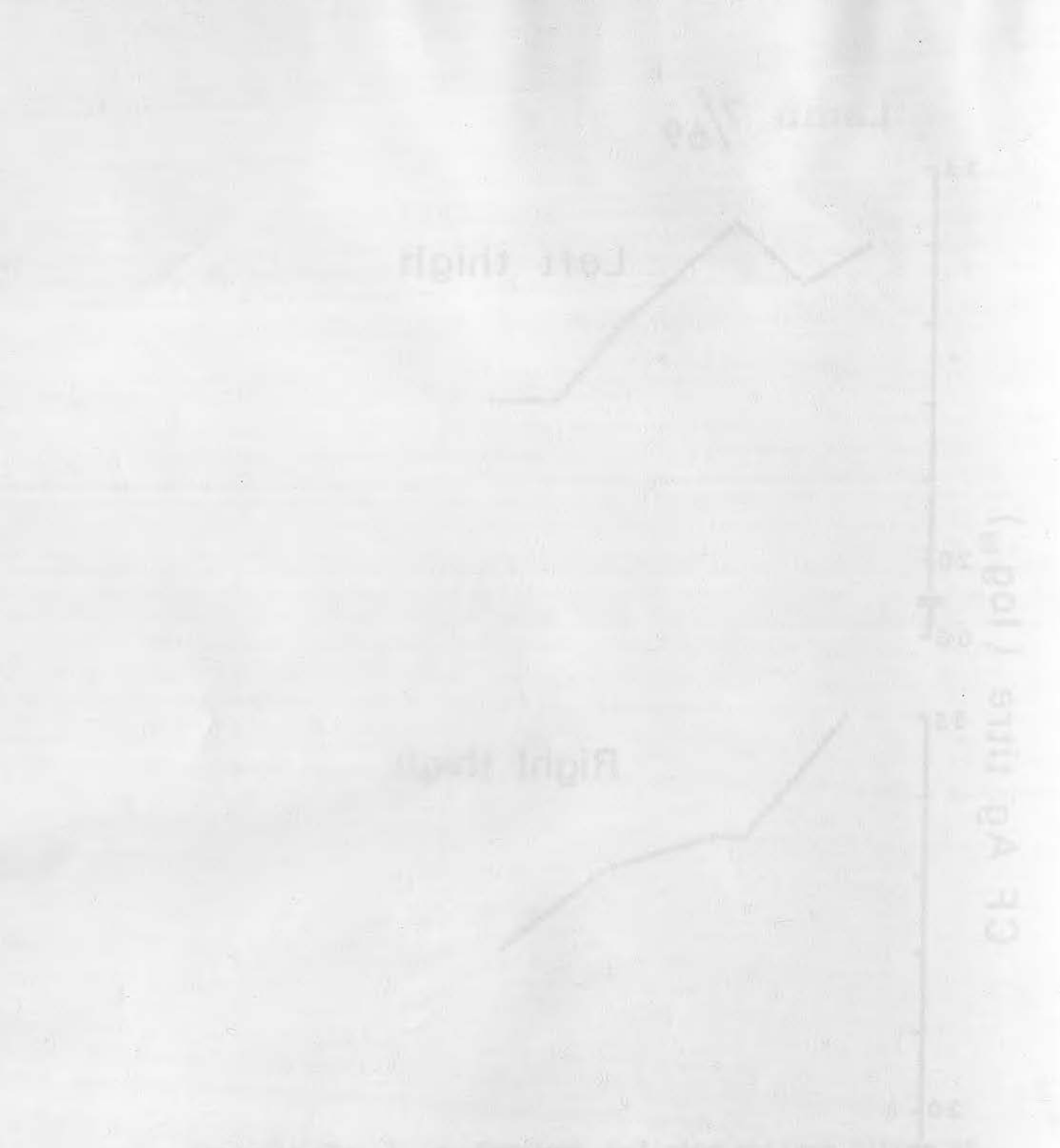


Appendix fig. 3 - Complement-fixing antigen titres of orf scabs collected from lamb 5/69.

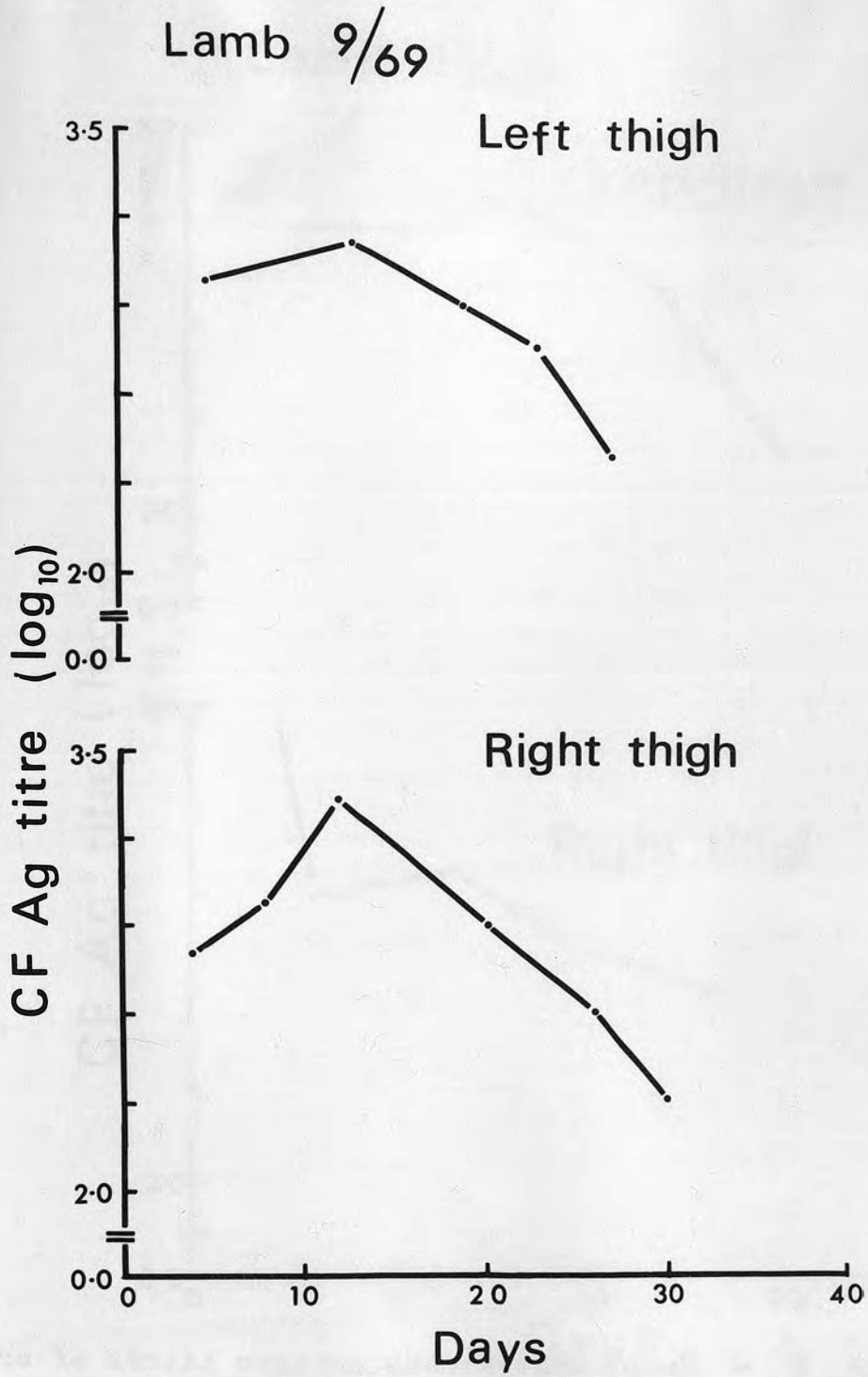

Lamb 5/69
3·8
2·0
0·0
Left thigh
CF Ag titre (log10)
3·5
Right thigh
2·0
0·0
0
10
20
30
40
Days

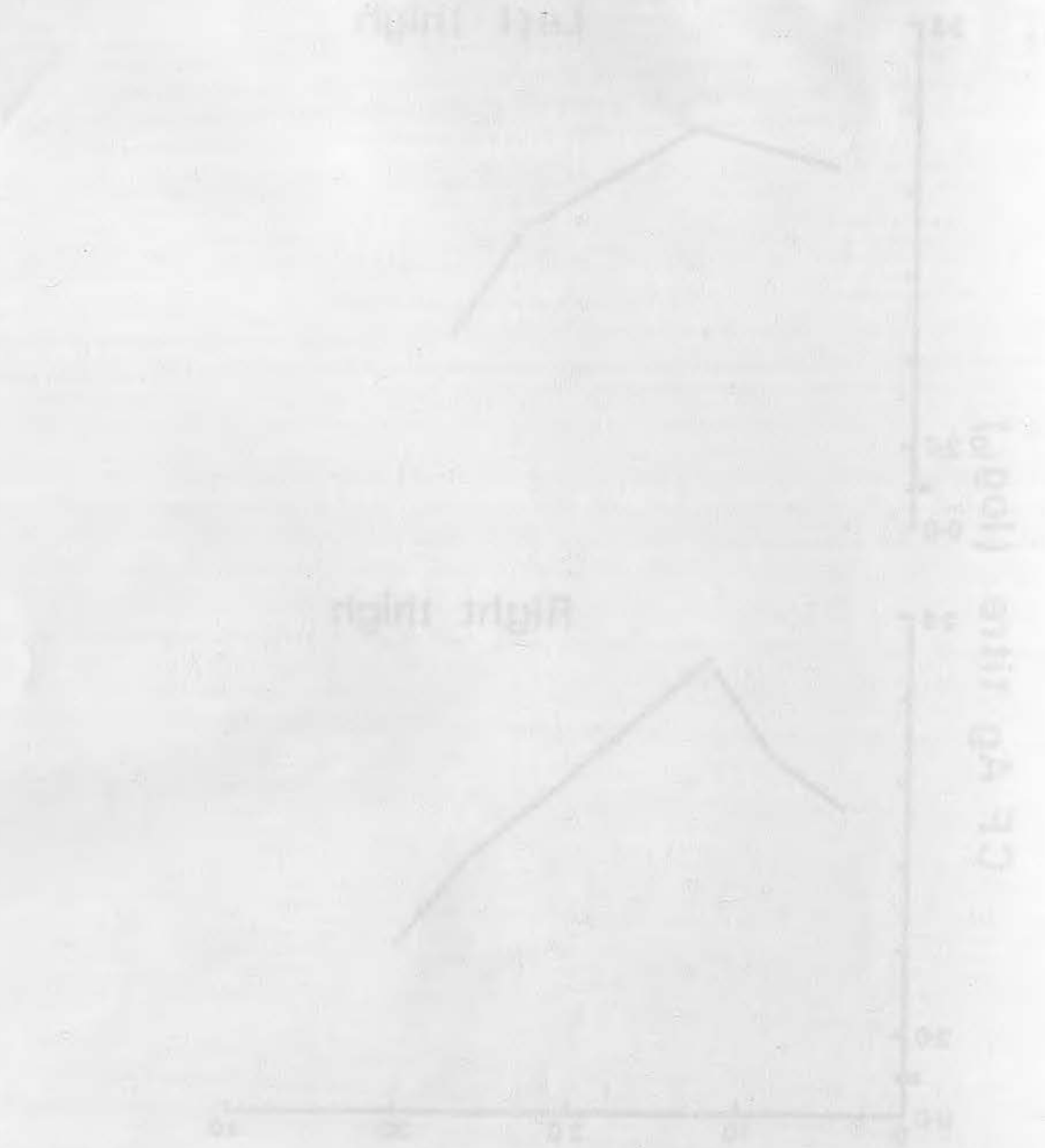
Appendix fig. 4 - Complement-fixing antigen titres of orf scabs collected from lamb 7/69.


Lamb 7/69
Left thigh
Right thigh
CF Ag titre (log10)
3·5
2·0
0·0
0
10
20
30
40
Days

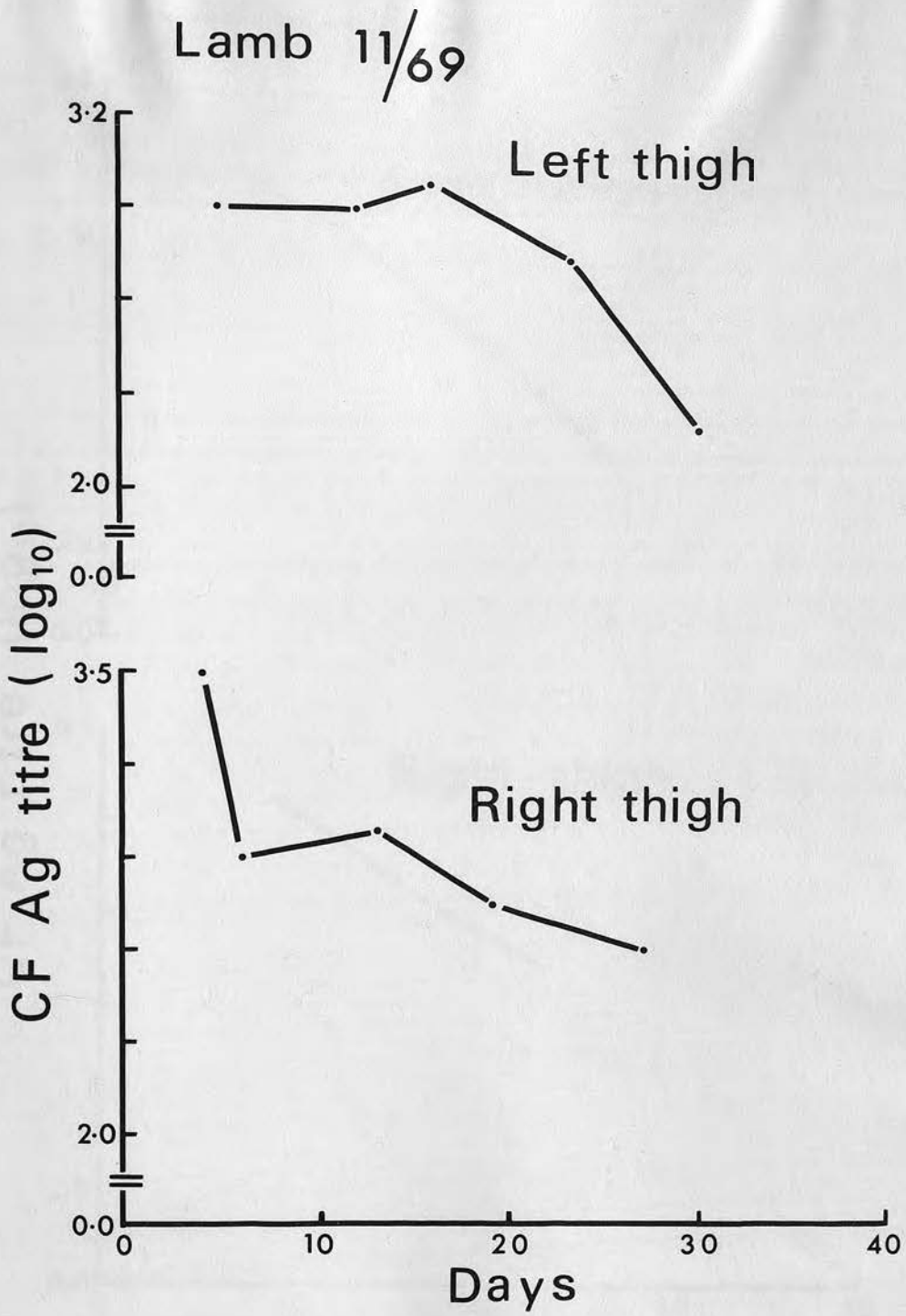


Appendix fig. 5 - Complement-fixing antigen titres of orf scabs collected from lamb 9/69.

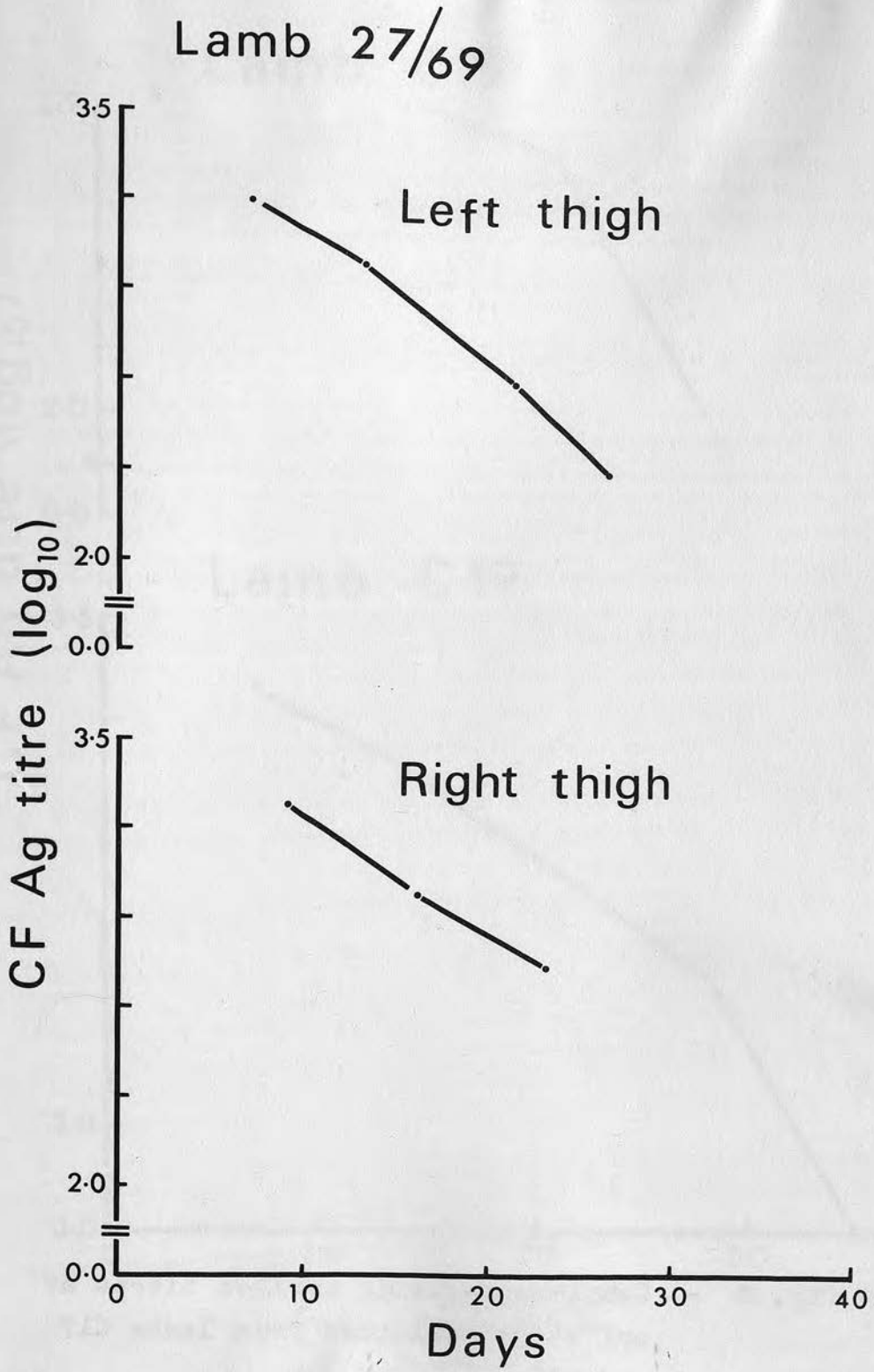

Lamb 9/69
Left thigh
Right thigh
CF Ag titre (log10)
3·5
2·0
0·0
0
10
20
30
40
Days

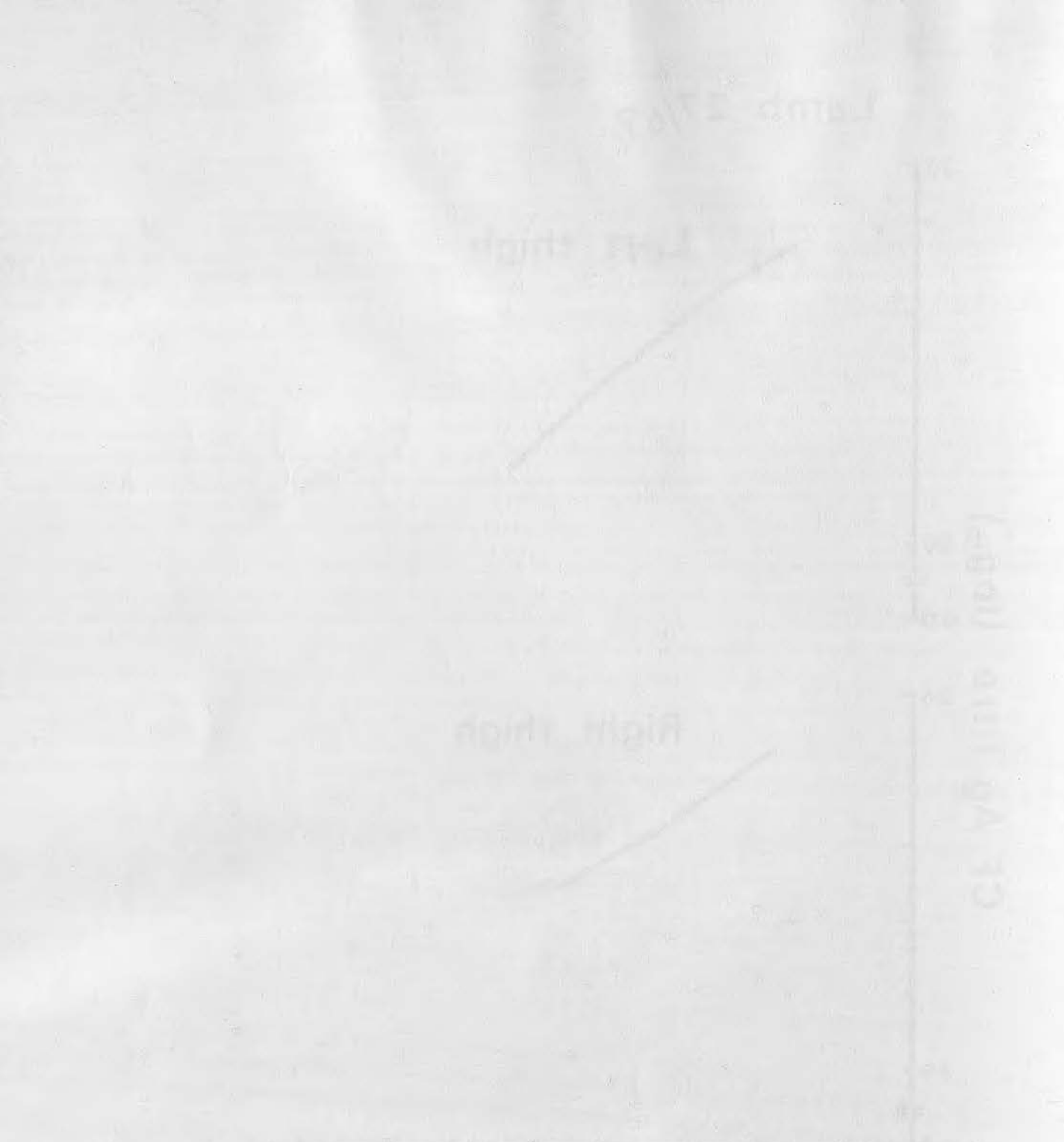


Appendix. fig. 6 - Complement-fixing antigen titres of orf scabs collected from lamb 11/69.

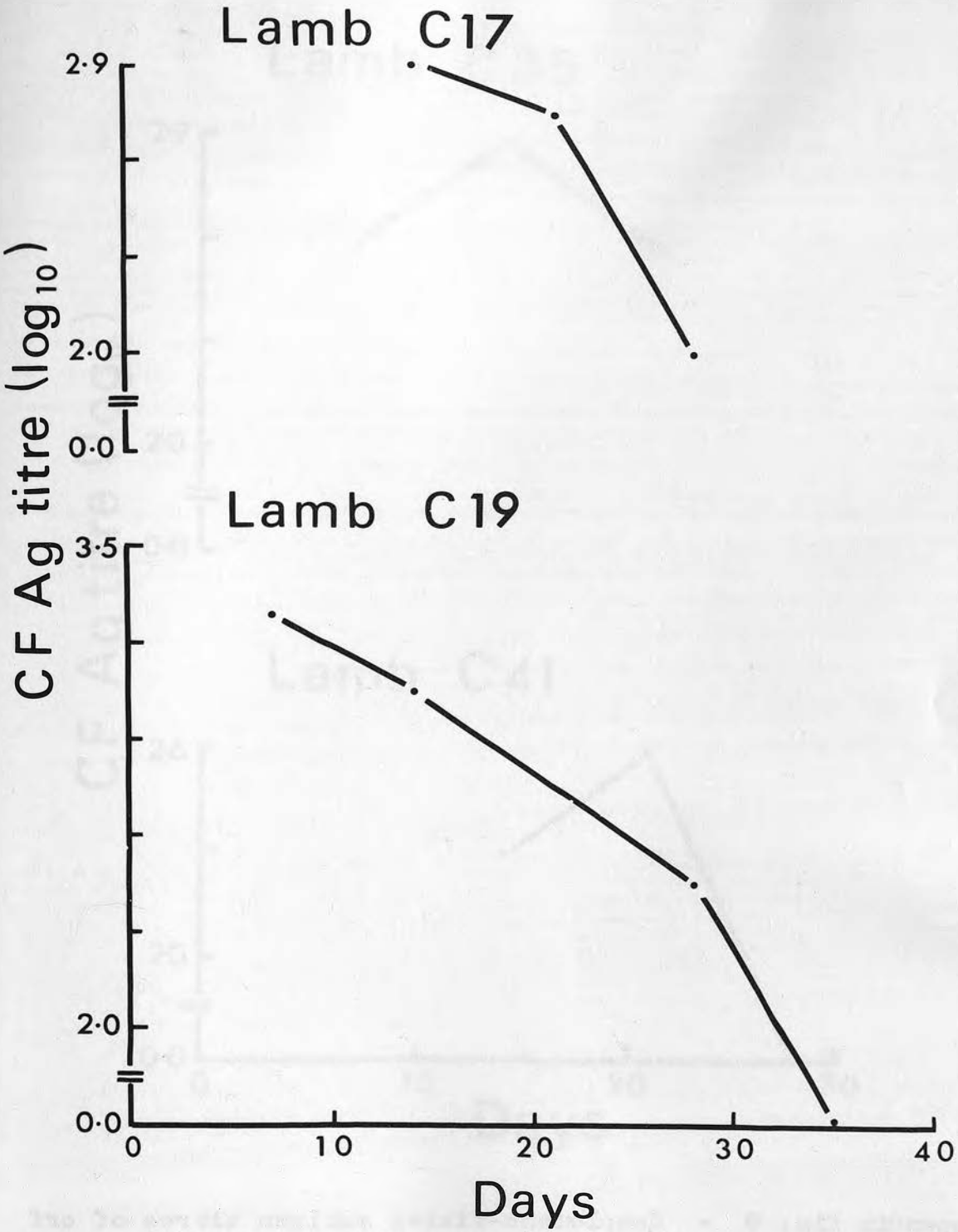

Lamb 11/69
Left thigh
Right thigh
CF Ag titre (log10)
3·2
2·0
0·0
3·5
2·0
0·0
0
10
20
30
40
Days

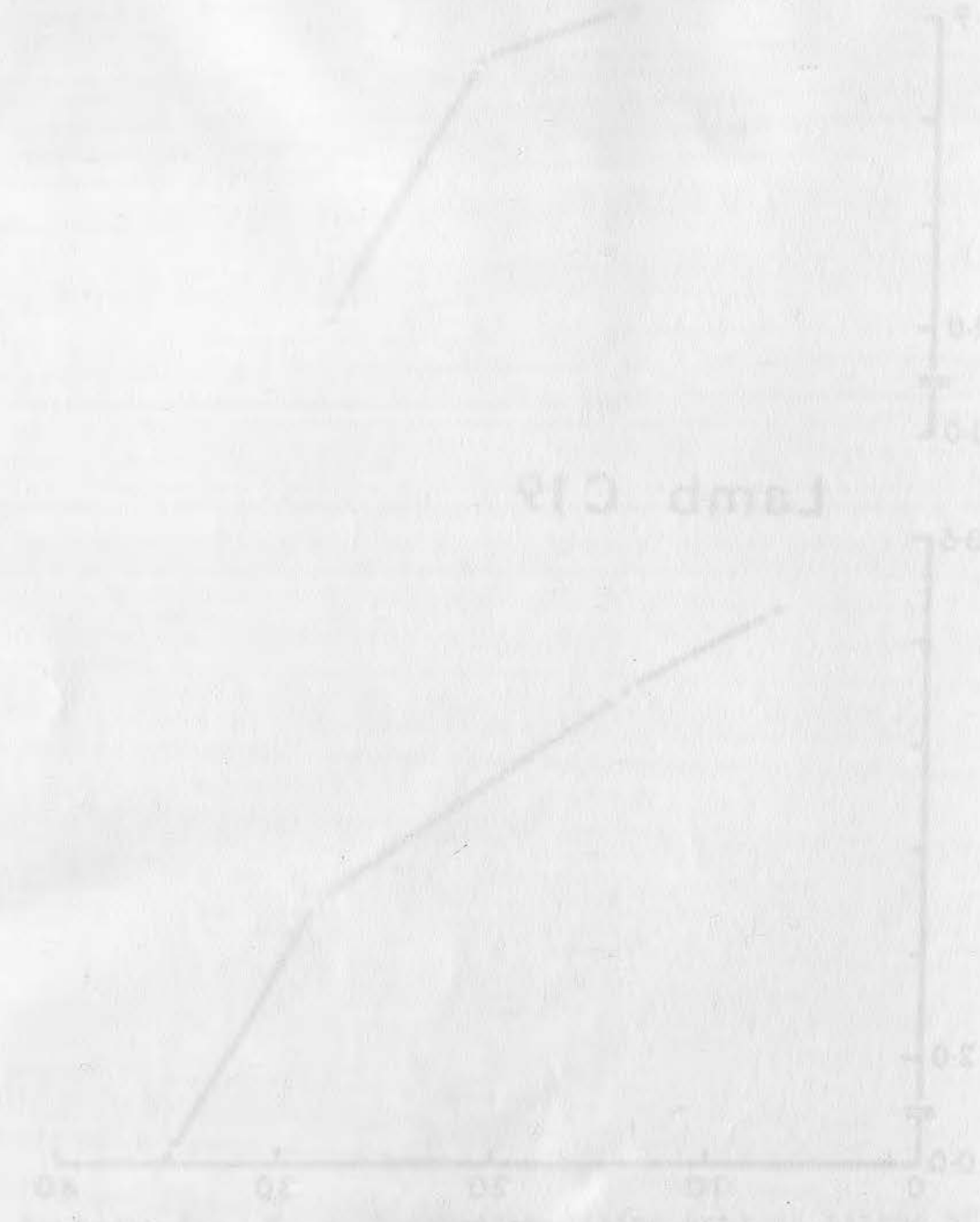
Appendix fig. 7 - Complement-fixing antigen titres of orf scabs collected from lamb 27/69.


Lamb 27/69
Left thigh
Right thigh
CF Ag titre (log10)
3·5
2·0
0·0
0
10
20
30
40
Days

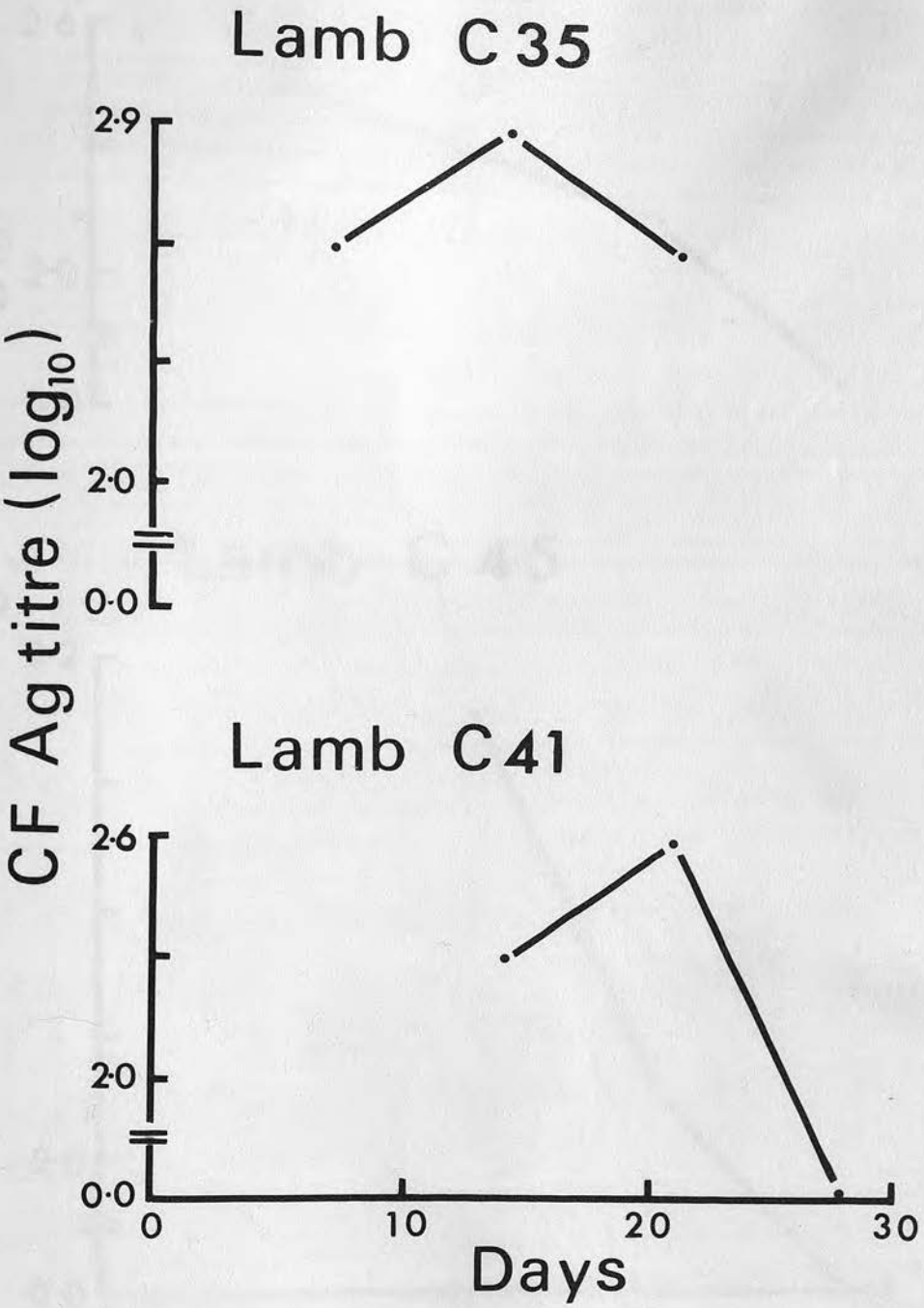


Appendix fig. 8 - Complement-fixing antigen titres of orf scabs collected from lambs C17 and C19.

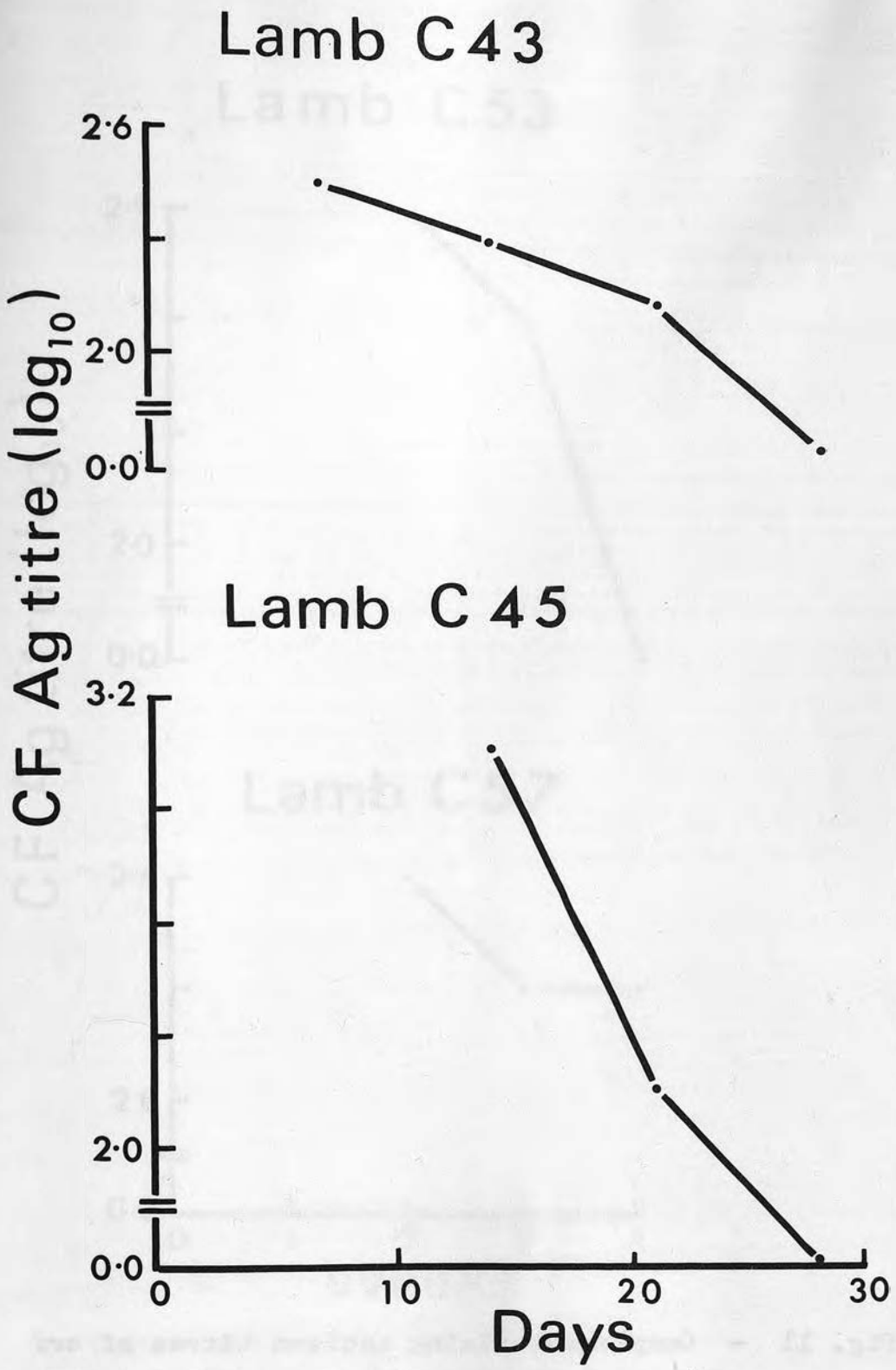

Lamb C17
2·9
2·0
0·0
Lamb C19
3·5
2·0
0·0
CF Ag titre (log10)
0
10
20
30
40
Days

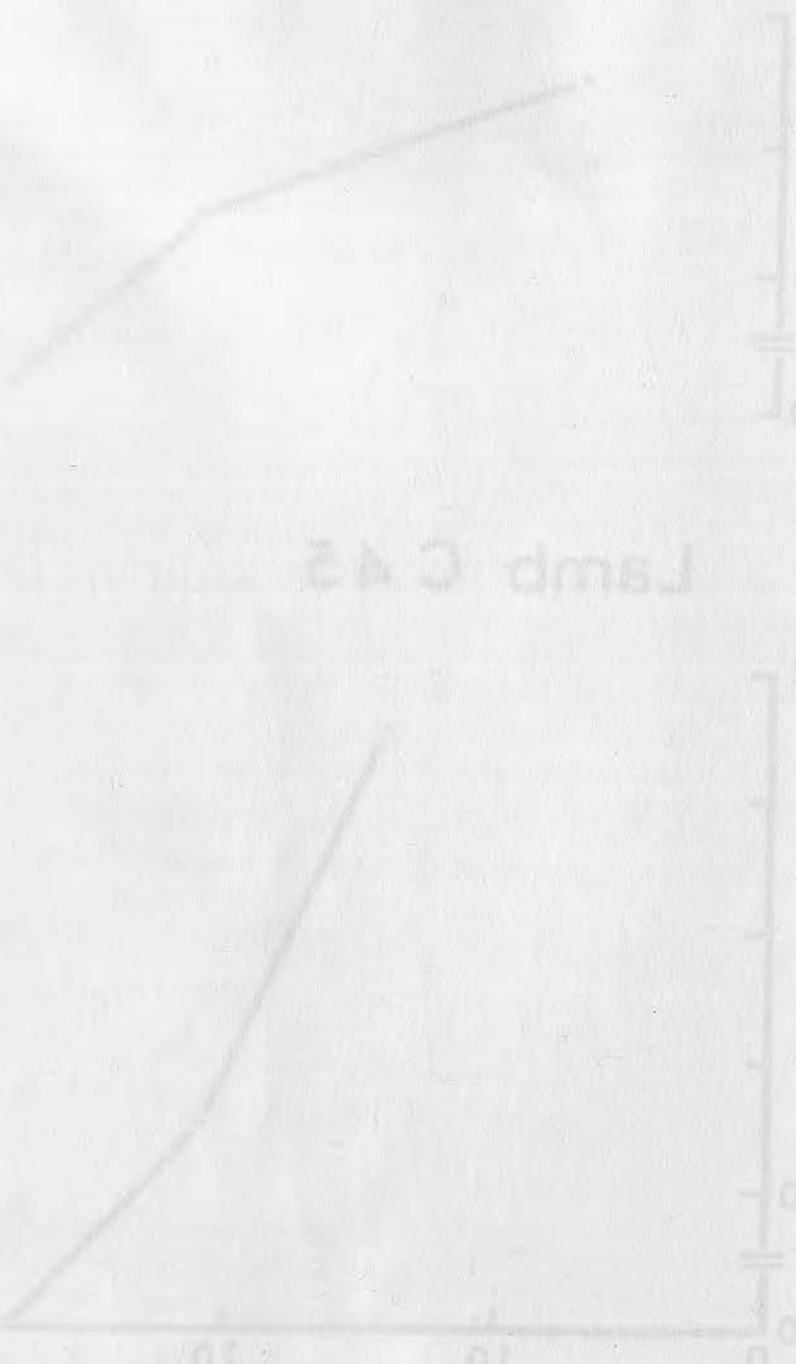


Appendix fig. 9 - Complement-fixing antigen titres of orf scabs collected from lambs C35 and C41

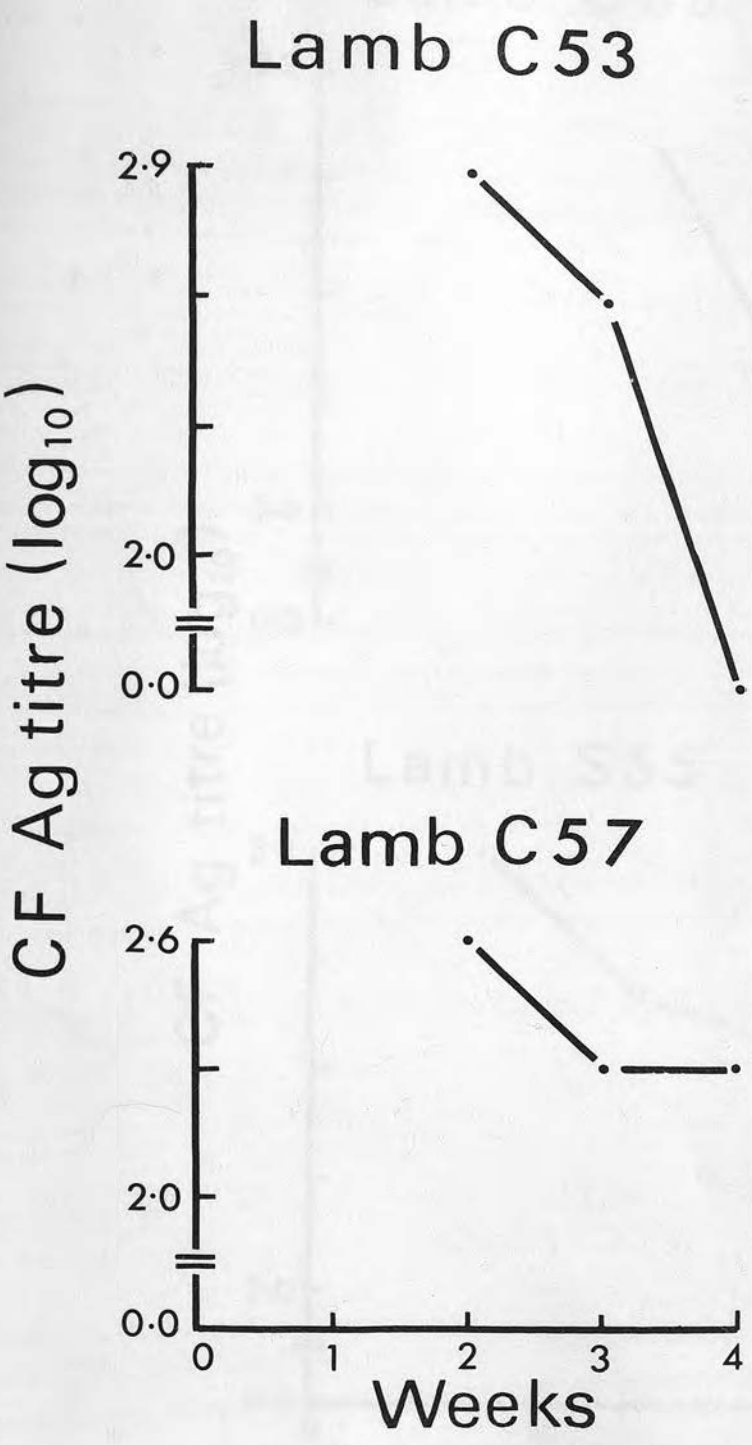

Lamb C35
2·9
2·0
0·0
Lamb C41
2·6
2·0
0·0
0
10
20
30
Days
CF Ag titre (log10)

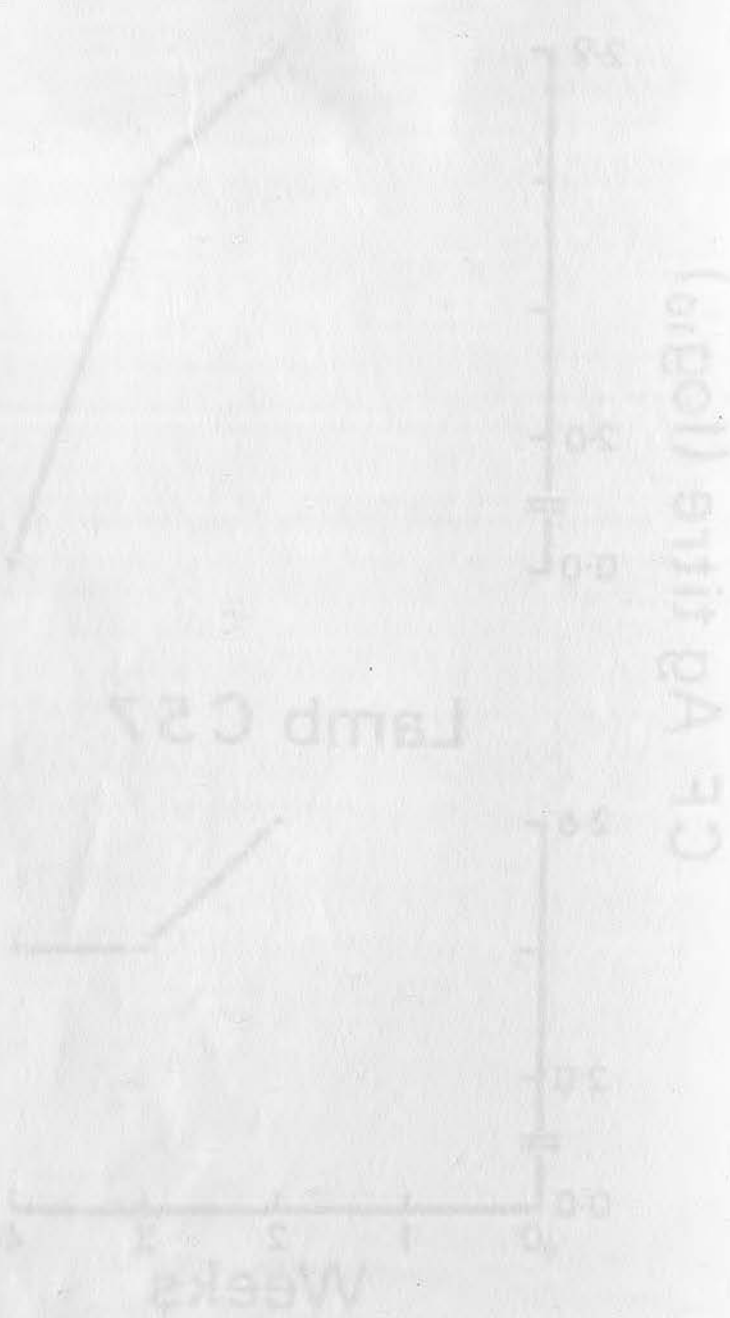
Appendix fig. 10 - Complement-fixing antigen titres of orf scabs collected from lambs C43 and C45.


Lamb C43
2·6
2·0
0·0
Lamb C45
3·2
2·0
0·0
0
10
20
30
Days
CF Ag titre (log10)

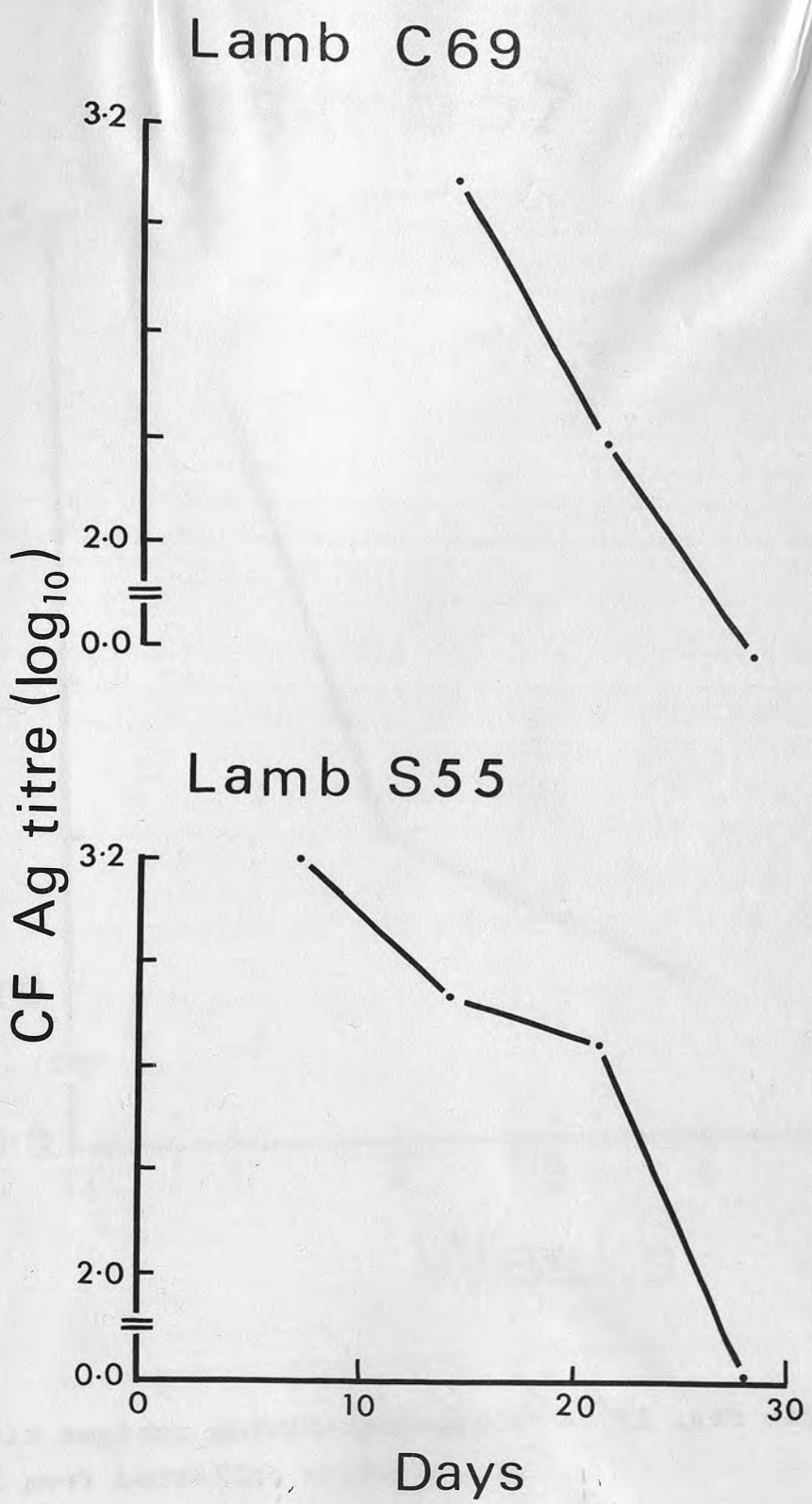


Appendix fig. 11 - Complement-fixing antigen titres of orf scabs collected from lambs C53 and C57.

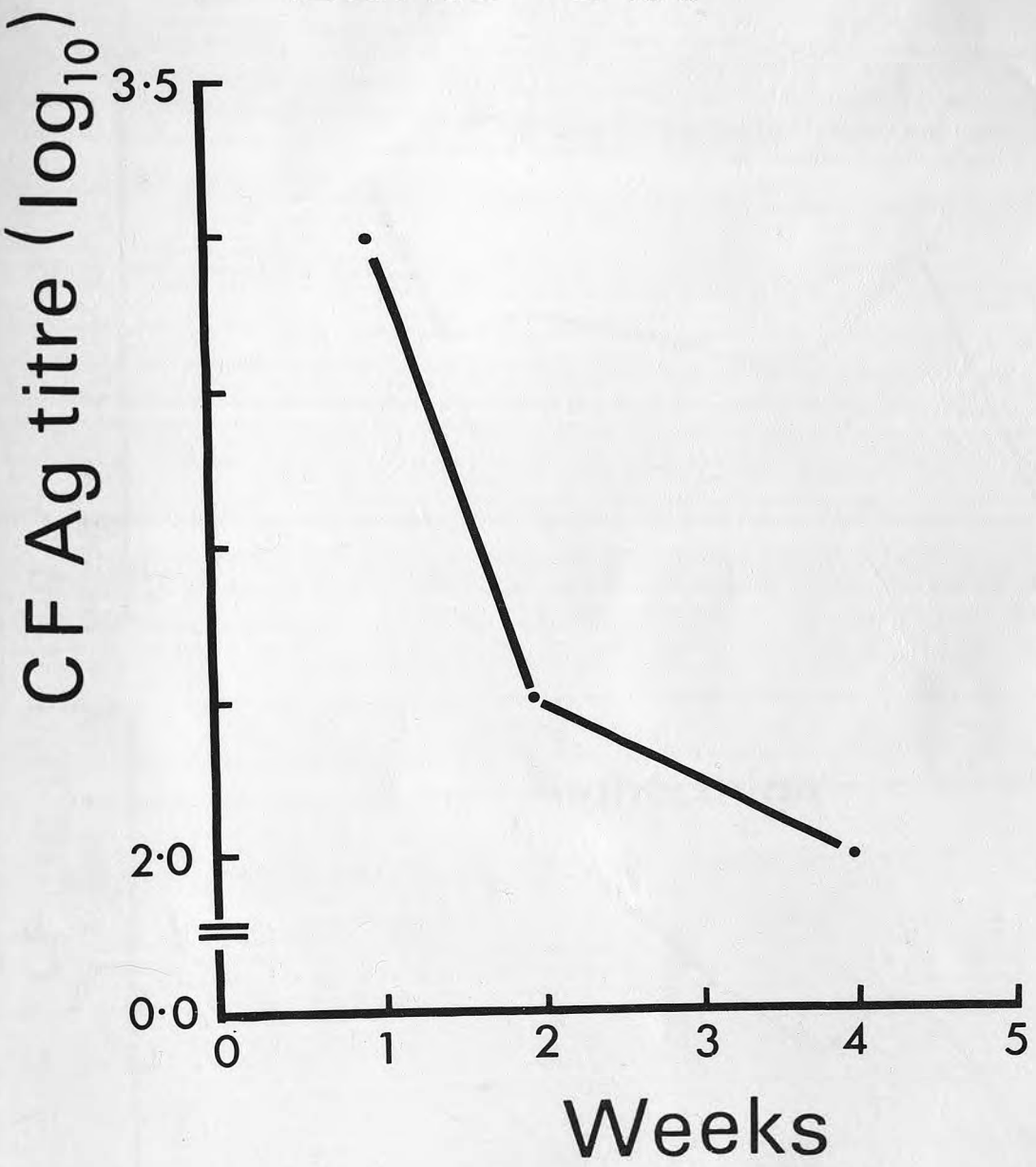

Lamb C53
2·9
2·0
0·0
Lamb C57
2·6
2·0
0·0
0
1
2
3
4
Weeks
CF Ag titre (log10)



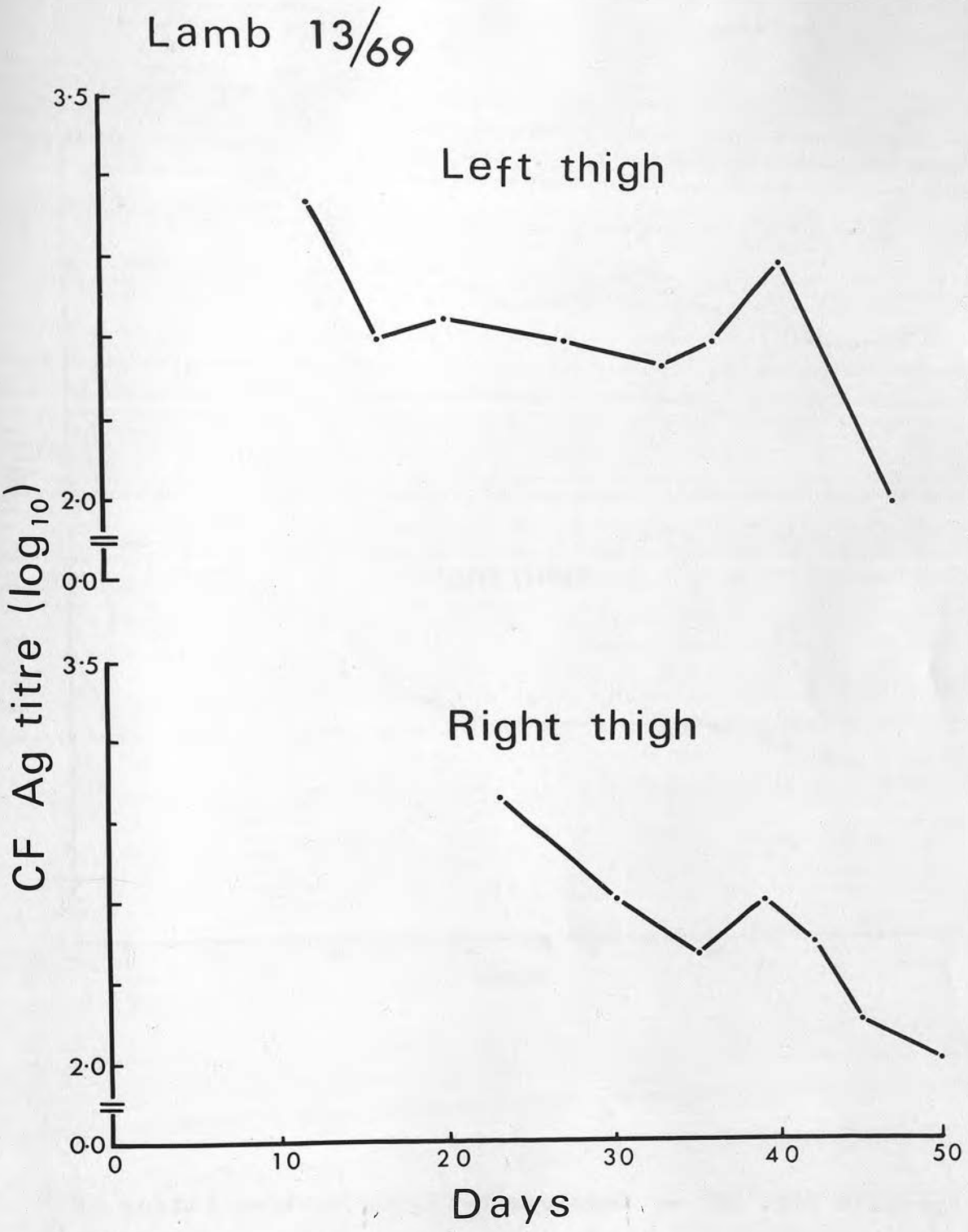
Appendix fig. 12 - Complement-fixing antigen titres of orf scab collected from lambs C69 and S55.


Lamb C69
3·2
2·0
0·0
Lamb S55
3·2
2·0
0·0
CF Ag titre (log10)
0
10
20
30
Days

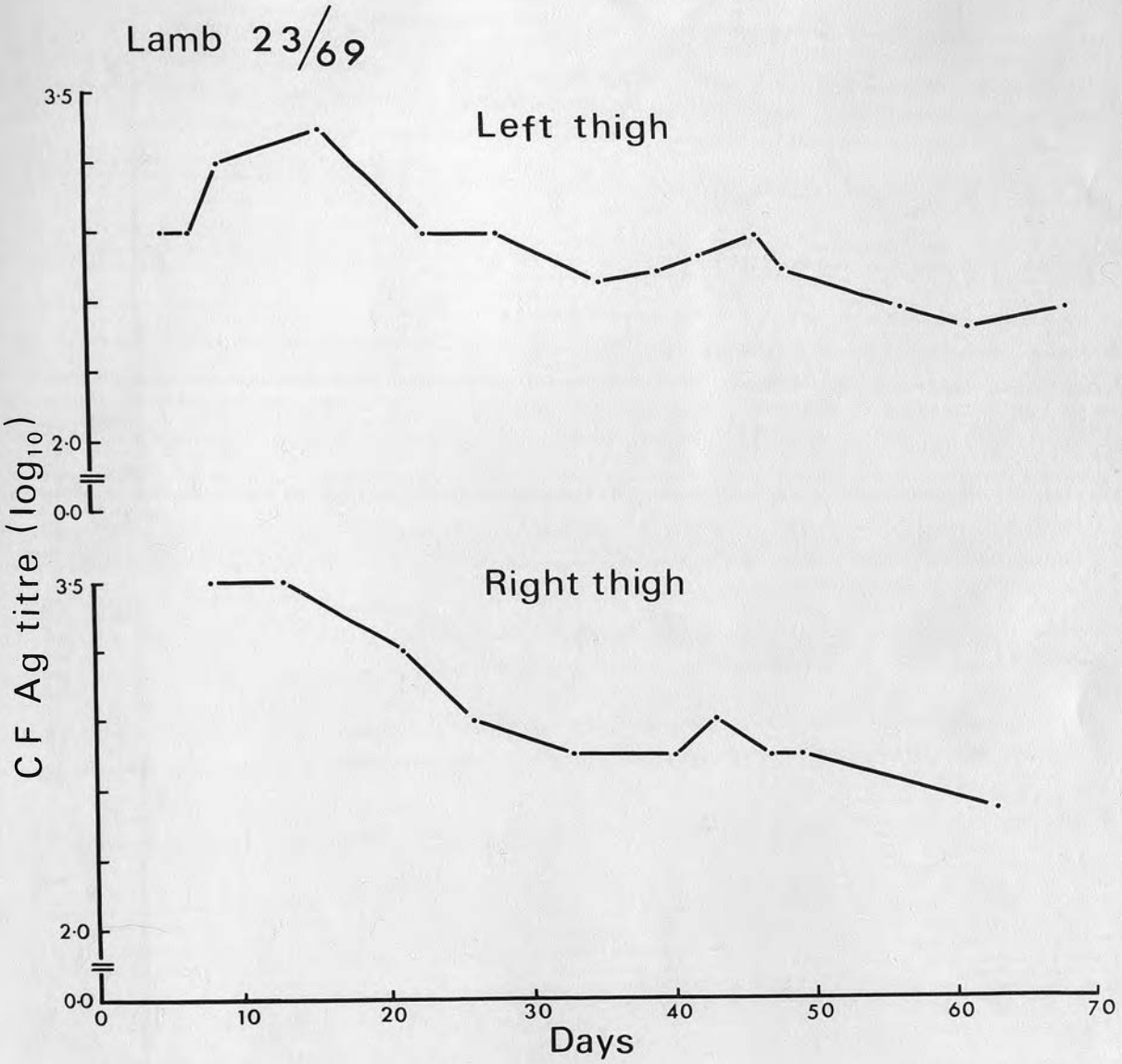
Appendix fig. 13 - Complement-fixing antigen titres of orf scabs collected from lamb S57.


Lamb S57
CF Ag titre (log_{10})
3·5
2·0
0·0
0
1
2
3
4
5
Weeks

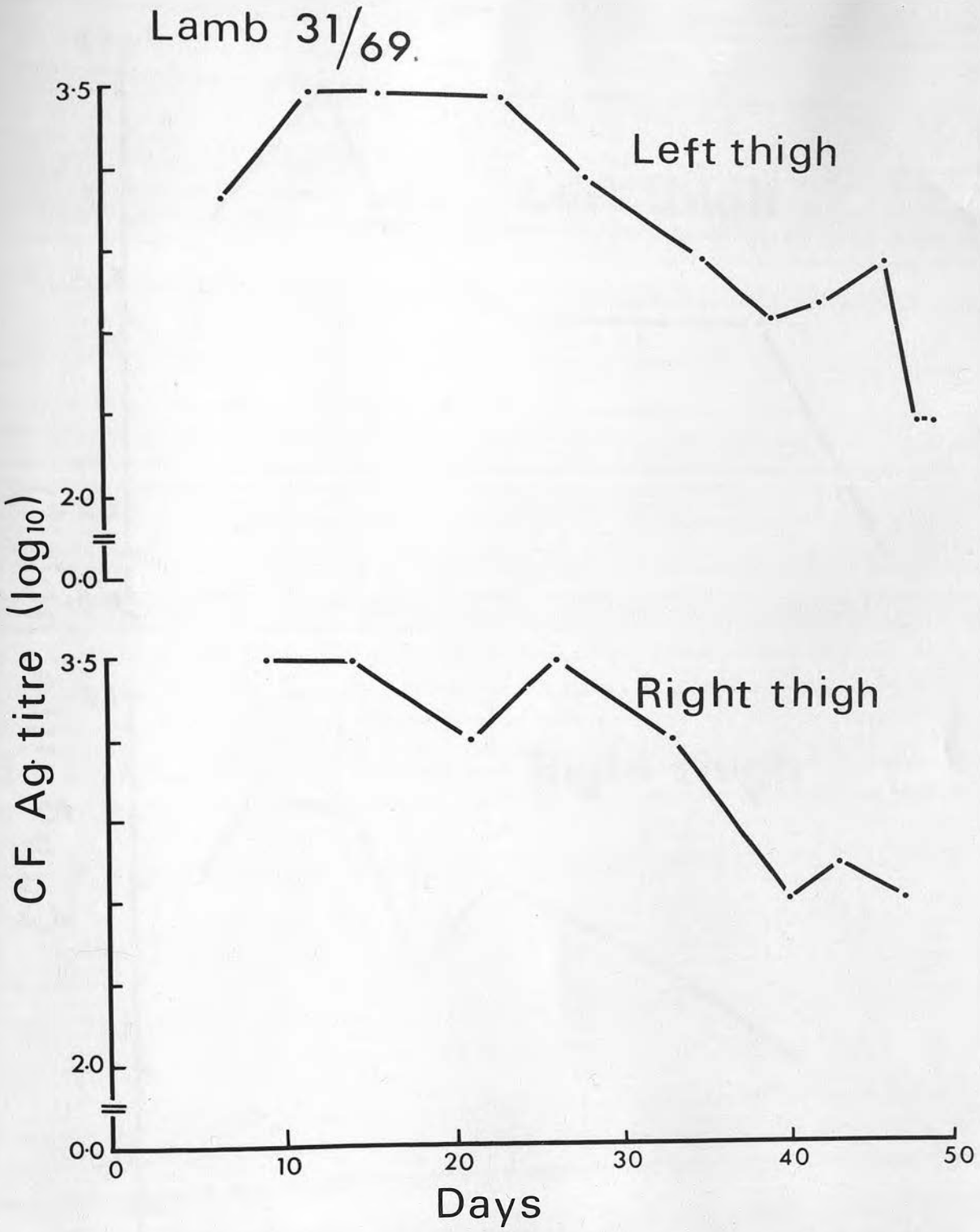
Appendix fig. 14 - Complement-fixing antigen titres of orf scabs collected from lamb 13/69.


Lamb 13/69
Left thigh
Right thigh
CF Ag titre (log10)
3·5
2·0
0·0
0
10
20
30
40
50
Days

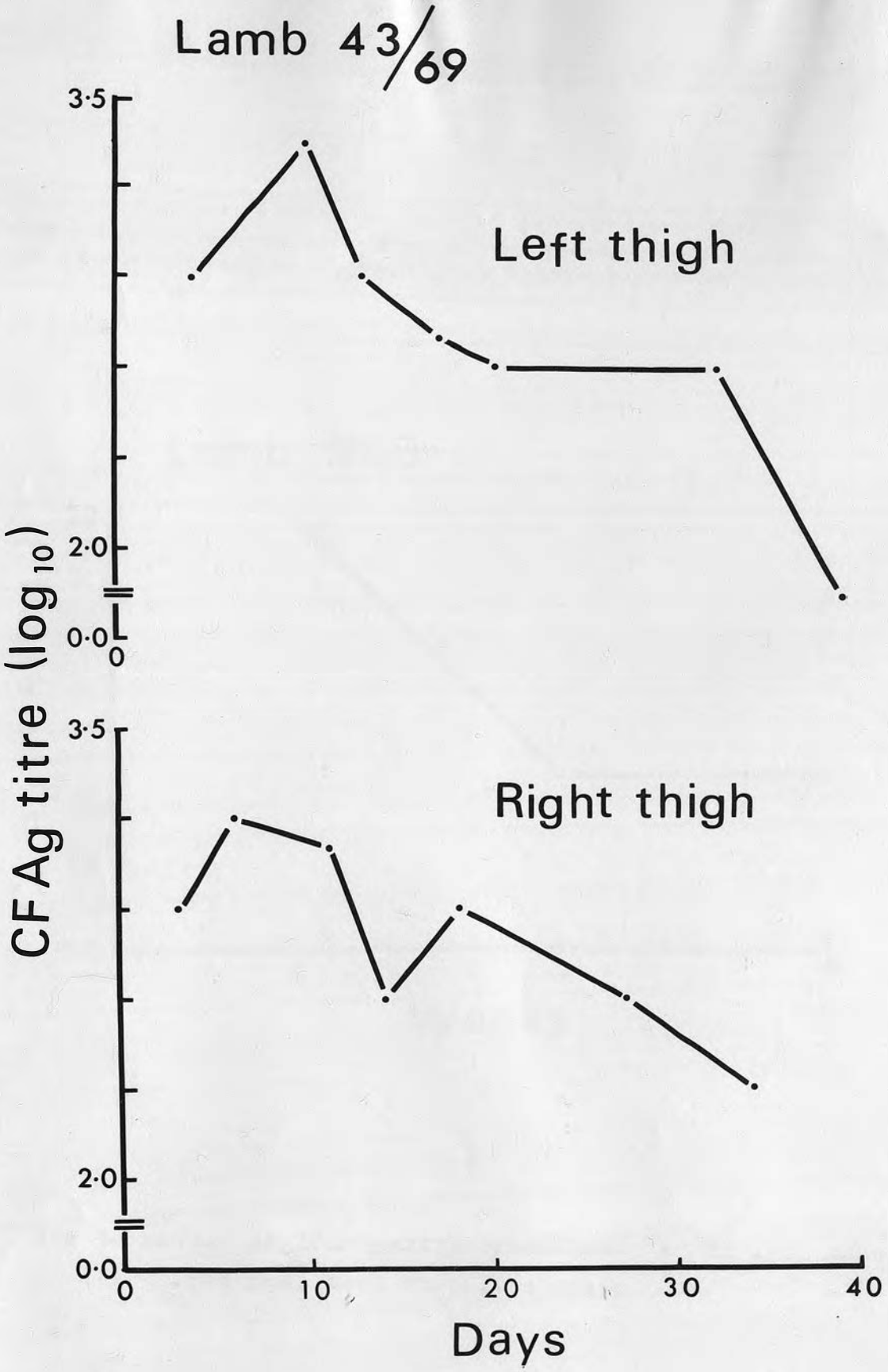
Appendix fig. 15 - Complement-fixing antigen titres of orf scabs collected from lamb 23/69.


Lamb 23/69
Left thigh
Right thigh
CF Ag titre (log10)
3·5
2·0
0·0
0
10
20
30
40
50
60
70
Days

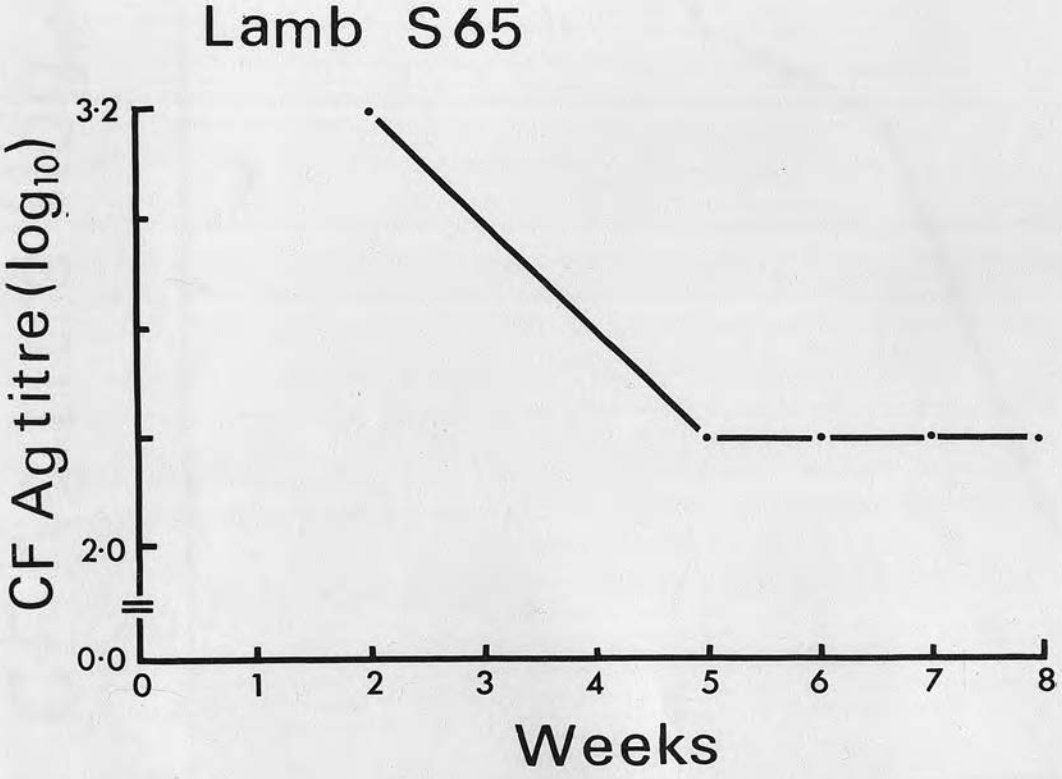
Appendix fig. 16 - Complement-fixing antigen titres of orf scabs collected from lamb 31/69.


Lamb 31/69.
3·5
2·0
0·0
Left thigh
CF Ag titre (log10)
3·5
2·0
0·0
Right thigh
0
10
20
30
40
50
Days

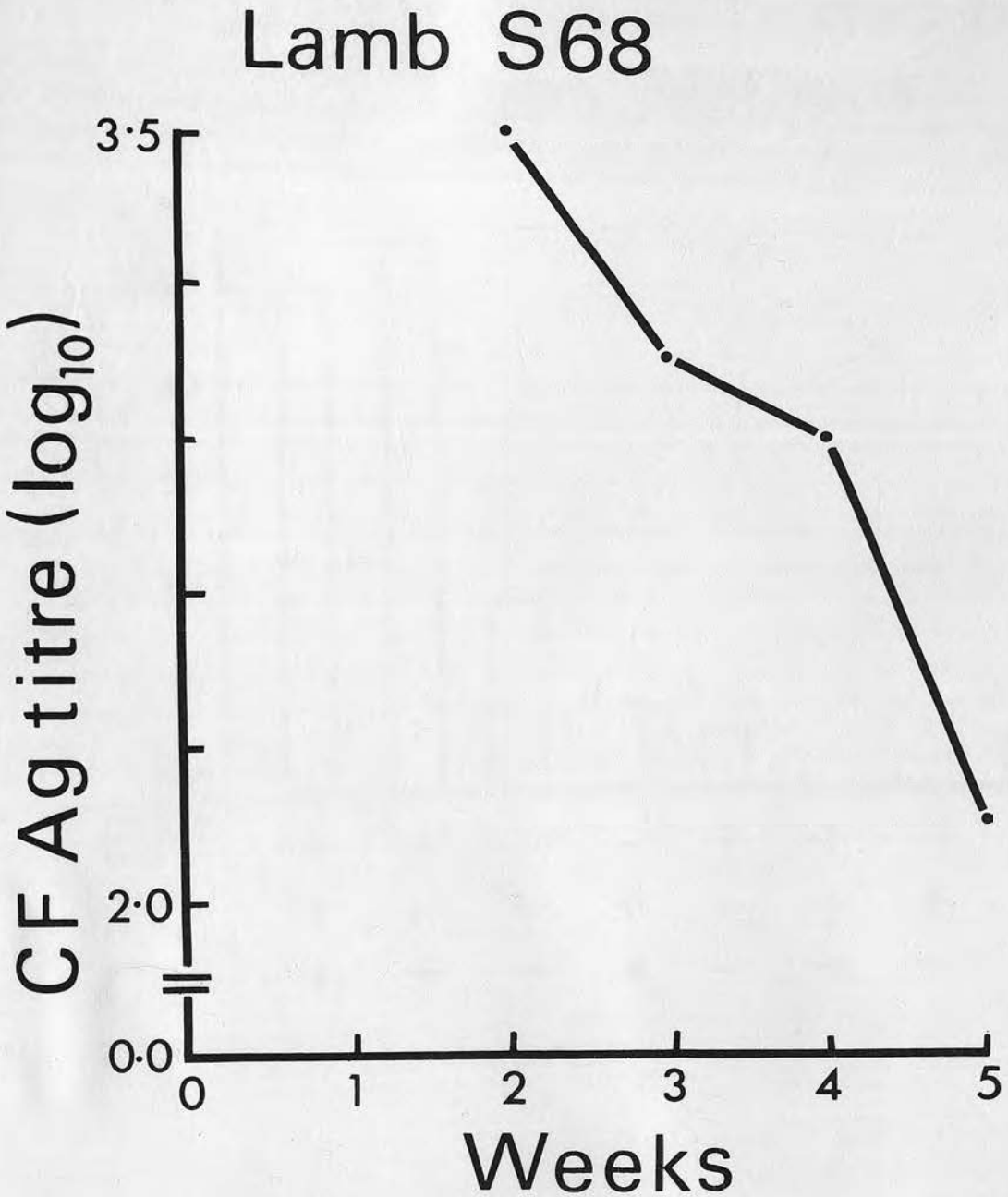
Appendix fig. 17 - Complement-fixing antigen titres of orf scabs collected from lamb 43/69.


Lamb 43/69
Left thigh
Right thigh
CF Ag titre (log10)
3·5
2·0
0·0
0
10
20
30
40
Days

Appendix fig. 18 - Complement-fixing antigen titres of orf scabs collected from lamb S65.

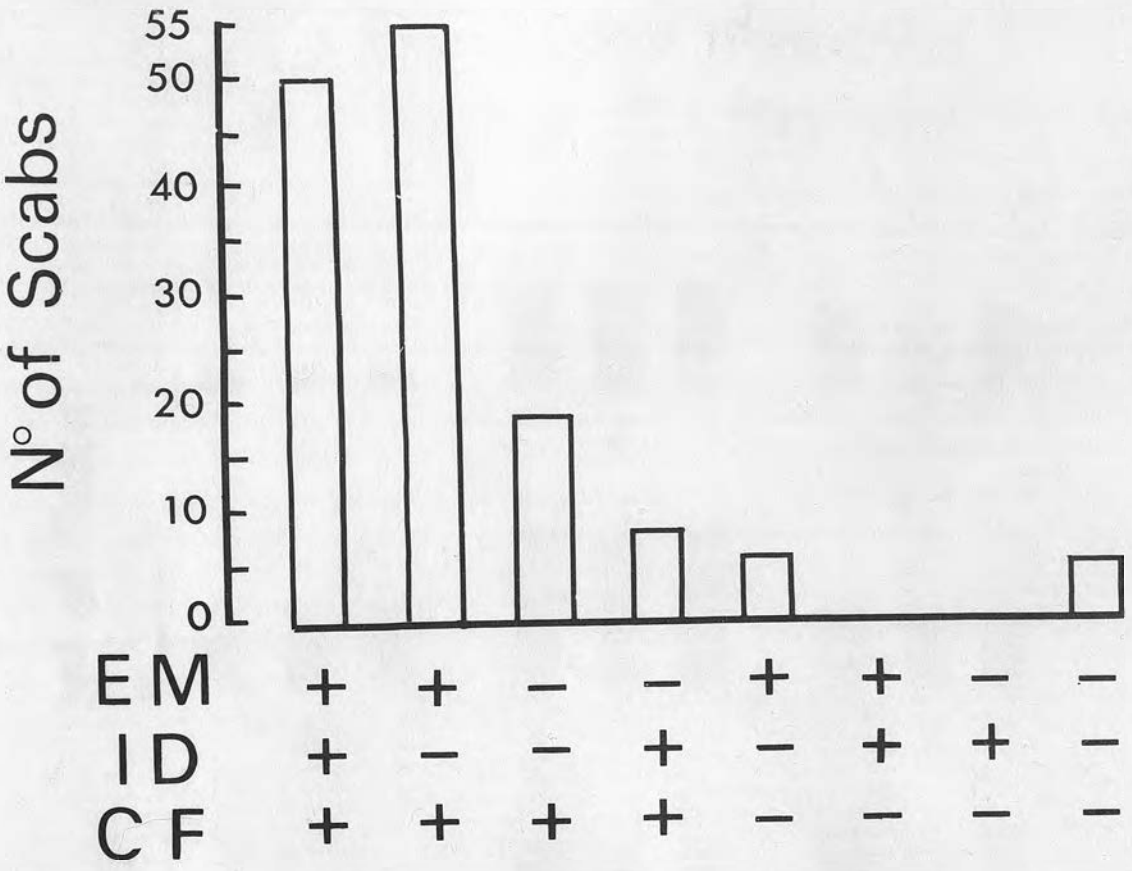

Lamb S65
CF Ag titre (log_{10})
3·2
2·0
0·0
0
1
2
3
4
5
6
7
8
Weeks

Appendix fig. 19 - Complement-fixing antigen titres of orf scabs collected from lamb S68.


Lamb S68
3·5
2·0
0·0
CF Ag titre (log10)
0
1
2
3
4
5
Weeks

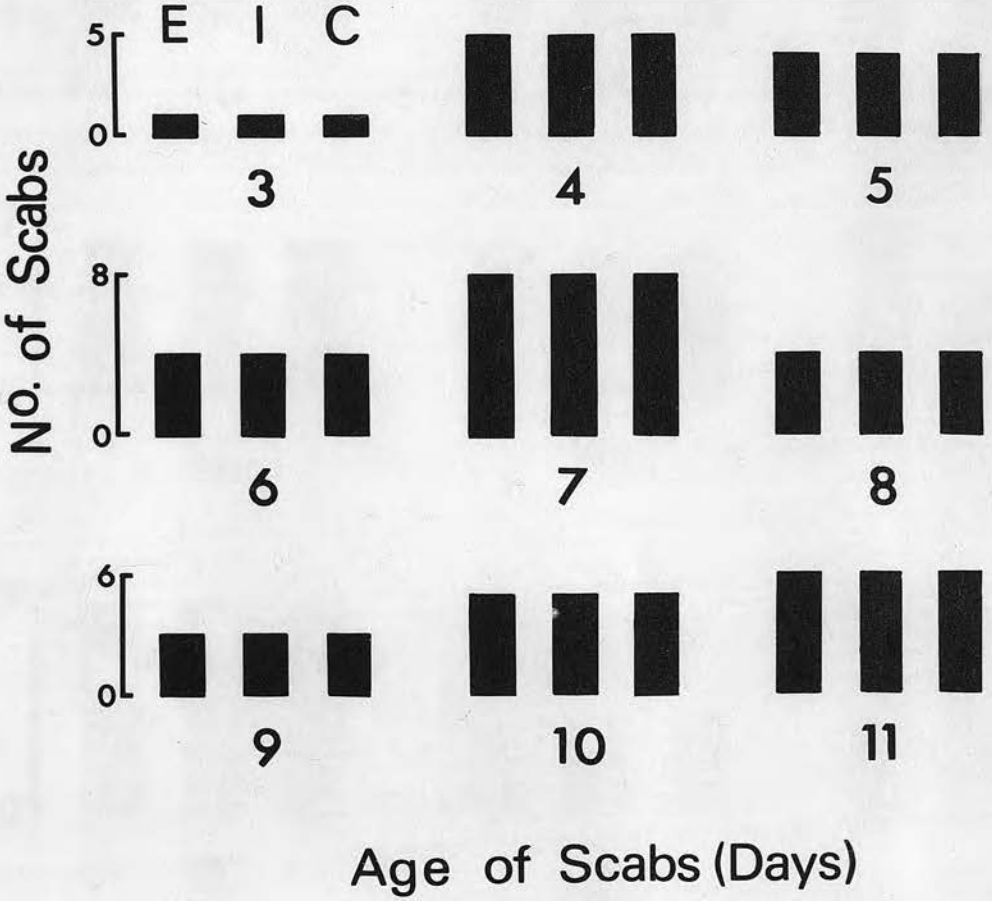
Appendix fig. 20 - Detection of virus particles, immuno-diffusion antigen and complement-fixing antigen in the same scabs.

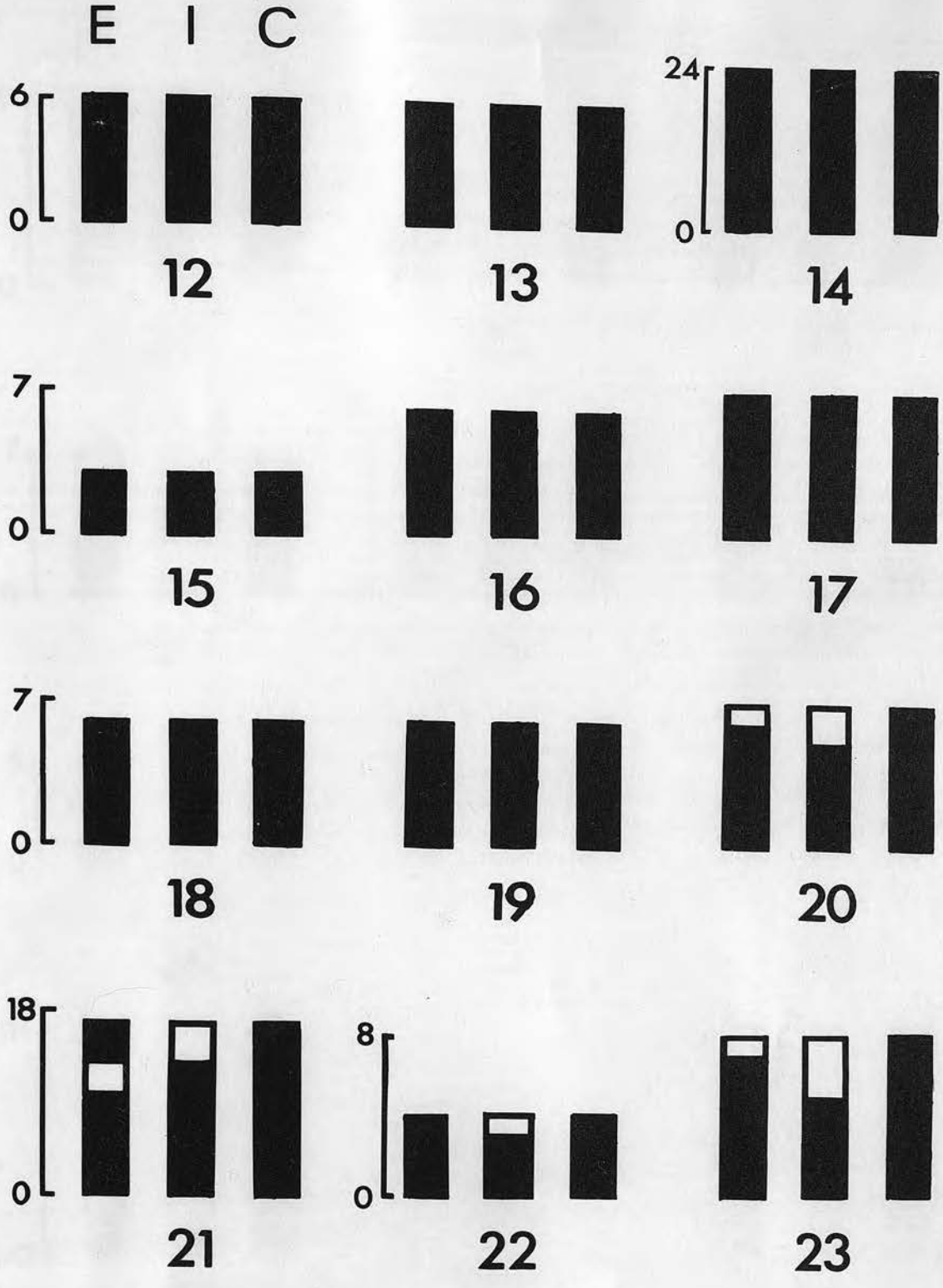
143 Orf Scabs Tested by Three Methods

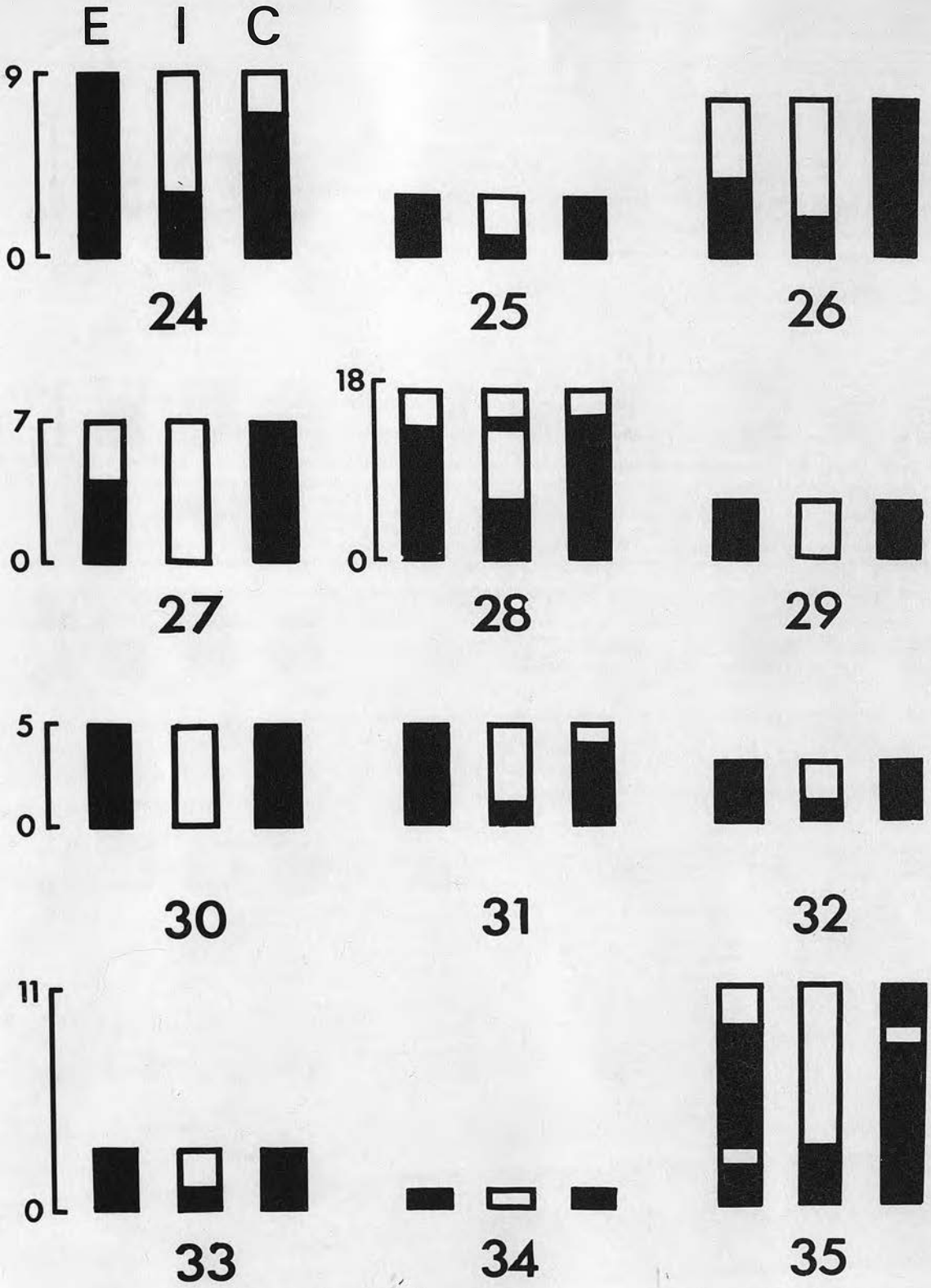


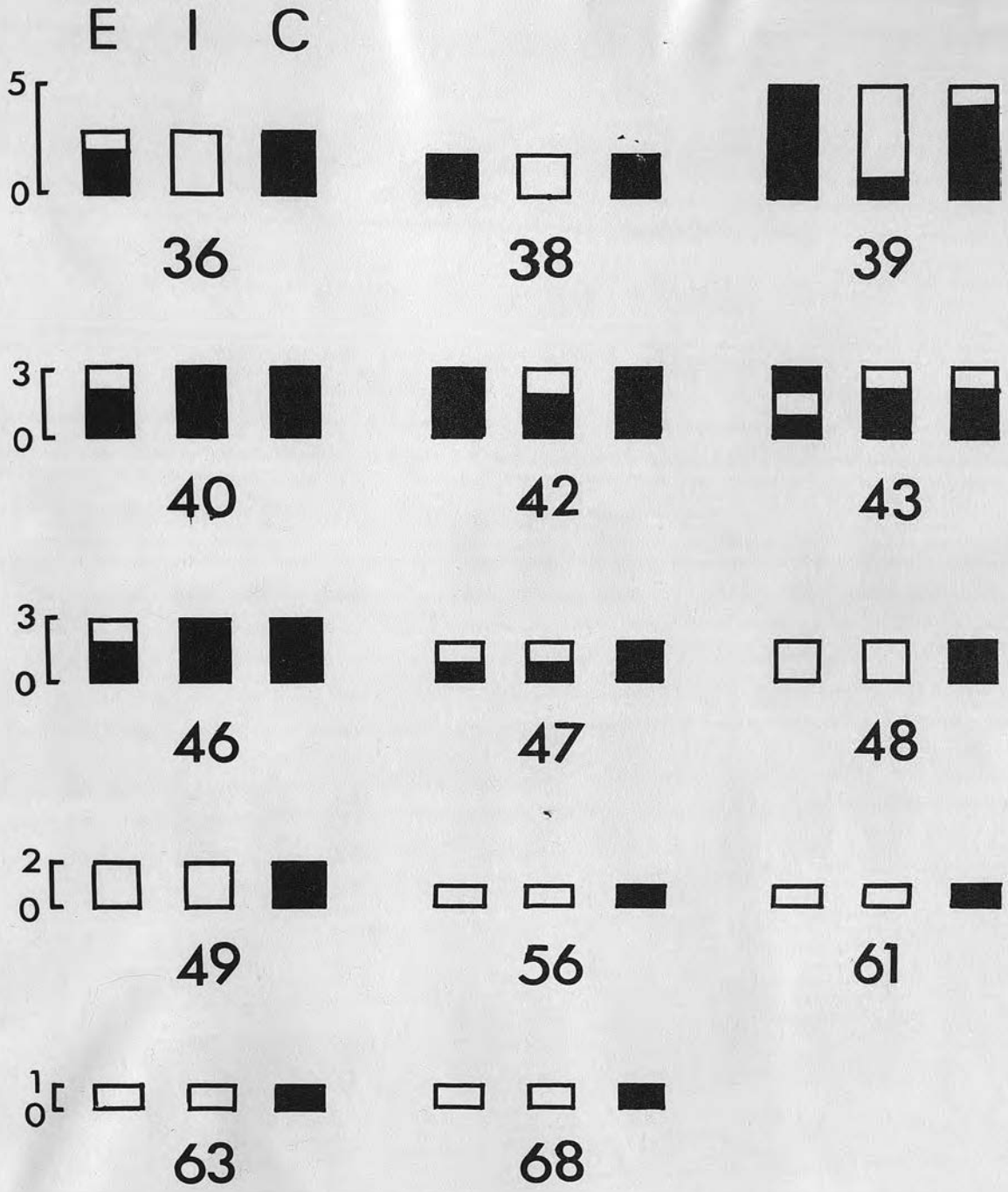
Detection of Virus Particles, Immuno-Diffusion Ag and Complement Fixing Ag. in the Same Scabs

E = Electron Microscopy
I = Immuno Diffusion Ag.
C = Complement Fixing Ag.
■ = Positive □ = Negative



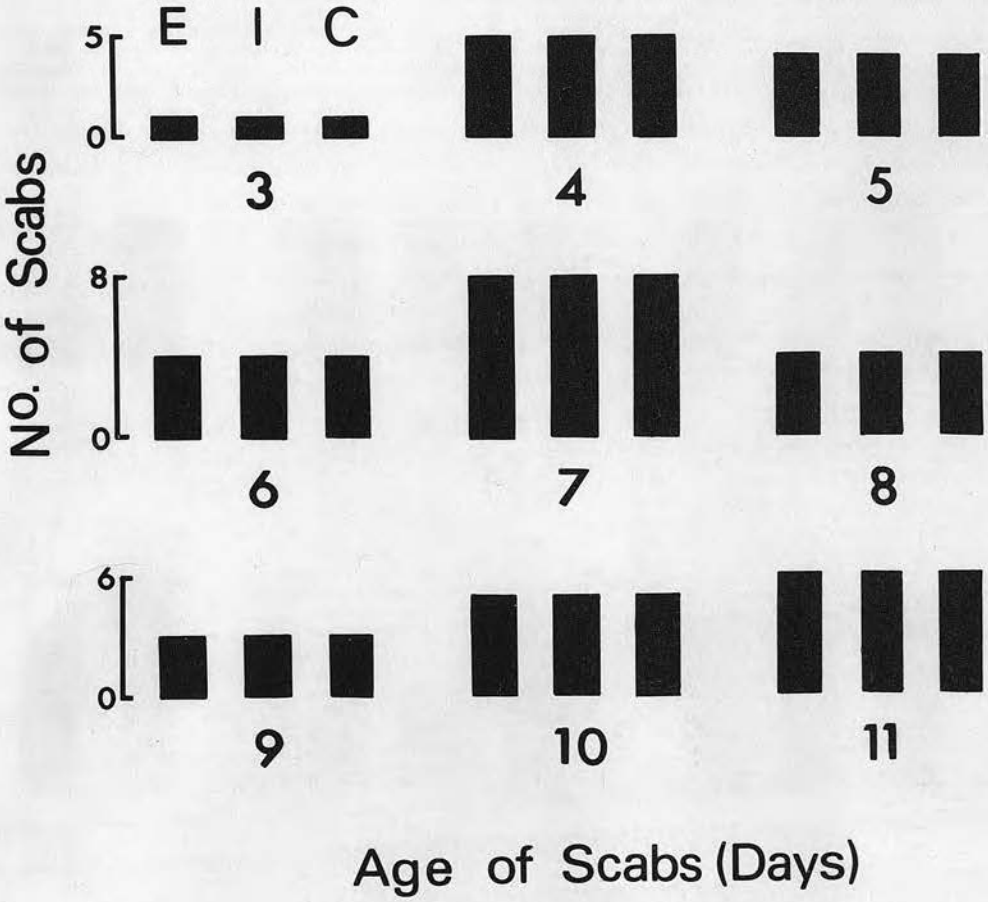

E I C
6
0
12
13
24
0
14
7
0
15
16
17
7
0
18
19
20
18
0
21
8
0
22
23

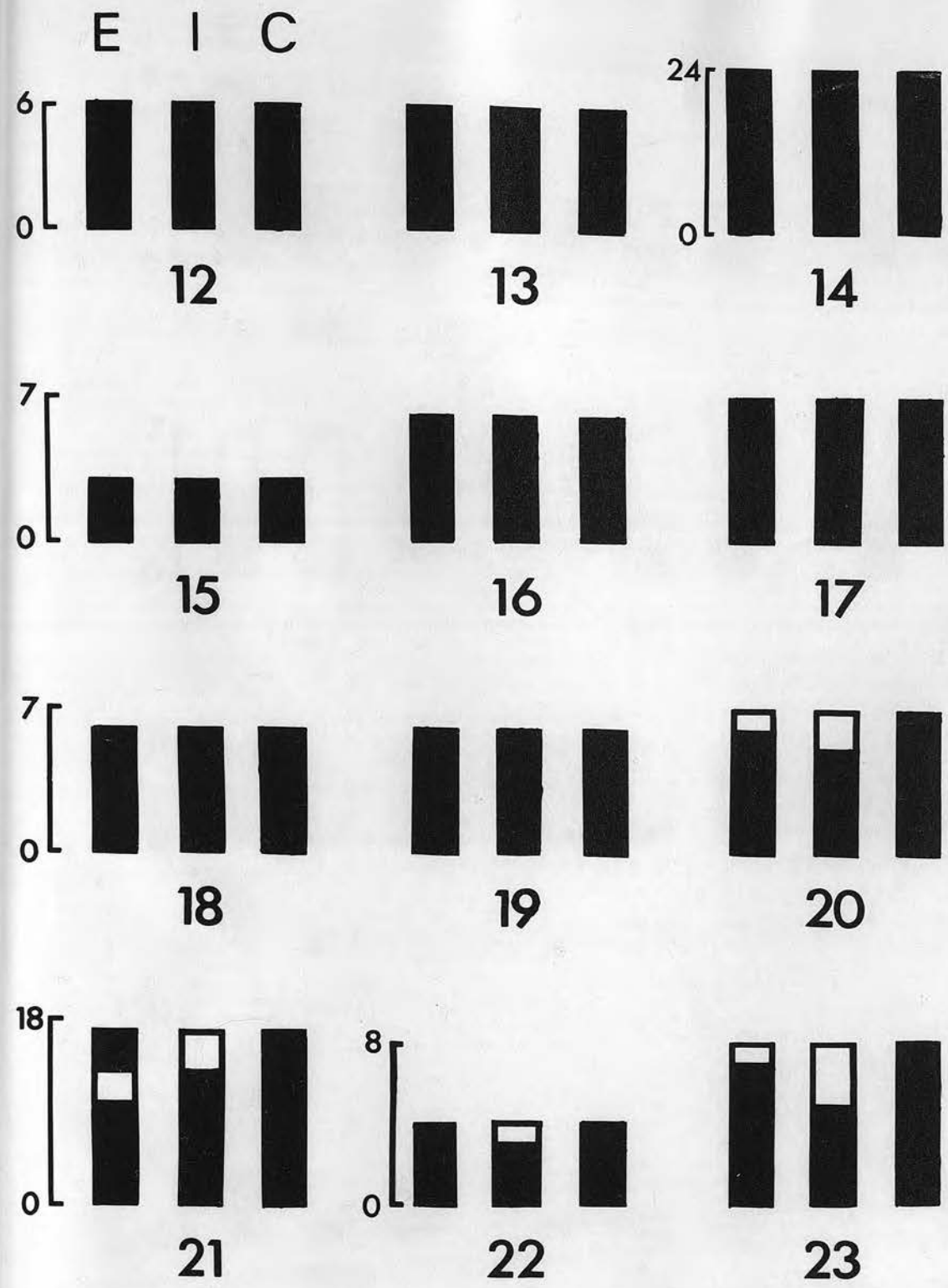

E I C
9
0
24
25
26
7
0
27
18
0
28
29
5
0
30
31
32
11
0
33
34
35

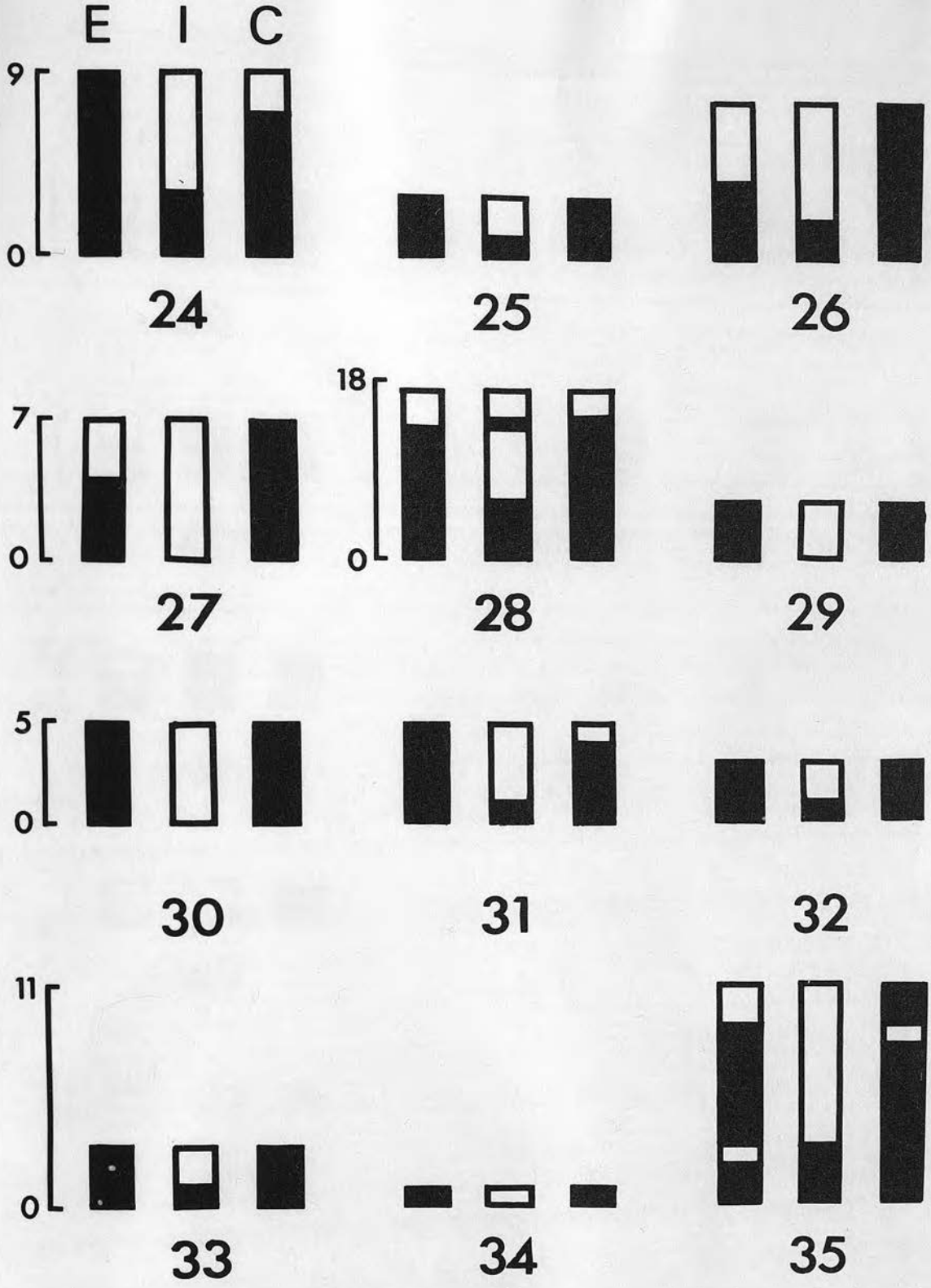

E I C
5
0
36
38
39
3
0
40
42
43
3
0
46
47
48
2
0
49
56
61
1
0
63
68

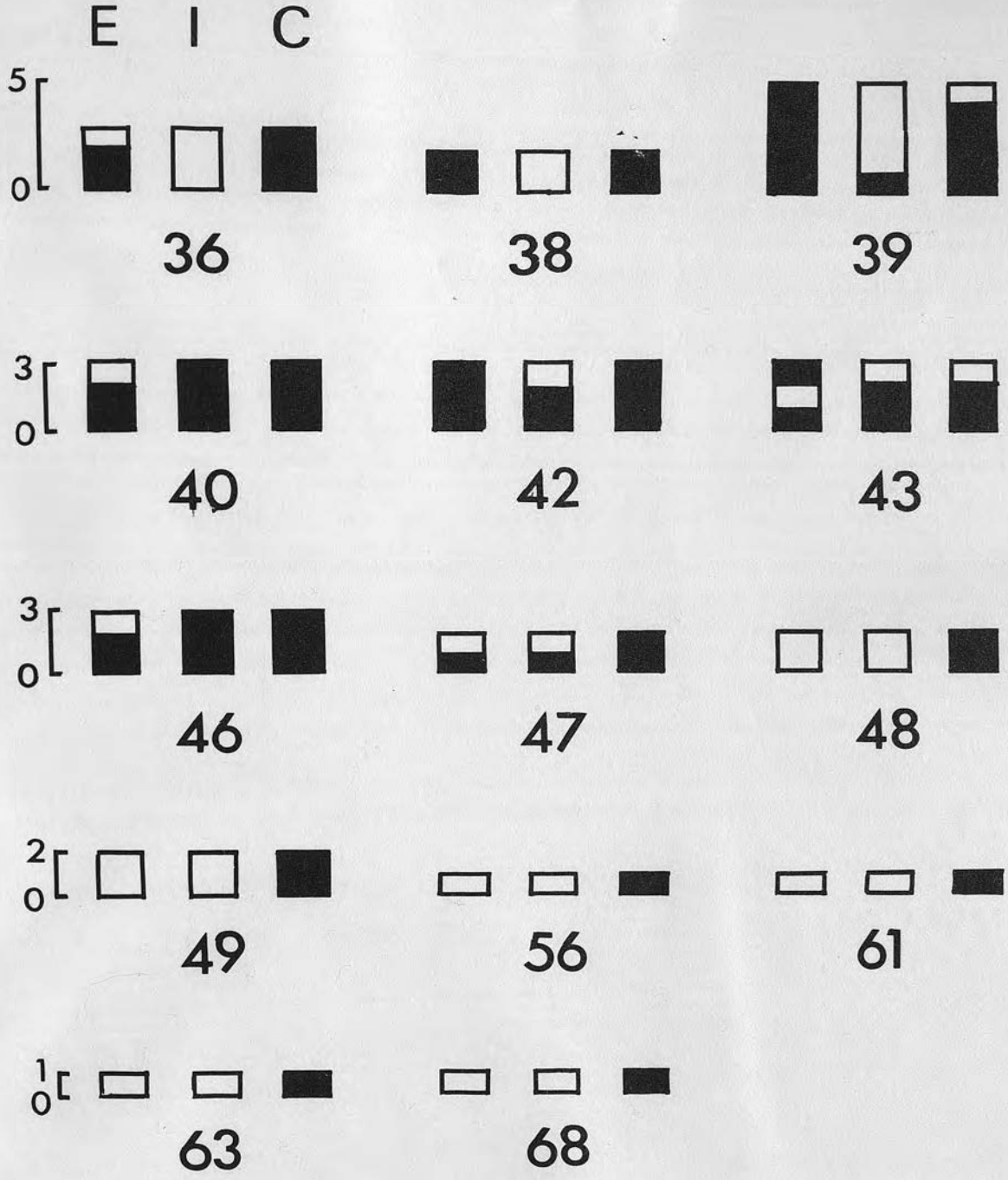
Detection of Virus Particles, Immuno-Diffusion Ag. and Complement Fixing Ag. in the Same Scabs

E = Electron Microscopy
I = Immuno Diffusion Ag.
C = Complement Fixing Ag.
■ = Positive □ = Negative

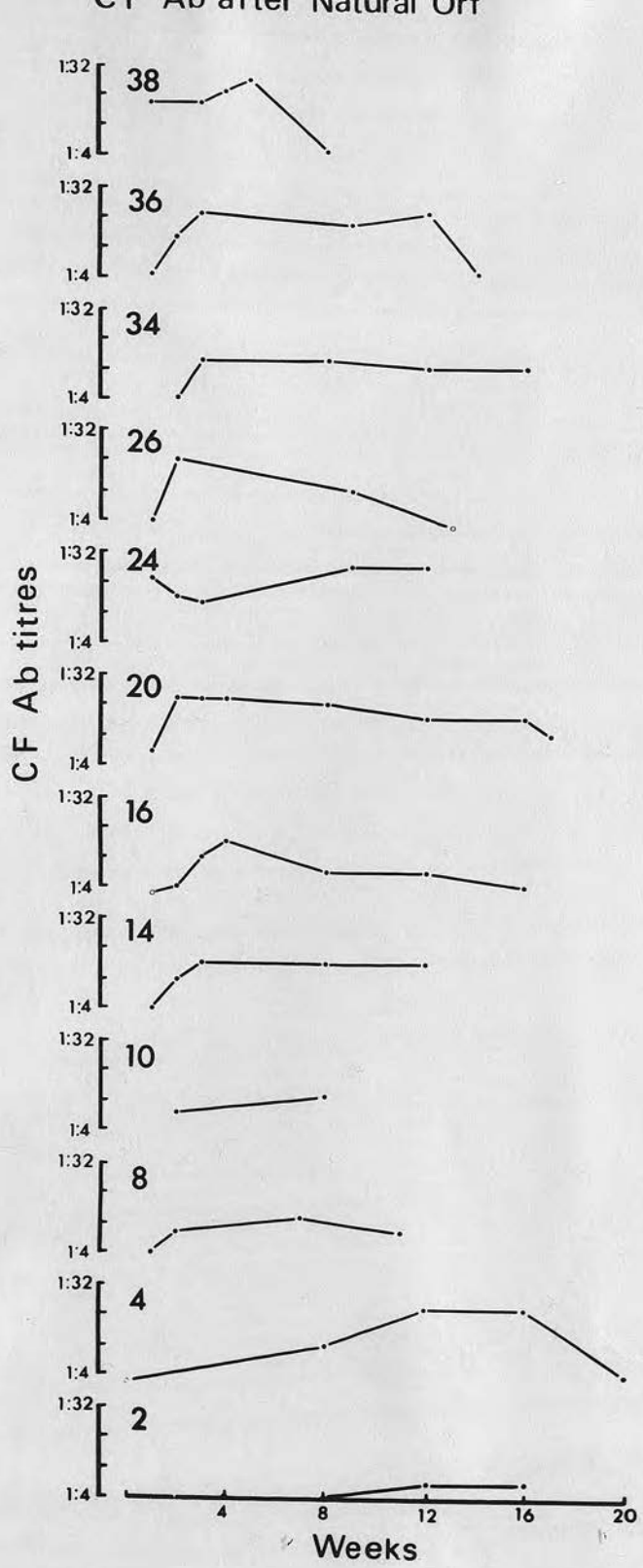



E I C
6
0
12
13
24
0
14
7
0
15
16
17
7
0
18
19
20
18
0
21
8
0
22
23

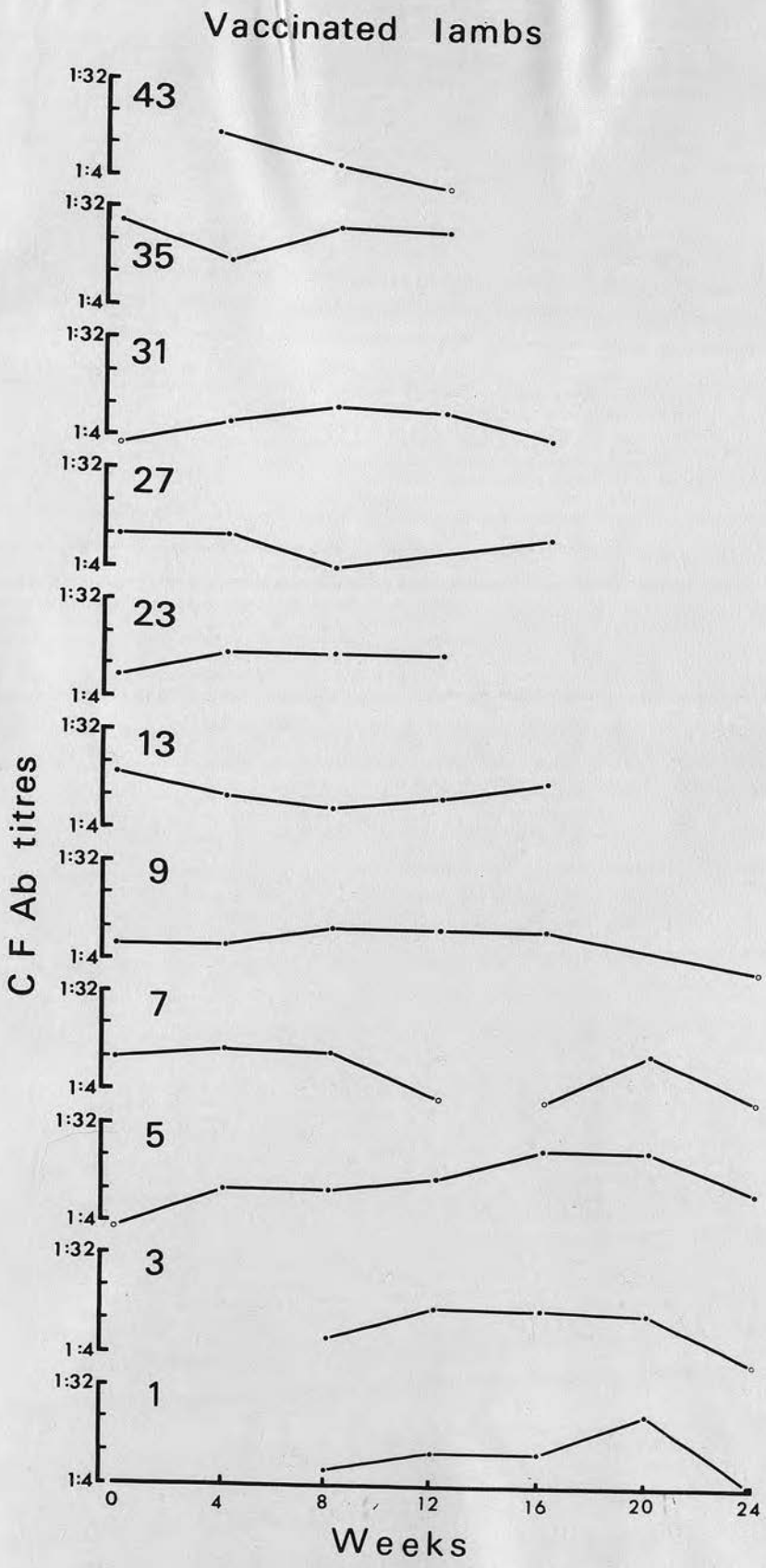

E
I
C
9
0
24
25
26
7
0
27
18
0
28
29
5
0
30
31
32
11
0
33
34
35


E I C
5
0
36
38
39
3
0
40
42
43
3
0
46
47
48
2
0
49
56
61
1
0
63
68

Appendix fig. 21 - Complement-fixing antibody titres in the sera of convalescent lambs.


CF Ab after Natural Orf
CF Ab titres
1:32
1:4
38
36
34
26
24
20
16
14
10
8
4
2
4
8
12
16
20
Weeks

Appendix fig. 22 - Complement-fixing antibody titres in the sera of vaccinated home-bred lambs.


Vaccinated lambs
1:32
1:4
43
35
31
27
23
13
9
7
5
3
1
C F Ab titres
0
4
8
12
16
20
24
Weeks