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STUDIES ON INFECTIOUS PUSTULAR VULVOVAGINITIS VIRUS

WITH PARTICULAR REFERENCE

TO THE GENITAL DISEASE IN BULLS

A Thesis

Presented to the School of Graduate Studies

of

The University of Toronto

by

MICHAEL JUSTIN STUDDERT

In partial fulfilment of the requirements

for the degree of

Master of Veterinary Science

1961
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Michael Justin Studdert, son of Justin Michael and Kathylene Margret Studdert, was born in Maitland, New South Wales, Australia, on 10th January, 1935. He received his primary education at the Marist Brothers School, Maitland and his secondary education at St. Gregory's Agricultural College, Campbelltown, N.S.W., where he gained his leaving certificate in 1952. He then studied at the University of Sydney and graduated with the degree, Bachelor of Veterinary Science in 1957. For a period of eighteen months he held the appointment of Teaching Fellow in the large animal clinic of the University of Sydney situated in Camden, N.S.W. In September, 1959, he came to Canada and commenced studies towards the degree of Master of Veterinary Science at the Ontario Veterinary College.
ACKNOWLEDGEMENTS

The author wishes to express sincere thanks to Dr. C.A.V. Barker who encouraged the pursuit of this work and was of invaluable assistance throughout its course. The sincere interest and invaluable guidance given by Dr. Milton Savan are gratefully acknowledged. The interest and criticism of Drs. G. Wills, H. Rowsell, Parvathi Basrur and K. V. Jubb were greatly appreciated. He wishes to express sincere thanks to Mrs. Florence Black for her excellent technical assistance and to Mrs. J. Boyle for her willing help. To Mr. J. Lambert and his associates who cared for the animals, thanks are due. The author wishes to express his appreciation to the Extension Photographic Service and to Dr. P. C. Kennedy for the preparation of the illustrative plates. The patience and care of Miss Elaine Wright for typing this thesis are acknowledged with thanks.
INTRODUCTION

Viruses have been implicated as a cause of bovine genital disease by workers in several countries. Recently attention has been focused on one such disease which has been defined as an acute, specific, viral infection of cattle affecting the vulva and vagina of the female and the penis and prepuce of the male and characterised first by pustules, later by ulceration of the affected mucous membrane.

The lack of precise knowledge concerning many facets of this disease is exemplified by the variety of synonyms which have been used in the past: vesicular venereal disease, vesicular vaginitis, coital exanthema, coital vesicular exanthema, genital cowpox. In Europe the term Bläschenausschlag is used. The inadequacy of this nomenclature may be illustrated by three pertinent facts: (a) Witte (1933) demonstrated that at no stage was the lesion vesicular and this author suggested the term exanthema pustulosum coitale. (b) North American experience, contrary to that of European workers has shown that most outbreaks of the disease are not related to coitus. (c) Exanthema strictly applies to eruptions of the skin (Gould, 1956). Although the term infectious pustular vulvovaginitis (IPV) introduced by Kendrick et al. (1958) circumvents several of these misnomers, acceptance of this term poses the problem of designating the disease in the bull.

Other viruses have been found associated with bovine genital diseases but the syndromes described do not resemble IPV.
These viruses include: a virus isolated from a catarrhal vaginitis in the USA (Kendrick et al., 1956). In South Africa McIntosh et al. (1952) have isolated a virus from outbreaks of vaginitis (the relationship of this virus to infectious bovine infertility (epi-wag) is uncertain). In England (Millar, 1955) suspected a virus as a cause of infertility. McClure (1956, 1957) in New Zealand also proposed that a virus was associated with vaginitis and lowered fertility in some herds.

In Canada an outbreak of an acute vaginitis in a herd of cattle in southern Ontario was observed in 1955; vaginal exudate collected from affected animals reproduced a similar disease when inoculated into the vagina of heifers (Barker, 1958). From the same vaginal exudate a cytopathogenic agent was isolated in foetal bovine skin and foetal bovine kidney tissue cultures and tissue culture fluid containing the agent reproduced the disease when inoculated into heifers (Grieg et al., 1958).

There are few detailed reports of infection of bulls with IPV virus and the present study has been primarily concerned with this aspect. Consideration has been given to the infectivity of the virus for sites other than the genitalia in cattle, infection of other species of domestic animal, and some of the fundamental properties of the infectious agent. Recently it has been demonstrated that IPV virus is indistinguishable from the agent which causes infectious bovine rhinotracheitis (IBR) (Gillespie et al., 1959; McKercher et al., 1959). Although IPV
is not considered to be an important disease by most author-
ities, the economic importance of IBR is well established. For
this reason it becomes necessary to have considered in full the
disease producing potential of the aetiological agent. More
importantly the inter-relationship of these conditions from an
epizootological viewpoint is basic to an understanding of both.
LITERATURE REVIEW

Occurrence:

The earliest reference to the disease, Bläschenausschlag, appears to be that of Brüchner, 1841 in Germany (cited by Witte, 1933) although it is likely that the disease was recognised before this time. By 1887 the disease was among those notifiable by law in Germany (Kampman, 1887). The annual incidence between the years 1886 and 1928 indicated that a maximum of 9,500 cases were reported in 1896 although the writer considered that these statistics omitted many unreported cases (Witte, 1933). The disease has been reported in Austria, Hungary and Scandinavia (Hutyra et al., 1938), in Yugoslavia (Debelic, 1936) and recently the infectious agent was isolated in tissue culture in Belgium (Bouters et al., 1960).

In the United States outbreaks have been reported by Steddom (1895); Parker (1900); Jones and Little (1927); Windrath (1935); Gibbons (1944); Roberts (1949); Loromore (1954); Kendrick et al. (1958); Wagner et al. (1959). A single outbreak has been reported in Canada (Barker, 1958).

Aetiology:

The infectious nature of the disease was recognised for many years before Reisinger and Reimann (1928) reproduced the disease with a bacteria-free filtrate and thus postulated that the disease was caused by a virus. Zwick and Gminder (1913) suspected a virus, but were unable to substantiate this experimentally.
From the vaginal discharge of an affected heifer a cytopathogenic agent was isolated in foetal bovine skin and foetal bovine kidney tissue cultures (Grieg et al., 1958). These workers reproduced the disease with 6th passage tissue culture fluid. The virus was also isolated by Kendrick et al. (1958) from an outbreak in the U.S.A. The Canadian isolate and three U.S.A. isolates of IPV virus have been shown, by reciprocal neutralization tests in tissue culture and cross-protection tests in animals, to be antigenically similar (Wagner & Gillespie, 1959).

Transmission:

The consensus of European opinion supports the view that coitus is the prime mode of transmission (Witte, 1933; Goetze, 1949; Wirth and Dieninhofer, 1950; Benesch, 1952). The disease has been observed in 16 out of 20 cows and heifers 5 to 9 days after artificial insemination with 24 to 48 hours-old chilled semen collected from a recently infected bull (Conradi et al., 1960). In European herds it is usual for only the animals bred to develop the disease (Witte, 1933), although Fenner (1890) observed 927 infected cows of which 749 had not been bred. An increased incidence of the disease was noted during the breeding season (Röder, 1913).

In North America outbreaks have been characterised by the rapid involvement of 75 to 100% of cows in the herd, unassociated with coitus (Gibbons, 1944; Roberts, 1954; Lormore, 1954;

A dog that licked the external genitalia of cows was suspected as transmitting the disease (Esser and Schütz, 1898; Gibbons, 1944). The latter author also suspected that grooming procedures may result in transmission. An extensive outbreak has been observed following routine caudal fold tuberculin testing (Jones and Little, 1927). Transmission has been reported to occur in animals at pasture by rubbing together of their hind quarters (Esser and Schütz, 1898). This view is supported by Dinter (cited by Hutyra et al., 1938) who also believed tail swishing to be important in dissemination of the infectious agent. The virus may be carried in straw and urine (Armbuster, 1886; Schliche (cited by Hutyra et al., 1938). Zwick and Gminder (1913) failed to induce the disease in healthy animals by placing them in contact with cows exhibiting the disease although Gibbons (1944) observed transmission under these circumstances. Faulty hygiene resulted in the infection of 22 out of 36 bulls in an artificial insemination centre when an attendant inadvertently washed the prepuces of bulls with the same sponge (Conradi et al., 1960).

In summary it may be stated that observations in north America support the view that infection can spread rapidly and randomly among female cattle standing in adjacent stalls, without the intervention of coitus while in Europe the disease is primarily venereal in origin.

Experimental transmission by inoculation of infective material into the vulva and vagina has been demonstrated by many workers
(Zwick and Gminder, 1913; Reisinger and Reiman, 1928; Witte, 1933; Gibbons, 1944; Barker, 1958; Grieg et al., 1958; Kendrick et al., 1958; McKercher et al., 1959). Conradi et al. (1960) has also shown that this is possible in bulls.

**Epizootiology:**

Most authorities consider that the female sheds virus only when lesions are present (Zwick and Gminder, 1913; Witte, 1933). This view is supported by Kendrick et al. (1958) who demonstrated that virus disappeared between the 8th and 12th day after infection. These authors also demonstrated that the maximum virus titre of vaginal exudate was attained on the 2nd day postinfection. Conradi et al. (1960) stated that in most cases the virus is not shed by bulls after healing of the lesions but occasionally bulls were suspected of harbouring the virus for much longer periods.

Recurrences of the disease each year in the same locality were observed frequently by Storch (1910) who considered that the agent must therefore be a facultative parasite that remains either in the animal or in the surroundings and becomes virulent under certain conditions. Outbreaks observed by Gibbons (1944) and Roberts (1949) were in the same herd but separated by an interval of 4 years. No cattle had been introduced for 3 months prior to each outbreak. (In both these outbreaks and in the one observed by Lormore (1954) the herd sire was examined and found to be free of the disease).

**Clinical Signs:**

In field outbreaks of the disease the incubation period averages
3 to 6 days but may be as short as 24 to 48 hours (Hutyra et al., 1938) or as long as 10 to 11 days (Friedberger and Fröhner, 1908; Diernhofer, 1953). In cases with a prolonged incubation it has been noted that the appearance of lesions frequently coincided with oestrus (Conradi et al., 1960). Animals of all ages are susceptible (Fenner, 1890; Reisinger and Reiman, 1928).

The clinical signs in experimentally infected heifers have been described by Kendrick et al. (1958) as follows:

Immediately after inoculation hyperaemia and a few petechial haemorrhages were noted on the vestibular mucosa. At 24 hours the mucosa was reddened and many fine, white areas approximately 0.5 mm in diameter were observed. On the 2nd day small white pustules approximately 2 mm in diameter appeared and the entire mucosa was intensely reddened. The pustules were soft and moist with a raised edge and a depressed centre. In some areas these pustules were covered by a loosely adherent exudate. The pustules tended to form over the lymphatic follicles and consequently were aligned in rows. A slight thick yellow exudate from the ventral commissure of the vulva and slight to moderate vulvar swelling was observed. Some animals exhibited pain when the vulva was examined. Others held the tail up constantly and strained for several minutes after urination.

Healing began on the 6th day and was completed between the 8th and 11th days. No scars remained after healing but in some animals nodules characteristic of so-called granular venereal disease appeared within a week.

Exudate first appeared at 24 hrs. and varied in amount from 7 to 20 ml. The cervix and vagina were reddened and white plaques of fibrinous exudate were noted on the vaginal mucosa. In some areas the vaginal surface was denuded of epithelium. In two animals the vaginal exudate persisted for a month and was considered to be the result of secondary bacterial invasion. Adhesions between the vaginal wall were observed in one animal. Increased temperatures (above 103°F) occurred on the 2nd day and persisted with fluctuations between 104 and 106.8°F for 3 to 7 days. Slight sluggishness and decreased appetite were noted. Many animals exhibited a slight nasal discharge and two animals had a purulent ocular discharge. Haemograms indicated a neutropenia occurred between the 2nd and 6th day in two animals.

With minor exceptions these observations are in agreement with those of others. Conradi et al. (1960) reported that typical lesions
did not develop anterior to the hymen (a view supported by Reidmuller, 1939; Gotze, 1949; Benesch, 1952; Küst-Schaetz, 1953) even though infective material was deeply instilled into the vagina; lesions developed on the skin of the vulvar labia in one animal. Other authors consider that the virus also attacks the vaginal epithelium (Zwick and Gminier, 1913; Reisinger and Reiman, 1928; Williams, 1943; Wirth and Dienhoffer, 1950). Kendrick et al. (1958) were able to demonstrate necrosis with inclusion body formation in the vaginal epithelium.

When lesions are closely settled on the vestibular mucosa they may coalesce resulting in the formation of a diphtheritic membrane. The necrotic tissue sloughs leaving irregular shallow erosions or somewhat deeper ulcers with a haemorrhagic centre and a raised border. Healing is rapid and white cicatrices, which in time become reddish yellow, mark the site of ulcer formation (Hutyra et al., 1938; Conradi et al., 1960).

Impaired milk production has been recorded (Law, 1912).

Clinical signs observed in animals infected by artificial insemination were less severe than in cows infected by natural service and it was suggested that decreased transmission of bacteria occasioned by the addition of antibiotics may have been responsible for the observed differences (Conradi et al., 1960). Intense pruritus resulting in severe damage to the vulva with secondary bacterial infection and septicaemia may occur (Hutyra et al., 1938). Uterine prolapse as the result of straining has been attributed to the disease (Späth, 1898). These severe secondary complications are
exceptional and most cases resolve in two weeks; indeed Mussil (1949) and Udall (1954) believe many cases of the disease may pass unrecognized.

Lesions similar to those seen in the female occur on the penis and prepuce. Both parietal and visceral layers of the prepuce are involved. Oedema of the glans penis, sometimes extending to involve the prepuce and scrotum, has been observed and may result in the development of phimosis or paraphimosis (Hutyra et al., 1938). These authors have also recorded the occurrence of strangury, prolonged erection of the penis, a yellow mucoid or purulent discharge from the urethra and painful enlargement of the inguinal and perianal lymph nodes. Deeply penetrating abscesses producing distortion of the penis have been recorded (Kampmann, 1887). Repair of the ulcers may result in adhesions, stricture or other disability of the penis (Hutyra et al., 1938). A seropurulent preputial discharge, which often contained blood, particularly after erection, as a result of haemorrhage from the ulcers was noted by Conradi et al. (1960). Reluctance or complete refusal to serve on the part of the bull during the acute phase has been observed by most authors.

An increased incidence of impotence in bulls in Belgium has been attributed to the occurrence of ulcers on the prepuce, frequently associated with acute balanoposthitis and a nasal discharge (Bouters et al., 1960). These authors induced typical lesions of IPV in heifers with both filtered and unfiltered preputial secretions collected from affected bulls. Serum collected from these heifers 2 to 3 weeks after infection neutralized a New York isolate of IPV.
virus. In 2 of 6 experimentally infected bulls, acute oedematous inflammation of the scrotum and testes occurred 50 and 300 days respectively after recovery from the balanoposthitis. Degeneration of the germinal epithelium was demonstrated histopathologically but the pathogenesis of these findings was uncertain (Bouters et al., 1960).

The role of the virus as a cause of sterility and infertility has been a subject of conjecture since the earliest reports. Development of secondary cervicitis and endometritis has been observed as sequelae in a small number of cows that failed to conceive (Benesch, 1952). The position has been somewhat clarified by Conradi et al. (1960) who induced the disease in 10 heifers by natural service to an infected bull and in 5 heifers by artificial inoculation; all 15 animals were acutely ill but 73% conceived and successfully completed pregnancy. Other European workers support this observation (Wirth and Dienhoffer, 1950). Neither repeat breeding nor abortion was observed in the outbreaks attended by Lormore (1954) and Roberts (1956).

There is general agreement that in most cases lesions in both sexes show an uninterrupted tendency to heal. The subsequent development of granular lesions - hyperplastic lymphoid follicles (Nieberle - Cohrs, 1952) - at the site of the healed ulcers has been noted by a number of workers (Witte, 1933; Gotze, 1949; Dienhoffer, 1950; Kendrick et al., 1958; Conradi et al., 1960). It is generally held that so-called granular venereal disease is the response of the lymphoid follicles of the vulva and vagina.
and the penis and prepuce to non-specific irritation, either infectious
(of which IPV is an example) or mechanical or chemical (Wirth and

Pathogenesis and Histopathology:

The basic and characteristic lesion produced by IPV virus is a
pustule. Examination of lesions as soon as they appeared and also
subsequently, demonstrated that at no stage were the lesions vesicular
(Witte, 1933). This author observed degeneration of the epithelial cells
over the pustule and diffuse round cell infiltration; the discrete
appearance of normal and hyperplastic lymphoid follicles was lacking.

The pathogenesis was considered by Kendrick et al. (1958) to be
the result of a progressive necrotising effect of the virus for
epithelial cells with accumulation of neutrophils in these necrotic areas
and infiltration of lymphocytes into the nearby connective tissue. These
authors described the histopathology as follows:

Characteristic inclusion bodies were observed in degenerating
epithelial cells at the periphery of the lesion. The submucosal
lymphoid follicles were the site of the necrotic areas and also of
the haemorrhages observed clinically. Diffuse lymphocytic accumulation
around the lymphoid follicles masked the sharp boundaries that
normally characterise these structures. Lymphatics were enlarged and
contained neutrophils, lymphocytes and macrophages and some degenera-
ting cells. Inclusion bodies were not demonstrable after the 2nd and
3rd day postinfection.

When infective material was instilled into the uterus, endometritis
and cervicitis resulted; the endometritis and cervicitis were character-
ised by loss of epithelium, inflammatory cell infiltration, enlargement
of the lymphatics and the absence of inclusion bodies. Fallopian
tubes were normal.

Two types of inclusion body were regularly observed in the vulvar
epithelial cells during the first three days of the disease and a third
less distinct type of nuclear change was observed infrequently. These
inclusions were described as follows:

(a) Nuclei at the periphery of the lesion contained an
eosinophilic mass. Clumps of chromatin were present in the space between the inclusion and the nuclear membrane.

(b) An homogenous light-blue staining mass completely filled some nuclei.

(c) The third and infrequently observed inclusion was a round, shrunken, dark staining body found in nuclei of the more superficial epithelia and also at the periphery of the pustule.

Immunity:

Because recurrence of the disease in the same animal had been observed, it was commonly held that specific immunity did not develop (Zwick and Gminder, 1913; Kalteyer and Kalb, 1901; Hamm, 1890; Witte, 1933). The latter two authors and Storch (1910) however, observed certain cows that were resistant to challenge when bred to infected bulls. Local immunity was postulated by Dedie (1955).

Serum antibodies, capable of neutralizing the virus in tissue culture have been demonstrated (Kendrick et al., 1958; McKercher et al., 1959; Bouters et al., 1960). Kendrick's group recorded a maximum serum titre of 1:12 when sera were tested against 100 to 500 tissue culture 50% infective doses (TCID$_{50}$); McKercher et al. (1959) obtained a titre of 1:70 when sera were tested against 100 TCID$_{50}$. Kendrick's group demonstrated that antibodies persisted for at least 30 days and during this time animals were resistant to challenge.

Antibodies to IBR virus have been shown to persist for at least 12 months. Exaggerated peaks recorded during this test period suggested an anamnestic response but no evidence of virus exposure at the time of the peak could be found. Antibodies were demonstrable as early as 12 days after exposure (McKercher et al., 1958).

Diagnosis:

Most workers familiar with the disease consider the lesions,
particularly in the early stages, to be pathognomonic (Hutyra et al., 1938; Conradi et al., 1960). The isolation of the virus and/or the demonstration of a rise in virus neutralizing antibodies would confirm the diagnosis.

Treatment:

The diversity of therapeutic agents recommended suggests that none, in most cases, is of any real value in altering the course of the disease. Symptomatic treatment with antiseptic agents to allay secondary bacterial invasion and the use of lubricants to prevent the possible development of adhesions have been recommended. Breeding should be suspended for the duration of the disease (Hutyra et al., 1938; Roberts, 1957; Conradi et al., 1960).

The Infectivity of IPV Virus for Other Species:

Knowledge concerning the infectivity of IPV virus for other species of animals is rudimentary and uncertain. It has been reported that the disease may be transmitted to horses, sheep and goats but that rabbits, swine and dogs are refractory (Zwick and Gminder, 1913). These authors succeeded in infecting one of four goats and one of two sheep. After 48 hours only a few pustules were apparent and these were similar to those seen in cattle. The hairless areas around the vulva, ventral surface of the tail and skin of the udder developed lesions and subsequently were covered with encrustations. Transmission to sheep and goats, with skin lesions predominant, has also been reported by Witte (1933); this author indicated that rabbits and guinea pigs were refractory.
Witte also cited references to French workers (Trasbot, Pench, Nocard, Leclanche) who believed the agent to be a pox virus and to another French author (Meyer, 1912) who reported successful transmission to a rabbit. Immunological studies have shown that IPV virus is distinct from the pox group (Zwick and Gminder, 1913).

Early literature suggested that infection of man may result from exposure to infective material (Röll, 1881; Pütz, 1882 (cited by Witte); Friedberger and Frohner, 1929). Reputedly, lesions which heal rapidly develop on the hands and face. Frohner and Zwick (1925) reported a case of a pox-like exanthema in which swelling of the arm and regional lymph nodes and a febrile reaction occurred. Transmission to other family members was reported (Hutyra and Marek, 1922). Witte (1933) concludes that there are no valid grounds for believing that man is susceptible and that reports of such infection should be questioned.

As far as is known the disease under natural conditions spreads only among animals of the same species and not from horses to cattle or conversely; however, the great similarity of the syndromes produced in different species suggests identity or at least a close relationship between the respective aetiological agents (Hutyra et al., 1938).

Transient leucopenia in a mare 48 hours after vaginal instillation and in a stallion after preputial inoculation of tissue-cultured virus has been reported, however no lesions occurred at the inoculation sites (Bouters et al., 1960).

Goats are reported to respond with pyrexia, to harbour the virus
and to develop antibodies when infected experimentally with IBR virus; horses, sheep and swine were refractory to experimental IBR inoculation, although a slight antibody response was elicited in each species (McKercher et al., 1958).

Relationship of IPV Infection to Other Diseases:

The relationship between IPV infection and so-called granular venereal disease has already been noted.

Infectious bovine rhinotracheitis (IBR) is an acute, contagious, febrile, viral infection of cattle characterised by intense inflammation of the upper respiratory passages and trachea and accompanied by dyspnoea, depression, nasal discharge and loss of condition; secondary bacterial complications may occasionally result in fatal pneumonia (McKercher, 1959). The disease was apparently first recognised in feedlots in Colorado in 1950 (Miller, 1955). According to figures released by the United States Department of Agriculture in 1957 the disease is known to occur in 16 states; IBR has recently been reported in Canada (Studdert et al., 1961) and a survey to detect the presence of IBR antibodies in cattle in southern Ontario has shown that 8.13% of serum samples, representing 18.9% of herds tested, were positive for IBR antibodies (Grieg, 1961). On the basis of behaviour in tissue culture, infectivity for calves, histopathology, cross-immunity and reciprocal serological tests, it has been shown that IBR (York isolate), all New York isolates of IPV and the one Canadian isolate of IPV are indistinguishable with the possible exception that IPV given intranasally
induces a more moderate clinical syndrome than IBR given by this route, (Gillespie et al., 1959; McKercher et al., 1959). The antibody response elicited by each virus was more marked following intranasal administration however, the febrile response to each was somewhat greater when given via the "recognised" route. Chance external transmission from one site to another may happen but haematogenous spread is believed not to occur (McKercher et al., 1959). The latter view is supported by Kendrick et al. (1957) who failed to isolate IPV virus from the blood stream during the febrile period of infectious pustular vulvovaginitis.

IPV virus has been shown to produce typical pustular lesions when inoculated onto the conjunctiva (Zwick and Gminder, 1913). These workers failed to produce lesions when infective material was inoculated intravenously, orally or cutaneously (onto the skin of the udder).

A catarrhal conjunctivitis has been reported in field outbreaks of IBR and the agent has been isolated from the lachrymal secretions, (McKercher et al., 1958; Abinanti and Plumer, 1961). A mild febrile response accompanied the conjunctivitis and a slight nasal discharge was also noted. In the IBR outbreak observed in Ontario, the earliest sign observed was conjunctivitis and this was exhibited by many animals that did not subsequently show respiratory tract involvement (Studdert et al., 1961).

A fatal disease has been produced by inoculation of IBR virus into newborn calves. The inoculum was given intravenously, orally and by contact. Five out of ten calves became moribund and three
of these died. At post mortem lesions were found on the mucosal surfaces of the mouth, oesophagus and fore stomachs and in the liver, spleen, kidneys and lymph nodes (Baker et al., 1960).

Inoculation of IBR-IPV virus into the teat cystern resulted in mastitis (Baker et al., 1960).

Characteristics of IBR-IPV Virus:

Reisinger and Reiman (1928) used a Reichl porcelain and Chamberlin L3 filter to obtain bacteria-free filtrate of the Blaschenausschlag virus; they transmitted the disease with both filtrates.

Ultracentrifugation studies have indicated that the diameter of IBR virus is smaller than 175 to 210 m\(\mu\) but equal to or larger than 148 to 151 m\(\mu\); by electron microscopy the particles were found to be spherical and the size was estimated to be 136 ± 10.8 m\(\mu\) (Tousimis et al., 1958).

The virus remained viable after 229 days storage in an icebox (Reisinger and Reiman, 1928). It was destroyed after 48 hours exposure to sunlight, 70°C heat, 1:1000 sublimat, 5% phenol and 10% lysol (Bihari, 1909).

The titre of IBR remained constant during 9 months storage at -60°C; at 4°C a loss of one logarithm occurred after 30 to 40 days; at 22°C complete inactivation occurred after 50 days; 10 days at 37°C completely destroyed the agent; at 56°C inactivation occurred after 21 minutes and a loss of one logarithm occurred after lyophilization (Griffin et al., 1958). These workers have also reported that IBR virus was inactivated within 24 hours by acetone
and ethyl alcohol; formalin also inactivated the agent but at a much slower rate. The virus survived at pH 6 to 9 but was quickly inactivated below pH 6.

The cytopathic (CP) changes induced by IPV virus in foetal bovine kidney (FBK) cell cultures have been described by Kendrick et al. (1958). Comparison of the CP changes produced in FBK monolayers by IBR and IPV viruses revealed no significant differences (Gillespie et al., 1958; McKercher et al., 1959). In an excellent and detailed study using phase contrast and light microscopy Stephens (1958) described normal FBK monolayers and the CP changes induced in them by IBR virus. These may be summarised as follows:

Two cell types were observed in normal FBK monolayers
(a) cells which grew in islands or whorls and which were relatively small, closely packed and sometimes layered on top of each other and (b) larger cells which were never layered and tended to be more spindle-shaped. Nuclei in both cell types were about equal size and contained two or more basophilic nucleoli. The appearance of mitotic figures and the presence of nucleoli attested to the rapidity with which cells multiplied. Chromatin appeared somewhat clumped in most nuclei. The cytoplasm demonstrated little that was distinctive except for the presence of numerous strands running parallel to the long axis of the cell. Unstained cytoplasmic vacuoles of various sizes were seen in some cells.

Nine hours after inoculation of IBR virus the first evidence of cytopathogenicity was noted. Small, sometimes multiple, strongly eosinophilic bodies, surrounded by a clear halo were recognisable within some nuclei.

By the 18th hour in addition to the small inclusions some nuclei contained a single large eosinophilic mass, that completely filled the nucleus except for a narrow halo between it and the nuclear membrane. Chromatin and nucleoli had aggregated and marginated to the nuclear membrane. In a few instances no halo was present. Cytoplasmic changes, in which the cells appeared more rounded and tended to contract leaving many holes in the monolayer, were also noted at this time. The nuclear membranes of those cells containing a single large inclusion were irregular. As infection progressed the cell wall became more indistinct and apparent dissolution of the cell membrane resulted in the formation
of multiple nucleated giant cells.

At 24 hours the cytoplasmic contraction was marked and nuclei were reduced to extremely basophilic bodies less than one quarter normal size. No definite inclusions were visible. Cytoplasmic contraction resulted in the appearance of long beaded processes that crossed the areas denuded of cells.

Thirty hours after infection individual cells appeared as small, extremely basophilic spheres with scant cytoplasm and at this stage they were released from the glass. At 72 hours all affected cells had fallen from the glass.

Using various histochemical techniques Stephens demonstrated that the cytoplasmic vacuoles observed in normal monolayers were neither lipid nor glycogen. He tentatively assumed the vacuoles to be aqueous solutions of various salts with small amounts of more complex chemical substances which had been imbibed by pinocytosis (cell drinking) originally shown by Lewis (1931) to be a normal process in cellular metabolism.

Under phase contrast and using Regaud's technique mitochondria, which are destroyed by the acetic acid in Bouin's Fixative, were found to be not visibly altered until the later stages of cellular degeneration, at which time they entirely disappeared.

A cytoplasmic mass observed under phase contrast consistently developed in infected cells in the later stages of degeneration. The mass was usually one quarter nuclear size, stippled in appearance, surrounded by a halo and situated in a position roughly corresponding to the Golgi complex. It was not observed in stained cultures or in control preparations under phase contrast. The significance of this mass was undetermined.
MATERIALS AND METHODS

Part A - Animal Experiments:

Eighteen bulls were experimentally infected with IPV virus. Fourteen of these animals were virgin and less than two years of age; the remainder were over two years of age and had been used for natural service to a variable extent.

During the observation period the animals were isolated in one wing of a barn. Special care was taken to contain infective material within this area: persons entering the area wore rubber boots which were disinfected on leaving; when contamination of the operators protective clothing occurred viz. when inoculating or examining the animals, this clothing was discarded; all contaminated instruments, drapes, rubber gloves and glassware were placed in a stainless steel container and autoclaved at 121°C for 20 minutes.

Rectal temperatures were recorded twice daily. Blood samples were collected from the jugular vein into citrated vials once daily. To obtain the total white blood cell count, two haemocytometer chambers were counted and a minimum of 100 cells were counted in the stained blood smears to obtain the differential count. Temperature and blood count data were recorded for three or four days preinfection and for 14 days postinfection or until they had returned to the preinfection level. Associated clinical findings including heart and respiratory rates, ruminal movements, appetite etc. were also noted and the inguinal lymph nodes were palpated for abnormalities.
Relaxation of the penis was accomplished by the intravenous administration of the ataractic - Acepromaxine maleate* (1 mgm per 10 lbs.). Occasionally the internal pudendal nerve block technique (Larson, 1953; Habel, 1956) was employed using Lidocaine hydrochloride 2%** as the anaesthetic agent. (The prolonged relaxation of the penis with consequent dehydration and distortion of the lesions accompanying this technique was undesirable and for this reason the former method was preferred). After administration of the ataractic manual exteriorization of the penis was usually necessary particularly in young bulls. The penis was grasped in the region of the glans penis with a cotton gauze sponge and complete exposure was obtained by gentle traction. Prior to infection the long hair was clipped from the preputial orifice and the mucous membrane of the penis and prepuce was examined for abnormalities.

Two types of inocula were used (a) vaginal exudate prepared by grinding in a TenBroeck tissue grinder and diluting 1:10 with phosphate buffered saline (PBS; Dulbecco, 1954) and (b) tissue culture fluid with a tissue culture 50% infective dose of $10^{-5.5}$ diluted 1:10 with PBS. The inoculum was contained in a 50 ml glass jar; a cotton gauze sponge held by forceps was soaked in the inoculum and then rubbed onto the mucosal surface of the penis and prepuce.

* Atravet-Ayerst, McKenna & Harrison Ltd., Canada.

** Xylocaine Hydrochloride 2%-Astra Pharmaceuticals (Canada) Ltd., Toronto, Ontario.
During the course of the disease biopsies of lesions were obtained using a pair of curved scissors. The tissues obtained in this manner were fixed for one hour in Bouin's solution and washed for 24 hours in running tap water. Sections, 0.6 microns thick, were cut from paraffin-embedded tissue and stained with haematoxylin and eosin.

Preputial washings were collected during the course of the disease. The washing fluid consisted of 10 ml of PBS to which 400 units of penicillin, 40 mg of streptomycin and 1000 units of Mycostatin* per millilitre had been added. The fluid was introduced into the prepuce by means of an intravenous injection apparatus and a Nielson metal catheter. The preputial orifice was clamped around the catheter with the left hand and the fluid massaged within the prepuce with the right hand. After thorough massaging the bottle was lowered to the floor and the fluid allowed to gravitate back. Approximately 80% of the fluid was recovered. Washings were stored at -60°C and subsequently examined for the presence of virus.

Serum samples were obtained aseptically at seven day intervals; the first sample was obtained on the day of infection. Fifty millilitres of blood were drawn from the jugular vein and allowed to clot overnight; the serum was drawn off, dispensed into vials and stored at -60°C until required for serum-virus neutralization tests.

Though exhaustive studies were not made, some cognizance was

* Mycostatin - E. R. Squibb and Sons, Montreal, P.Q.
taken of the bacterial flora of the prepuce both pre and post-infection. Samples were obtained with cotton-tipped applicator sticks which were then streaked onto blood agar and MacConkey plates. The isolation of pleuropneumonia-like organisms (PPLO) was attempted from four bulls. Two kinds of broth were employed (a) tryptose soy broth* containing 1 in 1,000 thallium acetate and 1% Bacto serum fraction ** (b) PPLO broth also containing 1 in 1,100 thallium acetate and 1% serum fraction. Both sets of tubes were incubated at 37°C for 96 hours before being streaked onto blood agar and PPLO agar plates. After 96 hours at 37°C the plates were examined under a dissecting microscope for the presence of PPLO colonies.

Nasal washings were collected for virus isolation from one animal that developed a nasal discharge; the washing fluid was similar to that used to obtain preputial washings. From the animals infected per conjunctiva, lachrymal discharge was collected with cotton-tipped applicator sticks; the end of the stick bearing the cotton pledget was broken off and allowed to steep in two millilitres of the washing fluid. Detailed examination of the eye was aided by topical anaesthesia using Butyn sulphate**.

Citrated blood samples and serum samples were collected during the first 48 hours of the febrile period; these were stored at -60°C and subsequently inoculated into cell culture tubes in an

* Baltimore Biological Lab., Baltimore, Md., U.S.A.
** Difco Labs. Detroit, Michigan, U.S.A.
*** Abbott Laboratories Ltd., Montreal, P.Q.
attempt to isolate the virus.

An area of skin on the side of the neck of one animal was clipped free of hair and tissue culture fluid containing the virus was scarified into the site.

Eight female cattle were inoculated intravestibularly. The inocula were similar to those used for bulls. Vaginal exudate was collected between the 3rd and 6th days postinfection by means of glass pipettes; the exudates were stored at -60°C.

Three two-month-old goats (two does and one buck) and a two year old ram were inoculated either intravestibularly or intrapreputially and cutaneously. Daily examinations for evidence of lesions were made. Pre and postinoculation serum samples were collected for serum-virus neutralization tests.

Eight bulls and two cows that had recovered two to three months from the original infection were included in a reinfection trial. One animal (#705) in this series was suspected of having orchitis; a unilateral castration was performed and sections of testicular tissue were prepared for histologic examination. Testicular material was also ground in a tissue grinder with PBS and inoculated into cell culture tubes in an attempt to isolate the virus.

During the course of this study an outbreak of an acute upper respiratory tract infection was observed in a nearby feedlot. Each nasal washing collected from three animals in this herd yielded a cytopathic agent when inoculated into cell culture tubes. By reciprocal neutralisation tests and infectivity for cattle this agent proved indistinguishable from the virus known to cause IBR.
Experiments designed to establish the identity of this new isolate included animal transmission studies. Two virgin bulls were acquired for this purpose and they were inoculated intranasally, intrapreputially and one of the animals was inoculated per conjunctiva (one eye). The inoculum used was 2nd passage TC fluid, TCID$_{50}$ $10^{-4.68}$. For comparison a second pair of virgin bulls were obtained and inoculated in a similar manner with IPV virus (TC fluid TCID$_{50}$ $10^{-4.5}$). The animals infected with the new isolate were held in individual isolation units while the animals infected with IPV virus were housed in adjacent stalls which were entirely separate from the former isolation units.

**Part B - Virological Procedures:**

At the commencement of this study two vials of vaginal exudate collected from experimentally infected heifers and several vials of 5th passage tissue culture fluid containing IPV virus were obtained*. The vaginal exudate was used to induce infectious pustular vulvo-vaginitis (IPV) in three heifers (#13, 30 and 31); vaginal discharge collected from #31 was further used to infect two other females (#10 and 11). The IPV virus was reisolated in foetal bovine kidney cell cultures from animal #10 and the 4th and 5th and 6th passages of this isolation were pooled. The pooled material was stored for 24 hours at $-60^\circ$C (to induce rupture of virus-containing cells), centrifuged at 1500 rpm for 15 minutes and the supernatant fluid

* Kindly supplied by Dr. A. S. Grieg, Animal Disease Research Institute, Hull, P.Q.
then dispensed into 1 ml glass ampoules which were heat sealed and stored at -60°C. This stock virus was used in all subsequent studies except those animal-infection trials in which vaginal exudate was used as inoculum.

Preparation of Foetal Bovine Kidney (FBK) Monolayer Cell Cultures:

The procedures adopted were modifications of those originally introduced by Younger et al. (1954) and modified for other animal tissues by Madin et al. (1954). Bovine foetuses varying in age from 3 to 7 months were obtained from a nearby packing plant. The kidneys were aseptically removed and placed in a sterile petri dish. Perirenal tissue and the capsule were stripped off. Pieces of kidney (2-4 mm) were taken from the cortical and peripheral medullary areas and these were further minced to smaller fragments using a pair of curved scissors. The instruments used were sterilized by dipping in alcohol and igniting the alcohol. The fragments of kidney tissue were placed in a trypsinizing flask and washed two or three times with PBS and once with 0.25% trypsin (1:250 Difco). Finally trypsin, to a depth of one inch over the kidney tissue, and a magnetic stirring bar were added to the flask which was then placed in a waterbath at 37°C on a magnetic stirrer and allowed to trypsinise for approximately two hours. The cell-containing trypsin was filtered through sterile gauze into a centrifuge tube and centrifuged at 800 rpm for 9 minutes. The supernatant fluid was poured off and the cellular deposit was resuspended in nutrient media (Madin et al., 1954) and recentrifuged as above. The super-
natant fluid was poured off and from the cellular deposit a measured quantity of cells was added to a quantity of nutrient media to give a 1 in 200 cell suspension. A Cornwall syringe* was used to dispense 1 ml aliquots of the cell suspension into 18 x 150 mm glass tubes. During dispensing cells were maintained in an even suspension by magnetic stirring. Racks containing the tubes were placed at an angle of 5° in an incubator at 37°C. Refeeding with fresh nutrient medium was necessary on the 3rd or 4th day and a further 2 to 4 days was required to establish a confluent monolayer. When required 6 oz medicine bottles and Leighton tubes containing a cover glass were prepared in a similar manner; the former received 12 ml of the cell suspension, the later 1.5 ml.

Normality of the cells during the trypsinisation procedure was assessed by staining a drop of the suspension with crystal violet-citric acid stain (Melnick, 1956) and examining it under a microscope. If the two hour trypsinisation did not provide sufficient cells, fresh trypsin was added and the procedure repeated.

Inoculation of the Monolayer Cell Cultures:

The following kinds of inocula were introduced into cell culture tubes:

(a) Vaginal exudate.
(b) Virus-containing cell culture fluid.
(c) Serum-virus mixtures.
(d) Preputial washings.
(e) Nasal washings.
(f) Ocular discharges.

* Becton, Dickinson and Company, Rutherford, N.J., U.S.A.
(g) Whole blood.
(h) Serum.
(i) Testicular tissue.

The nutrient fluid was poured off and either four or five tubes were each inoculated with 0.1 ml of the respective inoculum. The racks containing the inoculated tubes were gently rocked to distribute the inoculum over the monolayer and then placed in a near-horizontal position in an incubator at 37°C. After one hour 1 ml of maintenance media was placed in each tube and the racks containing the tubes were replaced in the incubator at the normal 5° slope. Maintenance media differed from nutrient media in having only 3% serum and 5% NaHCO₃ (2.8% solution).

Control tubes were included in each series. These were either uninoculated or received an inoculum of PBS similar to that used for the attempted isolation of the virus. Tubes were examined daily under the microscope. Typical cytopathic changes which resulted in complete degeneration of the monolayer were accepted as evidence of viral activity.

**Determination of the Tissue Culture 50% Infective Dose (TCID₅₀):**

Ten-fold dilutions of the virus-containing cell culture fluid were prepared using PBS as diluent (covering the range 10⁻¹ to 10⁻⁷). Five tubes were inoculated with each dilution and five tubes were left uninoculated as controls. The 50% end point was calculated by the Reed-Muench formula. Estimations of the TCID₅₀ of the stock virus were made frequently during the experimental period for two reasons: (a) to determine the dilution factor
required to prepare 200 TCID$_{50}$ for use in serum-virus neutralization tests, (b) to establish the stability of the virus when stored at -60°C over an extended period.

**Serum-Virus (S-V) Neutralization Test:**

The antibody response of animals to infection with IPV virus was measured in a cell culture system designed to assess the ability of sera to repress the cytopathic effect induced by the virus alone. Before testing, serum samples were heated at 56°C in a waterbath in an attempt to avoid nonspecific neutralization of the virus.

Doubling dilutions of sera were prepared using PBS as diluent and to 0.5 ml of each dilution 0.5 ml of a virus dilution containing 200 TCID$_{50}$ per 0.1 ml was added. Serum-virus mixtures were incubated for one hour at room temperature and 0.1 ml of each mixture was inoculated into four cell culture tubes. Each tube was thus challenged with 100 TCID$_{50}$ plus the prepared serum dilution (doubled again because of the addition of the virus dilution).

In each test series the following controls were included:

(a) Virus controls: four tubes inoculated with 100 TCID$_{50}$ of virus (b) Serum controls: randomly selected samples of the sera to be tested were inoculated to determine whether sera alone induced any changes in the monolayer. (c) Uninoculated controls:

**Detection of Local Antibodies:**

The test procedure to detect the presence of antibodies in the preputial washings was similar to that used for sera.
Cytopathic Effects:

Leighton tubes containing cover glasses upon which a monolayer was established were inoculated with 0.15 ml stock virus \((\text{TCID}_{50} 10^{-5} \text{ to } 10^{-6})\). Uninoculated controls were included in each series. Cover glasses were removed at various intervals after inoculation and were: (a) fixed in Bouin's solution for 30 minutes, washed in running tap water for 24 hours and stained with haematoxylin and eosin or (b) fixed in alcohol for 15 minutes and stained by the Feulgen technique. After mounting on glass slides the preparations were examined under the microscope.
RESULTS

Vaginal discharges collected from infected females and cell culture fluid with a titre of $10^{-4.5}$ proved equally effective in producing lesions when inoculated intrapreputially, intravestibularly, intranasally and into the conjunctival sac of bulls and cows.

The clinical syndromes manifest by the eighteen bulls may be divided into three types. The genital observations in each group will be discussed under separate headings and the associated clinical findings will be considered separately.

Group I: Acute Uncomplicated Cases:

The nine bulls in this group had been used sparingly, or not at all, for natural service. They were placed in the experimental area a short time (usually three days) before inoculation. The incubation period was considered to be the time between inoculation and the temperature rise that preceded the appearance of lesions by 6 to 12 hours. The interval varied between 40 - 72 hours (Table 1).

Hyperaemia and occasionally petechial haemorrhages were observed on the genital mucosa immediately after inoculation. The severity of these initial findings was considered a function of the degree of abrasion occasioned by the inoculation procedure. In most animals a mild transient 1 to 2° temperature rise was noted within 12 hours after inoculation. However, the mucosa and the temperature returned to normal within 24 to 36 hours.

Specific pustular lesions were evident 6 to 12 hours after a second, more severe and persistent temperature rise. Early lesions
were 1 to 3 mm in size, discrete, cream-coloured with a smooth, convex surface raised above the level of the surrounding mucosa. The number and distribution of these pustules varied; they were most plentiful within the preputial folds at sites corresponding to the submucosal lymphoid follicles. A dense cluster of pustules was usually evident at the ridge formed by the reflexion of the prepuce from the body of the penis, and a similar area was evident in the vicinity of the glans. Pustular lesions were in most instances sparsely scattered over the body of the penis (Plate 1).

After 24 hours the lesions appeared slightly flattened and in the dense areas a tendency for pustules to coalesce was noted, however most of the pustules remained discrete. A small (½ mm) slough from the centre of many of the pustules gave a crater-like appearance to the lesions. The mucosa was intensely reddened and inflamed (Plate 2). In more severely affected animals oedematous swelling of the penis and prepuce was marked. Pain was evident when the penis and prepuce were handled, although dysuria was not observed.

Sloughing of the necrotic pustular material between days 3 and 5 resulted in the formation of shallow, irregularly shaped but sharply defined (punched out) ulcers. Many ulcers were surrounded by a thin, intensely red halo. Spontaneous haemorrhage from the ulcers was common (Plate 3). A slight, mucopurulent discharge was usually evident at the preputial orifice.

Six to eight days after infection healing began. The process was associated with the accumulation of loosely adherent pus which although originating from the ulcer site, streaked the general
mucosal surface. The intense redness and inflammation showed signs of abatement at this time (Plate 4).

Resolution was complete 10 to 14 days after inoculation; the ulcer sites however were indicated by bright, hyperaemic areas which contrasted sharply with the surrounding mucosa and persisted for many months (Plate 7).

One animal in group 1 developed lesions at the muco-cutaneous junction at the preputial orifice. A thin diphtheritic membrane developed on the mucosa and adjacent skin.

**Group II: Acute Complicated Cases:**

The early manifestations of the disease in two animals (#980 and 67) were similar to those observed in Group I except that a greater number of pustules developed (Plate 5). Animal #980, a beef shorthorn bull normally had a flaccid penis and this was considered a major predisposing factor to the extreme severity of the resulting disease. At the stage of ulcer formation (day 5) profuse accumulations of pus, extensive and deep ulceration, haemorrhage and intense redness were observed. The flaccidity of the penis exacerbated the oedematous swelling to the point where phimosis developed. The tendency of the animal to lie down for much of the time resulted in the accretion of foreign material (faeces, bedding, etc.) to the exteriorized penis. A thick, necrotic, foul-smelling mass composed of foreign material cemented by pus, ensheathed the body and glans penis. Externally, the prepuce was grossly swollen and painful. Palpable enlargement of the external inguinal lymph nodes was
detected. On day 10 the necrotic sheath was shed but the glans and body remained covered by a diphtheritic membrane. Extensive haemorrhage from the ulcer sites was noted (Plate 6). A profuse mucopurulent preputial discharge was observed and urination was accompanied by pain.

Healing of the ulcers was not complete until day 18, and was associated with extensive fibrosis, which imparted an irregular surface to the normally smooth mucosa. Low grade catarrhal balanoposthitis characterised by reddening of the mucosa and a moderate amount of pus persisted for three months at which time the animal was slaughtered. No adhesions between the prepuce and penis developed. Because of the extreme complications in this animal treatment was undertaken on day 8. The penis and prepuce were bathed with warm antiseptic solution, antiseptic ointment applied locally and systemic antibiotics administered.

**Group III: Mild Form:**

Seven bulls manifested only mild evidence of the disease or were otherwise completely refractory to infection. The history of these animals included the following: (a) all were over two years of age and three had been used extensively for natural breeding (b) they had been housed in the barn used for all transmission studies but were separated from the experimental area by 40 ft of concrete flooring. (c) Hyperplastic lymphoid follicles were noted in four bulls before inoculation. (d) at the time of inoculation three animals (#8, 18 and 19) had a catarrhal balanoposthitis characterised by moderate reddness of the mucosa and the presence
of small amounts of cream coloured pus that streaked the mucosal surface (e) when preinoculation serum samples were subsequently tested for the presence of neutralizing antibodies, six of seven samples tested were found to be positive (Table 4).

Also to be included in group III and having in common the above history were six animals from groups I and II that were reinjected two to three months after recovery from primary infection. In the case of one animal (#355) reinfection was attempted on two occasions, three and two months respectively after the primary infection.

Only four animals (#90, 8, 18, and 3) developed specific pustular lesions after inoculation; these pustules however, were few in number and abortive in development and resolution was complete five to eight days after inoculation. The remaining nine animals were refractory and manifested no specific evidence of the virus infection. These refractory animals provided an opportunity to observe the effects of the inoculation procedures per se. The observations were similar to those observed immediately after inoculation in groups I and II i.e. inoculation was followed by hyperaemia and mild catarrhal inflammation of the mucosa and a transient temperature rise of one or two degrees. Petechial haemorrhages from the lymphoid follicles, due to abrasion occasioned by the inoculation procedures, were commonly observed in this group.

One animal in group III, (#705) exhibited a temperature of 107.6°C, 16 days after attempted reinfection. Clinical examination provided no explanation for the fever. It was noted that the scrotum was pendulous (a physiological response to be expected because of the
hyperpyrexia) and the testicles appeared moderately tense and swollen. Slight oedema of the scrotal skin was evident. Because of the possible significance of these findings a unilateral castration was performed and sections prepared for histologic examination. No significant findings were revealed when these sections were examined.

After recovery animals in groups I and II were examined for a period of three to four months before they were sent for slaughter. In most members of each group hyperplasia of the subepithelial lymphoid follicles of the penis and prepuce was observed. The site of these lesions corresponded to the areas of hyperaemia that persisted after recovery and marked the site of the pustular and ulcerative lesions observed during the acute infection. Three animals in group III, as indicated previously, exhibited the same lesions before inoculation and the remaining four developed them subsequent to inoculation. The above clinical findings have been summarised in Table 1.

**Associated Clinical Findings:**

The typical temperature response of animals in each group is shown in Figure 1. Most animals in the three groups exhibited a mild (1 to 2°F) but transient temperature rise within 12 hours after inoculation. The temperature returned to the preinoculation level 24 to 36 hours after inoculation. Animals in groups I and II showed a second and more severe (103 to 105.6°F) temperature response 40 to 72 hours after inoculation. In uncomplicated cases the hyperpyrexia persisted for four to five days while in one complicated case (#980) the temperature remained elevated for 12 days. Apart from the initial temperature response animals in group III showed no increase
in temperature.

Elevation of the heart and respiratory rates was noted during the febrile period. Heart rates of 100 per minute and respiratory rates of 60 per minute were common.

Inappetence, typified by refusal to consume the normal hay ration but consumption of the usual concentrate ration was noted during the febrile period. Anorexia was observed in two animals i.e. animal #980 in which severe secondary complications developed and animal #705 that developed a high fever after attempted reinfection. Ruminal movements were depressed but some degree of movement could be detected even at the peak of the temperature response. With the exception of animal #705, members of group III did not show aberrations of appetite or ruminal movements.

Daily total and differential white blood counts were undertaken in five animals in Group I. Neutropenia occurred in four of these animals although the degree and duration of the depression of the neutrophils was variable. Two animals (#355 and 356), which were less severely affected clinically, developed only a mild and transient neutropenia and the results were equivocal. The change was more obvious in #66 and 87 which were more severely affected clinically; in animal #66 a maximum depression to 374 neutrophils per cu mm was recorded and some degree of depression was demonstrable for four days. The onset of neutropenia coincided with the appearance of genital lesions. No significant haematologic alterations were demonstrated in members of groups II and III.

Pre and postinfection samples were obtained for bacteriological
examination from the preputes of 12 bulls. A variety of bacterial species were isolated including *E. coli*, *Proteus* spp., *C. pyogenes*, *Pseudomonas* spp., *Streptococcus* spp. and an unidentified diphtheroid. However, none of these were isolated consistently in the examinations that were made, nor were there any significant alterations in the bacterial flora between pre and postinfection samples.

Attempts to isolate pleuropneumonia-like organisms from preputial samples of four bulls were unsuccessful.

**Virus Isolation from Preputial Washings:**

In general, washings collected during the first eight or nine days after infection contained virus as evidenced by typical cytopathic changes when inoculated into cell culture tubes. Washings obtained at various intervals after this time failed to reveal the presence of virus. Results are summarized in Table III.

An exceptional recovery of virus was made from one animal (#41) 26 days after inoculation; four washings collected between the 8th and the 26th day were negative for virus, and samples obtained on the 30th, 32nd and 44th day were again negative. The TCID$_{50}$ of the day 26 washing was found to be approximately $10^{-1}$.

**Postmortem Findings:**

One bull (#349) was destroyed during the acute phase of the disease (84 hours after inoculation). Gross lesions were confined to the penile and preputial mucosa and there was no evidence that the virus had invaded the urethra or any other part of the genital tract.

At the completion of the experimental period the bulls were
slaughtered and the genital organs were examined post mortem. In all specimens examined the subepithelial lymphoid follicles of the penis and prepuce were hyperplastic. Observations tended to confirm the previous finding that viral activity was confined to the epithelial surfaces of the penis and prepuce and that invasion of the urethra did not occur. Scar tissue formation, the result of secondary bacterial complications, noted previously in bull #980 was confined to the more superficial layers of the penile epithelium.

Examination of the scrotal contents revealed the presence of fine, well organised fibrous tissue strands connecting the parietal and visceral layers of the tunica vaginalis. In addition, adhesions of the epididymis, particularly in the head and body regions were noted in most of the specimens examined. A moderate amount of dark-amber coloured, serous fluid was present between the layers of the tunica vaginalis. No significance was attached to these findings and no abnormalities of the testicular parenchyma were observed.

**Histopathology:**

Histologic examination of the biopsy specimens taken serially during the course of the disease indicated that the basic lesion was characterised by a progressive change that commenced as an intense inflammatory cell infiltration followed by degeneration of the epithelial cells with inclusion body formation and sloughing of the necrotic tissue resulting in ulceration; as a sequela hyperplasia of the subepithelial lymphoid tissue was observed.

Inflammatory cell infiltration was the earliest change observed; equal numbers of polymorphonuclear neutrophils and lymphocytes
invaded focal areas of the epithelium and diffusely infiltrated the subepithelial tissue. Histiocytes also became active but formed a minority of the infiltrating cells (Plate 9).

The cytoplasm of the epithelial cells was ballooned and appeared paler than normal. The nuclear contents became progressively disorganised and in many cells a nucleus was not recognisable (Plate 10). In sections prepared soon after gross lesions appeared, small, granular, eosinophilic inclusion bodies surrounded by a clear halo were noted in many nuclei particularly those at the periphery of the necrotic foci (Plates 11 and 12). In specimens obtained 24 and 48 hours later the inclusion bodies were larger and occupied one third to one half of the nucleus. The nuclear contents were displaced to the periphery and the zone between the marginated nuclear contents and the inclusion body was clear. Ulcer formation commenced as a small slough from the centre of the necrotic area (Plate 9); the fully developed ulcer is shown in Plate 11.

In contrast to these lesions the discrete, well circumscribed appearance of a hyperplastic lymphoid follicle observed as a common sequela to the above lesions is illustrated in Plate 13.

Infectious Pustular Vulvovaginitis:

Four of the six females inoculated with IPV virus developed acute manifestations of the disease. The syndrome in the females differed from that of the male in that it tended to be more severe temperatures of 106°F were not uncommon and the febrile period lasted 6 to 8 days. A greater number of lesions developed at the site of inoculation i.e. on the vestibular mucosa and pustules coalesced to
form a cream coloured diphtheritic, membrane which covered most of the mucosal surface.

Because of the presence of the diphtheritic membrane, ulceration with consequent haemorrhage was not apparent to the same extent as had been noted in bulls; ulcers however, could be demonstrated by removing the diphtheritic membrane. The amount of discharge was far greater than that in bulls. The linear arrangement of the early pustular lesions was characteristic particularly on the lateral vestibular walls where lesions were aligned in rows parallel to the long axis of the vagina. Lesions tended to be more profuse at the ventral commissure both on and around the clitoris; however, all areas of the vestibular mucosa were involved. Histologic examination of the affected vaginal mucosa was not undertaken and thus the pathogenesis of the vaginitis was not established. Other findings in females included strangury with frequent urination followed by prolonged straining, vulvar swelling and pain, deflection of the tail to one side, and a profuse but variable vaginal discharge.

The course of the disease in females was slightly longer than that observed in bulls. Resolution was accompanied by detachment of the diphtheritic membrane which revealed an intensely reddened but intact mucosal surface. The site of the initial pustular lesions could still be identified by the persistence of focal areas of hyperaemia.

Infectivity of IPV virus for Sites Other than the Genitalia:

The two animals (#85 and 67) that were inoculated intranasally, developed lesions on the nasal mucosa 44 hours after inoculation.
Part of the visible nasal mucosa was covered by an irregularly contoured, cream coloured diphtheritic membrane, which varied in thickness from 2 to 4 mm. In some areas small (2-4 mm) necrotic foci were visible. The rest of the visible nasal mucosa was intensely reddened. Coincident with the development of nasal lesions a serous nasal discharge was noted. This discharge increased progressively in amount and by the 5th or 6th day had become mucopurulent. Breathing became stertorous but dyspnoea was not a finding. Recovery was complete 10 to 14 days after inoculation.

The nasal lesions observed in these two animals infected with IPV virus were indistinguishable from those observed in two other bulls (#743 and 744) that received IBR virus via the same route. Similarly the lesions produced on the penile and preputial mucosa and on the conjunctiva by the two strains of virus were indistinguishable. Reciprocal cross-neutralization tests established that the two agents were indistinguishable and specific IBR antiserum neutralized both the IPV virus and the newly isolated "IBR" virus.

Nasal lesions were observed in one animal (#350) infected intrapreputially with IPV virus; a small necrotic area developed on the nasal mucosa just within the margin of the external nares. Nasal washings collected from this animal on day 4 postinfection did not yield virus. Several of the animals in groups I and II developed a serous nasal discharge but no specific lesions were found.

* Kindly supplied by Dr. T. L. Chow, Fort Collins, Colorado, U.S.A.
Three animals (#705, 703 and 85) inoculated by rubbing infective material onto the conjunctiva developed conjunctivitis characterized by the development of small (2-4 mm diameter), well circumscribed, white to cream coloured lesions. A few semi-translucent pustular lesions that were smoothly convex and raised above the surface, developed on the cornea. The conjunctiva was intensely reddened, the periscleral vessels were injected and a serous lachrymal discharge ran down the face causing matting of the hair. The palpebra were swollen and by day 4, the discharge was profuse and mucopurulent (Plate 8). Recovery occurred 10 to 14 days after inoculation but the sites of the original lesions were identifiable particularly on the pigmented areas of the conjunctiva by white foci of scar tissue. The sites of the corneal lesions were indicated by the persistence of small (\(\frac{1}{2}\) mm) white, semi-translucent opacities.

Reference has been made to skin lesions observed at the mucocutaneous junction of the preputial orifice of one animal (#356). Attempted infection by scarification of infective material into the skin at the side of the neck of one animal resulted in the development of oedematous swelling but no specific lesions.

**Infectivity of IPV Virus for Other Species of Domestic Animals:**

Limited trials to determine the infectivity of IPV virus for sheep and goats (one sheep and three goats) by inoculation onto the genital mucosa and cutaneously did not result in any lesions nor could neutralizing antibodies be demonstrated in postinoculation serum.
Characteristics of IPV Virus:

Stability:

The stock virus which consisted of tissue culture fluid stored at -60°C in sealed glass ampoules was titrated at frequent intervals during the course of the experiments to establish the tissue culture 50% infective dose. After 226 days storage, the TCID₅₀ had declined 1.5 logarithms. The results are shown in Table 2.

Cytopathic Effects:

Normal and infected foetal bovine kidney monolayers prepared on cover glasses were examined by light microscopy after they were (a) fixed in Bouin's solution and stained with haemotoxylin and eosin or (b) fixed in alcohol and stained by the Feulgen technique.

Two cell types were observed in normal, control preparations: (a) dense areas of cells arranged in whorls (b) larger oval shaped cells in the areas between the whorls. The nuclei of both cell types usually contained a single, large, round nucleolus which was stained either basophilic or slightly eosinophilic. In many cells, clumps of chromatin with similar staining affinity could not be distinguished from the nucleoli. The nucleoli were usually surrounded by an indistinct halo from which the smaller granules of chromatin had been partially dispersed. The chromatin pattern apart from that already referred to varied considerably from deeply stained, basophilic granules to fine, faintly basophilic dots that were evenly scattered throughout the nucleus. The cytoplasm of many cells contained structures which were uniformly round, sharply demarcated by a clear halo and deeply eosinophilic. In many
instances these bodies were in close apposition to the nuclear membrane. The normal monolayer is illustrated in Plate 14.

Eight hours after inoculation of the virus nuclear changes were recognisable. Some nuclei contained faintly eosinophilic, oval-shaped inclusion bodies which occupied most of the nucleus and were separated from the nuclear membrane by a narrow, unstained halo. These faintly stained inclusion bodies were precursors of those that were more readily visible 16 hours after inoculation. The distribution of the nuclear contents appeared little disturbed by the presence of the inclusion body. No abnormalities of the cytoplasm were apparent at this stage.

Sixteen hours after inoculation widespread disruption of the monolayer was evident and few normal cells were present. Many cells were round and contracted into deeply staining clusters in which little cellular detail was discernable. Large areas of the glass were devoid of any cells. Most of the cells in which nuclear detail could be seen contained a sharply defined, deeply eosinophilic inclusion body which occupied two thirds to four fifths of the nucleus. Chromatin was aggregated and displaced to the nuclear membrane and was distinctly separated from the inclusion body by a clear halo. These changes are illustrated in Plates 15 and 16.

In a series of monolayer preparations that were placed in Bouin's solution at 2 hour intervals postinoculation an attempt was made to determine the genesis of these inclusion bodies. The inclusion body was first recognisable approximately 8 hours after inoculation as faintly eosinophilic mass which in succeeding
preparations gradually became more deeply eosinophilic and more readily visible. The margination of the chromatin was also progressive and corresponded to the increasing eosinophilic intensity of the inclusion body. The marginated chromatin was readily visible in the Feulgen stained preparations in which the inclusion bodies were not stained and therefore did not contain deoxyribonucleic acid. A marked decrease in the mitotic rate of infected monolayers was also apparent in Feulgen preparations.

At 24 hours, many of the cells had fallen from the glass into the nutrient medium leaving large areas of the glass devoid of cells. Fine, lace-like, irregular, eosinophilic strands crossed these denuded areas of glass. The majority of cells were aggregated into deeply staining clusters resembling bunches of grapes; nuclear detail was distinguishable only in cells at the periphery of these clusters and such cells invariably contained an inclusion body. Inclusion bodies were also seen in the few cells not included in the clusters (Plate 17). The inclusion body underwent a reduction in size as a result of the contraction of the cell. Few cells remained on the glass at 32 hours. Both the cytoplasm and the nucleus of the cells that were still attached to the glass had undergone extreme contraction. The cells were reduced to about one quarter normal size and appeared as darkly stained spheres in which no detail was discernable. Cytoplasmic strands crossed the denuded areas of glass but the fine, lace-like reticulum seen at 24 hours had disappeared (Plate 18). Between 40 and 72 hours the remaining cells were released from the glass into the medium.
Serum Virus Neutralization Test: The serum-antibody titres of bulls infected with IPV virus are shown in Table IV.

The following represents additional findings observed in these serum-virus (S-V) neutralization tests:

(a) In a preliminary trial pre-inoculation serum collected from animal #11F neutralized 100 TCID$_{50}$ at a dilution of 1:4. The animal proved fully susceptible and manifested typical signs of the disease following inoculation. The pre-inoculation serum sample was heated to 56°C in a water bath and did not neutralize the virus when retested. The neutralizing effect was thus considered to be non-specific. All sera subsequently tested were inactivated by heating to 56°C and no further evidence of non-specific neutralization was noted.

(b) In a number of S-V neutralization tests, failure of the virus control tubes (that is those tubes receiving 100 TCID$_{50}$ only) to exhibit cytopathic changes was noted. This phenomenon was considered too frequent in occurrence to be due to faulty technique. Possibly analogous to this finding was the observation that tubes receiving higher dilutions (1/64, 1/128) of serum plus 100 TCID$_{50}$ of virus also failed to show cytopathic changes even though an end point had been established at lower serum dilutions.

(c) Cytopathic changes usually commenced near the bottom of the monolayer as several small foci in which the cells appeared round and dark. In tubes containing maximum amounts of free virus i.e. virus controls, the cytopathic foci were evident 16 to 24
hours after inoculation, while in tubes expected to contain minimal amounts of free virus i.e. postinfection sera close to the final end point, cytopathic changes were not evident until three or four days after inoculation. After recognition of the cytopathic foci, complete destruction of the monolayer was rapid and progressed until all cells had been detached from the glass (72 hours). Typical cytopathic changes which resulted in complete destruction of the monolayer was therefore used as the criterion for the presence of virus. All inoculated tubes were examined for 6 to 7 days at which time the final reading was made.
DISCUSSION

Contemporary nomenclature suggests that the term infectious pustular balanoposthitis would seem appropriate for the genital disease of bulls produced by IPV virus. The inadequacy of older nomenclature, with the exception of the German term Bläschenausschlag which may be translated as pustular eruption has already been alluded to. The perplexity of present day nomenclature pertaining to the virus and its diseases may be schematically represented as follows:

The Agent: IBR - IPV virus (Gillespie et al., 1959).

The Diseases:

- Infectious pustular vulvovaginitis (IPV) (Kendrick et al., 1957)
- Infectious pustular balanoposthitis (see above)
- Infectious bovine rhinotracheitis (IBR) (McKercher et al., 1955)
- Conjunctivitis
  - (a) pustular (Zwick and Gminder, 1913)
  - (b) catarrhal (McKercher et al., 1958)
- Fatal disease of newborn calves (Baker et al., 1960)
- Mastitis (experimental) (Baker et al., 1960).

It is tempting to suggest that what is required is a single, manageable name for the virus and then merely to qualify this term according to the site of the disease. It is however not within the province of this discussion to suggest any revision of nomenclature. Viral taxonomy in general is in a state of flux and in the absence of a generally accepted and soundly based system of
nomenclature such as immunologic characteristics, any new suggestion is apt to be disregarded and/or discarded in future years.

The genital disease induced in bulls by IPV virus differed from its counterpart in the female in a number of ways: (a) the number of lesions manifest on the penile and preputial mucosa was fewer than those produced on the vestibular mucosa even though approximately the same amount of inoculum was used. The dependent position of the penis and prepuce would presumably result in the loss of some inoculum. This is supported by the observation that in the male, lesions were most predominant within the folds of the prepuce where inoculum would presumably be more adequately retained. In the female, the close apposition of the opposing vestibular walls would provide better opportunity for the virus to gain entry to the epithelial cells.

(b) Coalescence of lesions in the female to form a diphtheritic membrane which covered most of the vestibular surface, was a constant feature. In bulls however, lesions coalesced and formed a diphtheritic membrane only at two sites that is at the reflexion of the prepuce from the body of the penis and at the glans (see Plate 1). During inoculation both of these sites were areas of maximum abrasion; the ridge formed by the preputial reflexion was more severely traumatized during inoculation and the glans area was used as a traction point in exteriorizing the penis.

(c) Ulcer formation was a constant and predominant clinical finding in bulls while in the female the diphtheritic membrane overshadowed the presence of ulcers. Ulcers were however demonstrable in the
female if the diphtheritic membrane was removed. Resolution in both sexes was characterised by the persistence of focal areas of hyperaemia. These areas marked the sites of the initial lesions and were associated with the subepithelial lymphoid follicles which subsequently became hyperplastic (Plate 7).

(d) Complications were not observed in the females infected during this study although they have been reported by Hutyra et al. (1938) and by Kendrick et al. (1957). Two bulls developed severe complications which were considered to be the result of secondary bacterial infection and contamination with foreign material. One animal developed phimosis. The course of the disease in these complicated cases was prolonged and resolution was accompanied by fibrosis but no adhesions between the penis and prepuce were observed.

(e) Females developed a profuse, mucopurulent vaginal discharge which was readily apparent even on cursory examination. Conversely in uncomplicated acute cases in the male the amount of discharge from the preputial orifice was minimal and was detected only on careful examination.

In the female the presence of a vaginal discharge together with vulvar swelling, a raised tail, dysuria followed by prolonged straining combined to make the disease readily apparent. Such obvious signs were not part of the syndrome in the male and unless the penis was exteriorized for examination, the disease would pass unnoticed under field conditions. This argument is augmented by the fact that systemic disturbances, other than the febrile
response, are minimal. Indeed it is surprising to find animals so little disturbed in demeanour, appetite etc. by a temperature rise of this magnitude.

In addition certain procedures used in this experimental study such as exteriorisation of the penis, the obtaining of biopsies, and abrasion aggravated the disease and would not be operative in natural infections by the virus. These procedures are worthy of further consideration. Exteriorization of the penis, for purposes of inoculation and daily examination, with consequent contamination of the affected epithelium with bacteria and foreign material was considered the prime factor responsible for the severe and very obvious signs that developed in two animals (#980 and 67). It appeared that the external genitalia were most susceptible to these secondary complications at the stage when the necrotic material sloughed to form ulcers (days 3 to 9). The aggravation of the disease occasioned by the collection of biopsies was in some instances considerable. A number of observations suggest that abrasion is of particular importance in the ultimate effect produced by the virus and these are as follows: (a) In field cases of IBR, conjunctivitis may be a predominant clinical finding (Abinanti and Plumer, 1961; Studdert et al., 1961). The inflammation observed in these instances was catarrhal and the former authors reproduced the disease experimentally simply by instilling infective material into the conjunctival sac. However, Zwick and Gminder (1913) reported that when infective material was rubbed onto the conjunctival surface specific pustular lesions resulted. This has also been
demonstrated in the present study (Plate 8). (b) The number of lesions observed on the epithelial surface of the penis and prepuce appeared to be a function of the thoroughness with which inoculation was pursued. (c) In four bulls (#980, 8, 18 and 19) a catarrhal balanoposthitis was observed at the time of inoculation; if this balanoposthitis was caused by chance contamination with the virus then the finding can be equated to the catarrhal form of conjunctivitis. (d) In Europe most cases of the disease in the past have been associated with coitus and this presumably would result in mild abrasion of the opposing epithelial surfaces. (e) At least two outbreaks have been associated with a dog that licked the external genitalia of recumbent cows (Esser and Schütz, 1898; Gibbons, 1944) and a third outbreak followed routine caudal fold tuberculin testing. In each instance cited above it can be seen that abrasion may have had a role in the precipitation of the acute pustular form of the disease.

The results obtained from the blood counts performed during this study have shown that in three bulls the neutrophil count fell sufficiently below the preinfection level to indicate that infection with IPV virus may induce a neutropenia. In three other bulls no neutropenia was demonstrated. These results are in agreement with those of Kendrick et al. (1957) who demonstrated a neutropenia in infected females and also with Studdert et al. (1961) who demonstrated a neutropenia in bulls infected intranasally and intrapreputially with IBR virus. Attempts to demonstrate viraemia during the febrile phase of IPV infection were negative and
Kendrick et al. (1957) were also unable to demonstrate IPV virus in the bloodstream. It may therefore be reasoned that the decrease in the number of circulating neutrophils was the end result of intensive infiltration into the affected areas and that the mechanism responsible for leucocytosis - a normal response to most inflammatory processes - was not operative in the early stages of infection when virus alone was active. If the neutropenia is the result of withdrawal of neutrophils from the bloodstream into the affected area then it would be in some measure explain the variability of the finding since it would therefore be dependent on the severity of the clinical syndrome.

Three bulls showed a slight leucocytosis between the fifth and ninth day when bacterial invasion of the ulcerated areas was believed to occur. The delicate balance between the neutropenia due to the virus on the one hand, and the leucocytosis due to bacterial invasion on the other would further explain the indefinite and erratic hematologic findings in the diseases caused by IBR - IPV virus. Additional critical work would be required to establish the true effect of the virus on the blood picture. Disease-free animals infected with the virus might provide the answer.

Results obtained from one animal (#355) indicated that serum antibodies to IPV virus persist for at least 18 weeks after infection. Although the serum sample obtained 18 weeks after primary infection from this animal was negative for neutralizing antibodies the animal was resistant to reinfection at this time. This finding suggests that it is possible for the antibody titre to fall to a non-detectable
level but for animals to remain resistant to infection. In any case animals are resistant even when the antibody titre is very low. Reinfection of #355 was again attempted 35 weeks after primary infection and a preinoculation serum sample obtained on this occasion did contain antibodies (see Table 4). The possibility of reinfection from the environment with consequent enhancement of a waning immunity cannot be excluded. McKercher (1959) has demonstrated antibodies to IBR 12 months after primary exposure to the virus.

Animal #6 possessed a low titre (1:2) in the preinoculation serum while serum obtained seven days postinoculation had a titre of 1:64. It is tempting to suggest that this rapid rise was an anamnestic response, however it can be seen from Table 4 that the antibody titres which develop as a result of IPV virus infection, are relatively low and the experimental error of the test considerable so that little may be said concerning the finding.

An attempt to demonstrate local antibodies in preputial washings, that had been shown not to contain virus, was negative. However the amount of preputial secretion which might be expected to contain antibodies is slight and the dilution occasioned by 10 ml of washing fluid is considerable. Greater refinement in the technique used would be required to exclude the occurrence of local antibodies.

The basic histopathologic change induced by the virus was hydropic degeneration of the affected epithelial cells, with consequent infiltration of neutrophils and lymphocytes in about
equal numbers. In none of the sections examined was lysis of these leucocytes demonstrated; the nuclear contents of the leucocytes were always sharply defined and normally stained. It may therefore be argued that the lesion is not a pustule since by definition the later requires the incorporation of dead, lysed leucocytes. The virus induced an inflammation of the lymphatic follicles and as a sequela hyperplasia of these structures invariably resulted.

The inclusion bodies that were demonstrable in the first three or four days of the disease differed from those observed in tissue culture preparations in that they were irregular in outline and generally somewhat shrunken in appearance.

Some controversy exists concerning the affect of the virus on the vaginal mucosa. Several authors have claimed that lesions do not extend beyond the hymen. However, Kendrick et al. (1958) considered that the virus does invade the vaginal epithelium and produce lesions similar to those observed on the vestibular mucosa; these authors were able to demonstrate inclusion bodies in vaginal epithelium. Sections of the affected vaginal mucosa were not examined histologically in this study.

A recent report (Bouters et al., 1960) indicated that orchitis may occur in bulls as a sequela to the primary genital disease as observed on the penile and preputial mucosa. Although one animal (#705) developed an acute febrile reaction (107.6°F) and orchitis was suspected, histologic examination of the testes did not reveal any indication of orchitis nor could virus be isolated from the
testicular tissue. Bouters et al. (1960) did isolate the virus from testicular tissue. Conradi et al. (1960) who observed the disease in 26 bulls did not record the occurrence of orchitis. These observations suggest that the occurrence of orchitis in association with IPV virus infection is uncommon. The role of the virus as a cause of orchitis is an important question worthy of further study.

The findings related to the infectivity of IPV virus when inoculated intranasally are in agreement with those of Gillespie et al. (1959) and McKercher et al. (1959). Accumulated data leaves little doubt that the agents causing IPV and IBR are identical. The observation by Zwick and Gminder (1913) that infective material inoculated onto the conjunctiva produced typical pustular lesions has been confirmed in this study (Plate 8). No outbreaks of this form of conjunctivitis have been reported in North America and it is believed that moderate epithelial damage is a prerequisite for these conjunctival lesions to develop.

Although cutaneous lesions at the preputial orifice were observed in one bull (#356) evidence of direct viral invasion of the skin was not noted. The single lesion observed in this animal was conjoined to a lesion on the adjacent mucosa i.e. the skin involved was close to the muco-cutaneous junction. When infective tissue culture fluid was scarified into the skin of one animal (#350) no lesions indicating viral activity were observed. It is suggested that skin lesions in association with IPV are a secondary and incidental finding.
The reports of earlier workers regarding the infectivity of IPV virus for sheep and goats have not been substantiated in this study however the number of trials was limited.

The eosinophilic intranuclear inclusion bodies observed in foetal bovine kidney cell cultures did not contain deoxyribonucleic acid (DNA) as evidenced by their negative Feulgen reaction. If virus is present in the IB it is possible that it does contain DNA which is not stained by this technique. Alternatively the agent may contain ribonucleic acid (RNA). In preliminary electron-microscopic examination of thin sections of infected monolayers spherical particles (40 to 50 m\(\mu\)) were observed within the nucleus and these were suggestive of immature virus particles (Grinyer et al., 1960). Stephens (1959) observed the sudden disappearance of inclusion body just prior to the detachment of cells from the glass and therefore considered that their sudden disappearance was related to the release of virus.

In the examination of infected monolayers, serially stained at intervals after infection, it was apparent that inclusion bodies first appeared as a faint eosinophilic precursor of the fully developed inclusion body which progressively stained more intensely with eosin. The extreme contraction of cells just prior to their detachment from the glass was associated with the disappearance of recognisable inclusion bodies although in many of these cells no nuclear detail was distinguishable.

Observations on the serum virus neutralization test were of particular interest from the following points of view. In cell
culture tubes containing 100 TCID$_{50}$ of virus plus a serum dilution that was close to the final end point i.e. the last serum dilution to neutralize 100 TCID$_{50}$, a considerable lag period, sometimes as long as three days, was noted before cytopathic changes were detected. The most obvious explanation for this lag is of course that most of the virus particles had been neutralized and therefore only those few unneutralized particles were free to attack the cells; these free virus particles had to produce several generations before obvious damage to the monolayer was observed. It has been pointed out however that after the first foci of cytopathic change was noted complete destruction of the monolayer followed rapidly. A possible alternate explanation for the lag period is that all the virus particles were neutralized when the serum and virus were first mixed and with subtle changes in the environment within the cell culture tube such as pH, virus was released from the antibody complex to invade the cells. No attempt was made to elucidate the real mechanism of this lag period, however the concept is important and worthy of further study.

The second observation made on the serum-virus neutralization tests worthy of comment was the failure of virus control tubes, i.e. tubes inoculated with 100 TCID$_{50}$ of virus, to show evidence of cytopathic changes. It would appear that the exposure of the virus to room temperature altered the virus particle in such a way as to prevent it from attacking the cells. It is unlikely that the virus was completely destroyed as observations already discussed have indicated that the agent is relatively resistant. If the virus
particle was altered then the presence of serum in the serum-virus mixtures must have a protective action which prevented the change. An alternate explanation that would partially explain the phenomenon would be that the serum potentiated the cytopathic effect of the virus after it was inoculated into cell culture tubes.

The relationship of IBR-IPV virus to other viruses is of importance and in keeping with a recent trend in virology in which relationships between viruses are rapidly being established. Moll et al. (1960) has indicated that IBR virus is immunologically related to certain bovine enteroviruses. There are at least some superficial similarities between IBR-IPV virus and human respiratory viruses particularly the adenoviruses. Acute outbreaks of human respiratory disease due to adenoviruses are most prevalent where large groups of people are congregated viz. military camps, orphanages, etc. (Ward, 1959); the agents may produce conjunctivitis in addition to involvement of the upper respiratory tract and it has been shown that the initiation and severity of the conjunctivitis is related to irritation and mild epithelial damage (Hogan, 1957); genital tract infection appears not to have been reported with the human agents. Although there is no evidence that animals are associated with human adenovirus infection, the possible relationships of the agents should be examined.

Perhaps the most important and intriguing questions related to IBR-IPV virus centre upon the epizootiology. Where does the virus exist in the absence of clinical disease? What factors incite the agent to produce disease? Why are there so few reports of field outbreaks of the genital form of the disease?
From animal #41 virus was isolated 26 days after infection; the animal had recovered from the disease and washings collected on the 12th, 16th and 19th days postinfection did not contain virus. Two explanations for the recovery of virus at 26 days are possible: (a) the virus had persisted in the environment and its presence in the washing was due to chance contamination (b) the virus had persisted in the animal during the 26 day period. Both possibilities are worthy of further considering for it is in their correct interpretation that many of the important epizoologic questions can be answered.

Evidence that the virus may persist in the environment, at least for short periods, was obtained during this study. Animals in group III (a) had been housed in the barn during infective trials on other animals. Three of these animals (#17, 18 and 19) were triplets and had been purchased and placed in the barn at three months of age. It is unlikely, though not impossible, that these three animals as well as all the others in Group III (a) had experienced exposure to the virus before entering the barn. Preinoculation sera from the animals contained neutralizing antibodies and they were refractory to the disease when inoculated. It has been pointed out that animals which were not included in an experiment were housed at the opposite end of the barn to that used to contain infected animals. The observation suggests that the virus can survive outside the animal and on gaining access to a suitable host may incite an antibody response if not clinical disease.
None of the animals that possessed antibodies in preinoculation serum had shown sufficient signs of illness to draw attention to them although four bulls (#980, 8, 18 and 19) did show evidence of a catarrhal balanoposthitis at the time of inoculation. In previous discussion, the factors believed to influence the severity of the disease have been considered and it can be readily appreciated that infection, particularly of bulls, could easily pass unnoticed. The survival of IBR-IPV virus in the environment is of considerable importance and worthy of further study.

The second possibility i.e. that the virus persists in the animal is a far more complex and important question. McKercher (1959) has stated that "it is difficult to avoid the conclusion that cattle themselves, either as subclinically infected animals or convalescent carriers or both, maintain the virus (IBR) and serve as the chief means of spread". What then are the possible modes by which the agent may persist in an animal carrier? There are at least two theoretical possibilities (a) the virus establishes itself within the epithelial cell as a relatively harmless commensal, protected from the neutralizing antibodies which have developed in response to its initial invasion. The concept is not new to virology; indeed, much current thought, particularly with regard to virus as a possible cause of cancer, is being given to the idea that viruses may establish themselves as an integral part of the nuclear apparatus. It might be that eventually the cell harbouring the commensal virus would be sloughed releasing its complement of virus. Alternatively, the
virus itself may result in the destruction of the cell and consequently release of the agent. The emancipated virus that escaped neutralization by antibody would then begin a new cycle of "Commensalism" in a new cell. Such might be termed a "low grade infection carrier state". (b) the virus exists extracellularly, after abatement of the acute signs of illness, in combination with antibody. Under certain conditions viable virus, which is not destroyed by union to antibody would escape and initiate a new outbreak of disease. Burnet (1955) believes that "to a near approximation in each case the virus antibody reaction resulting in inactivation is irreversible in the case of typical bacterial virus and completely reversible in the case of animal viruses". Mandel (1960) has advanced evidence that the virus antibody complex under certain variable conditions viz. temperature, dilution, pH and concentration undergoes dissociation with the release of cytopathogenic virus. This author considered therefore that the interaction between virus (poliomyelitis) and antibody leading to inactivation, that is neutralization, is a kinetic process. If this concept can be transposed to the in vivo activity of any animal virus, then elucidation of the factors operative in the release of virus in vivo could be considered as being analogous to the factors predisposing to disease and therefore of considerable economic importance.

Any theoretical concepts about the epizootiology of an infectious disease must be substantiated by the natural history of that disease. There are a number of important factors in the natural history of IBR-IPV virus worthy of mention: (a) the high incidence of
Bläschenaußschlag at the end of the last century in Europe was probably related to the use of community sires. (b) the reported incidence in modern times of the genital form of the disease from all parts of the world is surprisingly low. In Canada for example a single outbreak has been observed in the past 6 years, although Grieg (1961) found that 8.13% of sera from cattle in southern Ontario representing 18.9% of herds tested were positive for the presence of IBR antibodies. Gillespie et al. (1959) reported similar findings in a survey conducted in New York State. It is thus difficult to avoid the conclusion that the infectious agent is widespread in North America and that clinical manifestations of its presence are the exception rather than the rule. (c) Infectious bovine rhinotracheitis appears to be a disease peculiar to cattle under conditions providing for extremely close contact e.g. feedlots, although there are reports of its occurrence under range conditions (Chow et al., 1961). The rapid transfer of the infectious agent among animals in a feedlot may result in enhanced virulence of the virus and consequently the acute syndrome of IBR. Factors discussed previously, which affect the severity of the genital form of the disease such as abrasion, appear not to be as important in IBR outbreaks although McKercher (1959) has suggested that dust, which could conceivably abrade the respiratory epithelium, may be an important predisposing factor.

From the foregoing discussion it appears evident that the infectious agent is widespread and that exposure to it is more likely to result in subclinical infection and subsequent immunity.
In order to initiate clinical evidence of disease certain predisposing factors are necessary. Abrasion is considered important in the genital form and close confinement is at least one predisposing factor to ocular and respiratory manifestations of infection. It is of some importance to know what percentage of a cattle population is susceptible. The figures published by Gillespie et al. (1959) and Grieg (1961) indicate that less than 15% of cattle possess antibodies however the possibility that other cattle may not have a demonstrable titre and still be immune should be borne in mind (see table 4, Animal #18). The preliminary work of Moll et al. (1960) has suggested that other non pathogenic bovine enteroviruses are immunologically related to IBR virus. It may be possible that such viruses could confer a partial immunity sufficient to result in an abortive infection by the pathogenic IBR-IPV agent.

What then is the nature of subclinical infection? There are a number of possibilities but it is likely that subclinical respiratory infection could be prevalent in young cattle. The complexity of bovine respiratory diseases particularly in regard to the role of virus in their causation is well recognised. In bulls at least genital infection could readily pass unobserved.
CONCLUSIONS

1. The genital disease produced in bulls by IPV virus is an acute, specific disease and although basically similar to the disease in females differs in certain important respects which make diagnosis of the disease in bulls difficult under field conditions.

2. Abrasion of the epithelial surfaces is considered an important factor in the initiation and resulting severity of the genital disease produced by IPV virus.

3. Intranuclear eosinophilic inclusion bodies can be demonstrated in the affected penile and preputial mucosa during the first five days of the disease.

4. Bulls develop specific neutralizing antibodies after infection with IPV virus.

5. Subclinical infection is believed to be more common after exposure to IBR - IPV virus and clinical evidence of disease requires certain predisposing factors.

6. Infection may result in neutropenia.

7. The agent induces specific pustular lesions on the conjunctiva and cornea when inoculated by rubbing onto these surfaces.

8. The disease produced by intranasal inoculation of IPV virus is similar to infectious bovine rhinotracheitis.

9. The virus does not produce lesions when scarified into the skin of susceptible cattle.

10. Goats and sheep appear to be refractory to IPV virus
although the number of trials conducted in these species was limited.

11. There is some evidence that the virus may survive outside the animal body and on gaining access to susceptible cattle may result in an immunological response and perhaps clinical signs of infection.

12. When stored at -60°C in sealed glass ampoules for 226 days the TCID$_{50}$ of cell culture fluid declined 1.5 logarithms.

13. The inclusion bodies observed in infected foetal bovine kidney cell cultures do not contain desoxyribonucleic acid as evidenced by a negative Feulgen reaction.

14. The inclusion bodies observed in haematoxylin and eosin stained foetal bovine kidney monolayers after being fixed in Bouin's solution, are first seen approximately eight hours after infection as a faintly staining, eosinophilic precursor of the more intensely eosinophilic inclusion observed at 16 hours.
Eighteen bulls were experimentally infected with infectious pustular vulvovaginitis (IPV) virus and observations on the resulting genital disease were made. Six female cattle were infected intravestibularly with IPV virus and comparative observations on the disease in each sex were carried out. The infectivity of the virus when inoculated intranasally, cutaneously and into the conjunctival sac of cattle was examined. Three goats and a sheep were inoculated intrapreputially, intravestibularly and cutaneously and in all instances did not show evidence of disease. Observations on the cytopathic effects produced in foetal bovine kidney cell cultures have been made particularly with reference to the nature and genesis of inclusion bodies observed in infected monolayers. The stability of the virus when stored at -60°C has been recorded. The predisposing factors and some epizootological concepts pertaining to IBR - IPV virus infections are discussed.
REFERENCES


10. Debelic, S. Bläschenauusschlag auf den geschlechtsorganen bei
Vetmed., 59, 467, 1936).


12. Diernhofer, K. Zur mikrobiologie des geschlechtstraktes des

13. Dulbecco, R. and Vogt, M. Plaque formation and isolation of
pure lines with poliomyelitis viruses. J. Exptl. Med.

14. Esser, J. and Schütz, W. Mitteilungen aus den amtlichen veterinär-
sanitätsberichten 1898. (Cited by Kendrick et al., 1958).


16. Friedberger, F. and Fröhner, E. Veterinary Pathology. Translated

17. Gibbons, W.J. Interesting cases from the ambulatory clinic -
vesicular venereal disease. Cornell Vet., 34, 235 - 241,
1944.

Comparison of infectious pustular vulvovaginitis virus with
infectious bovine rhinotracheitis virus. Cornell Vet., 49,

   Cultivation in tissue culture of an infections agent
   from coital exanthema of cattle. A preliminary report.
22. Grieg, A.S. The detection of antibody to infectious bovine
   rhinotracheitis virus in Ontario cattle. Canad. J. Comp.
23. Griffin, T.P., Howells, W.V., Crandell, R.A. and Maurer, F.D.
   Stability of the virus of infectious bovine rhinotracheitis.
24. Grinyer, I., Savan, M., Studdert, M.J. and McKercher, P.D.
   Electron microscopic studies of infectious pustular
   vulvovaginitis virus in foetal bovine kidney cell cultures.
   Unpublished data.
   f. Thiermed., S 147, 1890. (Abs. in Jber. Vetmed., 10, 42,
   1890).
27. Hogan, M.J. Keratoconjunctivitis. The clinical characteristics
   of the Californian epidemic. Amer. J. Ophthal. 43, 41 - 44,
   1957.
28. Hutyra, F., Marek, J. and Manninger, R. Special pathology and
   therapeutics of the diseases of domestic animals. 4th Ed.,
   Bailliere, Tindall and Cox, 1938.


38. Lormore, R.E. Vesivular venereal disease of cattle. Cornell Vet.,


59. Späth. Infection mit Bläschenausschlag als Ursache von Uterus-


68. Williams, W.L. Diseases of the genital organs of domestic
animals. 3rd. Ed., Miss Louella Williams, Upland Rd.,
Ithaca, N.Y., 1943.

180, 1935.

70. Wirth, D. and Diernhofer, K. Lehrbuch der inneren
Krankheiten der Haustiere einschliesslich der Hautrankheiten
sowie der klinischen Seuchenlehre. Stuttgart: Ferdinand
Enke, 1950.

71. Witte, J. Untersuchungen über den Bläschenausschlag (exanthema
pustulosum coitale) des Rindes. Ztsch. f. Infektionskrank.,

72. Zwick und Gminder. Bestehen zwischen dem ansteckenden
Scheidenkatarrh und dem Bläschenausschlag der Rinder
ursächliche Beziehungen? Berlin. Tierärztl. Wchnschr., 29,
417 - 422, 1913.

73. Zwick und Gminder. Untersuchungen über den Bläschenausschlag
(exanthema vesiculorum coitale) der Rinder. Berlin.

74. Younger, J.S. Monolayer tissue cultures. I. Preparation and
standardisation of suspensions of trypsin-dispersed monkey
1954.
<table>
<thead>
<tr>
<th>Animal #</th>
<th>Age (yr)</th>
<th>Incubation Time (Hr)</th>
<th>Type of Syndrome</th>
<th>Inoculum</th>
<th>Clinical Course (days)</th>
<th>Maximum Temp. (°F)</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>355*</td>
<td>1</td>
<td>60</td>
<td>Acute</td>
<td>VD**</td>
<td>12</td>
<td>1025</td>
<td>Lesions observed on skin at preputial orifice. Nasal lesions.</td>
</tr>
<tr>
<td>356</td>
<td>1</td>
<td>60</td>
<td>Acute</td>
<td>VD</td>
<td>12</td>
<td>104.2</td>
<td>Cutaneous infection attempted-negative. Nasal lesions.</td>
</tr>
<tr>
<td>350</td>
<td>2/3</td>
<td>54</td>
<td>Acute</td>
<td>TC***</td>
<td>8</td>
<td>103.2</td>
<td>Attempted isolation from serum during febrile period—negative.</td>
</tr>
<tr>
<td>41*</td>
<td>1</td>
<td>40</td>
<td>Acute</td>
<td>VD+TC</td>
<td>10</td>
<td>104.9</td>
<td>Attempted isolation from citrated blood during febrile period—negative.</td>
</tr>
<tr>
<td>66*</td>
<td>1½</td>
<td>40</td>
<td>Acute</td>
<td>VD</td>
<td>15</td>
<td>104.2</td>
<td>Post-mortem 84 hr. after infection. No findings additional to those from clinical examination.</td>
</tr>
<tr>
<td>349</td>
<td>1</td>
<td>40</td>
<td>Acute</td>
<td>VD+TC</td>
<td>—</td>
<td>104.9</td>
<td>Fustular conjunctivitis after inoculation per conjunctiva; slight nasal discharge.</td>
</tr>
<tr>
<td>703*</td>
<td>1</td>
<td>72</td>
<td>Sub-Acute</td>
<td>TC</td>
<td>13</td>
<td>105.3</td>
<td>Infected intranasally—lesions typical of IBR. Fustular conjunctivitis after per conjunctival inoculation.</td>
</tr>
<tr>
<td>705*</td>
<td>1</td>
<td>72</td>
<td>Sub-Acute</td>
<td>TC</td>
<td>16</td>
<td>105.4</td>
<td></td>
</tr>
<tr>
<td>85</td>
<td>1</td>
<td>42</td>
<td>Acute</td>
<td>TC</td>
<td>14</td>
<td>106.1</td>
<td></td>
</tr>
<tr>
<td>Animal #</td>
<td>Age (yr)</td>
<td>Incubation Time (Hr)</td>
<td>Type of Syndrome</td>
<td>Inoculum</td>
<td>Clinical Course (days)</td>
<td>Maximum Temp. (°F)</td>
<td>Comments</td>
</tr>
<tr>
<td>----------</td>
<td>----------</td>
<td>----------------------</td>
<td>------------------</td>
<td>----------</td>
<td>-----------------------</td>
<td>-------------------</td>
<td>----------</td>
</tr>
<tr>
<td>67</td>
<td>2</td>
<td>42</td>
<td>Very Acute</td>
<td>TC</td>
<td>16</td>
<td>105.0</td>
<td>Developed marked oedema of penis and prepuce; profuse preputial discharge. Other findings as for #85.</td>
</tr>
<tr>
<td>980*</td>
<td>2½</td>
<td>?</td>
<td>Very Acute</td>
<td>VD</td>
<td>23</td>
<td>105.3</td>
<td>Catarrhal balanoposthitis at time of inoculation of undetermined aetiology.</td>
</tr>
<tr>
<td>90</td>
<td>4</td>
<td>-</td>
<td>nil</td>
<td>VD</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>2</td>
<td>54</td>
<td>nil</td>
<td>Coitus to infected female</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>4</td>
<td>-</td>
<td>nil</td>
<td>VD</td>
<td>8?</td>
<td>-</td>
<td>Catarrhal balanoposthitis at time of inoculation of undetermined aetiology.</td>
</tr>
<tr>
<td>18</td>
<td>4</td>
<td>?</td>
<td>Mild</td>
<td>VD</td>
<td>8</td>
<td>101.6</td>
<td>As for #8.</td>
</tr>
<tr>
<td>19</td>
<td>2</td>
<td>?</td>
<td>Mild</td>
<td>VD</td>
<td>7</td>
<td>102.2</td>
<td>As for #8.</td>
</tr>
<tr>
<td>3</td>
<td>3</td>
<td>70</td>
<td>Mild</td>
<td>VD+TC</td>
<td>6</td>
<td>103.4</td>
<td>Single lesion near glans.</td>
</tr>
<tr>
<td>452</td>
<td>3</td>
<td>-</td>
<td>nil</td>
<td>VD+TC</td>
<td>-</td>
<td>102.5</td>
<td></td>
</tr>
</tbody>
</table>

**Animals above are Marked**

**VD** = Vaginal Discharge.

**TC** = Tissue culture fluid TCID50 10^-4.5

**1** Group I: Acute Uncomplicated Cases.

**2** Group II: Acute Complicated Cases.

**3** Group III(a): Mild or Refractory to primary experimental infection.

**4** Group III(b): Mild or Refractory reinfection.
### Table 2: Stability of IPV Virus Stored at -60°C

<table>
<thead>
<tr>
<th>Days Storage</th>
<th>TCID&lt;sub&gt;50&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>6.07*</td>
</tr>
<tr>
<td>18</td>
<td>6.07</td>
</tr>
<tr>
<td>20</td>
<td>6.07</td>
</tr>
<tr>
<td>24</td>
<td>6.07</td>
</tr>
<tr>
<td>39</td>
<td>5.50</td>
</tr>
<tr>
<td>50</td>
<td>5.63</td>
</tr>
<tr>
<td>94</td>
<td>5.83</td>
</tr>
<tr>
<td>164</td>
<td>5.50</td>
</tr>
<tr>
<td>226</td>
<td>4.50</td>
</tr>
</tbody>
</table>

* Reciprocal of dilution of IPV virus causing cytopathic change in 50% (TCID<sub>50</sub>) of inoculated cell culture tubes.
<table>
<thead>
<tr>
<th>Animal</th>
<th>Days Postinfection</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>4  7  8  9  12  13  15  16  19  22  26  30  31  32  44</td>
</tr>
<tr>
<td>Group 1</td>
<td></td>
</tr>
<tr>
<td>355</td>
<td>+   -  +   -   -   -   -   o   -   -   o   -   -   o</td>
</tr>
<tr>
<td>356</td>
<td>+   -  +   -   -   -   -   o   -   -   o   -   -   o</td>
</tr>
<tr>
<td>41</td>
<td>+   -  +   -   -   -   o   -   o   -   o   -   +   -   o   o</td>
</tr>
<tr>
<td>66</td>
<td>+   -  +   -   -   -   o   -   o   -   o   -   -   o   o</td>
</tr>
<tr>
<td>703</td>
<td>-   o   -   -   -   -   -   -   o   o   -   -   -   -   o</td>
</tr>
<tr>
<td>705</td>
<td>-   +   -   -   -   -   -   -   -   o   o   -   -   -   -   o</td>
</tr>
<tr>
<td>Group 2</td>
<td></td>
</tr>
<tr>
<td>980</td>
<td>-   +   -   -   -   -   -   -   -   -   -   -   -   -   -   -</td>
</tr>
<tr>
<td>Group 3(a)</td>
<td></td>
</tr>
<tr>
<td>18</td>
<td>o   o   -   o   -   -   -   -   -   -   -   -   -   -   -   -</td>
</tr>
<tr>
<td>19</td>
<td>o   o   -   -   -   -   -   -   -   -   -   -   -   -   -   -</td>
</tr>
<tr>
<td>3</td>
<td>o   o   -   -   -   -   -   -   -   -   -   -   -   -   -   -</td>
</tr>
<tr>
<td>452</td>
<td>o   o   -   -   -   -   -   -   -   -   -   -   -   -   -   -</td>
</tr>
<tr>
<td>Group 3(b)</td>
<td></td>
</tr>
<tr>
<td>355</td>
<td>+   -   o   -   o   -   -   o   o   -   o   -   -   o   -   -   o</td>
</tr>
</tbody>
</table>

+ Virus isolated
o Virus not isolated
- Not tested
Table 4: Antibody Response in Bulls Experimentally Infected with IPV Virus

<table>
<thead>
<tr>
<th>Animal</th>
<th>Weeks</th>
<th>Postinfection</th>
</tr>
</thead>
<tbody>
<tr>
<td>#</td>
<td>0</td>
<td>1  2  3  4  5  6  9  17  18  19  20  21  22  24  35  38</td>
</tr>
<tr>
<td>Groups 1 and 3(b)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>355</td>
<td>0  0  4</td>
<td>-  4</td>
</tr>
<tr>
<td>356</td>
<td>0  -  2</td>
<td>-  8</td>
</tr>
<tr>
<td>41</td>
<td>0  0  0  2  2</td>
<td>-  32</td>
</tr>
<tr>
<td>66</td>
<td>0  2  4</td>
<td>-  8</td>
</tr>
</tbody>
</table>

Group 3(c)

| 90 8 | -  32 |
| 6    | 2  64  64  64 | -  128 |
| 8    | 8  -  -  32 | -  64 |
| 18   | 0  -  16 |
| 19   | 16  -  2 |
| 3    | 8  -  64  32 |

* Reciprocal of dilution which neutralized 100 TCID<sub>50</sub>

_ Reinfected
_ Not tested
Figure 1: Typical Temperature Response in Bulls Experimentally Infected

with IPV Virus

- **Group 1** Acute Uncomplicated Case
- **Group 2** Acute Complicated Case
- **Group 3** Refractory Case

Day infected

Time in Days
Plate I

Penis and prepuce of a bull illustrating lesions of acute IFV virus infection 48 hours postinoculation.

Plate 2

Acute IFV virus infection in a bull illustrating lesions 72 hours postinoculation. Note the small central slough from many of the pustules and the tendency for lesions at the preputial reflexion to coalesce.
Plate 3

Illustrating acute IPV virus infection in a bull 96 hours postinoculation. Note ulcers surrounded by narrow border of hyperaemia.

Plate 4

IPV virus infection in a bull 144 hours postinoculation illustrating haemorrhage and the accumulation of pus; resolution has commenced.
Plate 5

Acute complicated case of IPV virus infection in a bull illustrating extreme swelling of the penis and prepuce.

Plate 6

Acute complicated case of IPV virus infection in a bull 10 days postinoculation. Note extensive haemorrhage, deep ulceration and the formation of a diphtheritic membrane on the body of the penis.
Plate 7

Penis and prepuce of a bull recovered from IPV virus infection showing hyperaemic areas over the lymphatic follicles which mark the site of the initial pustular lesions.

Plate 8

Pustular conjunctivitis after inoculation of IPV virus. Note the profuse, mucopurulent discharge at the medial canthus.
Plate 9

Early lesions of IPV virus infection in a biopsy taken from the preputial mucosa. A small central slough from the area of leucocytic infiltration is shown and inclusion bodies can be seen in some adjacent epithelial cells.

Plate 10

Ballooning and hydropic degeneration of epithelial cells after infection with IPV virus.
Plate 11

Ulcerative lesion of the preputial mucosa of a bull infected with IPV virus. Note intense inflammatory cell infiltration, sloughing of degenerate epithelial cells and inclusion body formation.

Plate 12

Higher magnification of the epithelial cells shown in Plate 11 to illustrate the eosinophilic, intranuclear inclusion bodies.
Plate 13

Hyperplasia of the submucosal lymphoid follicle observed as a sequela to genital infection with IPV virus in cattle. The discrete appearance of these lesions contrasts with the diffuse leucocytic infiltration observed in the acute stages of IPV virus infection.
Plate 14

Normal foetal bovine kidney monolayer illustrating two cell types - cells which grow in whorls and the larger flatter cells observed in the areas between the whorls. Some cells contain a single large nucleolus while in others the chromatin is granular and the nucleolus cannot be distinguished.

Plate 15

Foetal bovine kidney monolayer illustrating cytopathic effects 16 hours after inoculation of IPV virus.
Plate 16

Foetal bovine kidney monolayer 16 hours after inoculation of IPV virus illustrating large, oval, eosinophilic inclusion bodies.
Plate 17

Foetal bovine kidney monolayer illustrating cytopathic effects induced by IPV virus 24 hours after inoculation. Note the clusters of deeply-staining, rounded cells; few normal cells are present.

Plate 18

Foetal bovine kidney monolayer illustrating cytopathic effects induced by IPV virus 32 hours after inoculation. Most of the cells have fallen from the glass and those which remain are small, round, deeply-staining spheres in which no cellular detail is discernable.
Date Due

NON-CIRCULATING UNTIL 1962

NOV 7 1961
JAN 2 2 1962
APR 30 1962
MAY 3 1962
AUG 28 1962
NOV 15 1962
NOV 15 1962
JAN 22 1963
OCT 15 1963
MAR 23 1964

Firm 9/5/64

BOOK CARD

—THIS CARD MUST BE KEPT IN THE BOOK POCKET

—THE BORROWER WILL BE RESPONSIBLE IF CARD IS MISSING OR DAMAGED.
THESIS:
SF 756.T
St 94
1961
Studdert, Michael J.
Studies on infections pustular vulvovaginitis virus with particular reference to the genital disease in bulls.