A STUDY OF THE ETIOLOGY AND PATHOLOGY OF BOVINE NODULAR VULVITIS (VAGINITIS)

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By

Duane E. Ullrey

A THESIS

Submitted to the School of Graduate Studies of Michigan State College of Agriculture and Applied Science in partial fulfillment of the requirements for the degree of

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Affectionately Dedicated To

My Family

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TABLE OF CONTENTS

TABLE OF CONTENTS (continued)

INTRODUCTION

Nodular vulvitis has been known as infectious vaginitis, contagious granular vaginitis, vaginitis folliculorum chronica contagiosa, and/or nodular venereal disease for many years. However, the primary site of inflammation in the female is the vulva. Thus, the author prefers to differentiate between the vulva and vagina by referring to Sisson and Grossman (1938) as the authority. These writers use as a line of demarcation, the introitus vaginae. This is the narrow entrance to the vagina slightly anterior to the external urethral orifice and is continuous with the hymen in the young animal. This distinction will be adhered to throughout the thesis.

The importance of nodular vulvitis in relation to the reproductive performance of cattle has been subject to serious controversy since it was first described by Isepponi in 1887. Some veterinarians consider it to be closely related to sterility and often to abortion; others believe that it is only moderately important in this respect and its influence on conception is only of short duration; still others feel that the importance of this disease has been greatly over-emphasized and deny any relationship to infertility whatsoever. Failure to

resolve this controversy is principally a result of ignorance concerning the disease rather than a conflict of experimental evidence. Very few reports of original investigations concerning nodular vulvitis were found during a perusal of the literature.

The disease in Michigan has come to the attention of veterinarians more vividly since the widespread acceptance of artificial insemination. It is doubtful that the disease is much more prevalent than it was before. However, during study of reproductive difficulties encountered both in artificial and natural service and in an effort to compare the breeding efficiency of the two methods, the bovine genitalia have been more closely examined from a pathological viewpoint. In many cases of infertility, no observable lesions have been noted other than the presence of nodules in the vulva often associated with inflammation and sometimes with a mucopurulent exudate.

Because of the lack of information concerning this disease, a brief study of its etiology and pathology was undertaken in the hope that the few facts which might be accumulated would contribute to an eventual comprehension of their effects upon the host.

HISTORICAL REVIEW

In 1886 Isepponi (1887), a veterinarian living in the Canton of Chur, Switzerland observed a condition occurring in the vulva of cows and on the penis of bulls which was described as nodular elevations in the mucosa. Often associated with this condition was a vulvar or preputial catarrhal exudate. Numerous cases of bovine sterility and abortion were studied, and these were attributed to this condition of the vulva.

As this observation was made a decade before Bang's discovery of Brucella abortus, and at the same time European veterinarians were troubled by an increasing prevalence of bovine breeding troubles, great interest in this announcement was aroused, and many atuhorities were prone to declare that infectious vaginitis was the crux of the difficulty. When Bang announced his discovery ten years later, a great controversy arose concerning the relative importance of Brucellosis and infectious vaginitis in causing sterility and abortion.

In 1898 a German worker, Ostertag (1901), received a heifer said to be affected with infectious vaginitis. The farmer who owned her, after many breeding attempts, believed that she had not conceived and requested Ostertag's advice. This veterinarian isolated what was

described as gram-negative diplococci or streptococci from the mucopurulent exudate of the vulva. Chains of six to nine organisms were often found. Using a pure culture of the organism, Ostertag inoculated the vulvae of cattle, sheep, goats, swine, horses, guinea pigs, and rabbits, producing symptoms of the disease only in cows. An attempt to transmit the disease to a bull was unsuccessful. The original heifer, after three months of investigation, was slaughtered and found to be four months pregnant. In a later instance, Ostertag isolated a gram-negative streptococcus from the uterus of a cow with infectious vaginitis.

In a study of the disease, Liebetanz (1901) noticed swelling and redness in the mouths of new born calves; pneumonia and an intestinal catarrh followed by death. This worker made the assumption that the etiological agent of vaginitis infected the calf as it passed through the diseased genital tract at birth. However, Frohner and Kitt (1901), editors of the Monatshefte fur Praktische Thierheilkunde, note in a subscript attached to Liebetanz's article that both Ostertag and Jensen (Denmark) bacteriologically examined a calf which Liebetanz believed was infected in this manner and diagnosed the condition as Kalberruhr or common calf scours.

Merchant (1950) states that in 1903, Hess edited a report of the Swiss Veterinary Surgeons' Society,

giving a complete summary of knowledge of the disease up to this time. Hess reported that the etiological agent was a gram-negative streptococcus with a delicate capsule, often occurring in chains of six to ten organisms. This bacterium produced typical colonies on agar (although they were not described), clouded bouillon, and did not acidify milk sufficiently for coagulation. If acids were produced in carbohydrate media at all, it was in an unappreciable quantity. The organism was often associated with colonies of micrococci and Bacterium coli, occurred extracellularly, and its presence in the depths of the mucosa was considered diagnostic. This streptococcus was apparently specific for the bovine and produced vaginitis by infection of the lymph follicles, followed by formation of a purulent exudate. The glans penis of the bull was similarly affected, and purulent material was often seen exuding from the prepuce.

Thoms (1906) reported that cattle of all ages could be affected by infectious vaginitis. This worker stated that it was always possible to demonstrate numerous diplococci and short streptococci in the vulvar discharge. It was believed that the infection was closely assoicated with coitus and that the incubation period was often as brief as twenty-four hours after breeding. A relatively complete histological study of the infected vulva was made.

Also studied were the vulvae of a seven year old cow and a calf of ten weeks which were considered normal. Several days (usually four to five) after experimental inoculation, small nodules appeared in the vulva. These occurred most prominently in the ventral wall where lymph follicles could normally be found in considerable abundance. The nodules were particularly numerous around the clitoris and often were arranged in rows upon the longitudinal folds of the vulvar mucosa. The nodules were one to two millimeters in diameter and consisted of masses of lymphocytes frequently intersected by small blood vessels. In the tissues surrounding these lymph follicles, a lymphocytic infiltration was observed.

Also presented in Thom's report were several photomicrographs which effectively demonstrated the varied histological picture. In one photograph, a single lymph follicle raised the mucosa. In another case, four follicles in close apposition caused a broad mucosal elevation. A section of the dorsal vulvar wall produced an instance where a series of nodules did not raise the mucosa at all.

Thom's believed that the nodules were formed by a mucosal infiltration of lymphocytes arising from the normal lymph follicles already present or from follicles which formed in the cytogenic tissue of the tunica propria.

After the disease had reached its climax, the nodules began to decrease in size. However, they did not always disappear completely. It was thus concluded that healing of the disease was not necessarily connected with complete disappearance of the nodules.

Raebiger (1907) and Juterbock (1909), as well as others, purportedly verified Ostertag's findings by reproducing the disease in cows with intravaginal inoculation using a similar streptococcus. These German workers knew infectious vaginitis as ansteckend Scheidenkatarrh, differentiating it from coital exanthema or Blaschenausschlag.

While these investigations were being made, the controversy waxed stronger and stronger between those who felt that Bang's disease was of greatest importance in causing sterility and abortion and those who believed that infectious vaginitis created the most difficulty. This conflict of opinion apparently reached its acme in 1909 when the two best known writers upon sterility vigorously supported the views of their countrymen. At the Ninth International Veterinary Congress in The Hague, Hess of Switzerland expounded the findings of Isepponi, while Albrechtsen of Denmark championed the convictions of Bang. The contribution of these two men at The Hague conference seemed to produce more harm than good for the vehemence initiated between later investigators often precluded scientific observation. Much of the dispute in

the writings of Williams (1914), McFadyean (1926), Edwards (1933), and others, was trifling as well as eristic.

During the same year as the Veterinary Congress, Blaha (1909) reported sickle-shaped bodies with eccentrically placed nuclei, located in the epithelial cells of the nodules. These resembled the trachoma bodies found in contagious granular conjunctivitis. Blaha concluded that the infectious agent was a chlamydozoan.

Williams (1909) announced that infectious vaginitis had been discovered in the United States. The disease was called to this investigator's attention in several New York state dairy herds where it was evidently producing considerable breeding trouble. Since little was known about the disease in North America, Williams embarked upon a rather extensive field study. The observations were reported five years later in a government bulletin (Williams 1914). One phase of this study included post-mortem examination of bovine genitalia on the killing floors of abattoirs in some of the major United States slaughtering centers. These included Chicago, Omaha, Kansas City, Denver, and Fort Worth. Williams' objective was to determine the incidence of the disease among cattle of beef type. Visible presence of nodules in the vulva was used as a basis of positive diagnosis. Out of 3,250

females examined, 2,806 exhibited visible nodules, while 444 were negative. Over 86% of the examined animals were diagnosed positive for infectious vaginitis. Williams also grouped the examined animals into age classes. The results of the study are shown in Table I.

TABLE I

WILLIAMS' STUDY OF THE INCIDENCE OF INFECTIOUS VAGINITIS IN CATTLE SLAUGHTERED FOR MARKET

% Diagnosed Positive	Class
61	Veal calves, 6 wks. - 1 yr.
68	Spayed heifers
95	Cows presumed bred, 1-4 yr.
85	Cows over 4 yrs.

No entire lot of slaughtered animals was found free of the disease, but occasionally an individual would appear negative. It was this group of occasional individuals that comprised the 14% exhibiting no infectious vaginitis. It was Williams' opinion that the incidence of the disease among beef cattle was considerably less than its occurrence among the dairy herds of New York. This was explained by the fact that dairy animals were usually kept in closer relation to one another, affording greater opportunity for disease transmission.

Dairy animals in New York, Nebraska, Arkansas, Pennsylvania, Ohio, Illinois, Missouri, Minnesota, and some European countries were examined but nowhere was a herd found completely free of this disease. From these examinations, Williams' concept of the development of infectious vaginitis was formulated, and this concept is herewith transcribed.

Heifer calves developed nodules at four to twelve weeks of age. Development of these nodules were forestalled for at least a year by feeding boiled milk from birth onward and by isolating the calf from infected animals. After the nodules developed and throughout the adult life of the individual, the disease exhibited vacillations of intensity so great that various writers designated these manifestations as acute, subacute, chronic, sound, cured, et cetera.

The nodules were one to two millimeters in diameter and were colorless to faint yellow in the center, and often presented the appearance of small, tense vesicles, although when examined closely, they contained no fluid. A zone of increased vascularity around the base of each nodule resembling a vascular girdle sometimes occurred.

In the early stages of the disease except for the nodules and vascular girdles, the mucosa was smooth, pale, rose colored and quite normal. There was no swelling or inflammation, and no mucous or mucopurulent discharge.

The number of nodules increased slowly with age up to puberty or estrum when increased vascularity and functional activity apparently favored a more rapid multiplication of nodules. The symptoms of the malady were intensified but did not become as severe in the virgin heifer as after copulation. The nodules had no particular arrangement until they became quite numerous, at which time they were formed into longitudinal, parallel rows, corresponding to the long folds of the vulva. They were situated principally upon the summits of the rugae and swelling of the vulva made these folds more prominent. The individual nodules increased little in size or projection, but the vascular girdles became more congested and an increased vascularity sometimes extended rather completely over the surface of the nodules, so that they appeared as bright red elevations or as petechiae on the vulvar mucosa. The mucosa between the nodules often became reddened and swollen.

Until copulation the disease behaved as though in a state of dormancy. However, under the stimulus of sexual contact, the mucosa became scarlet, swollen, tender, and in a large number of cases a very notable mucopurulent discharge was observed adhering to the vulvar tuft. The vulvar lips were frequently swollen and edematous. Covering the floor of the vulva were strings of a semiopaque pus. The nodules multiplied rapidly, often became semiconfluent, lost their transparency and became deep red in

color. Even this severe condition did not seem to affect the general health of the heifer. The intensity of the symptoms increased for a few days, remained static for a time, then tended to slowly recede. However, the appearance of the vulva never quite approached the condition before coitus.

If the heifer became pregnant, the nodules remained prominent, although a reduction in number sometimes occurred and the inflammation slightly abated. Essentially the disease remained static until just before parturition when edema of the mucosa partially or completely obscured the nodules. The nodules were still observed by digital palpation and usually reappeared a short time after birth of the calf.

If the heifer failed to conceive, and the sterility was refractory, repeat breedings aggravated the condition and sterile heifers were quite generally the worst clinical cases in the herd.

Symptoms of the disease acquired at the first pregnancy continued unabated through the second and third pregnancies when the condition began to subside. When the cows reached eight or nine years of age, the decrease in intensity of the lesions was quite marked. The nodules became fewer, were less prominent and more transparent, and the inflammation and any mucopurulent exudate had nearly or completely disappeared. Clinical evidence of the

disease vanished when the cow was twelve to fifteen years old.

Williams found that all cows recently bred could generally be identified by the severe vulvar lesions. This investigator did not believe that nodules in the vulva were normal structures and never observed them in a newborn calf. Furthermore, it was not Williams' belief that infectious vaginitis was primarily pyogenic. Rather, it was reasoned that the swollen vulvar folds rubbed together, denuding the epithelium and permitting entrance of streptococci and other pyogens. Williams' writings favor a protozoan etiology such as was proposed by Blaha, for this author states that the nodules disappear numerically while in bacterial infections, lesions usually subside volumetrically; thus, "such (a) conclusion is in agreement with the clinical character of the disease" (Williams (1943)).

Starr (1924) cultivated a streptococcus of the viridans type from the vulvar exudate of cows affected with vaginitis. He also noted that the nodules on the vulvar mucosa were produced by hyperplastic lymph follicles as a result of irritation.

McFadyean (1926) criticized those European veterinary writers who had given wide publicity to the alleged significance of infectious vaginitis as a cause of sterility. It was McFadyean's belief that the presence of nodules in the vulva was entirely normal. The censure of these authors

was so severe that Edwards (1933), who accepted McFadyean's report, was prompted to describe this publicity as "one of the most discreditable chapters in recent veterinary history".

During the next year, Jones and Little (1927) published a report of the first thorough clinical, pathological study of infectious granular vaginitis in North America. These workers found streptococci and micrococci in the vulvar exudate of diseased cows. However, the concentration of organisms was not considered sufficient to account for the lesions. When rapidly dried films of the exudate were fixed three to five minutes in methyl alcohol and stained with Giemsa, tiny delicate rods with polar granules were demonstrated. In many instances the cytoplasm between the granules stained feebly or not at all, so that the organisms resembled diplococci. When the exudate was cultured on ordinary media, streptococci, micrococci, B. coli, and long, slender, gram-positive rods appeared. Using agar slants prepared from veal infusion to which five tenths of a cubic centimeter of defibrinated horse blood was added after cooling, inoculating by the quantitative dilution technique, and sealing with wax, bipolar rods were obtained in pure culture.

This organism was found to be gram-negative and non-motile. Its morphology varied. Often it appeared as tiny coccoids and/or tiny rods with well defined granules. Sometimes it could be found in clumps or filamentous chains.

The bacilli in the exudate were one to two micra in length. The coccoids were approximately one third micron in diameter and were believed to be polar granules where the central zone and cell wall did not stain. This organism did not ferment dextrose, lactose, saccharose, maltose, or mannitol. Inoculated milk was unchanged, and there was no indole production. No pathogenic reaction was produced in rabbits when they were injected intravenously. However, two cubic centimeters of a blood, broth culture injected intraperitoneally into a three hundred gram guinea pig resulted in death.

In an attempt to reproduce the disease in the bovine, three to four months old heifer calves and two year old heifers were inoculated on the vaginal mucosa with a freshly isolated culture of the gram-negative rod. Acute inflammation resulted in two to three days followed seven to eleven days later by the appearance of nodules on the mucosa. An attempt to reproduce the disease in older cows was not successful and was explained on the basis of acquired resistance. Jones and Little were not able to produce symptoms of infectious granular vaginitis when a nonhemolytic streptococcus isolated from the exudate was used for inoculation.

These workers also described the disease process as it appeared to them. The bacilli attacked the vulvar mucosa in certain foci resulting in necrosis of the epithelium and some fibrinous exudation. Leucocytes

invaded the mucosa in large numbers while lymphocytes became particularly numerous in the submucosa. The exudate and mucosa sloughed, followed by regeneration and an accumulation of lymphocytes in follicle-like masses in the submucosa.

In a report of research into sterility of cattle in South Africa, Quinlan (1928) reported that infectious vaginitis was first recognized in Natal in 1912 or 1913. However, no intensive study of the disease was made until 1920 when the prevalence of sterility in some pure-bred herds again brought the disease to the attention of the South African Department of Agriculture.

Quinlan differentiated between a peracute and an acute stage where the exudate was quite profuse. A chronic stage was stated to follow in which there was little exudation and the nodules began to disappear. This transition to the chronic stage sometimes extended over a period of just a few weeks while at other times, the modification required several months. Quinlan did not believe the disease to be infectious during this chronic stage. Neither were the nodules considered of pathognomonic importance since they were said to occur in non-contagious forms of colpitis.

In an attempt to establish whether there was a relationship between infectious vaginitis and sterility, Quinlan reviewed some of the literature on the subject.

Schunhoff (Cited by Knell (1926)) believed that the spermatozoa failed to reach the ovum because irritation of the vagina during coitus produced a reflex action that resulted in cramplike closure of the ostium uterinum externum. Ellinger (Cited by Knell (1926)) apparently believed that irritation of the vagina caused premature ejaculation of the semen through straining. The spermatozoa were thus expelled before they could reach the uterus. Pfeiler (Cited by Knell (1926)) maintained that the endocrine system associated with conception and pregnancy was influenced by the disease. Wester (1921) and Knell (1926) were certain that the unphysiological secretion of infectious vaginitis destroyed the spermatozoa before they could reach the oviducts. Schlicte (Cited by Knell (1926)) secured normal spermatozoa from the vagina of a cow after service. The sperms were mixed with normal vaginal secretion of a healthy cow, with physiological saline, and with the vaginal secretion of a cow with acute infectious vaginitis. The results are presented in Table II.

TABLE II

SCHLICTE'S IN VITRO STUDY OF THE EFFECT OF PHYSIOLOGICAL SALINE AND VAGINAL SECRETIONS ON VIABILITY OF SPERMATOZOA

Tested Medium	Viability of Spermatozoa
Physiological saline	3 hrs.
Vaginal secretion (normal)	¾ - 1½ hrs.
Vaginal secretion (vaginitis)	½ hr.

Wester (1921) was reported to have produced the same results in a similar experiment.

Quinlan (1928) concluded that the acute form of infectious vaginitis associated with careless sexual hygiene and irrational treatment led to extension of the disease to the anterior vagina and the production of cervicitis. The result was often temporary sterility and frequent returning to the bull. This veterinarian believed that treatment of the disease was not indicated in the abscence of sterility or during pregnancy. If treatment seemed advisable, careful clinical and serological examination should be made as a precaution against other diseases which cause sterility and might concurrently exist.

Quinlan, Mettam, and Bisschop (1929) attempted to reproduce the disease experimentally using vaginal secretions and microorganisms isolated from the vagina but were unsuccessful.

Diplococci, indistinguishable from those found in the normal vagina, were isolated by Ispolatow (1929) from cows believed to be affected with infectious vaginitis. The local application or intravenous inoculation of these organisms was thought to produce inflammation of the vaginal follicles. However, this worker did not conclude that these diplococci were the primary cause of the naturally observed condition.

The antithetical nature of published opinion on the relation of infectious vaginitis to sterility was again demonstrated when during the same year and in the same publication, Pomayer (1930) stated that this disease produced a sensitiveness of the clitoris after coitus which caused straining and extrusion of the semen; and Abelein (1930) wrote that Pomayer's belief was ill-founded.

Webster (1932) claimed that the nodular or granular condition on the floor of the bovine vagina, especially prominent around the clitoris, was physiological and especially evident during preestrual periods. It was also stated that the condition was sometimes seen on the penis of bulls. This author believed that the frequent association of infectious vaginitis with sterility was due to the failure of earlier investigators to study sufficient herds where sterility was not a problem and other breeding disease did not prevail.

Edwards (1933) of the Lister Institute presented a paper before the Royal Society of Medicine in which infectious sterility in the larger domesticated animals was discussed. Included in the report was a critical review of some of the veterinary literature concerning vaginitis. Edwards was very uncompromising in the belief that the nodular condition of the vagina was entirely normal. Those veterinarians were severely censured who dared suggest that

infectious vaginitis possessed some pathological significance. This investigator was of the opinion that there were some morbid conditions affecting the bovine vagina which were generally mild and transient in nature and at times produced metritis and sterility. However, none of these conditions embraced the nodules in the vagina.

The same year, Mitchell et al. (1933) published a report of a bacterial study of acutely infected cattle in Canada. Protozoa were not found, although many other organisms were observed. A Leptothrix was at first believed to have produced the disease, but it was found that some other infective agent had apparently been carried along simultaneously during the first three generations of the fungus. These workers failed to transmit the disease by swabbing the normal vagina with porcelain filtered washings from the vaginas of infected cows. Infected material given orally and inoculated in the conjunctiva did not produce infectious vaginitis, nor did intravenous injections of blood from animals exhibiting the disease. Vulvovaginal swabbing of a normal animal with untreated infected material produced typical lesions as early as five to six days.

Sisson and Grossman (1938) referred to lymph nodules in the mucosa of the normal bovine vulva. The nodules were said to be especially prominent in the ventral floor and often large enough to cause visible prominences.

Brown (1942) believed that granular vaginitis was more serious than Bang's disease in 1,500 animals representing 16 herds in New York. The first indication of the disease was a vulvar discharge, sometimes yellow in color, but most often clear to white. This discharge seemed to increase in volume two to three days after breeding. Palpation of the vulva revealed groups of small pimples which extended over the vagina to the cervix in the more serious cases. Other symptoms were found, varying with the intensity of the disease, such as sterility of several cows, a sterile bull, irregular heat periods, and cows which had apparently conceived and then after a short time again exhibited heat. A few days before this heat period, there was a discharge of pus which was considered an indication of abortion. The abortion occurred two to three months after breeding and the embryo might or might not be found. Occasionally abortion of a mummified embryo occurred at four to six months of pregnancy.

This investigator gave credence to the probability that the bull aggravated the condition by service and was one means of spreading the disease. However, it was Brown's belief that vaginitis was spread in other ways, for the disease was noted in one new-born calf and calves of all ages.

Brown adapted a treatment for the disease from that used against vaginal trichomoniasis in humans. A compound

silver picrate powder (one per cent silver picrate dispersed in kaolin) was dusted into the vagina three times a week. A modified Shelanski insufflator with a veterinary tip found use as an applicator. Breeding was always halted during treatment, and the results obtained proved better than any other method studied.

Williams (1943) noted that occasionally nodules similar to those observed in infectious vaginitis of the bovine were observed in small numbers in the vulvae of swine and sheep.

Bryan (1944) reported that infectious vaginitis was difficult to completely eradicate from a herd because little or no immunity was produced by an attack of the disease.

Cunkleman (1948) stated that out of 683 cows with breeding troubles examined in Michigan, 594 or 86.9% of them had nodular vaginitis and 557 or 81.5% were severe enough to interfere with conception. It was observed that the inflammatory condition sometimes extended forward and involved the cervix, while a sympathetic metritis was often found on rectal examination.

In 1947 Durrell (1949) and his co-workers isolated a beta hemolytic streptococcus from both normal and infected cows. This organism was swabbed on both non-scarified and scarified vulvar mucous membrane of a normal animal, but they were unable to reproduce infectious vaginitis.

In another experiment, a sterile needle was used to prick the lesions of a diseased animal and then pricked into the normal vulvar mucosa of an Angus yearling which had not been bred and was isolated in a separate unit. In ten days, "pimple-like" elevations and ecchymoses in the mucosa was observed. This experimental case reached puberty two weeks after inoculation, and it was found that although the nodules and congestion subsided during diestrus they again became more severe with the advance of proestrus. During an interval of two months from the first observation of five or six "pin head" nodules, 182 nodules developed, all approximately the size of a small match head. The acute course of the disease lasted about three months, and then the nodules almost, but not entirely, disappeared. In cows, it was observed that edema of the vulva obscured the nodules in late pregnancy.

From reports received during a field study of the disease, Durrell observed that after the initial outbreak in a herd, it was usually just the heifers which exhibited infection annually, and these symptoms became especially evident shortly after the beginning of the breeding season. It was concluded that bulls or cows sometimes became chronic carriers, and a degree of local immunity was possibly induced.

Using figures compiled in 1946, Durrell calculated that the monetary loss, as measured by loss of milk, calves,

and cows, on each cow affected with sterility of an average duration of three and one half months, amounted to sixty-three dollars. Since this author believed that a herd problem of infectious vaginitis often delayed conception for about three months, the economic loss to the individual farmer was very severe.

Suggested measures for control were segregation, suspension of breeding for two to three months, and the use of artificial insemination to prevent further spread.

No specific attempt was made to include a historical review of treatments of infectious vaginitis in this report, since the thesis was not concerned with therapeutics. However, such a complete list of remedies was tabulated by Durrell, that this table was included on the following page for the reader's convenience. It should be recorded that this author did not consider infectious vaginitis very amenable to treatment and stated that a specific therapeutic agent had not been definitely recognized.

The Report of the Ontario Veterinary College for 1949 included a brief reference to an "H germ" frequently isolated from cattle with granular vaginitis, which appeared to be of an infectious nature. Also included was a compilation of data gathered by Dr. C. A. V. Barker, concerning 134 cows from 25 different herds with poor breeding histories. No animals serologically positive for brucellosis were studied. Clinical examination revealed

TABLE III

TREATMENTS FOR INFECTIOUS VAGINITIS (DURRELL (1949))

Treatment and Method	Reported Value[1]
Aqueous douches (1/2-1 gal. daily or weekly)	
Chlorine solution - Case (1935)	++
Zinc sulphocarbolate, 1:1000 Gould et al. (1942)	++
Potassium permangenate, 1:500[2]	++
Normal saline - Gould et al. (1942)	+
Normal saline with 0.25% Lugol's sol. of iodine or 0.50% carbolic acid - Williams (1943)	+
Sodium bicarbonate 1%[2]	+
Boric acid, saturated sol. - Knapp (1932)	-
Injections (2-3 ozs. in vagina 3 times daily)	
Azamine, 5 gms. in 1 gal. mineral oil - Knapp (1932)	+++
Argyrol (5%)[2]	++
Iodized oil - Cunkleman (1948)	+
Ichthyol, 10% in oil - Knapp (1932)	-
Powder insufflations (5 gms. 3 X weekly, 1-3 weeks)	
Silver picrate (1%) in kaolin - Brown (1942), Folger (1944)	++
Bismuth formic iodide - Bryan (1944), Cunkleman (1948)	++
Sulphanilimide - Bryan et al. (1944)	+
Iodochlorhydroxy-quinoline, e.g., "Vioform" (Ciba)[2]	+
Swabs (usually twice weekly, 3 weeks)	
Silver nitrate (1%) and glycerine, equal parts[2]	+++[3]
Gentian violet, saturated aqueous sol. once weekly, 3 weeks - Moore (1947)	+++
Potassium iodide 1, iodine crystals 1, distilled water 2, single treat. - Moore (1947)	+++
Acriflavine, 1:500[2]	++
"Vioform" in oil[2]	++
Lugol's sol. of iodine and glycerine, equal parts[2]	++
Silver nitrate 5% followed in a few days by 1% acriflavine ointment 2-3 times at 5-10 day intervals - Fincher (1948)	++
Acriflavine ointment 1% with vaseline and mineral oil, equal parts - Bryan et al. (1944)	+

[1] +++ indicates good results as reported by the author or practitioner.
- indicates no value as reported by the author or practitioner.

[2] Quoted from unpublished reports from practitioners.

[3] Causes severe straining.

TABLE III (continued)

Treatment and Method	Reported Value
Miscellaneous	
Autogenous bacterin[4] - Hornaday (1947)	+++
Benzoated H_2O_2 and colloidal silver proteinate in a glycerinated base - Groulade (1947)	+++
Kaolin quaternary ammonium adsorbate with an estrogenic substance, applied topically - Cunkleman (1948)	++

[4]Prepared by Ashe Lockhart, Inc., Kansas City 6, Mo. from the streptococcus originally described by Ostertag.

TABLE IV

STERILITY INVESTIGATIONS - CATTLE (DATA BY BARKER (1949))

Condition Observed	No. of individuals
Infectious vaginitis	55
Pregnant	18
Normal	18
Congenital	13
Cystic follicle	9
Metritis	8
Functional (no lesion)	5
Trichomoniasis	4
Retained corpus luteum	1
Vaginal prolapse	1
Vaginal stenosis	1
Infertile bull	1
Total	134

the conditions recorded in Table IV (page 26).

The sixty-second Annual Report of the Rhode Island Experiment Station (1949) made record of a study of vaginitis and sterility in cattle. All animals in the college herd were being routinely examined for the disease. Ninety-six per cent of the females beyond six months of age, and 33% under six months exhibited symptoms at some time or other. The condition had been noted in calves as young as six weeks of age.

An attempt was made to isolate an infectious agent from vaginal scrapings which would produce observable lesions in chicken embryos. The vaginal material was treated with streptomycin to destroy any bacteria and was injected into seven day old chicken embryos. Semen was also studied in the same manner. Some of the embryos in both cases were dwarfed or died although no bacteria were present. Yates (1950) in a personal communication indicated that sufficient evidence had not yet been gathered to incriminate a virus.

Crawley, Wills, and MacGregor (1950) reported that a systematic study was proceeding at the Ontario Veterinary College to establish the etiology and importance of nodular vaginitis.

Examinations for bacteria, protozoa, and spirochaetes were made in two herds of high breeding efficiency

to determine the normal microbiological population of the vulva and vagina. During this study some cows with nodular vaginitis were found, and by comparison with the normal, it was observed that a particular organism seemed to prevail. This organism was also isolated from calves and unbred heifers exhibiting nodular, inflamed vulvae, as well as from the sheath washings and semen of bulls.

This organism was difficult to classify for its pleomorphism and dependence on blood for primary isolation suggested the genus Hemophilus; while on subculture, the requirement for the X and V factors was not definite, and it appeared that other factors might be required either alone or in addition. Its morphology and carbon dioxide requirements suggested a relationship to Streptobacillus moniliformis (Levaditi), Haverhillia multiformis, and Actinomyces muris.

The organism was a gram-negstive, non-acid fast, medium sized rod, non-motile, and non-spore forming. It was very pleomorphic, appearing as bipolar rods, singly or in filamentous chains, or in coccobacillary or coccal forms. The bacterium underwent lysis rather quickly on artificial media.

Colonies of the organism were one half to one and one half millimeters in diameter. The smaller colonies were translucent, colorless, low convex or slightly conical, or sometimes umbonate. Larger colonies were usually similar

but sometimes semi-opaque and gray. Massive growth was grayish-white or yellow. Certain strains produced a slight clearing of blood agar directly under or near the colonies.

The organism produced lesions and death in mice and chicken embryos, but was non-pathogenic for rabbits, guinea pigs, and hamsters.

Penicillin, streptomycin, and aureomycin generally inhibited all strains of the organism in vitro.

A study was also made of the correlation between isolation of the organism and the symptoms of vaginitis. Table V illustrates the incidence of the organism in relation to inflammation and nodules.

TABLE V

INCIDENCE OF THE ORGANISM AND ITS RELATION TO INFLAMMATION AND NODULES

Classification	No Inflammation		Inflammation	
Nodules	Organism not isolated	Organism isolated	Organism not isolated	Organism isolated
None	50	7	3	7
1+	17	13	3	14
2+	5	3	4	12
3+	4	2	2	13
4+	1	1	3	13
Totals	77/103	26/103	15/74	59/74
%	75	25	20	80

Tables VI and VII compare herds of low breeding efficiency where vaginitis was present and herds of high breeding efficiency where vaginitis was not considered a herd problem. Table VI shows the relation of positive isolations to inflammation while Table VII compares isolation of the organism to the presence of nodules.

TABLE VI

CORRELATION BETWEEN ISOLATION OF THE ORGANISM AND INFLAMMATION

Herd	No Inflammation	Inflammation
"Normal"	3/47* - 6%	12/16 - 75%
"Vaginitis"	25/56 - 41%	47/58 - 81%

*Number positive isolations/number of cows.

TABLE VII

CORRELATION BETWEEN ISOLATION OF THE ORGANISM AND NODULES

Herd		No Nodules	1+	2+	3+	4+	Total
"Normal"	No.	3/36*	7/14	3/9	1/3	1/1	12/27
	%	8	50	33	---	---	44
"Vaginitis"	No.	11/31	20/33	12/15	14/18	13/17	59/83
	%	35	61	80	78	76	71

*Number positive isolations/number of cows.

By applying vulvovaginal scrapings from an infected animal to the lightly scarified vulvar mucosa of three

heifers, pus and inflammation were produced in three to nine days. Nodules began to appear between the fifteenth and twenty-first days. The intensity of these symptoms gradually increased, reaching a maximum in about eight weeks.

The disease was reproduced twice by using pure cultures of the previously described organism isolated from a heifer infected with vulvar scrapings. A saline suspension of this organism was applied to the lightly scarified vulvovaginal mucosa of two normal heifers. In one case pus appeared in four days and nodules were observed three weeks later. No pus was evident in the other heifer, but hyperemia was observed in ten days and nodules appeared on the fifteenth day. The organism could be reisolated in almost pure culture. Two control animals remained unaffected.

Histological examination of both natural and experimentally produced lesions revealed hyperplastic lymph follicles with an infiltration of mononuclears and eosinophils in the surrounding tissue. Increased vascularity and numerous mitotic figures were also noted.

These authors stated that no direct evidence had incriminated vaginitis as a cause of sterility, however it appeared that a temporary infertility was often associated with this condition.

MATERIALS AND METHODS

Clinical Observations

A field examination of 89 dairy cattle representing six different farms was conducted during the course of this study, to determine the incidence of nodular vulvitis in herds with recognized breeding difficulty. An attempt was made to swab the genital tracts of those animals which best represented the pathological picture in the herd. Some animals of necessity showed no visible lesions, while others were most severe.

Observations of the state of the vulva, whether diseased or normal, were made simply by parting the lips with the thumb and forefinger, while an assistant directed a flashlight beam on the area. The presence of few nodules was designated 1+, while semiconfluent nodules were given a 4+ rating. The remaining ratings, 2+ and 3+, were graded in between. Inflammation was described as being slight, moderate, or severe. Other lesions, such as suppuration or hemorrhage were also noted. A few animals were examined by rectal palpation and with a vaginal speculum, to establish the condition of the remaining genital tract.

Isolation Technique

Just before going into the field, 0.75 ml of sterile, tryptose broth was added to tubes containing sterile, cotton swabs. This broth seemed to be a more satisfactory medium for bacterial transportation than did vibrio diluent (Wilson (1949)).

The vulvae of 70 cows were examined bacteriologically by removing the sterile swabs from the tubes, revolving them slowly against all vulvar walls, withdrawing and replacing them in the stoppered tubes.

The bacterial population of the preputiae of two bulls was sampled by using a sterile, plastic, insemination tube fitted at one end with a rubber bulb. The tube was inserted into the prepuce, and a bit of smegma was aspirated. This smegma was then expirated into a culture tube containing the same amount of sterile, tryptose broth as used for the vulvar swabs.

The swabs and smegma were returned to the laboratory usually within three hours. Distribution of any bacteria on the swabs into the broth was assured by moderate shaking of the tubes. Fluid and mucous was removed from the swabs by pressing them against the side of the tube, and then the swabs were withdrawn and discarded.

During the initial portion of this investigation, semi-solid thiol, tryptose agar slants, and tryptose broth

were inoculated with the fluid, but these media proved impractical for isolation of pure cultures. Blood agar, broth base slants were inoculated serially through four tubes to dilute the amount of inoculum. However, this method also proved to be cumbersome and unreliable. Results with these media were not improved by incubation under anaerobic conditions. Jones and Little (1927) and Crawley, Wills, and Macgregor (1950) reported the presence of a microaerophilic bipolar rod frequently associated with infectious vaginitis. Isolation attempts were then directed to the use of blood agar plates incubated under reduced oxygen tension.

A two millimeter platinum loop was used to transfer a portion of the fluid to the blood plate. This was streaked in the conventional manner. The plates were inverted and placed in an air-tight jar together with an "alcohol burner". This "alcohol burner" consisted of a small can in which cotton and 15 to 20 drops of alcohol were placed. The burner was ignited and immediately the lid was placed on the jar and sealed. This resulted in the desired reduction of oxygen tension. The jar was incubated for 48 hours at 37° C., and the plates were examined for the presence of growth using a scanning microscope and reflected illumination as described by Huddleson (1946). If the desired organism was present, a single colony was transferred to a blood agar, broth

base slant. The cotton stopper was ignited and while it was still burning, a rubber stopper was inserted into the mouth of the tube. A moderate growth was evident after 48 hours of incubation at 37° C. The cultures were stored at room temperature and transferred every two to four weeks as the organism underwent lysis rather quickly.

Biochemical Reactions

Organisms isolated from the vulvae of three cows affected with nodular vulvitis and from the abomasa of two fetuses aborted from three to five months after conception, were tested for their biochemical and fermentation reactions. All media used were prepared in accordance with the Manual of Methods for the Pure Culture Study of Bacteria (1946). Two trials were conducted and for the second trial, 0.5 ml of sterile ovine blood serum was added to each culture tube before inoculating. The media was incubated for 14 days at 37° C. under microaerophilic conditions obtained by the method used for isolation.

Smear Preparation

Most of the bacterial smears were air dried, heat fixed, and stained with Hucker's modification of Gram's stain. On occasion, smears were either heat fixed or fixed in equal parts of ether and 95% alcohol for three to five minutes and stained with Giemsa stain or methylene blue. Wright's stain was also used for staining direct

smears of the vulva, vaginal mucous, and abomasal contents.

Curette Scrapings

Scrapings of the vulvar epithelium were made in four cases of nodular vulvitis in order that a bacterial stain might be employed to reveal possible intracellular organisms. Two normal vulvae were also curetted. Most of the scrapings were applied directly to slides. However, in one instance, a curetting was placed in 1.0 ml of vibrio diluent along with several sterile glass beads. This mixture was violently shaken in a paint mixer to macerate the tissue, and a smear was made of the suspension. All the curettings were fixed and stained using the procedures listed for smear preparation.

Biopsies

Three biopsies were performed on the vulvae of two different animals. The excised flesh was taken from the nodular area on the ventral floor, two to three centimeters anterior to the clitoris. One tissue was fixed in Zenker's fluid (Mallory (1938)), and the other two were fixed in FAA, a modification of Lavdowsky's mixture (Guyer (1936)). This latter fixative contained 500 cc. of 50% alcohol, 65 cc. of 40% formalin, and 25 cc. of glacial acetic acid, and was found to prevent undue hardening of the tissues.

Autopsies

The genital tracts of 14 cows and calves were examined at autopsy. Lesions were noted, as well as the presence of follicles or corpora lutea in the ovaries. If possible, the vulvae of these animals were observed before death so that the ante mortem condition could be better correlated with the post mortem findings. The standard procedure adopted was to take tissues from the following areas for histological examination: ovary, oviduct, uterine horn (caruncular and/or intercaruncular space), first annular ring of the cervix, anterior vagina (two to four centimeters posterior to the cervix), and the vulva. These tissues were fixed in either Zenker's fluid, 10% formalin, or FAA.

Tissue Preparation

The tissues were imbedded in paraffin, sectioned at seven micra, cleared in xylene, and stained with eosin and hematoxylin to reveal normal and pathological cell arrangement. McCallum's modification of Goodpasture's stain (McCallum (1919)) proved unsatisfactory for revealing gram-negative organisms, and a modification of Nicolle's carbol thionine solution (Wadsworth (1947)) was successfully employed. This modified solution consisted of 1 gm. thionine, 1 gm. phenol, and 100 cc. of 90% alcohol, and was used with the general staining procedure.

Chicken Embryo Inoculations

Virus investigation. Vulvar swabs and a biopsy specimen were used to study the possibility of a virus etiology in nodular vulvitis. A prepared inoculum was injected into 111 chicken embryos during nine serial passages. Swabs of the vulvae of diseased animals were placed in 1.0 ml of vibrio diluent and allowed to stand in the refrigerator at 4° C. for two hours. At the end of this time, the swabs were withdrawn from the tubes and discarded. The fluid remaining was treated with 1000 units of penicillin and 100 micrograms of streptomycin per milliliter and kept in the refrigerator for three more hours to obviate bacterial contamination. This fluid then constituted the swab inoculum. Bacterial sterility was determined by culturing on blood agar, broth base slants.

The biopsy specimen was macerated in a sterile mortar using sterile sand as a grinding agent. After maceration, the tissue was washed into a sterile tube with tryptose broth. This suspension of tissue and sand was treated with penicillin and streptomycin as previously described, and the sand was allowed to settle out. The supernatant fluid constituted the biopsy inoculum.

Embryos were inoculated at seven to ten days on the chorio-allantoic membrane with 0.2 ml of the inoculum using the technique described by Woodruff and Goodpasture (1931). The controls were inoculated in the same manner

with 0.2 ml of sterile tryptose broth. On each succeeding day, the eggs were candled for changes in activity or death of the embryo. Dead embryos were examined for lesions of the chorio-allantoic membrane and membranes worthy of histological examination were fixed in the shell using Helley's fluid (Mallory (1938)). Other membranes exhibiting lesions were frozen and stored at -40° C. for serial passage. Normal membranes from the controls were also saved for serial inoculation in this manner. At nineteen days of incubation all remaining embryos were examined and discarded.

Inoculum for the second and succeeding serial passages was prepared by allowing the frozen membranes to thaw at room temperature. The membranes were then placed in a Waring blendor with a micro head attachment, and enough sterile tryptose broth added to supply a 0.5 ml margin over the inoculum required (as determined by 0.2 ml for each embryo). The tissue was macerated in the blendor for five minutes and the suspension removed and centrifuged for ten minutes at 2250 r. p. m. The supernatant fluid was pipetted off to form the next inoculum.

Bacteria investigation. One hundred and twenty-six chicken embryos were used to study the pathogenic effect of an orgsnism commonly associated with nodular vulvitis when inoculated into the yolk sac, on the chorio-allantoic membrane, and into the amniotic cavity. The

seven to ten day old embryos were inoculated according to Cunningham (1948) with 0.2 ml of inoculum. In one instance, an attempt was made to isolate the organism by direct injection of the tryptose broth suspension taken from a vulvar swab of a cow with acute nodular vulvitis. Another study involved the injection of vibrio diluent in which a curette scraping of nodular vulvitis had been macerated. The third study consisted of inoculating the embryos with a 48 hour pure culture of the organism grown on a blood agar, broth base slant. Two milliliters of sterile tryptose broth were added to the slant; the organisms were mixed into the fluid with a transfer loop; and this fluid was removed with a sterile pipette.

The embryos were examined in the manner previously described. The chorio-allantoic membranes were fixed in Helley's fluid, and Zenker's fluid was used to fix the embryos.

Guinea Pig Inoculations

The pathogenicity of a gram-negative bipolar rod associated with nodular vulvitis for guinea pigs was investigated. Two different isolations of the organism were studied. An intraperitoneal injection of 2.0 to 3.0 ml of inoculum was made, the amount depending upon the weight of the guinea pig. The inoculum consisted of a tryptose broth suspension of a well grown 24 or 48 hour pure culture

incubated on blood agar, broth base slants at 37° C. The bacterial concentration was approximated in one instance by examining the inoculum microscopically, using a hemocytometer and the technique commonly employed for counting white blood cells.

Calf Inoculations

An attempt was made to reproduce nodular vulvitis in normal two to three months old calves by vulvar inoculation of a gram-negative, bipolar rod associated with the disease. Two reproduction trials were made.

First trial. In preparation for the trial, ten day old chicken embryos were inoculated into the yolk sac with 0.2 ml of a tryptose broth suspension containing the organism. The suspension was taken from a blood agar, broth base slant incubated for 48 hours at 37° C. Twenty-four hours after inoculation, two embryos were found dead. Amniotic fluid was withdrawn from the inoculated embryos, from a normal embryo, and then placed in the incubator at 37° C. for twenty hours. Microscopic and cultural examination assured a pure inoculum, and a hemocytometer count revealed approximately 512 million organisms per milliliter.

Three calves had been examined clinically and bacteriologically every three or four days for two weeks prior to inoculation, so that the absence of vulvar lesions and the organism could be established. One calf was separated from

the others and inoculated with amniotic fluid from the normal embryo, while the infected amniotic fluid was swabbed on the vulvae of the other two calves. Inoculation was achieved by lightly scarifying the vulvar mucosa with sterile "wet or dry", grit number 108c sandpaper attached to an applicator stick, and then swabbing 0.75 ml of the inoculum on each vulva.

The calves were fed and cared for by two men, one for the control and one for the experimental group.

The calves vulvae were clinically and bacteriologically examined every other day for ten days, and then every four to six days for the remainder of the trial. Thirty-one days after inoculation one infected calf and the control were killed. On the fifty-second day, the remaining infected calf was killed. Necropsies were performed and tissues excised and fixed in the standard manner.

Second trial. Inoculum was prepared from a pure culture of the organism reisolated from the vulva of an infected calf on the fifty-second day of the previous trial. Two blood agar, broth base slants inoculated with the organism were incubated for 24 hours at 37° C. Four tenths of a milliliter of a broth suspension of the organism was removed from each tube, the two samples were mixed together, and 0.5 ml of sterile tryptose broth added to the suspension. One tenth of a milliliter of the mixture was removed for a hemocytometer count and the remainder divided into two

inocula. The hemocytometer count revealed approximately one billion bacteria per milliliter. Six tenths of a milliliter of sterile tryptose broth was used as a control inoculum.

Three calves were examined clinically and bacteriologically every four days in the 12 day period preceding inoculation, and then one calf was isolated in a control pen. The calves were inoculated in the same manner as in the first trial.

Feeding and examination was also conducted in the same way, and the calves were autopsied and tissues taken from the genital tracts on the twenty-second day.

RESULTS AND DISCUSSION

Clinical Observations

The presence and severity of nodular vulvitis was subject to considerable variation in the 89 field cases studied. However, all but sixteen animals exhibited symptoms of the disease, and it was found in all six herds examined. Within the same herd, condition of the vulvae ranged from normal, exhibiting no nodules or inflammation, to very severely affected, exhibiting marked congestion and edema of the mucosa, petechial hemorrhage, suppuration, sloughing, and semiconfluent nodule formation. Most individuals manifested symptoms of an intermediate nature, usually 1+ to 3+ nodules and moderate inflammation. A major portion of this variation was undoubtedly due to freedom from the disease, differences in intensity of the initial or secondary infection, vacillations of acuteness and chronicity of unknown cause, possible acquired immunity, irritation of coitus, and/or the stage of the estrual cycle. If the statements made by Webster (1932) and Sisson and Grossman (1938) were correct, then some of the cows exhibiting but few small nodules and no inflammation were probably normal. It was also observed that during estrus and shortly before parturition, the vulvar mucosa often

became edematous and congested, and any nodules present were partially or completely obscured.

Many of the conditions described by other workers, were observed in this investigation. The nodules were usually one to two millimeters in diameter, although a large number of three millimeter nodules were observed. In certain animals where inflammation was slight to moderate, the nodules occasionally had a vesicular appearance. However, digital palpation revealed that they contained no fluid and were really quite firm. In several instances, a zone of increased vascularity surrounding the base of the nodules, gave the appearance of the vascular girdle described by Williams (1914). A common condition was the appearance of nodules in a three to four centimeter ring encircling the vulva one to two centimeters from the lips. The lesions were usually larger and more numerous in the area of the clitoris. In the more severe infections, they were diffusely scattered over all the walls of the vulva, sometimes becoming semiconfluent. Quite often the nodules formed longitudinal rows, extending from the clitoris to the external urethral orifice. In no instance were they found less than a centimeter from the rima vulvae or beyond the introitus vaginae. However, congestion and petechiae were observed on both the vaginal and cervical mucosa.

Severe edema and congestion often produced large folds in the vulvar walls, accompanied by abrasion of the surface and desquamation of epithelium. A mucous or gray to white mucopurulent exudate, as well as hemorrhage, could be observed on the mucosal surface and caused matting of the vulvar hairs. Straining, as a result of natural service, produced a copious discharge of this exudate.

Etiological Investigations

Attempt to Isolate a Virus

Inoculation of the chorio-allantoic membranes of chicken embryos with material from vulvar swabs and a biopsy specimen treated with antibiotics produced essentially negative results. Twelve out of 83 (14%) embryos inoculated with "infected" material died. Five of the deaths occurred within 24 hours after inoculation, probably due to trauma resulting from faulty technique, and one embryo which died later was contaminated with bacteria. No specific lesions were produced in the other six embryos (7.2%), although some fibrinous exudate on the chorio-allantoic membrane and congestion of the embryonic extremities was observed. Two of the 28 controls (7.1%) also died without any apparent reason.

Considering the small number of embryos involved, a one tenth per cent difference in the death rate between

"controls" and "experimentals" was very insignificant. These results did not preclude the possibility of a virus etiology, but the presence of a viable infectious agent in inoculated embryos, using the recorded technique, was very unlikely.

Attempt to Isolate a Bacterium

Isolation. Organisms commonly isolated from both normal and diseased vulvae were principally gram-positive streptococci (usually hemolytic) and micrococci. Also isolated were large gram-positive rods, long gram-negative rods, gram-negative diplococci, and small, hemolytic, gram-negative rods. However, a non-hemolytic, non-motile, gram-negative, bipolar rod was found commonly associated with nodular vulvitis. On the basis of this observation and the reports of Jones and Little (1927) and Crawley, Wills, and MacGregor (1950), this was the principle organism studied.

As previously described, 56 animals were cultured on the basis of representation of the general pathological picture in each herd. The percentages of positive isolations in five herds are shown in Table VIII.

TABLE VIII

ISOLATION OF "VULVITIS ORGANISM" ON HERD BASIS

Herd	No. Cultured	Positive Isolations	%
1	13	3	23
2	13	7	54
3	14	6	43
4	6	0	0
5	10	4	40

Variation in the isolation technique and per cent of normal and chronic individuals in each herd, as well as the chance factor involved in small numbers, probably accounted for most of the difference in positive isolations. The organism was isolated from ten out of fourteen individuals not included in the table, cultured on the basis of visible lesions.

The relationship between positive isolation of the organism and the presence of nodules and inflammation is shown in Table IX.

The organism was also isolated from the smegma of two bulls.

TABLE IX

ISOLATION OF ORGANISM IN RELATION TO NODULES AND INFLAMMATION

Nodules	No Inflammation		Inflammation	
	Not Isolated	Isolated	Not Isolated	Isolated
None	13	-	6	5
1+	4	2	7	6
2+	1	-	3	8
3+	-	-	3	6
4+	-	-	2	4
Total	18/20	2/20	21/50	29/50
%	90	10	42	58

Curette scrapings of the vulvae of four animals with nodular vulvitis revealed the presence of a bipolar rod both outside and inside the epithelial cells. Positive isolation of the organism commonly associated with this condition was achieved by culturing vulvar swabs of these four animals. In one instance, a curette scraping was macerated in a small amount of vibrio diluent and the suspension cultured on blood agar. The culture established positive isolation of the organism, while a smear of the suspension revealed intracellular invasion by a bipolar rod. No organisms were found in the curette scrapings of two normal animals.

Biopsy specimens of a cow in the experiment station herd affected with the disease, and tissues obtained at necropsy from calves experimentally inoculated with the organism, revealed a bipolar rod upon and within the superficial epithelium of the vulva. Cultures of vulvar swabs produced positive isolation of the organism in all cases.

Description of colonies. Although the organism showed scant growth on tryptose agar, blood and reduced oxygen tension were required for primary isolation. The colonies were very small, from .017 to .750 mm. in diameter, and were essentially transluscent, although occasionally gray with slight irridescence. Most of the isolations produced a round, slightly raised colony with an entire edge. The chief variations from this form were cratiform, umbonate, or cratiform plus umbonate. The organism was not generally hemolytic, although in one instance, a slight clearing of the blood agar was noted directly under or in a narrow zone around the colony. The same isolation of the organism in the preceding and succeeding transfers did not produce hemolysis.

Description of organism. The organism was very pleomorphic, occurring as single bipolar rods, as diplobacilli, in clumps, or in filamentous chains containing nine to sixteen organisms. Coccobacillary or coccal forms, swollen bodies and misshapen rods were also observed. Many of these malformed cells were probably due to

the rapid lysis typical of the organism. Average size of the rod was .50 to .75 micron by 2.0 micra. It was non-motile, gram-negative, and stained poorly with Gram's stain but reasonably well with Giemsa's, methylene blue, or Wright's. A halo, perhaps a delicate capsule, surrounding the rod form was noted.

Biochemical and fermentation reactions. Two comparisons were made of the biochemical and fermentation reactions of two isolations of this organism and an organism of similar morphology but which was motile and hemolytic. The hemolytic rod was isolated from the vulva of one cow exhibiting symptoms of nodular vulvitis and from the abomasa of two aborted fetuses. The results of this study are reported in Table X. The organism commonly associated with nodular vulvitis did not produce acid or gas in carbohydrate media; there was no hydrogen sulfide production; nor were nitrates or litmus milk reduced.

Pathogenicity for chicken embryos. A swab inoculum from the vulva of a diseased animal, when injected into the yolk sac of a chicken embryo, produced death within 24 to 72 hours, and the bipolar rod could be isolated from the yolk and amniotic fluid.

When a pure culture of the bipolar rod was used to infect the embryos, death was usually produced within 24 hours in yolk sac injection, within 24 to 48 hours in chorio-allantoic membrane inoculation, and within 24 to

TABLE X

BIOCHEMICAL AND FERMENTATION REACTIONS OF ORGANISMS ISOLATED FROM NODULAR VULVITIS AND ABORTED FETUSES

Media	AP-1	S-1	S-8	547X	580X
Arabinose	-	-	+	+	+
Dextrin	o	o	+	+	+
Dulcitol	-	-	-	+	-
Fructose	-	-	+	+	+
Galactose	-	-	+	+	+
Glucose	-	-	+	+	+
Glycerol	-	-	-	-	-
Inositol	o	o	+	+	+
Inulin	-	-	+	+	+
Lactose	-	-	+	+	+
Maltose	-	-	+	+	+
Mannitol	-	-	+	+	+
Raffinose	-	-	+	+	+
Salicin	-	-	+	+	+
Sorbitol	-	-	+	+	+
Sucrose	-	-	+	+	+
Trehalose	-	-	+	+	+
Xylose	-	-	+	+	+
Nitrate	-	-	-	-	-
Litmus milk	-	-	m	m	m
H_2S	-	-	s	s	s

AP-1 and S-1 ..... gram-negative rod associated with nodular vulvitis.
S-8 ..... hemolytic, motile rod isolated from diseased vulva.
547X and 580X ..... hemolytic, motile rod isolated from abomasa of aborted fetuses.

o ... no growth
- ... growth but no reaction
+ ... acid production

g ... gas production
n ... reduction of nitrates
m ... reduction of litmus milk
s ... production of H_2S

72 hours when injected into the amniotic cavity. Although it could be found in all parts of the embryo, the organism was recovered in greatest numbers from the amniotic fluid. Filamentous chains were commonly noted, and this live tissue seemed to produce much better growth than artificial media.

Gross examination revealed a congested and edematous embryo. If the embryo did not die until two or three days after inoculation, it was usually found to be smaller than the "controls" and was not feathering properly.

The organisms were easily found in the mesentary upon microscopic examination, but no specific lesions were observed.

Pathogenicity for guinea pigs. A 600 gm. guinea pig was injected intraperitoneally with three milliliters of inoculum taken from a 24 hour culture of the bipolar rod. Within two hours, there was a one degree increase in body temperature to 39° C. and then a gradual decline until 34.5° C. was reached. Pain was evident upon application of pressure to the abdomen. Lacrimation and dullness were observed six hours after inoculation, and diarrhea was noted at twenty-four hours. At twenty-eight hours, the guinea pig was nearly comatose and was killed.

Necropsy revealed considerable congestion of the parietal peritoneum, fibrin on the liver, patchy congestion of the lungs, and a white exudate in the peritoneal cavity. Soft yellow-green feces were found in the large

intestine. Microscopic examination of the peritoneal exudate revealed fibrin, many leucocytes, particularly polymorphonuclears, and large numbers of bacilli.

Inoculation of a second guinea pig weighing 300 grams, with a different isolation of the organism produced a two degree drop in temperature, accompanied by dullness and anorexia. However, the guinea pig recovered within 30 hours, and a second inoculation was made 11 days later with the isolation of the organism used in injection of the first guinea pig. No symptoms of disease appeared, perhaps as a result of immunity acquired from the first injection.

A third guinea pig weighing 765 grams, was injected intraperitoneally with three milliliters of a pure culture isolated from an experimentally infected calf, containing 560 million organisms per milliliter. The site of inoculation became swollen and warm within two hours. The body temperature rose to 39.4° C. and then declined slightly. Diarrhea and dullness appeared, but the disease terminated in recovery. The animal was killed 48 hours after injection and an autopsy performed, but no lesions were found.

The pathogenicity of this bipolar rod for the guinea pig was not definitely established. However, it appeared that in certain instances, perhaps as a result of lowered resistance, the organism was able to temporarily overcome the defense mechanisms of the host and produce symptoms of disease.

Attempt to Reproduce Nodular Vulvitis in the Bovine

First Trial

The calves used in this trial were assigned numbers by which they shall be known in this report. The control pen contained number 838; 837 and 840 comprised the experimental group.

On the day of inoculation and for the previous two weeks, no lesions were observed on the vulvar mucosa of any of the three calves. Two days later, the vulvae still appeared normal. However, on the fourth day, three pinpoint nodules were observed slightly anterior to the clitoris of 838. These nodules were not associated with inflammation, and except for the development of five more nodules posterior to the clitoris, the condition of this vulva remained essentially static throughout the investigation.

On the same day, slight congestion was noted on the dorsal vulvar wall of 837 and on the ventral vulvar wall of 840. Six days later, a slight inflammation of the dorsal wall of 840 was also noted. There was only a very gradual increase in hyperemia of the vulvae of the experimental group until 31 days after inoculation, when 840 was observed to have 1+ nodules ventrally and moderate diffuse hyperemia. The vulva of 837 also exhibited moderate inflammation and 2+ nodules both ventrally and dorsally.

Numbers 838 and 840 were killed and autopsied on this day, while 837 was examined periodically for three more weeks. During these three weeks, congestion of the vulva of 837 gradually increased until on the forty-third day of the experiment, inflammation was severe and 3+ nodules were present. From this day until the animal was killed, the inflammation gradually subsided to a moderate condition, but the number of nodules remained approximately the same.

The organism was recovered from both members of the experimental group, from the fourth day onward. Ease of isolation gradually increased until on the fifty-second day, it could be recovered in virtually pure culture. It was never recovered from the control.

The technique of slaughter, whereby the calves were stunned and bled, produced a blanching of the vulvar mucosa and made observation of the nodules difficult. The use of oblique lighting produced a tiny shadow by each nodule and increased the ease of examination. At necropsy, 838 exhibited only eight small nodules in the vicinity of the clitoris. Approximately two dozen nodules were scattered over the ventral wall of the vulva of 840. Number 837 had a five centimeter band of nodules completely encircling the vulva just anterior to the lips, as well as a few petechiae scattered over the ventral surface. Table XI.

Second Trial

A few nodules in the area of the clitoris were noted in the vulvae of all three calves when they were purchased for the trial. However, during the twelve days preceding inoculation, these nodules remained static, and there was no congestion of the mucosa. Number 221 was placed in the control pen, and 258 and 171 formed the experimental group.

The condition of the vulva of 221 did not change throughout the experiment. However, on the second day after inoculation, petechiae and congestion was noted in both members of the experimental group. Some of these lesions were undoubtedly due to the scarification process. On the fifth day, a mucopurulent exudate appeared in the vulva of 171, and on the seventh day was also noted in 258. The number of nodules began to increase on the sixth day and were very numerous by the end of the trial. Inflammation of the vulvar mucosa never exceeded a moderate condition, although it quite possibly would have if the animals had been kept for a longer period of observation.

The organism was recovered without difficulty from both 258 and 171 until the trial was terminated. It was not isolated from 221.

Three weeks after inoculation, the calves were killed. Twelve nodules were found on the ventral wall of the vulva of 221. Numerous nodules were scattered over all vulvar

walls of 258 and were very thickly distributed over the vulvar mucosa of 171. Where they were most numerous, approximately 36 nodules could be found for each square centimeter of surface. Many of them were very small and a few very large, ranging in size from .25 to 3.0 mm. in diameter. Table XI.

TABLE XI

SUMMARY OF
ATTEMPT TO REPRODUCE NODULAR VULVITIS IN THE BOVINE

Calf	Exposed	Lesions Produced	Organism Isolated
First trial.			
838	-	-	-
837	2/16	+	+
840	2/16	+	+
Second trial.			
221	-	-	-
258	4/11	+	+
171	4/11	+	+

Histopathological Study of Nodular Vulvitis and Comparison with Normal

A histopathological study was made of vulvar and vaginal tissues from both affected and apparently normal cows and heifers. Physiological changes in the vaginal and vulvar mucosa were noted during different periods of the estrual cycle, as described by Cole (1930) and Roark and Herman (1950). It was necessary to exercise great care to prevent confusion of these physiological changes with alterations of pathology. Lymphocytic and polymorphonuclear infiltration of the stratified squamous epithelium was observed in sections from normal animals obtained near the time of estrus. However, very few organized lymph follicles were observed in such sections. There was some vacuolization of the cytoplasm of the more superficial epithelial cells in most of the sections studied. However, this vacuolization or ballooning degeneration appeared to be more prominent in affected animals.

In sections from animals with nodular vulvitis, organized lymph follicles were commonly found in the lamina propria of the vulvar mucosa. Often they were somewhat edematous and appeared to compress the elastic tissue around them. Above the follicles, lymphocytes, plasma cells, and edematous fluid often pushed the basal layer of epithelial cells closer to the surface. In some instances, this basal layer was completely obliterated,

leaving an epithelial covering four cells thick. These remaining epithelial cells were thin and elongated, and characteristic of the superficial layers of stratified squamous epithelium.

The epithelium appeared vacuolated over other nodules and showed marked infiltration of lymphocytes to the uppermost layers. In some cases, the epithelium was seriously disrupted, and many of the cells were markedly atrophic. The papillae of the lamina propria were often larger, the capillaries more prominent, and the lymphocytes more numerous than in the normal vulva. Sometimes the epithelial cells surrounding these papillae were vacuolated and infiltrated with lymphocytes. Blood vessels in the submucosa were frequently distended with erythrocytes.

Tissues were taken from the vulva of a heifer which had experienced an acute attack of the disease three weeks previous to autopsy, during which time the symptoms receded. In sections where bipolar rods could be observed on the epithelial surface, polymorphonuclears were seen in considerable numbers, usually in the superficial layers of the epithelium. The lymphocytes in the follicle beneath were densely packed, with some infiltration into the surrounding tissues but not particularly directed toward the surface. A few lymphocytes could be seen migrating through the epithelium. Apparent desquamation of the superficial epithelial layers had occurred in some areas.

A similar condition was observed in tissues taken from a heifer during an acute attack. Polymorphonuclears and bipolar rods were observed in the superficial epithelial layers. However, the underlying lymph follicle was more loosely arranged with a very pronounced infiltration of lymphocytes into the epithelium and surrounding stroma.

The histopathological picture of experimentally produced nodular vulvitis was identical with that found in the field.

SUMMARY

Eighty-nine cows and heifers were examined in six herds with recognized breeding difficulty. All but sixteen animals exhibited symptoms of nodular vulvitis, and no herd was free from the disease. The symptoms found in the vulvar mucosa were varying degrees of congestion, edema, petechiae, suppuration, epithelial sloughing, and nodule formation.

An attempt to isolate a virus from infected vulvae was unsuccessful.

A gram-negative, bipolar rod was found to be commonly associated with nodular vulvitis and was isolated from fifty-two females and two males. The organism required blood and microaerophilic conditions for primary isolation. It grew as very small, transluscent or gray colonies, was non-motile, non-hemolytic, and very pleomorphic, occurring as single rods, diplobacilli, clumps, or in filamentous chains. Cocco-bacillary and coccal forms were also observed. Average size of the rod was .50 to .75 micron by 2.0 micra. It did not produce acid or gas in carbohydrate media; there was no hydrogen sulfide production; nor were nitrates or litmus milk reduced.

The organism was pathogenic for chicken embryos when inoculated into the yolk sac or amniotic cavity, or onto the chorio-allantoic membrane. Intraperitoneal injection of the organism produced a severely pathogenic reaction in one guinea pig and only mild or no reaction in two others.

Symptoms of the disease were experimentally induced in two to three months old calves by lightly scarifying the vulvar mucosa and then swabbing a pure culture of the organism on the surface.

Histopathological examinations were made of normal, naturally infected, and experimentally infected vulvae. Grossly visible nodules were found to be underlaid with one or more lymph follicles. Lymphocytic infiltration of the epithelium in these areas was commonly observed. There were no significant differences found between naturally and experimentally produced nodules.

Fig. 1. S-4 Nodules in vulva of two year old heifer showing linear arrangement on lateral vulvar wall. Also see Fig. 20.

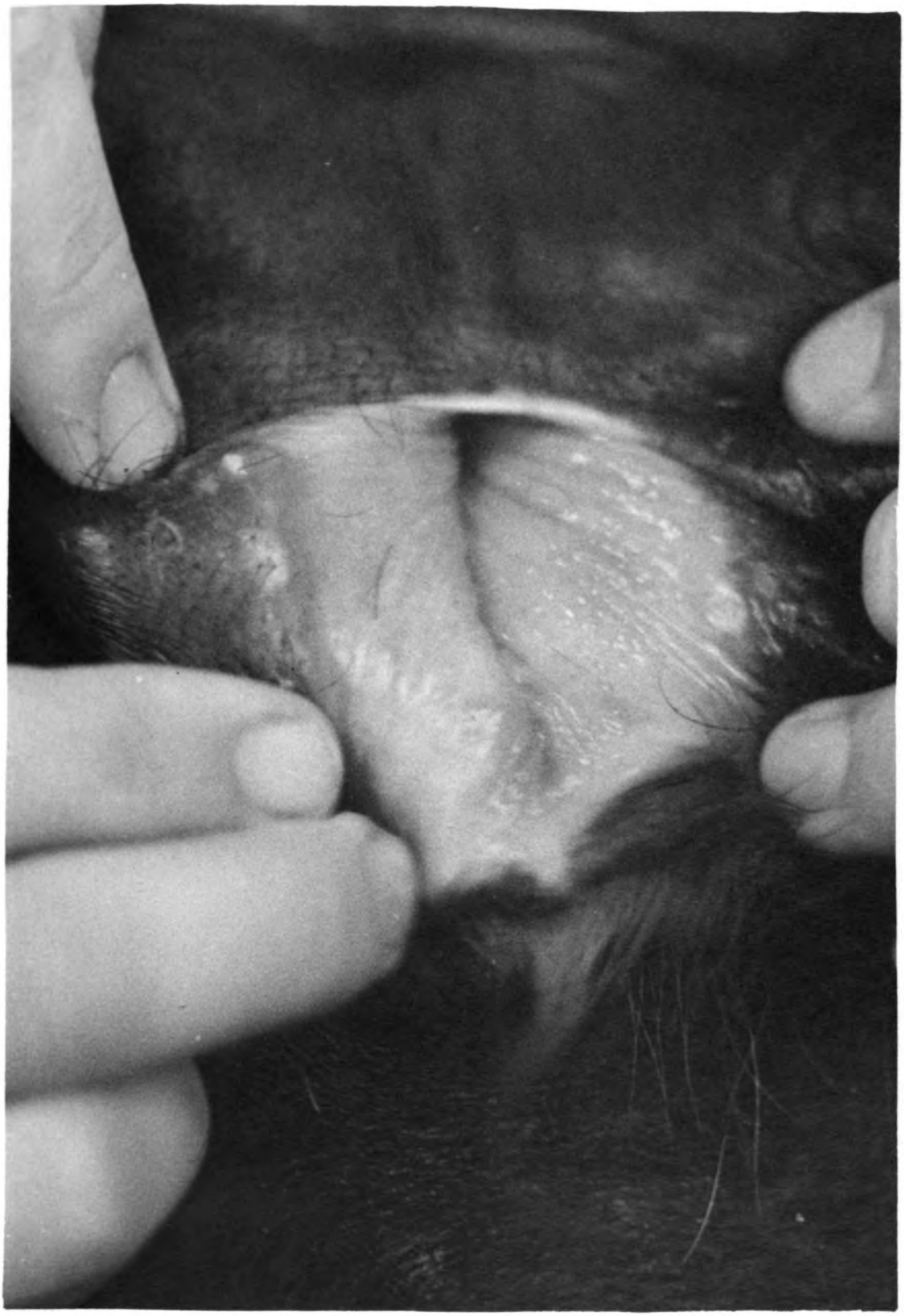


Fig. 1

Fig. 2. Same heifer as Fig. 1 in a view showing nodules heavily clustered about the clitoris.

S-4

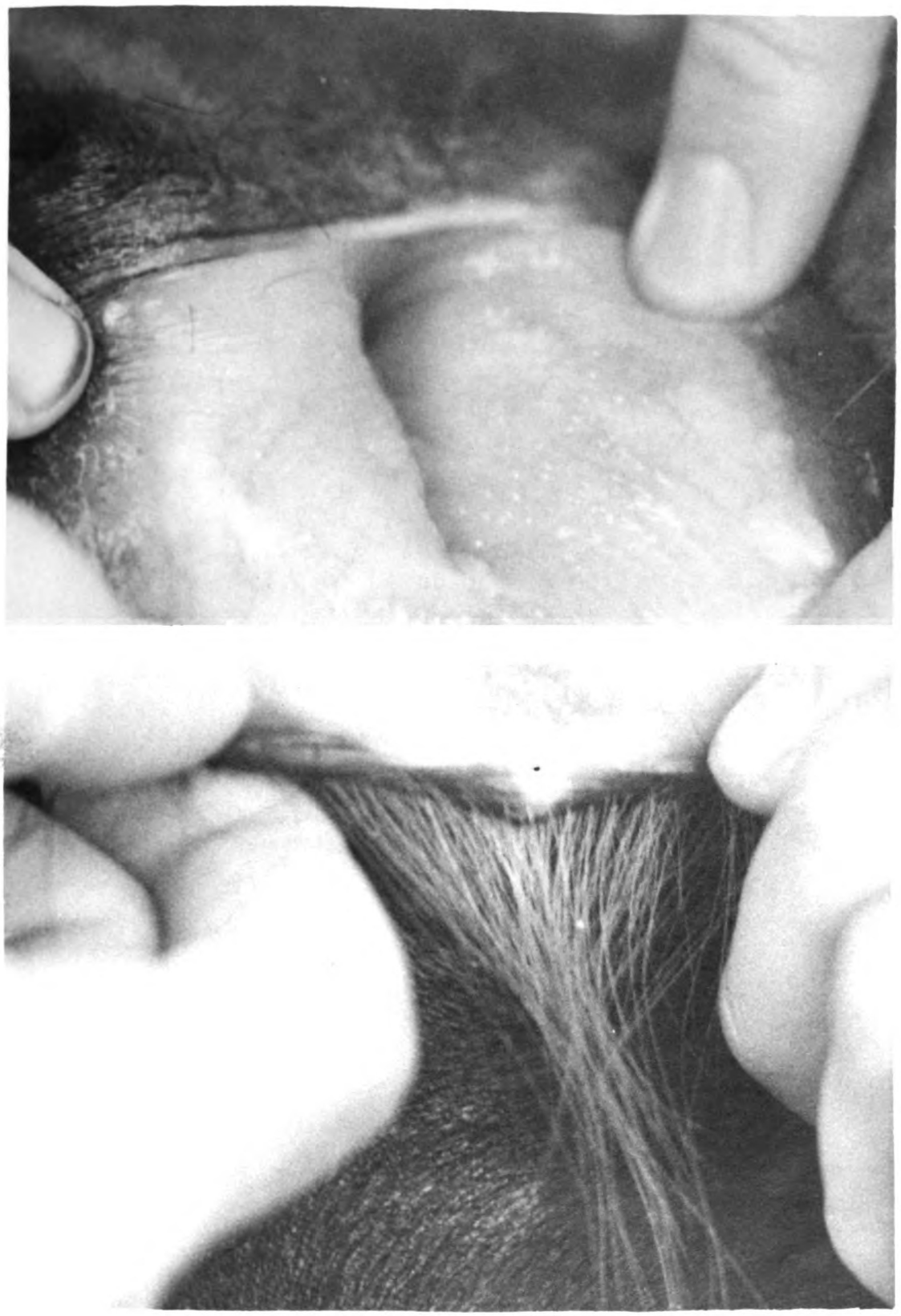
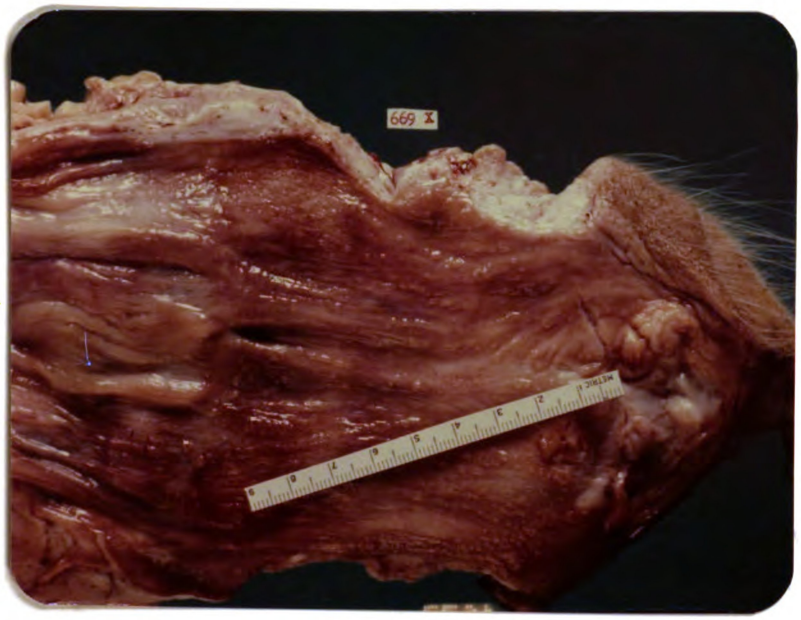


Fig. 2

Fig. 3. 669X Vulva of necropsied cow with a large number of nodules diffusely spread over all vulvar walls from the clitoris to the introitus vaginae. Linear arrangement of the nodules is also evident. Also see Figs. 4, 17, 18, and 19.

Fig. 4. 669X Magnified view of Fig. 3 in area of external urethral orifice. Each unit of scale equals one millimeter.


669 X

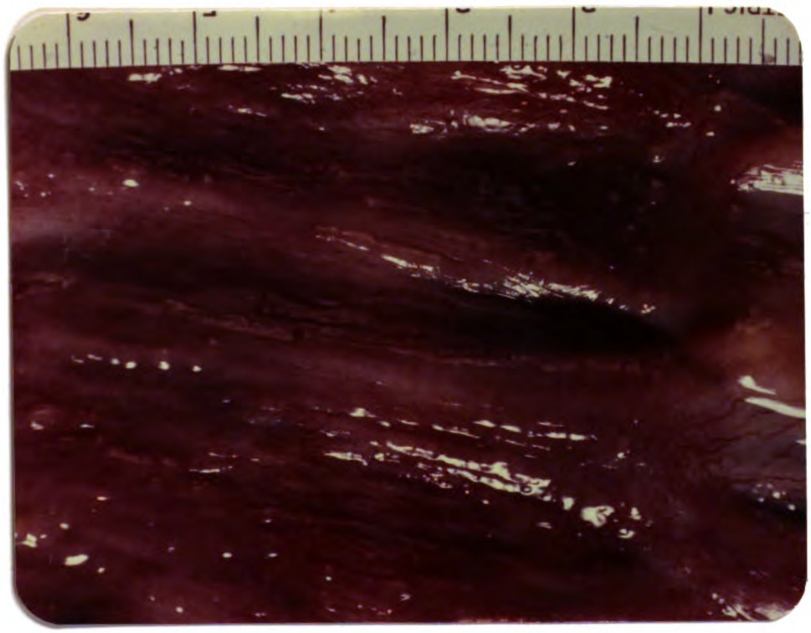


Fig. 3

Fig. 4

Fig. 5. AP-1 Curette scraping from vulva of cow with nodular vulvitis, revealing intracellular invasion of bipolar rods. This tissue was macerated in a small amount of vibrio diluent and a smear made of the suspension. Methylene blue. 670x.

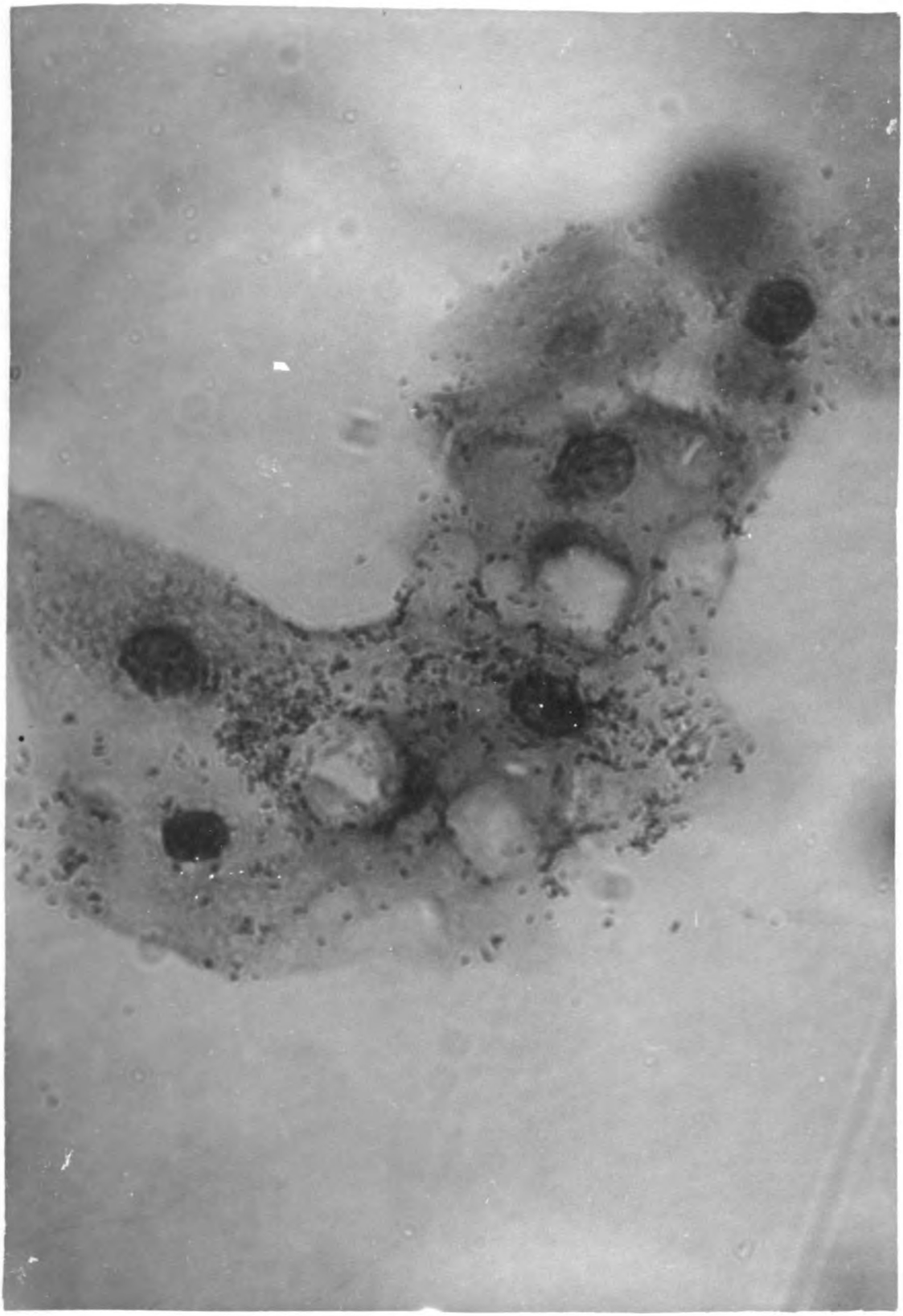


Fig. 5

Fig. 6. 784X Bipolar rods on the surface of superficial epithelium of vulva exhibiting symptoms of nodular vulvitis. Also see Fig. 7. Modified Nicolle's. 1800x.

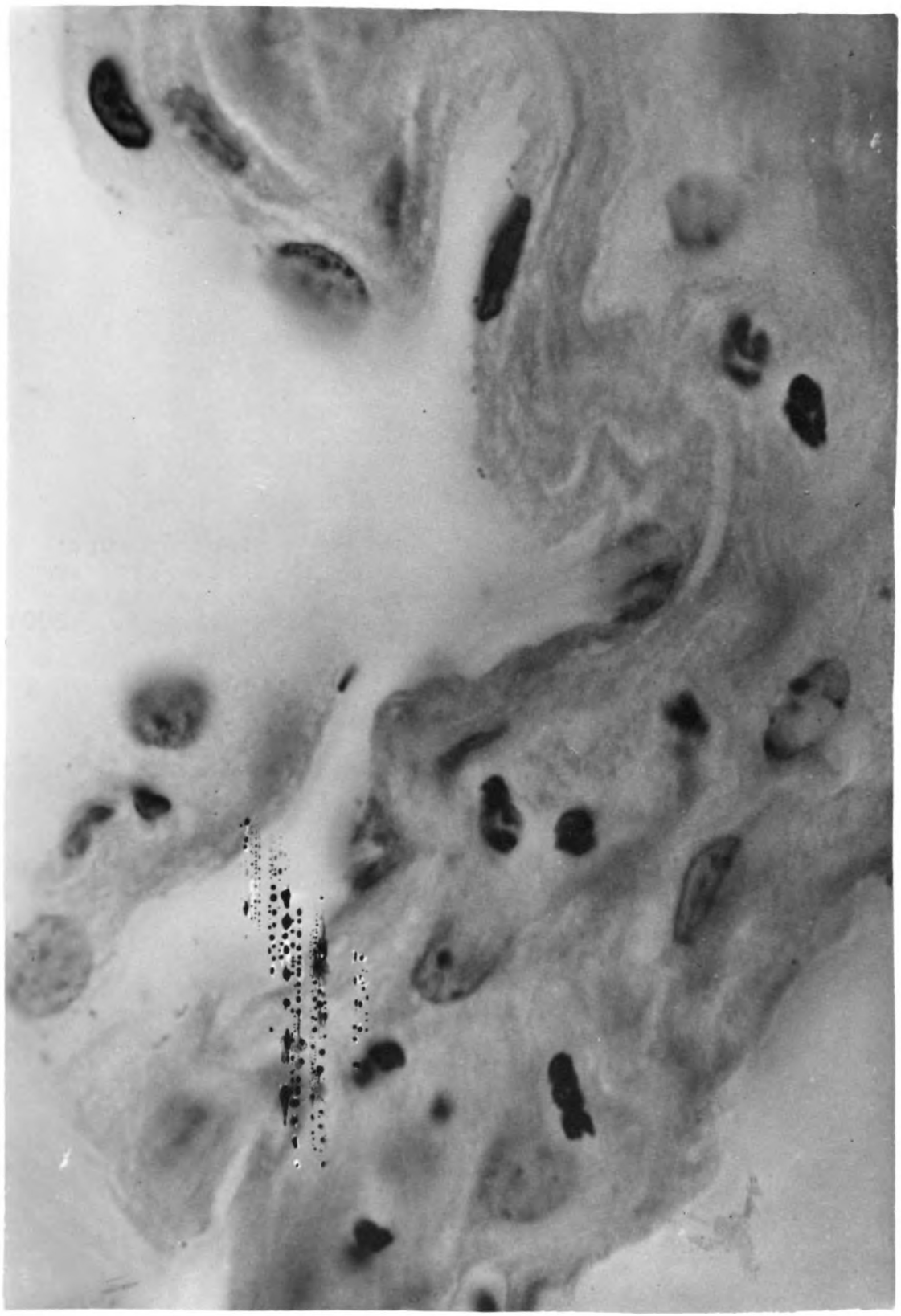


Fig. 6

Fig. 7. Low power view of area where bipolar
784X rods were found in Fig. 6. 120x.

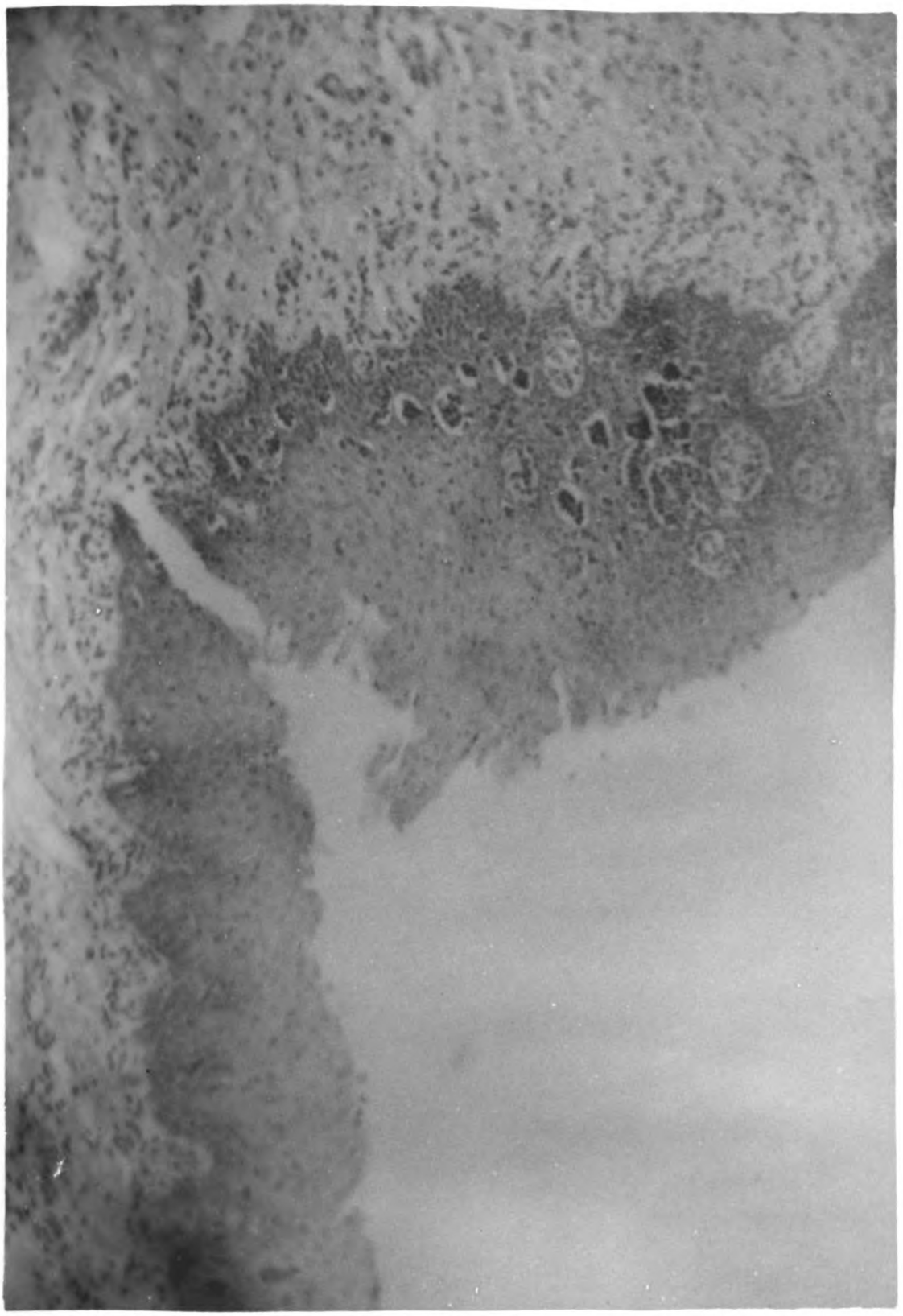


Fig. 7

Fig. 8. Apparatus used in this investigation. The air-tight chamber on the left was used for producing microaerophilic conditions in determination of biochemical and fermentation reactions of the organism. The glass jar in the center was used for isolation of the organism as described in text. The stoppered blood agar, broth base slant in the left end of the rack illustrates the method used for maintaining pure cultures. Second and third from the left are the "scarification sticks" used in preparing the vulvae of the calves for inoculation. The tube second from the right end contains a sterile swab of the type used in vulvar swabbing. At the extreme right is the curette used in obtaining vulvar scrapings.

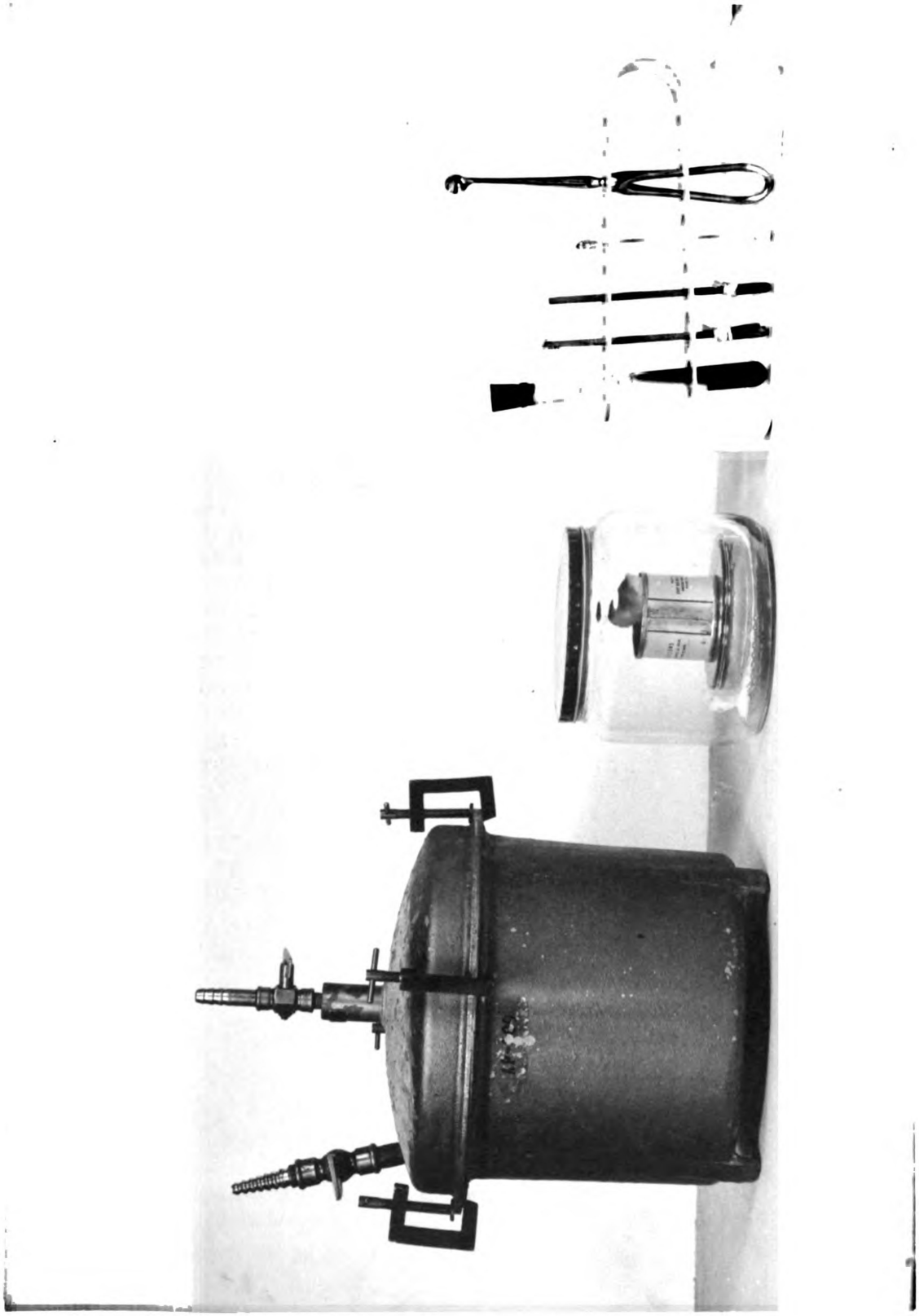


Fig. 8

Fig. 9. Round, cratiform colonies with slightly raised centers. The bipolar rod associated with nodular vulvitis isolated from a naturally infected animal. Blood agar. 35x.

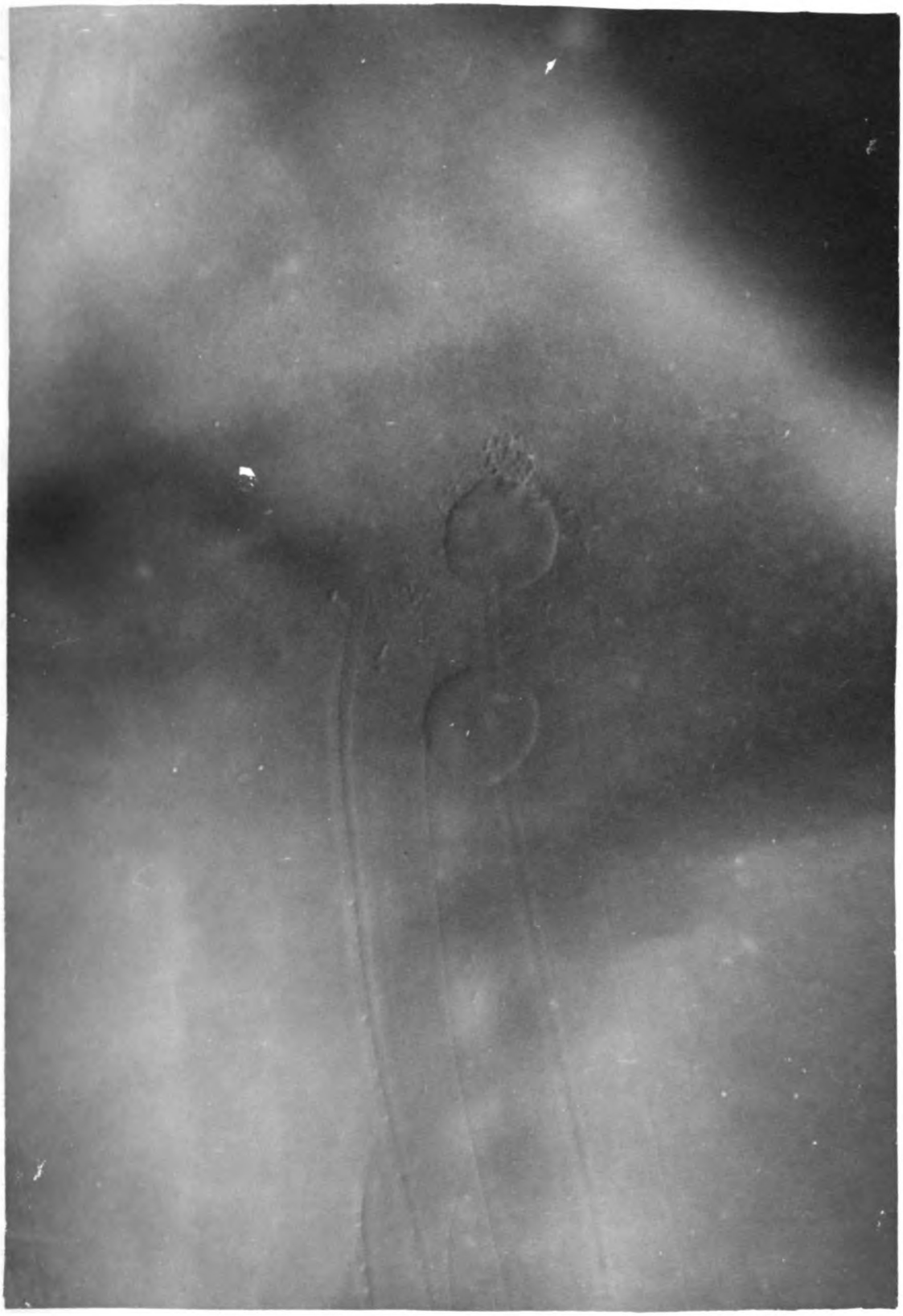


Fig. 9

Fig. 10. Flat to cratiform colonies of the bipolar rod. Isolated from a naturally infected animal. Blood agar. 35x.

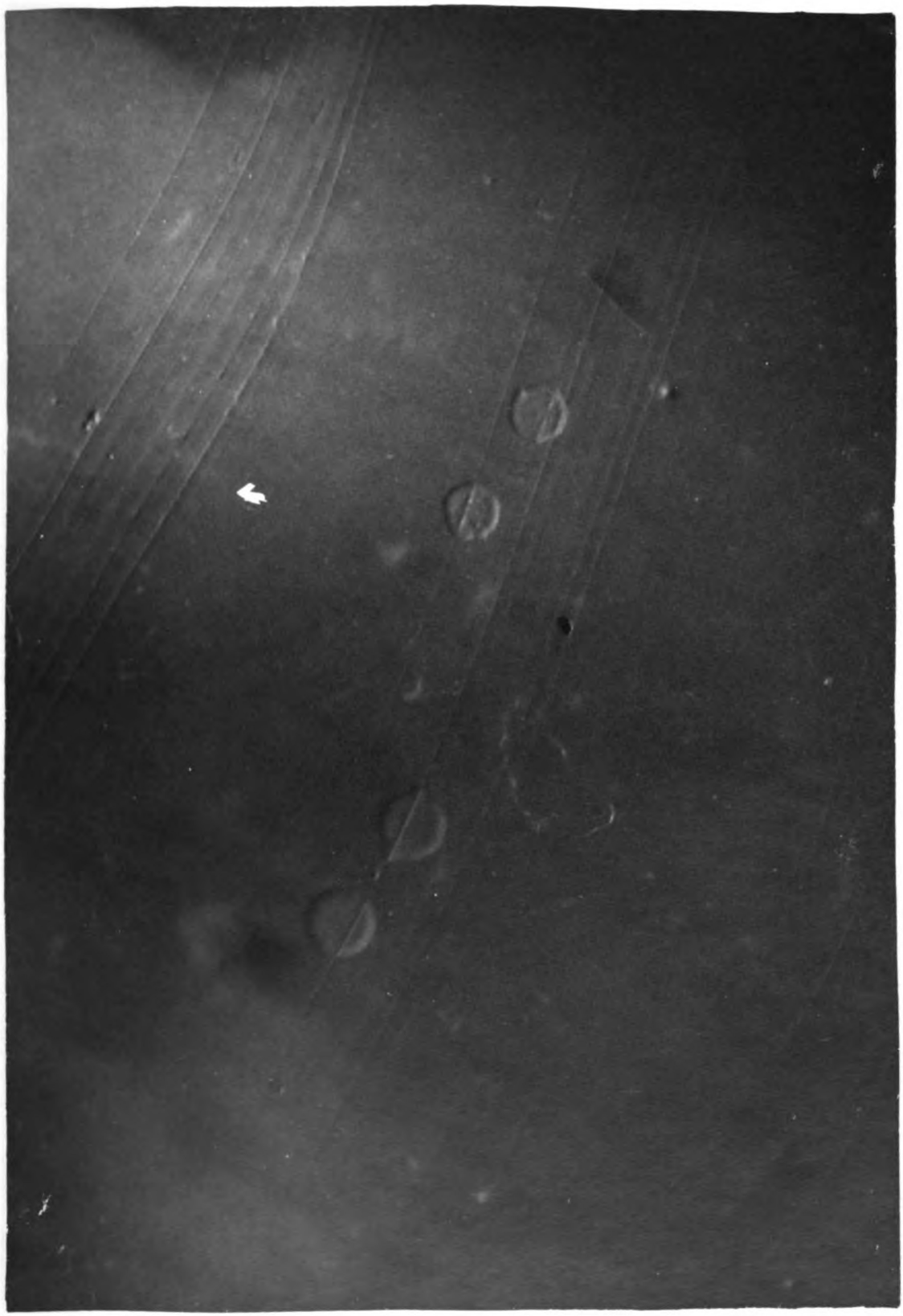


Fig. 10

Fig. 11. 827 Round, slightly raised colonies of the bipolar rod. Isolated from an experimentally infected calf. This was the most common colonial type. Blood agar. 35x.

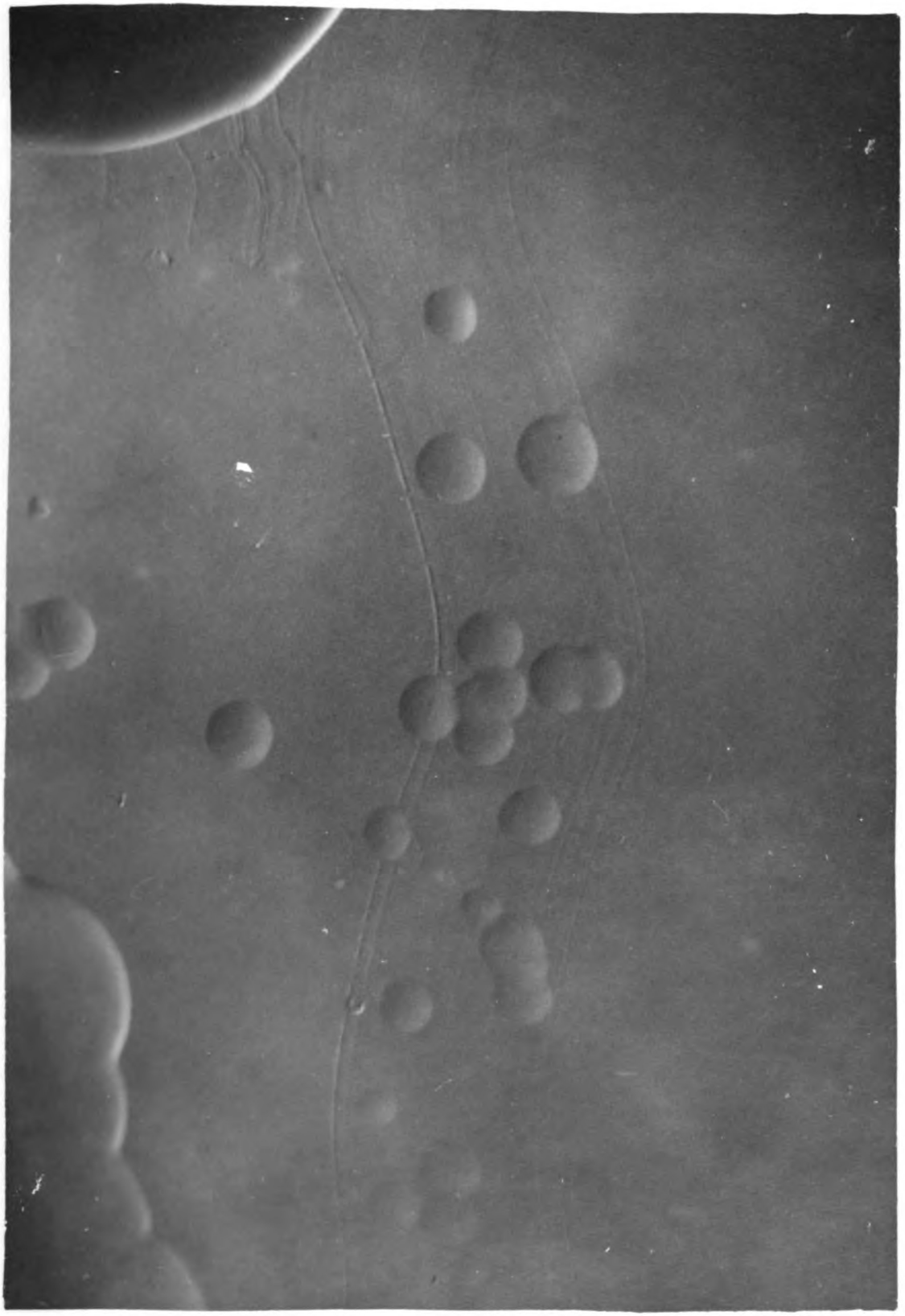


Fig. 11

Fig. 12. Smear of forty-eight hour culture of the bipolar rod on a blood agar, broth base slant incubated at 37° C. Hucker's Gram's stain. 1000x.

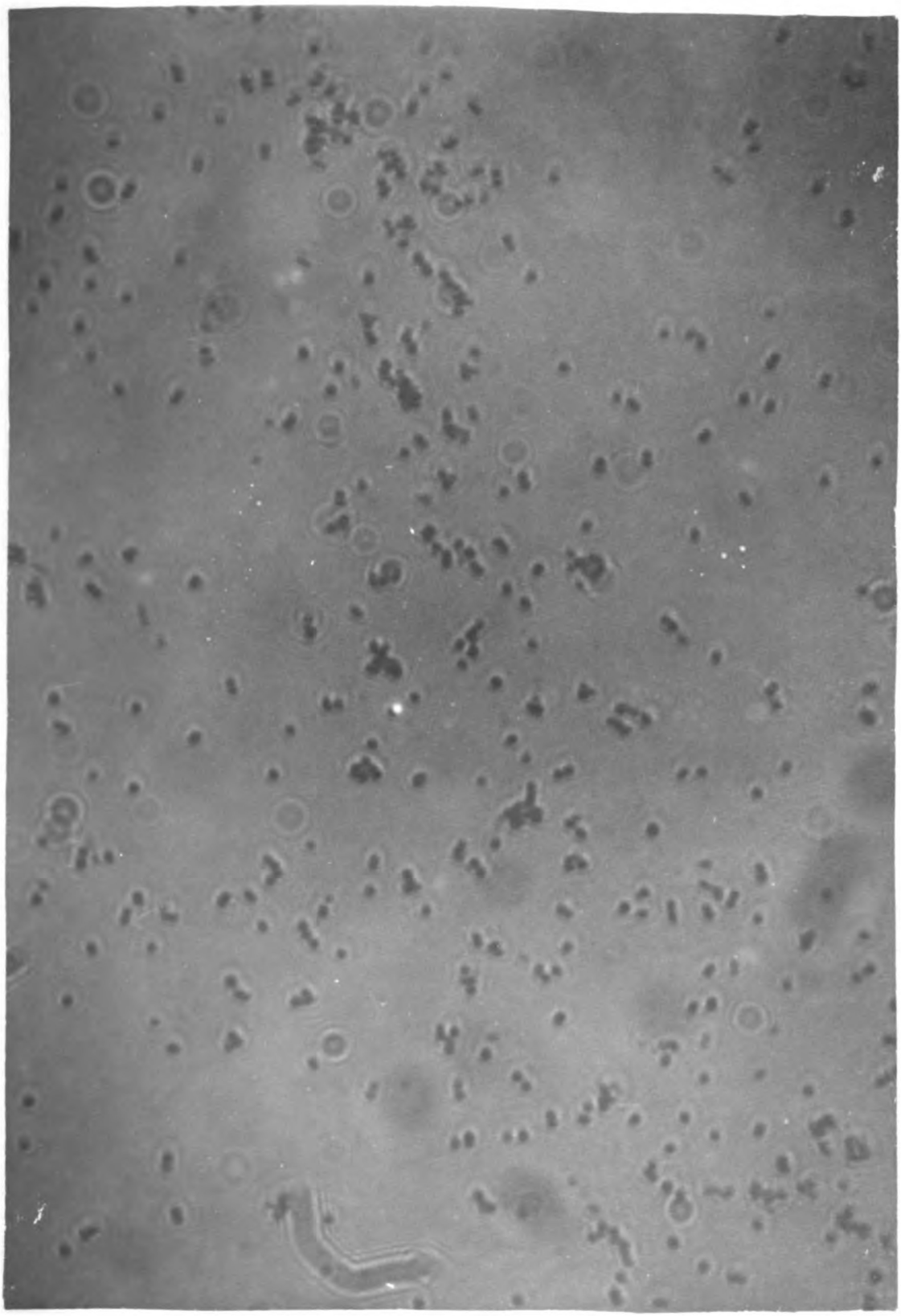


Fig. 12

Fig. 13. The effect of yolk sac inoculation of eleven day chicken embryos with the bipolar rod. The embryo on the left was the control.

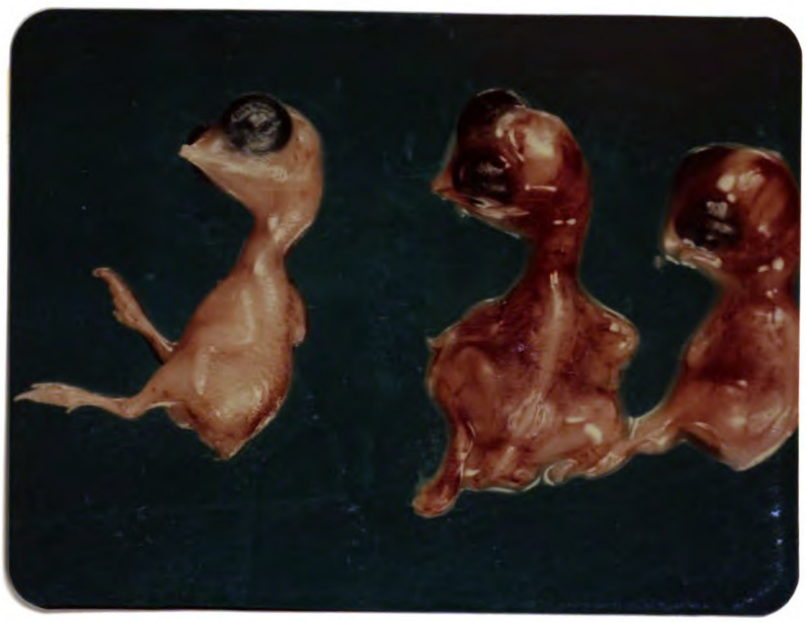


Fig. 13

Fig. 14. Bipolar rod in the mesentary of a chicken embryo following yolk sac inoculation of the organism. Modified Nicolle's. 1170x.

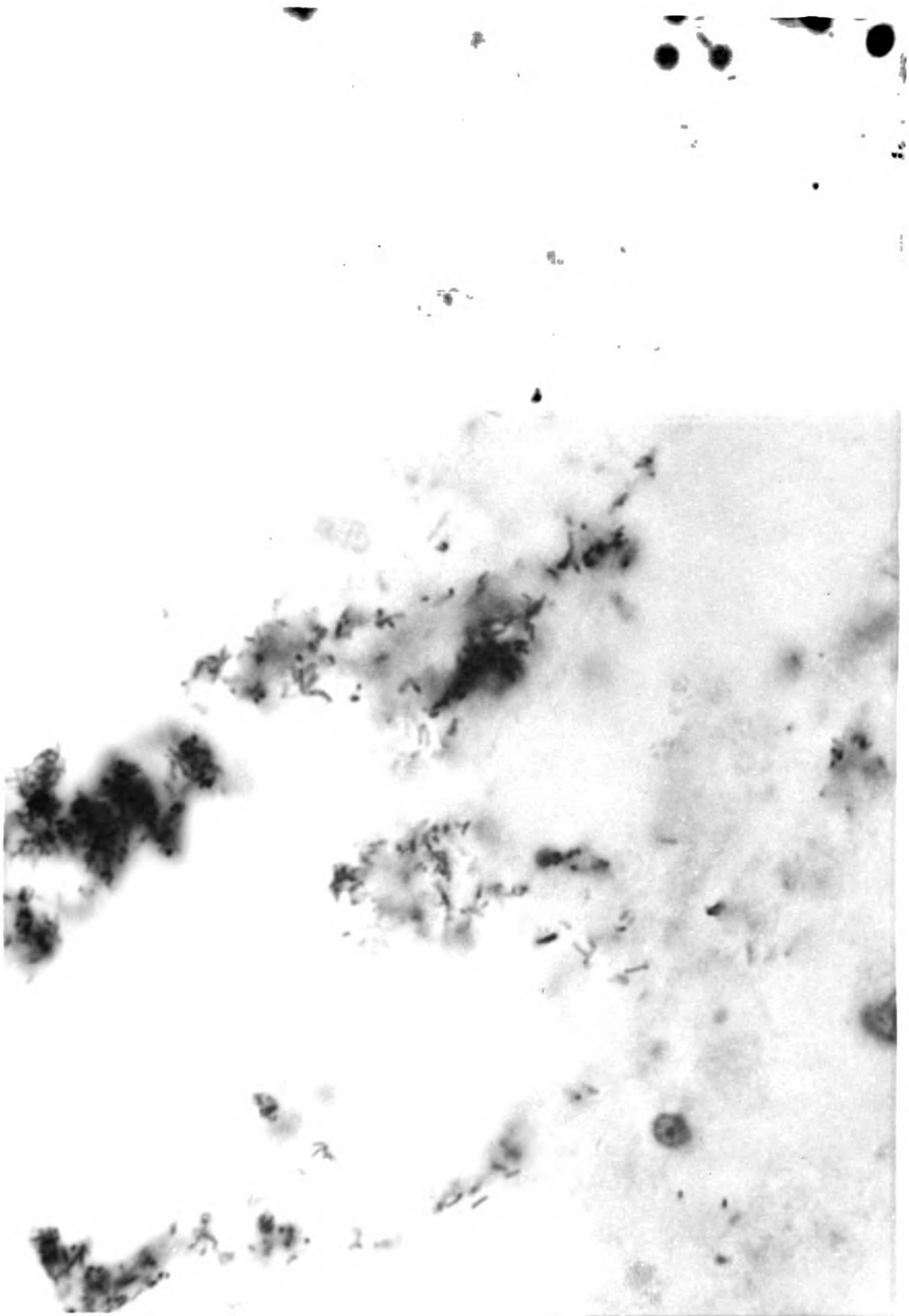


Fig. 14

Fig. 15. Bipolar rod in amniotic fluid of a chicken embryo following yolk sac inoculation. Methylene blue. 1170x.

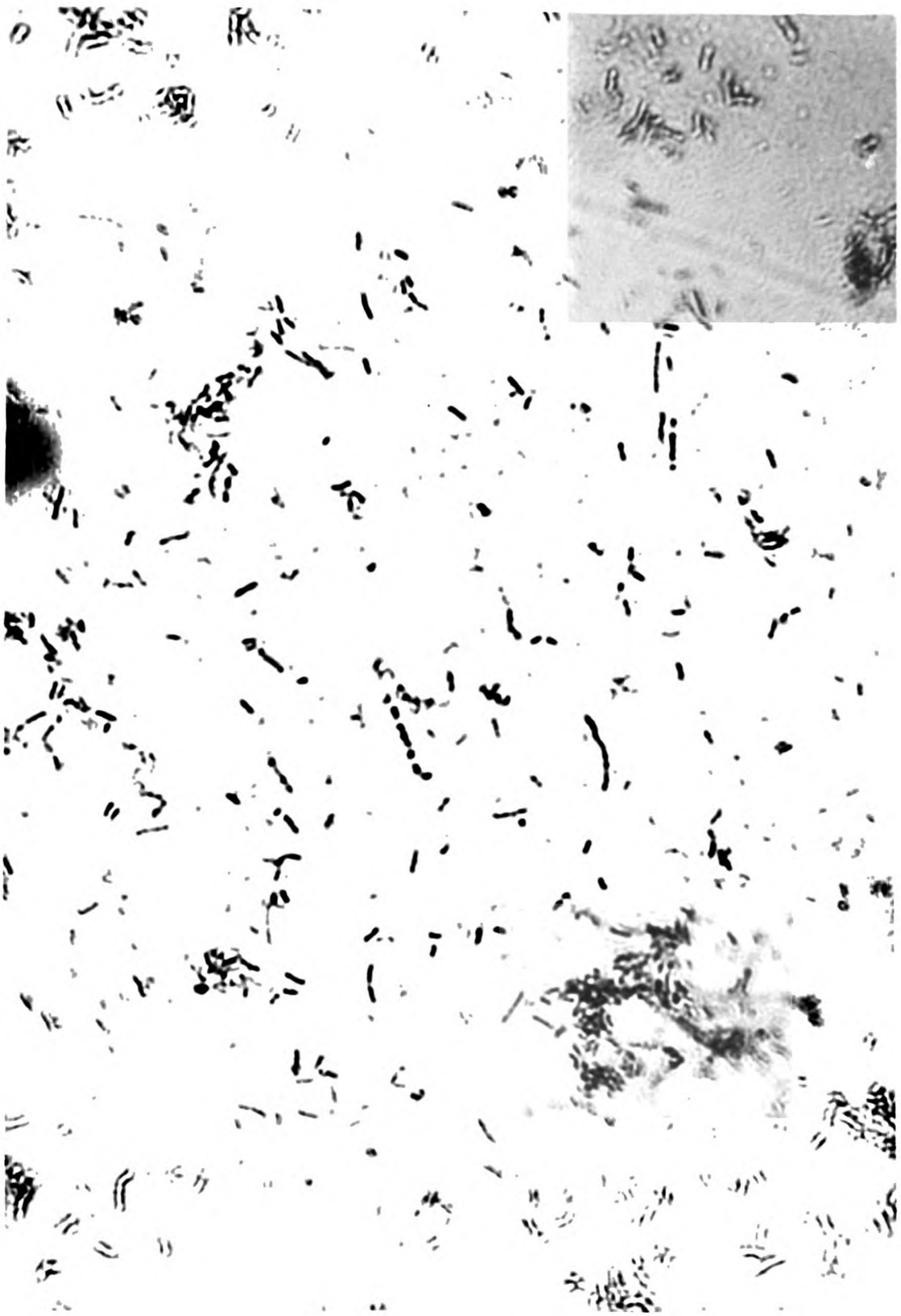


Fig. 15

Fig. 16. 540X

Numerous polymorphonuclears in peritoneal exudate from a guinea pig inoculated intraperitoneally with the bipolar rod. Methylene blue. 900x.

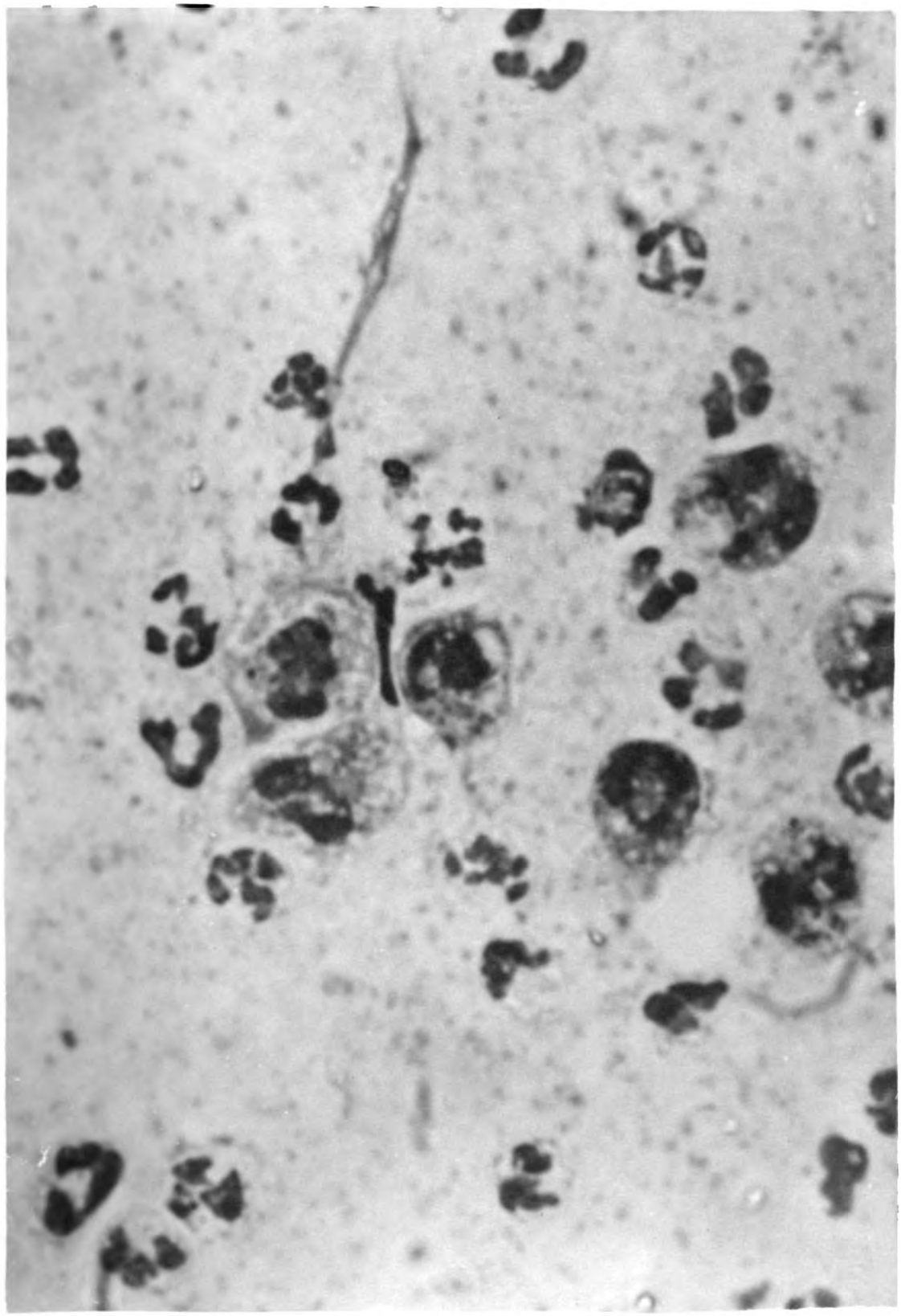


Fig. 16

Fig. 17. 669X Section through a vulvar nodule showing an organized lymph follicle and infiltration of lymphocytes into the epithelium. Tissue taken from naturally infected animal shown in Fig. 3. Also see Fig. 18. H & E. 170x.

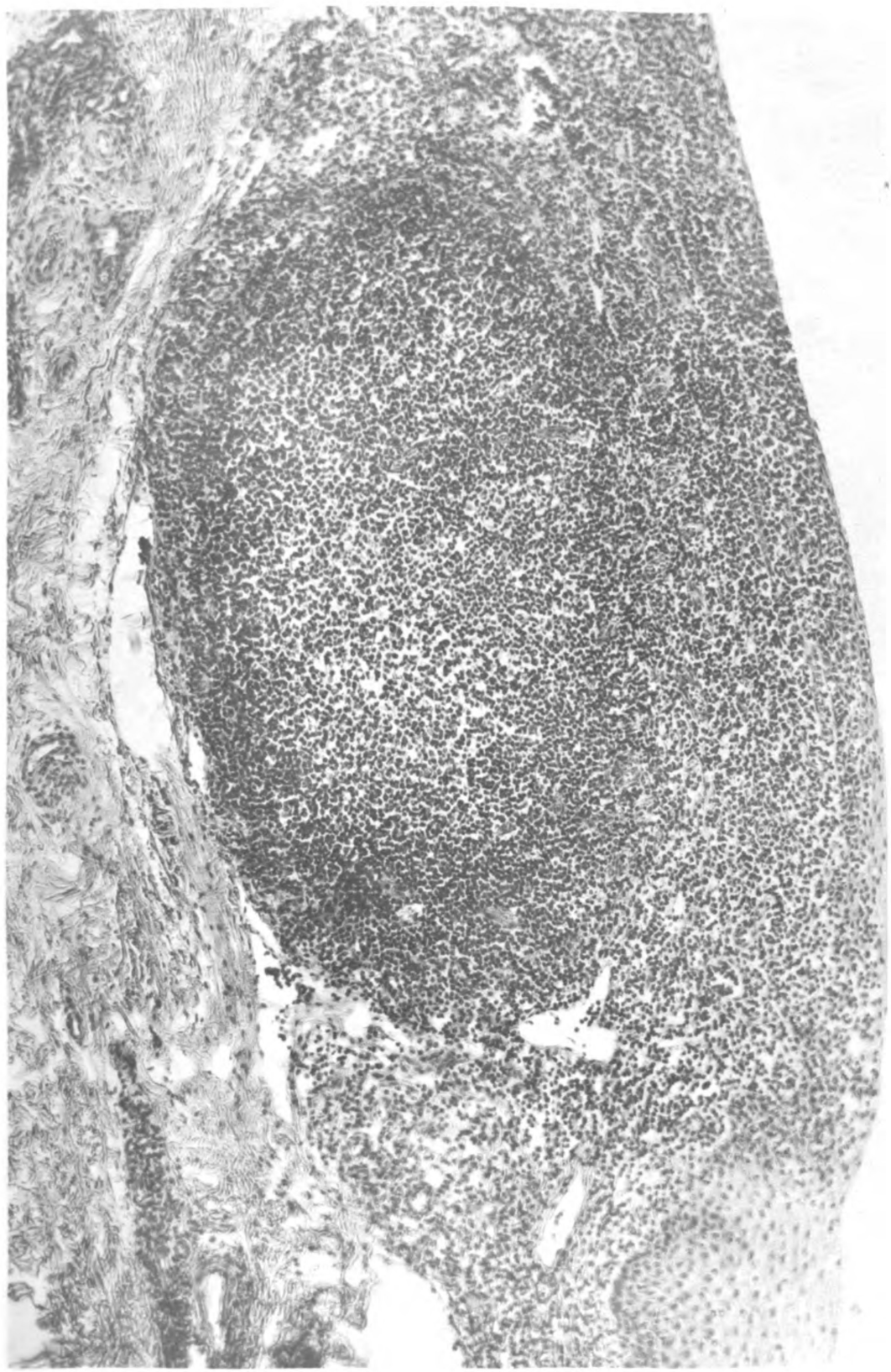


Fig. 17

Fig. 18. 669X High power of lyphocytic invasion of epithelium shown in Fig. 17. H & E. 900x.

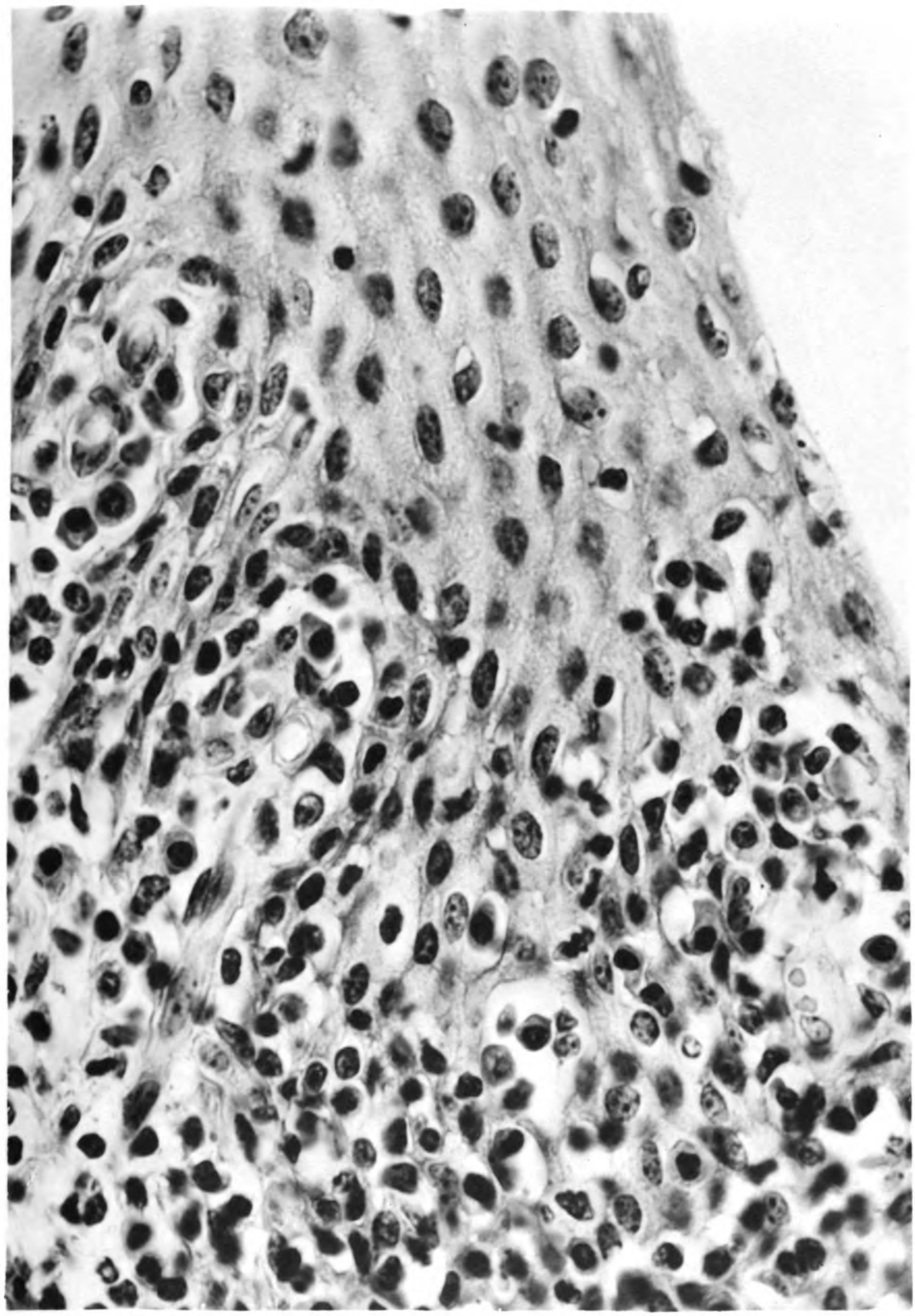


Fig. 18

Fig. 19. (69X) Section through a vulvar nodule showing a lymph follicle and displacement of basal layers of epithelial cells by infiltration of lymphocytes. Tissue taken from naturally infected animal shown in Fig. 3. H & E. 170x.

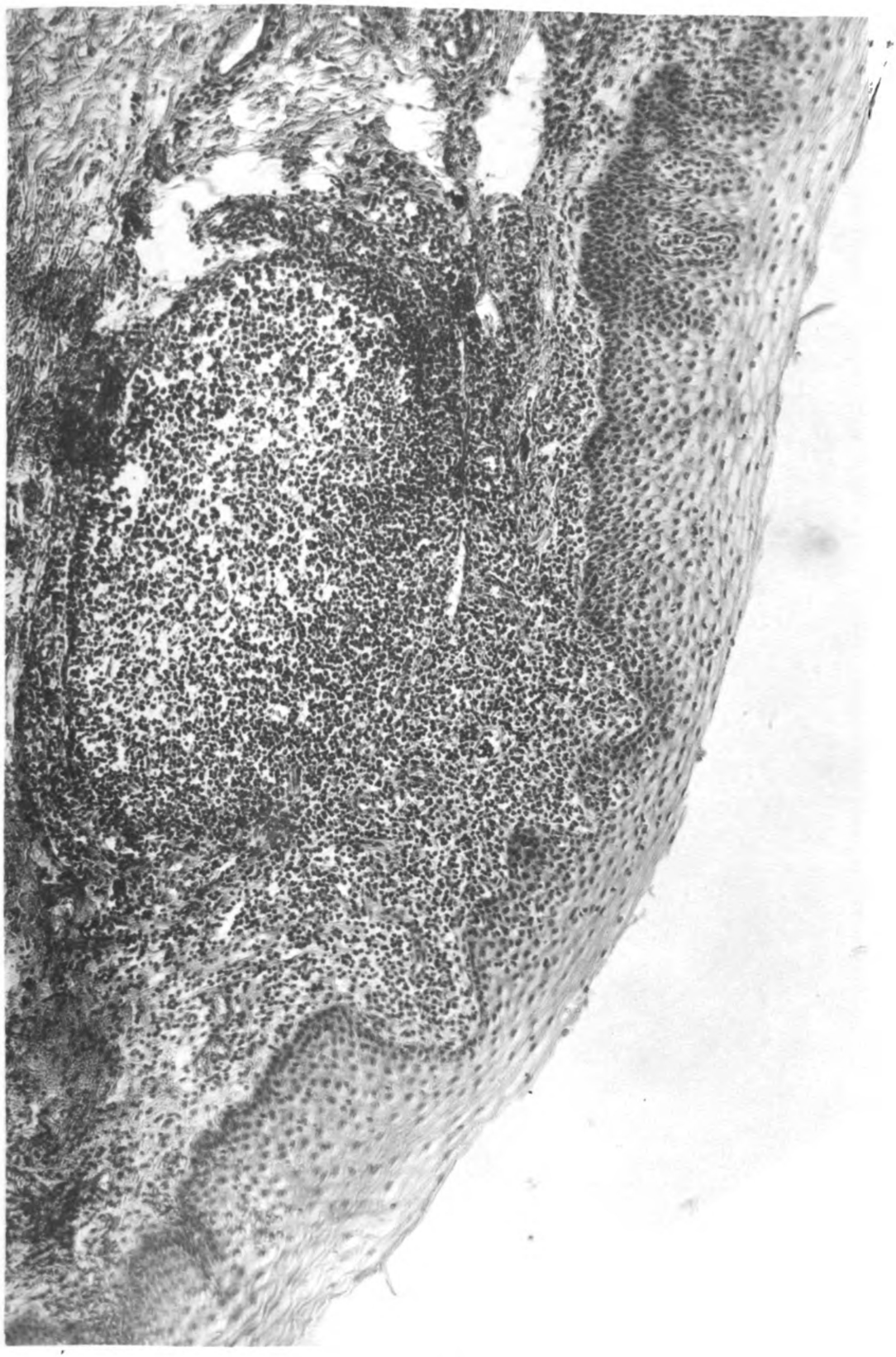


Fig. 19

Fig. 20. 500X Section of vulvar nodule composed of two lymph follicles with marked infiltration of lymphocytes toward the surface and obliteration of the epithelium above the lower follicle. Congestion is evident in the surrounding area. Tissue taken from animal shown in Figs. 1 and 2. H & E. 130x.

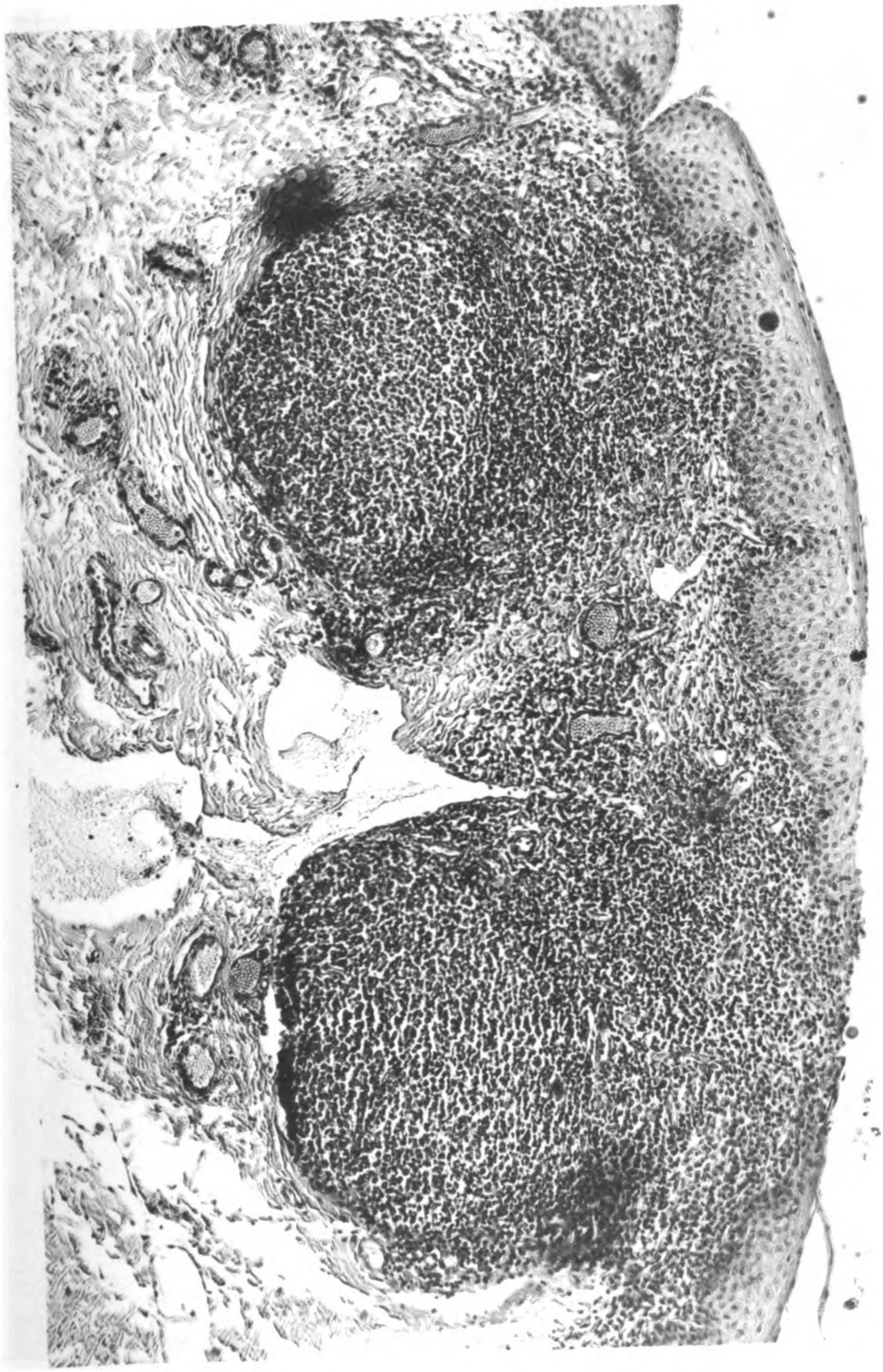


Fig. 20

Fig. 21. Section of two vulvar nodules in close proximity. Biopsy from naturally infected animal. Also see Figs. 22 and 23. H & E. 40x.
535X

Fig. 21

Fig. 22. 535X High power view of lower lymph follicle shown in Fig. 21. H & E. 130x.

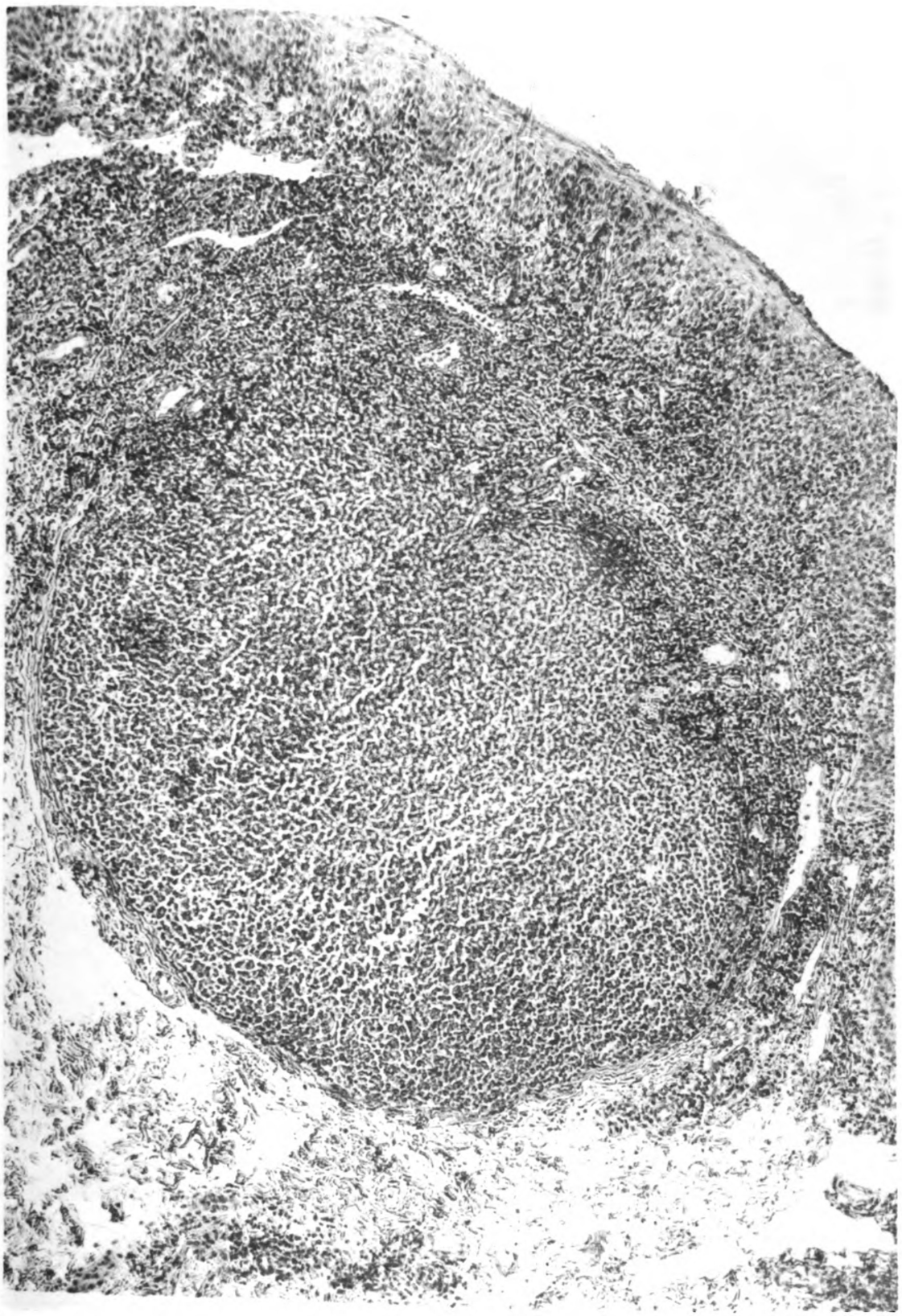


Fig. 22

Fig. 23. 535X High power view of upper lymph follicle shown in Fig. 21. H & E. 130x.

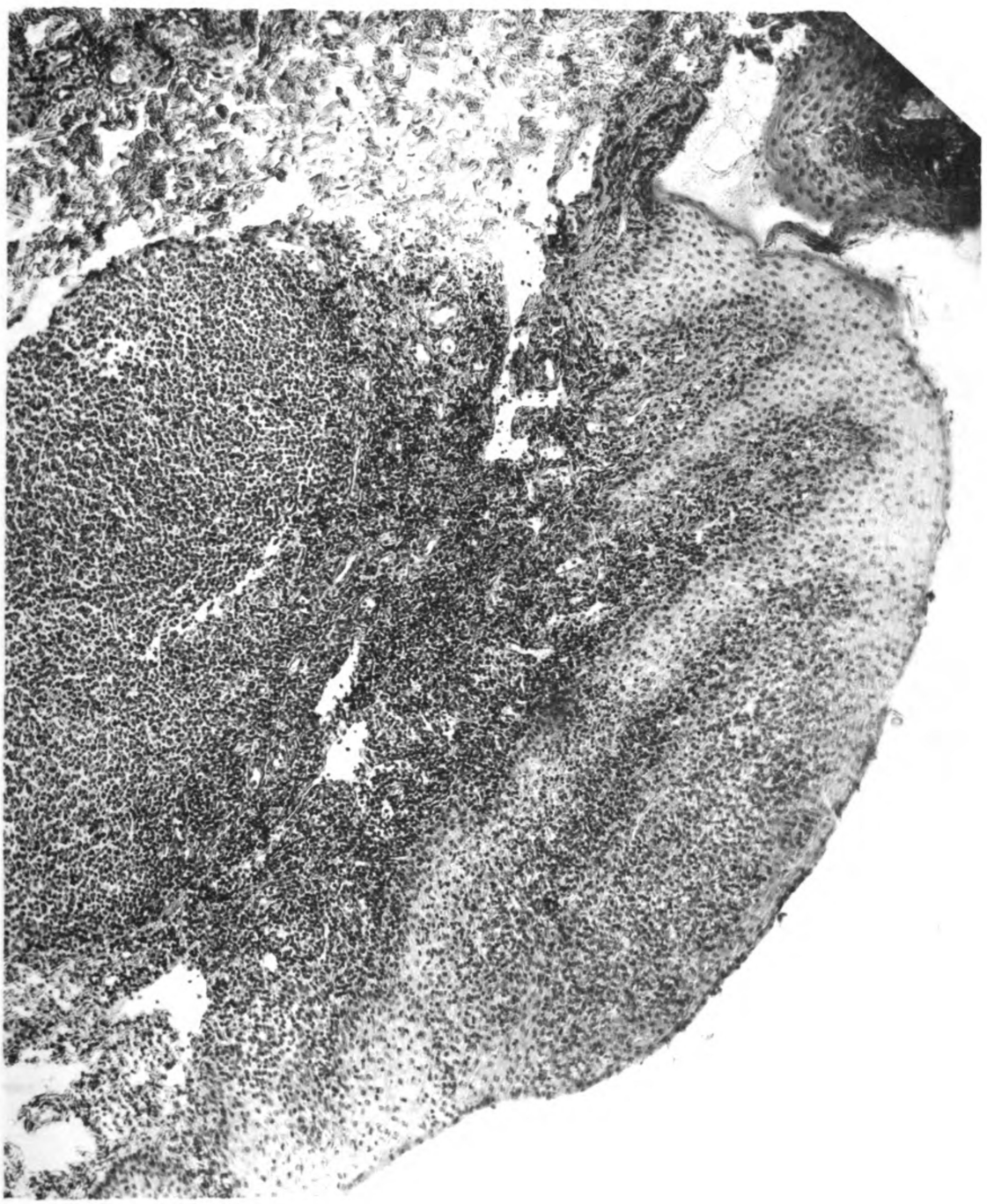


Fig. 23

Fig. 24. Matted vulvar hairs on the vulvae of two experimentally infected calves. Vulvae from a normal calf shown at top of page.

Fig. 24

Fig. 25. Experimentally produced nodules on the lateral vulvar wall of an artificially infected calf.

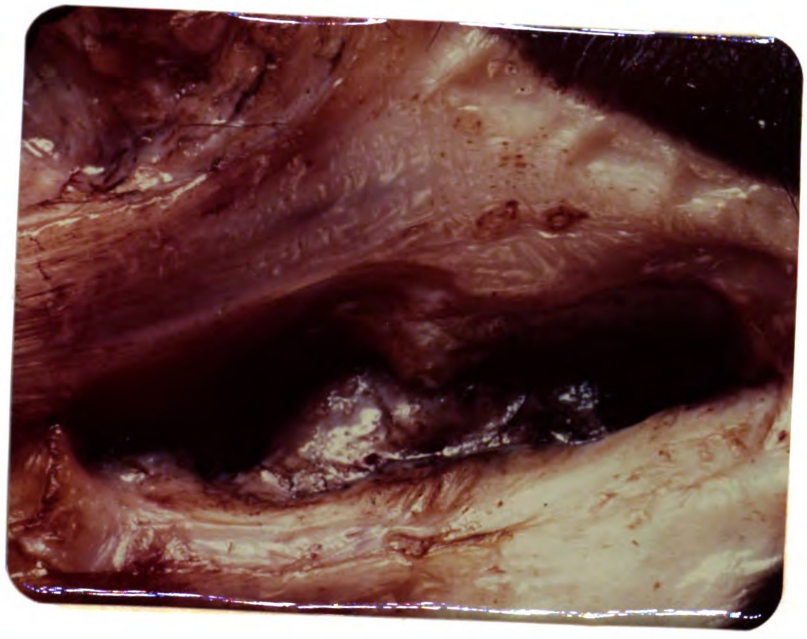


Fig. 25

Fig. 26. Section of normal vulvar mucosa from the control shown in Fig. 24. H & E. 150x.

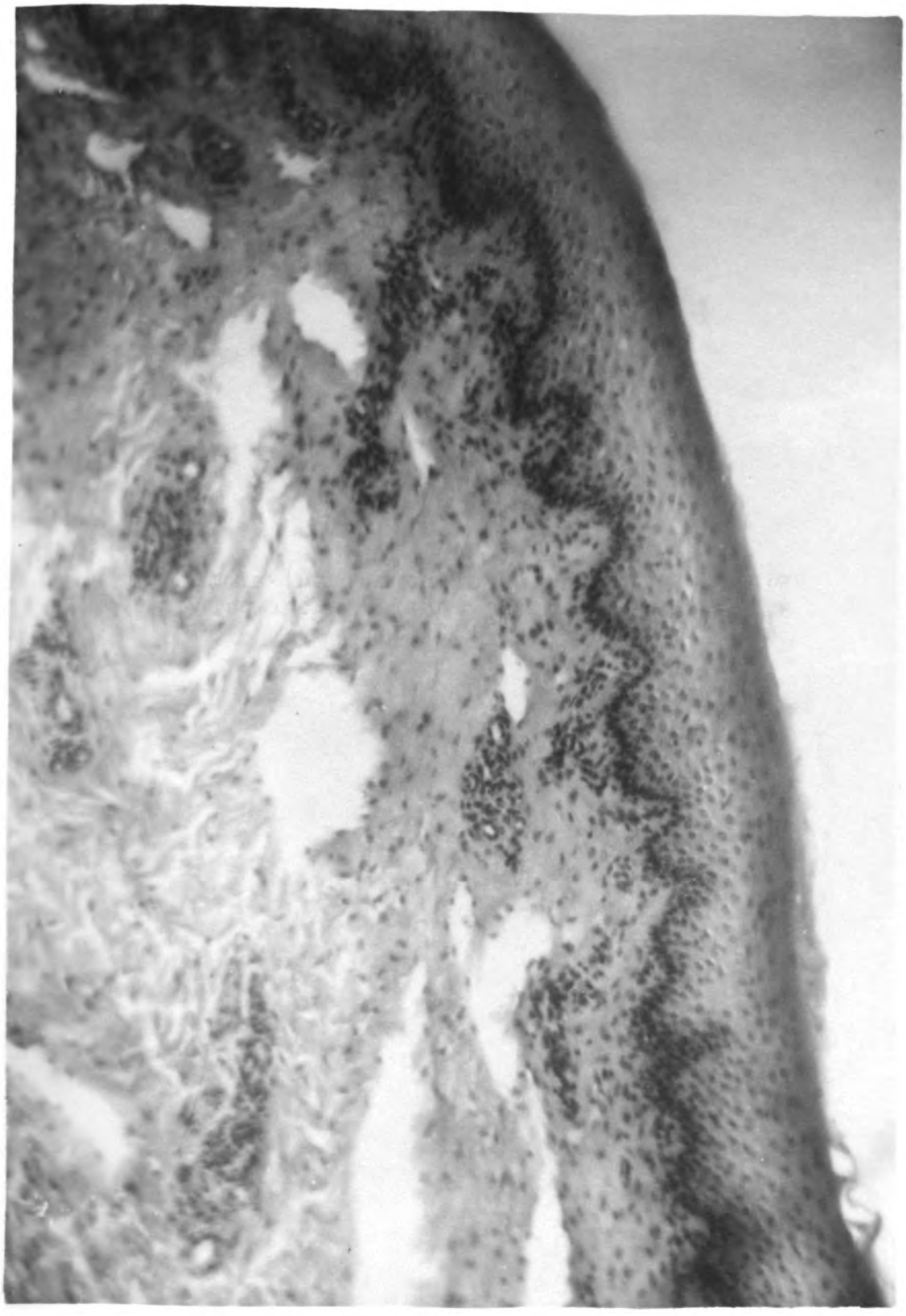


Fig. 26

Fig. 27. 834X Section through obliquely cut nodules showing infiltration of lymphocytes into the epithelium. Tissue taken from experimentally infected vulva shown in Fig. 24. H & E. 130x.

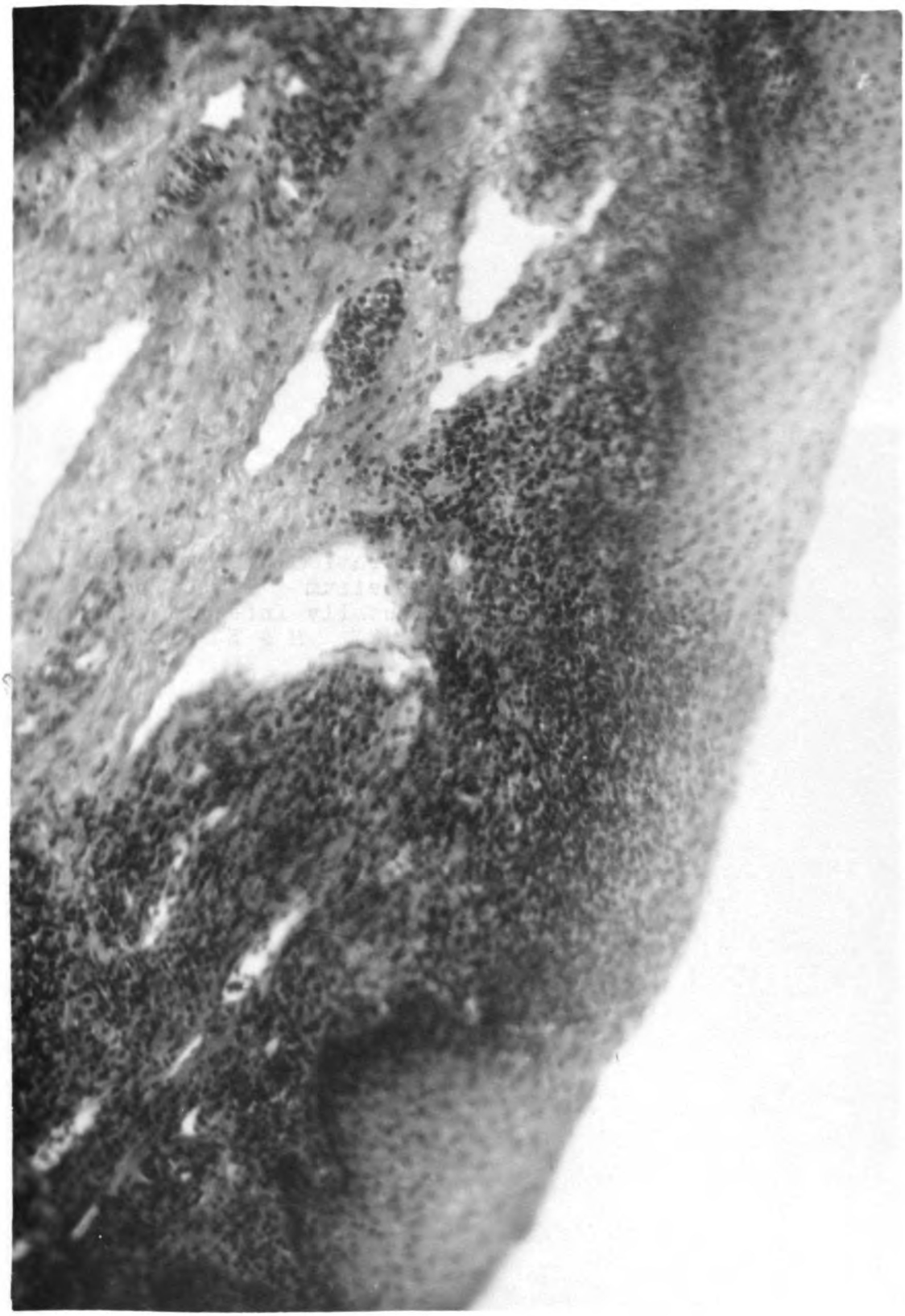


Fig. 27

Fig. 28. 835X Section of an experimentally produced nodule showing a loosely arranged lymph follicle and infiltration of lymphocytes into adjacent area. Tissue taken from infected vulva shown in Fig. 24. Modified Nicolle's. 170x.

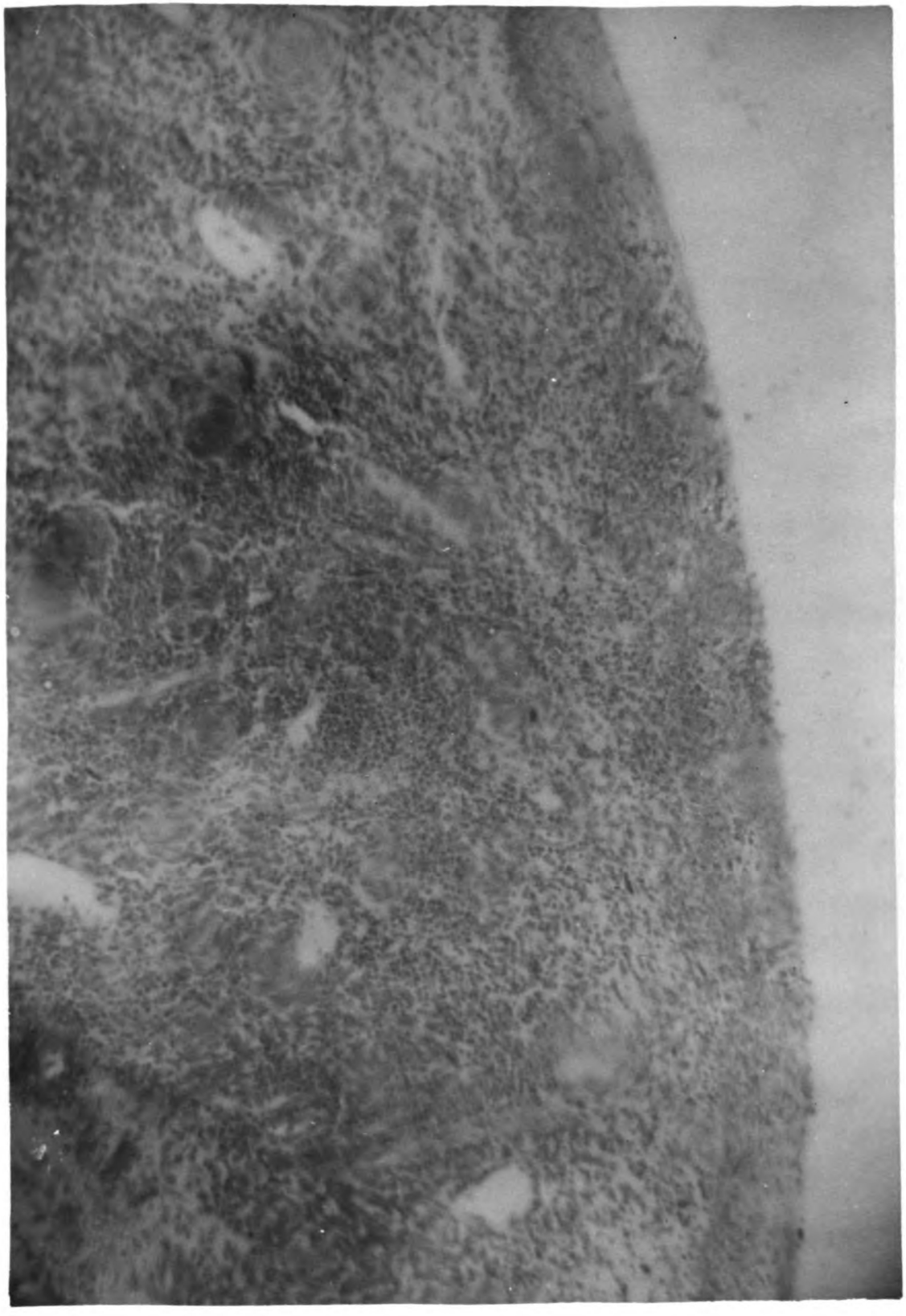


Fig. 28

Fig. 29. 691X Section taken from experimentally produced nodule showing an organized lymph follicle with moderate infiltration of lymphocytes toward the epithelial surface. Modified Nicolle's. 130x.

Fig. 29

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