AN INFECTIOUS GRANULAR VAGINITIS OF COWS.

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PLATE 15.

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An infectious disease of cows characterized by an acute inflammation of the vaginal mucosa and terminating in the formation of raised red nodules studding the mucosa has been recognized in many parts of the world. Hess\(^1\) reported that in certain sections of Switzerland over 60 per cent of the cows suffered with the disease. It is one of the common disorders of cows in this country.

Ostertag,\(^2\) Hecker,\(^3\) and others succeeded in cultivating a Gram-negative streptococcus from the mucopurulent exudate. The organism was described as extracellular and occurred in chains of from 6 to 9. In many instances it was associated with staphylococci and \textit{B. coli}. Ostertag inoculated the vagina of cattle, sheep, goats, pigs, and mares with the streptococcus and reproduced the disease. He points out that Raebiger,\(^4\) Jüterbock,\(^5\) and others reproduced the disease in cows by intravaginal inoculation with a similar streptococcus. Blaha\(^6\) observed in a series of cases bodies embedded in the epithelial cells similar in many respects to those observed in trachoma, which led him to believe that it was a Chlamydozoa infection.

Relatively little concerning the etiology of vaginal infections has been published in this country. Starr\(^7\) noted that the nodules resulted from hyperplasia of the lymph follicles as the result of irritation. He succeeded in cultivating a streptococcus of the \textit{viridans} type from the exudate.

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\(^{1}\) Hess, cited by Ostertag.\(^2\)
\(^{3}\) Hecker, \textit{Berl. tierärztl. Woch.}, 1900, 445.
\(^{4}\) Raebiger, W., \textit{Berl. tierärztl. Woch.}, 1907, 254.
\(^{5}\) Jüterbock, K., \textit{Z. Tiermed.}, 1909, xiii, 354.
INFECTIOUS GRANULAR VAGINITIS OF COWS

The disease we encountered resembled in many respects the usual granular vaginitis. However, it differed from the latter in that it was often more severe, and streptococci were not found in great numbers. It will be brought out later that the streptococci we isolated are not the microbic incitant of the condition.

History of the Cases.

A considerable proportion of our cases occurred during the months of November and December, 1925, and January and February, 1926. The disease became epidemic during November shortly after a tuberculin test when a large number of newly purchased cows and a considerable number of young native cows were introduced into the herd. In several instances all the newly purchased cows and the young native cows in certain barns were attacked about the same time. The epidemic subsided but sporadic cases continued to appear in native young cows introduced into the herd during the first 5 months of 1926. During this outbreak over 100 cases occurred.

In addition to this material we had access to several cases in cows originating in Ohio and purchased from a dealer, also to cases evidently originating in Oregon among cows shipped from there to New Jersey in special cars. This material convinces us that the infection with which we had to deal is one of considerable distribution in this country.

Characterization of the Disease.

The disease was severe among the newly purchased and young native cows. The vulva was greatly swollen and tender. The visible vaginal mucosa was deeply congested and swollen and the clitoris enlarged and bright red. The mucosa covering the floor and walls of the vagina was sprinkled with numerous, tiny, indistinct, grayish white areas which rapidly coalesced to form large plaques of grayish or yellowish white exudate (Fig. 1). When the exudate was forcibly removed a raw, bleeding, grayish red surface was exposed. Considerable thick mucopurulent exudate often gathered about the clitoris and on the floor of the vagina. The inflammation slowly subsided and the exudate sloughed exposing a granulating surface. The mucosa regenerated but tiny, round, red areas appeared embedded in
the mucous membrane. These enlarged and finally became round, raised red nodules 1 to 2 mm. in diameter. A little mucopurulent exudate frequently persisted about the clitoris for a considerable period.

The lesions in the cows originating in Ohio and Oregon were much less severe. In both groups the vaginal mucosa of a number of animals was sprinkled with the red granules similar to those observed in the severe cases. In other instances a more acute condition was observed, and here the vulva was swollen and tender. The vaginal mucosa was bright red and sprinkled with strings of loosely adherent, yellowish white, purulent exudate. At times small amounts of mucopurulent exudate accumulated on the floor of the vagina. With the subsidence of the acute inflammation the characteristic granules commenced to appear.

As far as we could determine the disease was confined to the vagina. The general health was not noticeably affected. The milk yield remained normal.

**Bacteriological Findings.**

We attempted to demonstrate the presence of organisms in films of the exudate from fresh cases by means of heat fixation and staining with methylene blue or Gram's method. By such procedures we were able to recognize a relatively few organisms, usually streptococci or micrococci, but in insufficient numbers to account for the lesions. When rapidly dried films were fixed for 3 to 5 minutes in methyl alcohol and then stained for 30 to 40 minutes with a solution consisting of Giemsa's stain 2.0 cc., methyl alcohol 1.5 cc., distilled water 20 cc., or stained with carbolfuchsin diluted 1:20 in distilled water for 1 to 2 hours, we were able to demonstrate a considerable number of tiny delicate rods with well developed polar granules (Fig. 2). In many instances the cytoplasm between the granules stained feebly or not at all, so that the organisms resembled tiny diplococci, shown in Fig. 3.

The exudate is composed largely of leucocytes, epithelial cells, a few endothelial phagocytes, and considerable mucus. It was possible by obtaining portions of the exudate on sterile swabs and bringing the material to the laboratory to cultivate on the ordinary media
certain well defined types of organisms, such as streptococci, staphylococci, *B. coli*, and long, slender, Gram-positive rods, but in no instance was an organism encountered which resembled the bacillus met with in the films. After a considerable number of failures we were successful in obtaining it in pure culture. The procedure finally adopted was to transfer the exudate directly from the cow into the condensation water of a blood agar slant. Agar slants were prepared from veal infusion, and when slanted and cooled, 0.5 cc. of defibrinated horse blood was added. From the first tube, three others were inoculated in series, care being taken to flame the loop between each tube. The tubes were then sealed with sealing wax and incubated for 5 days at 38°C. As a rule Tube 1 contained streptococci and other types of organisms. Tube 2 contained in addition to streptococci clumps of tiny coccoids and a few tiny bacilli with well defined polar granules. Tube 3 was often to outward appearances sterile, or showed an indistinct haze in a narrow zone about the level of the condensation liquid between the tube wall and the agar; examination of the stained films, however, revealed small numbers of tiny coccoids in clumps and occasional well defined rods (Fig. 4). Tube 4 contained a pure culture or remained sterile. Transfers from the tubes containing only the coccoids and rods are usually successful, but the organism is pretty apt to grow only in the condensation fluid or between the agar and glass for four or five generations; after this time delicate flattened colonies with slightly raised centers appear on the slant.

The organism stains poorly after heat fixation, but films fixed in methyl alcohol stain well with Giemsa. It is Gram-negative and non-motile. The morphology varies. A constant finding in cultures is the densely packed masses of tiny coccoids (Figs. 4 and 6) or tiny rods (Figs. 4, 5, 6) containing polar granules. Free forms more or less elongated with well defined granules are likewise present. In later cultures (Fig. 5), the bacilli are larger and stain more deeply. All cultures passed through a phase in which growth was apparently going on more rapidly, but a final adaptation to the medium had not been reached. Here large clumps of the coccoids are plentiful, as well as extremely long filamentous forms containing large masses of deeply stained protoplasm and the tiny granules (Fig. 6). This
phase passes and finally there is a reversion to the clumps of coccoids and the tiny rods with polar granules. Cultures in blood broth reveal the general variations as illustrated in Fig. 7. It is to be observed that considerable variation in size exists. In the films of exudate the bacilli measure from 1 to 2μ in length. Bacilli of this length are common in all the cultures. The coccoids are exceedingly small, 1/3μ, but the probabilities are that they comprise the polar granules of bacilli whose central zones and cell walls fail to stain. The filamentous forms referred to vary from 10 to 45μ in length. Many show a tendency to fragment near the ends. Others may stain irregularly throughout their entire length.

Once a culture is established on blood agar it is readily transferred to blood broth. Thus far it has not been possible to establish growth in coagulated horse serum to which sterile calf serum water has been added or in serum agar. It will grow, however, in the condensation water of plain agar or ascitic fluid containing fresh tissue such as guinea pig spleen or kidney.

In blood broth to which 1 per cent of dextrose, lactose, saccharose, maltose, or mannitol was added, no fermentation was observed after 10 days incubation. Milk heavily inoculated with blood broth culture remained unchanged. Indole was not produced in sugar-free broth containing blood.

It seemed possible from the size of the coccoids that the organism might readily pass through the coarser Berkefeld filters. On four occasions we attempted filtration through candles V and N but the filtrates remained sterile. Inoculations from the filtrates after suitable incubation were also negative.

**Pathogenicity of the Bacillus.**

Rabbits weighing 2000 gm. withstand 2 cc. of blood broth culture injected intravenously. Guinea pigs of 300 gm. remain well when injected intraperitoneal with 0.5 cc. of culture. 1 cc. may cause death or produce a febrile reaction lasting several days. The injection of 2 cc. has always resulted fatally. Death results from peritonitis in 24 hours. The bacilli are found in the exudate in enormous numbers and can be cultivated from the heart's blood.

Heifer calves, 3 or 4 months old, and 2 year old heifers were in-
oculated into the vagina with culture. In every instance acute inflammation resulted. Granules similar to those observed in the spontaneous disease were always observed after the acute inflammation had subsided. The following experiment affords an example.

The mucosa of the vagina of unbred Heifer 1116 was brushed with a swab immersed in the condensation water of a 3 day blood agar culture of the bacillus in the third culture generation. There was no reaction during the first 24 hours. On the 2nd day the vulva was swollen and tender. The vaginal mucosa was bright red and swollen. Strings of yellowish white, purulent exudate adhered to the mucosa covering the floor and sides. On the 3rd day the swelling was more marked and there was considerable tenderness on manipulation. The whole mucosa was bright red and bled when brushed lightly with a sterile swab. Blood agar inoculated with exudate on this day resulted in pure cultures. The films of the exudate (Fig. 8) showed necrotic epithelial cells, leucocytes, mucus, and a moderate number of the characteristic bacilli. On the 4th, 5th, and 6th days the congestion and swelling were pronounced, and considerable exudate was present about the clitoris and adhered to the walls. Cultures made on the 5th day contained the bacilli. On the 9th day there was more exudate and the whole mucosa appeared to be granulating. After 11 days the mucosa was studded with barely visible, indistinct, grayish white areas. These were a little larger and more red in color on the 12th day, and on the 13th day were recognizable as distinctly visible, raised, red nodules. The nodules increased in size and finally on the 19th day appeared round, sharply raised, firm, 1 to 1.5 mm. in diameter. Cultures made on this day contained the bacilli.

The heifer was slaughtered 89 days after the inoculation. The granules were still visible in the mucosa of the vestibule and walls of the vagina. They did not extend into the uterus. Examination of material fixed in Zenker's fluid and stained with methylene blue revealed that the lymph follicles in the submucosa were hyperplastic. Some follicles were discrete, others were joined by bands of round cells. Over the smaller, more discrete, round celled accumulations the epithelium was normal, but that overlying the larger follicles was heavily invaded with round cells (Fig. 9).

Mention has been made that many of the cultures from the spontaneous cases contained streptococci. These were all of the non-hemolytic or green-producing type and resembled those described by F. S. Jones as the type usually found in the vagina of healthy cows. We inoculated four heifer calves with the cultures. No inflammation resulted, but on subsequent inoculation with pure cultures of

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the rods from vaginitis acute inflammation resulted followed by the formation of typical granules. In these experiments the granules appeared from 5 to 7 days after infection.

We were unable to obtain material for histological study from acute spontaneous cases. On several occasions old cows leaving the herd for various reasons were inoculated into the vagina with material from severe cases, but the animals failed to contract the disease. They had probably passed through an attack of the disease and were resistant. Calves as a rule respond moderately only to inoculation with infectious material. In certain instances such inoculations induced severer inflammation and afforded some insight into the nature of the acute process.

The mucosa of the vagina of Calf 1240, 4 weeks old, was brushed with a swab containing exudate from two spontaneous cases. The usual type of acute inflammation followed. 4 days later the calf was slaughtered. At antemortem examination the mucosa was scarlet and sprinkled with strings of tenacious, yellowish white exudate. Evidently the method of slaughter, similar to that used in abattoirs, caused a blanching of the vaginal mucosa, since it was of a pale yellowish pink color along the floor and walls. Anterior to the clitoris was a red area situated within the mucosa. Other portions of the mucosa contained a few tiny, sunken, irregular, red patches. A little mucopurulent exudate was present on the mucosa of the floor. Inoculation of blood agar with this material developed cultures of the characteristic bacilli. The uterus was normal.

Histological examination of fixed and stained material revealed well defined necrosis of portions of the epithelium. In portions most of it had apparently sloughed so that the surface was covered with a thin layer of necrotic epithelial cells, degenerated leucocytes, and a little fibrin. The submucosa was edematous and infiltrated with leucocytes and round cells. The blood vessels were moderately engorged with red cells and contained excessive numbers of leucocytes and round cells. Other portions of the epithelium were intact. Lesions were not found in sections of the uterus.

From clinical examination of cases, exudate from such cases, and the histological material, we feel that the process may in part be pieced together. The bacilli attack the mucosa in certain foci. Here necrosis of the epithelium results. A little fibrin may exude beneath the epithelium. Leucocytes in large numbers invade the mucosa. The submucosa is invaded by round cells and leucocytes. The exudate and mucosa slough, followed by regeneration accompanied by
large accumulations of round cells in follicle-like masses in the submucosa. The large amount of exudate in the outbreak may be explained by a heavy infection with the bacilli so that the necrotic areas occurred close together and gave the appearance of a continuous membrane.

DISCUSSION.

It is apparent that in the large outbreak we had to deal with a severe type of inflammation of the vagina. In certain respects the type of disease differed from that usually considered typical of granular vaginitis and that encountered in our cases drawn from other sources. From each group, however, we succeeded in isolating a similar organism. The acute inflammation in all cases terminated in the appearance of the characteristic granules in the submucosa.

In the outbreak several factors contributed to exalt the severity of the infection. The disease could easily be spread by thermometers during a tuberculin test. There had been a large number of young cows recently introduced into the herd and these animals with young native cows represented a large number of susceptible individuals. The method of spread was apparently direct from cow to cow, since in this herd animals are brushed and curried before milking. It appeared that all cows on one side of a barn were infected at about the same time. A favorable opportunity was thus created for the rapid spread of the inciting organism to a large number of relatively highly susceptible cows.

With the culture isolated from the severe cases it was not possible to produce the severe type of disease. We simply reproduced a condition similar to that found in the cows from Oregon and Ohio. It must be remembered, however, that we used for the purposes of inoculation relatively small doses of a feebly growing culture. The experimental disease was always well pronounced, accompanied by a mucopurulent exudate, and the acute process terminated in the formation of the characteristic granules. In sharp contrast are the entirely negative results after inoculation with non-hemolytic streptococci also isolated from the vaginal exudate.

Although the question of immunity produced by an attack is not
definitely proved, yet considerable resistance seems to result. We have noted on several occasions that old cows standing between severe cases did not contract the disease although infectious material must have been frequently brushed into the vagina. In two instances cows which had been exposed during the outbreak were inoculated intravaginal with material from severe cases and failed to develop the disease.

The bacillus isolated is apparently one not described before. That its distribution is widespread is indicated by its presence in cows from Oregon, Ohio, and New Jersey. Morphologically it resembles in certain respects the bipolar group in having polar granules and giving rise to the long involution forms during certain phases. However, it differs in many respects from usual organisms of this type. It fails to ferment dextrose, or any of the carbohydrates, produces no indole, and possesses relatively no pathogenicity for rabbits. It grows only in media containing blood or bits of tissue, and then only in the parts of the tubes containing little free oxygen. It is not an anaerobe. Thus far it has failed to grow in the unsealed tube. It possesses no hemolytic or proteolytic properties. At present its identification rests largely on morphological criteria, the difficulty with which it stains, and the inability to grow in media which do not contain fresh blood or tissue.

SUMMARY.

A disease of cows characterized by swelling of the vulva, acute inflammation of the vaginal mucosa, accompanied by a more or less profuse mucopurulent exudate is described. After the acute inflammation subsides the mucosa becomes studded with tiny, round, raised, red nodules which persist 2 or 3 months or longer. The acute lesion consists in necrosis of the epithelium and accumulations of leucocytes and round cells in the edematous submucosa. The nodules in the later stages are made up of densely packed masses of lymphocytes in the submucosa which force the epithelial layer outward.

A Gram-negative bacillus with tiny polar granules was found in the exudate. It measures 1 to 2μ in length and stains with difficulty. The organism was obtained in pure culture by inoculating the exudate
into tubes of slanted agar to which defibrinated horse blood had been added. Growth occurs only in sealed tubes. The organism possesses slight pathogenicity for guinea pigs. When freshly isolated cultures were introduced into the vagina of heifers or young calves, acute inflammation resulted which terminated in the characteristic granular stage of the disease.

EXPLANATION OF PLATE 15.

Fig. 1. Natural infection. Severe inflammation of the vagina. Note patches of exudate on the mucosa. About 1/5 natural size.

Fig. 2. The bacilli along the border of heavily stained mass of exudate. Spontaneous case. Methyl alcohol fixation. Dilute carbolfuchsin stain. × 1000.

Fig. 3. Exudate from spontaneous case, showing two large leucocytes and two bacilli with polar granules. Methyl alcohol fixation. Giemsa stain. × 1000.

Fig. 4. The condensation fluid from an original blood agar culture 5 days old. Note the clump of coccoids, a single well stained bacillus, and an elongated form. A red blood cell is also present. Giemsa stain, after methyl alcohol fixation. × 1000.

Fig. 5. The same culture as Fig. 4, in the fourth generation. Condensation fluid of a 5 day blood agar culture. The bacilli are larger and stain more intensely. A red blood cell is included in the field. Giemsa stain. × 1000.

Fig. 6. The same culture as Figs. 4 and 5, in the fourteenth culture generation. Condensation fluid of a 3 day blood agar culture. Three forms are illustrated, a large clump of short coccoids, a few individual bipolar forms, and two long filaments, one of which shows a tendency to fragment. Giemsa stain. × 1000.

Fig. 7. A blood broth culture, in the third generation, 3 days old. Giemsa stain. × 1000.

Fig. 8. The bacilli in the vaginal exudate from Heifer 1116, 3 days after intravaginal inoculation with culture. Giemsa stain. × 1000.

Fig. 9. Section of the mucosa of the vagina of Heifer 1116, 89 days after inoculation with culture. Note the infiltration of round cells in the mucosa and the dense accumulation of round cells in the submucosa. Zenker's fixation. Eosin-methylene blue stain. × 66.
(Jones and Little: Infectious vaginitis of cows.)