THE ORGANISM ASSOCIATED WITH SPECIFIC INFECTIOUS CYSTITIS AND PYELONEPHRITIS OF COWS.

BY F. S. JONES, V.M.D., AND RALPH B. LITTLE, V.M.D.

(From the Department of Animal Pathology of The Rockefeller Institute for Medical Research, Princeton, N. J.)

PLATE 1.

(Received for publication, March 31, 1926.)

In a previous communication we described a specific infectious cystitis and pyelonephritis of cows apparently not observed previously in this country. Owing to the inclusion of cases of other types of infections of the kidneys in the reports of certain writers and the slight comment on the bladder lesions, considerable confusion seems to have arisen both in regard to the organism and its method of reaching the kidney. The organism most frequently obtained in Europe thus far has failed in the hands of several observers to reproduce the disease.

Höflich and Enderlen cultivated from affected kidneys a Gram-positive diphtheroid. Enderlen named it B. pyelonephritis houn. Ernst, who obtained pathologic kidneys from 24 cows slaughtered at the abattoir, found diphtheroids similar to those cultivated by Höflich and Enderlen in 11 instances. The others were associated with B. pyogenes, B. coli, and the tubercle bacillus. These workers agree that the diphtheroid is not pathogenic for the smaller animals. They obtained only negative results by intravenous injection of cultures into cows. Ernst first injected sterile sand into the bladder of a cow and on the following day 100 cc. of broth culture. Violent inflammation ensued but subsided after 9 days. This experiment, with the negative experiments reported by himself and others, led him to question the relationship of the diphtheroid to pyelonephritis.

In our first communication little was reported concerning the bacteriological findings. In all our cases we were able to readily cul-

2 Höflich, D., Monatsch. prakt. Tierheilk., 1891, ii, 337.
tivate a diphtheroid from the urinary sediment and from the pelvis of affected kidneys. As a rule the cortex was not invaded by the bacillus although in a few instances it was obtained from cortical lesions of advanced cases. The bacilli were usually found in practically pure culture, but at times cocci and streptococci were encountered in small numbers. From the morphology and lack of pathogenicity for small animals as noted by Enderlen, Hoříček, and Ernst, it is evident that our cultures resemble those obtained by these workers. From 6 cases we cultivated the organism from the kidney. From the urine of 20 definitely affected cases the same organism was obtained. In the main it is true that the bacilli usually occur in the pelvis of the kidney in pure culture. They always predominate in the urine and frequently occur in pure culture, although at times cocci of various sorts are found. These bacteriological findings associated with lesions of uniform character led us to conclude that we were dealing with a specific infectious disease.

**The Morphological and Cultural Characters of the Organism.**

The bacillus is non-motile and in the urinary sediment is found in dense masses. The rods are slender and vary from 2 to 3μ in length. They stain well with the ordinary stains and are Gram-positive. Those from the tissues are not as pleomorphic as those from the earlier transfer cultures, although many may show polar granules or swollen ends. The morphology of cultures grown in broth is more variable; short coccoid forms, beaded rods with swollen ends, as well as the usual slender rods are abundant (Fig. 1). Occasionally a stalk with rudimentary branches may be observed.

The bacillus is readily grown on ordinary media. In liquids, such as broth or urine, aggregates of considerable size are found at the bottom of the tube. On the surface of agar or serum the growth is raised, grayish white, and dry. On potato the growth at first is usually grayish white, but later it becomes a dingy yellow and the potato turns brown. Blood serum and gelatin are not liquefied and hemolysin is not produced. In litmus milk the growth reaction is characteristic. The litmus at the bottom of the tube is reduced, here the casein coagulates, but the upper stratum is deep blue and coagulates slowly. The casein is digested slowly. Of the carbohydrates tested only dextrose has been attacked. No gas is produced, but a final hydrogen ion concentration of pH 4.9 to 5.0 is a regular finding. Lactose, saccharose, maltose, and mannitol are not attacked, since only alkali is produced in tubes containing them. These findings were constant for the 26 strains studied.
TABLE I.

The Agglutination Affinities of Various Strains of the Diphtheroid Organism.

<table>
<thead>
<tr>
<th>Case No. and source of the strain</th>
<th>Antiserum Strain 1132 (kidney), Herd A.</th>
<th>Antiserum Strain 1143 (kidney), Herd B.</th>
<th>Antiserum Strain 1151 (urine), Herd B.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1132. Kidney, Herd A.</td>
<td>C*</td>
<td>C.</td>
<td>+++</td>
</tr>
<tr>
<td>273. Urine, Herd A.</td>
<td>C.</td>
<td>C.</td>
<td>C.</td>
</tr>
<tr>
<td>139B. Urine, Herd A.</td>
<td>C.</td>
<td>C.</td>
<td>C.</td>
</tr>
<tr>
<td>1143. Kidney, Herd B.</td>
<td>C.</td>
<td>C.</td>
<td>C.</td>
</tr>
<tr>
<td>1151. Urine, Herd B.</td>
<td>C.</td>
<td>C.</td>
<td>C.</td>
</tr>
<tr>
<td>1154. Urine, Herd B.</td>
<td>C.</td>
<td>C.</td>
<td>++++</td>
</tr>
<tr>
<td>1243. Urine, Herd B.</td>
<td>C.</td>
<td>C.</td>
<td>C.</td>
</tr>
<tr>
<td>1223. Urine and kidney, Herd C.</td>
<td>C.</td>
<td>C.</td>
<td>++++</td>
</tr>
<tr>
<td>1225. Urine, Herd C.</td>
<td>+++++</td>
<td>++++</td>
<td>+</td>
</tr>
<tr>
<td>K.M. Urine, Herd D.</td>
<td>C.</td>
<td>C.</td>
<td>C.</td>
</tr>
<tr>
<td>K. Urine, Herd E.</td>
<td>+++++</td>
<td>+++</td>
<td>+</td>
</tr>
</tbody>
</table>

* The degree of agglutination has been recorded as follows: C., complete clearing of the fluid as result of agglutination; +++++, a heavy clumping without complete clearing; ++++, well defined clumping; ++, less well defined clumping; +, a definite deposit on the bottom of the tube; ±, a slight deposit on the bottom of the tube.
Since it was possible to show that we were dealing with a group of organisms whose morphological and cultural characters were identical, it was desirable to know whether strains from different herds were immunologically related.

Our material was drawn from five herds in this vicinity and, since there was no exchange of cows between the herds, we were presumably dealing with fairly independent strains. To establish their immunological relationship, rabbits were immunized with cultures of the various strains. After the serum had reached a maximum agglutinin titer the animals were bled. The serum was tested against salt solution suspensions of all the cultures. The tendency of certain strains to clump spontaneously was largely overcome by rapid transfer on artificial media and mechanical shaking of the suspension with glass beads. Inasmuch as the 26 strains reacted to about the same degree, the details concerning only a few from each herd are given in Table I.

In the main it can be said that all cultures reacted to a greater or less degree to three agglutinins prepared by immunizing rabbits with single strains. It is of further interest to note that those isolated from the kidneys at autopsy or from the urine of cows in Herds A, B, C, and D, behaved in an almost identical manner to all agglutinins. The strain from Herd E also agglutinates with the specific sera but to a less degree.

Pathogenicity for Animals.

Our cultures, like those described by the European workers, are not pathogenic for rabbits or guinea pigs. Rabbits easily withstand an intraperitoneal inoculation with 10 cc. of a 48 hour bouillon culture. Guinea pigs and white mice are equally resistant. We were unable to show that the organism produces a soluble toxin. Young guinea pigs injected intraperitoneally with filtrates of unheated 48 hour and 5 day broth cultures fail to react. In our first communication mention was made of experiments in which broth cultures filtered through Berkefeld candles were injected into and beneath the skin and instilled on the conjunctiva of infected cows. In no instance were allergic reactions obtained.

A number of experiments were made with cows. In two instances cows were injected intravenously with 5 cc. and 10 cc. of freshly isolated broth cultures. Slight temperature reactions occurred shortly
after the injections, but the organism never appeared in the urine. At autopsy the urinary system failed to show abnormalities. On two occasions sterile swabs moistened with cultures were introduced into the vagina. The organism failed to develop in the vagina, and as far as we could determine the bladder never became invaded. The findings at autopsy were negative. In two instances cows were fed large quantities of freshly isolated broth cultures. One developed typical cystitis associated with the diphtheroid. The other remained normal. The experiment was repeated with entirely negative results. The feces were examined a number of times after the cows had been fed the cultures but the organism could not be found.

Instillation of small quantities of culture into the bladder led to definite infection. Since this is the first recorded experiment in which infection with pure culture of the diphtheroid has been established the details are given.

**Cow 1100.**—A Holstein that had been under observation at the Institute for over 10 months. Samples of urine were obtained for examination on several occasions before the injection. The urine was normal and contained only a few cocci. On Jan. 24, 1925, the bladder was emptied by means of a catheter and 3 cc. of a 24 hour broth culture in the 3rd generation was introduced. On Jan. 26, the urine contained a little albumen; the sediment contained a few red blood cells, leucocytes, cocci, and diphtheroids. The next day the bacilli were much more numerous, but the urine presented no gross abnormalities. On Jan. 28, the sample was slightly turbid. It reacted to HNO₃. The sediment contained epithelial cells, leucocytes, and bacilli in enormous numbers. Plate cultures contained the organism in practically pure culture. On this day the cow strained considerably at the close of urination. Daily examinations were made until Feb. 4. On this day the urine was dark brown in color. It reacted markedly with HNO₃ and gave an Esebach reading of ¼ per cent albumen. There was considerable sediment composed of red cells, leucocytes, and large numbers of bacilli. For the next 3 days red cells were always observed in the sediment. From this time until Mar. 10 the urine was examined daily. It always reacted to tests for albumen. The amount and character of the sediment varied. At times red cells made up the bulk of it, at others leucocytes predominated. The bacilli were always present in large numbers (Fig. 2). On Mar. 11, 1925, the cow was slaughtered at an abattoir. The kidneys and ureters presented no gross abnormalities. The bladder, however, showed characteristic lesions (Fig. 3). The mucosa of the ventral half was swollen. It was thrown up into broad raised folds. The color of the involved portion was yellowish pink and mottled with scattered, well defined, irregular, deeply reddened areas. Under slight mag-
The reddened areas were composed of points, lines, and patches of deep red extravasations located within the mucous membrane. The bacilli were readily cultivated from the mucosa overlying the congested areas. Microscopic examination of fixed and stained sections from the kidney and ureter failed to show abnormalities. Those from the bladder revealed definite lesions. The mucosa was degenerated. The nuclei were swollen and stained faintly; many were observed to lie within a clear vacuole. In certain instances the nuclei were shrunken, granular, and stained deeply. Beneath the mucosa there was a band of fibrinous exudate containing many round cells and a few leucocytes. The submucous connective tissue was edematous. Aggregates of round cells were scattered throughout the submucosa; they were particularly numerous just beneath the mucosa. The edema extended into the muscular coat.

Another cow was inoculated into the urethra with 5 cc. of broth culture. Albumen appeared in the urine 3 days after injection and the bacilli were present in the sediment in practically pure culture. The cow was under observation for over 3 months. The urine was examined frequently. The bacilli were always present. Albumen could usually be detected; leucocytes and red cells were usually present in the sediment and at times in considerable quantities. The cow was slaughtered 100 days after the inoculation. The kidneys and ureters failed to show gross changes. About five-eighths of the mucosa of the bladder was swollen. It was thrown up into broad folds and rounded hummocks. The involved portions varied from yellow to orange in color and were sprinkled with red areas varying from points barely visible to hemorrhages 1.5 to 2 cm. in diameter. Microscopic examination of fixed and stained sections (Fig. 4) revealed degeneration of the mucosa, hemorrhage into the mucous layer, accumulations of round cells and leucocytes just beneath the mucosa, and numerous accumulations of round cells scattered throughout the edematous submucosa.

From our experiments it becomes clear that the bacillus is not pathogenic when introduced into the blood stream. In our first communication we placed particular emphasis on the bladder lesions, since cystitis was observed in all "spontaneous" cases of the disease. Instillation of pure cultures of the bacilli obtained from the urine or kidney into the bladder of normal animals results in a well defined cystitis. It is true that the cystitis encountered under natural conditions is more severe, but the character of the urine and the gross and microscopic pathology are the same.

Ritzenthaler called attention to the fact that the means by which cows become infected is unknown. We attempted to throw some light on this phase of the problem. Since we recognized that the

Ritzenthaler, M., J. Comp. Path. and Therap., 1910, xxiii. 33.
process is an ascending one, it seemed possible that the organism might inhabit the vagina of the normal cow or that it might be associated with metritis. The vaginal secretions of a considerable number of normal cows were examined bacteriologically. Diphtheroids were found, but in no instance could it be said that an organism identical with the pyelonephritis and cystitis bacillus was cultivated. The experimental inoculations of culture into the vagina confirmed these observations, for it was shown that the organism soon died when implanted on the vaginal mucosa.

It is conceivable that the bull during copulation might readily transmit the organism. The urine from 11 bulls was collected in rubber bags and examined. Diphtheroids were encountered in several samples but all differed culturally and immunologically from the specific bacillus. It seemed possible that the penis of the bull might become contaminated with the bacillus and thus the organisms gain access to the vagina of other cows. From the herd records we were able to obtain the dates on which known cases of the disease were bred, together with the record of the males' subsequent breedings. In this way we found three bulls that had bred 26 cows shortly after copulation with an infected cow. Urine examinations of these cows showed that one was infected. This cow occupied for several months an adjoining stall to a severe clinical case of the disease. In an isolated observation one bull served a cow suffering from a well marked cystitis. Within the next half hour the bull served three other cows. One of the cows was disposed of shortly after the breeding, but the other two remained uninfected. It is clear then that the bull is not a ready agent of transmission.

It is customary in the herd where most of our observations have been made to curry and brush the cows. Vigorous currying and brushing of the regions about the anus and vulva are done twice daily. This suggested itself as a means of transmission, especially since the bacilli might be implanted in tiny wounds or in exuded urine adhering to the vulva. An opportunity was afforded for observations on this point. Well defined cases of the disease were placed among four uninfected cows in such positions that the infected cows were brushed first. Two cows were disposed of within 1 month of exposure. From the urine of the third the bacilli were
isolated. This cow recovered. The fourth cow was found infected 5 months after the exposure was started.

DISCUSSION.

We concur with the opinion expressed by Joest\(^6\) that pyelonephritis of cows is a specific infectious disease. The organisms that we have encountered in cases occurring on five different farms have been identical in morphology and cultural characters. This organism when introduced in small quantities into the bladders of normal cows promptly sets up cystitis which becomes chronic. The experimental disease is on the whole milder than that usually encountered in the herd, but the bacilli will persist in the urine for a period of months. In the case of the cows of our experiments it must be borne in mind that the animals were not pregnant and were fed only a moderate grain ration. Many have noted that certain forms of nephritis in cows may occur shortly after parturition. We have previously pointed out that statistics in the matter are liable to considerable error because of the possibility that there may have existed a long standing but unnoticed infection. However, we are inclined to believe that pregnancy may play a considerable part in increasing the severity of the disease. During the dry period the concentrates in the ration are diminished and a considerable bulk of dry matter is substituted. The amount of urine excreted is considerably less. The organisms will grow readily in normal cow's urine and abundantly in urine containing a little blood serum. Under the conditions of pregnancy, then, there is an ample opportunity for the bacilli to multiply and exert a relatively prolonged action on the bladder mucosa. Late in pregnancy the pressure on the bladder, and perhaps injury to the bladder during parturition, may serve to influence the disease unfavorably.

In certain respects the causative organism is unique. It possesses no pathogenicity for the smaller animals. As far as we can learn it inhabits only the urinary tract of cows, unlike other organisms associated with renal infection, such as \textit{Bacillus coli} and \textit{Bacillus pyogenes}. The organism cannot be said to be an inhabitant of the

\(^6\) Joest, E., Spezielle pathologische Anatomie der Haustiere, Berlin, 1924, iii.
normal vagina or uterus and, as far as can be learned, plays no part in metritis. In our experiments in which large quantities of broth cultures were fed to cows, the bacilli never appeared in the feces. The lymph glands of the head and neck of a cow fed the organism were cultured with negative results. On the whole it can be said that it possesses but little pathogenicity for cows unless it finds its way into the urine where it readily grows and attacks the structures lining the bladder. The large numbers of bacilli in the urine in even mild cases strengthen the view that this is the method whereby the injury comes about.

Our experiments have thrown little light on the mode of dissemination of the organism. However, we feel that certain portions of the work are suggestive. In the first place, the bacilli attack only the mucosa of the urinary tract. They are not concerned in metritis. The disease is apparently independent of genital conditions. Once the bacilli gain access to the bladder they multiply and produce the characteristic cystitis. We feel certain that they do not reach the bladder by way of the blood stream, since cows injected intravenously with culture failed to develop cystitis. The experiments in which small amounts of culture were introduced into the vagina were negative. None of the cows became infected. Nevertheless, under most favorable conditions, organisms deposited in the vagina in the region of the urethra might very well find a favorable medium for growth and thence reach the bladder. When intimate contact occurs over long periods it has been possible to observe transient and prolonged infection, as in the experiment in which cows were placed in stalls adjoining clinical cases of the disease. Under these conditions the cows were curried and brushed vigorously twice a day and doubtless as a result the bacilli were mechanically deposited in the vagina at frequent intervals. From our observations we feel that the bull does not often act to disseminate the disease during copulation. Males are relatively insusceptible to the disease.

SUMMARY.

A Gram-positive diphtheroid isolated from spontaneous cases of cystitis and pyelonephritis of cows on five farms has been studied.
All the strains obtained were of the same general morphology. They possess similar cultural characteristics and to a great degree similar agglutination affinities. The organism is not pathogenic for the ordinary laboratory animals. It produces no toxin. Cows injected intravenously with broth cultures failed to develop the disease. On the other hand, small quantities of cultures introduced into the bladder by way of the urethra gave rise to a typical persistent cystitis.

There is evidence for a transmission of the disease by contact infection, as when the same individual curries both normal and infected cows.

EXPLANATION OF PLATE 1.

Fig. 1. A 48 hour broth culture of the diphtheroid showing one of the characteristic aggregates. Methylene blue. $\times 880$.

Fig. 2. The bacilli in the urinary sediment of Cow 1100, 17 days after inoculation of 3 cc. of broth culture into the bladder. Methylene blue. $\times 880$.

Fig. 3. A portion of the bladder of Cow 1100, 43 days after artificial infection with culture. Note the swollen mucosa studded with dark areas of hemorrhage. About $\frac{1}{4}$ actual size.

Fig. 4. A section of the bladder of Cow 1166 inoculated into the urethra with broth culture 100 days before autopsy. There is a marked infiltration of round cells in the lower portion of the mucosa and a well defined accumulation of round cells in the submucosa. The submucous connective tissue is hyperplastic and a proliferation of blood vessels has occurred. $\times 73$. 
(Jones and Little: Cystitis and pyelonephritis of cows.)