A SYSTEMATIC STUDY OF THE PATHOGENICITY OF SIXTY-TWO STRAINS OF CORYNEBACTERIUM RENALE FOR LABORATORY WHITE MICE

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Corynebacterium renale is the etiological agent of a specific cystitis and pyelonephritis of cattle. Aberrant C. renale infections have been reported in a horse by Foss (1944), in horses and sheep by Boyd and Bishop (1937), and in a dog by Olafson (1930).

Ernst (1906) and Jones and Little (1926) were unable to infect mice with C. renale. Lovell (1946) reported that C. renale was pathogenic for mice provided an adequate number were given intravenously. Morse and Morgan (1951) were able to infect mice by intravenous and intraperitoneal inoculations of strains of C. renale which were isolated from the urine or posterior urogenital tracts of apparently normal cattle. Morse and Wipf (1951) studied the epizootiology, pathogenicity, and pathogenesis of a diphtheroid which, although it resembled C. renale in cellular and colonial morphology, differed from it in biochemical reactivity.

The investigations cited stimulated the authors to investigate the pathogenicity for white mice of a number of C. renale strains isolated from pyelonephritis-infected as well as apparently normal cattle. Further information on pathogenicity might well aid in the identification and classification of members of the genus Corynebacterium.

MATERIALS AND METHODS

Sixty-two strains of C. renale were used of which 50 were isolated from cases of bovine pyelonephritis, while 12 were from urine or vaginal swabs of apparently normal cattle.

The organisms were grown on slants of Difco blood agar base medium to which citrated cow blood was added to make a final blood concentration of 5 per cent. The cultures were incubated for 24 to 36 hours at 37 C, and the resultant growth was washed from the slants with sterile 0.85 per cent sodium chloride solution. The growth obtained from 3 to 4 slants constituted a single lot of inoculum. The approximate number of bacteria which were inoculated were determined by plate counts made on Difco blood agar medium. Plates were inoculated in triplicate with each tenfold dilution. The number of bacteria injected into the mice

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2 In addition to cultures isolated by the authors, strains were kindly furnished by Dr. R. Lovell, Royal Veterinary College, London, England, Dr. I. A. Merchant, Iowa State College, Ames, Iowa, and Dr. E. S. Feenstra, Michigan State College, East Lansing, Michigan.
ranged from a minimum of 5.3 million for one culture to a maximum of 6.75 billion for another. A standard volume of inoculum, 0.5 ml, was introduced into the large dorsal tail vein.

Ten mice, 8 to 12 weeks of age and of either sex, were given a single inoculation of the test strain. Five or fewer mice, depending upon the number of losses which occurred, were sacrificed at 2 weeks. The remaining mice were killed at the end of the fourth week. All mice including those sacrificed at the end of two and four weeks, and those mice which died or were killed when visible symptoms were observed were subjected to autopsy examinations. Gross pathological alterations in the urinary tract were recorded and studied. Bacteriological cultures were made of both kidneys. The organs were minced and streaked on 5 per cent cow blood agar (Difco) plates, which were incubated at 37 C for 48 hours and examined. Identification of C. renale strains which were recovered from the experimental mice was accomplished by determination of cellular and colonial morphology, and the ability to hydrolyze urea rapidly (Christensen, 1946).

RESULTS

Thirty-four of the 50 strains which were isolated from clinical cases of bovine pyelonephritis produced the disease in mice. Three of the 12 strains recovered from the urine or posterior urogenital tract of apparently normal cattle also proved to be pathogenic for mice.

Infections were observed when as few as 3.1 \( \times \) 10^7 bacteria were inoculated. However, one strain which was isolated from a case of bovine pyelonephritis failed to produce infection with a calculated dosage of 275 \( \times \) 10^9 organisms. Eleven strains failed to infect the mice in numbers of 5.3 \( \times \) 10^6 to 3.0 \( \times \) 10^7 bacteria.

Of the 620 mice which were inoculated, 14.83 per cent were positive on bacteriological examination conducted at autopsy. The infection rate of individual strains varied from 10 to 70 per cent. A 10 per cent rate of infection was observed for 12 strains, a 20 per cent rate for 10 strains, a 30 per cent rate for 8 strains, a 40 per cent rate for 3 strains, a 50 per cent rate for 2 strains, a 60 per cent rate for 1 strain, and a 70 per cent rate was obtained with 1 strain. Approximately 97.8 \( \times \) 10^7 bacteria of one strain produced infection in 70 per cent of the mice. A 20 per cent rate of infection was observed with one strain when 675 \( \times \) 10^7 bacteria were inoculated, while another strain produced similar results with 5.63 \( \times \) 10^7 bacteria. Within the limits of the experiment, the number of organisms of the different strains inoculated did not appear to influence directly the infection rate.

Bilateral kidney involvement was observed in 65 per cent of the bacteriologically positive mice. Significant differences were not demonstrable between the occurrence of right or left kidney involvement in unilateral renal infections. Approximately 34 per cent of the kidneys which yielded cultures of C. renale appeared normal on gross examination.

The first deaths in most of the series of mice occurred 5 to 14 days following
inoculation. The number of organisms injected did not appear to influence the length of time the experimental animals survived.

Marked alterations of the kidneys and bladder were observed in mice which died or were killed when visibly ill as early as the fifth day after inoculation. The gross changes appeared to be of a severity comparable to those seen in mice killed at 14 or more days after inoculation. The lesions consisted of a hemorrhagic cystitis and a glomerular and pyelonephritis. The bladder contained an admixture of blood and exudate of a mucuslike consistency. Numerous inflammatory cells and cellular detritus were present. The urocyst often contained as much as 1 ml of this semisolid material. Resultant occlusion of the urethra was observed in some of the male mice. The distended pelvises of the kidneys contained debris similar to that observed in the bladder. The ureters were enlarged and prominent. Necrotic areas, 1–5 mm in size, were seen in the cortical and medullary areas of the kidneys. When such kidneys were minced with scissors, a slight grating sound was produced indicating the presence of minute calculi. A similar condition, with the presence of calcareous deposits, has been observed in bovine pyelonephritis.

DISCUSSION

Diphtheroid bacilli which, on the basis of morphological and cultural characteristics, appear to be similar to or identical with *C. renale* were recovered frequently from the urogenital tract of apparently normal cattle. However, only 25 per cent or 3 of the strains from normal cattle produced infection in mice as compared to 68 per cent or 34 of the strains from bovine pyelonephritis cases. Dissociation may occur with *C. renale* comparable to that observed with *Brucella* and other bacteria, and this may account for alterations in pathogenicity. Some diphtheroids may be nonpathogenic, close relatives of the pathogenic *C. renale*.

Of the 10 strains recently isolated from normal cows, only 1 was pathogenic for mice. Unfortunately, freshly isolated cultures from cattle pyelonephritis cases were not available for comparative study.

Difficulty was experienced in the preparation of inocula inasmuch as some strains did not suspend well in saline solution. It is possible that too few bacteria were employed in the inoculations, hence infection was not produced.

Morse and Wipf (1951) found that 14 intravenous serial passages of an atypical *C. renale* strain in mice did not alter its pathogenicity for mice or for cattle. The culture, prior to mouse passage, had been carried on blood agar medium for a number of transfers and had been stored for approximately 2 years in the lyophilized state. Our cultures were handled similarly, and it is assumed that the effect upon such characteristics as pathogenicity was comparable.

In order to clarify the status of the *C. renale*-like diphtheroids and the corynebacterium group in general, additional studies seem desirable especially from the approaches of serology and pathogenicity.

SUMMARY

Sixty-two strains of *Corynebacterium renale* of diverse origins were tested for their pathogenicity for laboratory white mice following intravenous inoculation.
Thirty-seven strains produced a pyelonephritis which appeared to be identical with the macroscopic lesions that are characteristic of bovine pyelonephritis. Sixty-eight per cent of the strains which were obtained from field cases of bovine pyelonephritis produced the infection in mice, while only 25 per cent of those isolated from apparently normal cattle were virulent. Reference is made to some of the problems of the classification of the diphtheroid bacteria.

REFERENCES

Christensen, W. B. 1946 Urea decomposition as a means of differentiating Proteus and Paracolon cultures from each other and from Salmonella and Shigella types. J. Bact., 52, 461-466.