GLOMERULONEPHRITIS OCCURRING IN EXPERIMENTAL BRUCELLOSIS IN DOGS *

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The production of experimental nephritis has been a problem to which much attention has been given. It has always been difficult to produce, and the results have seldom been consistent with the disease as it is seen in man. Much of the experimental work done in recent years has been directed toward hypersensitivity of renal tissue to some sensitizing agent. Irradiation, high protein diets, and various other forms of injury also have been attempted with varying measures of success.[1-4]

Longcope [5] used horse serum and egg white to produce glomerular lesions in experimental animals, and suggested that nephritis might occur as the result of an allergic reaction.

MacNider [6] thoroughly reviewed the literature up to 1924.

Duval and Hibbard [7] produced acute glomerular lesions in immune and nonimmune rabbits by single large injections of the endotoxic product of the scarlatinal streptococcus, obtained either from the peritoneal lysate of immunized rabbits, or from cultures of the organism treated with activated homologous immune serum. The kidneys in these animals were enlarged, mottled, and showed endothelial proliferation, necrosis, and hyaline thrombi in the glomerular capillaries.

Long and Finner [8] reported the occurrence of proliferative lesions in the endothelial and epithelial elements of glomeruli, with atrophy of the tubules in kidneys of sensitized swine, following the injection of tuberculin into the renal arteries.

Blackman, Brown, and Rake [9] used autolysates of type I pneumococcus to produce lesions in the kidneys of rabbits.

Lukens and Longcope [10] noted the occurrence of hyaline thrombi in the capillary loops with epithelial proliferation in the glomeruli of rabbits in which there was injected into the renal artery a heat-killed vaccine of beta hemolytic streptococcus. Lesions in their animals occurred in 74 per cent of rabbits which had been previously sensitized by intradermal injections of the organism, while lesions occurred in 28 per cent of the nonsensitized rabbits.

Bell and Clawson [11] reported the case of a monkey (*Macacus rhesus*) into which were injected intravenously suspensions of live

streptococci over a period of 4 years. This animal showed in its kidneys involvement of nearly all glomeruli. The glomerular capillaries were narrowed and occluded by endothelial proliferation and by thickening of the basement membrane of the glomerular loops. There was little fat in the tubules, but atrophy was marked.

VonGlahn and Weld [12] found, in the kidneys of rabbits injected with staphylococcus toxin, congestion, distention of the glomerular capillaries, endothelial necrosis with fibrin thrombi, and changes in the proximal convoluted tubules which varied from cloudy swelling to complete necrosis.

Extensive hemorrhagic cortical necrosis was noted by Rigdon, Joyner, and Ricketts [13] in the kidneys of rabbits injected intravenously with *Staphylococcus aureus* toxin. They found in the less involved kidneys of their animals proliferation of the endothelial lining of the glomerular loops and, in some, hyaline thrombi. The involvement of the proximal convoluted tubules and Henle's loops was extensive, ranging from cloudy swelling to complete cellular disintegration.

More recently investigation of experimental renal lesions has emphasized the nephrotoxic or anti-kidney immune aspect. However, Lindemann [14] had in 1900 produced marked albuminuria, cast formation, and tubular degeneration in rabbits by injecting the serum of guinea-pigs which had been immunized to rabbit kidney. Other experiments along the same line followed.[15] In 1936 Smadel [16] reviewed the literature and reported his work in which anti-kidney serum was prepared by immunizing rabbits with perfused rat kidney and injecting the rabbit serum into rats. He found that specific kidney antibodies, as well as antibodies to other tissues, resulted in lesions or organs other than the kidney.

Horn [17] reviewed the literature up to 1937, covering most of the experimental work done since MacNider's review in 1924. Schwentker and Comploier [18] were the first to prepare a nephrotoxic substance from homologous species. They used both *Staph. aureus* and beta hemolytic streptococcus mixed with rabbit kidney and succeeded in demonstrating antibodies to kidney in rabbits into which this kidney-bacterial mixture was injected. They also demonstrated antibodies to kidney in the blood of 37 of 40 scarlet fever patients tested. They offered the interesting explanation concerning the pathogenesis of glomerulonephritis that the combination of the bacteria or their products with kidney tissue results in some alteration of the renal protein, and that this altered protein constitutes a new antigen as it is released into the circulation during the process of repair. The antibody thus produced is capable of reacting with the altered kidney and possibly with nor-

mal kidney. They thus explain the delay in the human species between infection and the onset of nephritis. Kay [19] explained on this basis the delayed appearance of nephritis in rabbits following injection of the serum of ducks immunized to rabbit kidney.

Cavelti and Cavelti [20-22] also produced glomerular and tubular lesions and demonstrated by serologic methods antibodies to kidney in rats and rabbits injected with homologous kidney and bacterial suspensions.

Simonds and Hepler [23] in recent papers have described the kidneys of dogs following injections of heavy metals (potassium dichromate, mercuric chloride, and uranyl nitrate); snake venoms; diphtheria, staphylococcus, and streptococcus toxins.

The production of specific renal lesions by organisms of the brucella group has not been described in reports on canine brucellosis nor in reports on experimental nephritis. Feldman, Bollman, and Olson,[24] in reporting experiments in brucellosis of dogs, noted in many of their animals the clinical symptoms of disease of the kidney, bladder, and prostate, but were inclined to discount its relation to brucellosis, inasmuch as spontaneous infection in the genitourinary tract is frequent in the dog. At autopsy diffuse cortical scarring was found in the kidneys of these animals. Multiple yellowish nodules in the kidney were described by Thomsen,[25] but no microscopic description is included in his monograph. The kidneys were not found to be involved by others who have described the anatomic lesions of brucellosis in dogs.[26-28]

During the course of experiments performed in this laboratory to determine the effect of organisms of the brucella group on the reticuloendothelial system of dogs and to determine the length of time after infection that the organisms could be recovered from the blood stream, viscera, and lymph nodes, there were found in the kidneys of 4 of 9 animals unusual and interesting lesions. The general histopathologic and bacteriologic observations in these experiments have been reported elsewhere.[29,30] The experimental procedures may be reviewed briefly.

MATERIALS AND METHODS

Two strains of *Brucella suis* were selected for inoculation into the test animals. Strain A (ABF 36) was isolated from the spleen of a naturally infected hog. It was virulent for guinea-pigs, producing gross lesions and often death in 3 to 4 weeks after intraperitoneal inoculation. Strain B was obtained at autopsy from a case of Hodgkin's disease, and its virulence for guinea-pigs at the time of these experiments was slight. This has been previously referred to as the Brody strain.[31]

Nine healthy dogs were employed. Seven were used for a study of the infection produced by strain B. In 4 of this group the organisms were inoculated intravenously, while in the other 3 the inoculation was intraperitoneal. In the remaining 2 dogs strain A (ABF 36) was inoculated intravenously in one and intraperitoneally in the other. Each injection consisted of 10 billion organisms.

The inoculations were made at intervals of about 1 week, and in some instances they were given in two series, with time allowed for recovery of the animal in order to prolong the experiment, which varied in the individual animals from 186 to 487 days. One animal included here died from ingesting pine shavings on the 38th day.

The dogs were bled frequently from the jugular vein preceding inoculations. Blood cultures, brucella agglutination titers, and opsonocytophagic indices were determined at each bleeding. The details of the bacteriologic observations were fully described previously.[80]

Of the 4 dogs under consideration here, 2 were severely ill at the time of their deaths, on the 198th and 261st day, respectively, of the experiment. In both animals the Brody organism was inoculated intravenously, and inoculations were continued until death in order to produce an overwhelming disease. The first animal (dog 1) received 21 injections, the last injection 14 days before death. Twelve positive blood cultures were obtained from this animal, the last 7 days before death. In the other animal (dog 4) 28 injections were given, with 23 positive blood cultures. The last injection and the last positive blood culture occurred 7 days before death. At autopsy positive cultures of *Br. suis* were obtained from the spleen, liver, and kidney in each animal and from the lymph nodes of dog 1 and the testis of dog 4.

The other 2 animals (dogs 2 and 3) offered a contrast to the first 2 in the degree of clinical illness. Dog 2 received 35 intravenous inoculations of the Brody organism over a period of 37 weeks, during which time it was severely ill. The injections were discontinued and were followed by clinical recovery until the animal was killed on the 487th day of the experiment. Altogether, 18 positive blood cultures were obtained from this animal, the last 233 days before death. At autopsy *Br. suis* was isolated from the lymph nodes and kidney.

In the next animal (dog 3) 39 intraperitoneal injections were given over a period of 45 weeks. The animal was killed on the 461st day of the experiment, 143 days after the last injection. Only 4 of 41 blood cultures were positive, the last 270 days before death. No positive cultures were obtained from the organs at autopsy. During life this dog showed no signs of peritonitis and was not clinically ill at any time.

RESULTS

The general lesions have been described previously, and only the kidneys will be considered here.

In dog 1 focal epithelioid granulomata attributed to the brucella infection were found in the kidney. There were also widespread glomerular lesions, which consisted of extensive fibrosis and hyalinization of the capillary loops with proliferation of the capillary endothelium, and occlusion of some of the glomerular loops. Necrosis was prominent, with an exudate of polymorphonuclear leukocytes and lymphocytes into the basement membrane of many of the fibrotic glomerular loops. In some instances fibrin, albuminous fluid, and cellular débris were found in the glomerular spaces. Adhesions were numerous, and there was usually a definite thickening of Bowman's capsule. Epithelial proliferation was not prominent, but it did occur minimally in many of the glomeruli. In a moderate degree, dilatation and atrophy were found in the tubules.

The lesions found in the kidneys of dog 4 were similar to those just described. They were, however, more acute, with more prominent necrosis and cellular exudation. Hemorrhage into the glomeruli was occasionally found. Cloudy swelling, dilatation, and atrophy of the tubules were prominent. In addition to the changes described there were several linear collections of mononuclear cells, such as are found in spontaneous nephritis in the dog. These appeared to be unrelated to the glomerular lesions.

In the other 2 animals the glomerular lesions were somewhat different from those just described. In dog 2, capillary dilatation with hyaline capillary thrombi and glomerular hemorrhage were found. Endothelial proliferation and increased cellularity of the glomerulus were prominent. Adhesions between glomerular loops and capsule were present but were not numerous. There were many glomeruli which showed a marked diffuse sclerosis, sometimes with thickening of Bowman's capsule, though more often with no capsular change evident. Epithelial proliferation was not a prominent feature. Cloudy swelling in the tubular epithelium was of moderate degree. Vascular changes were not evident. In dog 3 the changes were similar to those just described. No interstitial inflammation was found in either.

COMMENT

The renal lesions which occurred in these 4 dogs are interesting from the standpoints of experimental nephritis and of chronic brucellosis in the dog. It was demonstrated that *Br. suis* can remain viable over

periods of 5 to 8 months in the tissues of an animal highly refractive to it, and only by repeated massive infection can it produce the disease.

In 2 animals with severe and active disease, active inflammatory lesions were found in the glomeruli. The recent lesions here were superimposed on chronic glomerular scarring, which was obviously not of recent occurrence. In these animals the nature of the experiment was such that one would expect to find a focal embolic glomerulonephritis, such as occurs in bacteremias in man. The lesions here were similar. They probably were the result of repeated sublethal injury to the glomerulus, as is indicated by variations in age and type of the changes in the capillary loops. Some were sclerotic, while others in the same glomerulus were of more recent occurrence with cellular exudation and necrosis. The changes in the tubules were those usually associated with long-standing glomerular injury.

The lesions in the glomeruli of the other 2 dogs are more difficult to interpret. These dogs had had repeated injections early in the course of the experiment; and, although one (dog 2) had been severely ill, it had recovered clinically and had received no injections of the organisms for 8 months prior to death. The other (dog 3) had received 39 intraperitoneal injections, the last injection 5 months prior to death. This animal had not been clinically ill and no organisms were recovered from its tissues at autopsy. The organisms were recovered from lymph nodes and kidneys of the former. There apparently was a progressive renal lesion, histologically more nearly analogous to that of an acute and subacute glomerulonephritis such as is seen in man, and different in structure from the lesions found in the kidneys of the first 2 dogs. It should be emphasized that these lesions differ from the spontaneous interstitial nephritis so common in the dog.[32]

In dog 2 a persistent brucella infection was demonstrated in the kidneys and lymph nodes, while no such infection could be found in the tissues of dog 3. Five of the 9 dogs showed no glomerular lesions. There was no correlation between the level of the agglutination titers to *Br. suis* or the opsonocytophagic index and the occurrence of renal lesions. All titers were high during the entire experiment. Moreover, no correlation could be found between the nephritic lesions and the positive blood cultures obtained in the last 2 animals. Such was not the case with the severely ill and repeatedly infected dogs showing the embolic glomerular lesions. In the last 2 animals (dogs 2 and 3) the mechanism of renal injury is probably that of renal injury of similar type in man, whether it be direct or due to an altered reactivity of the tissues.

There are certain data which would be of interest but which are not

available here. The serologic investigation of antibodies to kidney in the circulating blood during the experiment, and biopsies of the kidneys from time to time should be a part of any future work along these lines.

SUMMARY

1. In experimental brucellosis of dogs there occurred in 4 of 9 animals renal lesions of a type not usually found in dogs.

2. In 2 of the animals necrotizing acute inflammatory lesions involving portions of the glomerulus were found to be superimposed on an older sclerotic glomerulonephritis, such as is seen in focal acute glomerulonephritis in man.

3. In other dogs the lesion was a progressive, active, subacute glomerulonephritis, somewhat similar in structure to the lesion found in man.

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[*Illustrations follow*]

DESCRIPTION OF PLATE

PLATE 145

FIG. 1. Dog 1. Extensive acute inflammatory reaction in the lower glomerulus, with scarring and obliteration of the capillary loops, particularly evident in the upper glomerulus. $\times$ 265.

FIG. 2. Dog 4. The necrotic lesion with fibrinoid changes, seen in many of the glomeruli of this kidney. $\times$ 265.

FIG. 3. Dog 2. Thickening of the capillary basement membrane and endothelial proliferation are characteristic of the changes found in this animal. $\times$ 265.

FIG. 4. Dog 3. The changes here are those of endothelial proliferation, scarring, and focal necrosis with glomerular hemorrhage. They are similar to the changes shown in Figure 3. $\times$ 325.
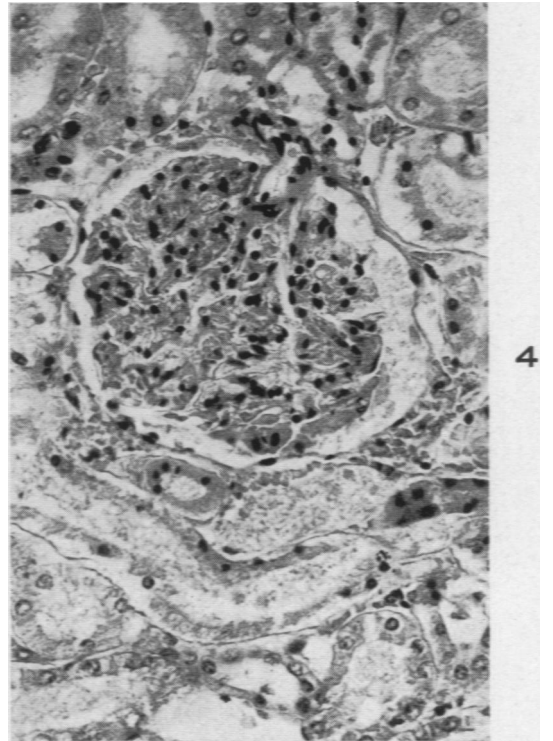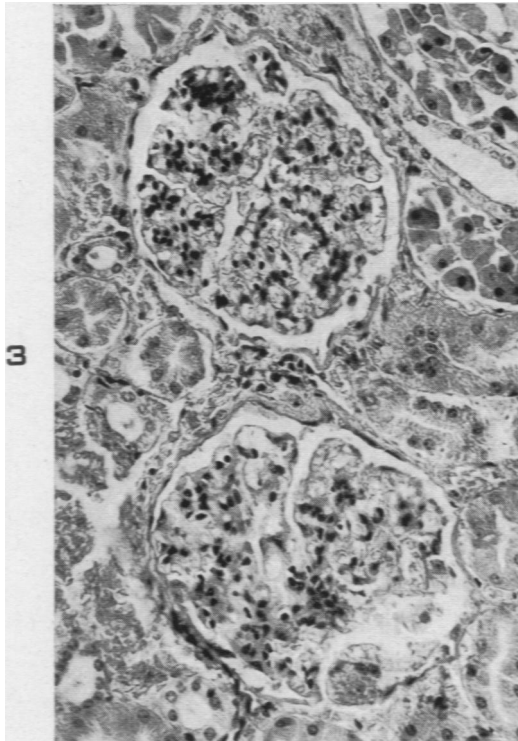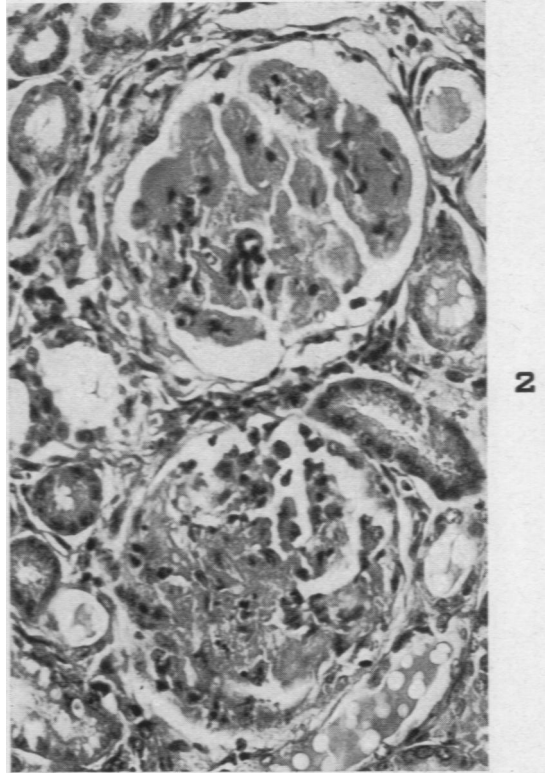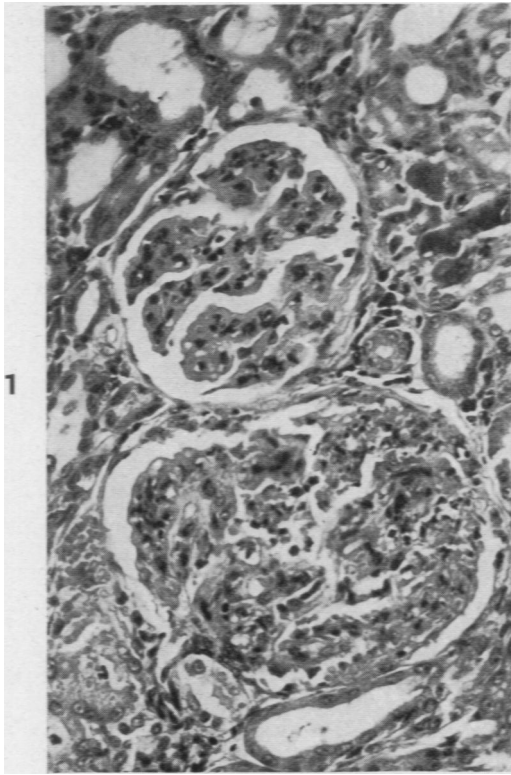
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