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EXPERIMENTAL PYELONEPHRITIS. IX. THE BACTERIOLOGICAL COURSE AND MORPHOLOGICAL CONSEQUENCES OF STAPHYLOCOCCAL PYELONEPHRITIS IN THE RAT, WITH CONSIDERATION OF THE SPECIFICITY OF THE PATHOLOGICAL CHANGES OBSERVED¶

INTRODUCTION

Although coliform bacteria are the organisms most commonly recovered from the urine of patients with pyelonephritis, they seldom cause infection in the absence of some kind of renal injury.[1] These bacteria are not suitable, therefore, for experiments designed to study alterations of structure and function attributable to infection alone. For these purposes it would seem preferable to employ microorganisms capable of establishing infection in normal kidneys, e.g. staphylococci.

The present series of experiments was carried out in the rat since renal physiology and pathology have been studied extensively in this animal. Other investigations of staphylococcal pyelonephritis have been performed in mice or rabbits.[2,3,4] Such species were considered unsatisfactory for these investigations since they are not well suited for studies of renal physiology and since the mortality is appreciable following production of extensive pyelonephritis with virulent staphylococci.

This report describes the bacteriological and morphological course of staphylococcal pyelonephritis in normal rats. The alterations in renal function produced by this infection will be described in the paper which follows.

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MATERIALS AND METHODS

Male Sprague-Dawley rats weighing 200 to 250 grams served as the experimental animal in all studies.

The *Staphylococcus aureus* "Giorgio" was the test organism. It is coagulase-positive, hemolytic, and has been described in full by Smith and Dubos.[4] This organism has been used in experiments previously reported from this laboratory.[5]

The techniques employed for determining the number of bacteria in culture media and tissues of the rat have been given in detail in previous pulications.[6] One half of each kidney was taken for culture and the remainder used for histological study. Urine was cultured by streaking a loopful on a blood agar plate. All inocula were obtained from overnight cultures prepared in beef heart infusion broth.

The tail vein of the rat was the site of intravenous injections.

Histological studies were carried out on 3-4 slides obtained from a longitudinal hemisection of the kidney.

RESULTS

Production of pyelonephritis following intravenous injection of staphylococci

The injection of 10^8 staphylococci resulted in the production of renal infection in approximately 100 per cent of animals. The course of this infection is shown in Table 1. Urine cultures did not become positive until 24 hours after inoculation at a time when bacterial multiplication could be detected in the kidney. Bacteremia persisted for two to four days. Approximately 10-20 per cent of animals died at intervals from two weeks to three months following injection. At autopsy these animals had extensive bilateral renal infection.

Size of inoculum necessary to produce renal infection

The inoculation of 10^7 organisms into ten rats resulted in renal infection at one week in only one animal. Both kidneys of this rat were heavily infected, and the urine contained a large number of bacteria. One other kidney contained 2,000 organisms and the remainder less than 100. All blood cultures were sterile. All urine cultures were sterile save for the sample obtained from the infected animal.

Course of renal infection

Reference to Table 1 reveals that there was a natural tendency for the pyelonephritis to subside. Although occasional kidneys were found to be sterile or contain low numbers of organisms between 4 and 12 weeks, it was not until 15-22 weeks after inoculation that the majority of kidneys had become sterile. Starting 24 hours after the injection of organisms, the

TABLE 1. THE COURSE OF RENAL INFECTION FOLLOWING THE INTRAVENOUS INJECTION OF 10^8 STAPHYLOCOCCI IN NORMAL RATS

Time interval	*Kidney*		*Blood*	*Urine*
	Rt.	*Lt.*		
2 hrs.	18,000*	21,000	+	0
	21,000	22,000	+	0
	22,000	18,000	+	0
	24,000	1,000	+	0
	31,000	73,000	+	0
	26,000	11,000	+	0
6 hrs.	19,000	20,000		0
	21,000	18,000		0
	20,000	22,000		0
	23,000	14,000	+	0
	24,000	37,000	+	0
	51,000	21,000	+	0
1 day	> 1,000,000	> 1,000,000	+	+
	97,000	> 100,000	+	+
	> 100,000	> 100,000	+	+
	> 1,000,000	> 1,000,000	+	+
	28,000	2,530	+	0
	> 100,000	83,000	+	0
2 days	> 1,000,000	> 1,000,000	0	+
	20,000	> 1,000,000	0	+
	11,000	> 1,000,000	0	+
	> 1,000,000	> 1,000,000	+	+
	53,000	> 1,000,000	+	+
	2,330	> 1,000,000	0	+
4 days	39,000	> 1,000,000	+	+
	> 1,000,000	> 1,000,000	+	+
	> 1,000,000	> 100,000	+	+
	0	> 1,000,000	+	+
	> 1,000,000	> 1,000,000	+	+
1 week	> 1,000,000	> 100,000	0	+
	> 1,000,000	> 1,000,000	0	+
	> 100,000	> 100,000	0	
	> 1,000,000	37,000	0	+
	> 1,000,000	> 1,000,000	0	+
	> 1,000,000	> 100,000	0	+
2 weeks	> 100,000	100,000		
	100,000	> 100,000		
	500,000	90		
4 weeks	> 100,000	90		
		0		
	> 100,000	> 100,000		
6 weeks	> 100,000	4,000		
	> 1,000,000	0		
	0	0		
8 weeks	> 1,000,000	> 1,000,000		
	> 100,000	100,000		
	> 100,000	> 1,000,000		

TABLE 1. (CONTINUED)

Time interval	Kidney		Blood	Urine
	Rt.	Lt.		
11-12 weeks	0	0		
	> 100,000	> 1,000,000		
	> 1,000,000	> 1,000,000		
	> 1,000,000	> 1,000,000		
	11,000	0		
15-18 weeks	0	0		
	0	0		
	> 1,000,000	> 1,000,000		
	0	0		
	0	0		
	0	0		
	0	0		
	0	0	0	0
22 weeks	11,000	0	+	+
	> 1,000,000	> 1,000,000	0	+
	0	0	0	0
	12,000	9,000	0	+
	0	0	0	0
	0	0	0	0
	0	0	0	0
	> 100,000	0	0	
30 weeks	0	60	0	0
	0	0	0	0
	0	0	0	0
	> 100,000	60,000	0	+
	2,000	> 100,000	0	+
	30,000	30,000	0	+
	0	0	0	0
	790	0	0	0
	> 100,000	0	0	+
	0	0	0	0
	0	700	0	0
	0	0	0	0
	20	10	0	+
	0	0	0	+
	0	30,000	0	+
	25,000	0	0	+
	6,000	0	0	+
	0	620	0	+
	0	0	0	0
	0	0	0	0
51 weeks	0	0	0	0
	0	0	0	0
	0	0	0	0
	0	0	0	0
	0	0	0	0

*Colonies staphylococci per one-half kidney.

general rule was for the urine to contain staphylococci at a time the kidney contained staphylococci.

Inoculation of staphylococci into bladder urine

The urinary bladder of six rats was exposed through a midline abdominal incision and the bladder urine inoculated with 10^8 staphylococci in a volume of 0.25 ml. of broth culture medium. The kidneys and ureters were not exposed or manipulated in any way. After an interval of four days the animals were examined. No gross abnormalities were seen in the kidneys or bladder. As shown in Table 2, significant numbers of bacteria were recovered from four kidney cultures of two animals, although only one of these kidneys contained 300,000 viable units, a number indicative of infection. It is evident that staphylococci can make their way from bladder urine to the kidney in a normal animal.

TABLE 2. BACTERIOLOGICAL STUDY FOUR DAYS AFTER INOCULATION OF 10^8 STAPHYLOCOCCI INTO BLADDER URINE OF NORMAL RATS

	Kidney		*Urine*	*Blood*
	Rt.	*Lt.*		
1.	0	10*	0	0
2.	0	0	0	0
3.	0	0		0
4.	1,600	15,000	+	0
5.	300,000	4,000	+	0
6.	0	0	+	0

*Colonies staphylococci per one-half kidney.

Pathology of staphylococcal pyelonephritis
Two hours—24 hours

The earliest change detectable in the kidney was marked hyaline droplet formation in renal tubular epithelial cells located primarily in the cortex. Most tubules contained large droplets similar to those described by Oliver[7] and Sheehan and Davis.[8] They were seen up to one week after the injection of organisms but not to any significant degree after that time.

Two days—1 week

During this interval, foci of acute inflammation became visible both on gross and microscopic examination. The changes conformed with those described by others as occurring in mice and rabbits at similar intervals of time following the intravenous inoculation of staphylococci.[2,4,9]

The most prominent finding was pus-filled tubules, and regions of tubular destruction with abscess formation. These changes were confined to wedge-shaped areas, the apices of which were located in the papilla (Figs. 1, 2). Often three or four such wedges could be seen in one kidney, separated by normal renal tissue. Papillary involvement was sometimes so severe as to produce visible necrosis of a section of the papilla (Fig. 3). The limitation of areas of inflammation to discrete wedges was striking, resembling the infections produced in this laboratory previously by the inoculation of bacteria directly into the renal medulla.[5,10] Microscopically, the lesions were characterized by dense polymorphonuclear infiltration within and around cortical and medullary tubules (Fig. 4). Abscess formation within the wedges was accompanied by the loss of recognizable elements of renal structure. Isolated abscesses were occasionally seen in the medulla, surrounded by normal tissue, and encased in a thick wall of inflammatory cellular exudate. Colloid casts were sometimes seen in tubules at the corticomedullary junction surrounded by polymorphonuclear leucocytes. No abnormalities were seen in glomeruli or large or small blood vessels.

Two weeks—1 year

At two weeks the interstitial inflammatory cellular exudate was composed of polymorphonuclear leucocytes and lymphocytes. At four weeks only a few polymorphs could still be seen. Within distended tubules at four weeks, however, polymorphonuclear leucocytes persisted in large numbers. From this time on there was progressive atrophy of tubules within the areas of suppuration with consequent crowding of glomeruli (Fig. 5). Condensation of connective tissue in association with atrophic tubules around normal glomeruli presented a picture similar to periglomerular fibrosis. These microscopic changes were accompanied by gross depression and contraction of areas previously the site of bulging suppuration (Fig. 6). Abscesses in early stages of development were not seen in any kidneys containing areas of resolving inflammation. No glomerular or vascular abnormalities were seen in any of the kidneys examined.

A lesion often considered characteristic of healed pyelonephritis in man, which was observed in rabbits by Mallory, Crane, and Edwards, is that of colloid cast formation within dilated tubules.[11] These casts were encountered only twice in the present study and each time to a slight degree. These animals were examined 1 and 12 weeks after infection.

A peculiar lesion noted in one rat was extreme cystic dilatation of the medullary portion of a group of nephrons within an area of healed pyelonephritis (Fig. 7). This cyst formation is most likely a result of tubular

obstruction by scar tissue in the medulla and resembles the cysts produced by mechanical medullary obstruction in the rabbit.[12]

Acute polymorphonuclear leucocytic infiltration of the renal pelvis was frequently encountered from 6 to 30 weeks after inoculation of organisms (Fig. 8). These lesions were not seen in kidneys taken prior to six weeks or one year after infection. The occurrence of similar lesions in mice is mentioned by Gray *et al.*[9]

Correlation of histology with bacteriological findings

Animals examined between one day and one week after inoculation of organisms were virtually all found to have pyelonephritis histologically and bacteriologically. At two and six weeks sterile kidneys were encountered in which there was no morphological evidence of prior infection. After six weeks, however, almost all of the sterile kidneys demonstrated unequivocal morphological evidence of previous infection thereby indicating that healing had occurred.

The lesion of active (polymorphonuclear exudate) pyelitis was first encountered six weeks after injection. Between six and 22 weeks over half the kidneys which contained viable staphylococci had this microscopic lesion. At 30 weeks all kidneys which were positive on culture had active pyelitis except two. The finding of active pyelitis microscopically was associated in all instances with the recovery of staphylococci from the kidney. All but two kidneys with active pyelitis had, in addition, evidence of cortical and medullary scars.

Extent of morphological damage

It was very difficult to estimate the extent of renal damage upon either gross or microscopic examination of these kidneys due to the patchy nature of the inflammation. By gross inspection one could often detect large scars which might not appear in the microscopic section. Conversely, papillae which appeared normal on gross examination might reveal large areas of scarring microscopically. The only certain way of not missing morphological damage would be by serially sectioning whole kidneys. By the techniques used, however, scars might easily have been missed on both gross and microscopic examination. Despite these difficulties morphological evidence of infection was seen in most kidneys.

It was difficult to compare the extent of damage in the cortex with that in the medulla. It did appear, nevertheless, particularly during the early stages of the infection, that relative to its size, the papilla contained more inflammatory exudate than the cortex (Figs. 2, 9).

"Pyelonephritic" lesions seen during the course of sterile hydronephrosis in the rat

In an attempt to distinguish the pathological changes, described by others, of pyelonephritis in the presence of obstruction from those changes due to pyelonephritis alone, a group of six rats was examined three weeks after ligation and division of the left ureter. Marked hydronephrosis was evident. There was no gross evidence of infection, and culture of one half the obstructed kidneys was sterile. Despite the absence of infection, a number of lesions seen in these kidneys bore a remarkable resemblance to those described with staphylococcal infection in this report and in the study of coliform infection of the obstructed rabbit kidney by Mallory, Crane, and Edwards.[11]

Numerous examples were seen of dilated tubules filled with polymorphonuclear leucocytes and nuclear debris (Fig. 10). The similarity between these lesions and those which were seen in the early stages of staphylococcal pyelonephritis is evident (Fig. 4). Such tubular lesions have been described previously by Helmholtz and Field in the rabbit with hydronephrosis.[18] There was inflammatory exudate scattered through the interstitial tissues (Fig. 11), and dilated tubules filled with colloid casts were also seen (Fig. 12). Formation of colloid casts was more pronounced in these hydronephrotic kidneys than at any stage examined during the course of staphylococcal pyelonephritis. It was also possible in these kidneys to identify glomerular crowding (Fig. 13) and glomeruli in various stages of hyalinization (Figs. 14, 15).

DISCUSSION

The rat seems to be as susceptible to renal infection with virulent staphylococci as the mouse and rabbit, but, in contrast to these species, the attendant mortality is negligible. A large number of bacteria is necessary to produce hematogenous pyelonephritis in the rat since reducing the inoculum by one log resulted in a sharp decrease in the percentage of animals infected. Despite the ability of staphylococci to infect normal kidneys, the natural tendency of the infection was toward healing, most kidneys being sterile 5-22 weeks after inoculation.

It is well known that the concentration of bacteria in blood is much higher immediately after intravenous injection than it is later, yet no bacteria were recovered in the urine up to six hours after injection. It was only when bacterial multiplication was detected in the kidney that these organisms could be recovered from the urine. It is evident that staphy-

lococci are not excreted into the urine by normal kidneys. Positive urine cultures later in the course of the infection occurred in those animals whose kidneys still contained culturable organisms in all but one instance, and since hemisections of the kidney were examined histologically, it is possible that a localized focus of infection was present in the single exception. The kidneys of animals with negative urine cultures were sterile save for three kidneys which contained 60, 700, and 790 bacteria.

The lesions of staphylococcal pyelonephritis in the rat kidney were similar to those previously described in the mouse and rabbit and, in addition, resembled those produced by *E. coli* in a kidney with intrarenal hydronephrosis.[2,4,10] The areas of suppuration were wedge-shaped with the apex in the papilla.

Particular attention was directed to the extent of damage produced in the renal papilla as compared with cortex in this infection. The impression was gained that *proportional to its size,* the papilla was more extensively involved by the process of inflammation than was the cortex. These observations will be cited in considering the alterations of renal function which result from this infection and are particularly relevant in comparing the functional effect of pyelonephritis in an animal without a well-formed papilla (dog) with its effect in the rat or man.

There did not appear to be any tendency for lateral spread of infection within the kidney. This is remarkable in view of the ability of the staphylococcus to infect the normal kidney and has been commented upon previously in the discussion of this disease in rabbits.[5] This impression is supported also by the absence of any new abscess formation in kidneys with healing lesions. Even in those animals with active pyelitis and positive cultures for 30 weeks, no evidence of recent invasion of the parenchyma was seen. Further support for this comes from the fact that kidneys examined six months and one year after inoculation of bacteria did not seem in general to be more severely scarred than those examined just a few weeks after injection. It would appear then that the model of pyelonephritis under study does not produce a lesion which is progressive; damage of the renal parenchyma did not increase with the passage of time.

Correlation of morphological and bacteriological data shows that disappearance of viable bacteria from a previously infected kidney usually takes at least six weeks, since only after this time was definite morphological evidence of previous infection detected in a sterile kidney. Active pyelitis began to be seen six weeks after injection of organisms. At 30 weeks this renal lesion was closely associated with viable bacteria in the kidney. In two kidneys with severe active pyelitis, no other renal lesions were detected.

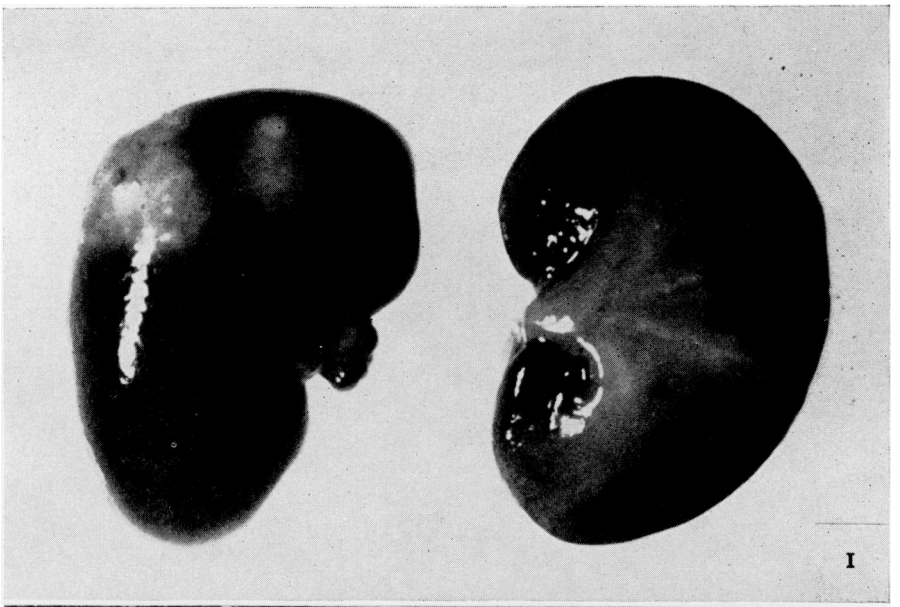
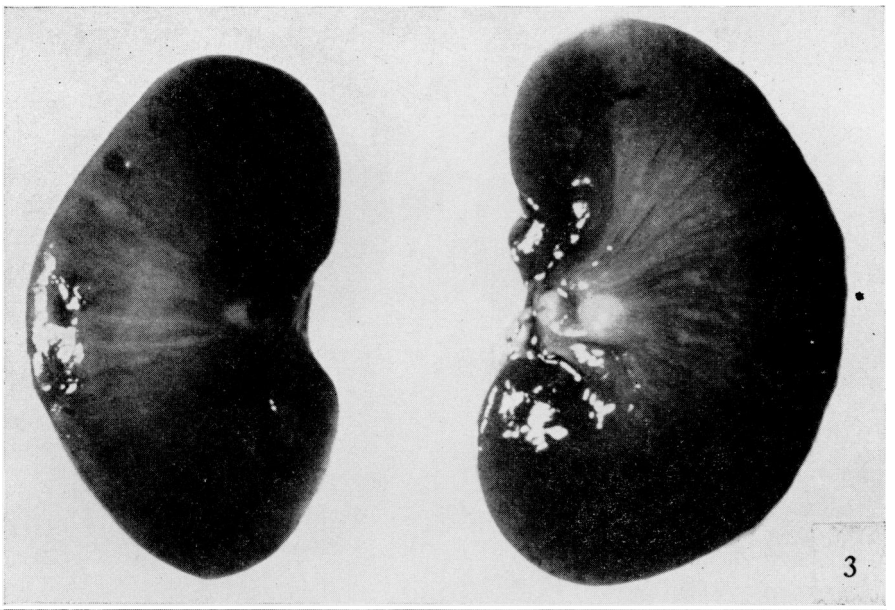


FIG. 1. Gross appearance of kidneys one week after the intravenous inoculation of 10^8 staphylococci showing wedges of inflammation extending from medulla to cortex.

FIG. 2. Low power view of kidney examined at the same interval as kidneys shown in Figure 1. Abscess involving over one-half the diameter of the papilla in this section. Cortical inflammation limited to abscesses shown.



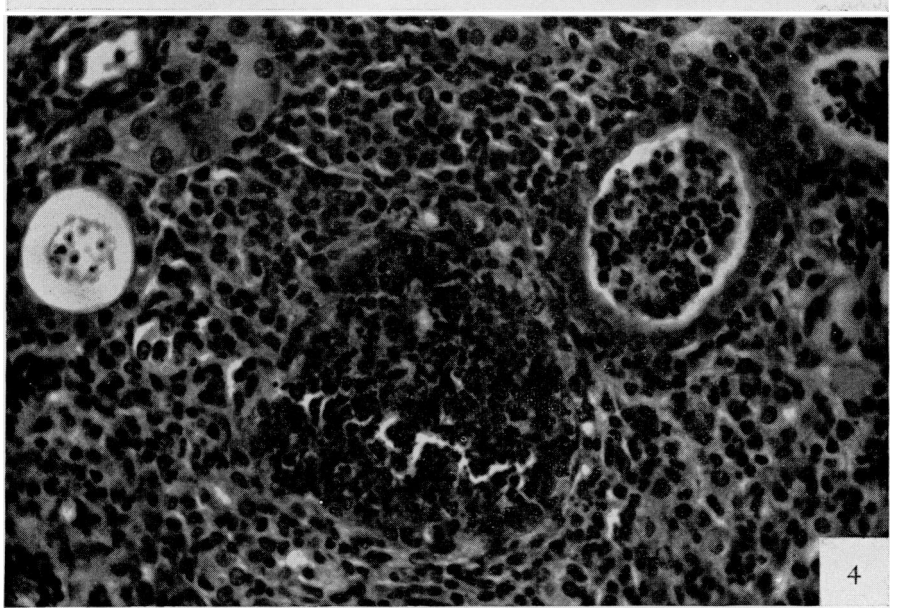
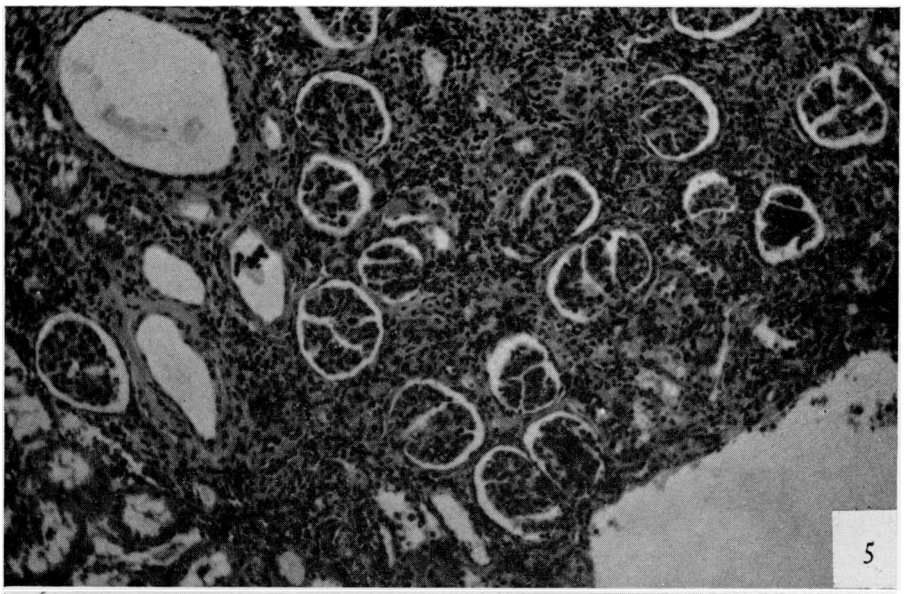


FIG. 3. The necrotic tip of the papilla has fallen out of both halves of this kidney examined one week after the inoculation of organisms.

FIG. 4. Microabscess one week after inoculation of bacteria. Polymorphonuclear leucocytes are visible in tubular lumina.



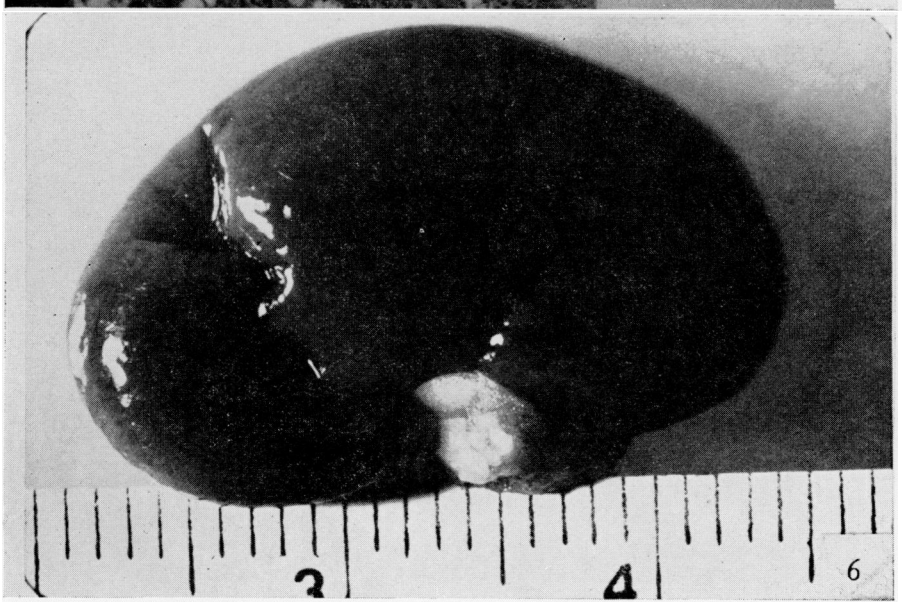
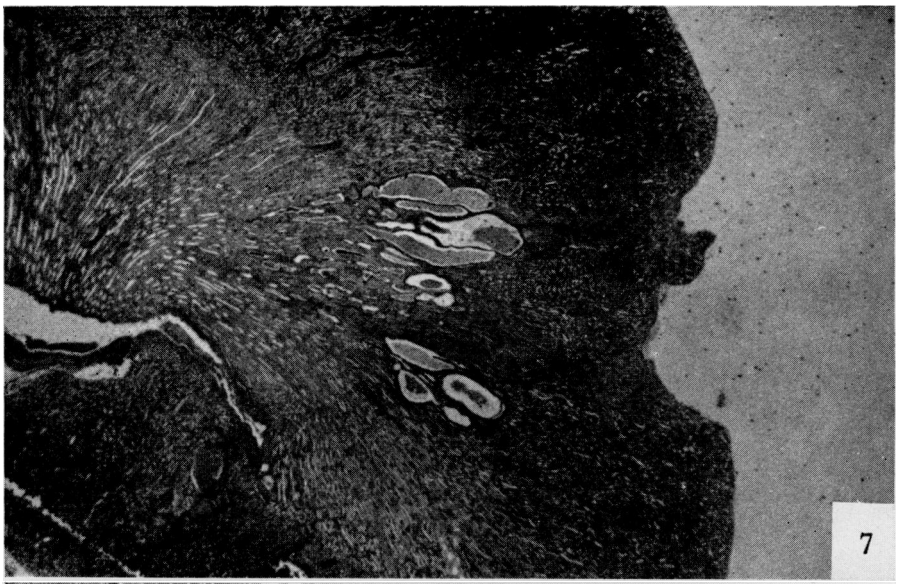


FIG. 5. Typical cortical scar four months after infection. The glomerular capillary tuffs are normal.

FIG. 6. Gross appearance of cortical scars five months after infection.



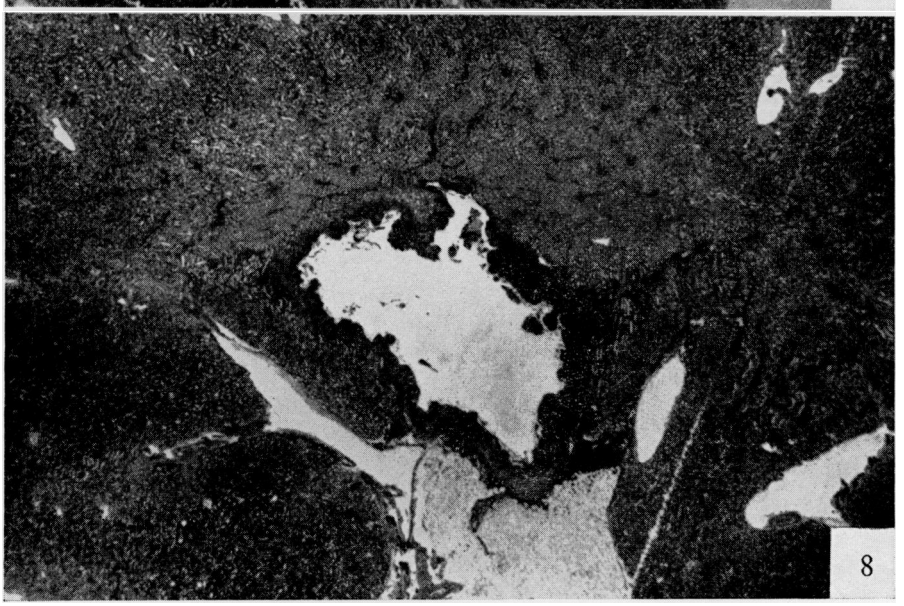
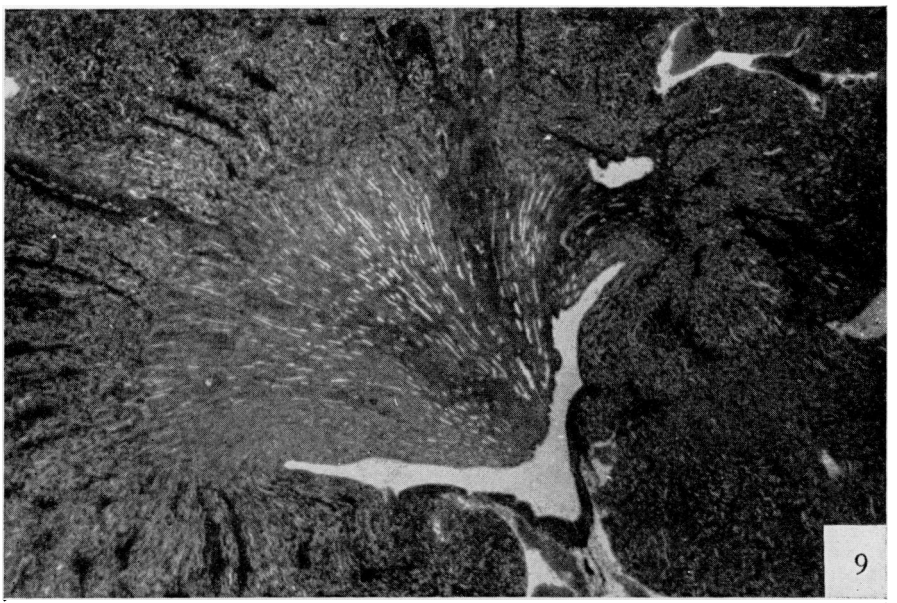


FIG. 7. Medullary cyst formation underlying cortical scar. Papillary scarring is not visible in this section.

FIG. 8. A dense, dark layer of polymorphonuclear leucocytes is seen lining the renal pelvis. No other lesions were found in this kidney either on gross or microscopic examination.



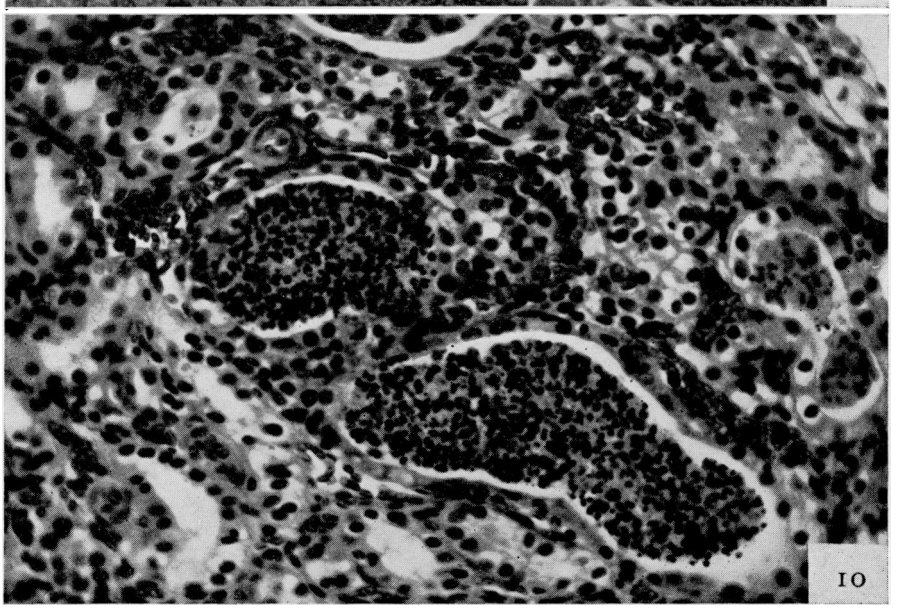
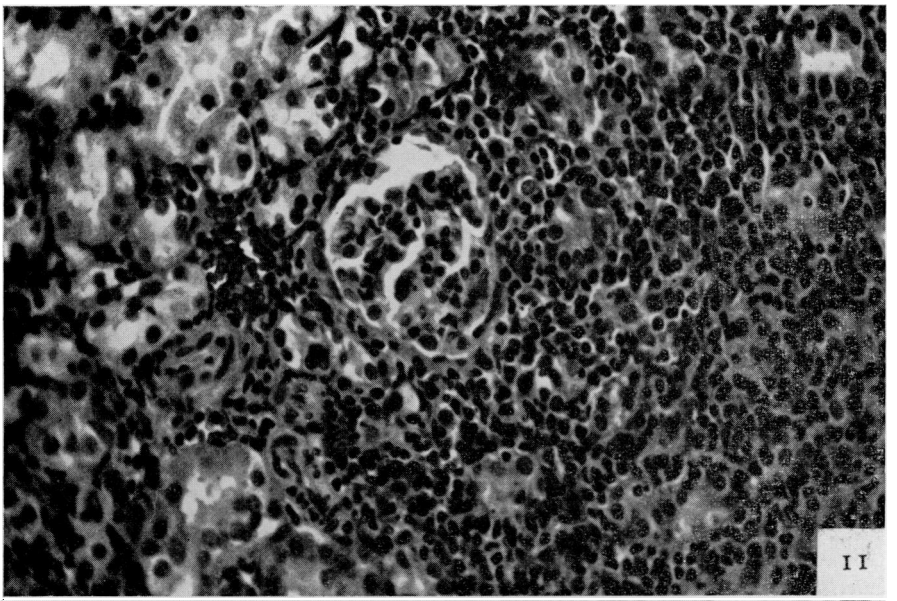


FIG. 9. Darker zones in papilla indicate scar remaining five months after infection.

FIG. 10. Dilated tubules filled with polymorphonuclear leucocytes in a kidney whose ureter was ligated for three weeks. No infection. Compare with Figure 4.



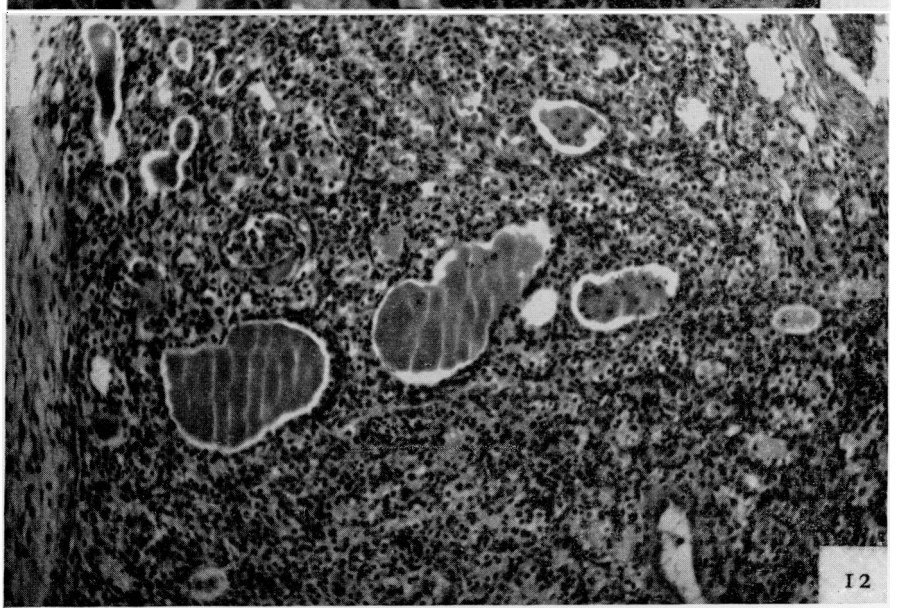
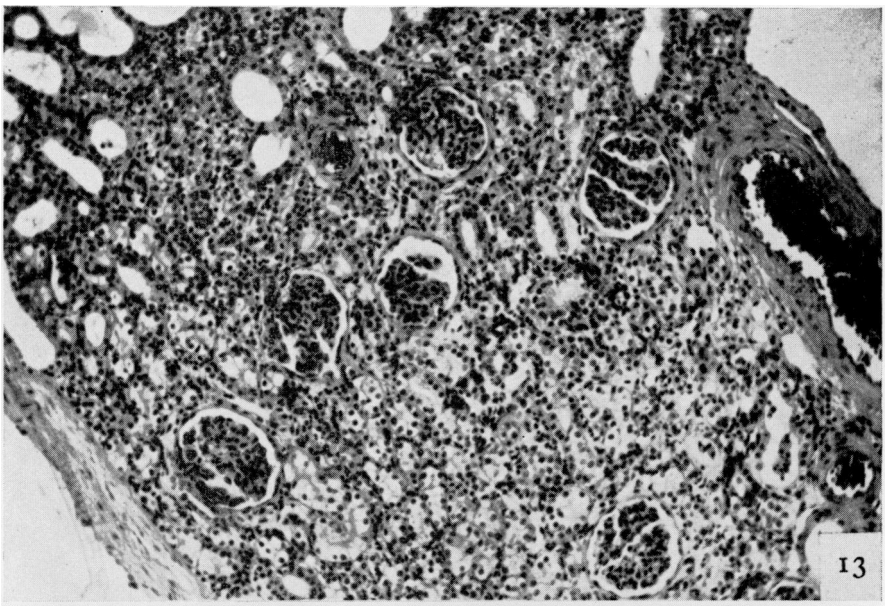
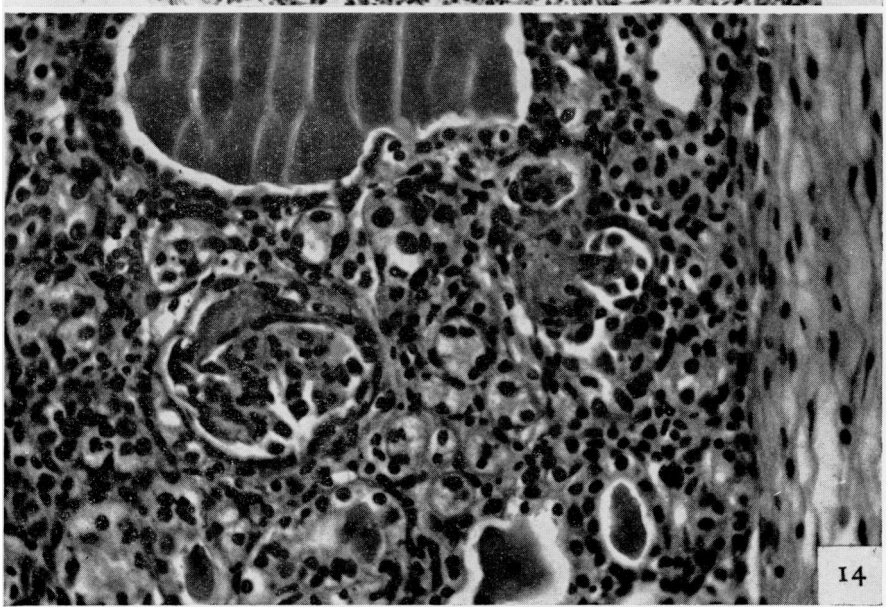


FIG. 11. Cellular exudate composed of polymorphonuclear leucocytes, lymphocytes and plasma cells, three weeks after ureteral ligation without infection.

FIG. 12. Colloid cast formation three weeks after ureteral ligation without infection.





FIGS. 13, 14, 15. Glomerular crowding and hyalinization three weeks after ureteral ligation without infection.

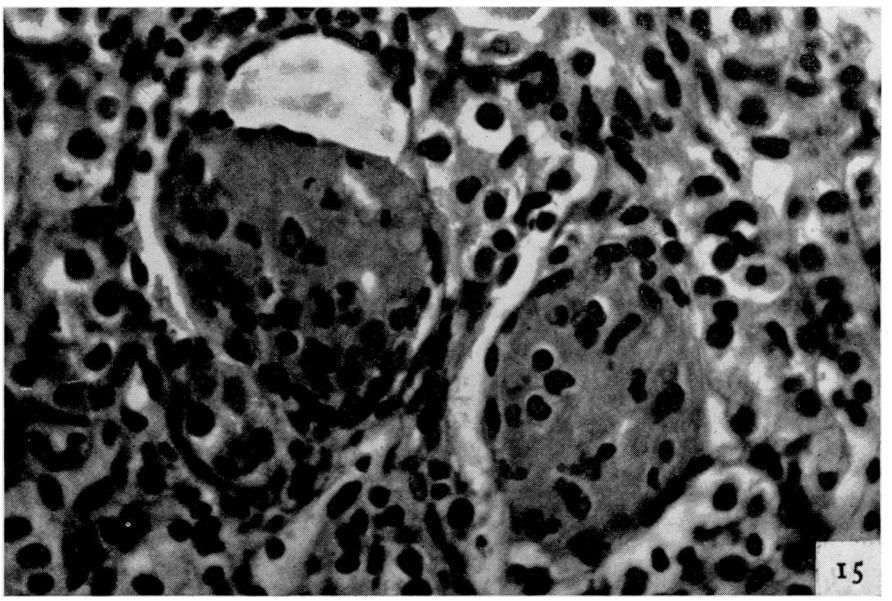


FIG. 15. See legend Figs. 13, 14.

It is apparent that pyelitis is a common finding in hematogenous staphylococcal pyelonephritis in rats. Occasionally it may be the only detectable morphological abnormality of the kidney.

The morphological findings in the present study do not differ significantly from previous descriptions of staphylococcal pyelonephritis in the mouse and rabbit. However, since ureteral obstruction was not a necessary first step in the production of this infection, it is pertinent to examine the differences that exist between the present disease and the one produced with *E. coli* following ureteral ligation. The studies using *E. coli* were carried out in the rabbit, a fact which in itself may explain some of the differences. Nevertheless, it is important to try to distinguish the changes due to obstructon and infection from those due to infection alone.

The presence of colloid casts in the kidney is considered to be one of the most characteristic histological features of chronic pyelonephritis. Mallory, Crane, and Edwards have stated: "If our interpretation of the formation of these colloid casts is correct as given, their presence should be the most reliable criterion for the diagnosis of the healed lesion (of pyelonephritis)... Acute pyelonephritis is probably the only lesion of the kidney which could produce such a combination of circumstances."[11] These observations have been confirmed by Heptinstall and Gorrill, in experiments which were also carried out with *E. coli* using ureteral obstruction.[14] It was striking, therefore, that such casts were only rarely seen in rats with staphylococcal renal infection in any stage of disease up to one year after infection. It is of interest that Heptinstall and Gorrill also observed infrequent cast formation in rabbits given staphylococci without ureteral ligation.[14] Most authors have agreed that colloid cast formation was dependent upon obstruction of nephrons in addition to infection. Cast formation *was* observed, in the present study, however, in rats whose ureters had been ligated for three weeks in which there was *no evidence of infection.* It would appear that the formation of colloid casts in dilated nephrons is dependent primarily upon obstruction, not infection.

The distention of tubules with leucocytic casts was also seen in sterile obstruction along with crowding of glomeruli, tubular atrophy, and marked lymphocytic interstitial infiltration. Helmholtz and Field have commented on this.[18] This tissue reaction is no doubt a result of increased tissue pressure and anoxia, both of which are well known to accompany obstruction.[15]

Glomerular hyalinization is another of the changes which has been described in infected obstructed kidneys. Although these lesions were not seen in rats with staphylococcal pyelonephritis, it might be argued that they were missed, or that species difference accounted for a transformation of

glomeruli which made them difficult to identify. These glomerular changes were seen, however, in obstructed kidneys which were sterile. Thus another lesion of pyelonephritis may result from obstruction without infection.

It is apparent that many of the histological features of infection combined with obstruction may be duplicated by obstruction alone. Sometimes, however, infection itself is the cause of obstruction. Furthermore, since many effects of obstruction are related to changes in blood flow and tissue pressure, it is possible that primary vascular abnormalities might also produce tissue changes indistinguishable from those of pyelonephritis.

SUMMARY

The present report describes the bacteriological course and morphological consequences of pyelonephritis following the intravenous injection of large numbers of virulent staphylococci into normal rats. There is only slight mortality accompanying this infection, the natural tendency of which is to heal in 15-22 weeks. Bacteria were not recovered from the urine until their multiplication was demonstrable in the kidney after which time there was good correlation between the presence of bacteria in the urine and kidney.

Active pyelitis was frequently seen in kidneys six weeks or more following the intravenous inoculation of staphylococci. At 30 weeks this lesion was usually found when viable bacteria were recovered from the kidney. Occasionally, pyelitis was the only lesion seen microscopically. It is evident that inflammation of the renal pelvis is not useful in determining the route by which bacteria reach the kidney.

Histological features considered typical of experimental pyelonephritis, such as colloid casts, polymorphonuclear leucocytic casts, and glomerular hyalinization, were reproduced in sterile obstructed kidneys. Colloid casts were rarely seen and hyalinized glomeruli not detected in the healing stages of staphylococcal pyelonephritis, an infection which does not require obstruction for its initiation. It is suggested that many of the histological changes thought to be typical of renal infection may be produced by obstruction of nephrons.

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