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EXPERIMENTAL PYELONEPHRITIS XV. INCREASED SUSCEPTIBILITY TO *E. COLI* INFECTION IN OLD RATS†

One of the most interesting features of the epidemiology of urinary tract infections is an increasing prevalence with advancing age.[1-3] Infections are exceedingly uncommon in men below age 50 and the rates in elderly women are two to three times the rates in women below age 40.

There are a number of possible explanations for this pattern. Urinary infections might confer a survival advantage on those infected; persons without infections would then have higher death rates and the result would be increasing rates of positive urine cultures with advancing age. This possibility is unlikely since all the clinical features with which urinary infections have been correlated would tend to decrease survival.

The course of infections in the elderly may be more prolonged. Thus, at any given time there would be more infections in older women. This too, is not likely to be the explanation for the increased rates in old age since, despite some disagreement, analyses of various features (including response to treatment) does not indicate significant differences between old and young women.[1,4]

It is possible that treatment of urinary infections has been improving over the years. If this were so, older subjects who developed their infections 20 or 30 years ago might have had less chance to be treated effectively at the onset, whereas, young persons developing infections for the first time might be more likely to receive effective therapy. This, too, would result in higher rates of urinary infection in older women. Unfortunately, there is little evidence that even today the long-term effect of antibiotic treatment is very satisfactory, and there are some data to suggest that antibiotic treatment may not influence the outcome of infection four years later.[4]

The most likely explanation for the increased rates of urinary infections in older persons is that there is an increasing susceptibility to urinary tract infections with advancing age. Increased susceptibility up to now has been considered a result of the increasing rates of lower urinary tract abnormali-

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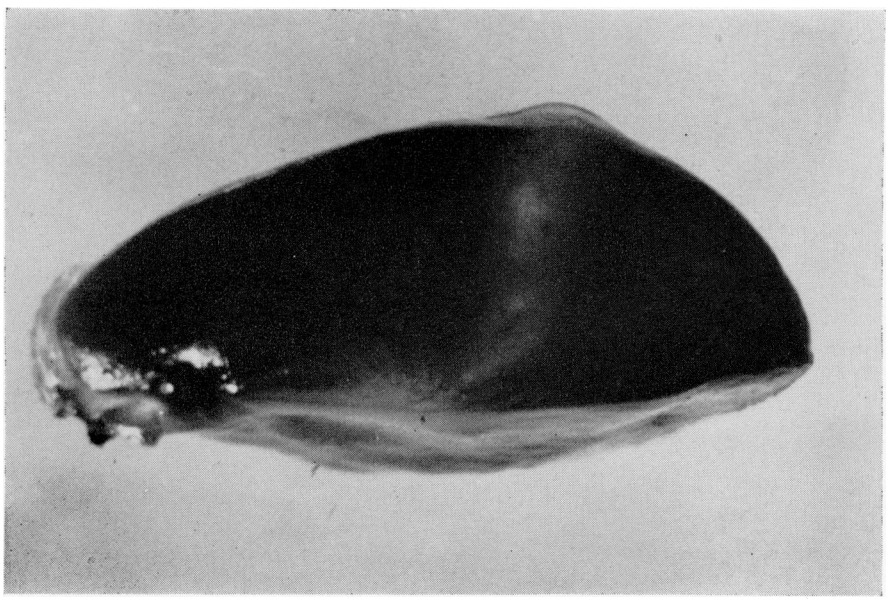

FIG. 1. Wedge-shaped zone of infection resulting from the intravenous inoculation of 10^8 *E. coli* in a one-year old rat.

ties that are known to arise in older persons: cystocele in women, prostatism in men.

Little attention has been directed to the possibility that increased susceptibility to bacterial infection in old age might be a consequence of tissue changes or altered host defense mechanisms. These are not unreasonable possibilities, however, since studies in man have demonstrated that vascular abnormalities at autopsy, abnormalities of the renal papilla, hypertension, and rising levels of BUN are all known to accompany the aging process.[5,6] Similar changes in the renal parenchyma have been found to accompany aging in rats.[7] It was reasoned, therefore, that if the tissue changes of aging were responsible for increased susceptibility to infection in man, the occurrence of similar tissue changes with age in rats might be accompanied by the same changes in susceptibility to infection.

The present series of experiments was designed to compare the susceptibility of young and old rats to *E. coli* infection of the urinary system.

MATERIALS AND METHODS

The test animals were male Sprague-Dawley rats. Young animals weighing 175-250 grams (approximately 40-60 days old) were compared with animals that had been obtained from the same supplier and had been housed individually in the laboratory for approximately one year. The weights of the old rats varied from 550 to 750 grams. The animals were permitted *ad libitum* water and were fed Purina lab chow. All animals appeared in excellent health at the time of testing.

The bacterium employed and the techniques for quantitative bacteriologic study of the animal tissues have been described previously.[8] The bacterial inoculum was obtained from a 4½-hour broth culture containing 100-300 million viable units as determined by serial dilution and agar pour plate counts. Saline dilutions were used as indicated in appropriate experiments.

Intravenous injections were given into the tail vein of the rats and inoculations of bacteria into the bladder lumen were accomplished through an abdominal incision as described previously.[9] Both kidneys, blood, and urine were obtained at laparotomy and cultured quantitatively. The urine volumes available for culture varied from 0.1 to 0.5 ml.

A small group of rats had been nephrectomized through abdominal incisions six months prior to testing when their weights were 150 to 200 grams. The rats were kept in a manner similar to the normal animals. Their weights at the time of testing varied from 350 to 450 grams and they too appeared in excellent health.

RESULTS

Intravenous inoculation of bacteria in old and young normal rats

The first series of experiments was designed to test the susceptibility of young and old rats to pyelonephritis following the intravenous injection of bacteria. The results of kidney and urine cultures obtained two to three days after the intravenous inoculation of 10^8 *E. coli* are shown in Table 1.

TABLE 1. INTRAVENOUS INOCULATION OF 10^8 *E. coli* INTO YOUNG RATS AND OLD RATS

Young rats			*Old rats*		
Left kidney	*Right kidney*	*Urine*	*Left kidney*	*Right kidney*	*Urine*
0	0	0	0	0	0
0	0	0	10^1	10^4	10^8
10^2	10^8	0	0	0	0
10^2	0	0	0	0	0
10^4	10^4	0	10^4	10^8	10^4
10^2	10^2	0	10^2	10^8	0
10^2	10^2	—	10^5	10^5	10^4
10^8	10^4	0	10^2	0	0
0	0	0	0	10^2	0
0	0	0	10^8	10^1	0
0	0	0	0	10^2	0
10^8	10^2	0	0	0	0
0	0	0	0	10^2	0
10^2	0	0	10^2	10^1	0
10^2	0	0	0	10^2	0
10^8	10^8	0	0	0	0
0	0	0	10^2	10^2	0
0	0	0			
10^2	10^8	0			
0	0	0			
0	0	0			
10^2	0	0			
0	0	0			
0	0	0			

Although two of the young rats had as many as 10^4 viable units in their kidneys per gram of tissue, in no instance in any of the 23 animals was bacteria recovered from the urine. This is consistent with what has been observed in this laboratory during the past 10 years in hundreds of rats inoculated with the same bacteria.[8,10]

Infections were considered to have been established in three of the 17-year old rats. In one of them, a typical wedge-shaped area of abscess formation was visible grossly (Fig. 1). In all three instances, large numbers of bacteria were recovered from the urine. Cultures of the blood, urine, and kidneys of six old rats not given bacteria were sterile.

Small yellow stones (less than 1.0 mm. in diameter) were found in the fornices of the renal pelves of four old rats. Only one of these animals was infected. Histological sections were not made routinely. A piece of tissue was taken, however, from the infected rat whose renal pelvis contained

"sand." Changes consistent with renal infection were confined to the papilla where there were collections of polymorphonuclear leucocytes beneath the pelvic epithelium. No areas of inflammation were seen elsewhere in the kidney. Stones were never found in young rats.

Although the increased susceptibility to pyelonephritis in old rats is slight, even with the small number of animals tested, the difference between old and young rats is significant ($.05 > p > .01$). If one considers the long experience with this species of *E. coli* in rats, the increased susceptibility to infection with age is impressive.

Intravenous inoculation of bacteria in unilaterally nephrectomized rats

Since it is inconvenient to wait one year to conduct an experiment on an old rat, it was decided to take advantage of the effect of unilateral nephrectomy in accelerating the changes associated with aging in the kidney. *E. coli* (10^8) were inoculated intravenously into eight old rats that had been nephrectomized six months previously when they weighed 175-250 grams and 12 young rats nephrectomized one week previously.

Infections did not occur in the young unilaterally nephrectomized rats. All urine cultures were sterile. In one of the old rats unilaterally nephrectomized six months earlier more than 10^5 viable bacterial units were recovered from the kidney and the urine contained $> 10^4$ colonies of bacteria per milliliter. No gross abnormalities were seen in the kidney. In another animal with tiny calcific granules in the renal pelvis, large numbers of bacteria were recovered in the urine (10^5 ml.) but the kidney was sterile.

Thus, as was shown in normal rats, aging following nephrectomy is also associated with a slight increase in susceptibility to pyelonephritis. The magnitude of the increased susceptibility six months after nephrectomy is similar to that found after one year in normal rats.

Clearance of bacteria from the lumen of the urinary bladder

Since it is well known that *E. coli* are rapidly cleared from the bladder cavity of young rats,[9] it was decided to test this clearance mechanism in old normal rats and old unilaterally nephrectomized rats. Injections of 10^3 and 10^5 *E. coli* were made into the lumen of the urinary bladder. After four to five days, cultures of the urine and both kidneys of 29 normal young rats were sterile. This was similar to results obtained in previous studies in this laboratory.[9] On the other hand, 7 out of 14 old rats had large numbers of *E. coli* recovered from the bladder urine or kidneys (Table 2). In similar experiments carried out in unilaterally nephrectomized rats, 2 out of 23 young rats (nephrectomized one week previously) had more than 10^2 viable bacterial units in the bladder urine whereas 6 out of 9 old rats

TABLE 2. INOCULATION OF *E. coli* INTO THE BLADDER CAVITY. BACTERIOLOGICAL EXAMINATION AFTER 4-5 DAYS

Inoculum	*Young rats*			*Old rats*		
	Left kidney	*Right kidney*	*Urine*	*Left kidney*	*Right kidney*	*Urine*
10^8	0	0	0	0	0	0
	0	0	0	0	0	0
	0	0	0	0	10^5	10^5
	0	0	0	0	0	10^5
	0	0	0	10^5	10^5	10^5
	0	0	0	0	0	0
	0	0	0	0	10^4	10^5
	0	0	0	0	0	0
	0	0	0	0	0	0
	0	0	0			
	0	0	0			
	0	0	0			
	0	0	0			
	0	0	0			
	0	0	0			
	0	0	0			
	0	0	0			
	0	0	0			
	0	0	0			
	0	0	0			
	0	0	0			
10^5	0	0	0	0	0	0
	0	0	0	0	0	10^2
	0	0	0	10^3	0	10^5
	0	0	0	10^4	10^4	10^5
	0	0	0	10^3	10^3	10^5
	0	0	0			
	0	0	0			

(nephrectomized six months previously) had this number of bacteria in the urine (Table 3). The urine and both kidneys of 9 additional control rats were cultured and were sterile. These animals had been unilaterally nephrectomized but were inoculated with saline into the bladder cavity.

It was apparent, therefore, that there was a decrease in the efficiency of clearance of bacteria from the urinary bladder in old animals as well as increased susceptibility of the kidney to infection.

TABLE 3. INOCULATION OF 10^8 *E. coli* INTO THE BLADDER CAVITY ONE WEEK OR SIX MONTHS AFTER UNILATERAL NEPHRECTOMY. BACTERIOLOGICAL EXAMINATION AFTER 4-5 DAYS

One week after nephrectomy		*Six months after nephrectomy*	
Right kidney	*Urine*	*Right kidney*	*Urine*
0	10^1	0	0
0	10^2	10^4	10^5
0	0	0	10^3
0	0	10^5	10^5
0	0	0	10^5
0	0	0	10^5
0	0	0	0
0	0	0	0
0	0	10^5	10^5
10^5	10^5		
0	10^1		
0	0		
0	0		
10^5	10^5		
0	0		
0	0		
0	0		
0	0		
0	0		
0	0		
0	0		
0	0		
0	0		

Histology

The kidneys of old rats frequently had a granular surface as compared to the smooth surface of kidneys from young rats. On microscopic examination the most prominent changes seen in the kidneys of old rats consisted of foci of dilated tubules filled with colloid casts and fusion of capillary loops of glomeruli. In addition, in some kidneys there were collections of atrophic tubules in the cortex around which there were varying numbers of mononuclear cells. In some instances the mononuclear cell infiltrates were marked and in one animal there were giant cells seen in the midst of these dense infiltrates. The histological appearance of the kidneys of old rats not given bacteria was the same as that of animals inoculated with *E. coli* save for the findings of foci of acute polymorphonuclear inflammation in the renal parenchyma in rats given bacteria intravenously and in the renal pelves of

rats with bacteriuria, from whose kidneys large numbers of bacteria were recovered.

DISCUSSION

Epidemiological studies in man have clearly demonstrated an increase in the prevalence of urinary infections with increasing age in both sexes.[1-8] Although there are many possible explanations for this finding, the one considered most likely is that there is increased susceptibility to urinary infections as a consequence of the known increase in abnormalities of the lower urinary tract with advancing age.

The present series of experiments demonstrates that rats are more susceptible to urinary infections in old age and that there are at least two mechanisms involved: increased susceptibility to pyelonephritis following the intravenous inoculation of bacteria and decreased effectiveness of bacterial clearance mechanisms in the bladder lumen.

The first series of experiments utilizing intravenous inoculation of bacteria demonstrated a small but definite increase in susceptibility to pyelonephritis in old rats. The outcome of studies in normal rats kept in the laboratory for one year (3/17 infected) was similar to that in nephrectomized rats kept for six months (2/8 infected). This study was undertaken to see if the known effect of nephrectomy in accelerating the renal changes associated with aging would increase susceptibility to infection, thus permitting experiments to be conducted over a shorter time interval. The results indicated that six months after nephrectomy susceptibility to infection was similar to that demonstrable after one year in normal rats. These experiments do not give any information as to whether nephrectomy influences the increased infectivity associated with aging. They are useful only in permitting studies of this phenomenon to be conducted over a shorter time period, the lower limits of which have not been defined.

If the histological changes in kidneys of aged rats explain the increased susceptibility of the kidneys to infection, it was surprising to be able to demonstrate this. Previous studies in rabbits have shown that a lesion capable of being infected when tested one to two weeks after its production was not infected when tested three months later.[11] The rate of production of kidney damage and the interval between damage and testing might also explain the surprisingly small increase in susceptibility to infection resulting both from hydronephrosis brought on by the gradual occlusion of the ureter and from congenital hydronephrosis.[12] Since the histological lesions in aged rats are likely to have developed slowly, it may well be that the increased susceptibility to pyelonephritis in old rats is not a consequence of the obvious morphological consequences of aging.

The decreased efficiency of bladder clearance mechanisms was more easily demonstrable in old rats than was the increased susceptibility of the kidney to infection. This is not surprising since previous studies in this laboratory have demonstrated the delicate balance that exists between bacterial multiplication in the bladder lumen and host defense mechanisms. By merely establishing chronic water diuresis in normal rats, the ability of host defense mechanisms to clear the bladder urine of bacteria is decreased about 1,000,000 times.[9]

As was true in previous studies of ascending infection with this strain of bacteria, large numbers of bacteria were recovered from the kidneys without morphological evidence of pyelonephritis. Nephrectomy was, therefore, combined with aging to see if pyelonephritis resulted. The data showed that clearance was somewhat impaired, even in young rats, after nephrectomy. The rats tested six months after nephrectomy had even greater impairment of clearance mechanisms, but pyelonephritis was not observed.

Whereas it is at least possible to consider kidney damage as an explanation for the infections produced following the intravenous inoculation of bacteria in old rats, the reason for the decreased clearance of bacteria from the bladder lumen is unknown. Whether the decreased effectiveness of bladder defense mechanisms is related to altered white cell function,[13] abnormal mechanics of the urinary tract in aged rats, or other bladder defense mechanisms[14] is not known.

SUMMARY

In view of the increased prevalence of urinary infections in men and women with advancing age, experiments were undertaken in rats to test whether differences in susceptibility to infections with *E. coli* were demonstrable within the kidney parenchyma and bladder lumen in old male rats.

Intravenous inoculation of *E. coli* produced pyelonephritis in a small percentage of one year old rats whereas infection was never noted in young rats.

When *E. coli* were injected into the bladder cavity of old rats, there was a decreased ability of these animals to rid the urine of bacteria as compared with young rats.

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