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EXPERIMENTAL PYELONEPHRITIS: Observations on mixed infection†

Uncomplicated pyelonephritis in man is almost always produced by a single species of bacteria, ordinarily a Gram-negative organism. Mixed infection of the urinary tract is usually associated with chronic obstruction or instrumentation. In animals the production of pyelonephritis results in an acute process, and in most models a single species of bacteria has been used as the infecting agent. It has been observed, however, that when the urinary tract is first damaged and bacteria are inoculated in numbers sufficient to induce experimental infection, kidney cultures sometimes reveal pure growth of a second spontaneous invader.[3,5] It seems as if the previously occupied organ could not support the multiplication of another species of bacteria. The experimental studies reported here were designed to evaluate the possibility of two kinds of bacteria infecting the same kidney in animals subjected to different procedures known to increase the susceptibility to pyelonephritis.

MATERIALS AND METHODS

Animals. Sprague-Dawley rats, weighing 180 to 250 gm. and albino rabbits, weighing 2 to 3 kg., were used.

Methods used to increase susceptibility of kidneys to infection. In all rabbits a thermo-cautery injury of the renal medulla was performed as previously described.[4] Rats were subjected to the following procedures: a) complete left ureteral ligation,[3] or b) left kidney massage through the intact abdominal wall for five minutes.[1]

Bacteria. Three different organisms were used: a strain of *E. coli* already employed in previous experiments;[3] a strain of *Ps. aeruginosa* isolated from a patient with

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urinary tract infection, and a strain of coagulase negative *Staphylococcus albus* isolated from a spontaneous infection in a hydronephrotic rat kidney. The test organism before inoculation was cultured in trypticase soy broth (BBL) for four hours in the case of *E. coli* and pseudomonas, and overnight for the staphylococcus. These cultures were used for intravenous injection (0.5 ml.). For mixed infections, 0.5 ml. of equal portions of the selected cultures constituted the inoculum. In each instance the number of bacteria injected was counted (approximately 5 x 10^7 organisms for the Gram-negatives and 5 x 10^8 for the staphylococcus).

Demonstration of kidney infection. Animals were sacrificed at various time intervals from 1 to 4 weeks following the inoculation of bacteria. The kidneys were removed aseptically through a large midline incision and transected by sterile methods. Half of

TABLE 1. PRODUCTION OF PYELONEPHRITIS IN RABBITS WITH MEDULLARY THERMO-CAUTERY INJURY FOLLOWED BY INTRAVENOUS INOCULATION OF ONE OR TWO SPECIES OF BACTERIA

	Bacteria injected (I.V.)		*Kidney infection*	
No. of rabbits	*1st week*	*2nd week*	*No. of animals*	*Bacteria recovered*
6	*E. coli*	—	5	*E. coli*
6	*Ps. aeruginosa*	—	5	*Ps. aeruginosa*
4	*E. coli*	*Ps. aeruginosa*	4	*E. coli*
5	*Ps. aeruginosa*	*E. coli*	5	*Ps. aeruginosa*
7	*E. coli* + *Ps. aeruginosa*	—	6	*E. coli* (4); *Ps. aeruginosa* (2)

the kidney was homogenized in sterile saline and the bacteria were counted by performing serial pour plate dilutions.[4] The other half was fixed in 10 per cent formol and sectioned for histologic study. Bladder urine was cultured at the same time, and in all cases the same flora were recovered from the urine as from the infected kidney. To demonstrate the existence of mixed infection, 0.1 ml. of 10^{-1} and 10^{-8} dilution of the kidney homogenates were spread with a glass rod on blood agar and desoxycholate plates. The plates were read after overnight incubation. The selective inhibition of staphylococcus by desoxycholate and differences in colony characteristics of *E. coli, Ps. aeruginosa,* and *Staphylococcus albus* in these media allowed the recognition of either organism in a concentration of 100 or more per gram of tissue. When infection was present, the number of organisms was usually in the order of 10^5 or more per gram. An *in vitro* control was done to show that it was possible by the methods used in the experiment to demonstrate the presence of a second organism even when it constituted only one per cent of the total number of organisms. Although colonies may have been so numerous that they were not discrete, the change in color of the media called attention to the presence of the second organism which was recoverable by subculture. It was also shown *in vitro* that the organisms grow well in each other's presence and do not inhibit each other's growth.

RESULTS

Kidney infection in rabbits with thermal injury of the medulla

In a total of 28 rabbits, a cautery wire was inserted to a depth of about 1.5 cm. in the lower pole of the left kidney. A single thermo-cautery lesion was produced using 25 volts for 1 to 2 seconds. One and two weeks after the cautery, the animals were challenged intravenously with bacteria. All animals were sacrificed one week after the last injection of bacteria.

TABLE 2. PRODUCTION OF PYELONEPHRITIS IN RATS WITH URETERAL LIGATION FOLLOWED BY INOCULATION OF ONE OR TWO SPECIES OF BACTERIA

		Kidney infection	
No. of rats	*Bacteria injected (I.V.)*	*No. of animals*	*Bacteria recovered*
10	*E. coli*	10	*E. coli*
16	*Ps. aeruginosa*	13	*Ps. aeruginosa*
9	*Staph. albus*	5	*Staph. albus*
18	*E. coli* + *Ps. aeruginosa*	16	*E. coli* (11) *Ps. aeruginosa* + *E. coli* (2) *Staph. albus* (3)*
9	*E. coli* + *Staph. albus*	8	*E. coli* (6) *Staph. albus* (2)
10	*Ps. aeruginosa* + *Staph. albus*	7	*Ps. aeruginosa* (6) *Staph. albus* (1)

* Animals "spontaneously" infected.

Over 80 per cent of the animals showed evidence of kidney infection subsequent to an intravenous injection of either *E. coli* or *Ps. aeruginosa* (Table 1).

No instance of mixed infection was detected following the simultaneous intravenous inoculation of these two bacteria. When one was injected, followed a week later by the other, only the first organism inoculated could be recovered. This was not due to decreased susceptibility of the kidney to infection the second week after cautery as this did not differ significantly from susceptibility during the first week. The infection rate was 70 to 80 per cent in both cases and only declined after four or more weeks. The presence of infection usually could be detected by macroscopic inspection because of the large number of abscesses. The histologic appearance of

the infection was similar to what has been described previously.[4] No gross or histologic difference was found between the infection produced by *E. coli* and *Ps. aeruginosa.*

Pyelonephritis in rats following the injection of one or two species of bacteria

1. *Rats subjected to ureteral ligation.* Seventy-two rats were subjected to complete left ureteral ligation, followed 5 to 7 days later by the intravenous injection of *E. coli, Ps. aeruginosa, Staphylococcus albus,* or a mixture of two of these bacteria (Table 2). All three strains were able

TABLE 3. PRODUCTION OF PYELONEPHRITIS IN RATS SUBMITTED TO RENAL MASSAGE SUBSEQUENT TO INTRAVENOUS INOCULATION OF ONE OR TWO SPECIES OF BACTERIA

		Kidney infection	
No. of rats	*Bacteria injected (I.V.)*	*No. of animals*	*Bacteria recovered*
10	*E. coli*	2	*E. coli*
10	*Ps. aeruginosa*	3	*Ps. aeruginosa*
15	*E. coli* + *Ps. aeruginosa*	9	*Ps. aeruginosa* (6) *E. coli* (2) *Ps. aeruginosa* + *E. coli* (1)*

* Very few colonies of *E. coli.*

to induce pyelonephritis in a high percentage of rats. When a mixture of two of these bacteria was injected, only one could be recovered from the infected kidney in most instances. In only 2 of 37 rats injected with a mixture of two species of bacteria, was a mixed flora detected; *Ps. aeruginosa* was the predominant organism, but a few colonies of *E. coli* were also present. The infected kidneys displayed the macroscopic and microscopic appearance already described.[3] When *Staphylococcus albus* was the infecting agent, however, multiple abscesses were infrequently found.

2. *Rats subjected to renal massage.* Left renal massage was performed on 35 rats subsequent to intravenous inoculation of *E. coli, Ps. aeruginosa,* or a mixture of both bacteria (Table 3). There was 40 per cent incidence of pyelonephritis in the total group. Of 15 rats receiving a mixture of *E. coli and Ps. aeruginosa,* only one of the species inoculated could be recovered from the kidneys in eight animals. In only one rat,

a few colonies of *E. coli* could be found in a heavily infected kidney with *Ps. aeruginosa.*

DISCUSSION

It seems apparent that when the intravenous route was used to induce experimental pyelonephritis, very few instances of mixed infection could be detected following the inoculation of a mixture of two species of bacteria. This was observed in rabbits with a medullary thermo-cautery injury and in rats subsequent to several procedures known to increase the susceptibility to kidney infection. Previous observations by Gorril[3] have shown that in spite of injecting mice with mixtures of two or three different strains of staphylococcus, over 90 per cent of kidney abscesses, when tested, yielded pure cultures. Morever, Guze and Beeson[3] reported pure staphylococcal growth from several hydronephrotic rat kidneys which had received a recent previous intravenous injection of *E. coli.* Those animals were "spontaneously" infected with the staphylococcus prior to the inoculation of the test organism. Similar observations have also been made in dogs submitted to a thermal injury of the renal medulla.[4]

Shapiro and Kobernick,[6] however, reported experimental mixed infection in rats subjected to renal massage following repeated intravenous injections of different bacterial species. The inoculations of *E. coli,* proteus, or enterococcus were separated by a long interval in the experiment (4 to 6 weeks). The available bacteriological data reveal, however, the isolation of only one species of bacteria from most of the kidneys at the time of sacrifice. The authors mention the "replacement" of one type of bacteria by another, but the incidence of mixed kidney infection is not reported.

An explanation for the very low incidence of mixed infection, in animals receiving an intravenous inoculation of two bacterial species is not apparent at this time. Local tissue changes induced by multiplication of one microorganism in the kidney may possibly result in inadequate environment for the localization of second invaders.[8] It is also possible that other causes of bacterial antagonism may play some role. The phenomenon of bacterial interference has been documented in several other situations. Guinea pigs infected with *B. suis* may develop resistance or may exhibit attenuated patterns of infection when challenged with certain doses of *P. pestis, B. anthracis,* and also *M. tuberculosis.*[8] The phenomenon, however, has many variables, and similar results could not be documented in mice.

Certainly those observations do not exclude the possibility of different results by using bacterial species or even strains other than those employed

in the present study. The nature of kidney infection in rats may differ a great deal according to the organism used.[7] Also, the isolation of only one bacterial species following the inoculation of two, does not preclude the possibility of the other remaining quiescent in very small quantity (<1 per cent) in the organ. The bacteria may be able to multiply subsequent to an effective inhibition of the invader. The major point, however, is that one species is multiplying whereas the other, if multiplying at all, is doing so to a very limited extent. These hypotheses, and also the local tissue changes in the kidney induced by the multiplication of one organism are worthy of further study.

SUMMARY

An attempt was made to induce mixed infection of the urinary tract in rats and rabbits following the intravenous inoculation of *E. coli, Ps. aeruginosa,* and a coagulase negative staphylococcus. The increase in susceptibility to kidney infection was obtained by ureteral ligation, medullary thermo-cautery injury, and renal massage.

When kidney infection was induced either by simultaneous intravenous injection of two species of bacteria, or by the injection of one followed a week later by the other, only one type of organism could be isolated in practically all instances.

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