HEMATOGENOUS PYELONEPHRITIS IN RATS. I. ITS PATHOGENESIS WHEN PRODUCED BY A SIMPLE NEW METHOD [1, 2]

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The study of experimental pyelonephritis has been generally restricted to that form of the disease which develops after ligation of the ureter (1–3). When pyelonephritis is complicated by ureteral ligation, however, a clear distinction cannot be made between the effects of urinary obstruction and those of infection. We have attempted, therefore, to establish experimental pyelonephritis in the absence of hydronephrosis and have succeeded by subjecting rats to a simple new technique requiring only two steps: 1) Injection of bacteria into the blood and 2) Massage of the kidneys through the intact abdominal wall. This method has provided an experimental model which appears to reproduce closely that form of human pyelonephritis occurring in patients with no manifest hydronephrosis. In addition, it dispenses with the cumbersome surgical procedures previously required for the production of experimental pyelonephritis.

This report describes the pathogenesis of pyelonephritis in rats when renal massage is combined with the inoculation of strains of *Escherichia coli* isolated from human infections.

METHODS

I. *The effect of renal massage on the incidence of pyelonephritis in rats inoculated with E. coli*

Sprague-Dawley rats weighing 150 to 200 gm. were divided into two groups of 15 each, and inoculated intracardially with 0.5 ml. of an 18-hour tryptose broth culture of *E. coli,* or approximately 10^8 living bacterial cells as determined by plate counts. This strain of *E. coli* had been isolated from the blood of a patient a few days before the experiment and had been subcultured only twice. Immediately after the bacterial inoculation, one group of 15 rats were subjected to bilateral renal massage. The kidneys of each rat were grasped by thumb and forefinger through the intact abdominal wall (Figure 1) and gently but firmly massaged during ether anesthesia for exactly 5 minutes. In order to maintain a uniform degree of pressure on the massaged kidneys, each period of 5 minutes was divided between two of us instead of confining the total period of massage for each animal to one of several persons. The 15 rats in the second group were anesthetized under ether for 5 minutes but their kidneys were not massaged. The kidneys of a third group were massaged for 5 minutes under ether anesthesia but the rats were not inoculated with bacteria. This third group also served as controls for the experiments in section 3b and are described there.

Within 48 hours, 4 rats in each group had died after the inoculation of *E. coli.* The survivors recovered fully and appeared in excellent condition. At the end of two weeks, the rats were sacrificed by exposure to ether and exsanguination. Approximately 1.0 ml. of blood was divided between trypticase soy broth and blood agar for culture and 0.5 ml. was used for determining urea nitrogen. The kidneys were examined grossly and then each divided into several representative parts for culture and microscopic examination. For culture, the kidney was ground to a pulp with sterile sand, suspended in water, and all of the remaining tissue cultured on blood agar, EMB agar, and tryptose broth. The kidneys were fixed in 10 per cent formalin and the sections stained with hematoxylin and eosin and with Schiff's periodic acid stain.

The procedures described in this experiment for renal massage, bacterial inoculation, and postmortem examination of tissues was that used in all experiments which follow, unless otherwise stated.

II. *Effect of massage on localization of E. coli in kidney as measured by bacterial counts*

From a group of 9 rats inoculated intracardially with *E. coli* (same strain as in I), 3 each were sacrificed at 4 minutes, 1 hour, and 2 hours after unilateral renal massage had been completed. The massage was applied to the right kidney of 4 rats and to the left kidney of the 5 other rats. Immediately before sacrifice by excessive exposure to ether anesthesia, blood was drawn from the heart for plate counts. Each kidney was weighed, ground to a pulp, and suspended in 9.0 ml. of sterile water. Bacterial plate counts were then made on each aqueous suspension of kidney tissue. The entire procedure was carried out aseptically.

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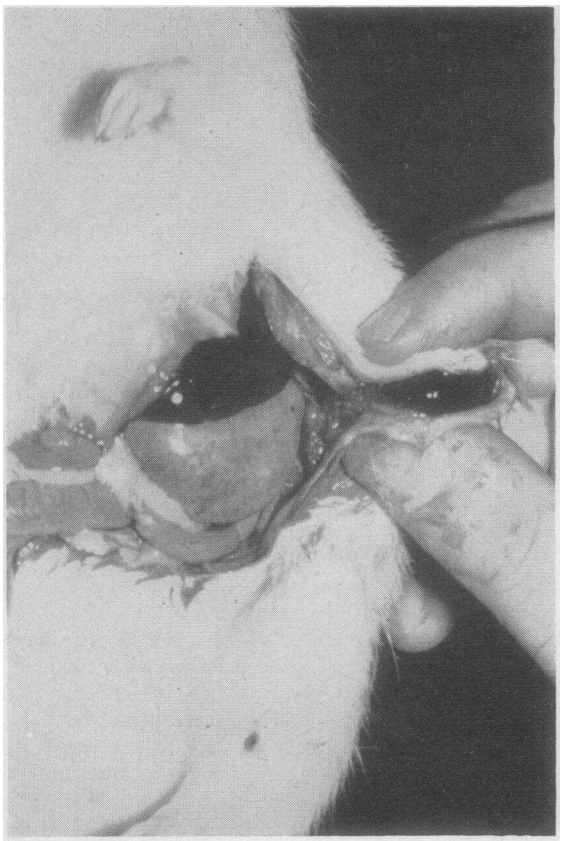


FIG. 1. TECHNIQUE FOR RENAL MASSAGE ILLUSTRATED IN A SACRIFICED RAT

An incision was made to allow visualization of the kidney as it is grasped between the thumb and forefinger.

An additional procedure was carried out with the kidneys of one rat in each group of 3 sacrificed at the specified time intervals. Before suspensions of ground tissue were prepared for plate counts, these kidneys were divided in half and the cortex and medulla from one half were separated. Then each separated portion (intact half, cortex, medulla) of the six kidneys was weighed, ground, and suspended in water.

III. *Serial studies of pathologic changes in massaged kidneys after inoculation of E. coli*

a. *Changes produced by a strain of high virulence.* From a group of 22 rats, 5 each were sacrificed at 2, 24, and 48 hours and the remaining 7 at 7 days after inoculation of approximately 10^8 *E. coli* and bilateral renal massage. Each rat was inoculated with either of two strains of *E. coli* which had been isolated from the urine of patients with pyelonephritis. The two strains appeared to be of equal pathogenicity. Cultures were made of the urine, heart blood, and kidneys; the urinary sediment and the kidneys were examined microscopically; and urea nitrogen was measured on samples of heart blood drawn immediately before sacrifice.

b. *Changes produced by a strain of low virulence.* Rats were divided into 5 groups of 10 each. One group of 10 received bilateral and another group unilateral (right kidney only) renal massage immediately after each animal had been inoculated intracardially with approximately 10^8 *E. coli.* This strain had been isolated from the urine of a patient with pyelonephritis and its virulence was reduced by repeated subculture. Of the three remaining groups of 10 each which served as uninfected controls, one group received bilateral renal massage, a second unilateral renal massage, and the third no massage. Two rats from each of the five groups were sacrificed at 1, 11, 17, 24, and 42 days after inoculation and the same examinations were carried out as in IIIa. In addition, blood pressures were measured just before sacrifice by the tail-plethysmographic method of Williams, Harrison, and Grollman (4).

IV. *Serial studies on urine obtained from living rats by cystotomy*

The experiment in IIIb was repeated but instead of sacrificing the animals, urine was obtained from them by cystotomy during anesthesia. The urines from 2 or more rats in each group were obtained at 2, 3, 5, 6, 8, 10 and 15 weeks after inoculation and then cultured and examined microscopically; but no rat was subjected to cystotomy more than once for the first 6 weeks. After 6 weeks, each of the animals examined was subjected to its second cystotomy. In addition, each of the inoculated rats had received two additional intracardiac injections of the same strain of *E. coli* as follows.

7 weeks—Intracardiac injection of 10^3 *E. coli* with no renal massage. This was done to determine whether the initial renal infection had increased the vulnerability of the kidneys so that pyelonephritis could now be established with relatively small numbers of *E. coli.*

9 weeks—Intracardiac injection of 10^6 *E. coli* accompanied by a second bilateral or unilateral renal massage. This was done to determine whether prior renal infection had rendered the kidneys relatively immune to reinfection.

Cystotomies were done aseptically on rats anesthetized with ether. The bladder was exposed by a small suprapubic incision and urine withdrawn by syringe through a 26 gauge needle. The abdominal wall was repaired with a few silk sutures. At the time of second cystotomies, complete healing of the original cystotomy was usually found.

Before each cystotomy, cardiac blood was drawn from each anesthetized rat for culture and urea nitrogen determination. Blood pressures were measured every other week.

RESULTS

I. *The effect of renal massage on the incidence of pyelonephritis in rats inoculated with E. coli*

The results summarized in Table I disclose that 95 per cent of the massaged kidneys were infected in contrast to only 23 per cent of the control kid-

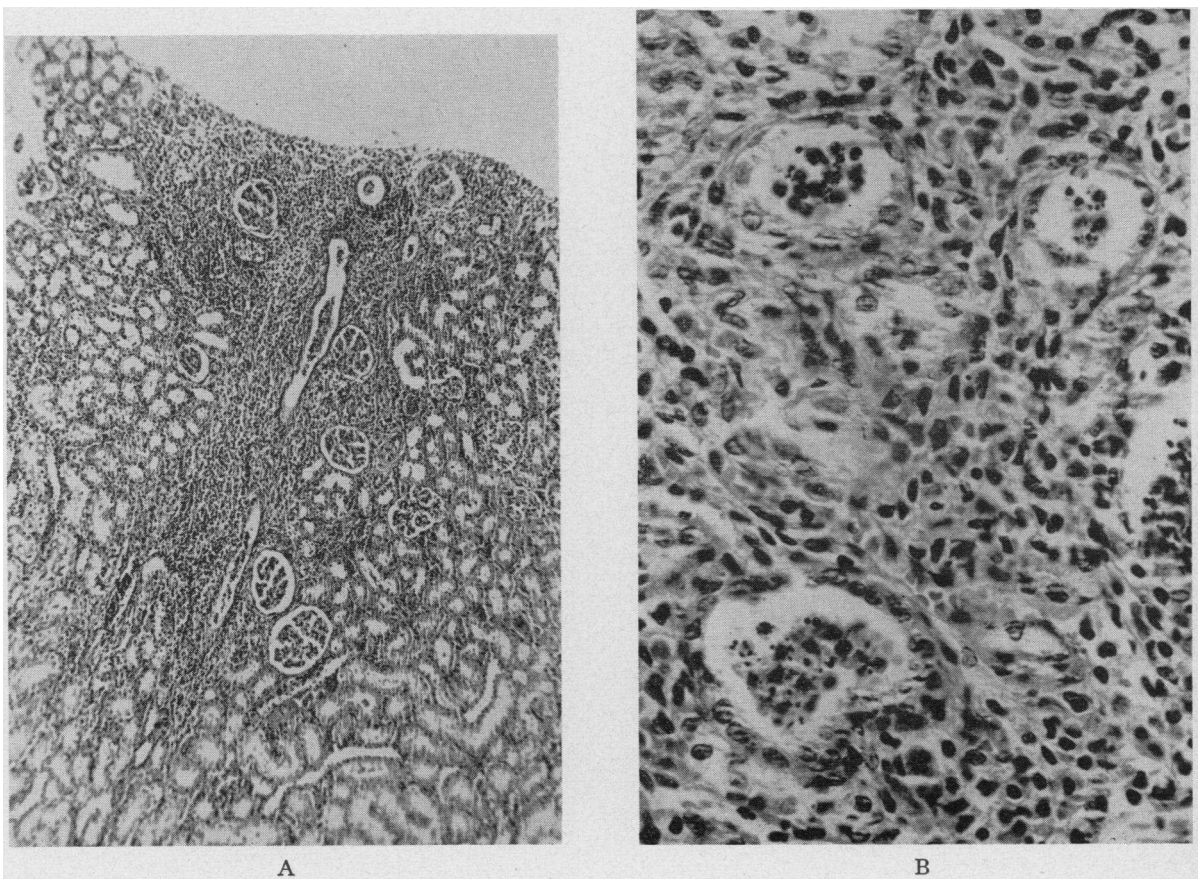
TABLE I*

EFFECT OF RENAL MASSAGE ON THE DEVELOPMENT OF PYELONEPHRITIS AFTER INTRACARDIAC INJECTION OF E. COLI IN RATS

	TOTAL NUMBER OF RATS	E. COLI ISOLATED FROM		GROSS PATHOLOGIC CHANGES	MICROSCOPIC EVIDENCE OF PYELONEPHRITIS
		BLOOD	KIDNEY		
NO MASSAGE	11	0	5	0	0
BOTH KIDNEYS MASSAGED	11	0	21	14	20

* Four rats in each group of 15 died within 48 hours after inoculation of *E. coli*.

neys after inoculating rats with *E. coli*. Even more striking was the observation that although no pathologic changes were found in any control kidneys, they were so extensive in the massaged group that gross evidence of pyelonephritis was found in 64 per cent and microscopic evidence in 91 per cent. Massaged kidneys of rats receiving no inoculation of *E. coli* were invariably sterile and only rarely displayed any sign of trauma from renal massage. Such trauma in non-infected mas-



A B

FIG. 2. MICROSCOPIC CHANGES IN PYELONEPHRITIC KIDNEY INFECTED WITH VIRULENT STRAIN OF *E. Coli* AND SACRIFICED AT 2 WEEKS

A. WEDGE SHAPED AREA OF INTERSTITIAL INFLAMMATION EXTENDING FROM CORTEX TO MEDULLA (× 100)

B. DILATED TUBULES ARE FILLED WITH POLYMORPHONUCLEAR CELLS, EVEN THOUGH CELLS COMPRISING THE INFLAMMATORY EXUDATE IN THE INTERSTITIAL TISSUES ARE VIRTUALLY ALL MONONUCLEAR (× 700)

Only polymorphonuclears are sufficiently motile to move into the tubules in large numbers.

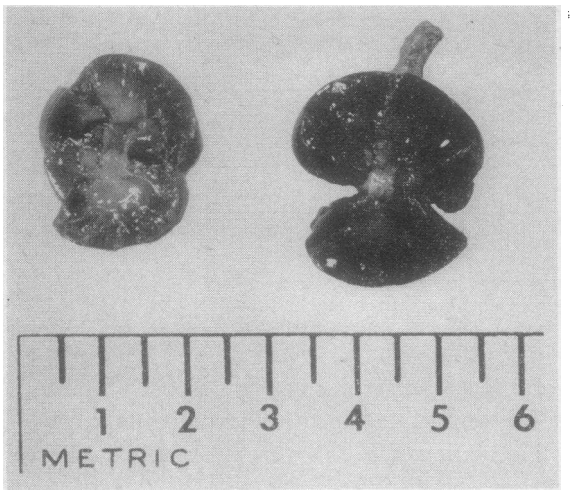


FIG. 3. COMPARISON OF MASSAGED KIDNEY AND CORRESPONDING NON-MASSAGED KIDNEY FROM SAME RAT PHOTOGRAPHED IMMEDIATELY AFTER COMPLETION OF 5-MINUTE PERIOD OF MASSAGE

Note marked hyperemia and enlargement of massaged kidney.

saged kidneys was never manifested by more than a minute hemorrhage or very small areas of tubular necrosis.

The kidneys which developed pyelonephritis were usually enlarged and sometimes showed small abscesses beneath the capsule. On section, there were frequently diffuse areas of suppuration visible. The microscopic appearances of these kidneys were identical to that seen in human pyelonephritis (5). There were wedge shaped areas of interstitial inflammation with its base in the cortex and the apex extending down into the medulla. The process usually spread up to the glomerulus but did not invade it. Although the great majority of cells comprising the inflammatory reaction in the tissues at two weeks were mononuclear, those in the tubules were almost all polymorphonuclear. Many areas containing dilated tubules filled with pus cells were found in association with the interstitial inflammation. The microscopic changes are illustrated in Figure 2.

II. *Effect of massage on localization of E. coli in kidneys as measured by bacterial counts*

Immediately after massage, the kidneys appeared to contain an increased amount of blood and were much heavier than the kidneys on the opposite side which were not massaged (Figure 3). This difference in weight between the massaged and non-massaged kidneys disappeared within two hours as the hyperemic appearance subsided. The number of bacteria per gram of massaged kidney remained about 10 to 100 times greater than that of the control and exhibited a bacterial concentration of 10 to 100 times greater than that of the blood. The higher bacterial count in the massaged kidney resulted from an initial heavy localization of *E. coli* in the cortex alone as illustrated by the 100 fold increase in bacterial count observed in the cortex of massaged over control kidney. Later, the bacterial counts in the medulla of the massaged kidney also increased over that of the control. These results are given in detail in Figure 4 for the 3 rats whose kidneys were separated into cortex and medulla and in Table II for the remaining 6 rats.

III. *Serial studies of pathologic changes in massaged kidneys after inoculation of E. coli*

a. After inoculation of a highly virulent strain of *E. coli* no inflammatory cells appeared in the kidneys or urine during the first 2 hours even though cultures of all kidneys and urines yielded large numbers of *E. coli*. At 24 hours pyuria was observed only in the 2 rats in which the early microscopic signs of pyelonephritis were also found.

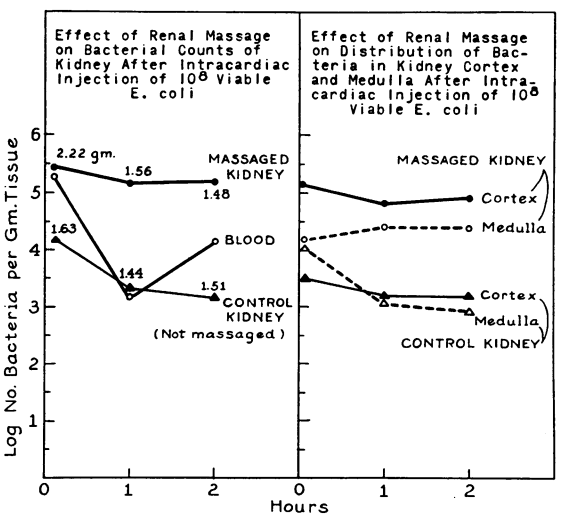


FIG. 4. EFFECT OF RENAL MASSAGE ON BACTERIAL COUNTS OF KIDNEYS AFTER INTRACARDIAC INOCULATION OF *E. Coli*

TABLE II

EFFECT OF RENAL MASSAGE ON BACTERIAL COUNTS OF KIDNEY AFTER INTRACARDIAC INJECTION OF 10^8 VIABLE E. COLI

TIME ELAPSED (MINUTES) AFTER INOC.	IDENTIFICATION NUMBER OF RAT	MASSAGED		TOTAL NO. BACTERIA PER KIDNEY	WEIGHT OF KIDNEY (Gm.)	NO. BACTERIA PER Gm. OF KIDNEY	NO. BACTERIA PER Ml. OF BLOOD
4	1	No	L	85,400	1.0154	84,138	
		Yes	R	740,733	1.2821	577,800	2,330,666
	2	Yes	L	254,866	1.3204	193,080	
		No	R	29,500	1.1294	25,243	2,620,666
60	3	No	L	59,133	1.0081	58,547	
		Yes	R	327,866	1.1782	278,325	15,225
	4	Yes	L	145,400	1.2113	120,165	
		No	R	8,550	1.1695	7,307	11,580
120	5	No	L	35,533	1.1671	30,370	
		Yes	R	449,733	1.2057	371,680	1,173
	6	Yes	L	72,700	1.2619	57,700	
		No	R	18,400	1.2996	14,154	3,013

L = Left Kidney
R = Right Kidney

There were no pus cells in the urine of the other rats examined at 24 hours, and there was no pyelonephritis. Similarly, at 48 hours, the finding of pyuria was limited to those rats whose kidneys displayed the typical gross and microscopic findings of acute pyelonephritis. Yet the urine culture from all rats yielded *E. coli* during the first and second days. By one week, pyelonephritis was present in 13 kidneys of all 7 rats and pyuria was present in the urine of all rats. The results are summarized in Table III.

The earliest evidence of pyelonephritis was found at 24 hours when neutrophils began to attack bacterial masses situated between the tubules. By 48 hours, interstitial abscesses composed of dense accumulations of neutrophils, were

TABLE III *

CORRELATION OF CULTURES AND CELLULAR CHANGES IN EXPERIMENTAL PYELONEPHRITIS

	TIME ELAPSED AFTER INTRACARDIAC INJECTION VIRULENT STRAIN 10^8 E. COLI															
	2 hours				1 day				2 days				7 days			
	U	B	RK	LK	U	B	RK	LK	U	B	RK	LK	U	B	RK	LK
Rat 1	+	+++	+++	+++	+	+	+++	+++	+ P+++	+	+++ PN+++	+++ PN+++	+ P+	0	++ PN++	+ PN++
Rat 2	+	+++	+++	+++	+	+	+++	+++	+	+	+++	+++	+ P++	±	++ PN+++	+ PN+++
Rat 3	+	+++	+++	+++	+	+	+++	++	±	++	+++	+++	++ P++	0	+++ PN+++	+++ PN+++
Rat 4	+	+++	+++	+++	+ P+	++ PN ±	+++	+++	+ P++	±	+++ PN+++	+++ PN+++	+ P++	0	+++ PN++	+
Rat 5	+	+++	+++	+++	+ P+	+ PN ±	+++	+++	++ P++	+	+++ PN+++	+++ PN+++	++ P++	0	+++ PN+++	+ PN+++
Rat 6													++ P++	0	+++ PN+++	+++ PN++
Rat 7													+ P++	0	+++ PN+++	++ PN++

P = Pyuria
PN = Pyelonephritis

* Cultures were made of blood (B), urine (U), right kidney (RK), and left kidney (LK). (P = pyuria; PN = pyelonephritis; O = sterile.)

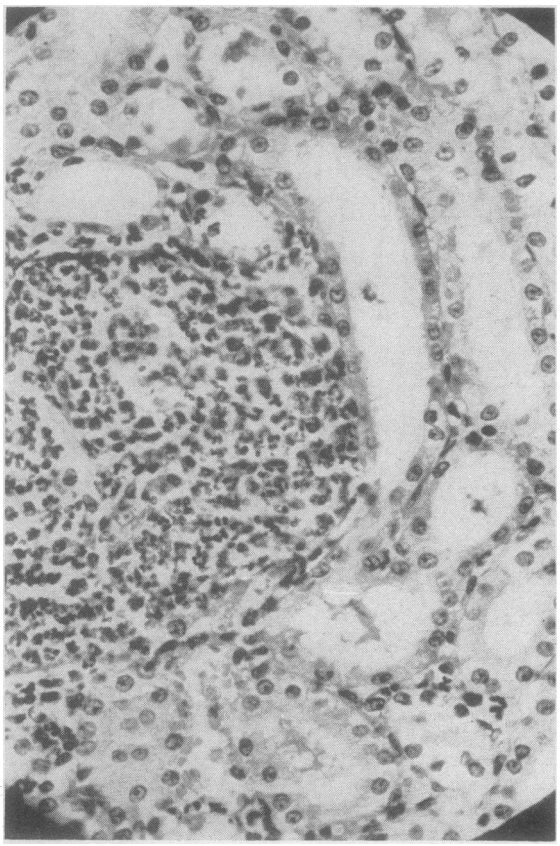


FIG. 5. MICROSCOPIC APPEARANCE OF INTERSTITIAL SUPPURATION IN PYELONEPHRITIC KIDNEY OF RAT SACRIFICED 48 HOURS AFTER INOCULATION OF *E. Coli*

observed grossly and microscopically in the cortex and medulla (Figure 5). At one week, however, many lymphocytes and plasma cells began to participate in the pyelonephritic process. These mononuclears, accompanied by an equal number of neutrophils, comprised a dense infiltrate which usually extended from cortex to medulla in a wedge shaped fashion. The glomeruli were usually spared although the tubules were frequently destroyed or filled with large clumps of neutrophils.

The values for B.U.N. and blood pressure are given in Table IIIA. The B.U.N. ranged from 26 to 46. A few of these values, when compared with those of normal controls given in Table VA, are slightly elevated. The blood pressures on 5 rats taken at 1 week were all normal.

b. After inoculation of a strain of low virulence, the infection is characterized by very few signs of inflammation at any time. The infection was self-limited to about 3 weeks. Despite repeatedly positive cultures of kidney tissue, no renal inflammation was found during the first 17 days and no pyuria developed. In addition, urine cultures were sometimes negative even though the kidneys were infected with *E. coli.* It is particularly noteworthy, however, that microscopic evidence of pyelonephritis sometimes appeared after the infection had disappeared. This late inflammation, which was entirely mononuclear in character, was not accompanied by pyuria. The results are summarized in Table IV. The systolic blood pressure of one animal was 130 mm. Hg at 42 days after bilateral renal massage and inoculation *E. coli.* The blood pressures of all other animals receiving renal massage were 115 or less. The blood pressures in normal control rats were 120 or less. The B.U.N. ranged from 24 to 31 in normals and from 20 to 33 in the animals receiving renal massage with inoculation of *E. coli.* No cultural or pathologic sign of pyelonephritis appeared in any of the control rats.

IV. *Serial studies on urines obtained from living rats by cystotomy*

Table V summarizes the results of serial examinations of urines obtained by cystotomy from rats after renal massage and inoculation of a strain of *E. coli* of low virulence. As in Section IIIb, pyuria was uncommon and renal infection caused by this strain appeared to be self-limited; in these animals it subsided after about 6 weeks. After subsidence of infection, reinoculation with

TABLE IIIA *

TIME ELAPSED AFTER INTRACARDIAC INJECTION 10^8 E. COLI

RAT NUMBER	2 HRS.	1 DAY	2 DAYS	7 DAYS	B.P.
1	44	ND	34	26	ND
2	36	ND	ND	34	ND
3	32	28	30	46	110
4	32	ND	38	32	115
5	34	46	31	42	100
6	34			42	100
7				38	105

Blood urea nitrogens of rats sacrificed serially. Blood pressures just before sacrifice are also given for those examined at 7 days. (ND = No determination)

* These results were obtained on rats listed in Table III.

TABLE IV *

RESULTS OF CULTURE OF URINE (U), BLOOD (B), RIGHT KIDNEY (RK) AND LEFT KIDNEY(LK) UPON SACRIFICE OF RATS

		DAYS ELAPSED AFTER INTRACARDIAC INOCULATION OF 10^8 E. COLI																	
		1				11				17				24				42			
		U	B	RK	LK	U	B	RK	LK	U	B	RK	LK	U	B	RK	LK	U	B	RK	LK
MASSAGE BOTH KIDNEYS	Rat 1	0	+	+	+	0	0	+	+	0	0	0	0	0	0	0	0	0	0	0	0
	Rat 2	+	+	+	+	+	0	+	0	0	0	+	0	0	0	0	0 PN	0	0	0 PN	0
MASSAGE RIGHT KIDNEY ONLY	Rat 3	+	+	+	+	0	+	+	0	0	0	0	0	0	0	0	0	0	0	0	0
	Rat 4	0	+	+	+	0	0	+	0	0	0	0	0	0	0	+ PN	0	0	0	0 PN	0

PN = Pyelonephritis
No Pyuria observed in any animal.

* + = isolation of *E. coli*; O = sterile. Isolation of *E. coli* from left kidneys, 1 day after inoculation of rats receiving renal massage only on right side, was attributed to the presence of *E. coli* in the blood contained in those kidneys.

a relatively small number of *E. coli* without renal massage did not lead to recurrent signs of renal infection; but bacteriuria was reestablished at 10 weeks by renal massage in conjunction with the inoculation of larger numbers of *E. coli*. The latter results suggest that upon subsidence of pyelonephritis, the kidneys were neither unduly susceptible to reinfection by small bacterial inoculums, nor immune to reinfection by the usual inoculums. No sign of urinary infection appeared at any time in urines obtained from control rats. The highest blood pressure obtained in any animal was 120 mm. Hg. The B.U.N. in rats subjected to renal massage and inoculation of *E. coli* ranged from 25 to 35, while the B.U.N. of normal controls varied from 26 to 34. These results are given in Table VA.

DISCUSSION

The pathologic appearance of the kidneys of rats, infected with virulent strains of *E. coli*, was almost identical to that seen in human pyelonephritis caused by *E. coli*. In both cases, the inflammation is interstitial and extends in a wedge shaped fashion from its base in the cortex down to the medulla (5). In rats, as in man, it was also found that urinary findings may be normal despite extensive infection in the interstitial tissue of the kidney (6). The acute pyelonephritis in

TABLE V *

RESULTS OF URINE CULTURE FOR E. COLI OBTAINED BY CYSTOTOMY WITHOUT SACRIFICING RATS

		WEEKS ELAPSED AFTER FIRST INTRACARDIAC INJECTION OF 10^8 E. COLI								
		2	3	5	6	7	8	9	10	15
MASSAGE BOTH KIDNEYS	RAT 1	++++	+ P	0	0	Intra cardiac injection 10^3 E. Coli NO MASSAGE	0	Intra cardiac injection 10^6 E. Coli and kidneys massaged	+	0
	RAT 2	++++	+	0	0		0		0	+
MASSAGE RIGHT KIDNEY ONLY	RAT 3	++	0	0	0		0		0	+
	RAT 4	++	0	+	+		0		+ P	0

P = Pyuria
All blood culture sterile.
BUN and Blood pressure normal on all animals.

* The designations "Rat 1, Rat 2, Rat 3, Rat 4" are used only to identify rats within the group examined on a given day; no rat was subjected to cystotomy more than once for the first 6 weeks. In addition to those listed, urines were also sterile from 2 rats receiving bilateral renal massage at 6 weeks and from 2 rats receiving unilateral renal massage at 15 weeks.

TABLE VA *

	Inoc. with E. Coli at 0, 7, & 9 wks.	WEEKS ELAPSED AFTER FIRST INTRACARDIAC INJECTION OF 10^8 E. COLI: 2	3	5	6	8	10	15
Massage both kidneys	Yes	25 117	29 111	27 112	30 112	26 107	35 109	ND 110
Massage right kidney	Yes	28 116	25 107	30 109	25 112	33 110	ND 107	ND 112
No massage of kidneys	Yes	30 112	31 106	27 106	29 104	30 103	31 106	ND 109
Massage both kidneys	No	29 108	29 109	26 107	31 113	27 109	31 115	ND 115
No massage (normal controls)	No	34 109	29 104	28 106	32 110	ND 109	26 108	ND 107

Blood urea nitrogens and blood pressures of rats examined periodically by cystotomy.

* The B.U.N. is the upper value, and the blood pressure the lower value given at each time interval for each group of rats. The values given are averages for all rats examined in a group. (N.D. = no determination.) These results were obtained on rats listed in Table V and on the controls.

rats and in man also resemble each other in that hypertension and usually uremia are absent in both cases. These similarities between pyelonephritis in humans and in these rats suggest that the experimental disease may be used as a model for studying certain aspects of human pyelonephritis.

Renal massage appears to favor the development of hematogenous pyelonephritis in bacteremic rats by causing increased numbers of bacteria to localize in the kidney. It is not clear from the present data whether this heavy localization of *E. coli* resulted from renal damage, urinary obstruction, or from the delivery of more bacteria through increased renal flow of infected blood. Although no obstruction of the urinary tract and only rare renal damage were evident at postmortem examination of the massaged kidneys, these abnormalities might have been detectable if function studies had been carried out. Likewise, in the absence of hemodynamic studies, it cannot be established whether the hyperemic appearance of the massaged kidneys represents increased blood flow or simple engorgement and stasis. It is also possible that the relatively poor lymphatic supply of the kidney was damaged and not adequate to remove the excessive numbers of bacteria which could then establish infection. Pierce (7) has demonstrated that in contrast to skin, intestine, or uterus, there are very few renal lymphatics present to take over as emergency units to handle any additional load over that normally present. In the present experiments, bacteria which lodged in the kidneys remained there even though they were of such low virulence that they induced no necrosis or inflammatory reaction. This would account for the persistence of *E. coli* in the kidney and urine for several weeks in the absence of inflammatory cells.

Once the experimental infection was established, its manifestations varied with the virulence of the organism. Highly virulent strains led to suppuration and pyuria. Strains of lower virulence produced a mild self-limited infection with sometimes little or no sign of renal inflammation. Upon analysis of these two types of infectious processes in the experimental animals, several points become evident which help explain certain clinical findings in human pyelonephritis which are not well understood. For example, it was found in suppurative experimental pyelonephritis that, although the cellular reaction in the tissues was predominantly mononuclear by two weeks, virtually all the cells in the tubules were polymorphonuclears. This difference is accounted for by the great motility of polymorphonuclears which enables them to move out of the inflamed areas into the tubules. This experimental observation is useful in explaining

why the polymorphonuclear is almost the only leukocyte found in the urinary sediment in human pyelonephritis and why the urinary sediment in chronic pyelonephritis frequently contains no cells after the renal inflammation becomes lymphocytic in character.

During the experimental renal infection by strains of low virulence, there was a different reason for the absence of pyuria. Here the cellular reaction in the kidney itself was so slight, if present at all, that there was often no significant focus of polymorphonuclears to supply the tubules. In such an infection, only motile bacteria were present in the urine and even these were often absent. Whether a comparable form of acute non-suppurative pyelonephritis occurs in human beings can only be conjectured, however, because pathologic and cultural examinations of such kidneys would be almost impossible to secure. Yet there is abundant evidence to suggest that a clinical entity of this type exists. The syndrome of fever, chills, sweats, and bacteriuria occurring in the absence of flank pain, costovertebral angle tenderness, and pyuria is not uncommonly produced by *E. coli*. The course of this disease is generally self-limited to a few days or weeks, but may recur for many years and lead to chronic pyelonephritis (6). In this connection, it is of interest that in the experimental infection, there was not only a lack of immunity against subsequent attacks of *E. coli* pyelonephritis, but also evidence which suggested that the pyelonephritic changes may first appear as the infection vanishes (Table III). Thus it may be possible for repeated infections by the same bacterial species to occur and initiate inflammatory reactions which perpetuate themselves after the bacteria disappear.

These phenomena and others relating to chronic pyelonephritis are under investigation in rats whose renal infections have persisted for over 6 months. It is anticipated that these studies now in progress may illuminate some of the problems related to chronic human pyelonephritis.

CONCLUSIONS

Renal massage appears to favor development of hematogenous pyelonephritis in bacteremic rats by causing large numbers of bacteria to localize in the kidney. The severity of the pyelonephritis depends on both renal massage and bacterial virulence. Renal infection (proved by positive cultures of kidneys) may exist in the absence of pyuria and bacteriuria.

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