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Hematogenous Pyelonephritis in Rats. II. Production of Chronic Pyelonephritis by *Escherichia coli.** (22157)

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(Introduced by Carleton B. Chapman.)
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Development of chronic pyelonephritis in man is obscure because usually the disease is examined only in its end stages. The present experiments were undertaken therefore to provide an experimental model for a controlled study of processes which culminate in chronic pyelonephritis. A major objective was to observe effects of chronic infection on the kidney in the absence of hydronephrosis or other structural abnormalities. We described previously a simple, non-surgical technic for producing acute hematogenous phylonephritis in rats, involving 2 steps; injection of *Escherichia coli* into blood, and massage of kidneys through intact body wall(1). The resulting disease was similar to acute pyelonephritis without manifest hydronephrosis in man, but self-limited, and subsided in approximately 6 weeks. To produce chronic pyelonephritis, rats from this study were subjected repeatedly for several months to bacterial injection and renal massage. Special interest was directed to the following problems: 1) relationship of chronic pyelonephritis to vascular disease and hypertension, 2) progression of chronic renal inflammation after cessation of active infection.

Materials and methods. Sixty-one male Sprague-Dawley rats, weighing 150 to 200 g each, were divided into 5 groups of 12.† All were fed rat chow‡ and tap water *ad lib.* Treatments for each group are described in Table I. During the first 30 weeks, urine from rats in each group was obtained peri-

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† Group V consisted of 13 rats.

‡ Uncle Johnny Mills, Houston, Texas.

TABLE I. Schedule of Inoculations and Renal Massage.

Group	Time of inoculation of *E. coli* (wk)	Total No. of *E. coli* inoculated	Time of massage (wk)
I	0	10^8	Bilateral at 0, 9, 26
	7	10^5	
	9	"	
	26	10^8	
II	0	10^8	Unilateral (right side) at 0 and 9; bilateral* at 26
	7	10^5	
	9	"	
	26	10^8	
III	None		Bilateral at 0, 9, 26
IV	0	10^8	None
	7	10^5	
	9	"	
	26	10^8	
V	None		"

Inoculations made with 0.5 ml of 18 hr tryptose broth culture of *E. coli*. Strain used at 0, 7, and 9 wk was isolated from urine of patient with pyelonephritis and its virulence somewhat reduced by repeated subculture. The strain inoculated at 26 wk had been freshly isolated from blood of a second patient with pyelonephritis.

* Six of the 12 rats were given an additional inoculation of 10^8 *E. coli* and bilateral massage at 12 wk.

odically by cystotomy for culture and microscopic examination. Blood was removed by cardiac puncture at these times for culture and measurement of urea nitrogen. Blood pressure of each rat was determined every 2 weeks by tail plethysmographic method described by Williams, Grollman, and Harrison (2), with the rat lightly anesthetized with ether. Surviving animals were sacrificed after 42 weeks and at autopsy, studies of urine and blood were repeated. In addition, half of each kidney was ground to a pulp with sterile sand and cultured on blood agar, EMB agar, and in tryptose broth. The remaining half, as well as portions of heart, liver, spleen, and bladder were fixed in 10% formalin. Sections were stained with hematoxylin-eosin and Schiff's periodic acid.

Results. Serial studies before sacrifice. Twelve rats from Groups I and II failed to survive to 42 weeks; causes of death were anesthesia accidents in 5, bacteremic shock in 3, and unknown in 4. *E. coli* was isolated from urine at least once during life in all but 3 of the 12 rats which survived to 42 weeks

TABLE II. Summary of Bacteriologic and Pathologic Findings at Time of Sacrifice (42 Weeks).

Group‖	No. surviving rats	Positive urine cultures	Positive kidney cultures (No. of kidneys = 2 × No. of rats)	Positive blood cultures	BUN range and avg		Pyelonephritis†		Pyuria No. with pyuria/No. of rats
					During life	At sacrifice	Gross	Microscopic	
I & II—r.m. and inoc. of *E. coli*	12	1 *E. coli* 3 *P. inter.***	1 *E. coli*‖ 6 *P. inter.*	0	17-42 (28.6)	24-38 (29.7)	24/24	24/24	6/12§
III—r.m. only	10	0	2 *P. inter.**	1 *P. inter.*	25-33 (28.3)	26-34 (30.3)	0/20	1/20‡	0/10
IV—inoc. of *E. coli* without r.m.	6	0	0	0	20-33 (28.5)	Not done	1/12	3/12	1/ 6
V—controls; no inoc. or r.m.	13	0	4 *P. inter.**	2 *P. inter.*	17-40 (28.9)	24-27 (25.5)	0/26	1/26‡	0/13

* Isolation of *P. intermedium* probably resulted from blood in the kidney and not from renal tissues themselves since urines were negative and pyelonephritis was not present in these animals.
† Numerator = No. of kidneys with pyelonephritis; denominator = total No. of kidneys examined.
‡ Questionable pyelonephritis only (see text).
§ Includes the 4 rats with positive cultures.
‖ r.m. = renal massage.
¶ One of the kidneys in this animal was sterile.
** *P. inter.* = *P. intermedium.*

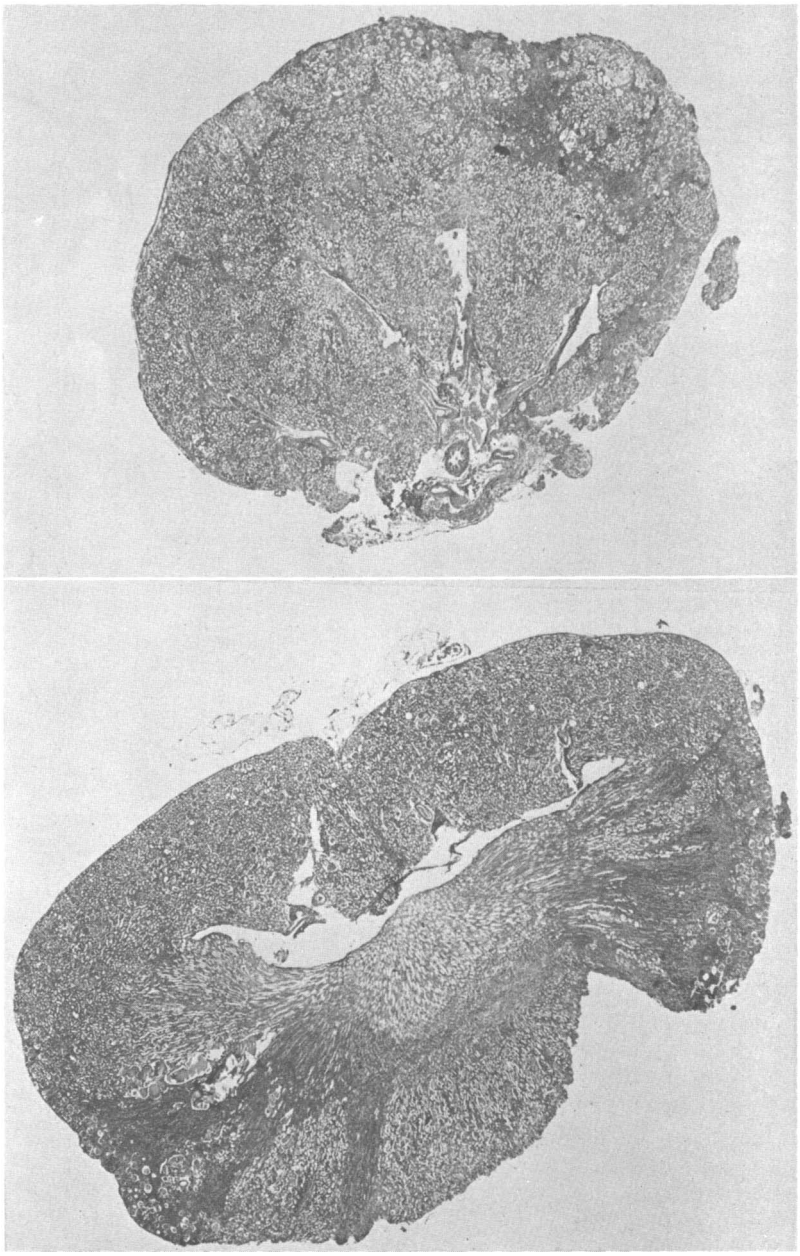

FIG. 1. Hilar cross sections of both kidneys from the same animal in Group 1 (×4). Note marked differences in size, gross distortion produced by scarring, and loss of cortical substance. Urine culture had been positive for *E. coli* at 5 wk but both urine and kidney cultures were negative at 42 wk.

and in 6 dying earlier. Six rats in Group IV survived 42 weeks after the first inoculation of *E. coli*. Urines from 3 animals yielded positive cultures after first inoculation, but all subsequent urines from these and remainder of the group were negative. Two rats died of bacteremic shock and 2 died of unknown causes. Two were sacrificed at 36 weeks and negative cultures obtained from both urine and kidneys. In Group III, 2 deaths occurred from unknown causes; in Group V, all survived. All urine cultures in

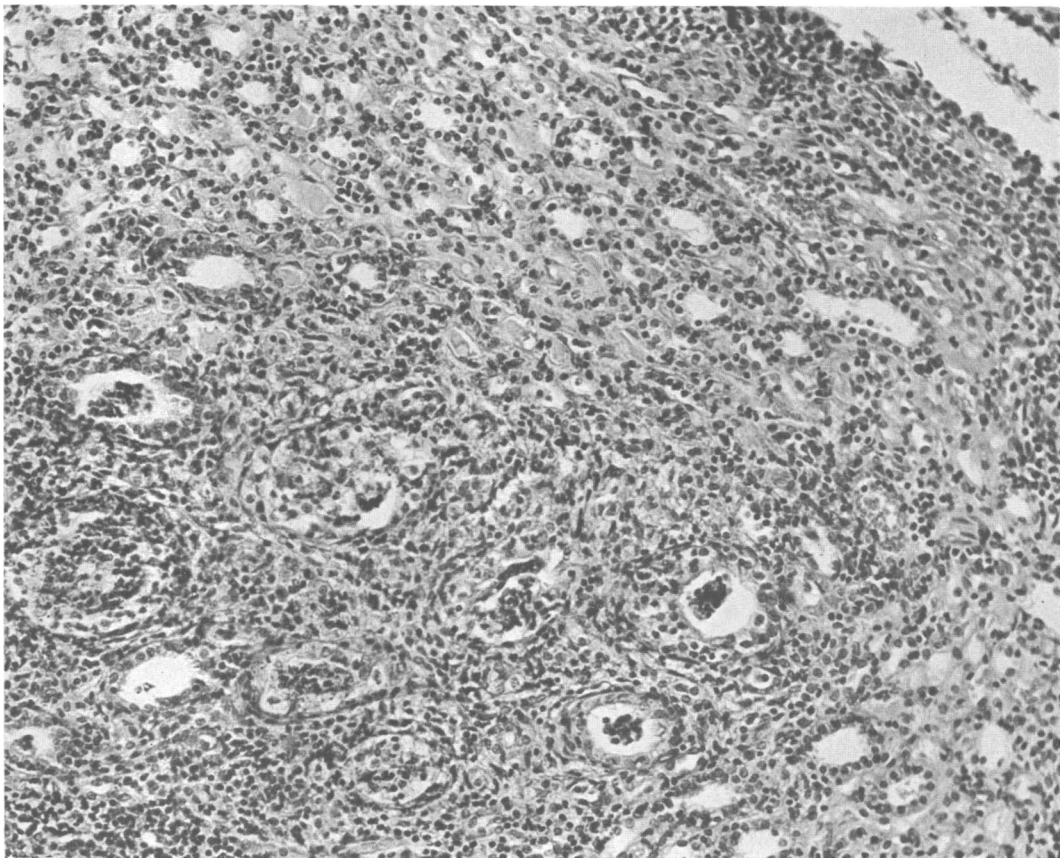

FIG. 2. Microscopic section of papillary tip of pyelonephritic kidney. Note fibrosis, mononuclear infiltration, and dilated tubules containing pus casts (×140). Urine cultures were positive for *E. coli* at 10 and 30 wk; *P. intermedium* was cultured from urine and kidneys at 42 wk.

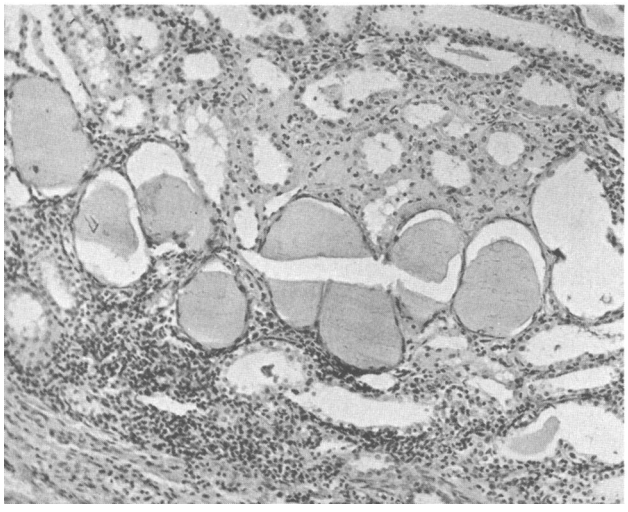

FIG. 3. Microscopic section from medullary area of pyelonephritic kidney revealing dilated, colloid-filled tubules. At the lower left, an area of fibrosis and mononuclear infiltration extends through medulla (×65). Urine culture positive for *E. coli* at 5 wk and negative at 15 and 42 wk; kidney culture negative at 42 wk.

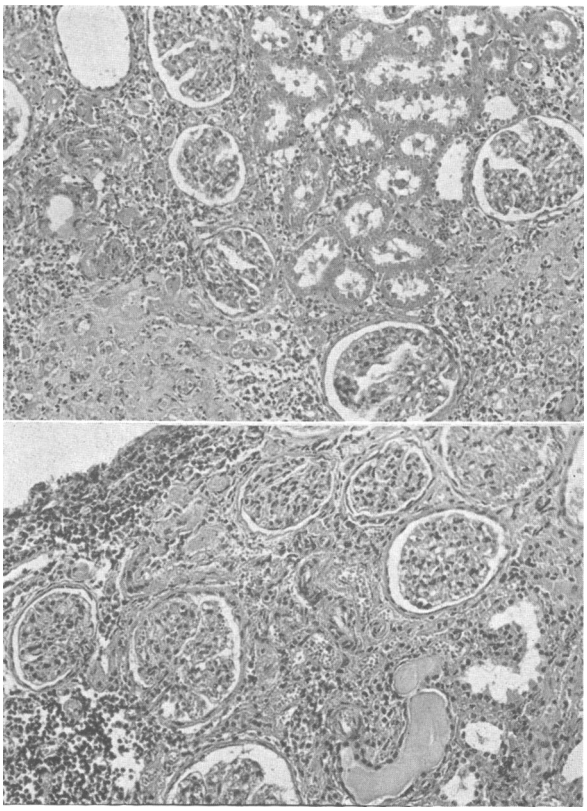

FIG. 4. Microscopic sections from 2 areas of cortex of pyelonephritic kidneys. A. Intact glomeruli with periglomerular fibrosis and marked interstitial fibrosis. Note interstitial accumulation of lymphocytes and plasma cells, and normal appearance of arterioles (×65). Cultures of urine and kidneys negative at 42 wk. B. Marked interstitial infiltration with mononuclear cells and widespread periglomerular fibrosis. Glomeruli themselves are normal (×70). Urine culture positive for *E. coli* at 5 wk, negative at 15 and 42 wk. Culture of kidney negative at 42 wk.

both groups were negative. Blood cultures repeatedly were negative in all five groups. Blood urea nitrogen was normal; values are listed in Table II.

Studies at completion of experiment. Bacteriological and pathological findings in all animals when sacrificed (42 weeks) are summarized in Table II. The 12 surviving animals in Groups I and II have been considered as a single group because all were subjected ultimately to bilateral renal massage. *E. coli* could still be isolated from only one rat in this combined group. From pyelonephritic kidneys and urine of 3 additional animals in the group, *Paracolobactrum intermedium* was cultured.

Pathologic findings. 1) Kidneys (Fig. 1-4). Changes of chronic pyelonephritis were found in all 12 rats in Groups I and II which survived 42 weeks (Table II). Four of the 12 animals dying earlier were examined, and 2 displayed pyelonephritic changes. The 2 in whom changes were absent had died after 12th week of the experiment. Kidneys were often adherent to surrounding organs and usually differed in size. They were distorted, lobulated by scars, and displayed loss of cortical substance and increased thickness of pelvic walls. Microscopic examination revealed areas of interstitial inflammation consisting of lymphocytes and plasma cells which extended throughout cortex and medulla. The tubules were dilated and many contained large colloid casts ("thyroid kidney"). Numerous "pus casts" were observed in kidneys from which positive cultures were obtained,

but only a few in absence of demonstrable infection. There was extensive interstitial and periglomerular fibrosis but glomeruli themselves usually appeared normal. Significant vascular lesions were absent. Thickness of media of arterioles never exceeded that in controls. There was no intimal proliferation or medial necrosis. Rarely, an arteriole in the midst of a dense scar showed slight hyalinization. In 2 rats from Group IV, minimal findings of pyelonephritis were present, consisting of scattered infiltrations with lymphocytes and plasma cells and slight fibrosis; a single, small scar was observed grossly in one kidney. Among the remaining control animals (Groups III and V), occasional tiny depressions on surface of kidney were seen grossly, which corresponded to small areas of cellular infiltration beneath the capsule. A few tubules were dilated and contained colloid, but never to the degree seen in Groups I and II. In 2 rats with sterile kidneys, rare areas of cellular infiltration without fibrosis suggesting pyelonephritis were observed in cortex and medulla. 2) *Other organs.* Tiny well-healed scars were present in the hearts of many animals at site of needle puncture, but there was no other abnormality. Bladders in Groups I and II were often slightly thickened. Significant changes in spleen and liver were absent, except for splenic enlargement in animals with septicemia. *Blood pressure.* Mean systolic blood pressures and standard deviations of each group were: Group I and II: 111 $\pm$ 6; Group III: 116 $\pm$ 4; Group IV: 109 $\pm$ 5; Group V: 113 $\pm$ 6 mm Hg, respectively. Average blood pressures of pyelonephritic rats were thus not significantly higher than controls and hypertension was not observed in any individual animal, including those which died prior to completion of the study. Single determinations as high as 140 mm Hg§ were noted rarely in animals from all groups but these were not sustained.

Discussion. Pathologic changes in kidneys of rats in Groups I and II closely resembled those seen in chronic pyelonephritis in man (3). The technic therefore provides an experimental model for examining problems related to the human disease.

The data suggest that recurrent, self-limited renal infections lead to permanent renal damage and inflammation which may persist even after infection has terminated. Although polymorphonuclear infiltration of kidneys and pyuria tended to diminish upon disappearance of active infection, an inflammatory reaction remained, characterized by infiltration of plasma cells and lymphocytes and accompanied by proliferation of connective tissue. Studies are presently underway to determine whether these sterile inflammatory lesions gradually subside or progress in the absence of reinfection and lead to renal failure.

Bacteremia due to *Paracolobactrum intermedium* noted in 3 control animals failed to produce pyelonephritis or infected urine. In 3 pyelonephritic rats in Groups I and II, however, *P. intermedium* was associated with pyuria and infected urine. These data suggest that the diseased kidney is rendered more vulnerable to infection by secondary invaders.

In contrast to the impression gained by investigators from clinical-pathologic correlations in the human(3), there was no evidence in these animals that hypertensive vascular disease develops as a direct sequel to the inflammatory process. Although extensive pathological damage was produced in these rats, areas of normal kidney were present and sufficient renal function remained to prevent azotemia. Observations over longer periods should help to determine whether more severe impairment of renal function must develop or some other factor affecting arterioles must be imposed before vascular disease appears.

Summary and conclusions. 1) Chronic bilateral pyelonephritis without manifest hydronephrosis was produced experimentally in the albino rat by a simple non-surgical technique. This experimental disease provides a model for study of human pyelonephritis. 2)

§ The average systolic blood pressure of normal Sprague-Dawley rats as determined with the tail plethysmographic method in this laboratory is 110 mm Hg with a standard deviation of $\pm$ 10. A rise of 5 to 10 mm Hg occurs with age. Rats in whom experimental hypertension is produced by renal ischemia reveal blood pressures of 150 mm Hg and above, with this method.

Renal inflammation tended to persist after infection had disappeared. 3) Vascular disease and hypertension did not develop; however, the lesions were of insufficient severity to produce azotemia.

We are indebted to Dr. Leo Weiss, Chief of the Pathology Service, Dallas Veterans Administration Hospital, for his assistance in interpretation of microscopic findings.

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Purification of Hyaluronidase from *Clostridium perfringens.** (22158)

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Although several investigators(1-4) have prepared highly purified hyaluronidase from bovine testicular tissue, bacterial hyaluronidases have not usually been purified to a comparable degree. Most authors(5) have used either simple filtrates of the bacteria or crude $(NH_4)_2SO_4$ precipitates. The notable exception was the discovery by Rogers(6) that crude precipitates from ammonium sulphate treatment of filtrates of *Clostridium perfringens*, Type A, Strain 107, could be absorbed on aluminum oxide at pH 4.6 and eluted at pH 7.0 to give a 16-fold increase in potency of hyaluronidase preparations. This method is difficult to reproduce and Rogers was not always able to get a sharp separation with his preparations. For this reason we initiated an alternate method for purification of the bacterial hyaluronidases. This report deals with the preparation of highly purified hyaluronidase from *Cl. perfringens, culture 2278.*

Methods. The hyaluronidase unit was based on the unit of testicular hyaluronidase previously reported(7). Due to instability of the bacterial filtrates at pH 5.5, the enzyme was incubated with hyaluronic acid at pH 7.0. Fig. 1 shows that turbidimetric curves coincided in the range of 2 to 5 units. Fractions for assay were diluted with a buffered solution of 0.1% bovine serum albumin(8) until they fell within these limits. Nitrogen content of various fractions was determined by micro-Kjeldahl digestion with selenium-sulfuric acid followed by nesslerization. Three strains of *Clostridium perfringens* were studied. A lyophilized stock strain, #47-E-2, Walter Reed Army Institute of Research collection, represented the so-called laboratory strains. It had been held in dried state since 1944, but there were no records of its source. A second culture, #4209, isolated in 1951,

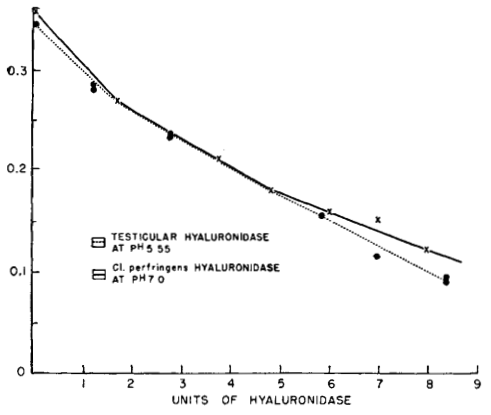

FIG. 1. Comparison of relationship of optical density to *Cl. perfringens* hyaluronidase and testicular hyaluronidase.

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