Pyelonephritis in the Mouse. I. Infection Experiments. (31481)

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Most of the recent experimental work in pyelonephritis has been concerned with the development of an experimental model in the rat beginning with the report of Braude *et al* (1). Mice and rabbits have been used to a limited extent, kidney infections in these species being produced by means of intravenous inoculation with large doses of microorganisms. In early reports mice were infected with Gram-positives [refs. in (2)]. Gorrill(2) using a Pseudomonas, departed from this trend and, more recently, with coworkers also has employed Escherichia and Proteus cultures(3). Phillips(4) while studying Proteus strains of animal origin in mice observed kidney lesions after intravenous inoculation and recovered the infecting organism.

We wished to produce kidney infections in mice with the Gram-negative organisms most commonly implicated in human disease and having established a procedure, to study urea N levels, the effect of therapy and vaccination and to observe the development of antibody. Some preliminary experiments were performed in rats using the method of Andersen and Jackson(5). Concurrent experiments in mice, however, were found more satisfactory, this species being easier to handle in quantity and yielding more information in a shorter time. This communication concerns results obtained during the past few years on the pathogenicity and pattern of infection of several organisms for the first 30 days after intravenous challenge; with the duration of infection in Proteus- and Pseudomonas-challenged mice sacrificed up to a year after inoculation and with the urea N results obtained.

Methods. CF_1 female mice 18 to 20 g were infected by the intravenous route with 0.25 ml of 20-hour cultures of various Gram-negative species grown in brain heart broth and sacrificed at intervals usually in groups of 10. Kidneys were observed for gross cortical damage, crushed with forceps and each pair dropped into one tube of brain heart broth.

TABLE I. Mortality in Mice After Intravenou Infection.*

Organism	Culture dilution	% Dead	Days' interva to deat
Aerobacter aerogenes	Undil.	5	1–2
	10^{-1}	0	—
Escherichia coli	Undil.	0	—
	10^{-1}	0	—
Klebsiella pneumoniae	10^{-2}	100	1
	10^{-3}	79	1–4
	10^{-4}	71	3–6
	10^{-5}	25	3–7
	10^{-6}	0	—
	10^{-7}	0	—
Proteus mirabilis	Undil.	100	1
	10^{-1}	10–15	1–10
	10^{-2}	0	—
Proteus morgani	10^{-1}	100	1–2
	2×10^{-2}	50	1–2
	10^{-2}	12	2–4
	10^{-3}	0	—
	10^{-4}	0	—
Pseudomonas aeruginosa	Undil.	100	1
	2×10^{-1}	30	1–3
	10^{-1}	10–15	1–11
	10^{-2}	0	—
	10^{-3}	0	—

* 0.25 cc-20 hr cultures, 8 to 50 mice/dilution.

Identification of positive cultures was mad using brain heart agar, Endo's, urea broth and other media where indicated.

The organisms used were stock laborator strains of *Aerobactor aerogenes, Escherichi coli, Klebsiella pneumoniae, Proteus mirabilis Proteus morgani* and *Pseudomonas aeruginosa* Pathogenicity titrations starting with un diluted cultures were carried out to arriv at a dose that would be optimal for yieldin positive kidney cultures without being to lethal. Urea N determinations were per formed by the Urograph* method on oxalate blood or serum obtained by cardiac puncture

Results. Titrations of the 6 cultures re vealed great differences in pathogenicit (Table I). Undiluted *A. aerogenes* and *E coli* gave very low mortality. Undiluted in oculum of the other 4 organisms was 100%

* Warner-Chilcott, Morris Plains, N. J.

TABLE II. Relation of Kidney Damage and Positive Cultures to Day of Sacrifice During First 30 Days After Intravenous Infection.*

Organism	Challenge dose/mouse	Day of sacrifice	% Gross damage	% Positive kidney cultures
Aerobacter aerogenes	60×10^7	4	0	83
		8	0	46
		14	0	10
		21	0	0
	60×10^6	13	0	0
		21	0	10
Escherichia coli	13×10^8	5	0	100
		9	0	43
		18	0	0
	13×10^7	22	0	0
Klebsiella pneumoniae	15×10^7	9	0	0
		15	0	0
		20	0	70†
		30	0	80†
Proteus mirabilis	$(23 \text{ to } 52) \times 10^6$	5	50	100
		7	25	80
		14	36	64
		17	38	76
		27	33	45
		30	30	40
	6×10^6	8	20	20
Proteus morgani	$(2 \text{ to } 4) \times 10^6$	8	0	66
		13	25	75
		19	25	50
		21	50	33
	1×10^6	14	0	41
		18	0	20
Pseudomonas aeruginosa	60×10^6	4	0	50
		12	100	75
		7	20	36
	$(16 \text{ to } 40) \times 10^6$	14	35	48
		17	35	51
		25	32	50
		28	46	33

* 8 to 50 mice/group.

† Only atypical colonies of Klebsiella recovered.

fatal. The relation of kidney damage to the organism used for infection and the time of appearance and disappearance of positive kidney cultures during the first 30 days are depicted in Table II. No gross damage was noted in the kidneys of mice infected with *A. aerogenes, E. coli* or *K. pneumoniae* during a period of 3 weeks after inoculation and the animals appeared to rid themselves of these infections in a short time. The Proteus and Pseudomonas infections, on the other hand, resulted in a high proportion of damaged kidneys and positive cultures that persisted. Accordingly, *P. mirabilis* and *Ps. aeruginosa* were selected for further experiments. Several hundred mice were infected with 10^{-1} dilution of these 2 cultures to permit long-term observation. Mortality usually was between 10 and 15% with both infections, deaths seldom occurring after 10 days. In large groups of mice studied during the first 6 weeks after infection, there was about 20% kidney damage at 7 days, this figure increasing during succeeding weeks. Damage was evenly divided between right and left kidneys. Visible lesions in both kidneys were observed in about 6% of the mice, usually within the first 14 days after infection. Per cent of damage was higher in Pseudomonas infected mice whereas the per cent of positive kidney cultures was greater in those infected with Proteus. (The reverse was true in Gorrill and De Navasquez' experiments(3). The explanation may lie in the fact that we were using different strains of organisms and a lighter infecting dose.) For example, it can

TABLE III. Relation of Positive Kidney Cultures to Presence of Visible Damage. *Proteus mirabilis*-Infected Mice.

	Damage positive Culture positive		Damage positive Culture negative		Damage negative Culture positive		Damage negative Culture negative	
Time*	No.	%	No.	%	No.	%	No.	%
7	13/72	18.0	0/72	0	41/72	56.9	18/72	25.0
14	52/172	30.2	0/172	0	53/172	30.8	67/172	38.9
28	28/116	24.1	7/116	6.0	11/116	9.4	70/116	60.3
42	8/27	29.6	1/27	3.7	2/27	7.4	16/27	59.3
Totals	101/387	25.5	8/387	2.4	107/387	26.1	171/387	45.8

* Time: Day of sacrifice after intravenous infection.

TABLE IV. Relation of Positive Kidney Cultures to Presence of Visible Damage. *Pseudomonas aeruginosa*-Infected Mice.

	Damage positive Culture positive		Damage positive Culture negative		Damage negative Culture positive		Damage negative Culture negative	
Time*	No.	%	No.	%	No.	%	No.	%
7	16/76	21.0	1/76	1.3	11/76	14.4	48/76	63.2
14	43/180	23.9	16/180	9.9	44/180	24.4	77/180	42.7
28	41/113	36.2	20/113	17.7	6/113	5.3	46/113	40.7
42	10/49	20.4	10/49	20.4	4/49	8.2	25/49	51.0
Totals	110/418	25.4	47/418	12.3	65/418	13.0	196/418	49.4

* Time: Day of sacrifice after intravenous infection.

be calculated from the data in Table III and IV, where the results for the 6 weeks are averaged, that 27.9% of the *P. mirabilis* infected mice showed gross kidney damage but 37.7% of the *Ps. aeruginosa* infected mice showed this damage. The figures for positive cultures are 46.6% and 38.4% respectively. In groups of 10 to 25 mice sacrificed over a period of 12 months, the per cent of positive cultures obtained gradually declined. By the 200th day Pseudomonas mouse kidneys were sterile while 5% of Proteus mice yielded positive cultures. The amount of gross abnormality observed was more erratic and persisted at a fairly high level in the absence of positive cultures indicating that some of the animals were capable of clearing their infections (Table V). No stones were ever found, confirming over a longer period, Gorrill's observation in mice followed for 8 weeks(6). Macroscopic damage included pallor, scarring and almost complete obliteration of a kidney, with compensatory hyperplasia of the opposite kidney (Fig. 1). In view of functional hyperplasia these mice looked well and could survive; those mice in whom both kidneys were damaged apparently died within 2 weeks.

Urea N test results for normal and infected mice are shown in Table VI. A value of 17 to 20 mg% was obtained in normal mouse serum or plasma and in most infected animals during the first 3 weeks. Values gradually shifted to higher levels in mice tested at later periods, but no correlation was found between the urea N level and the presence of damage or positive cultures.

TABLE V. Kidney Damage and Positive Cultures in Mice Sacrificed Later Than 30 Days After Intravenous Infection:* *Proteus mirabilis* and *Pseudomonas aeruginosa*.

	Proteus mirabilis†		*Pseudomonas aeruginosa*‡	
Day after infection	% Gross damage	% Positive cultures	% Gross damage	% Positive cultures
42	37	41	41	27
46	20	30	45	30
60	20	30	28	12
75	—	—	30	10
92	30	10	60	10
120	39	12	52	13
186	—	—	12	0
203	22	5	—	—
228	40	5	20	0
260	20	0	50	0
300	20	0	20	0
365	44	0	32	0

* 10 to 25 mice/group.
† Challenge (34 to 65) $\times 10^6$ organisms/mouse.
‡ Challenge (16 to 31) $\times 10^6$ organisms/mouse.

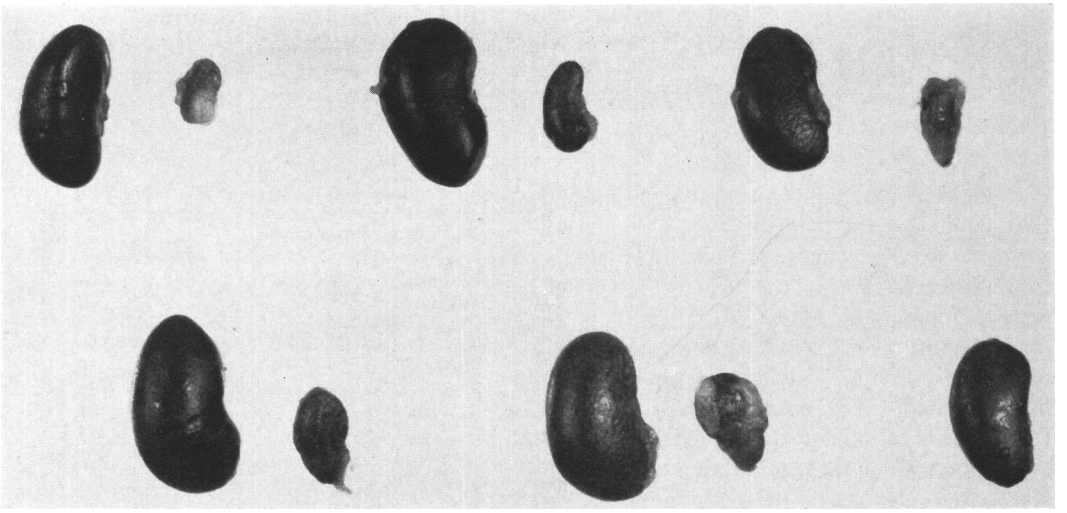

FIG. 1. Pairs of scarred and hypertrophic kidneys removed 6 months after intravenous infection with *Pseudomonas aeruginosa*. Normal kidney, lower right.

TABLE VI. Urea N in *P. mirabilis*- and *Ps. aeruginosa*-Infected Mice.

Day post infection	*P. mirabilis* infection				*Ps. aeruginosa* infection				No. of bloods tested
	No. of mice giving urea N mg % values of								
	17-20	21-30	31-35	>35	17-20	21-30	31-35	>35	
7	4	1	0	0	3	2	0	0	5
14	10	0	0	0	9	1	0	0	10
21	6	6	0	0	7	5	0	0	12
28	6	10	1	1	10	8	0	0	18
46	4	5	1	0	2	8	0	0	10
58	0	10	0	0	1	7	1	1	10
90-100	3	5	1	1	4	6	0	0	10
255	9	6	0	1	—	—	—	—	16
298	—	—	—	—	7	3	0	0	10
365	4	12	8	1	2	18	4	1	25
Normal	12	0	0	0	—	—	—	—	12

Summary. Mice were inoculated intravenously with one of 6 Gram-negative bacilli. Kidneys were removed at intervals for gross examination and culture. *A aerogenes* and *E. coli* were the least lethal and gave no visible damage during 21 days of observation. *K. pneumoniae* was the most virulent but did not produce gross lesions. *P. morgani* was more lethal than *P. mirabilis,* but the latter resulted in a higher percentage of gross damage. *Ps. aeruginosa* resembled *P. mirabilis* in virulence but resulted in more kidney damage, and slightly lower per cent of positive cultures. In *P. mirabilis* and *Ps. aeruginosa* mice examined over a period of a year there was no mortality after the first 2 weeks. Gross damage persisted while cultures became sterile at about 200 days for *Ps. aeruginosa* and 260 days for *P. mirabilis*. Urea N levels rose gradually from a normal value of 17-20 mg% as infection progressed, but there was no correlation with damage or positive cultures.

The competent assistance of Arthur Girard is gratefully acknowledged.

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Received April 4, 1966. P.S.E.B.M., 1966, v123.