Pyelonephritis in the Mouse. II. Vaccination Studies* (32746)

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In studies unrelated to the experiments to be described here, it was found that mice developed very good immunity against several gram-negative infections (*Aerobacter aerogenes, Escherichia coli, Klebsiella pneumoniae, Salmonella schottmuelleri* and others) following a single dose of vaccine. We therefore explored the possibility of vaccination as a means of protecting mice against the development of kidney infection. One laboratory strain of *Proteus mirabilis* and 2 of *Pseudomonas aeruginosa* were selected for most of these experiments since these species are frequently found in human pyelonephritis. Other *Proteus* species and *Pseudomonas* strains were used in cross-vaccination tests. In addition, we studied the effect of endotoxin on pyelonephritis and the distribution of organisms in vaccinated mice after challenge.

Methods. CF_1 female mice 18–20 gm were used throughout. In the vaccination experiments they were immunized by the intraperitoneal (ip), intravenous (iv) or subcutaneous (sc) route with one dose of 0.25 ml of *Proteus* or *Pseudomonas* vaccine. One week later, they were infected by the iv route along with controls, with 0.25 ml of a 20-hour culture, 10^{-1} dilution for *P. mirabilis* and one *Pseudomonas* strain, 2×10^{-2} for the other. Following challenge, groups of vaccinated and control animals were sacrificed at intervals of from 1 to 6 weeks. The kidneys were observed for gross cortical damage and cultured as previously reported (1).

Vaccines were prepared by washing the 20-hour growth from slants with 0.85% NaCl containing 0.2% formalin. Suspensions were adjusted to about 300 in the Klett-Summerson colorimeter using a green filter. Vaccines of this density contained $(4–6) \times 10^9$ organisms per ml and will be referred to as undiluted vaccine. They were usually sterile after 48–72 hours at room temperature.

Difco *Escherichia coli* 026:B6 lipopolysaccharide (LPS) was the endotoxin used. It was administered by various routes before or after infection.

For studying the distribution of organisms, mice vaccinated 1 week prior to challenge and unvaccinated, infected animals were sacrificed at intervals of: 4 hours, 1 day, 3, 6, 8, 10, and 17 days after iv inoculation. Until it became evident that bloods were sterile sooner than other tissues, mice were bled out before the liver, spleen, and kidneys were removed for culture. Subsequently, they were bled out only at the 4-hour and 1-day sampling times. In qualitative tests with 8 or more mice, 0.2 ml of blood, ¼ liver, whole spleen, and both kidneys each were placed in brain heart infusion broth tubes, and presence or absence of growth was recorded after 48 hours' incubation. Quantitative results with groups of 4 mice were obtained using serial 10-fold dilutions of weighed organs in brain heart infusion agar pour plates starting with the 10^{-1} dilution.

Results. Vaccination experiments. Initially, vaccines were administered by the ip route. Undiluted vaccine 1 week prior to challenge was highly effective in protecting mice against visible damage to and infection of the kidneys with both organisms. Moreover, survival was complete in contrast to controls. Because of variation in culture virulence and condition of the mice, control mortality occasionally rose to 50% with the *P. mirabilis* and *Pseudomonas* cultures used in most of these experiments. The beneficial effect of vaccination on the kidneys was evident at 1 week after challenge and at 2, 4, and 6 weeks after, the longest interval tested (Table I). Vaccinations by the iv and sc routes were then done and found to give results similar to ip immunization. Once it had been established that the sc route of vaccination was sucessful, it was used in all subsequent experiments. (It is interesting to note that pooled serum from

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TABLE I. Effect of Vaccination on Development of Pyelonephritis.[a]

Time[b] (weeks)	Mouse group	*Proteus mirabilis*[c] Kidney damage (%)	*Proteus mirabilis*[c] Positive kidney cultures (%)	*Pseudomonas aeruginosa*[d] Kidney damage (%)	*Pseudomonas aeruginosa*[d] Positive kidney cultures (%)
1	Vaccinated	5	35	0	10
	Controls	35	90	20	40
2	Vaccinated	5	5	10	25
	Controls	35	60	25	45
4	Vaccinated	0	0	20	5
	Controls	40	50	60	30
6	Vaccinated	10	10	30	5
	Controls	30	35	45	25

[a] Two hundred mice vaccinated ip 1 week before challenge iv; 20 sacrificed at each interval.
[b] Interval between challenge and sacrifice.
[c] Challenge dose: 59×10^6 organisms/mouse.
[d] Challenge dose: 25×10^6 organisms/mouse.

TABLE II. Immunizing Ability of Various Quantities of Vaccines.[a]

Vaccine dilution	Mouse group	*Proteus mirabilis*[b] Gross damage (%)	*Proteus mirabilis*[b] Positive kidney cultures (%)	*Pseudomonas aeruginosa*[c] Gross damage (%)	*Pseudomonas aeruginosa*[c] Positive kidney cultures (%)	No. of mice/group
1:4	Vaccinated	5	15	2.5	7.5	40
	Control	25	50	37.5	52.5	
1:8	Vaccinated	5	15	0	5	20
	Control	35	55	30	45	
1:16	Vaccinated	15	20	10	20	20
	Control	37.5	53.0	52	55	18
1:32	Vaccinated	10	20	10	10	20
	Control	37.5	53	52	55	18

[a] Mice vaccinated sc 1 week before challenge iv; sacrificed 2 weeks after challenge.
[b] Challenge dose: $(46–70) \times 10^6$ organisms/mouse.
[c] Challenge dose: $(20 \times 34) \times 10^6$ organisms/mouse.

vaccinated mice at a dose of 0.025 ml protected 97% of normal mice challenged by the ip route with *P. mirabilis* or *Ps. aeruginosa*.)

To determine whether fewer killed organisms than those contained in the undiluted vaccines would immunize, vaccines were administered subcutaneously at dilutions of from 1:2 to 1:32. From the results presented in Table II, it is apparent that we had been using an excess of antigen. At 2 weeks after challenge, with both organisms, at all dilutions, gross kidney damage and positive cultures were less than in controls. There was no mortality in vaccinated mice while control deaths averaged 27% for *P. mirabilis* and 31% for *Ps. aeruginosa*.

Since the foregoing experiments were performed with only one species of *Proteus* and 2 strains of *Pseudomonas*, it was important to determine whether other strains and species of these organisms would make good immunizing agents and whether there was cross-protection. Vaccines were made from 3 additional *Pseudomonas* strains (of human origin), *P. morganii* and *P. vulgaris*. All immunized well against homologous challenge. In

TABLE III. Cross-Protection with Vaccines of 3 *Proteus* Species.[a]

Vaccine	Challenge[b]								
	P. mirabilis			*P. morganii*			*P. vulgaris*		
	Dead	Gross damage	Positive cultures	Dead	Gross damage	Positive cultures	Dead	Gross damage	Positive cultures
P. mirabilis	0	0	0	0	0	0	0	0	0
P. morganii	0	10	50	5	0	10	0	0	0
P. vulgaris	0	0	0	10	0	0	0	0	0
None, controls	6.6	20	40	83	60	60	8.3	10	30

[a] Mice vaccinated sc with 0.25 ml of vaccine (diluted 1:4) 7 days before challenge iv; sacrificed 2 weeks after challenge; 10–30 mice/group.

[b] Values are percentages.

TABLE IV. Cross-Protection with Vaccines of Four *Pseudomonas* Strains.[a]

Vaccine strain no.	Challenge strain no.	Mortality (%)		Kidney damage (%)		Positive cultures (%)	
		Controls	Vaccinated	Controls	Vaccinated	Controls	Vaccinated
547	547	0	0	40.0	10.0	40.0	10.0
	2616	6.6	0	50.0	30.0	85.0	60.0
	2836	70.0	10.0	70.0	26.0	80.0	38.0
	2837	0	0	20.0	5.0	25.0	15.0
2616	547	10.0	0	40.0	10.0	50.0	10.0
	2616	10.0	0	50.0	0.0	60.0	0.0
	2836	43.3	20.0	45.0	35.0	55.0	40.0
	2837	0	0	20.0	25.0	20.0	35.0
2836	547	6.6	0	35.0	5.0	45.0	10.0
	2616	13.0	10.0	45.0	33.0	70.0	50.0
	2836	44.0	0	33.0	10.0	77.0	10.0
	2837	0	0	15.0	15.0	15.0	30.0
2837	547	22.6	0	21.0	11.0	46.6	21.6
	2616	13.0	0	50.0	10.0	85.0	40.0
	2836	20.0	5.0	45.0	10.0	70.0	20.0
	2837	0	0	60.0	10.0	70.0	20.0

[a] Mice vaccinated sc with 0.25 ml of vaccine (diluted 1:4 or 1:8) 7 days before challenge iv; sacrificed 2 weeks after challenge; 8–28 mice/group.

cross-challenge experiments, with a 0.25 ml dose of a 1:4 or 1:8 dilution of vaccine by the sc route, there was considerable protection as shown in Tables III and IV. *P. morganii* vaccine did not protect against *P. mirabilis* challenge, but both *P. mirabilis* and *P. vulgaris* vaccines immunized completely against heterologous challenge as evidenced by sterile kidney cultures.

The *Pseudomonas* strains varied considerably in virulence, strain no. 2836 causing 20–70% mortality in controls and some deaths in mice that had received heterologous vaccine. All 4 vaccines gave some cross-protection shown by lower mortality compared with unvaccinated controls except that mice vaccinated with no. 2836 vaccine and challenged with strain no. 2616 had approximately the same mortality as unvaccinated controls. The most striking benefit on kidney damage and infectiousness resulted from vaccination with no. 2837. The 4 groups of mice receiving this vaccine and challenged with the homologous and 3 heterologous strains all had lower per-

TABLE V. Endotoxin Tolerance in *P. mirabilis* Pyelonephritis.[a]

LPS (μg) ip	Interval (days)[b]	Survivors (%)	
		Uninfected controls	*P. mirabilis* infected
500	+1	70	55
1000		10	0
500	+4	60	90
1000		3.0	35
500	+14	30	70
1000		5.0	25
500	+21	50	95
1000		25	40

[a] Difco *E. coli* LPS .026:B6; 20–30 mice/group.
[b] Day after iv infection when LPS was given.

centages of kidney damage and positive cultures than their respective unvaccinated controls.

It was not anticipated that vaccination *after* infection would be successful in reducing infectiousness of mouse kidneys and no striking results of late vaccination were noted. At intervals of from 1 to 10 days following infection, mice were given *P. mirabilis* or *Ps. aeruginosa* vaccine by the sc route and sacrificed 7 days after vaccination. In general, vaccinated mice resembled controls in percentage mortality and positive kidney cultures.

Effect of endotoxin. Pretreatment with *E. coli* LPS by various routes followed by infection iv with *P. mirabilis* at intervals of from 2 to 21 days did not exert any host-stimulating activity nor was there obvious harmful effect on the amount of gross damage or kidney infection in treated mice. The results of treatment with LPS on days 1, 4, 14, and 21 *after* iv challenge with *P. mirabilis* are depicted in Table V where it may be noted that infected mice developed tolerance, evident on the fourth day after infection. The survival rate for normal mice given 500 μg of LPS was 60%; for mice with pyelonephritis, 90% after day 4.

Distribution of organisms. We found that mice cleared organisms from their tissues at different rates following iv inoculation with these cultures of *P. mirabilis* and *Ps. aeruginosa*. In both infections bloods became sterile first as noted by Gorrill after iv *Pseudomonas* inoculation (2). Spleens and livers of *Pseudomonas*-inoculated mice yielded negative cultures about 10 days earlier than those from mice infected with *P. mirabilis*. When vaccinated mice were challenged and their tissues compared to those of infected, unvaccinated controls removed at the same intervals after inoculation, it was observed that they also yielded positive cultures but in smaller numbers (Table VI). In the *P. mirabilis* vaccinated and challenged mice, cultures were positive for a longer time than in those vaccinated and challenged with *Ps. aeruginosa,* the same pattern that obtained after infection of normal mice with these cultures. At the 4-hour sampling period, vaccinated and unvaccinated groups were indistinguishable. One and 3 days after infection, vaccinated mice in the *Pseudomonas* groups had fewer positive cultures than controls, and on subsequent days, tissues of vaccinated mice were almost uniformly sterile. In mice vaccinated with *P. mirabilis,* some difference from controls was seen on the sixth day. By the eighth day there was a real difference. Theoretically, the presence of one organism could have been detected in the organs by this method of culture. In the more quantitative tests,—serial dilutions of weighed organ homogenates, 10^{-1} or higher—less than 10 organisms would have been missed. By the first day after infection, mice that had received *Pseudomonas* vaccine had fewer positive tissues than controls (Table VII). In *P. mirabilis* vaccinated mice, no great difference in number of positive cultures was seen until day 6 post infection. Of those tissues that were positive, average counts were similar for both groups of mice in both infections.

Discussion. Vaccination of mice with whole organisms for the purpose of protecting them against the development of pyelonephritis has not heretofore been done and results are more encouraging than those reported by other investigators in other species: Weyrauch and co-workers in rabbits with heat-killed *E. coli* (3); Arana and associates (4) in rats with the same antigen; Sanford *et al.* with boiled *P. mirabilis* and *E. coli* vaccines in rats and rabbits (5,6); Niijima and Hinman with

TABLE VI. Distribution of Organisms in Vaccinated and Control Mice; Qualitative Results.[a]

		P. mirabilis[b]				*Ps. aeruginosa*[c]			
	Day[d]	Blood	Liver	Spleen	Kidney	Blood	Liver	Spleen	Kidney
C[e]	4 hours	8/8[f]	8/8	8/8	8/8	8/8	8/8	8/8	8/8
V		8/8	8/8	8/8	8/8	8/8	8/8	8/8	8/8
C	1	8/8	8/8	8/8	8/8	7/8	7/8	5/8	6/8
V		4/8	8/8	8/8	8/8	0/8	7/8	5/8	0/8
C	3	3/8	8/8	8/8	7/8	3/8	6/8	8/8	7/8
V		3/8	8/8	8/8	8/8	0/8	1/8	4/8	3/8
C	6	1/8	8/8	8/8	7/8	0/8	4/8	0/8	7/8
V		0/8	8/8	6/8	4/8	0/8	0/8	0/8	0/8
C	8	0/10	9/10	10/10	7/10	1/8	4/8	4/8	6/8
V		0/10	5/10	3/10	2/10	0/8	0/8	0/8	0/8
C	10	0/15	12/15	10/15	6/15	1/15	1/15	3/15	6/15
V		1/15	10/15	5/15	3/15	0/15	1/15	0/15	2/15
C	17	1/15	10/15	8/15	10/15	0/10	1/10	0/10	2/10
V		0/15	2/15	0/15	3/15	0/10	0/10	0/10	0/10

[a] Mice infected iv 7 days after vaccination by the sc route with 0.25 ml 1:4 dilution of vaccine.

[b] Challenge (18–27) $\times 10^6$ organisms/mouse.

[c] Challenge (20–40) $\times 10^5$ organisms/mouse.

[d] Day of sacrifice after infection.

[e] C = control; V = vaccinated mice.

[f] Number of positive cultures out of total number of mice cultured.

Pseudomonas (7). One dose of *P. mirabilis* or *Ps. aeruginosa* vaccine 1–6 weeks before iv infection with homologous cultures resulted in complete survival, unless challenge was lethal to more than 50% of controls, and in 65–100% negative kidney cultures. With suitable cultures such as those used in these studies, iv inoculation in mice, if not lethal, results in a generalized infection at first followed by clearing of organisms from the blood and later from spleen and liver. Without chemotherapy or prior vaccination however, organisms persist in the kidneys inducing the changes characteristic of pyelonephritis and positive cultures are obtained for many months (1). Although our aim was to see if vaccination prior to iv challenge would prevent development of infection in the kidney, the question might be asked: Did vaccination protect the whole mouse, or particularly the kidneys? Our results could be interpreted as a direct effect on the kidney, since antibody was present before challenge, or as an effect on the generalized infection. Further work is needed to determine this point.

In contrast to the successful results with vaccine, we were unable to demonstrate any beneficial effect on *P. mirabilis* pyelonephritis from treatment with 1 dose of *E. coli* LPS before infection. Braude and Siemienski (8) found that in rats, kidney lesion severity and the number of organisms were reduced when 3 doses of *E. coli* LPS were given before pyelonephritis with this organism was induced, but *P. mirabilis* LPS with homologous challenge was much less effective. We did not test *P. mirabilis* LPS and it is possible that in the mouse this or other endotoxins might have been protective.

Tolerance to endotoxin in *P. mirabilis* pyelonephritis mice was demonstrated at 4, 14, and 21 days after infection. Infected mice had a higher rate of survival than normal controls. In this connection, McCabe reported (9) pyrogen tolerance in rabbits given heterologous endotoxin following *E. coli* infection of the kidneys.

The results obtained in the qualitative

TABLE VII. Distribution of Organisms in Vaccinated and Control Mice; Quantitative Results.[a]

		P. mirabilis[b] (organisms/gm of tissue)				Day after infection	*Ps. aeruginosa*[c] (organisms/gm of tissue)			
		Blood	Liver	Spleen	Kidney		Blood	Liver	Spleen	Kidney
C	Average count	87×10^1	14×10^5	43×10^4	41×10^2	4 hours	6.5×10^1	43×10^3	14×10^3	53×10^2
	Mice positive	4	4	4	4		1	4	4	4
V	Average count	3×10^1	13×10^5	91×10^3	15.9×10^2		0	13×10^4	52×10^2	24×10^1
	Mice positive	3	4	4	4		0	4	4	3
C	Average count	2×10^1	24×10^5	27×10^2	14×10^2	1	0	42×10^1	40×10^1	56×10^3
	Mice positive	2	4	3	4		0	4	2	4
V	Average count	29×10^1	37×10^3	23×10^2	57×10^1		0	8×10^1	37×10^1	0
	Mice positive	1	4	4	3		0	2	1	0
C	Average count	2×10^1	12×10^4	7×10^1	5×10^1	3	0	4×10^1	0	57×10^4
	Mice positive	1	4	1	4		0	1	0	3
V	Average count	0	21×10^2	0	0		0	12×10^1	61×10^1	25×10^3
	Mice positive	0	4	0	0		0	1	1	1
C	Average count	0	64×10^1	59×10^1	19×10^3	6	0	13×10^2	0	26×10^4
	Mice positive	0	2	1	3		0	1	0	2
V	Average count	0	0	0	0		0	0	0	14×10^5
	Mice positive	0	0	0	0		0	0	0	1
C	Average count	0	28×10^1	0	51×10^5	8	0	28×10^1	13×10^1	36×10^7
	Mice positive	0	1	0	1		0	1	1	3
V	Average count	0	0	0	11×10^4		0	0	0	0
	Mice positive	0	0	0	1		0	0	0	0
C	Average count	0	0	0	16×10^6	10	0	11×10^1	27×10^1	43×10^7
	Mice positive	0	0	0	2		0	3	2	3
V	Average count	0	0	0	36×10^6		0	0	0	0
	Mice positive	0	0	0	1		0	0	0	0
C	Average count	0	0	0	25×10^6	17	0	3×10^1	4×10^1	94×10^7
	Mice positive	0	0	0	1		0	1	1	2
V	Average count	0	0	0	0		0	0	0	0
	Mice positive	0	0	0	0		0	0	0	0

[a] Groups of 4 mice sacrificed after infection at intervals shown. C = control; V = vaccinated.

[b] Challenge: (23×10^6) organisms/mouse.

[c] Challenge: (10.3×10^6) organisms/mouse.

method of studying the distribution of organisms, in which whole organs were cultured, agreed in general with those using plate counts of weighed tissue homogenates though it must be kept in mind that a larger quantity of tissue was cultured by the former than by the latter method in which less than 10 organisms could not have been counted. Organisms were cleared with greater rapidity from the tissues of vaccinated than unvaccinated mice. When whole organs were cultured, the difference in clearance rate between vaccinated and control mice was not conspicuous until the third day for *Ps. aeruginosa* and the eighth day for *P. mirabilis*. By the quantitative procedure, the difference in rate of clearance between vaccinated and control mice was observed as early as the first day after iv infection for *Pseudomonas* and the third day for *Proteus*.

Summary. Mice were vaccinated with 1 dose of 0.25 ml of *P. mirabilis* or *Ps. aeruginosa* antigen by various routes 1 week before iv challenge with homologous culture. The incidence of gross damage and positive kidney cultures compared with controls was greatly reduced whichever the route of immunization. Vaccination after pyelonephritis had become established was ineffective. Cross-vaccination studies showed considerable protection among 4 strains of *Pseudomonas* and 3 species of *Proteus*. LPS administered before *P. mirabilis* pyelonephritis was induced did not alter the outcome of the infection; when given after the disease was established, tolerance was noted. Qualitative and quantitative cultures of tissues of normal and vaccinated mice at various intervals following iv infection revealed that *P. mirabilis* and *Ps. aeruginosa* were cleared with greater rapidity from the blood, spleen, liver, and kidneys of vaccinated than of unvaccinated mice.

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Toxic Effects of Intraperitoneal Juglone in Rats* (32747)

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Juglone, 5-hydroxy 1:4 naphthoquinone, originally extracted from the Black Walnut tree, *Juglans nigra,* was found to be depressant when administered to several species of animals (1), and toxic in larger doses. Preliminary investigations of toxicity indicated that juglone caused accumulation of fluid in the abdominal cavity of rats when administered intraperitoneally (ip), and this study was done to determine the possible mechanisms involved.

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Materials and Methods. Adult Sprague-Dawley rats of both sexes and approximately 350–400 gm in weight were used. They were divided into 3 groups of which 1 group served as controls and the remaining 2 as experimentals. Juglone, obtained commercially and repurified prior to use by sublimation, was dissolved in corn oil in concentrations of 2 or 3 mg/ml. Experimental Group A received 2 mg/kg of juglone and Group B, 3 mg/kg. Controls received corresponding amounts of corn oil. All animals were maintained on food and water *ad libitum*. Parameters measured