in the histamine-phenomenon. Studies in progress are emphasizing phosphorylase *b* as a possible site of adrenergic blockade.

Summary. The injection of 3′,5′-AMP elicited an increase in blood glucose concentration of both the CFW and CFI strains of mice and had no effect on the histamine sensitivity of either strain. Administration of 5′-AMP was followed by hyperglycemia and histamine hypersensitivity in the CFW mice; the CFI animal was unresponsive to 5′-AMP in terms of both phenomena.

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Pyelonephritis in the Mouse. III. Therapeutic Experiments (32904)

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As part of our study of pyelonephritis in mice we have investigated the therapeutic effects of agents used to treat the disease in man and have explored the possibilities of serotherapy.

Methods. The strains of *Proteus mirabilis* and *Pseudomonas aeruginosa* were those used previously as were the techniques for infection and kidney culture (1). The following therapeutic agents were tested: streptomycin, neomycin, kanamycin sulfate, nalidixic acid, 3,4-dimethyl-5-sulfanilamidoisoxazole (Gantrisin), 2-sulfanilamidopyrimidine (sulfadiazine), Ampicillin, penicillin G, polymyxin, oxytetracycline (Terramycin), D-4-amino-3-isoxazolidone (Oxamycin), and chloramphenicol (Chloromycetin). Antibiotics were given by the intraperitoneal (i.p.) or subcutaneous (s.c.) route, chemical agents by the i.p. or oral (p.o.) route. Many different schedules were tried. In some experiments chemotherapeutic treatments were begun from 1 to 5 days after i.v. infection and given for 5 or 7 days, in 2 doses on weekdays, one half daily dose on Saturdays and Sundays. In other tests a one dose i.v. treatment was administered 1 hour after challenge. Mice were sacrificed from 1 to 7 days after discontinuance of therapy. The sources of materials used in serotherapy tests were: (i) pooled serum from mice that had survived i.v. challenge with *P. mirabilis* or *Ps. aeruginosa* (MS); (ii) serum from rabbits that had received 3 i.v. doses of formalin killed organisms (RS); and (iii) commercial human gamma globulin (GG). For therapy, immune serum and GG were given by various routes in 1 or more 0.25 ml doses before or soon after i.v. challenge. Kidney cultures were done a week after infection.

Results. Chemotherapy experiments. In 67 chemotherapy tests in which duplicate sets of 10 controls were employed, the percentage of positive kidney cultures was identical 32 times, differed by 10% 25 times, and by 20%

5 times. In 5 instances, the difference was 30% or greater. In the latter case, experiments were considered unsatisfactory. If in treated mice the percentage of positive kidney cultures was 30% less than in controls, the results were considered encouraging enough to warrant further testing.

When therapy was initiated 5 or even as soon as 3 days after i.v. infection with *P. mirabilis* or *Ps. aeruginosa,* continued for 5 days and the kidneys cultured the third day after treatment was discontinued, only streptomycin, neomycin, and kanamycin reduced kidney infectiousness. The other agents tested

TABLE I. Effect of Sulfadiazine Therapy Begun 1, 3, or 5 Days after Infection.

	Mouse group[a]	Dose[b] (mg/day)	Kidney damage (%)	Positive culture (%)	Day therapy began[c]	Day of sacrifice[d]	Infection	No. of mice
1.	Treated	10.0	20	70	+5	5		10
	Controls	—	50	90	—	—		10
2.	Treated	10.0	20	70	+3	3		10
	Controls	—	40	60	—	—	*P. mirabilis*	10
3.	Treated	10.0	29	33	+1	1		20
	"	5.0	10	50	+1	1		10
	Controls	—	37	82	—	—		18
4.	Treated	5.0	30	40	+5	5		10
	Controls	—	30	40	—	—		10
5.	Treated	10.0	35	45	+3	3		20
	Controls	—	35	70	—	—	*Ps. aeruginosa*	20
6.	Treated	10.0	33.3	16.7	+1	1		30
	"	2.5	16.7	36.7	+1	1		30
	Controls	—	74.0	77.8	—	—		27

[a] Groups 1, 2, 4, and 5: 5 days' therapy; Groups 3 and 6: 7 days' therapy.
[b] Divided in 2 doses on weekdays; 0.5 daily dose Saturdays and Sundays.
[c] i.e., day after i.v. infection.
[d] Mice sacrificed 1, 3, or 5 days after cessation of therapy.

TABLE II. Effect of Polymyxin Therapy Begun 1, 3 or 5 Days after Infection with *Ps. aeruginosa.*

	Group[a]	Dose (μg/day)[b]	Kidney damage (%)	Positive culture (%)	Day therapy began[c]	Day of sacrifice	No. of mice
1.	Treated	50	40	50	+5	3	20
	Controls	—	35	45	—	—	20
2.	Treated	100	10	30	+5	3	10
	Controls	—	30	50	—	—	10
3.	Treated	250	35	50	+3	3	20
	Controls	—	50	55	—	—	20
4.	Treated	250	10	10	+1	1	20
	Controls	—	55	55	—	—	20

[a] Groups 1, 2 and 3: 5 days' therapy; Group 4: 7 days' therapy.
[b] Divided in 2 doses weekdays; 0.5 daily dose Saturdays and Sundays.
[c] i.e., day after i.v. infection.
[d] Mice sacrificed 1 or 3 days after cessation of therapy.

(enumerated under "Methods") that might have been expected to be beneficial showed little if any favorable effect indicating that the treatment schedules were too rigorous. When treatment was begun 1 day after infection, continued for 7 days, and the mice

TABLE III. Effect of Streptomycin Treatment on Pyelonephritis.

	Group[a]	Dose/day[b]	Day therapy began[c]	*P. mirabilis* Kidney damage (%)	*P. mirabilis* Positive culture (%)	*P. mirabilis* No. of mice	*Ps. aeruginosa* Kidney damage (%)	*Ps. aeruginosa* Positive culture (%)	*Ps. aeruginosa* No. of mice
1.	Treated	10.0	+5	20.7	20.7	29	14.8	11.1	27
	Controls	—	—	43.5	60.0	30	45.0	35.0	20
2.	Treated	10.0	+3	8.0	13.8	87	17.7	20.0	45
	Controls	—	—	43.3	67.0	88	36.7	57.1	49
3.	Treated	5.0	+3	6.7	16.7	30	25.0	15.0	20
	Controls	—	—	40.0	56.7	30	50.0	40.0	20
4.	Treated	10.0	+1	5.2	12.1	58	0	10.0	20
	Controls	—	—	55.2	72.4	58	47.4	52.6	19
5.	Treated	5.0	+1	7.5	16.9	80	10	15.0	50
	Controls	—	—	44.7	79.4	90	60	65	70
6.	Treated	2.5	+1	22.2	33.3	18	20.6	5.5	28
	Controls	—	—	61.1	72.2	18	47.2	52.2	28

[a] Groups 1–3: 5 days' therapy, mice sacrificed 3 days after last dose; Groups 4–6: 7 days' therapy, mice sacrificed 1 day after last dose.

[b] Divided in 2 doses weekdays; 0.5 daily dose Saturdays and Sundays.

[c] i.e., day after i.v. infection.

TABLE IV. Effect of Neomycin Treatment on Pyelonephritis.

	Mouse group[a]	Dose (mg/day)[b]	Day therapy began[c]	*P. mirabilis* Kidney damage (%)	*P. mirabilis* Positive culture (%)	*P. mirabilis* No. of mice	*Ps. aeruginosa* Kidney damage (%)	*Ps. aeruginosa* Positive culture (%)	*Ps. aeruginosa* No. of mice
1.	Treated	5.0	+5	20.0	10.0	10	10.0	0	10
	Controls	—	—	50.0	90.0	10	50.0	30.0	10
2.	Treated	5.0	+3	3.3	0	30	23.1	11.5	26
	Controls	—	—	35.0	55.0	20	65.0	70.0	20
3.	Treated	2.5	+3	10.0	10.0	20	10.0	0	20
	Controls	—	—	40.0	55.0	20	45.0	40.0	20
4.	Treated	1.25	+3	20.0	15.0	20	40.0	50.0	20
	Controls	—	—	30.0	55.0	20	70.0	70.0	20
5.	Treated	5.0	+1	40.0	5.0	20	0	0	10
	Controls	—	—	55.0	75.0	20	45.0	30.0	20
6.	Treated	2.5	+1	20.0	10.0	10	25.0	20.0	20
	Controls	—	—	40.0	60.0	10	47.3	52.6	19

[a] Group 1: 5 days' therapy, mice sacrificed 5 days after last dose; Groups 2, 3, and 4: 5 days' therapy, mice sacrificed 3 days after last dose; Groups 5 and 6: 7 days' therapy, mice sacrificed 1 day after last dose.

[b] Divided in 2 doses weekdays; 0.5 daily dose Saturdays and Sundays.

[c] i.e., day after i.v. infection.

sacrificed 1 day after cessation of therapy, sulfadiazine (in both infections), and polymyxin (Pseudomonas only) reduced the number of positive kidney cultures. The other agents did not. The increased sensitivity of this treatment schedule with sulfadiazine and polymyxin is illustrated in Tables I and II, where it may be noted that only when treatment was begun 1 day after infection and continued for 7 days was there good reduction in percentage of positive kidney cultures. Streptomycin, neomycin, and kanamycin, on the other hand, were effective agents when treatment was delayed beyond the first day (Tables III, IV, and V). Table III depicts the effects of streptomycin at 3 dose levels on kidney damage and infection in mice treated for 5 to 7 days beginning the fifth, third, or first day after infection. Neomycin (Table IV) reduced kidney damage and the number of positive cultures obtained at each of the 3 dose levels used whether therapy was instituted 1, 3, or 5 days after infection. Kanamycin treatment for 5 days which began 3 days after i.v. infection, also resulted in a reduction of gross damage and positive cultures in mice sacrificed 3 days after cessation of therapy (Table V). Earlier treatment and sacrifice did not alter greatly the results obtained with these 3 antibiotics. In experiments in which a single i.v. treatment was administered 1 hour after infection—the method used by Harrison and Zygmunt (2) in *Staphylococcus* pyelonephritis—streptomycin, polymyxin, and sulfadiazine, as might have been expected, were effective in reducing kidney damage and infectiousness as they had been when treatment was delayed (Table VI). Nalidixic acid, Gantrisin, chloramphenicol, oxytetracycline, Oxamycin, Ampicillin and penicillin G gave no encouraging results.

Serotherapy experiments. Preliminary to the therapeutic use of GG and the antisera, it was established that these substances contained antibody. Agglutinins were present at 1:320 dilution or higher in the sera, at 1:10 in GG. The protective capacity of the mouse antisera was such that over 90% of normal mice that received 0.0025 ml i.p. survived challenge with *P. mirabilis* or *Ps. aeruginosa* by the same route. The GG protected at 1.25 mg for *P. mirabilis*, 0.312 mg for *Ps. aeruginosa*.

Pretreatment, 6 or 24 hours before infection, treatment at the time of infection (0 hours), or a combination of pre- and post-i.v. challenge therapy with the 3 types of antibody exerted a favorable effect on the kidneys. One dose of RS s.c. 24 hours before i.v. infection reduced the percentage of positive kidney cultures in the *P. mirabilis* infection by 80% and in the Pseudomonas infection by 70%. On dose of 1.25 mg of GG by the ip route given at 0 hours reduced kidney infec-

TABLE V. Effect of Kanamycin Treatment on Pyelonephritis.

				P. mirabilis			*Ps. aeruginosa*		
	Group[a]	Dose/day[b]	Day therapy began[c]	Kidney damage (%)	Positive culture (%)	No. of mice	Kidney damage (%)	Positive culture (%)	No. of mice
1.	Treated	10.0	+3	10.0	10.0	20	10.0	40.0	10
	Controls	—	—	40.0	55.0	20	50.0	70.0	10
2.	Treated	5.0	+3	15.0	12.5	40	50.0	40.0	10
	Controls	—	—	37.5	60.0	40	80.0	80.0	10
3.	Treated	2.5	+3	15.0	20.0	20	20.0	15.0	20
	Controls	—	—	40.0	60.0	20	45.0	40.0	20
4.	Treated	5.0	+1	25.0	15.0	20	10.0	10.0	10
	Controls	—	—	45.0	71.0	36	45.0	30.0	20

[a] Groups 1–3: 5 days' therapy, mice sacrificed 3 days after last dose; Group 4: 7 days' therapy, mice sacrificed 1 day after last dose.

[b] Divided in 2 doses weekdays; 0.5 daily dose on Saturdays and Sundays.

[c] i.e., day after i.v. infection.

TABLE VI. Effect of Single iv Treatment on Development of Pyelonephritis.[a]

Treatment	Dose	No. of mice with: Kidney damage	No. of mice with: Positive culture	Infection
Streptomycin	1.0 mg	2/50	8/50	*P. mirabilis*
Controls	—	22/50	41/50	
Streptomycin	0.5 mg	1/20	4/20	
Controls	—	7/20	13/20	
Polymyxin	50 μg	0/10	0/10	*Ps. aeruginosa*
	25 μg	2/20	3/20	
Controls	—	16/30	16/30	
Streptomycin	1.0 mg	0/30	0/30	
	0.5 mg	6/30	5/30	
Controls	—	22/40	20/40	
Sulfadiazine	1.0 mg	1/10	0/10	
Controls	—	5/10	4/10	

[a] Mice treated 1 hour after iv infection; sacrificed 7 days later.

tion in treated mice by 50%. The MS given by the i.v. route at 0 and at 24 hours after infection resulted in a 40% reduction of positive kidney cultures. In most experiments a favorable effect on gross kidney damage was noted. When immunotherapy was delayed beyond 24 hours, treated mice resembled controls.

Discussion. The assessing of chemotherapeutic agents by the method we employed—sterility or growth after 48 hours' incubation of broth containing both mouse kidneys—was rigorous. We felt that from a practical standpoint, this criterion was a necessary aim. If organisms are not eradicated completely, relapse will probably occur. In the delayed treatment experiments, the tremendous doses needed of those agents that were active attest to the fact that in mice as in man, infection of the kidney is very difficult to cure. The reasons for this are not known at present. Much smaller amounts of drug were effective in the simpler experimental model which can be regarded as a means of selecting agents specific for the organism used to produce the infection. Less material and fewer man hours are required than when 2× daily treatment is carried out for several days. On the other hand, therapy instituted so soon after challenge may eradicate the infecting organism and is therefore not aimed at an infected kidney where, in our experience, bacteria do not become established for 3–6 days after the i.v. inoculation. In practice, the one dose method should then be followed up with a delayed treatment test.

If circulating antibody is important in protecting the host against pyelonephritis—and our results with vaccine would suggest that it is (4)—the administration of specific antiserum or of GG should be of benefit. Sanford and associates have reported protecting rats against *P. mirabilis* and other infections by pretreating with specific antisera (3). The i.v. would probably be the most effective route but species differences presented a problem in our tests in that undiluted RS and GG were toxic for mice by this route. Further studies are required to see whether concentration of mouse antiserum and daily i.v. dosing would improve our results.

Summary. Mice infected by the i.v. route with *P. mirabilis* or *Ps. aeruginosa* were treated with several chemotherapeutics commonly used in human pyelonephritis or recommended for gram-negative infections. Streptomycin, neomycin, and kanamycin were the most effective agents, and with sulfadiazine, the only ones active against both

infections. When therapy was delayed as long as 5 days, the 3 antibiotics substantially reduced kidney damage and the number of positive cultures obtained if given in large doses. Sulfadiazine was not beneficial for either infection if treatment was delayed more than one day after inoculation. The same was true for polymyxin (Pseudomonas infection). When one treatment i.v. was given 1 hour after challenge, only those agents found active in the delayed therapy experiments gave good results. The other chemotherapeutics were ineffective by any test method. Gamma globulin as well as specific antisera reduced the incidence of gross kidney damage and infection when administered before or up to 24 hours after i.v. inoculation.

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The Estimated Size of *Pseudomonas aeruginosa* β-Galactosidase (32905)

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Variations in structure of the enzyme β-galactosidase provide an index for natural relationships among bacteria (1). Previous studies comparing *Bacillus megaterium* and *Escherichia coli* β-galactosidases suggest no relatedness (2); this is exemplified by the difference in size of their respective enzymes (mol. wt. 150,000 and 518,000). A similar comparison revealed the β-galactosidases of *E. coli* and *Aeromonas formicans* to be identical in size, but very different in amino acid composition (3). Because the *Escherichia* and *Aeromonas* enzymes alone are antigenically similar, size may be employed as a major distinction.

Members of the genera *Pseudomonas* and *Aeromonas* are taxonomically allied by similarities in morphology, i.e., polar flagellation. The natural relationship of *Aeromonas* species to pseudomonads, or to enteric organisms (e.g., *E. coli*), is unresolved despite the use of Adansonian analysis for overall similarity and methods for testing nucleic acid homologies (4). The isolation of β-galactosidase from *P. aeruginosa* provides new evidence for the reexamination of this controversy.

Materials and Methods. Ps. aeruginosa strain X was provided for this study by R. G. Doggett; it is one of 28 strains capable of hydrolyzing *o*-nitrophenyl-β-D-galactopyranoside (ONPG). The basal culture medium (Vogel and Bonner, medium E) was prepared by adding to 670 ml of distilled water 10 gm of $MgSO_4 \cdot 7H_2O$, 100 gm of citric acid $\cdot H_2O$, 500 gm of K_2HPO_4, and 175 gm of $Na(NH_4)$-$HPO_4 \cdot 4H_2O$; these ingredients diluted 50-fold will support growth of the organism and the addition of 0.2% (w/v) lactose insures enzyme production. Cells for extract preparation were grown in 2-liter Erlenmeyer flasks containing 1 liter of basal medium plus lactose. The flasks were incubated on a rotary shaker for 18 hours at 37°C. The final cell density was 150 units in a Klett-Summerson colorimeter (660-mμ filter). Cells were harvested by centrifugation and washed once with five times their wet weight of basal medium.

The extraction and enrichment procedures for *Ps. aeruginosa* β-galactosidase were performed at temperatures of 4–10°C. A 20% (w/v) suspension of cells in 0.1 *M* sodium phosphate buffer, pH 7.0, containing 10^{-3} *M* β-mercaptoethanol was sonified for 10 min; cellular debris was removed by centrifugation at 30,000*g* for 20 min. The assay of β-galactosidase employing ONPG has been described (3); the Lowry Folin–phenol method was used to determine total protein (5). The specific activity of the cell-free extract was 0.37 units/mg of protein. Fractionation