YALE JOURNAL OF BIOLOGY AND MEDICINE 46, 203–211 (1973)

Pyelonephritis XVI. Correlates ot Parasite Virulence in Acute Ascending *Escherichia coli* Pyelonephritis in Mice Undergoing Diuresis[1,2]

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Received for publication 30 January, 1973

In a preceding publication (1), a long-term study of ascending pyelonephritis in mice with *Escherichia coli* strain Yale, confirmed and extended the findings of Keane and Freedman (2). The availability of this model has permitted us to study factors influencing the virulence of *E. coli* in ascending pyelonephritis. The present paper presents data on 22 strains of *E. coli* relevant to various factors affecting virulence.

MATERIALS AND METHODS

Techniques for the cultivation of bacteria, media used, production of pyelonephritis, and examination of kidneys have been described in the preceding publication (1).

Bacteria. In addition to the Yale strain, the following strains of *E. coli* were used: CL-1B, CL-135, CL-136, CL-143, CL-185, P33, P67, P92, P98, OB51, OB248, and Lowell, furnished by Dr. K. L. Vosti; strains CF1, W1895 and 113, obtained from Dr. A. Braude; strain 0127:B8 obtained from Dr. M. Landy; strain H obtained from Dr. A. Hershey, and strain MP and J5 from Dr. D. Medearis. Strains 214 and 946 were clinical isolates from patients with urinary tract infection.

A standardized procedure was used for cultivation of the bacteria for *in vitro* studies. Frozen cultures of thrice mouse-passed bacteria were thawed at room temperature and one loopful inoculated into 9 ml of broth and incubated overnight. A second transfer was made the following day, 18 hr before use.

[1] Supported in part by United States Public Health Service Grants No. 02257 and 03310.
[2] Excellent technical assistance by J. Kazlowski, M. Kubota, and J. Hardin is gratefully acknowledged.

Serotype. Strain MP was typed by Dr. D. Medearis; strain J5 is a mutant thereof. All of the remainder of the strains were kindly typed by Dr. E. Ewing at the Communicable Disease Center, Atlanta, Georgia. Some proved to be untypable.

K antigen. The presence of K antigen in each strain was determined by the method of Glynn *et al.* (3). K antigen was measured 10 times in all 22 strains to demonstrate its presence at a confidence level of 0.05.

Fermentation reactions. Fermentation of dulcitol lactose, sucrose, glucose, galactose, salicin, raffinose, inulin, maltose, mannitol and glycerin, were determined by the use of Key Tablets (Key Scientific Products Co.) and in phenol red broth base (BBL) to which the various sugars were added.

Motility. Bacteria were cultured successively three times in motility agar (Difco) to maximize motility. Test of motility was done in U tubes with motility agar, and progress of motility noted at intervals.

Growth in urine. The bladder was exposed by a lower abdominal incision and urine was collected aseptically into a syringe directly from bladder of mice undergoing diuresis and non-diuresed tap water controls. The samples were individually checked for sterility and the urines of diuresed mice and control animals pooled. The specific gravity was determined, and aliquots stored at —20°C until used. At time of test, urines were thawed at room temperature, control (non-diuresed) urine was diluted with sterile distilled water to equal the specific gravity of diuresed urine. Urine from diuresed mice (specific gravity 1.002, pH 5.2), from nondiuresed mice (specific gravity 1.070, pH 5.0), and urine from non-diuresed diluted to the specific gravity of that of diuresed mice, 1.002 (pH 5.0), were inoculated with an 18-hr culture of 20 strains of *E. coli* to final concentration of approximately 1.0×10^4/ml. Growth at 37°C was assayed by plate count at intervals of 1, 4, 6 and 24 hr.

Growth in minimal medium. The minimal medium consisted of 0.22 M glucose as sole source of carbon and salts (4). Overnight broth culture was washed with saline and passed twice in minimal medium, once at 37°C and the second time at 30°C. Incubation at 30° rather than 37° was used in this study to slow growth and thus possibly accentuate growth rate differences among the strains of *E. coli.* The 30° minimal medium culture in Wolff-Kaplan Flasks was centrifuged when turbidity at 520 nm reached 0.3–0.4 (Bausch and Lomb Spectronic R Spectrophotometer using 13 mm diameter tubes). The cells were washed three times with saline, resuspended in saline to original volume and minimal medium inoculated at a 1:30 dilution. A plot of turbidity versus time of incubation at 30°C was constructed for each organism and the slope at the maximum rate of growth (which was essentially constant for 3–4 hr) determined.

Phagocytosis. The method of Medearis *et al.* (5) was used except that 0.8 ml of 20% aqueous solution of beef extract (Oxoid) was injected intraperitoneally to induce polymorphonuclear leucocyte response. Animals were sacrificed 18–20 hr later and leucocytes recovered by washing the peritoneum. The washed leucocytes were mixed with log-phase *E. coli* on a glass slide and incubated for 30 min. The suspension was then washed off the slide and centrifuged; smears were made from the sediment, dried overnight, and strained with alkaline methylene blue.

Serum bactericidal activity. A pool of normal mouse serum was obtained by bleeding from the heart directly onto a Petri dish on an ice bed. Serum was separated as soon as clotting occurred and maintained in small aliquots at —70°C.

Study of relation of resistance to serum bactericidal activity to virulence was

first attempted with normal mouse serum. Efforts to demonstrate bactericidal activity with a known serum-susceptible strain, using mouse blood obtained in several ways, did not succeed. Accordingly, study of the 22 strains' serum bactericidal resistance or susceptibility was done with a pool of normal human serum. Human serum was obtained from several normal donors, pooled and frozen in aliquots within three hours; the sera were also stored at —70°C. The serum bactericidal test was done as described in the preceding paper (1). Percent surviving bacteria at 1 hr was plotted against amount of serum on log probit paper, and the dilution killing 50% was extrapolated and used as the endpoint.

RESULTS

Two parameters were used to examine virulence of the *E. coli* strains. (A) The ability of the *E. coli* to produce pyelonephritis as measured by bacterial infection of the kidneys. A rank order of virulence was obtained for renal infection by giving equal weight to renal bacterial population and the proportion of kidneys infected. (B) Virulence was also measured by the lethality of the *E. coli*. Deaths following infection with *E. coli* were frequent and all occurred in the first few days; the majority died within 24 hr. Bacteriologic studies were not carried out on mice that died.

The results (Table 1) show marked differences among the 22 strains. The virulence ranks determined by the presence of renal infection and by the proportion

TABLE 1

RENAL MICROBIAL POPULATION, PROPORTION OF KIDNEYS INFECTED AND DEATHS IN DIURESED MICE INFECTED VIA BLADDER WITH 22 STRAINS OF *Escherichia coli*

	Renal infection		Deaths	
Strain	Log No. *E. coli*/g/ kidney[a]	Proportion infected kidneys	Proportion	Percent
214	7.53 ± 0.12	44/46	5/28	17.9
946	7.59 ± 0.20	24/34	11/28	39.6
P92	6.51 ± 0.16	34/36	10/28	35.7
Yale	6.82 ± 0.11	44/52	2/28	7.1
P98	6.32 ± 0.13	53/54	1/28	3.6
P67	7.07 ± 0.16	38/62	67/88	76.1
CF1	6.48 ± 0.13	36/56	15/48	31.5
113	5.78 ± 0.14	45/52	2/28	7.1
CL-135	6.48 ± 0.28	25/50	3/28	10.7
MP	5.90 ± 0.39	22/34	3/20	15.0
P33	6.21 ± 0.29	21/42	86/108	79.6
Lowell	6.31 ± 0.11	31/66	16/48	33.3
0127:B8	5.00 ± 0.25	37/56	0/28	0.0
J5	5.40 ± 0.44	17/38	1/20	5.0
W1895	4.11 ± 0.29	13/54	1/28	3.6
CL-136	4.07 ± 0.43	10/56	0/28	0.0
OB248	3.30 ± 0.16	17/46	5/28	17.9
OB51	3.65 ± 0.36	7/81	2/48	4.2
CL-185	3.48 ± 0.22	6/52	2/28	7.1
CL-143	2.69 ± 0.54	3/50	6/28	21.5
H	1.95 ± 0.35	3/54	1/28	3.6
CL-1B	1.47 —	1/52	2/28	7.1

[a] Infected kidneys only, mean and standard deviation of the mean.

TABLE 2

SEROTYPE, K ANTIGEN, FERMENTATION OF DULCITOL AND MOTILITY OF 22 STRAINS OF *Escherichia coli* IN RELATION TO VIRULENCE

Strain	Virulence rank[a]		Serotype	K antigen[b]	Fermentation of dulcitol[c]	Motility[d]
	Renal infection	Proportion of deaths				
214	1	8.5	O undet:H6	−	+	96
946	2	3	rough	−	+	not motile
P92	3	4	O:75	+	+	not motile
Yale	4.5	13.5	O undet	−	+	not motile
P98	4.5	19	rough:H5	+	+	47
P67	6	2	O6:H1	+	+	72
CF1	7	6	O undet:H7	−	+	72
113	8	13.5	O113:H10	−	+	144
CL-135	9.5	11	rough:H1	+	+	23
MP	9.5	10	O111:B4	−	(+)	not motile
P33	11	1	OH:H1	+	+	69
Lowell	12.5	5	rough	−	−	not motile
O127:B8	12.5	21.5	O127:B8	−	−	not motile
J5	14	16	O111:B4	−	(+)	not motile
W1895	15	19	rough:H48	−	(+)	47
CL-136	16	21.5	rough:H48	−	−	38
OB248	17	8.5	O12:H27	−	+	144
OB51	18.5	17	O18:H14	−	(+)	90
CL-185	18.5	13.5	rough:H48	−	(+)	not motile
CL-143	20	7	rough:H48	−	−	54
H	21	19	rough	−	(+)	not motile
CL-1B	22	13.5	rough	−	−	not motile

[a] In descending order of virulence.
[b] "+" Means present, "−" means absent.
[c] "+" Means fermentation within 24 hr; "(+)" delayed for 48–72 hr.
[d] Hours to travel 128 mm in a U tube in motility agar.

of deaths are shown in Table 2 together with the serotype, K antigen, fermentation of dulcitol and motility of the 22 strains of *E. coli*. To determine if virulence correlated with the presence of K antigen, carbohydrate fermentation, motility or roughness, the incidence of each of these factors was compared in the 11 most virulent and 11 least virulent strains. The ability of the strains of *E. coli* to grow in urine and minimal media, their resistance to phagocytosis and their resistance to serum bactericidal activity was measured and the relationship to virulence correlated by rank order.

Differences in virulence which could be related to ability to grow in urine were studied in 20 strains of *E. coli*. Growth in urine from diuresed and control (non-diuresed) mice, as well as urine from control mice diluted to the specific gravity of the urine of diuresed mice was compared at 1, 4, 6 and 24 hr (Table 3). Growth of all 20 strains was better in diuresed than non-diuresed urine at 4, 6 and 24 hr. Further, if non-diuresed urine was diluted to the specific gravity of diuresed, growth rate was completely restored to the level of that in the diuresed urine. These data suggest that improved growth in urine from diuresed animals was due to dilution of an inhibitory factor, possibly urea. Correlation of virulence (as measured by renal infection) with ability to grow in diuresed urine was significant at 6

TABLE 3

GROWTH OF 20 STRAINS OF *Escherichia coli* IN URINE FROM DIURESED MICE AND NON-DIURESED MICE, AND URINE FROM NON-DIURESED MICE DILUTED TO SPECIFIC GRAVITY OF URINE FROM DIURESED MICE

	Diuresed urine				Non-diuresed urine				Diluted non-diuresed urine			
	Time (hr)				Time (hr)				Time (hr)			
Strain[a]	1	4	6	24	1	4	6	24	1	4	6	24
214	4.18[b]	6.72	8.72	9.41	4.28	4.18	4.38	8.74	4.20	6.89	8.67	9.46
946	4.30	7.36	8.66	9.08	4.08	3.89	3.81	8.84	4.32	7.47	8.90	9.32
P92	3.77	6.00	8.45	9.00	3.73	3.41	4.30	8.46	3.80	6.48	8.72	9.03
Yale	4.18	4.96	7.08	8.80	4.20	4.15	3.83	8.30	4.03	5.00	6.91	8.78
P98	4.26	5.92	7.89	9.11	4.15	4.23	4.26	8.65	4.23	7.04	8.23	9.11
P67	3.96	5.53	6.79	8.84	3.89	3.00	2.54	6.32	3.92	5.55	6.62	8.91
CF1	4.30	7.04	7.08	8.81	4.20	4.36	4.96	8.00	4.36	7.05	8.27	8.74
113	4.23	7.00	7.91	8.83	4.43	4.41	4.34	7.65	4.38	7.40	8.21	8.79
CL-135	4.15	6.96	8.04	8.36	4.04	3.96	3.54	7.08	4.11	7.10	8.15	8.70
P33	4.11	6.46	7.93	8.78	4.11	4.11	4.00	6.00	4.24	6.93	8.18	8.82
Lowell	3.99	6.32	8.46	8.90	3.88	3.78	3.87	8.39	3.98	5.72	8.21	8.94
0127-B8	4.00	5.08	6.28	8.91	4.00	3.91	3.72	6.74	3.98	5.58	6.98	9.00
W1895	4.45	6.43	7.66	8.69	4.32	3.98	3.93	7.39	4.45	6.37	7.64	8.68
CL-136	4.41	5.45	5.79	8.20	4.41	4.00	3.76	3.69	4.30	5.56	6.29	8.42
OB248	3.95	5.04	6.36	9.00	4.08	4.26	4.08	6.53	3.94	4.95	6.89	9.53
OB51	4.18	5.81	7.73	8.18	3.88	3.76	3.98	7.45	4.17	6.26	8.13	8.11
CL-185	4.23	4.98	5.11	5.00	4.00	3.60	3.26	4.00	4.31	4.49	4.94	5.04
CL-143	4.28	4.54	5.34	7.96	4.18	3.79	3.54	4.15	4.14	5.51	6.02	8.33
H	3.97	6.76	7.89	8.81	3.91	3.70	3.57	5.80	3.93	6.78	7.83	8.79
CL-1B	4.15	6.51	8.34	9.26	4.00	3.52	3.20	7.48	4.19	6.51	8.56	8.81

[a] In descending order of virulence as measured by renal infection.

[b] Log number bacteria/ml urine.

and 24 hr. Interestingly, correlation was also significant for non-diuresed urine at the same time intervals.

Growth of 17 strains of *E. coli* in minimal media correlated with virulence as measured by renal infection and lethality (Table 4).

The relationship between resistance to phagocytosis and virulence, by rank order correlation used both percent leucocytes with bacteria and number of bacteria ingested per leucocyte. Virulence measured by renal infection or lethality correlated with percent leucocytes containing bacteria and with number of bacteria per leucocyte (Table 5). Serum bactericidal activity correlated with virulence as measured by renal infection but not with lethality.

Table 6 shows the correlation of virulence of *E. coli* as measured by bacterial infection in the kidney and by proportion of deaths with the various parameters studied. Renal infection correlated with resistance to phagocytosis, the presence of K antigen, resistance to serum bactericidal activity, dulcitol fermentation and growth in urine or minimal media. Lethality of the bacteria correlated with resistance to phagocytosis and growth in minimal media. Renal infection did not correlate with the proportion of deaths.

DISCUSSION

The model of *E. coli* pyelonephritis used in these studies to examine virulence factors avoided manipulation of the kidney or urinary tract which has usually been

TABLE 4

RELATION OF GROWTH RATE IN MINIMAL MEDIUM TO VIRULENCE OF 17 STRAINS OF *Escherichia coli*[a]

Strain	Virulence Rank[b]		Growth rank
	Renal infection	Proportion of deaths	
214	1	6.5	8
P92	2	2	6
Yale	3.5	10	5
P98	3.5	14.5	4
CF1	5	4	2
CL-135	6	8	11
P33	7	1	3
Lowell	8.5	3	1
0127:B8	8.5	16.5	7
J5	10	12	10
W1895	11	14.5	17
CL-136	12	16.5	15
OB248	13	6.5	12
OB51	14	13	13
CL-185	15	10	14
CL-143	16	5	16
CL-1B	17	10	9

[a] Correlation coefficient: renal infection vs growth, 0.67 $p < .01$; proportion of deaths vs growth, 0.454 $p < .05$.

[b] In descending order of virulence.

necessary to establish *E. coli* pyelonephritis in experimental animals. Previous studies of virulence factors of *E. coli* pyelonephritis have used ureteric obstruction (6) and renal massage (7) to produce renal infection. The ascending route of infection was used in this study because this most closely parallels the majority of clinical urinary tract infections.

Marked differences in the severity of pyelonephritis produced by different strains of *E. coli* injected into the bladder of mice prompted us to examine some of the factors which may account for the differences in nephropathogenicity. Nephropathogenicity as measured by the ability of *E. coli* to produce bacterial infection correlated with the presence of K antigen, resistance to phagocytosis and serum bactericidal activity, ability to ferment dulcitol and ability of the *E. coli* to multiply in urine and minimal media.

Studies by others have correlated capsular (K) antigens with virulence. Vahlne (8) found slightly more strains with K-antigens from urinary tract infections than from feces as judged by inagglutinability. More recently, Glynn *et al.* (3) found that strains of *E. coli* from urine of patients with renal involvement had K-antigens more frequently than those from patients in whom infection was confined to the bladder or in fecal strains. On the other hand, McCabe and Jackson (7), and Brumfitt and Heptinstall (6), were unable to demonstrate a relation between K-antigen and nephropathogenicity. The mechanism by which presence of K-antigen may promote virulence is uncertain but probably is related to interference with body defense mechanisms (9, 10).

Nephropathogenicity also correlated with resistance to phagocytosis and serum bactericidal activity. These host defenses are well established as the cornerstones

TABLE 5

RELATIONSHIP OF VIRULENCE TO RESISTANCE TO PHAGOCYTOSIS AND RESISTANCE TO SERUM BACTERICIDAL ACTIVITY AMONG 22 STRAINS OF *Escherichia coli*

Strain	Virulence rank[a]	Phagocytosis		Serum bactericidal activity[b]
		Percent leucocytes with bacteria	Number of bacteria/ leucocyte	
214	1	41	3.1	>0.25
946	2	38	3.5	0.012
P92	3	22	1.8	0.25
Yale	4.5	42	3.7	>0.25
P98	4.5	35	2.4	0.046
P67	6	35	3.25	0.09
CF1	7	33	3.3	>0.25
113	8	63	3.5	>0.25
CL-135	9.5	35	2.1	0.028
MP	9.5	17	1.7	0.065
P33	11	33	2.4	0.044
Lowell	12.5	37	1.9	0.013
0127:B8	12.5	57	4.2	0.012
J5	14	64	6.0	0.008
W1895	15	81	5.5	0.033
CL-136	16	58	3.4	0.010
OB248	17	54	3.6	0.026
OB51	18.5	78	5.3	0.005
CL-185	18.5	76	8.6	0.001
CL-143	20	73	6.0	0.008
H	21	61	3.7	0.016
CL-1B	22	64	4.2	0.001

[a] Descending order of virulence as measured by renal infection.
[b] Volume (ml) of serum killing 50% of inoculum.

TABLE 6

FACTORS INFLUENCING VIRULENCE OF *E. coli*

	Measure of virulence	
	Renal infection	Proportion of deaths
Phagocytosis	+[a]	+
Growth in minimal media	+	+
Growth in urine	+	−
K antigen	+	−
Serum bactericidal activity	+	−
Dulcitol fermentation	+	−
Rough mutation	−	−
Motility	−	−
Proportion of deaths	−	

[a] + = Significance at a confidence level of 0.05 or less.

of resistance to infection by many infectious agents. However, it may be surprising to note this correlation with renal infection since the kidney milieu has been shown to have a deleterious effect on phagocytosis (11) and serum bactericidal activity (12). Even though both these activities are depressed in the kidney, they may still operate sufficiently to affect outcome of infection.

Examination of the carbohydrate fermentation showed that nephropathogenicity was positively related to fermentation of dulcitol. Others have examined carbohydrate fermentation in relation to the virulence of *E. coli* for the kidney and urinary tract. McCabe and Jackson (7) noted that strains of *E. coli* isolated from patients with pyelonephritis had a high proportion that did not ferment sucrose or salicin. Several possibilities exist to explain the relation of dulcitol fermentation to virulence. We considered that the cell wall may contain or incorporate dulcitol since the nature of the cell wall may determine resistance to phagocytosis (5, 13). In preliminary experiments the cell wall of one of the more virulent *E. coli* strains (214) was examined for dulcitol by gas–liquid chromatography. No dulcitol was found. By use of internal standards, it was calculated that less than one saccharide residue in 100 could have been dulcitol and escaped detection. Dulcitol fermentation would also be a potential advantage if dulcitol was present in the kidney. We were, however, unable to detect dulcitol in the kidneys. By internal standards, a maximum of 200 ng/mg dry weight could have been present but not detected. Thus, as yet we have been unable to account for the correlation between dulcitol fermentation and virulence.

Better growth of more virulent *E. coli* in urine appeared to be a manifestation of a greater growth "vigor." This was shown by a study of 17 strains in a minimal medium with only glucose as energy source. There have been a number of studies of the factors permitting growth of bacteria in the urine (14) but to our knowledge there have been no previous studies correlating growth of *E. coli* strains in urine with nephropathogenicity. Brumfitt and Heptinstall (6) examined the average generation time of *E. coli in vitro* in nutrient broth and concluded that the generation time did not vary significantly for any of the four *E. coli* strains used.

It has been suggested that motility may be an important factor in the pathogenesis of ascending pyelonephritis (15), and our strains were examined for motility through soft agar. Neither motility itself nor speed of motility among motile strains correlated with nephropathogenicity.

The mutation smooth to rough has been correlated with increased phagocytosis of *Shigella sonnei* and *S. typhi* (16) and we might have expected a correlation of smooth strains with nephropathogenicity or lethality. However, our studies did not show any correlation. This conclusion was based on rough and smooth strains of different serotypes and other factors may have overriden the importance of the rough mutation.

Our studies showed that *E. coli* strains which produced a high rate of pyelonephritis did not necessarily produce a high death rate. This failure to correlate virulence as measured by production of pyelonephritis with virulence as measured by the proportion of deaths has been observed in two previous studies (6, 7). McCabe and Jackson (7) found that the proportion of deaths with ascending infection of the kidney in rats did not correlate with renal infection. They considered that the renal massage (used to establish infection in the kidney) may have obscured strain differences in nephropathogenicity. Brumfitt and Heptinstall (6) carried out mouse virulence tests by LD_{50} following intraperitoneal injection of *E. coli* in hog mucin and found that this did not correlate with the ability of the *E. coli* to produce pyelonephritis in the rat. Most studies of virulence of *E. coli* have examined virulence as measured by death after intraperitoneal injection in mice. The LD_{50} by the method of Reed and Meunch has been the technique most frequently used. Those studies have correlated virulence with K antigen (9) and resistance to

phagocytosis and serum bactericidal activity (5, 17). In our studies, lethality of the *E. coli* correlated with resistance to phagocytosis and growth in minimal media but not with serum bactericidal activity or the presence of K antigen. Others, however, have also failed to correlate K antigen with lethality in mice (18).

Deaths following infection with *E. coli* in our studies occurred within the first few days and the majority were within the first 24 hr. Because of the rapidity of the deaths we have considered these deaths were due to endotoxin, although, because we did not carry out bacteriologic studies on the mice that died, we could not exclude the possibility that deaths were due to extensive systemic infection.

The present study has examined 22 strains of *E. coli* and found that the virulence of these strains as measured by their ability to produce pyelonephritis correlated with a number of factors: The presence of K antigen, resistance to phagocytosis and serum bactericidal activity, ability to ferment dulcitol and ability of the strain of *E. coli* to grow in urine and minimal media. The means by which these factors increase the virulence of the organism is not clear. The surface (K) antigens may alter phagocytosis and serum bactericidal activity (9, 10). It is difficult to see how this might relate to the growth rate of organisms which is probably a separate unrelated factor increasing virulence. Growth rate obviously would be of importance in the establishment and maintenance of an infection. The means by which dulcitol fermentation may increase virulence of *E. coli* is more difficult to explain. Dulcitol is the alditol of galactose which is an important constituent of the cell wall and this remains a possible means by which dulcitol fermentation influences virulence. Because of the absence of dulcitol in the kidney and the adaptive nature of the enzyme which ferments dulcitol it is difficult to see how dulcitol fermentation relates directly to virulence. Further studies are in progress to examine this interrelationship.

REFERENCES

1. Kalmanson, G. M., Montgomerie, J. Z., Hubert, E. G., Barajas, L., and Guze, L. B. *Yale J. Biol. Med.* **46,** 196–202 (1973).
2. Keane, W. F., and Freedman, L. R. *Yale J. Biol. Med.* **40,** 231–237 (1967).
3. Glynn, A. A., Brumfitt, W., and Howard, C. J. *Lancet* **1,** 514–516 (1971).
4. Wolff, J. B., and Kaplan, N. O., *J. Bacteriol.* **71,** 557–564 (1956).
5. Medearis, D. N. Jr., Camitta, B. M., and Heath, E. C. *J. Exp. Med.* **128,** 399–414 (1968).
6. Brumfitt, W., and Heptinstall, R. H. *Brit. J. Exp. Pathol.* **41,** 552–558 (1960).
7. McCabe, W. R., and Jackson, G. G. *In* "Biology of Pyelonephritis" (E. L. Quinn and E. H. Kass, eds.), p. 39–52. Little, Brown, Boston, 1960.
8. Vahlne, G. *Acta Pathol. Microbiol. Scand. Suppl. 62,* **22,** 1–127 (1945).
9. Wolberg, G., and DeWitt, C. W. *J. Bacteriol.* **100,** 730–737 (1969).
10. Glynn, A. A., and Howard, C. J. *Immunology* **18,** 331–346 (1970).
11. Chernew, I., and Braude, A. I. *J. Clin. Invest.* **41,** 1945–1953 (1962).
12. Hubert, E. G., Montgomerie, J. Z., Kalmanson, G. M., and Guze, L. B. *Amer. J. Med. Sci.* **253,** 225–228 (1967).
13. Nakano, M., and Saito, K. *Japan J. Microbiol.* **12,** 471–478 (1968).
14. Kaye, D. *J. Clin. Invest.* **47,** 2374–2390 (1968).
15. Pazin, G. J., and Braude, A. I. *J. Immunol.* **102,** 1454–1465 (1969).
16. Skurski, A., Slopek, S., Michalska, E., and Obst, B. *J. Hyg. Epidemiol. Microbiol. Immunol. (Prague)* **3,** 389–392 (1959).
17. Meynell, G. G. *In* "Microbial Reaction to Environment" (G. G. Meynell and H. Gooder, eds.), p. 174–195. University Press, Cambridge, 1961.
18. Jacks, T. M., and Glantz, P. J. *J. Bacteriol.* **93,** 991–995 (1967).