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William R. McCabe

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ENDOTOXIN TOLERANCE. II. ITS OCCURRENCE IN PATIENTS WITH PYELONEPHRITIS *

By WILLIAM R. McCABE

(From the Research Laboratories, Veterans Administration West Side Hospital, and the Department of Medicine, University of Illinois College of Medicine, Chicago, Ill.)

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The possibility that endotoxin tolerance occurred in patients with chronic pyelonephritis was suggested from a clinical study of gram-negative bacteremia (1). Subsequent studies have demonstrated that the induction of pyelonephritis with endotoxin-producing bacteria resulted in tolerance to heterologous endotoxin in the rabbit (2, 3) and tended to confirm the clinical findings. Since these findings appear to offer at least a partial explanation for the relatively asymptomatic state of patients with chronic pyelonephritis, it seemed desirable to confirm them further in man. The present study was undertaken to compare the febrile response of subjects with chronic pyelonephritis and age-matched, uninfected control subjects to intravenous *Salmonella typhosa* vaccine. In addition, peripheral blood leukocyte counts, timed leukocyte excretion in the urine, and plasma 17-hydroxycorticosteroid (17-OHCS) levels were measured before and after pyrogen challenge to assess the effects of endotoxin tolerance on these parameters.

MATERIALS AND METHODS

Subjects. The subjects in this study were 26 adult males ranging in age from 27 to 72 years. Clinical data pertaining to these patients are shown in Table I. A variety of medical conditions was present, but no subjects were included who had diseases previously recognized to alter the febrile response to pyrogens (4-7). Twelve subjects without renal infection, hospitalized for a variety of reasons, were selected as controls. One additional control subject had chronic pyelonephritis with *Streptococcus faecalis* and was intentionally selected as an example of pyelonephritis due to nonendotoxin-producing species of bacteria. Thirteen patients had active urinary tract infections with gram-negative bacteria as evidence by "pale cell" pyuria and bacteriuria in ex-

cess of 10^5 bacteria per ml of urine in multiple urine specimens. All were adjudged to have pyelonephritis, although four of the patients are listed as having asymptomatic bacteriuria only because of the absence of symptoms at the time of examination and for identification. Three of these patients had abnormalities demonstrated by intravenous pyelography, consisting of varying degrees of calyceal dilatation or caliectasis, compatible with a diagnosis of pyelonephritis. The fourth patient (H.L.) died several months later of unrelated causes, and both gross and histologic evidence of unilateral pyelonephritis was observed at necropsy. The nine patients listed as having chronic pyelonephritis had experienced recurrent symptomatic attacks of pyelonephritis, and all had had either persistent or intermittent bacteriuria documented for at least one year. These nine patients had received 33 recorded courses of antimicrobial therapy during hospitalizations prior to the study. Intravenous pyelograms in eight of these patients demonstrated abnormalities of the renal collecting system attributable to pyelonephritis. Renal biopsy had been performed in one patient (E.M.) and revealed active pyelonephritis. Additional renal biopsies were not performed because of the typical histories and urinary findings and because previous studies in this center have demonstrated almost uniform occurrence of biopsy evidence of pyelonephritis in symptomatic patients with pyuria and bacteriuria (8).

All subjects were ambulatory and none had blood urea nitrogen concentrations in excess of 25 mg per 100 ml. At least two and usually three urine specimens were obtained from each subject prior to pyrogen challenge to insure their accuracy. The number of bacteria present in the urine were quantitated and identified by standard methods (9-11).

Pyrogen challenge. All pyrogen studies were begun at 8:00 a.m. No antipyretic agents were administered on the day of pyrogen challenge or the preceding day, and no patient received antibiotics for 2 weeks prior to testing. Temperatures were determined rectally with the thermometer being kept in place for 5 minutes and with one of the ward personnel in attendance. After basal temperatures were recorded at 8:00, 9:00, and 10:00 a.m., 0.1 ml of typhoid-paratyphoid vaccine (10^7 phenol-killed bacteria)¹ was administered intravenously at 10:00 a.m. Temperatures were obtained every 30 min-

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¹ Lederle Laboratories, American Cyanamid Co., New York, N. Y.

TABLE I

Clinical and laboratory data on patients with pyelonephritis and control patients

Patient	Age	Diagnosis	Urine culture	Initial temperature	Maximal temperature rise
				Degrees F	Degrees F
Patients with pyelonephritis					
1. H.L.	29	Rheumatoid arthritis; asymptomatic bacteriuria	$>10^6$ <i>E. coli</i>	98.6	1.0
2. E.M.	33	Chronic pyelonephritis	$>10^7$ <i>Klebsiella-Aerobacter sp.</i>	99.4	0.6
3. L.W.	42	Acute alcoholism; asymptomatic bacteriuria	$>10^7$ <i>E. coli</i>	100.0	1.2
4. W.G.	44	Benign prostatic hypertrophy; asymptomatic bacteriuria	10^6 <i>Klebsiella-Aerobacter sp.</i>	99.2	1.0
5. R.C.	45	Hypertension; chronic pyelonephritis	8×10^6 <i>Klebsiella-Aerobacter sp.</i>	98.6	2.4
6. C.H.	62	Bronchogenic carcinoma; chronic pyelonephritis	10^6 <i>Klebsiella-Aerobacter sp.</i>	100.2	2.0
7. J.P.	63	Chronic pyelonephritis	$>10^6$ <i>Klebsiella-Aerobacter sp.</i>	99.2	1.0
8. J.S.	64	Duodenal ulcer; renal calculi; chronic pyelonephritis	$>10^6$ <i>Pseudomonas aeruginosa</i>	99.0	2.0
9. J.H.	65	Hypertension; congestive failure; chronic pyelonephritis	10^6 <i>E. coli</i>	98.6	2.6
10. J.A.	65	Diabetes; benign prostatic hypertrophy; asymptomatic bacteriuria	10^6 <i>Klebsiella-Aerobacter sp.</i>	97.4	1.8
11. E.H.	70	Multiple sclerosis; chronic pyelonephritis	$>10^6$ <i>Proteus mirabilis</i>	99.4	2.2
12. H.K.	70	Benign prostatic hypertrophy; chronic pyelonephritis	10^7 <i>Klebsiella-Aerobacter sp.</i>	98.8	1.6
13. W.S.	72	Benign prostatic hypertrophy; chronic pyelonephritis	10^6 <i>E. coli</i>	99.0	3.0
Mean	56 \pm 15			99.0 \pm 0.7	1.73 \pm 0.71
Control patients					
1. J.S.	27	Secondary syphilis	0	98.8	1.6
2. M.G.	39	Hypertension	0	97.9	2.5
3. J.F.	42	Pulmonary blastomycosis	0	99.9	2.0
4. R.K.	46	Hypertension	0	98.0	1.6
5. F.B.	47	Duodenal ulcer	0	99.6	3.0
6. W.B.	57	Hypertension; bronchogenic carcinoma	0	98.0	2.4
7. J.C.	62	Hypertension; bronchogenic carcinoma	0	98.0	2.0
8. S.S.	65	Hypertension; chronic pyelonephritis	2×10^6 <i>S. faecalis</i>	98.8	4.0
9. F.S.	66	Syncope, etiology undetermined	0	99.0	3.0
10. H.B.	66	Inguinal hernia; hypertension	0	99.6	3.8
11. J.H.	67	Chronic pancreatitis	0	97.8	2.4
12. A.O.	68	Hypertension; congestive failure	0	98.2	3.8
13. E.L.	70	Duodenal ulcer; rheumatoid arthritis	0	98.9	2.5
Mean	56 \pm 14			98.6 \pm 0.61	2.66 \pm 0.81

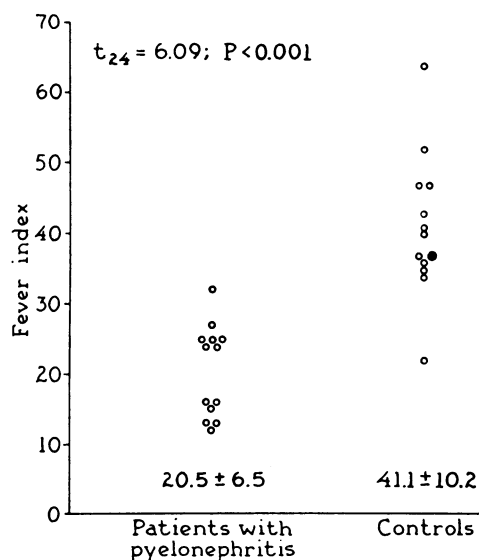


FIG. 1. FEVER INDEXES IN SUBJECTS WITH PYELO-NEPHRITIS AND CONTROL SUBJECTS AFTER 0.1 ML OF TYPHOID VACCINE (10^7 BACTERIA).

utes for 4 hours and hourly for an additional 4 hours. None of the patients' temperatures varied more than 0.5° F during the initial observation period. The last control temperature (10:00 a.m.) was used as the base-line temperature. Temperatures were recorded on 1.0 cm graph paper with each centimeter representing 0.5° F or 30 minutes. The fever curve was closed by dropping a perpendicular to the base line when the temperature had not returned to control levels within 8 hours. The area enclosed by the fever curve was measured by a planimeter. Each unit on the vernier was termed a fever unit (F.U.), and the number of units representing the area above the base line was recorded as the fever index.

Blood specimens were obtained for total leukocyte and differential leukocyte counts immediately preceding and 2 and 4 hours after endotoxin administration. Standard technics were used for the enumeration of leukocytes and differential counts (12). Counts were performed in duplicate and agreed within 5%.

Urinary leukocyte excretion. Timed urinary leukocyte excretion was determined by a modification of the method of Houghton and Pears (13). All urine was collected for the 3-hour periods preceding and following the injection of typhoid vaccine. Urine was obtained from an indwelling catheter in the four patients in whom residual urine and prostatic hypertrophy had been demonstrated, but voided specimens were collected from the other subjects. Urine volumes were recorded and 10-ml samples were spun at 2,000 rpm. A 9.1-ml volume of the supernatant fluid was discarded, and 0.1 ml of crystal violet-safranin supravital stain (14) was added to facilitate cellular identification. The remaining supernatant fluid, sediment, and stain were carefully mixed with a Pasteur pipette and added to a Spencer counting chamber. The

number of leukocytes within 0.9 mm^3 were counted, and epithelial cells were disregarded. The percentage of "pale staining" and "dark staining" leukocytes was determined (15). All counts were performed in triplicate.

17-OHCS response. Forty ml of venous blood was collected in heparinized syringes immediately before and 4 hours after the administration of typhoid vaccine. The blood was centrifuged and the plasma separated and frozen within 1 hour. Plasma 17-OHCS levels were determined on duplicate specimens by the method of Peterson and associates (16).

RESULTS

Data pertaining to the age, underlying diseases, results of urine cultures, control temperatures, and maximal temperature rise observed after intravenous challenge with typhoid vaccine of each subject is shown in Table I. None of the patients had received antimicrobial therapy recently. Four of the patients (E.M., C.H., H.K., and J.B.) had been admitted with febrile episodes of pyelonephritis. These episodes responded well to symptomatic therapy, and these patients were not treated with antibiotics until after the completion of pyrogen challenge. None of the patients in either group had had rectal temperatures exceeding 100° F for 48 hours prior to the morning of the study, but two did have basal rectal temperatures of 100.0° F and 100.2° F, respectively, on the morning of the study. The remaining subjects all had temperatures of less than 100.0° F. The mean prechallenge temperature, $99.0 \pm 0.7^\circ$ F, of subjects with pyelonephritis did not differ significantly from the mean reading, $98.6 \pm 0.61^\circ$ F, observed in the control subjects. Only four of the 13 subjects with pyelonephritis had maximal temperature elevations of more than 2.0° after the administration of pyrogen, while nine of the 13 control subjects had a rise of greater than 2.0° . The mean maximal temperature elevation after typhoid vaccine in the controls ($2.66 \pm 0.81^\circ$ F) was significantly greater ($t_{24} = 3.11$; $p < .01$) than the mean maximal elevation (1.73 ± 0.71) in patients with pyelonephritis.

The measurement of both the magnitude and duration of the fever, fever index, provides a more accurate assessment of the febrile response to endotoxin than either of these parameters alone (17, 18). The fever indexes of both groups of subjects are shown in Figure 1. The fever indexes of the subjects with pyelonephritis ranged from 12

to 32 F.U., while control subjects had fever indexes ranging from 21 to 64 F.U. Only one of the patients with pyelonephritis (R.C.) had a fever index of more than 30 F.U., and only one of the controls (J.S.) had a fever index of less than 30 F.U. The fever index of the subject with enterococcal pyelonephritis is depicted by the solid circled dot. The mean fever index of the controls (41 ± 10.2 F.U.) was significantly greater ($t_{24} = 6.09$; $p < .001$) than the mean fever index of subjects with pyelonephritis (20.5 ± 6.5 F.U.).

Differences in the fever indexes of both groups were even more striking when the febrile response was correlated with the patient's age. The fever index for each subject in both groups is plotted against his age in Figure 2 and regression lines, ± 1 SD, are shown for both controls ($Y = 8.01 + .594X$) and subjects with pyelonephritis ($Y = 4.5 + .302X$). Both the controls ($r_{11} = .801$; $p < .01$) and pyelonephritics ($r_{11} = .710$; $p < .01$) demonstrated a significant correlation between increasing age and enhanced responsiveness to the pyrogenic effect of endotoxin.

Mean total polymorphonuclear leukocyte counts, ± 1 SD, obtained from both groups before and 2 and 4 hours after typhoid vaccine are shown in Figure 3. Considerable variation of counts occurred among individuals in both groups. Although the controls exhibited a mean increase in total polymorphonuclear leukocyte count of 3,700

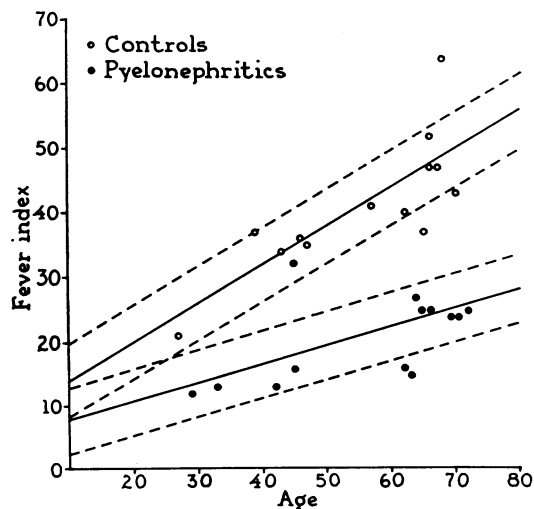


FIG. 2. FEBRILE RESPONSE TO TYPHOID VACCINE BY AGE IN CONTROL SUBJECTS AND PATIENTS WITH PYELONEPHRITIS.

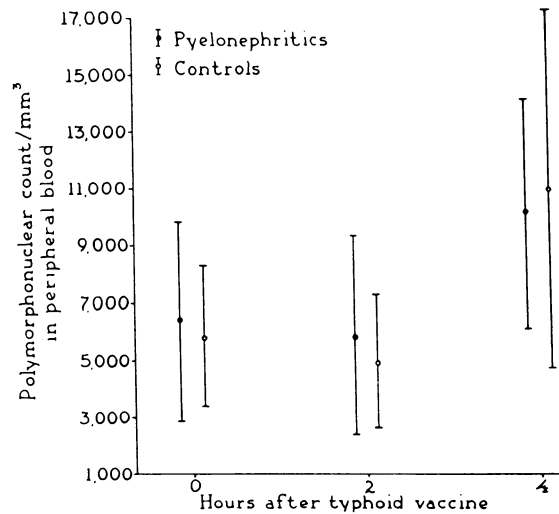


FIG. 3. PERIPHERAL BLOOD TOTAL POLYMORPHONUCLEAR LEUKOCYTE COUNTS BEFORE AND AFTER ENDOTOXIN CHALLENGE.

per mm^3 4 hours after endotoxin in comparison with a rise of 2,500 per mm^3 in pyelonephritics, this difference was not statistically significant. The decrease in polymorphonuclear leukocytes observed 2 hours after challenge also was similar in both groups.

Timed leukocyte excretion rates before and after the administration of typhoid vaccine were determined for 12 subjects with pyelonephritis and 12 control subjects. Counts performed in triplicate varied 10% or less. The excretion rates of leukocytes for both study periods are shown in Table II. Patients with pyelonephritis all had excretion rates of 5×10^5 leukocytes per hour or greater, while only one of the controls, the patient with enterococcal pyelonephritis, had initial excretion rates exceeding 2×10^5 cells per hour. Even including the patient with enterococcal pyelonephritis, only six of the 13 patients with pyelonephritis had an increase in leukocyte excretion in the urine of 75% or greater after endotoxin. Five of 11 patients without pyelonephritis of any type also had a 75% or greater increase in urinary leukocyte excretion after typhoid vaccine. Fifty to eighty per cent of the leukocytes in the urine of patients with pyelonephritis were of the "pale staining" variety, and no significant change in their frequency was observed after the injection of typhoid vaccine. The leukocytes excreted by controls were almost exclusively "dark staining," and their rela-

TABLE II
Changes in urinary leukocyte excretion and plasma corticosteroid levels after endotoxin

Patient	Fever index	Urinary leukocyte excretion			17-Hydroxycorticosteroid	
		Prechallenge	Postchallenge	Per cent change	Prechallenge	Postchallenge
					<i>µg/100 ml</i>	
<i>Cells per hour</i>						
Patients with pyelonephritis						
H.L.	12	1.2×10^5	3.2×10^5	+167		
E.M.	13	5.7×10^6	1.3×10^7	+128	7.3	17.7
L.W.	13	5×10^6	8×10^4	- 84	14.3	18.3
J.P.	15	1.5×10^7	7×10^6	- 53		
W.G.	16	4×10^8	7×10^8	+ 75	20.3	23.3
C.H.	16	2.3×10^6	5×10^6	+120		
H.K.	17	5.4×10^7	6.8×10^6	- 87	13.0	19.3
E.H.	24	4.6×10^6	1.8×10^7	+289		
J.H.	24					
W.S.	25	9×10^6	8.5×10^6	- 5	10.6	24.6
J.A.	25	8×10^6	1.7×10^6	- 80	9.0	22.0
J.S.	27	4×10^8	2.1×10^8	- 47		
R.C.	32	1.5×10^6	6.8×10^6	- 55	12.4	25.7
					Mean 12.4 ± 4.24	21.5 ± 3.2
Control patients						
J.S.	21	1.5×10^4	1.2×10^5	+ 80		
J.F.	34	2×10^6	9×10^4	- 55	15.7	20.7
F.B.	35	1×10^5	3×10^4	- 70	10.3	23.7
R.K.	36	2.2×10^4	8.4×10^4	+282	19.3	31.5
S.S.*	37	8.4×10^5	1.8×10^6	+115		
M.G.	37	7×10^4	5×10^4	- 30	16.3	21.3
J.C.	40	1.7×10^4	5×10^3	- 70	12.0	21.3
W.B.	41	2.7×10^4	2.2×10^4	- 20	22.9	33.0
E.L.	43	1.5×10^4	3×10^3	- 80	12.0	18.3
F.S.	47	4×10^4	1.9×10^6	+350		
J.H.	47				9.3	26.0
H.B.	52	3.9×10^4	4×10^4	+ 3		
A.O.	64	2.2×10^4	2.7×10^6	+113		
					Mean 14.7 ± 4.7	24.5 ± 5.0

* *Streptococcus faecalis* pyelonephritis.

tive frequency also was unaltered by endotoxin administration.

Plasma 17-OHCS levels were measured immediately before and 4 hours after the injection of typhoid vaccine in seven subjects with pyelonephritis and in eight control subjects. These values also are given in Table II. Prechallenge 17-OHCS levels were similar in both groups of patients, and similar rises occurred in both after endotoxin challenge.

No apparent relationship was observed between the degree of febrile response, the magnitude of the rise in plasma 17-OHCS, and changes in urinary leukocyte excretion in patients in either group.

DISCUSSION

The above studies describe two factors influencing the response of humans to bacterial endotoxin that had not been recognized prior to this study.

Neither the occurrence of endotoxin tolerance in humans with pyelonephritis nor the relationship of age to susceptibility to the pyrogenic effects of endotoxin in humans has been described previously. The demonstration of endotoxin tolerance in patients with chronic pyelonephritis confirms a clinical impression (1) gained from a study of gram-negative bacteremia. An earlier study demonstrated quite conclusively that the induction of pyelonephritis with gram-negative bacteria produced tolerance in rabbits known to respond normally to endotoxin and that the eradication of renal infection or reticuloendothelial blockade completely reversed the tolerant state (3). It was desired to confirm these observations in humans, and although it was not possible to reproduce these manipulations in the present study, it was shown that patients with chronic pyelonephritis due to gram-negative bacteria responded to a fixed dose of typhoid vaccine with significantly less fever than

age-matched controls. Typhoid vaccine was selected as the challenging agent, since it represented an endotoxin unrelated antigenically to the bacterial species producing pyelonephritis and because of its previous use in similar studies. The dose used in this study was somewhat smaller than that used by others (4, 5), but was deliberately selected because of the steep dose-response relationship in man (19) and the suspicion that the degree of tolerance was not great and could be masked by a relatively large challenge dose. Even with this dose of vaccine, almost all of the controls experienced chills and complained of headache and muscular pains during the procedure.

It has long been recognized that chronic pyelonephritis may lead to serious renal damage in patients with only minimal signs and symptoms of infection (20, 21), and it has appeared enigmatic that the clinical course of this disease could be so benign despite persistent renal infection and tissue damage by virulent pyrogenic bacteria. The present data suggest that this relatively benign clinical course may partially result from tolerance to the effects of endotoxin in addition to the development of specific antibodies to the infecting organism (22, 23). The time of the onset of tolerance in pyelonephritis is uncertain, but it seems likely that the continued renal infection serves to maintain the tolerant state. The explanation for symptomatic exacerbations is not readily apparent, but may be related to a lapse of tolerance, increased bacterial multiplication, or the release of larger amounts of endotoxin than usual. Since the tolerant state is quite dose dependent, it is readily overcome by larger doses of endotoxin (17). Similarly, the development of tolerance may also explain defervescence in infections caused by gram-negative bacilli, as has been suggested by Neva and Morgan (6). More precise methods for the measurement of serum levels of endotoxin would greatly enhance understanding of the importance of endotoxin in clinical infections and the kinetics of the development of endotoxin tolerance.

Variations in the susceptibility to endotoxin of animals of different ages have been reported (24). McDonald, Solomon, and Shock also have demonstrated significantly greater decreases in renal vascular resistance in older patients after pyrogen challenge than in younger controls (25). These investigators postulated greater vascular reactivity

to endotoxin in the elderly, and this increased susceptibility of older patients to endotoxin may partially explain the increased lethality of gram-negative bacteremia in the elderly (26).

The diagnostic value of an increase in the excretion of leukocytes in the urine after pyrogen challenge for the recognition of pyelonephritis has been the subject of a number of reports (27-29). The value of this test could not be confirmed, however, in the present investigation. The patients in this study were carefully selected as examples of documented chronic pyelonephritis. Although four of the patients had no symptoms at the time of examination, pyelograms and necropsy findings demonstrated that they had renal involvement in addition to bacteriuria. The latter group responded similarly to the other patients with pyelonephritis both with regard to pyrogen response and urinary leukocyte excretion. Increased leukocyte excretion was equally frequent in control subjects and in subjects with pyelonephritis, but basal urinary leukocyte excretion was considerably higher in subjects with pyelonephritis. Other investigations have demonstrated that the increased leukocyte excretion is not specific for pyelonephritis and may be observed in patients with hypertension and glomerulonephritis (28). A more recent study demonstrated that only two of 12 patients with chronic pyelonephritis responded to pyrogen challenge with a significant increase in urinary leukocyte excretion (30). Although the pyrogen used in the present study was derived from a different bacterial species than that used in the above reports, the similarity of the effects of all endotoxins (31-33) makes this an unlikely explanation for these differences.

Since pyrogen stimulation has been recommended as a method for the assessment of pituitary-adrenal function (34), it appeared important to insure that endotoxin tolerance did not lead to a diminution of 17-OHCS release. Study of plasma 17-OHCS levels before and after pyrogen administration in controls and tolerant patients failed to demonstrate significant differences between the two groups. A similar failure of tolerance to affect adrenal steroid excretion after pyrogen administration also has been demonstrated in experimental animals (35). Since tolerance has been considered to result from enhanced endotoxin clearance (36-38), it was anticipated that a lesser

rise in 17-OHCS would be observed in the subjects with pyelonephritis. Whether the failure of tolerance to affect adrenal steroid secretion represents extreme sensitivity of the pituitary to pyrogens is uncertain, but it does point out another area of uncertainty in understanding the actions of endotoxin.

SUMMARY

The febrile response to bacterial endotoxin was studied in 13 control subjects and 13 subjects with pyelonephritis. Patients with pyelonephritis exhibited significantly less fever after the administration of heterologous endotoxin than did age-matched controls. In both groups of patients a distinct correlation between the patient's age and the magnitude of the febrile response was observed. No significant differences were observed in the magnitude of changes in peripheral blood leukocyte counts, plasma 17-hydroxycorticosteroid elevation, or urinary leukocyte excretion between the two groups after endotoxin administration.

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