BACTERIAL PYROGEN IN DIAGNOSIS OF PYELONEPHRITIS

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Pears and Houghton (1958, 1959) reported an increase in the urinary excretion of leucocytes after the intravenous administration of bacterial pyrogen in 17 subjects with chronic pyelonephritis. No such rise was demonstrated in six normal subjects or in three patients with non-infective renal disease. The authors suggested that this phenomenon might be employed as a diagnostic test for the presence of chronic infection of the urinary tract.

The present study was made for two reasons: to assess the value of this phenomenon as a diagnostic procedure in chronic pyelonephritis and, if possible, to simplify the procedure involved.

Material and Methods

A total of 166 tests were carried out on 144 patients. These included 21 control subjects with no past or present evidence of renal disease, 24 patients in whom pyelonephritis was established or strongly suspected, and 30 patients with past or present infection of the urinary tract but in whom investigation yielded no other evidence of pyelonephritis. Others studied included 30 patients with hypertension but without evidence of pyelonephritis, 11 with glomerulonephritis, 7 with the nephrotic syndrome, 3 with orthostatic albuminuria, 3 with renal failure of uncertain origin, 3 with renal tuberculosis, and 1 with a history of urethritis. In 11 cases classification was not possible, and these have been excluded from the present review.

Urine was collected from males by normal voiding after cleansing of the external urinary meatus. In females, to avoid contamination the urine specimens were collected via a sterile glass tube placed over the urethral orifice (Leather and Hutchings, 1960) or in some cases by catheter. When a catheter was used prophylactic tetracycline was given orally for the next 48 hours.

The rate of urinary excretion of leucocytes was determined by the method of Houghton and Pears (1957) with the following modifications. All specimens were stained with malachite green (1%) by adding 1 drop of the stain to 9 drops of urine concentrated tenfold by centrifugation, and were examined under 1/6-in. (4 mm.) objective. In addition, as malachite green lyses red blood cells, an unstained specimen from each sample of urine was examined and a red-cell count was made if simple scanning of the preparation suggested any increase in red-cell excretion.

All studies were made during the morning, commencing usually at 8 o'clock. A fluid intake of 350 ml./hour was started one hour beforehand and continued

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throughout the test unless the patient became nauseated after the pyrogen. The pyrogen preparation used throughout was "pyrexal" (0.007 µg./kg. body weight), a purified lipopolysaccharide prepared from *Salmonella abortus-equi*. At first three half-hourly urine collections were made before and four half-hourly specimens after the pyrogen. Later the number was increased to six half-hourly specimens after pyrogen. All specimens were refrigerated on collection and were examined within two hours.

In the first 70 subjects proteinuria was estimated quantitatively by adding 1.5 ml. of 3% salicylsulphonic acid to 0.5 ml. of each specimen of urine and reading photometrically in a grey wedge photometer. In the remainder proteinuria was determined by boiling, acidifying with glacial acetic acid, and reboiling.

All urine specimens were cultured on blood-agar and MacConkey medium, using the quantitative loop technique of Gillespie *et al.* (1960). Thus any increase in the bacterial content following the pyrogen was evident. Infection was considered to be present when the bacterial content exceeded 3,000 organisms/ml. (Gillespie *et al.*, 1960).

In the first 36 subjects endogenous creatinine clearance was measured before and after pyrogen. Plasma and urine creatinine levels were estimated by a modification of the method of Hare (1950).

Pyrogen Test: Technical Considerations

1. *Are Half-hourly Specimens Really Necessary?*—(a) *Before Pyrogen*:—In each of 129 tests the mean of three counts has been compared with that half-hourly count which differed most from this mean (Table I). In 84 cases (65%) the difference was less than 20,000 cells/hour: in the 34 (26%) in whom the difference was greater than 50,000 cells/hour this increase was usually

TABLE I.—*Greatest Difference Between the Mean Cell Count of Three Resting Specimens and Individual Half-hourly Levels in 129 Cases*

Difference between Average and Individual Specimens in W.B.C. Hour	No. of Cases
<5,000	41
6,000–10,000	24
11,000–20,000	19
21,000–30,000	5
31,000–40,000	5
41,000–50,000	1
>50,000	34

only a small proportion of the total output, which in 17 of the 34 cases exceeded 1 million cells/hour. There were only two cases in which one half-hourly count greatly exceeded the mean figure. (b) *After Pyrogen*:—In 37 of 121 patients the white-blood-cell excretion after pyrogen was double the resting excretion and rose above 100,000 cells/hour during one or more of the half-hourly specimens. If the increase was only transient, half-hourly specimens might be necessary to detect it. The figures in these 37 cases have been analysed to determine whether this was so. The results are shown in the Chart. It would appear that the advantage of making half-hourly cell counts does not justify the extra effort involved, for cell counts on hourly specimens yield almost as much information.

2. *For How Long after Pyrogen should Cell Counts be Made?*—In 2 out of 82 cases in which post-pyrogen specimens were collected for two hours it was noted that the last specimen yielded the highest cell count. The duration of post-pyrogen examination was therefore

extended to five half-hourly periods (36 cases) and later to six half-hourly periods (24 cases). In 4 of these additional 60 cases the peak excretion occurred during the third hour after pyrogen, though in three of these four the test had already reached a "positive" level of excretion (see below) by the end of the second hour. We

Results in 37 patients in whom, after pyrogen, W.B.C. excretion doubled and exceeded 100,000/hour. At each level of W.B.C. excretion is shown the number of patients who reached this level for (A) entire post-pyrogen period, (B) maximum hour after pyrogen, (C) maximum half-hour after pyrogen.

are at present examining six one-hourly specimens after pyrogen, following the report from Montgomerie and North (1961) that the peak excretion may not occur until the fourth hour after pyrogen.

3. White-blood-cell Excretion in Normal Subjects.—In 19 out of 21 subjects without evidence of renal disease the white-blood-cell excretion levels were 60,000 cells/hour or less (Table II). The remaining two excreted 164,000 cells and 2,700,000 per hour. After pyrogen the white-cell excretion remained unchanged or fell in

TABLE II.—Resting Level of White-blood-cell Excretion in 21 Subjects Without Evidence of Renal Disease

No. of Subjects	Average Excretion W.B.C. Hour
5	10,000
4	11,000–20,000
5	21,000–30,000
3	31,000–40,000
1	41,000–50,000
1	51,000–60,000
1	164,000
1	2,700,000

seven cases, rose by 1,000 to 10,000 cells/hour in seven cases, and by 12,000 to 37,000 cells/hour in six cases. In one case a marked rise from 3,000 to 190,000 cells/hour occurred.

4. Criteria for a "Positive" Pyrogen Test.—From a consideration of the findings in normal subjects an arbitrary decision was made to regard as positive a test in which the white-blood-cell excretion rate during the hour of maximum excretion after pyrogen was double that of the control period; and during that period exceeded 300,000 cells/hour. It seemed probable that such an increase was not due to chance and could reasonably be attributed to the effect of the pyrogen.

5. Relation between Cell Excretion, Urine Flow, and Renal Blood Flow.—In 121 cases the percentage increase in cell excretion during the maximal hour after pyrogen was compared with the percentage change in urine flow during the same period. No relation between white-blood-cell excretion and urine flow was noted. In 36

cases studied there was no relation between alteration in creatinine clearance following pyrogen and white-blood-cell excretion.

6. Reproducibility of Pyrogen Test.—In 10 cases a second test was carried out without intervening chemotherapy (Table III). In seven the first test was positive,

TABLE III.—Reproducibility of Pyrogen Test. Results in 10 Patients in Whom More than One Test was Made. (Figures = W.B.C. Excretion in Thousands/Hour)

First Test		Time Interval between 1st and 2nd Tests	Second Test	
Resting Level	After Pyrogen		Resting Level	After Pyrogen
43	951	2 days	261	124
620	1,448	5 months	22	27
0	4,350	3 "	42	79
9	18	21 "	20	24
9	20	6 "	17	14
128	115	1 day	24	22
11	1,832	1 week	11	1,235
3,970	3,975	1 "	15,000	22,000
63	688	1 day	63	298
1,516	21,000	11 days	2,115	4,775

and in three of these seven when the test was repeated it became negative. Constitutional reaction to the pyrogen had been the same on both occasions. In the three cases negative at the first test the result at the second test was likewise negative.

7. Effect of Antibiotic Treatment on Result of Pyrogen Test.—In eight patients the test was carried out before and after a course of antibiotics (Table IV). In four

TABLE IV.—Effect of Antibiotic Treatment on Urinary W.B.C. Excretion Before and After Pyrogen in Eight Patients. (Figures = W.B.C. Excretion in Thousands/Hour)

First Test			Interval between Tests	Second Test		
Before Pyrogen	After Pyrogen	Culture		Before Pyrogen	After Pyrogen	Culture
32,000	20,000	<i>E. coli</i>	7 months	0	55	Sterile
3,200	11,000	"	3 weeks	30	137	"
10,000	7,000	<i>B. paracolon</i>	3 "	34	97	"
477	1,593	<i>E. coli</i>	7 "	45	168	"
21	153	Sterile	3 "	27	23	"
41	153	"	3 months	53	142	"
14	153	"	9 "	34	116	"
61	425	"	3 weeks	3	180	"

cases with a heavy urinary infection the white-blood-cell excretion rate at the first test was very high both before and after pyrogen. After chemotherapy the resting white-blood-cell excretion was normal, but in all four cases after pyrogen the output of white blood cells rose, though in none did it reach the levels accepted as a positive response. Four other cases gave a past history of infection of the urinary tract but the urine was now sterile. The resting white-blood-cell output was normal, but it rose after pyrogen, though in only one did it reach a positive level. After antibiotic treatment similar results were obtained in three of the four cases.

8. Albuminuria and Haematuria After Pyrogen.—The effect of pyrogen on albuminuria was studied in 133 patients. In 49 albuminuria was present before the pyrogen; in two of these some increase followed the pyrogen. In 84 patients no albuminuria was present before the pyrogen; in 11 of these traces appeared afterwards; this usually occurred one to two hours after the injection. The effect of pyrogen on red-cell excretion was studied in 132 cases. In 88 haematuria was not present before the pyrogen and none developed afterwards. In 11 out of 44 cases with microscopic haematuria (>1 R.B.C./high-power field) before the test an

increase in urinary red-cell excretion occurred, usually one to two hours after the pyrogen. In five of these the urine became frankly blood-stained. In some this persisted for 24 to 72 hours. In none of these 11 was any rise in blood urea noted after the test.

9. *Effect of Pyrogen on Output of Organisms.*—Quantitative bacterial counts were made on every specimen in 154 tests—118 were sterile, in 16 contamination was present (<3,000 organisms/ml.) in some specimens, and in 20 infection (>3,000 organisms/ml.) was present throughout. In only two of these 20 did a rise in the bacterial count follow pyrogen. In one other patient in whom all specimens in the test were sterile a coliform infection with bacterial counts in excess of 10,000 organisms/ml. developed within 24 hours of the test. No instrumentation had been used.

Results of Pyrogen Test in Various Disorders Infection of Urinary Tract

Patients ranged from those in whom the diagnosis of chronic pyelonephritis was firmly established to those with a past history of infection of the urinary tract but who now had no clinical evidence of renal disease and were in hospital with some unrelated disorder.

(a) Chronic Pyelonephritis

The diagnosis of chronic pyelonephritis may be very difficult to establish. Many cases occur in which the history and clinical findings are suggestive but in whom convincing proof of the diagnosis is not obtainable. In the present study chronic pyelonephritis was considered proved when there was histological evidence of pyelonephritis obtained from biopsy, nephrectomy, or eventually at necropsy, which included interstitial fibrosis, round-cell infiltration, periglomerular fibrosis, and tubular atrophy with eosinophilic cast formation. Chronic pyelonephritis was also considered proved when a history of infection of the urinary tract was associated with changes in excretory urography (or retrograde pyelography) in which caliceal clubbing and distortion were present together with focal reduction in renal parenchyma.

Chronic pyelonephritis was diagnosed as "probable" when most but not all of the histological criteria were present. It was also considered "probable" in subjects in whom a history of infection of the urinary tract was associated with some but not all of the above radiological changes.

In 13 cases the diagnosis of pyelonephritis was proved (Table V). The pyrogen test was positive in 5 of the 13 cases. In three of these and in four others the resting level of white-blood-cell excretion was normal.

In 11 cases the diagnosis of chronic pyelonephritis was thought probable. The test was positive in 6 of the 11 cases. In four of these six and in two others the resting level of white-blood-cell excretion was normal.

In these 24 cases of pyelonephritis there was a history of recent symptoms suggesting active infection in 5 out of 11 with a positive test and in 5 out of 13 who were negative. In three out of six with a blood urea of 130 mg./100 ml. the test was positive. The only factor which appeared to influence whether or not a test was positive was previous antibiotic treatment. Thus 10 out of 15 cases with no such treatment within the previous three months were positive, while only one out of nine who had had such treatment was positive.

(b) Acute Infection of the Urinary Tract

Eight cases had an active infection of the urinary tract with high bacterial counts at the time of the pyrogen test. Five had symptoms of acute infection of the urinary tract. Excretory urography was carried out in all and biopsies were taken in two cases; in none was evidence of pyelonephritis obtained. The resting level of white-blood-cell excretion was above normal in six, and in five of these a fall in excretion followed pyrogen. In only one case was the pyrogen test positive.

There were six other patients who had recently suffered from acute infection of the urinary tract and who had received chemotherapy before the pyrogen test was carried out. Symptoms were still present in five of the six, though in all the urine was sterile. The test was positive in two cases. In both of these excretory urography suggested localized infection of one kidney.

TABLE V.—Chronic Pyelonephritis

Sex	Age	History	B.P.	Proteinuria at Test	Blood Urea	Pyrogen Test W.B.C. Excretion (10 ⁹)		
						Pre-pyrogen	Post-pyrogen	Culture
Diagnosis Established (13 Cases)								
F	60	Episodes frequency and dysuria for 30 years	190/100	Nil	30	15	42	Sterile
M	40	Nil	195/145	+	33	56	518	"
F	48	Nephrolithotomy 16 years ago; dysuria and frequency followed	210/110	Nil	40	41	153	"
F	34	Episodes frequency and dysuria for 7 years	140/90	+	—	206	159	<i>E. coli</i>
F	43	" " " " 20 years ago	210/130	+	170	202	266	Sterile
F	37	Episodes fever and frequency for 18 months	200/120	+	42	1,500	16,000	"
F	23	Nil	165/120	Nil	200	63	550	"
F	27	Episodes frequency and dysuria since pregnancy 7 years ago	135/85	+	56	10,000	7,000	<i>B. paracolon</i>
M	6	Episodes fever, frequency and dysuria	105/75	Nil	30	12	435	Sterile
F	33	Fever and dysuria age 7 years; nil since	210/130	"	29	9	18	<i>E. coli</i>
M	45	Episodes frequency and dysuria 15 years ago	160/115	+	150	60	102	Sterile
F	37	Frequency dysuria 17 years ago; also recently	170/100	+	141	1,825	5,450	"
F	63	No urinary symptoms. Diabetes mellitus	170/100	+	—	5,000	1,000	<i>E. coli</i>
Diagnosis Probable (11 Cases)								
F	39	Recent frequency and dysuria	145/90	Nil	24	61	425	Sterile
M	41	Nil; proteinuria at age 23 years	145/70	+	250	40	49	"
F	26	Frequency and dysuria for 1 year	130/80	Nil	20	360	10	"
F	23	Episodes frequency and dysuria for 7 years	125/80	"	27	89	231	"
M	67	" " " " 20 years. Urethral stricture	160/90	+	34	353	412	<i>E. coli</i>
F	48	Episodes frequency and dysuria for many years	190/120	Nil	35	87	1,858	"
F	25	" " " " 7 years	120/70	"	—	1	1,992	Sterile
F	23	" " " " 8 "	130/75	"	22	23	633	"
M	39	Nil	220/170	"	100	6	24	"
F	23	" " " " " "	230/135	+	360	628	16,000	<i>E. coli</i>
F	25	Dysuria and frequency recently	130/80	Nil	30	26	688	Sterile

(c) *Past History of Infection of the Urinary Tract but without Histological or Radiological Confirmation of Pyelonephritis*

Sixteen female subjects with a past history of infection of the urinary tract were studied. All were symptom-free at the time of the test. Eight were hypertensive (diastolic blood-pressure 100 mm. Hg or over). Excretory urography (15 cases) was normal in 12, and in three some abnormality was present but was not diagnostic of pyelonephritis. Renal biopsy (four cases) showed slight focal cortical scarring in two.

Resting levels of white-blood-cell excretion were normal in 14 of the 16 cases. The pyrogen test was positive in three cases.

Hypertension

In 19 subjects with hypertension the history, clinical examination, and investigation failed to reveal a cause for the hypertension. In all excretory urography was normal. Aortography was normal in four in which it was performed. Biopsies (nine cases) were normal in two and revealed varying degrees of nephrosclerosis in the other seven. The pyrogen test was positive in four and in three others a rise to over 200,000/hour occurred (in five of these seven biopsy was carried out).

In 11 cases with hypertension the cause was known. These included renal artery stenosis in two cases, phaeochromocytoma in two, systemic lupus erythematosus in one, and hypertension following toxæmia of pregnancy in one. In the remaining five cases essential hypertension was diagnosed on the basis of negative investigation together with a strong family history of hypertension. In all 11 the pyrogen test was negative.

Glomerulonephritis

There were 11 cases of glomerulonephritis at various stages of the disease (Table VI). The diagnosis was confirmed histologically in seven. In 3 of the 11 the pyrogen test was positive. Two of these three gave a history of recurrent focal nephritis and in both histological evidence of glomerulitis was present. After pyrogen both developed macroscopic haematuria which persisted in one for several days. The remaining patient with a positive test was a woman of 52 who had had acute nephritis 16 years previously. Hypertension had persisted since. Renal biopsy revealed no abnormality.

Other Patients Studied

Seven patients with the nephrotic syndrome were studied. Five had undergone biopsy—in two no abnormality was seen and in three basement membrane thickening was present. Resting levels of white-blood-cell excretion were normal in four and raised in three. The pyrogen test was negative in all.

In three subjects with orthostatic albuminuria the test was negative. In three with renal tuberculosis high resting levels were present but no rise after pyrogen occurred. In three subjects with renal failure (one case associated with bilateral hydronephrosis) the resting levels were high but no rise developed after pyrogen. In one subject with a past history of urethritis resting levels were normal and the pyrogen test was negative.

Discussion

We do not yet know the full mechanism by which pyrogen affects the white-blood-cell excretion in certain diseases. It is therefore important to keep an open mind on any suggestion that it can be used as a specific test of any one disease.

In their original description Pears and Houghton (1958) examined three half-hourly urine specimens before and four half-hourly specimens after pyrogen. Our results indicate that very little accuracy is lost if only one two-hourly control specimen and hourly specimens after pyrogen are examined. It is necessary to examine urine specimens for more than two hours after pyrogen because in some cases an increase in cell excretion is delayed. Montgomerie and North (1961) have also stressed the importance of cell counts beyond a two-hour period. In 12 cases of pyelonephritis they found the peak excretion occurred in the fourth hour and suggested that counts should be made for six hours after pyrogen. Our subsequent work has indicated that counts for four hours are probably sufficient (Leather and Gault, 1962).

The levels of excretion in normal subjects and in many patients in this study were lower than in previous reports (Houghton and Pears, 1957; Hutt *et al.*, 1961). This was probably because non-squamous epithelial cells were omitted. These cells, which vary in number from patient to patient and specimen to specimen, may be present in large numbers. Staining with malachite green has made these and other cells in the urinary deposit

TABLE VI.—*Glomerulonephritis*

Sex	Age	Presenting Features	B.P.	Proteinuria	I.V.P.	Histology	Blood Urea	Pyrogen Test W.B.C. Excretion ($\times 10^3$)		
								Before Pyrogen	After Pyrogen	Culture
F	23	Recurrent haematuria	145/80	+	N	Focal glomerulitis	27	266	1,000	Sterile
F	16	Acute nephritis	180/110	+	—	—	28	8,000	4,000	"
F	22	Hypertension. Acute nephritis 8 years previously	170/110	+	—	—	60	11	22	"
M	25	Recurrent haematuria	145/85	+	N	Focal glomerulitis	38	0	8,000	"
M	23	Renal failure. Nephrotic syndrome 8 years previously	250/160	+	Poor contrast	Chronic glomerulonephritis	121	160	180	"
M	52	Headache; blurred vision	255/175	Nil	—	"	37	54	45	"
M	48	Renal failure	190/110	+	Retrograde pyelogram N	Chronic glomerulonephritis (at necropsy)	440	310,000	24,000	<i>E. coli</i>
F	45	"Headache"; blurred vision.	170/100	+	—	"	280	22	33	Sterile
M	22	Acute nephritis 3 years previously	210/160	+	Poor contrast	Chronic glomerulonephritis	94	715	697	"
M	60	Headache. Acute nephritis 10 years previously	240/120	+	—	—	80	124	145	"
F	63	Headache. Acute nephritis 15 years previously	280/130	Nil	N	Hypertensive changes only	25	12	2,246	"

easier to recognize and has also had the advantage of reducing counting-time.

Hutt *et al.* (1961) regarded the response to pyrogen to be not only of diagnostic value but as some indication of effectiveness of antibiotic therapy. Our findings also suggest that antibiotic treatment may influence the results of the pyrogen test. However, in 3 out of 10 cases in which tests were duplicated without intervening antibiotic treatment, a different result was obtained at the second test from that at the first, and no good reason for this could be found. As the response to pyrogen may apparently change spontaneously, the value of the test as a means of assessing the results of treatment must always be doubtful.

In assessing the practical value of the pyrogen test one cannot dismiss the finding in some cases of haematuria following pyrogen. Haematuria never developed where none was present beforehand. It appeared that bleeding, already present, was exacerbated by the increase in renal blood flow known to follow intravenous bacterial pyrogen (Chasis *et al.*, 1938). Though no subsequent rise in blood urea was noted, the possibility that the pyrogen damaged the kidney cannot be excluded. Taylor and Page (1943) attempted to treat hypertensive subjects with bacterial pyrogen, and reported the occurrence in two cases of frank haematuria followed by a rise in blood urea. Moreover, the generalized Schwartzman reaction classically initiated by two intravenous injections of bacterial pyrogen separated by an interval 18 to 24 hours is characterized by bilateral renal cortical necrosis (Bennett and Cluff, 1957). Katz and Moore (1960) failed to find any evidence of the Schwartzman reaction in renal biopsy specimens taken three to four hours after the injection of pyrogen in pyelonephritic dogs, but it would seem prudent to avoid the pyrogen test in subjects with microscopic haematuria.

Bacteriological Findings

Pears and Houghton (1958) found that in 2 out of 17 cases organisms were present in a Gram smear taken from post-pyrogen urine specimens when none had been present beforehand. In two others the number of organisms was "greatly increased" after pyrogen. In the present study quantitative counts were made on all urine specimens in order to determine how often this occurred. In only two cases did bacterial counts increase in the post-pyrogen specimens. In another case, in which all the nine specimens taken at the test were sterile, a coliform infection of the urine with pyuria was present 24 hours later. These findings suggest that intravenous pyrogen only rarely reveals the offending organism in an occult infection of the urinary tract.

Results of Pyrogen Test in Different Disorders

Pears and Houghton (1959, Fig. 2 of their communication) found that in 8 out of 17 cases of pyelonephritis the increase in cellular excretion after pyrogen was double that of the control period. In the series of Hutt *et al.* (1961) positive results were present in 16 out of 26 cases of chronic pyelonephritis, and in the present study in 11 out of 24 cases.

In cases with acute infection of the urinary tract or with a recent history of such infection results were positive in only 3 out of 14. In patients with a past history of infection of the urinary tract the test was positive in 3 out of 15, and the possibility of pyelonephritis was raised by the radiological or histological abnormality in

two of these three. Positive tests also occurred in 4 out of 19 subjects with hypertension of unknown cause, whereas none were positive in 11 cases in which a cause for hypertension was demonstrated. This raises the suspicion that pyelonephritis may often be the explanation of hypertensives of "unknown cause." However, in 3 out of 11 cases of glomerulonephritis the pyrogen test was likewise positive, and Hutt *et al.* noted similar results. The possibility cannot be excluded that these three subjects were suffering from superimposed pyelonephritis, but there was no evidence that this was so. These findings in glomerulonephritis clearly lessen the value of pyrogen as a specific test for the presence of pyelonephritis.

Pears and Houghton and Hutt *et al.* have drawn attention to the occurrence of a normal urine in chronic pyelonephritis. This point cannot be overemphasized. In the present series in 8 of the 24 cases in which pyelonephritis was either proved or highly likely the urine at the time of the test was protein-free, the deposit normal, and culture sterile. The white-blood-cell excretion rate was under 65,000/hour. In six of them the pyrogen test was positive. It is under these circumstances that the pyrogen test is most useful.

Summary

Pyrogen tests have been made in 144 subjects. The following modifications of this test as described by Pears and Houghton are suggested: (1) examination of urine should be made hourly for at least three hours after pyrogen; (2) examination of half-hourly specimens is unnecessary; and (3) specimens should be stained with malachite green; this helps to distinguish white cells from epithelial cells in the urinary deposit; non-squamous epithelial cells should not be counted.

From our findings in normal subjects the following criteria of a positive test are suggested. After pyrogen (a) the resting white-blood-cell excretion is doubled, and (b) during the first, second, or third hours it exceeds 300,000 cells/hour.

Possible disadvantages of the pyrogen test were: (1) results were not always reproducible; (2) an increase in quantitative bacterial count after pyrogen rarely occurred; and (3) in 11 out of 44 cases with microscopic haematuria an increase in haematuria followed pyrogen; in five the urine became frankly blood-stained.

The pyrogen test was positive in 11 out of 24 cases of chronic pyelonephritis, in 3 out of 14 subjects with acute infection of the urinary tract or with a recent history of such infection, in 3 out of 16 with a past history of urinary infection, and in 4 out of 19 cases of hypertension of unknown origin. It was also positive in 3 out of 11 cases of glomerulonephritis.

It is concluded that the pyrogen test is positive in only half the established cases of pyelonephritis. It is more likely to be positive if no recent antibacterial treatment has been given. The test is of greatest diagnostic value in those cases in which the urine is apparently normal.

It is sometimes positive in glomerulonephritis and cannot therefore be considered a specific test for pyelonephritis.

It should not be employed if haematuria is present.

We wish to thank Professor C. Bruce Perry, Dr. J. E. Cates, and Mr. Ashton Miller, of the Bristol Royal Infirmary, for permission to study patients under their care; Dr. Cates

for valuable advice in the preparation of the paper; and Professor T. F. Hewer for assistance in the interpretation of histological material. We gratefully acknowledge the generous help received from the Medical Research Fund of the United Bristol Hospitals towards the expenses of this study.

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BACTERIOLOGICAL EXAMINATION OF URINE

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In recent years there has been a reappraisal of the bacteriological methods in use for the diagnosis of infection of the urinary tract. Appreciation of the risk of infection following the catheterization of female patients has led to several techniques being developed to allow collection of mid-stream samples of urine from women (Hart and Magee, 1957; Boshell and Sanford, 1958; Wood *et al.*, 1960; Leather and Hutchings, 1960). In conjunction with these, quantitative urine culture has been advocated as a means of distinguishing between contaminated urine and genuine infection (Kass, 1955, 1956; Sanford *et al.*, 1956; MacDonald *et al.*, 1957; Jackson *et al.*, 1958; Monzon *et al.*, 1958).

Unfortunately most of the methods suggested for the collection of mid-stream specimens are elaborate, time-consuming, and often impracticable because of age or infirmity on the part of the patient. The standard method for performing bacterial counts on urine is expensive in time and material, and demands either immediate culture of the specimen or refrigeration at 4° C. There is also some doubt about the need for elaborate bacterial counts for the detection of significant bacteriuria (100,000 organisms/ml.).

In this paper the different methods recommended for the collection of "clean" specimens of urine from women have been re-examined, and a simplified procedure is suggested. In addition a rapid method for performing quantitative urine culture is described and compared with the standard technique. Finally, the results obtained by quantitative culture and from routine laboratory reports are compared.

Material and Methods

Urine specimens were obtained by the nursing staff of busy general medical wards. So far as possible these were obtained from consecutive admissions, irrespective of the reason for admission or the presence or absence of suspected renal disease.

Collection of Specimens

Several methods were attempted, but eventually two were adopted for collecting urine specimens. Clean specimens were obtained by preparing the patient as for catheterization. The perineum was first washed with soap and water. This was followed by cleansing of the vulva with a sterile swab soaked in 1% cetrimide solution, the labia being separated and cleansed from before backwards. The patient was then sat on a sterile bed-pan and encouraged to pass urine. The sample

collected in the bed-pan was used for bacteriological examination. No attempt was made to obtain a mid-stream specimen. The second method employed a sterile glass tube as described by Leather and Hutchings (1960). The same preparation was carried out, but this was followed by placing the bell-shaped end of the tube over the urethral orifice. The patient was encouraged to void urine, and a mid-stream specimen was collected in a sterile container. All samples collected were divided between two sterile specimen jars and immediately taken to the laboratory or refrigerated at 4° C. until this was possible.

Where the results of urine culture indicated infection, catheter specimens were obtained within 24 hours, and before any treatment was begun.

Bacteriological Methods

One-half of each specimen was examined in the routine manner. This involved seeding a blood-agar plate with a urine inoculum of variable size, depending on the number of bacteria seen on the Gram film of the centrifuged deposit. The inoculum was distributed with a glass rod spreader. The plate was incubated at 37° C. overnight and then reported in the conventional way. The other half of each specimen was taken to a separate laboratory and quantitative cultures were performed. Two methods were employed. In the first of these nutrient-agar pour plates were prepared, using undiluted urine and urine diluted 1 in 100 in normal saline. Colony counts were made after overnight incubation at 37° C. The second method involved the use of a fused platinum loop of 2-mm. internal diameter. This loop was immersed vertically in the undiluted urine and discharged fully on to the surface of a well-dried blood-agar plate. This inoculum was distributed with a glass spreader, was incubated at 37° C. overnight, and the surface colonies were counted.

Since bacterial counts were performed on specimens which were also being examined independently in the routine manner, a comparison of the results obtained by the two methods was undertaken. It has been the practice here to report the results of urine cultures on a roughly quantitative basis thus: sterile; scanty growth; mixed growth of doubtful significance; pure growth of "X." In this study such reports were analysed by a clinician familiar with this practice and allocated to one of the three groups representing sterile or contaminated urine, infected urine, or urine which yielded an