The relationship between urinary infection, cystoscopic appearance, and pathology of the bladder in man

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I Lymphocytes in the lamina propria

SYNOPSIS Studies of bladder histology were made in patients with recurrent urinary infection or symptoms of cystitis, with a view to establishing its relationship to symptoms, cystoscopic appearance, and bacilluria. Bladder biopsies were taken from the patients and compared with control material obtained at necropsy. Acute inflammatory changes were inconstant and sometimes sparse. Chronic inflammatory changes were often marked, and were ranked by severity into four grades. There was a statistically significant correlation between the more severe grades and the finding of bacilluria, which was not dependent on sex or age. The grades in patients with recurrent non-bacterial dysuria ('urethritis') lay between those of the controls and the infected patients, and were statistically different from both. There was no correlation between these grades and either symptoms or the finding of trigonal hyperaemia during cystoscopy. However, heavy lymphocytic infiltration, and particularly germinal follicle formation, frequently resulted in macroscopic tubercle-like nodules which were visible on cystoscopy. Such germinal follicles may contribute to local antibacterial defences by their production of immunoglobulins, and may explain the high antibacterial antibody titres sometimes found in the sera of subjects with infection confined to the lower urinary tract.

During a prospective study of women with recurrent urinary infection we could not relate the cystoscopic appearance of the bladder either to the patients' symptoms or to the presence of bacilluria. Because of this, and because the literature concerning bladder pathology in cystitis is scanty and inadequate, we performed bladder biopsies in an attempt to relate clinical, cystoscopic, bacteriological, and immunological findings to the histological changes. We report here our findings concerning lymphocytes in the lamina propria.

Clinical Material and Methods

Patients
Fifty bladder biopsies were taken, after full explanation and discussion, from 37 female and four male patients suspected or known to have urinary infections. Thirty-one patients had recurrent cystitis, five painless frequency, and one abdominal pain.

Three others had symptoms associated with neuropathic bladders and a fourth had a vesical calculus. The patients' ages ranged from 21 to 76 years.

Cystoscopy was performed under general anaesthesia. After examination of the bladder a biopsy was taken with a resectoscope or Riches forceps, usually from the middle of the interureteric bar. If any local abnormality was noted, this was biopsied instead. In a few cases the biopsy was taken from the vault or bladder neck in the absence of any local abnormality. Haemorrhage immediately after the initial biopsy made a second attempt at the same cystoscopy impossible. Repeat biopsies were made in some patients at a later date. Although transient postoperative haematuria was common the only potentially serious complication was the development in one patient of abdominal pain with signs of peritoneal irritation, which subsided after two days of catheter drainage.

Patients with bacilluria were given antibiotics for 48 hours before cystoscopy, unless they were also undergoing ureteric catheterization to locate infection.

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In this event, chemotherapy was commenced immediately afterwards. Non-bacilluric patients were not routinely given antibiotics.

CONTROLs
Adequate human control material for histological study is difficult to obtain, for bladder biopsy of patients with no urological complaints would be unjustified. Therefore we studied vertical sections of the trigone taken at necropsy as soon after death as possible, so as to reduce changes due to autolysis. In some cases additional sections were taken from the bladder vault. Material was obtained from 30 subjects (17 female, 13 male) whose ages ranged from 0 (stillbirth) to 76 years, whom we called 'non-infected controls'. There had been no clinical evidence of urinary infection in these. They had not received steroids or immunosuppressives, or been catheterized before death, and at necropsy had no evidence of any condition predisposing to urinary infection.

Similar tissue from seven female and five male subjects (aged 21 to 79 years) with documented clinical or necropsy evidence suggesting antemortem urinary infection was also examined. These were called 'infected controls'.

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Fig 1  Section of bladder from uninfected control showing grade I subepithelial histology. The transitional epithelium is partly autolysed. H & E × 140.

Fig 2  Bladder biopsy from patient showing grade II subepithelial changes (oedema of the lamina propria with a doubtful increase in lymphocytes). H & E × 140.

Fig 3  Bladder biopsy from patient showing a diffuse increase in the number of lymphocytes in the lamina propria, and, to the right, a focal accumulation of lymphocytes without a germinal centre (grade III). H & E × 140.

Fig 4  Bladder biopsy from patient showing a subepithelial germinal follicle (grade IV). H & E × 53.
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HISTOLOGICAL CLASSIFICATION
The degree of lymphocytic infiltration in the lamina propria was assessed as falling into one of four grades:

I Scattering of lymphocytes (fig 1)
II Subepithelial oedema + a minor increase in the number of lymphocytes (fig 2)
III Definite focal or diffuse lymphocytic infiltrate (fig 3) but no germinal centres
IV Germinall follicle formation (fig 4).

ASSESSMENT OF THE BACTERIOLOGICAL STATE OF THE PATIENTS
Patients were seen, and their urine cultured, monthly. If they developed symptoms of urinary infection, cultures were made before they took antibacterial agents. Bacterial urinary infections were diagnosed in 24 of the patients (bacilluric patients), who provided 29 of the biopsies, according to any of the following criteria: (1) two successive midstream urine specimens culturing over 100,000 of the same bacteria (not serotyped) per ml of fresh or refrigerated urine; (2) one midstream specimen culturing over 100,000 bacteria per ml, with over 10 leucocytes per cmm of uncentrifuged urine; (3) one midstream urine culturing over 100,000 bacteria per ml urine, collected when there was pain during micturition; (4) any number of bacteria cultured from a suprapubic aspirate.

Fifteen biopsies were taken from 11 patients who were regarded as non-bacilluric because they exhibited none of the above features during follow up for at least six months. Six biopsies came from six patients who were regarded as of uncertain bacilluric status because, although no evidence of bacilluria had been obtained, the duration of follow up was less than six months.

SEROLOGICAL STUDIES
Serial agglutination studies were performed in 21 patients using sera stored at −20°C, tested in batches by the method of Percival, Brumfitt, and de Louvois (1964). The normal range of titre in our laboratory was up to 1:320. 0 antigens from 11 E. coli (0—1, 2, 4, 5, 6, 7, 9, 11, 18, 39, 75), including those known to cause urinary infections frequently (Rantz, 1962), were used to determine titres in non-bacilluric patients, but in bacilluric ones antigen prepared from the homologous infecting E. coli was used.

STATISTICAL METHODS
Chi square, Fisher's exact probability, and Mann-Whitney U tests were used where applicable (Siegel, 1956; Finney, Latscha, Bennet, and Hsu, 1963). Analysis of the ranked data concerning the degree of lymphocytic infiltration was performed by the allotment of normal scores (David, Barton, Ganeshalingham, Harter, Kim, Merrington, and Walley, 1968) and calculation of a t statistic with infinite degrees of freedom.

Results

URINARY INFECTION (TABLE I)
Significantly higher grades of lymphocytic infiltration were found in bacilluric than in non-bacilluric patients, and in non-bacilluric ones than in non-infected controls. Sections from the trigones of infected controls were graded significantly higher than those from non-infected controls. Germinall follicles (grade IV) were found in 12 of the 29 biopsies from bacilluric patients and in two of 12 infected controls but were not found in any sections from non-infected controls or non-bacilluric patients. The differences between groups were still highly significant when only sections from female subjects were considered.

The grading of 31 biopsies taken from 24 women with recurrent cystitis who had undergone close follow up for over six months is also shown in table I. In this more homogeneous group, bacilluric patients again tended to have higher grades than non-bacilluric ones (0-005 > p > 0-0025), who in

<table>
<thead>
<tr>
<th>Origin of Biopsy</th>
<th>No. of Biopsies</th>
<th>Subepithelial Grade</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>I</td>
</tr>
<tr>
<td>A Uninfected controls</td>
<td>30 (17)</td>
<td>23 (13)</td>
</tr>
<tr>
<td>B Infected controls</td>
<td>12 (7)</td>
<td>3 (2)</td>
</tr>
<tr>
<td>C Non-bacilluric patients</td>
<td>15 (12)</td>
<td>1 (0)</td>
</tr>
<tr>
<td>D Bacilluric patients</td>
<td>29 (19)</td>
<td>2 (1)</td>
</tr>
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</table>

Table I  Histology and urinary infection

1Figures not in parentheses refer to trigonal sections from controls or (in patients) to biopsies from those of known bacilluric status, whether male or female. Figures in parentheses refer to trigonal sections from female controls or biopsies from female patients of known bacilluric status who had recurrent cystitis.

For figures not in parentheses: A v B, t = −3·550, p < 0·0005; A v C, t = −3·7154, p < 0·0005; A v D, t = −5·6333, p < 0·0005; C v D, t = −2·9624, 0·0025 > p > 0·0005 (α = 0·05).
irrespective controls

Degree of to regard chemotherapy.

la = No. of controls

The bacilluric control subjects in none of the patients, bacilluric biopsies from sections of site were statistically significant. However, biopsies taken of site in the one case. The reason for this was uncertain, as the biopsy was taken outside the trigone, the difference being statistically significant. However, as the ratio of trigonal to extratrigonal biopsies in bacilluric and non-bacilluric patients was almost identical this did not invalidate our conclusions concerning the

Table II Histology and sex

Males v females: group A t = 0.2749; group B t = -1.5224, group C t = -1.4935; p > 0.05 (2α = 0.05).

Table III Histology and site of biopsy in patients

1 The biopsy site was uncertain in one case.

t = -2.2681, 0.025 > p > 0.02 (2α = 0.05)

relationship between infection and lymphocytic infiltration.

SYMPTOMS

In 25 women (33 biopsies) symptoms present at the time of biopsy were recorded by one observer on a six-point scale, allowing one point for each of the following: pain during voiding, pain after voiding, frequency, urgency, nocturia, and suprapubic pain. The mean score per biopsy in each histological grade, and the distribution of scores, did not suggest any relationship between symptoms and histological classification.

The frequency of the above symptoms during the pre-biopsy observation period was considered in relation to the histological grade. Two points were awarded for each symptom present at 50% or more of pre-biopsy visits; one for each symptom present at 25 to 49% of visits; and none for symptoms

Table IV Focal abnormalities and trigonal hyperaemia on cystoscopy

1a = Absent, b = moderate, c = severe. (a) v (b + c): t = 0.2639, p > 0.05 (α = 0.05).
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occurring less frequently. Consideration of the mean scores and the distribution of scores did not suggest that such symptoms occurred more frequently in patients with the highest grades.

Cystoscopic Appearance (Table IV)
The appearance of the trigone was allotted to one of three grades, according to the presence and severity of hyperaemia as seen on cystoscopy. The higher histological grades were commonly found in bladders without trigonal hyperaemia and vice versa. Neither were trigonal hyperaemia and subepithelial histology related when only biopsies taken from the trigone itself were considered.

A special note was made of the presence or absence of focal abnormalities. The finding of small pink or grey nodules on cystoscopy corresponded to histological grades III and IV, but the latter were often found when no local abnormality had been found at cystoscopy.

SEROLOGICAL STUDIES
Only patients with biopsies graded II, III, and IV were studied.

Sera from all five non-bacilluric patients gave normal titres when tested against the 11 standard O antigens.

Sixteen bacilluric patients were studied. Three of the seven whose biopsies showed grade III changes had high titres at the time of infection but two of these had clinical evidence of upper urinary tract infection. Agglutination titres in the third patient (S.G.) rose from 1:640 to 1:1280 at the time of biopsy; she had no clinical or radiological evidence of upper urinary tract infection, and bilateral ureteric catheterization performed according to Stamey, Govan, and Palmer (1965) suggested that the infection was confined to the lower urinary tract.

Seven of the nine bacilluric patients whose biopsies showed grade IV changes had abnormal titres (high, or not less than a four-fold rise in titre within the 'normal' range); however, all except one had good evidence of upper urinary infection. The exception was the patient (S.G.) referred to above, who had a second biopsy.

Thus in only one patient, from whom two biopsies were taken, was there a suggestion that heavy lymphocytic infiltration of the bladder by itself could result in abnormal agglutination titres in serum.

Observer Error in Histological Classification
Two authors independently classified 32 of the biopsies from patients, the source of the biopsy being concealed. They agreed over all but three biopsies, differing in their assessment of these by only one grade.

Blind examination of sections from control subjects mixed with similarly sized biopsies from patients provided no evidence that knowledge of the origin of control sections (which had sometimes seemed identifiable by their larger size) had favoured bias to a lower grade assessment.

Discussion
There has been disagreement for many years about the significance of collections of lymphocytes in the bladder wall. Some authors have thought them normal (Hamburger, 1879; Weichselbaum, 1881; Alexander, 1893; Asschuff, 1894; and Böhm and Von Davidoff, 1895) but Chiari (1881) and Przewoski (1889) believed that they signified infection. This is implied in the term 'cystitis follicularis', used to denote subepithelial lymphoid follicles and considered by some to be a common sequel to prolonged urinary infection (Hinman and Cordonnier, 1935; Stirling and Ash, 1941). However, Kretschmer (1952) believed that infection was common, but follicular changes were rare. Mitchell and Andrews (1953) found lymph follicles in most patients with bladder neck obstruction but did not attribute them to infection. Pugh (1967) grouped cystitis follicularis with other varieties of 'hyperplastic cystitis' which he believed were not genuine types of cystitis.

These opinions were based on inadequate bacteriological methods, for the importance of quantitative cultures of urine was not recognized. Our results suggest that the lamina propria of the normal bladder contains relatively few lymphocytes. However, biopsies from bacilluric patients nearly always contained a heavy diffuse or focal lymphocytic infiltrate and germinal follicles were seen in almost half. Biopsies from patients with a long history of 'cystitis', but without significant bacilluria during prolonged follow up (often said to have a 'urethritis'), showed a degree of lymphocytic infiltration which was intermediate in severity between that of the uninfected controls and the bacilluric patients. This might suggest a less frequent or intense antigenic stimulation than occurs in bacilluric patients, which might be of unrecognized bacterial, or even viral, origin (Numazaki, Shigeta, Kumasaka, Miyazawa, Yamanaka, Yano, Takai, and Ishida, 1968).

Comparison of necropsy material with that obtained from patients could lead to fallacious conclusions. Although there were more males in our control than in our patient groups, we found no evidence that women have more lymphocytes in the lamina propria of the bladder than do men, other than as a result of their increased susceptibility to urinary infection. Age distribution might be impor-
tant, for old age impairs ability to synthesize antibody (Raffel, 1961). We found no evidence of any reduction in lymphocytic infiltration with increasing age, and the distribution of ages in patient and control groups was similar. The ages of the uninfected subjects (35.7 ± 22.4 years) were significantly lower than those of infected subjects (46.4 ± 17.1 years; 0.05 > p > 0.025), a tendency commented on elsewhere (Marsh, Murray, and Panchamia, 1972). The process of dying might produce some lymphocyte depletion. However, the modes of death of the infected controls were comparable to those of the uninfected controls, although their sections showed much more lymphocytic infiltration.

We found no evidence that the histological changes discussed were directly associated with symptoms, either at the time of biopsy or when considered over a period of time before biopsy. Neither were they related to the degree of trigonal hyperaemia observed at cystoscopy. However, the changes were those of chronic inflammation (predominantly lymphocytic, although plasma cells were occasionally prominent) rather than acute inflammation.

Cystoscopy of bladders whose biopsies showed marked lymphocytic infiltration or germinal follicles (grades III and IV) often revealed pink or pinkish-grey nodules, presumably composed of focal collections of lymphocytes, although the latter were often found without cystoscopically visible nodulation. Similar nodules were described at necropsy by Chiari (1881), Przewoski (1889), and Alexander (1893). Hinman and Cordonnier (1935) noted them to be particularly prominent over the trigone at cystoscopy, with which we agree. Ziegler (1886) noted that such nodules may resemble tubercles. They led to a suggested diagnosis of tuberculosis in one of our patients, and we know of another in whom their visualization at cystoscopy resulted in antituberculous chemotherapy being incorrectly instituted.

The function of these lymphocyte collections is not established. If bursa-dependent, they may be concerned in the production of humoral antibody and its transfer into urine (via the luminal surface of the bladder) or blood. The epithelium overlying the germinal follicles was often thinned, and sometimes apparently absent (fig 4). IgA (s) (including antibodies to E. coli) has been found in normal urine (Bienenstock and Tomasi, 1968). Its concentration is significantly higher in urine from children with recurrent urinary infection than from those without infection (Uehling and Steihm, 1971), and it can be produced in the bladder (Kaufman, Katz, and McIntosh, 1970) where it might be locally protective. There is also evidence that a secretory form of IgE may be produced locally in the urinary tract (Burdon, 1973). Our findings provide a histological basis for these concepts. Although protection against lower urinary tract infection may predominantly involve hydrokinetic mechanisms, many other factors can be invoked (Marsh, 1973). The antibacterial activity of intact bladder tissue may inhibit bacterial multiplication between voiding. However, its mechanism and its importance are uncertain. The local antibacterial activity of the bladder is not related to serum agglutination titres against the infecting bacteria (Norden, Green, and Kass, 1968).

Although renal parenchymal infection is reputed to cause high agglutination or haemagglutination titres to the 0 antigen of homologous infecting E. coli, while lower urinary tract infection is said to result in low titres (Percival et al, 1964; Andersen, Hanson, Lincoln, Ørskov, Ørskov, and Winberg, 1965; Vosti, Monto, and Rantz, 1965), unless other tissues such as prostate are involved, this has been found unreliable (Gower, Taylor, Koutsianamis, and Roberts, 1972) and it cannot be the whole story. E. coli infection in the isolated bladder pouch of the dog results in 0 haemagglutination titres comparable to those reached in renal infections (Darwish, Staubitz, Scheuller, Rubin, and Neter, 1968). In one patient out of those whose sera we studied using serial agglutination techniques, the abnormally high titres found could not be attributed to renal infection. This case suggests that bacterial cystitis in man may sometimes cause high titres in serum.

References

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II Squamous change in the bladder epithelium

SYNOPSIS The occurrence of squamous change in the bladder epithelium was studied in bladder biopsies from patients with recurrent urinary infections or symptoms of cystitis and comparisons were made with sections taken at necropsy from control subjects. Two types of squamous change, termed 'vaginal' and 'cornified', are described. Vaginal change was frequent in females, and seemed to be a normal finding in them, but was not observed in males. It did not seem to be caused by urinary infection. Cornified change was also common in females and was found in one (infected) man. Like vaginal change it was most often found in sections from the trigone. Urinary infection may predispose to its formation but we were unable to prove this. Squamous change did not seem to cause symptoms, and could not usually be recognized at cystoscopy. The varieties described are so common that they are unlikely to be markedly precarcinomatous.

The literature concerning the significance of squamous epithelial change in the bladder is inadequate. During a prospective study of women with recurrent urinary infections we took 50 bladder biopsies (usually from the trigone) from 41 patients who presented with recurrent or persistent symptoms suggesting infection. Twenty-four developed urinary infection during follow up but in 11 there was no evidence of infection throughout prolonged observation; six were regarded as of uncertain bacilluric status as they were observed for too short a time. Only one biopsy was taken at each cystoscopy. The frequency with which squamous change was found in these biopsies, and its nature, was compared with that in sections made at necropsy from the trigones, and in some cases the vaults also, of bladders from 42 control subjects. We could find no evidence of previous urinary infection in 30 of these but 12 had suggestive evidence of antemortem infection.

Details of patients and controls, and our bacteriological and statistical methods, have been given elsewhere (Marsh, Banerjee, and Panchamia, 1974).

Histological Classification

Two types of squamous epithelium were found:

1 VAGINAL SQUAMOUS EPITHELIUM (Fig 1) This thick epithelium was composed largely of polygonal cells with clear cytoplasm; the epithelial
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