CHRONIC INFLAMMATORY DISEASE OF THE MALE LOWER GENITO-URINARY TRACT

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"The greater our knowledge increases

the greater our ignorance unfolds"

John F Kennedy, 1962

CHRONIC INFLAMMATORY DISEASE OF THE MALE LOWER GENITO - URINARY TRACT

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SUBMISSION FOR MS THESIS

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DISCUSSION

ABSTRACT

The underlying aetiology and pathophysiology of chronic abacterial prostatitis is poorly understood.

The study of patients with chronic prostatitis and normal controls by transrectal ultrasound identified seven signs associated with a diagnosis of chronic prostatitis. A cohort of sixty patients with chronic abacterial prostatitis (CABP), based on standard localisation criteria, was constructed. These patients underwent transrectal ultrasound and subsequent guided biopsy of any parenchymal abnormalities, thereby overcoming the problem of urethral contamination. The tissue so obtained was submitted for microbiological, histological and immunological study.

Within the cohort no organism was isolated consistently from either prostatic secretion or tissue. In particular Chlamydia trachomatis, Mycoplasma hominis and Ureaplasma urealyticum could not be identified. A chronic inflammatory infiltrate was detected in 85% of the cohort, yet no controls, thereby vindicating the biopsy technique. However, no specific histological pattern could be attributed to CABP.

Immunological analysis of the prostatic tissue suggested the inflammatory process was stimulated by a persistent

antigen and was in keeping with a cell mediated, type IV hypersensitivity reaction. Urinary flow rates were subnormal in 27% of the cohort. In selected cases, intraprostatic urinary reflux was demonstrated, and postulated, as being responsible for the transportation of the inciting antigen, whose nature remains unknown, yet probably is non-organismal.

Serum PSA was unhelpful in diagnosis and management of CABP. No evidence of a psychological role in the aetiology of CABP was identified.

A possible link between acute epididymitis and inflammatory prostatic disease was noted on transrectal ultrasound; intraprostatic and vasal reflux being a proposed unifying factor. In acute epididymitis the role of Chlamydia trachomatis and Enterobacteriaceae was confirmed, and Ureaplasma urealyticum discovered.

Thus CABP appears to be an active immunological reaction in response to a persistent antigen whose nature, although unknown, is possibly non-organismal and transported into the prostate by urinary reflux.

INTRODUCTION

The lack of progress in understanding the nature of chronic prostatitis as evidenced by the quotations "Chronic prostatitis... is relatively of much more frequent occurrence than is the acute form of the disease but is much less understood", (White & Wood, 1898) and "except for true bacterial prostatitis... little more is known about prostatitis than was reported by Hugh H. Young and associates in 1906" (Stamey, 1981), stimulated my research into the subject.

The prostate gland features prominently in the clinical practice of any urologist, yet most research into this organ has dwelt on benign hyperplasia and carcinoma. Inflammatory diseases of the prostate gland, in particular chronic prostatitis, although common, have in general been neglected. The reason for this omission appears to be the lack of specificity of the symptoms, absence of physical signs and the diagnostic difficulties encountered in prostatitis. Patients therefore comprise a heterogeneous collective and are subsequently managed poorly, as little attempt has been made to correct the problems in diagnosis and define aetiology; not surprisingly much of the treatment, through being empirical, is unsuccessful.

The prime difficulty in diagnosis is the apparently inescapable dilemma of urethral contamination of prostatic

secretions. Thus, the idea of obtaining prostatic tissue by means of biopsy was proposed. However, the prostate of a young man is not only small but relatively mobile, making "blind biopsy" both imprecise and hazardous. In view of the advent of transrectal ultrasound and thus accurate biopsy, could abnormalities be detected in the chronically inflamed gland? If so, could accurate biopsy answer outstanding questions regarding aetiological organisms in chronic abacterial prostatitis? With the tissue obtained would histological and immunological analysis provide some clues to aetiology and enable more appropriate therapy?

This research first investigated a group of normal controls before studying a cohort of patients with chronic abacterial prostatitis and concentrated on attempts to define the aetiology and immunopathology of this condition. In addition other avenues were explored to asses the role played by mechanical and psycho- logical factors in the natural history of chronic abacterial prostatitis.

GENERAL STATEMENT OF PROBLEMS TO BE INVESTIGATED

This thesis is directed towards determining the aetiology of chronic abacterial prostatitis by posing the following questions:

1. In patients with chronic abacterial prostatitis are there any identifiable abnormalities on transrectal prostatic ultrasound? Do they alter with time and can these abnormalities be used as an aid to diagnosis?

2. By performing transperineal biopsy of abnormal areas of the prostate under ultrasound control, and thereby overcoming urethral contamination, is it possible to culture any organisms, in particular chlamydiae, mycoplasmas and ureaplasmas which may be involved in the aetiology of chronic abacterial prostatitis?

3. Does routine histological analysis of tissue obtained by ultrasound guided biopsy from abnormal areas of the prostate provide any useful information about the aetiology and natural history of chronic abacterial prostatitis?

4. Does the presence of tissue antibodies within prostatic tissue, obtained by ultrasound guided biopsy, provide any information regarding the

aetiology of chronic abacterial prostatitis?

5. By means of monoclonal antibodies applied to prostatic tissue, obtained by ultrasound guided biopsy, can the immunopathology of chronic abacterial prostatitis be defined?

6. Does estimation of prostate specific antigen (PSA) within the serum aid diagnosis in chronic abacterial prostatitis?

7. Do free urinary flow rates in chronic abacterial prostatitis provide any prognostic or aetiological information in chronic abacterial prostatitis. What role does intra-prostatic urinary reflux play?

8. Do patients with chronic abacterial prostatitis have a greater tendency towards the development of neurotic psychiatric illness compared to patients with prostatodynia or the general urological outpatient population?

9. Is there any link between epididymitis and inflammatory prostatic disease as assessed by transrectal ultrasound, serological studies and epididymal aspiration?

10. Is it possible to construct an animal model of chlamydial prostatitis?

HISTORICAL REVIEW

This review of the literature on chronic prostatitis has been broken down into sub-headings so that it corresponds to the subsequent clinical and experimental studies that comprise this thesis.

Introduction

The first reference to the prostate was attributed to Herophilus in 350 BC (von Lackum, 1933). However, his description was of a bifid organ and therefore probably referred to the seminal vesicles rather than the prostate. Indeed Versalius, in his description of the prostate also mentioned a bilobed structure which may reflect that the subject material was animal, such as a dog, rather than human (Werschub, 1970).

In 1536 Nicola Massa detailed an organ in the male pelvis equivalent to the prostate, but failed to make any sketches or afford it a name (Werschub, 1970). Versalius in Tabula 1 of Tabula Anatomica Sex, drew a bilobed structure below the bladder, but it was not until Fabrica by the same author, that this organ was named: corpus glandulosum and assistens glandulosus. The term prostate was first used by Casper Batholin in 1611.

Although a detailed knowledge of this organ has only been available over the past 400 years, the ancients were aware

of the effects of inflammatory diseases. In Mesopotamia (3400-1200 BC), two groups were involved with medical care: the Asutu (physicians) and the Asiputu (magical experts). "If a man in his sleep (or) in his walking has seminal discharge and he does not know that he went to his wife and his penis and his cloth are full of seminal fluid... he will recover" ascribed to the Asutu, may be a reference to the spontaneous discharge of a prostatic abscess. Furthermore, "when he walks his semen flows out without his knowing it as if he had gone into a woman...," may be a description, again by the Asutu, of either prostatorrhoea or indeed an urethral discharge (Werschub, 1970).

Naturally, little objective information about prostatitis was available until the anatomy was appreciated. In his treatise on venereal diseases, (Hunter, 1786) describes prostatic inflammation as arising from distention and irritation of the urethra secondary to a stricture, instillations or violent gonorrhoea. Brodie (1832) later described the management of prostatic abscess.

The close proximity of the bladder and prostate made the distinction between cystitis and prostatitis difficult, a problem that persisted until the localisation studies of Stamey and colleagues (1965). Legneau in 1815 was the first to describe prostatitis as a frequent complication of urethritis, but it was not until 1838 that Verdier provided an accurate statement on the pathology of

prostatitis (von Lackum, 1933). A detailed account of chronic prostatitis appeared in Sir Henry Thompson's, Diseases of the Prostate (1861) and this heralded a steady supply of articles in the world literature, many of which have served to cloud rather than elucidate understanding of the condition.

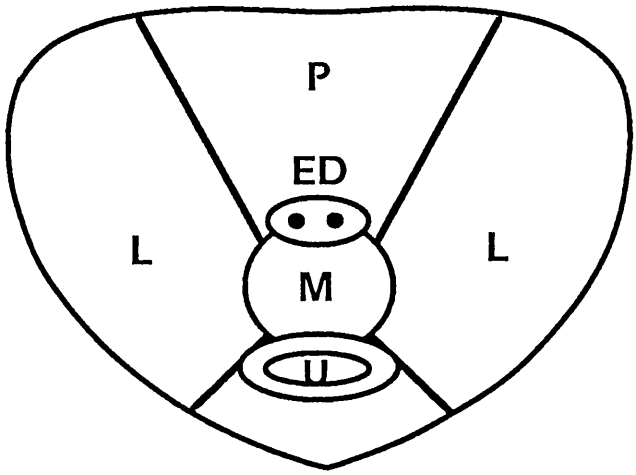
SECTION 1 - PROSTATIC ANATOMY

The earliest description of detailed prostatic anatomy by Lowsley (1912) divided the gland into a series of lobes depending on their relation to the ejaculatory ducts, (Fig 1.1). Subdivisions were based on embryological studies (Lowsley, 1912; 1930), but the "lobes" become distorted by disease and ageing and are unidentifiable as separate entities in the adult and thus, have little clinical relevance. A legacy of this work, however, is the concept that the periurethral glands are not part of the prostate but separate structures which are completely encased by the prostate gland. A simplified sub-division of the gland, as a result of the effect of oestrogens on the various regions, was devised by Huggins (1948) (Fig 1.2). The central area, degenerated under the influence of oestrogens, whereas the posterior area, designated the posterior lobe, was oestrogen resistant. This latter region was noted to be the part of the gland most susceptible to carcinoma. A number of studies (Franks, 1954; Strahan, 1963) threw doubt on the validity of this subdivision, by finding no difference in the incidence of carcinoma between the anterior and posterior lobes. As a result of interest in the pathogenesis of benign modular hyperplasia, Franks (1954) described the inner prostate glands, in truth the periurethral glands, and the outer prostate glands. These latter structures would be regarded as the whole prostate in the currently accepted classification. Amidst much semantic confusion McNeal

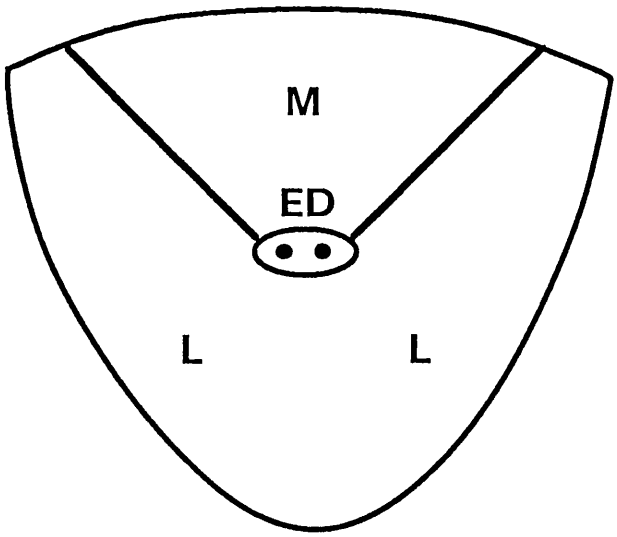
Fig. 1.1

Lobar Theory of Prostate Anatomy - after Lowsley

Transverse section



Sagittal section posterior to urethra.



Key:

P - Posterior lobe.

M - Middle lobe.

L - Lateral lobe.

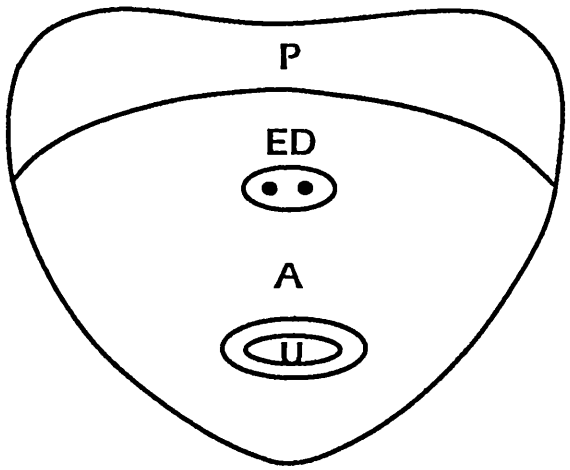
ED - Ejaculatory ducts.

U - Urethra.

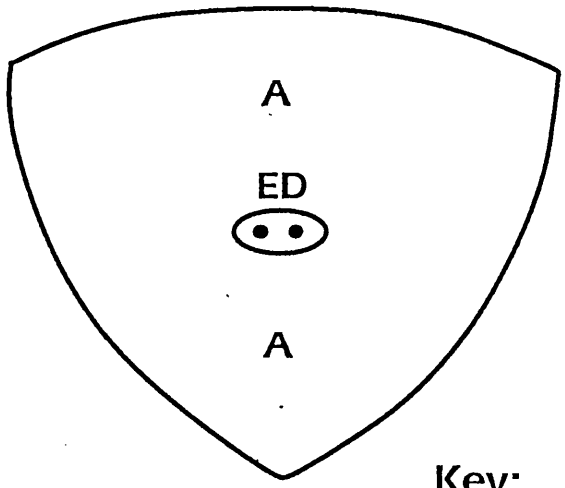
Fig. 1.2

Lobar Theory of Prostate Anatomy - after Huggins

Transverse section



Sagittal section posterior to urethra



Key:

P - Posterior lobe.

A - Anterior region.

ED - Ejaculatory duct.

U - Urethra.

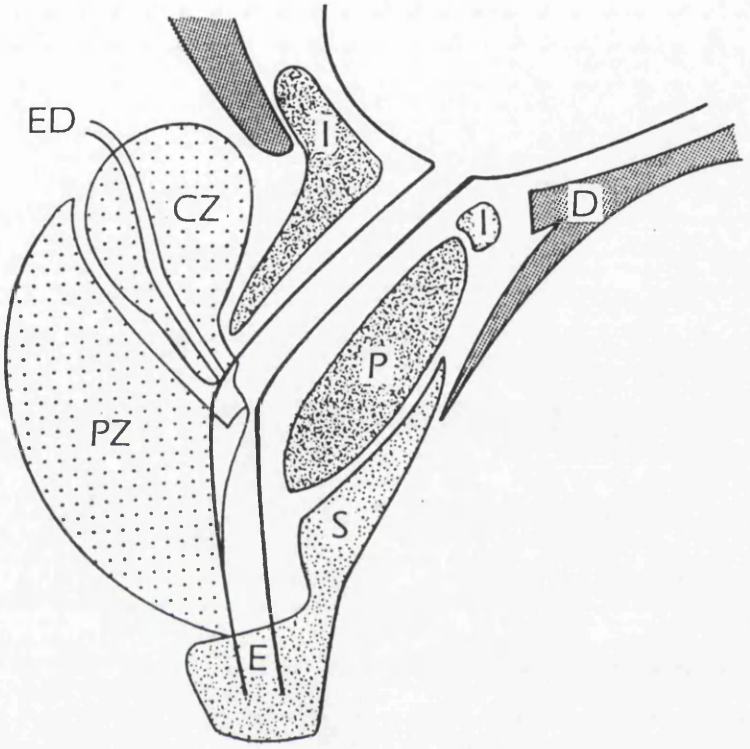
(1968) clarified the situation with the terms central and peripheral zone, (Figs 1.3 and 1.4). The differentiation was noted after sectioning the gland not only in the transverse and coronal planes but also in the plane of the ejaculatory ducts. The latter section had the advantage of exposure of more glandular tissue in one cut than any other section and as duct branches were cut in longitudinal section it enabled their course to be traced; the probability of identifying focal changes within the gland was thereby increased.

The classification of McNeal (1968) consisted of a central zone and peripheral zone. The former comprises an inverted conical structure with it's apex at the verumontanum and base directed towards the bladder. It surrounds and extends superiorly to the ejaculatory ducts and is separated from the peripheral zone, which lies inferiorly and laterally, by a narrow band of stroma. The zones of the prostate are thus like stacked cups, with the central urethra and periurethral glands lying within the central zone which in turn lies within the peripheral zone.

The analogy is incomplete however, as below the verumontanum the central zone is absent and the peripheral zone does not extend above the ejaculatory ducts. The zonal theory has achieved further credibility in the finding of histological differences between the two zones. The central area contains elaborately branching ducts

Fig. 1.3

Sagittal Section of Human Prostate (after McNeal)



D - Detrusor

I - Internal sphincter

P - Preprostatic sphincter

CZ - Central zone

PZ - Peripheral zone

ED - Ejaculatory duct

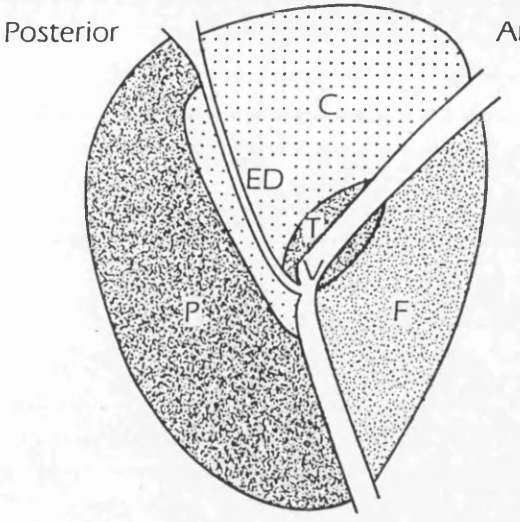
S - Prostatic sphincter

E - External sphincter

Fig. 1.4

Schematic Representation of Human Prostate (After McNeal)

Sagittal



U - Urethra

V - Verumontanum

C - Central zone

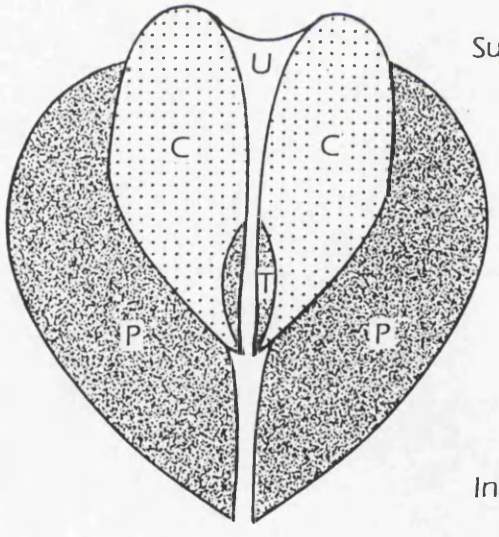
T - Transitional zone

P - Peripheral zone

F - Anterior fibromuscular septum

ED - Ejaculatory duct

Coronal



with terminal sacculations, comprising large rectangular structures with prominent intralunminal partitions. In contrast the peripheral zone has simple branching ducts and smaller less partitioned sacculations such that the latter appear strung out along a greater length of the duct's course (McNeal 1968). In addition, the stroma appears less dense in the central zone.

The periurethral glands, which never fully develop due to their encasement in the pre-prostatic sphincter muscle, comprise approximately 1% of prostatic glandular tissue. However, the most distal of the glands, close to the verumontanum, are free to develop beyond the confines of the pre-prostatic sphincter by escaping under it's lower margin. Although histologically very similar to the peripheral zone, this tissue develops in the long axis of the sphincter and the urethra towards the bladder neck. This region, the transition zone (McNeal, 1981) accounts for approximatley 5% of normal prostatic glandular tissue.

The arrangement of the sphincter musculature has also been studied extensively. The bladder neck muscle is felt to be simply an extension of the detrusor muscle which fans out around the urethra to provide an encasing muscular tube (Tanagho & Smith, 1966; Hutch 1967; Hutch & Rambo, 1967; Tanagho & Smith, 1968). The internal sphincter composed of two muscles, neither of which completely encircle the urethra, is neither a true anatomic sphincter nor anatomical entity (Hutch, 1965; 1966; Tanagho & Smith,

1966; Hutch, 1971). The muscle of the trigone forms a wedge posterior to the meatus and a portion of the middle layer of the detrusor passes anteriorly to fuse with the trigonal muscle laterally; this composite structure comprises the internal sphincter. McNeal's view of the urethra and bladder neck (McNeal, 1972) varied slighty from the above description. In his opinion the urethral mucosa is encased in a stroma containing scattered longitudinal muscle fibres most notable anteriorly and laterally. The majority of these fibres fail to reach the bladder, but those that do, extend as a sub-mucosal layer continuous with the detrusor. In the region of the internal meatus, McNeal regarded the muscle fibres as forming two sheets with parallel transverse fibres positioned either side of the meatus and containing abundant interdigitations between the sheets. The muscle fibres form compact bundles that completely encircle the urethra, in the deep layers of the sphincter and although of detrusor origin, contrast markedly with the woven architecture normally attributed to this muscle.

Distal to the bladder neck the urethra is encased by cylindrical muscle (Tanagho & Smith, 1966; Hutch, 1967) termed the pre-prostatic sphincter by McNeal (1972). Although contiguous with the internal sphincter, it's fibres, a compact array of concentric rings around the urethra, are neither continuous nor interdigitated with the detrusor fibres. Anteriorly these concentric fibres are less densely packed, and longitudinal fibres

proximally yet fibrous tissue distally, occupy the space.

The distal sphincter complex, is referred to as the prostatic sphincter proximal to the apex of the prostate and the external sphincter distal to this point. Skeletal muscle fibres pass transversely and insert into the lateral part of the peripheral zone. As these fibres pass proximally they become progressively more attenuated and fade out at verumontanum level. The pre-prostatic and prostatic sphincters overlap, the former extending along 75% of the urethra and lying posterior to the prostatic sphincter in it's distal extent.

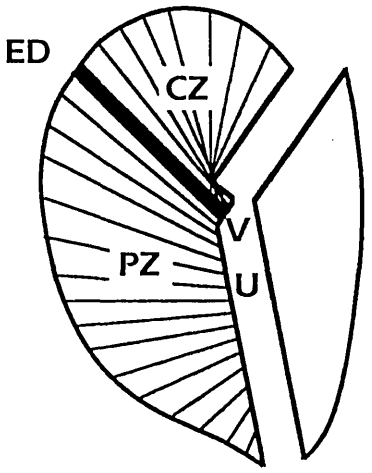
As with it's gross structure, the orientation of the draining ducts differs within the various zones of the prostate. Those draining the central zone empty onto the verumontanum clustered around the ejaculatory ducts, whereas those of the transition and peripheral zones empty via ducts whose orifices form two parallel lines from bladder neck to apex and pass either side of the verumontanum (McNeal, 1981). The ducts possess no sphincters at their exits into the urethra and in the central zone are shorter, wider and directed obliquely in the direction of urine flow. In contrast, those of the peripheral zone have a longer, more tortuous course and are orientated either horizontally or obliquely against the urine flow (Blacklock, 1974). It has been postulated (Blacklock, 1974) that the difference in duct direction predisposes the peripheral zone to retention of secretions

and debris, and thus a greater susceptibility to infection.

Fig. 1.5

Prostatic Duct Orientation - after Blacklock

Sagittal section.



Key:

CZ - Central zone.

PZ - Peripheral zone.

ED - Ejaculatory duct.

V - Verumontanum.

U - Urethra.

SUMMARY

The zonal theory of prostate anatomy has now achieved general acceptance and needs to be considered when imaging the prostate as well as in studying the pathology of the gland, as different zones appear susceptible to different disease processes. Furthermore, the ductal orientation of the various zones may provide a clue to why the zones have differing susceptibilities to involvement in inflammatory disease processes.

SECTION 2 CHRONIC PROSTATITIS - CLASSIFICATION, DIAGNOSIS AND AETIOLOGY

The incidence of prostatitis is very difficult to assess. On the basis of clinical experience, Stamey (1981) loosely gauged the incidence of acute and chronic bacterial prostatitis as very low, since recurrent bacteriuria in the male is uncommon, in contrast to the greater incidence of both chronic abacterial prostatitis and prostatodynia. If autopsy data is used as a guide (Moore, 1937), with the risk of over estimation if histological criteria alone are used, then approximately 5% of men have some form of prostatitis. As no clinical data was available in this study, the figures are of little value.

Also, Kohnen and Drach (1979) detected inflammatory changes in 98% of surgically resected prostates. In a study of over 300 consecutive new patients presenting to an urology clinic (Schaeffer et al, 1981), 10% of normal people and patients with non-inflammatory urological disease were found to have evidence of prostatitis using the generally accepted criteria of Anderson and Weller (1979). Employing these criteria, 13% were diagnosed as having prostatitis, 12% of whom had acute or chronic bacterial prostatitis and 88% abacterial prostatitis; 63% of the latter group were symptomatic (Schaeffer et al, 1981).

CLASSIFICATION

Verdier undoubtedly opened a Pandora's box with the first accurate description of prostatitis in 1838 (von Lackum, 1933). Thompson (1861) classified the prostatitides into acute and chronic forms; the latter prompted the comment "it's existence is barely recognised by some of the best known writers on prostatic disease and by some it is not even named" (Christian, 1899). It is likely that at this stage true ignorance was to blame for these omissions rather than the denial commonly practiced at the present time. Thompson (1861) saw chronic prostatitis in three phases which differed in degree rather than kind: subsequent to an acute prostatitis with no resolution, the same but with a slow resolution and de novo chronic inflammation.

The need for a classification of inflammatory prostatic diseases was evident: "the term prostatitis is often loosely used to denote several different pathological conditions, each of which is characterised by more or less distinctive signs" (Durand, 1898). Several authors referred to the terms catarrhal, follicular and parenchymatous prostatitis (Durand, 1898; Christian, 1899; Martin, 1899), which described theoretical pathological processes based on the findings of prostate palpation per rectum. These views were endorsed by Keyes (1917), in his textbook of urology when he loosely defined the forms of prostatitis: catarrhal - prostatic ductal inflammation,

follicular - inflammation of the ducts, acini and surrounding stroma, parenchymatous - intense inflammation of the whole gland involving wide areas of stroma with a predilection for abscess formation.

The diagnosis rested mainly on clinical criteria, in particular palpation of the prostate and the finding of pus and/or organisms within the prostatic fluid. However, as the symptomatology of perigenital pain, irritative voiding, voiding dysfunction and urethral discharge, was non-specific and thus unable to localise the inflammation to specific sites in the uro-genital tract, it was impossible to distinguish between cystitis, prostatitis and urethritis on clinical criteria alone; a method of localisation was required. Several authors (von Sehlen, 1893; Krotoszyner & Spencer, 1894) described the three glass test, consisting of a first void urine representing the urethra and a mid-stream urine representing the bladder, followed by prostatic massage and collèction of secretion from the external urethral meatus. Each specimen was examined microscopically and cultured. The differentiation between acute and chronic forms was made on clinical grounds alone: speed of onset and degree of systemic illness. This clinical differentiation persists to this day. It was soon apparent that despite attempts at localisation studies, urethral contamination was a perennial problem. Thus, a variety of sampling techniques were employed, including irrigation of the urethra with saline and antiseptics

(Hitchens & Brown, 1913; Culver, 1916) and the use of elaborate endoscopes (Player et al, 1923) in order to obtain "pure", uncontaminated prostatic fluid.

Little additional progress was made in the classification of prostatitis until the work of Stamey and colleagues (1965), who stressed the importance of segmenting the voided bladder urine into three aliquots, namely, first void (VB1), mid-stream (VB2) and post-prostatic massage urine (VB3); the differentiation between urethral and prostatic infection in the presence of sterile mid-stream urine thus became possible. If however, the mid-stream urine is infected, the study is repeated after sterilisation of the urine with a suitable antimicrobial that does not enter the prostate readily; the true prostatic inflammatory pathology is thereby exposed. On the basis of this localisation technique (Fig. 2.1) a practical classification of prostatitis was devised (Drach et al, 1978) such that patients with clinical prostatitis fell into four groups: acute bacterial prostatitis (ABP), chronic bacterial prostatitis (CBP), chronic abacterial prostatitis (CABP) and prostatodynia (Pd), (Fig. 2.2). The patients are categorised according to the cytological and microbiological findings in both the urine and expressed prostatic secretion (EPS).

The acute and chronic bacterial forms of prostatitis are distinguished readily despite similar findings in the urine and EPS by the former group of patients being

Fig. 2.1

Segmented Urine Localisation Technique (after Meares and Stamey)

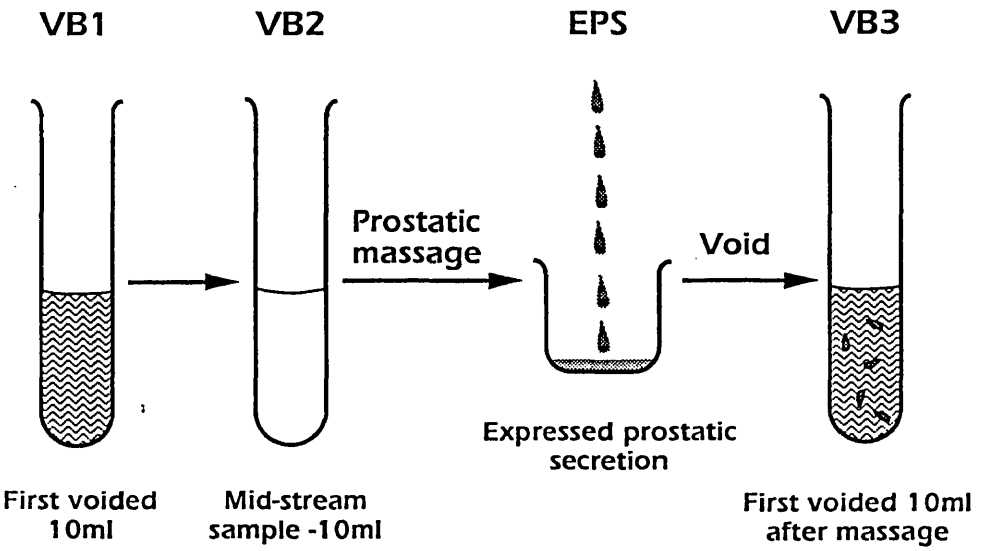


Fig 2.2 THE CLASSIFICATION OF PROSTATITIS BASED ON PROSTATIC LOCALISATION STUDIES

Category	MSU or VB2		EPS		Organism
	WCC	Culture	WCC	Culture	
ABP	++	+	++	+	Enterobacteriaceae
CBP	+	+	+	+	Enterobacteriaceae
CABP	-	-	+	-	Nil
Pd	-	-	-	-	Nil

KEY: ABP - Acute bacterial prostatitis

CBP - Chronic bacterial prostatitis

CABP - Chronic abacterial prostatitis

Pd[1] - Prostatodynia

WCC - White cell count

MSU - Mid-stream urine

EPS - Expressed prostatic secretion

(After Drach et al, 1978)

systemically unwell and having an exquisitely tender prostate on palpation; prostatic massage is felt to be inadvisable in ABP in view of the risks of inducing bacteraemia. Of greatest importance in the classification of the chronic form is the differentiation of those patients with an infective aetiology (CBP), those with inflammatory disease but no apparent infective aetiology (CABP) and those who, although symptomatic, do not appear to have any inflammatory disease in their prostate (Pd). It is impossible on clinical grounds alone to differentiate between the three groups as the symptoms of perigenital pain, irritative voiding and voiding dysfunction, are broadly similar.

Another variant of chronic prostatitis is the granulomatous form: this is a histological diagnosis characterised by foci of classically non-caseating granulomata with nodular aggregates of histiocytes and multinucleated giant cells. The majority of cases are associated with a recent urinary tract infection, though recent transurethral resection or needle biopsy may be the stimulus, while tuberculous infection and systemic granulomatous disease are only responsible occasionally.

DIAGNOSIS

In the early days following it's recognition, the diagnosis of prostatitis was clinical, with particular emphasis laid on the findings of rectal palpation of the prostate. Symptoms were described as varied, but broadly consisted of perigenital pain, most commonly perineal, sacral, thigh and testicular, irritative voiding, voiding dysfunction and urethral discharge or prostatorrhoea (White & Wood, 1898). The differentiation between acute and chronic prostatitis was made on the speed of onset of symptoms and degree of prostration. Keyes (1917) in his textbook of urology pointed out the difficulty of distinguishing chronic posterior urethritis from chronic prostatitis, but felt moved to describe the classical symptom of perineal pain thus: "the patient who suffers from this symptom cannot sit still for any length of time, though as a rule he prefers a hard seat to a soft one. He is debarred from the theatre and church, and on the railroad[1] he either sits obliquely on one hip or paces the aisle."

In addition to the classical symptoms, more peripheral complaints were recognised: painful or incomplete erections, premature ejaculation, profound mental depression and melancholia. These symptoms were apt to be greatly magnified by the patient (White & Wood, 1898).

The observation that many cases of chronic prostatitis

were symptomless was noted by several authors (Krotoszyner & Spencer, 1894; Young et al, 1906; Hume, 1910; Keyes, 1917). This conclusion was based, in part, on autopsy studies performed on patients without symptoms of prostatitis but in whom histological evidence of prostatitis was detected. Hume (1910) however, found incidental prostatitis in six out of eleven patients who presented with varicocele or phimosis. More recently Meares (1980) expressed the opinion that chronic bacterial prostatitis was the commonest cause of relapsing urinary tract infection in men and that many were asymptomatic.

The early workers were fully aware of the need for objective information and Swinburne (1898) observed the need for repeated examination of expressed prostatic secretion, a point reaffirmed by Fair (1984), as initial specimens may be nonrepresentative. The problem of urethral contamination was a real conundrum and remains so, although recently a four-channel, two-balloon catheter has been devised (Jin & Guagi, 1987) in order to isolate the prostatic urethra and thereby reduce contamination produced by prostatic secretion travelling along the urethra; this device theoretically conveys advantages but has yet to be evaluated fully in clinical practice. However, as mentioned above, the present classification of prostatitis is based on the Stamey localisation procedure (Meares & Stamey, 1968) which takes full account of the influences of the urethra and urine on prostatic

secretions. Although far from perfect, this technique has yet to be bettered or superceded and is a mandatory investigation for any patient with symptoms of chronic prostatitis.

The diagnosis of prostatitis rests on finding excessive numbers of white cells in the expressed prostatic secretion (EPS) and/or post-prostatic massage urine (VB3), over and above that found in urethral (VB1) and bladder (VB2) urine. As with much in prostatitis, there is disagreement over the level at which the EPS leucocyte count is deemed pathological. Normal individuals undoubtedly possess leucocytes in their EPS and the range of upper limit of normal varies from two white cells per high powered field (Anderson & Weller, 1979) to twenty cells per high powered field (Drach et al, 1978). Other authors have submitted upper limit of normal values within this range (Blacklock & Beavis, 1974; Pfau et al, 1978; Stamey, 1980; Schaeffer et al, 1981). However, to achieve some form of consensus a compromise has been struck: by means of a Fuchs-Rosenthal chamber, Anderson and Weller (1979) estimated the leucocyte count in the EPS of a group of normal men and concluded that ten leucocytes per high powered field was the upper limit of normal. This value is now the generally accepted level for a diagnosis of prostatitis, although with some reservation, as the leucocyte count in the EPS has been shown to vary between estimations in both normal individuals and prostatitis patients (O'Shaugnessy et al,

1956; Colleen & Mardh, 1975). As early as 1894, the effect of increasing the prostatic fluid leucocyte count by recent ejaculation was noted (Krotoszyner & Spencer, 1894), and was reiterated more recently (Jameson, 1967). Thus, it is advisable for patients to refrain from sexual activity for five days prior to prostatic fluid collection.

All too commonly, EPS cannot be obtained and the diagnosis therefore rests on finding excessive numbers of leucocytes in VB3, over and above that found in VB1 and VB2. In centrifuged urine, a leucocyte count of greater than or equal to four cells per high powered field (x 400), in excess of the count in VB1 and VB2 is highly suggestive of prostatitis, whereas greater than ten cells per high powered field is pathogmonic (Weidner & Ebner, 1985). In cases of a dry expressate, semen can be used in place of EPS, provided it is collected as part of a localisation study (Mobley, 1975). This technique has been advocated in cases of acute prostatitis, where prostatic massage is inadvisable, and cases where a dry expressate is encountered or there is poor patient compliance to prostatic massage. Although a close correlation of the bacteriological data existed between semen and EPS in this report, the technique has drawbacks as only 20% of semen volume arises from the prostate and also cytological interpretation is hampered by the immature spermatozoa closely resembling leucocytes (Ulstein et al, 1976). In order to implicate an organism in the aetiology of

prostatitis strict criteria have to be followed in view of the problem of urethral contamination. A great breakthrough was made by Meares and Stamey (1968) with a longitudinal study of patients with chronic bacterial prostatitis. The method has stood the test of time and it's value was reiterated in a review (Stamey, 1981), which included data on patients with a ten year follow-up period. The author advised at least a ten fold increase in the bacterial colony count obtained from the VB3 (post prostatic massage) aliquot and/or expressed prostatic secretion (EPS) compared to that obtained from VB1 (urethral urine) when the VB2 (mid-stream urine) is sterile. In practice, the bacterial colony counts in the EPS, when available, are often one to five hundred fold greater than VB1 and VB2 (Stamey, 1981). If the mid-stream urine is infected or results are equivocal, a short course of an antimicrobial such as penicillin or nitro-furantoin, which fails to penetrate the prostate, often sterilises the urine and urethra, and enables a 'pure' culture within the prostatic fluid to come to light.

These criteria for diagnosing bacterial prostatitis (Meares & Stamey, 1968) differ from those of Drach (1975), another researcher of renown in the field of prostatitis. The latter author is prepared to accept absolute colony counts as diagnostic: namely 5000 organisms per ml in the EPS or VB3 provided the count in VB1 and VB2 is less than 3000 organisms per ml. No cytological data on leucocyte

counts accommpanied the bacteriological data and thus there was no corroborative evidence to support actual infection by the organism cited (Drach, 1975). Stamey (1981) was critical of Drach's conclusions in view of the low colony counts and lack of data on the effect of antimicrobials, such as penicillin or nitrofurantoin, on the organisms isolated, and held the view that the organisms in Drach's work (1975) were simply contaminants.

AETIOLOGY

Although John Hunter (1786) in his treatise on venereal diseases described prostatic inflammation as a result of distention and irritation of the urethra secondary to a stricture, instillations or violent gonorrhoea, Legneau in 1815 was attributed with the observation that prostatitis was a complication of urethritis (von Lackum, 1933).

The gonococcus occupied centre stage in the aetiology of prostatitis, both acute and chronic, for many years (Thompson, 1861; Montagnon, 1885; Finger, 1893; Krotoszyner & Spencer, 1894; Martin, 1899; Young et al, 1906; Keyes, 1917). At the turn of this century it was felt to be responsible for 70 to 94% of cases of prostatitis (Montagnon, 1885; Eraud, 1886; Young et al, 1906). However, Notthaft (1904) observed increasing difficulty in isolating Neisseria gonorrhoeae with increasing chronicity of disease. The concept of the gonococcus reducing the resistance of the prostate and thereby allowing secondary infection by organisms such as streptococci was broached by Steinfeld (1918).

As a result of closer examination of prostatic secretions further organisms were implicated: bacillus coli (Friedman, 1919; Nelken, 1922; Wolbarst, 1925), staphylococci and non-faecal streptococci (Friedman, 1919; Richards, 1920; Nelken, 1922; Baker, 1925), faecal

streptococci (Rosenow, 1921) and diphtheroids (Nelken, 1922). At this early stage urethral contamination of prostatic secretions was recognised as a problem and irrigation of the urethra with antiseptics (Hitchens & Brown, 1913; Culver, 1916) prior to collection of prostatic fluid, and elaborate endoscopes (Player et al, 1923), were employed. As a result support for staphylococci, streptococci and coliforms as the causative organisms of prostatitis developed.

At this time two concepts became established, firstly the role of the prostate as a focus of infection within the genito-urinary tract and secondly, the systemic effects of prostatic infection. The prostate was felt to be a focal source of sepsis, resulting in lower and upper urinary tract infection, by several authors (Crabtree & Cabot, 1917; Culver, 1918; Strachstein, 1921; Wolbarst, 1925). Experimental studies at this time showed that organisms isolated from human prostates became localised to the prostate in experimental animals (Nickel, 1926).

The systemic effects of prostatitis were manifest primarily in the musculoskeletal system. Richards (1920) diagnosed prostatitis due to Streptococcus viridans in two patients with arthritis. Several authors also noted an association between prostatitis, arthritis and eye diseases, particularly iritis (Baker, 1925; Holloway & von Lackum, 1925; Benedict et al, 1927; Nickel & Stuhler, 1930). The arthritides associated with prostatitis

commonly affected the axial skeleton, such that two authors independently viewed prostatitis as the commonest cause of backache (Player, 1925; Wesson, 1927). Shea (1922) postulated that the systemic effects were due to the release of toxins by the offending organisms.

The precise role of organisms isolated from urine and prostatic secretions was not resolved until Stamey and colleagues (1965) indicated the value of segmenting the voided urine, as described earlier, and later highlighted the importance of quantifying organisms by colony counts in the urine and prostatic secretion samples (Meares & Stamey, 1968). It was appreciated from this point forward that the gram negative organisms and faecal streptococci were the most commonly implicated bacteria and the concept of subdividing chronic prostatitis into bacterial and abacterial forms was born.

The mode of transmission of infection in prostatitis was regarded generally as haematogeneous or via lymphatics (Hume, 1910; Nelken, 1922; Baker, 1925), with infected teeth and sinuses as possible foci of organisms. Preceding urethritis (Archibald, 1903; Hume, 1910) was seen as important by some authors and this notion of ascending infection via the urethra being a possible aetiological mechanism (Frederick, 1940; Ill, 1951; Meares, 1980) has persisted. The concept of de novo infection without predisposing factors was also appreciated (Hume, 1910).

During the early part of this century masturbation and sexual irregularities (Archibald, 1903; Leusman, 1905) and trauma, such as excessive exposure to cold or saddle, and instrumentation (Archibald, 1903) were regarded as predisposing factors in prostatitis. Similarly, prolonged prostatic congestion, as a result of sexual irregularities, such as coitus interruptus, was thought to lay the prostate open to infection (Bryant, 1910; Lowsley, 1912).

The aetiological organisms in acute and chronic bacterial prostatitis are well established and identical for the two conditions. The majority of cases are caused by the gram-negative Enterobacteriaceae family or gram-positive Streptococcus faecalis (Meares, 1987). Organisms such as Staphylococcus aureus (Giamarellou et al, 1982), Neisseria gonorrhoeae and Mycobacterium tuberculosis, are isolated rarely from patients with chronic prostatitis. In immunocompromised hosts, atypical bacteria and fungi are more likely to be causative (Lief & Sarfarazi, 1986; Clairmont et al, 1987). Protazoal infection within the prostate by Trichomonas vaginalis has also been described (van Laarhoven, 1987), but is uncommon.

Although mixed infections may occur, a single aetiological organism is isolated in approximately 80% of cases, being most commonly Escherichia coli, followed by Klebsiella species, Pseudomonas aeruginosa and Proteus species, in order of prevalence (Meares, 1987). The role of gram-positive organisms in chronic prostatitis has been controversial since the beginning of this century (Young et al, 1906; Friedman, 1919; Nelken, 1922; Baker, 1925), and continues to be a subject of intense debate. There is little doubt that Streptococcus faecalis has a definite aetiological role and not uncommonly, (in approximately half the cases in which it is isolated), in partnership with another organism (Meares, 1987). The main point of

discussion centres around organisms such as Staphylococcus epidermidis, micrococci, non group D streptococci and diphtheroids. As these organisms are skin and urethral commensals, unless scrupulous attention to detail is observed in the collection of samples during the Stamey localisation procedure (Meares & Stamey, 1968), they may cloud the diagnostic picture. Although the consensus of opinion views these gram-positive organisms as commensals and contaminants when isolated from patients with chronic prostatitis (Meares & Stamey, 1968; Meares, 1973; Stamey, 1981; Thin & Simmons, 1983; Jimenez-Cruz et al, 1984; Pfau, 1986; Meares, 1987), some authors (Drach, 1975; Greenberg et al, 1985; Drach, 1986) regard these organisms as pathogenic. The reason for this diversity of opinion may be the level at which a bacterial colony count is regarded as significant. Both Meares and Stamey (Meares & Stamey, 1968; Stamey, 1981) advocate a ten fold increase in the bacterial colony count obtained from VB3 (the post-prostatic massage urine aliquot) and/or EPS (expressed prostatic secretion) compared to that obtained from VB1 (urethral urine) when VB2 (mid-stream urine) is sterile. Drach (1975) however, is prepared to accept pure numbers of bacteria, namely 5000 organisms/ml in EPS and/or VB3 provided the count is less than 3000 organisms/ml in VB1 and VB2 (vide supra); these values leave little margin for error. Furthermore Meares and Stamey (1968) suggest sterilising the urine by means of nitrofurantoin or penicillin G, both of which have very poor prostatic penetration, if there is either an active

urinary tract infection at the time of testing or if bacterial colony count data are equivocal. Repeat localisation studies one week after such action usually clarifies the diagnosis. The data presented by Drach (1975), was not supported by leucocyte counts in the urine and prostatic secretion samples and thus there was no corroborative evidence that the gram-positive organisms were actually pathological and causing infection.

ABACTERIAL PROSTATITIS

By definition this form of chronic prostatitis is associated with an inflammatory response in the absence of any definite aetiological organism.

The possibility that Chlamydia trachomatis may be involved in the aetiology of chronic abacterial prostatitis was first raised by Màrdh and colleagues (1972) on the basis of serological studies. A serum complement fixing antibody against Chlamydia trachomatis was found at a titre of $\geq$ 1:5 in 33% of men with chronic prostatitis compared to only 3% of an age matched group of controls drawn from a blood donor population. However, serial serological estimations were not performed and neither was there any attempt to culture the organisms from prostatic secretions. A later study by the same group of workers (Màrdh et al, 1978) were unable to isolate C. trachomatis from the EPS of any of the 53 patients studies. Furthermore, significant titres of IgG and IgM antibodies to pooled chlamydial antigen, using a modified micro-immunofluorescence technique on this occasion, were detected in only 14% and 8% of cases respectively. In contrast, chlamydiae have been unequivocally established as a cause of non-gonococcal urethritis, accounting for 30-70% of cases, (Oriel et al, 1976; Alani et al, 1977). Conflicting information then ensued from a number of uncontrolled studies. In a review of Chlamydia trachomatis in genito-urinary diseases in general, Bruce

and colleagues (1981) claimed to have cultured the organism in 56% of patients studied with prostatitis, though the criteria for diagnosing prostatitis relied solely on clinical methods. In the same year another group (Nilsson et al, 1981) isolated chlamydiae from the prostatic secretion of a group of patients with acute urethritis, and concluded that this organism played an important role in prostatitis, although urethral contamination was not assessed or considered. The support grew for a positive role for Chlamydia trachomatis when Weidner and co-workers (1983) isolated the organism from urethral swabs after prostatic massage in 19% of cases of abacterial prostatitis versus 8% of normal controls. Although 71% of cases had serological titres supporting a chlamydial infection, only 47% of the group diagnosed as having prostatitis, had cytological evidence to support the diagnosis. A study of infertile men with seminal leucocytosis (Suominen et al, 1983) found chlamydial IgA antibodies within the semen in 51% of cases and as the authors felt seminal leucocytosis was a marker of chronic prostatitis, concluded that C. trachomatis was a common cause of prostatitis. It appeared that Poletti and co-workers (1985) had clinched the debate by isolating C. trachomatis from 33% of patients with chronic prostatitis who had undergone blind transrectal aspiration biopsy of the prostate. However, in every patient the organism was also detected in their urethra, an uncommonly high percentage even in non-gonococcal urethritis, where C. trachomatis undoubtedly plays a significant role; in this

study doubt must exist over the accuracy of the diagnosis of chronic abacterial prostatitis and thus the validity of the findings.

There is a body of opinion (Berger et al, 1987; 1989) that supports the conclusion of Màrdh and colleagues (1978) in their later study, namely that C. trachomatis has little or no influence in the aetiology of chronic abacterial prostatitis. This prospective study failed to isolate C. trachomatis in any of the patients who had been accurately diagnosed by standard localisation procedures.

The last word on this subject goes to the pro C. trachomatis lobby. Schurbaji and colleagues (1988) in a retrospective study of patients with histological evidence of prostatitis identified chlamydial antigen using a monoclonal antibody against C. trachomatis in 31% of cases. These data are undoubtedly interesting, but the group studied were by no means homogeneous and no details of criteria for diagnosis were available.

It has now been established that both Ureaplasma urealyticum (Shepard, 1970; Taylor-Robinson et al, 1977; Taylor-Robinson & McCormack, 1980) and Mycoplasma hominis (Hill et al, 1973; Taylor-Robinson & McCormack, 1980) play an aetiological role in non-gonococcal urethritis, but quantitative studies are essential, as both these organisms are found in approximately 20% of men aged less than 50 years attending hospital for a non-venereal

condition (Furr & Taylor-Robinson, 1987). Initial reports on these organisms in chronic prostatitis (Meares, 1973; Màrdh & Colleen, 1975) suggested that they had little or no aetiological role in the condition, as no difference in isolation rates were detected between patients and controls; there were no quantitative data in these reports. The first quantitative assessment of Ureaplasma urealyticum in chronic prostatitis (Brunner et al, 1983) found 13.7% of cases, in a series of over 500 patients, with colony counts indicative of prostatitis on standard localisation studies. Mycoplasma hominis showed the same pattern in less than 2% of cases, but was always accompanied by other organisms and thus was less clearly implicated. In contrast, Berger and colleagues (1989), in a prospective study of patients with proven chronic prostatitis, were unable to isolate Ureaplasma urealyticum at higher titres in the prostate than in the urethra in any of the patients, but did so in 26% of controls. The role of these organisms in chronic prostatitis remains unresolved.

The search for other aetiological agents in chronic abacterial prostatitis has met with greater unanimity. No evidence of a role for anaerobes (Meares, 1973; Nielsen & Justesen, 1974; Màrdh & Colleen, 1975) or viruses (Gordon et al, 1972; Nielsen & Vestergaard, 1973; Màrdh & Colleen, 1975) including herpes virus, cytomegalovirus, rubella, varicella-zoster and adenoviruses has been found. Similarly a search for fungi has proved unrewarding

(Màrdh & Colleen, 1975).

A major problem in assigning aetiology to an organism in prostatitis is urethral contamination of EPS. The localisation studies of Meares & Stamey (1968) certainly go a long way to eliminating this problem, but the viscid nature of prostatic secretions and their resultant slow passage along the urethra may "recruit" significant numbers of misleading urethral contaminants (Stamey, 1981). In an attempt to overcome this problem, a number of researchers have employed prostatic biopsy in their search for aetiological organisms (Schmidt & Patterson, 1966; Meares, 1973; Nielsen & Justesen, 1974; Poletti et al, 1985). All these studies employed "blind" biopsy techniques and as prostatitis is a focal disease (McNeal, 1968), it is not surprising that two groups (Schmidt & Patterson, 1966; Nielsen & Justesen, 1974) concluded that prostatic biopsy was of no consistent value in chronic prostatitis; it is equally surprising that Poletti and co-workers (1985) gained so much apparently conclusive data from their prostatic biopsies. Drach (1975) used cold-punch biopies of the prostate taken endoscopically and found an organism identical to that recovered from prostatic fluid in 84% of patients. Rather than supporting the aetiological role of an organism this latter technique serves only to indicate the ease of contamination of samples.

SUMMARY

The diagnosis of the four prostatitis syndromes, acute bacterial (ABP), chronic bacterial (CBP), chronic abacterial (CABP) and prostatodynia (Pd) is based on the findings of the Stamey localisation procedure, a mandatory investigation. The currently accepted diagnostic level of leucocytes within the samples is ten per high powered field (x1000) on examination of the expressed prostatic secretion (EPS) or four per high powered field (x400) in the post-massage (VB3) urine, over and above that found in the first void (VB1) and mid-stream (VB2) urine specimens.

The organisms responsible for chronic bacterial prostatitis are generally members of the gram-negative Enterobacteriaceae family, most commonly Escherichia coli, or the gram-positive Streptococcus faecalis. A number of organisms, particularly Chlamydia trachomatis and Ureaplasma urealyticum have been cited as aetiological organisms in chronic abacterial prostatitis, however the data are conflicting and many of the studies supporting their role fail to exclude urethral contamination of prostatic fluid by these agents. As yet no organism has been proven unequivocally to be responsible for this condition.

Prostatic biopsy to date has failed to yield useful information concerning the role of infectious agents in chronic prostatitis, due chiefly to the "blind" nature of the procedure and the focal distribution of inflammatory changes.

SECTION 3 TRANSRECTAL PROSTATIC ULTRASOUND - HISTORY, BIOPSY TECHNIQUES, NORMAL GLAND AND INFLAMMATORY PROSTATIC DISEASE.

HISTORY

The first attempts to visualise the prostate by means of ultrasound used the transrectal route (Wild & Reid, 1955). The device, an intrarectal transducer mounted on a screw driven gantry, could be rotated and withdrawn from the rectum simultaneously. However, only images of the rectal wall could be obtained and the technique fell from favour. During the following decades several authors reported the use of an A mode scanner (Pell, 1964; Gotoh & Nishi, 1965), which created images of the prostate, but the resolution was such that the various disease processes within the gland could not be differentiated.

In order to achieve more accurate tissue definition a topographic presentation was necessary and the first radial scanning probe was therefore developed by Takahashi and Ouchi (1963). Unfortunately poor image quality again precluded it's clinical application. Undaunted, research continued (Watanabe et al, 1974) and with it improved image quality, such that the Aloka chair (Watanabe et al, 1975) came into commercial use in 1975. This device consisted of a probe that was inserted into the rectum with the patient in the sitting position. Stable images were obtained, aided by the settling of the pelvic organs

with gravity, but the equipment's lack of mobility and flexibility, and the inablilty to biopsy the prostate were disadvantages. Many refinements followed on from these prototypes including the use of grey scale (Harada et al, 1980) real time scanning and imaging the prostate in the sagittal plane (Sekine et al, 1982). In order to improve flexibility, the later models employed hand held probes and the use of higher frequency transducers, namely 5.5 and 7 megahertz (MHZ), achieved better tissue definition.

The development of readily manoeuvreable transducers enabled imaging of the prostate by ultrasound to be performed with the patient in either the lateral decubitus or supine positions. With the probe mounted on special gantries, the hands of the operator were then free for biopsy purposes. The concept of ultrasound guided biopsy was introduced by Harada and colleagues (1979) and later refined (Holm & Gammelgaard, 1981; Saitoh et al, 1981). The major feature of this technique is the accurate placement of biopsy needles, made possible by the visualisationn of the needle on the ultrasound scan, into areas of the prostate. Thus, small discrete lesions can be biopsied and analysed, thereby providing access to the pathology of the prostate.

ULTRASOUND FEATURES OF THE NORMAL PROSTATE GLAND

The ultrasonographic features of the normal prostate gland have been well described. The gland appears as a symmetrical structure with a well defined, echogenic, circumferentially continuous capsule (Peeling et al, 1979; Brooman et al, 1981; Spirnak & Resnick, 1984). In the young man the gland is triangular, (Harada et al, 1979), (Fig. 3.1) and often distorted by an anterior bulge which has a sonopattern of low echogenicity, representing the periurethral glands (Brooman et al, 1981; Peeling & Griffiths, 1984; Spirnak & Resnick, 1984), but the separate zones, namely central, transitional and peripheral, can be identified by their individual echoes (Shinohara et al, 1989). With advancing age, the gland adopts a more rounded, globular shape. The seminal vesicles are readily visualised and appear as tubular, often asymmetrical structures, located postero-superiorly to the prostate and have a hypoechoic echo pattern traversed[1] by structures of increased echogenicity representing the septae (Brooman et al, 1981; Fornage, 1986).

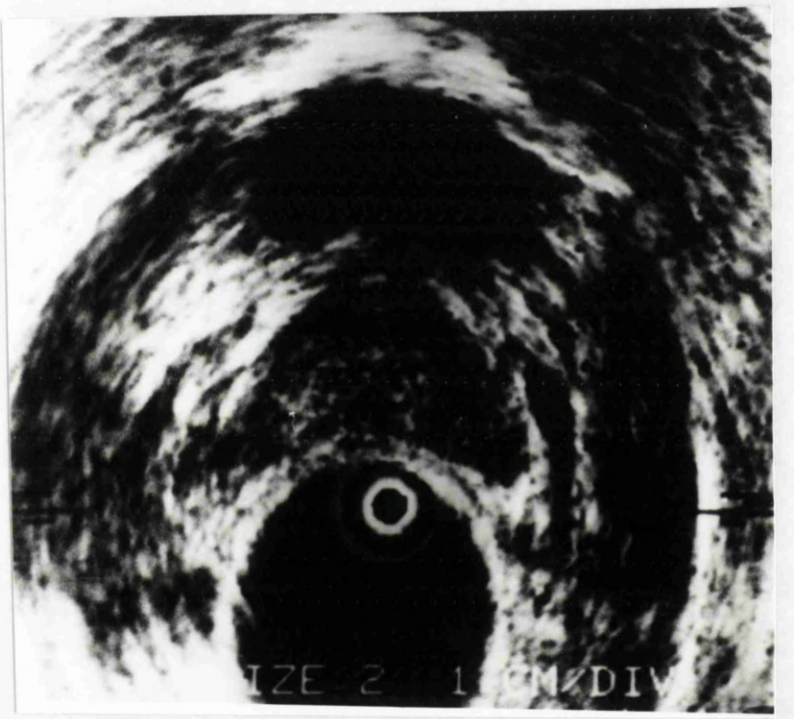


Fig. 3.1 Normal prostatic ultrasonograph

PROSTATIC ULTRASOUND IN INFLAMMATORY DISEASE

To date clinical interest in prostatic ultrasound (Spirnak & Resnick, 1984) has centred mainly on the study of cancer. Initial reports on the precise echo pattern of prostate cancer were conflicting (Peeling et al, 1979; Resnick, 1980; Brooman et al, 1981; Rifkin, 1983), but the debate has now been settled by comparative studies of sonographic findings with the pathology of whole prostates (Lee et al, 1985). Little attention has been paid to the role of ultrasound in the diagnosis and management of inflammatory prostatic disease.

As with prostate cancer, the ultrasound features of chronic inflammatory prostatic disease have caused confusion from the outset. The early bistable scanners produced indistinguishable echo patterns in chronic prostatitis and prostatic cancer (Watanabe et al, 1971). Despite the technological improvements in the latest generation of ultrasound probes, certain features of inflammatory prostatic disease and prostatic cancer continue to be similar, (Harada et al, 1980; Brooman et al, 1981; Rifkin, 1987), although the patient's age and clinical presentation may make the latter condition less likely. The early reports of prostatic ultrasound detailed the features of the normal gland and concentrated on prostatic cancer; little mention was made of prostatitis (Watanabe et al, 1974; Watanabe, 1975; Harada et al, 1979; Peeling et al, 1979; Brooman et al, 1981).

When chronic prostatitis was discussed in these studies, details of the criteria adopted for diagnosis were not given and thus no firm conculsions could be drawn from the findings.

The ultrasound features of chronic prostatitis were first addressed specifically by Griffiths and colleagues (1984) in a group of patients identified on the basis of the Stamey localisation procedure (Meares & Stamey, 1968). The investigators noted three features of significance, namely a low amplitude "halo" around a slightly echogenic central area in the periurethral zone (100% of patients), multiple low amplitude regions in the peripheral zone of the prostate with an ill definced capsule (80%) and curvilinear tubular, echo-free regions immediately adjacent to the gland on it's anterolateral aspect (20%). It was postulated that the peripheral zone abnormalities represented infection and the curvilinear echoes adjacent to the capsule, prominent periprostatic veins. No pathological explanation for the "halo sign" was advanced. Harada and co-workers (1980), in an earlier study, found a hypoechoic area in the periurethral region in 69% of normal glands but in only 50% of patients with prostatitis and concluded that this was a non-specific sign.

Another study (Wiegand & Weidner, 1986) investigated 62 patients with prostatitis and 53 with prostatodynia and identified two ultrasound signs that displayed significant differences between the two groups. Heterogeneous

echopatterns were present in 62.9% of patients with prostatitis, but in only 16.5% of those with prostatodynia ($p<0.001$). Solitary prostatic calculi (type A) were noted in 29% of the prostatitis group compared to 15% of the prostatodynia patients ($p<0.05$) and diffuse calculi (type B) in 43.6% and 7.5% of patients, respectively ($p<0.001$). The authors proposed that these calculi are responsible for chronic infection, although this view is not universally held (Peeling & Griffiths, 1984). The hypoechoic halo sign (Griffiths et al, 1984) within the periurethral region was noted in 54.9% of the prostatitis and 24.5% of the prostatodynia patients in this study.

In the studies discussed above opinions are expressed on the possible histological features displayed by the various ultrasound echopatterns. These are purely speculative as no study to date has performed ultrasound guided biopsy of these areas.

The correlation between echopattern and histology has been carried out by a number of authors mainly with regard to prostatic cancer (King et al, 1973; Harada et al, 1980; Resnick, 1980; Lee et al, 1985; Dahnert, 1986), but also the normal gland (Shinohora et al, 1989). No such study has been performed on patients with proven prostatitis as defined by standard localisation techniques (Meares & Stamey, 1968).

Griffiths and colleagues (1984) observed a decrease in

prostatic volume as determined by ultrasound in patients whose prostatitis showed clinical improvement after treatment, whereas no change was seen when symptoms remained unaltered. No details of post treatment prostatic secretion cytology and microbiology were provided and therefore some doubt must exist as to the validity of this finding.

Few imaging studies have examined the seminal vesicles specifically in chronic prostatitis, although enlargement and thickening of the septi (Di Trapani et al, 1988) has been observed. Seminal vesicle size in normal individuals differs greatly and therefore would appear to be a difficult sign to assess objectively.

Although somewhat outside the remit of this thesis, the ultrasound features of acute prostatitis will be briefly mentioned, for the sake of completeness. Acute prostatitis is a rare condition and clinical diagnosis is usually 'straight forward. The sonographic features of acute prostatitis are well documented (Harada et al, 1980; Peeling et al, 1984; Spirnak & Resnick, 1984; Weidner, 1985) and comprise an enlarged, rounded gland whose capsule is symmetrical, with decreased echogenicity and increased sound transmission within the gland parenchyma; features thought to represent oedema. After treatment, the gland reverts, not uncommonly, to a normal configuration.

SUMMARY

Clinically useful prostatic ultrasound is a relatively new modality. It is clear that amid the euphoria of it's application in prostatic carcinoma, it's role in the inflamed prostate gland has been ignored, despite there being a desperate need to improve diagnostic methods and define the pathology in chronic prostatitis. Although a few studies have made tentative inroads into the problem, no accurate data are available on the correlation between transrectal prostatic ultrasound abnormalities and the leucocyte count within the expressed prostatic secretion. Any progress can only be anticipated when such data are available.

SECTION 4 PATHOLOGY OF PROSTATITIS - NORMAL HISTOLOGY, PATHOLOGY, SEROLOGY AND IMMUNOPATHOLOGY.

HISTOLOGY

The early accounts of prostatic anatomy also described the features of normal histology (Lowsley, 1912; 1930). The inner gland had a loose supporting stroma, compared to the individual investment by smooth muscle cells, of the outer gland acini. Further details were provided by Franks (1954) and McNeal (1968); the latter author observed that the gland was adapted for the function of slow accumulation and rapid expulsion of small volumes of fluid (McNeal, 1988) and that the prostatic ducts and acini both appear to function as distensible secretory reservoirs.

Within the transition and peripheral zones, ducts and acini, in the order of 0.15 - 0.3mm in diameter, have rounded contours with corrugated epithelial borders in order to provide an expansile reservoir. In contrast, the central zone ducts are larger in diameter, approximately 0.6mm, and the acini become progressively larger and more elaborate towards the base of the prostate (McNeal, 1988).

The stroma differs between the zones also, containing bands of smooth muscle fibres in the central zone, compact interlacing smooth muscle bundles in the transition zone

and loosely woven, randomly arranged muscle fibres in the peripheral zone, where the stroma to epithelium ratio is greater (McNeal, 1988). The epithelium lining the acini of the central zone consists of columnar cells with dark, granular cytoplasm and large pale nuclei, in contrast to the more uniform columnar cells with distinct cell borders, pale cytoplasm and dark basally situated nuclei, in the peripheral and transition zones (McNeal, 1981; 1988). In addition all zones of the prostate contain a randomly situated population of paracrine cells (Di Sant'Agnese & de Mesy Jensen, 1984; Di Sant'Agnese et al, 1985) which are rich in serotonin granules and neurone-specific enolase. Sub populations of these cells also secrete a variety of peptide hormones: somatostatin, calcitonin and bombesin; their function remains unclear. The secretory portion of the prostate produces an enzymic cocktail. The central zone exclusively secretes pepsinogen II (Reese et al, 1986) and tissue plasminogen activator (Reese et al, 1988) and thus it has been postulated that the central zone secretes enzymes whose substrates are produced by the peripheral zone.

PATHOLOGY

The first accurate description of the pathology of prostatitis was attributed to Verdier in 1838 (Von Lackum, 1933). The gross morbid anatomy was observed, by Sir Henry Thompson (1861), as a soft gland, containing excessive fluid with a dirty hue and deposits of pus in advanced cases. These abscesses varied in size from "a grain of pearl sago "to that of a pea" and in some cases communicated via dilated ducts, with the urethra; periprostatic abscesses were also recorded.

Three forms of chronic prostatitis were recognised by Keyes (1917), namely catarrhal - affecting mainly ducts rather than acini and stroma, follicular - involving acini which become distended with pus, and parenchymatous - involving acini and stroma to a greater degree. The demarcation between these forms was by no means clear cut and cases displayed a marked overlap. Microscopically periacinous inflammation was invariably present and regarded as "the essential lesion of chronic prostatitis" (Young et al, 1906). The prostatic tubules and ducts were dilated, sometimes cystic, often containing pus and surrounded by round cell infiltration (Young et al, 1906; Friedman, 1919). In long standing cases prostatic ducts and acini were surrounded and replaced by fibrous tissue (Young et al, 1906).

The focal distribution of inflammatory cells in prostatitis, first eluded to by Young and Colleagues (1906), was reiterated by McNeal (1968) who also observed, in an autopsy study, that only 5% of cases involved the central zone alone, compared to 60% involving solely the peripheral zone. The latter author also felt that those cases involving both zones represented "spillover of inflammatory changes into the central zone from a peripheral zone origin".

Inflammatory changes within the prostate are not confined to patients with prostatitis. In a study of 581 adult prostates (Moore, 1937) from unselected autopsies, 6.3% displayed histological evidence of acute and chronic inflammation, two thirds of which had evidence of ascending ductal inflammation. This prevalance of inflammatory changes increased with age to 16.3% in patients of their 80's. Similarly, Kohnen and Drach (1979), detected inflammatory changes in 98.1% of surgically resected prostates, which were classified into six morphological patterns: segregated glandular, periglandular, diffuse stromal, isolated lymphoid nodular, acute necrotising and localised granulomatous. However, no significant morphological differences were observed between those with a demonstrable bacterial prostatic infection and those without. In studies involving patients with a clinical diagnosis of prostatitis, who underwent biopsy of the prostate in search of aetiological organisms (Schmidt & Patterson, 1966; Nielsen & Justesen,

1974), approximately 60% displayed a chornic inflammatory infiltrate. On the basis of these data, the focal nature of prostatitis and the low specificity of periductal and periacinar lymphocytic infiltration (Cameron, 1974), it is generally agreed that prostatic biopsy provides no useful histoligical information in chronic prostatitis (Meares, 1980).

There are a number of specific inflammatory conditions of the prostate that are difficult, on clinical grounds alone, to differentiate from carcinoma. Granulomatous prostatitis, first described by Tanner & McDonald (1943), occurs mainly in the benign prostatic hyperplasia age group and microscopically consists of lipid laden histiocytes, multinucleated giant cells, lymphocytes and plasma cells. Occasionally, this inflammatory infiltrate encompasses duct and acinar remnants, implyig that duct rupture may be of relevance in aetiology. In a recent review (Stillwell et al, 1987), the incidence of granulaomatous prostatitis was estimated at 0.8% in resection specimens from benign glands and was associated with a recent urinary tract infection of 71% of cases, previous transurethral resection in 17% and prostatic needle biopsy in 7.5%. Rarely, tuberculous infection or systemic granulomatous disease is causative.

Malakoplakia, first described by Michaelis and Gutmann (1902) is characterised by aggregates of large histiocytic cells, with abundant foamy cytoplasm. A lymphocytic and

plasma cell infiltrate, and the characteristic calcospherules, the pathogmonic Michealis/Gutmann bodies (Cazzaniga et al, 1987). The stimulus for inflammation is thought to be an urinary tract infection, most commonly Escherichia coli, associated with a defect of phagocytosis due to low levels of cyclic guanine monophosphate, (Abdou et al, 1977).

PROSTATIC CALCULI

A brief discussion on prostatic calculi is pertinent in view of transrectal prostatic ultrasound providing a greater degree of image resolution than existing image modalities.

The first reference to prostatic calculi by Donatus in 1586 described prostatic concretions in elderly autopsy subjects. In the 19th century clinical reports of prostatic calculi associated with urinary tract obstruction appeared in the literature and in 1861 Sir Henry Thompson gave an account of corpora amylaceae within prostatic acini and suggested they were precursors of prostatic calculi. The development of prostatic calculi occurs in part due to precipitation of prostatic fluid components and also from a nidus provided by corpora amylaceae (Vilches, 1982). However, urinary constituents are incorporated in approximately 50% of calculi (Sutor & Wooley, 1974; Kim, 1982).

The incidence of prostatic calculi in the study of whole prostates is very dependent on techniques adopted. However, if a meticulous search is employed figures of 98-100% are achieved (Fox, 1963; Sondergaard et al, 1987). There is no particular association between prostatic calculi and any specific disease process, though they are commonly found in the peripheral zone (Huggins & Bear, 1944) and between hyperplastic nodules and compressed

peripheral zone tissue (Sondergaard et al, 1987). They have also been termed "infection stones" in association with recurrent urinary tract infections (Meares, 1979). The calculi appear, in part, to set up a vicious cycle of ductal and acinar obstruction with resultant prostatic calculi formation, resulting in further obstruction, inflammation and fibrosis and as a consequence further calculus formation (Klimas et al, 1985).

SPECIALISED HISTOLOGICAL TECHNIQUES

The historical aspects of the specialised histologic techniques adopted in this thesis are discussed below.

The mast cell has been shown to play a role in inflammatory reactions (McGovern, 1957) via mediators contained in their granules (Riley, 1966). There is evidence that mast cells have a role in the pathology of interstitial cystitis (Simmons & Bunce, 1958; De Juana & Everett, 1977) and have been found in increased numbers in bladder biopsies of patients with this condition (Simmons & Bunce, 1958; Collan et al, 1976; Larsen et al, 1982). No such information is available in inflammatory prostatic disease.

Mast cells have been detected within the normal and pathological prostate (Gupta, 1970). These cells were located more commonly in the stroma than around the acini and their absolute numbers increased with age to be most marked in the stromal tissue of benign prostatic hyperplasia, some sections of which, also possessed inflammatory changes.

SEROLOGY

Immunoglobulins within the expressed prostatic secretion (EPS) were first noted, in normal subjects, in 1963 (Chodirker & Tomasi). The findings of antibody coated bacteria in the urine of patients with chronic bacterial prostatitis (Jones, 1974; Thomas et al, 1974), stimulated great interest in the immunoglobulins within prostatic fluid. A group of patients with a diagnosis of prostatitis, though no details of localisation studies were given, were found to have elevated levels of the immunoglobulins IgA, IgG and IgM within the EPS compared to controls (Gray et al, 1974). Serum antibody titres to various strains of Escherichia coli were found to be significantly elevated in 82% of patients with chronic bacterial prostatitis compared to both a control group and patients with urethritis, using a direct bacterial agglutination test (Meares, 1977). Later studies in patients with both chronic bacterial and abacterial prostatitis, diagnosed by the Stamey localisation procedure, have detected increased levels of IgA and IgG in prostatic fluid (Fowler & Mariano, 1984; Shortliffe & Wehner, 1986); greater values were obtained in patients with the chronic bacterial form and a satisfactory resolution of the inflammatory response, was accompanied by a return of the immunoglobulins to normal levels,

albeit over a long period (6-24 months). Another study (Shimamura, 1979) of chronic bacterial prostatitis, detected IgM in EPS, along with IgG, which followed this same pattern of decreasing immunoglobulin levels associated with resolution.

Both the total and organism specific immunoglobulins in EPS have been measured by several investigators (Shortliffe et al, 1981; Fowler et al, 1982; Wishnow et al, 1982; Fowler & Mariano, 1984; Shortliffe & Wehner, 1986) and the general consensus is that levels of IgA and IgG are elevated in prostatitis compared to controls. The IgA response is most marked and thought to represent local antibody production within the prostate rather than transudation from the serum. The immunoglobulin levels in chronic abacterial prostatitis are lower than those in chronic bacterial prostatitis and not organism specific for the Enterobacteriaceae (Shortliffe & Wehner, 1986). It thus appears that chronic abacterial prostatitis is not an occult infection, caused by these organisms, that has escaped detection. Recently, Shortliffe and colleagues (1989) reported the use of an enzyme-linked immunosorbent assay (ELISA) to measure total urinary immunoglobulin and specific antibodies to common gram-negative organisms in pre- and post-prostatic massage urine samples. In patients with chronic bacterial prostatitis elevated levels of IgA and IgG were detected in the post-massage urine. This method not only provides a simple test for diagnosing bacterial prostatitis, but also enables a

diagnosis to be made in cases with an active urinary tract infection or in whom free EPS cannot be obtained. In contrast to chronic bacterial prostatitis, where the antigen specific immunoglobulins of IgA and IgG classes only appear at elevated levels in the EPS, acute bacterial prostatitis is also associated with elevated levels of such immunoglobulins within the plasma (Meares, 1987); IgG is elevated in both compartments at the onset of infection and declines over a period of six to twelve months with adequate treatment. In contrast, there is disparity with the IgA response in the EPS and serum, with the serum levels following those of IgG, but the decline in EPS levels not occurring until approximately twelve months after successful treatment.

As with urine, antibody coated bacteria have been detected in the ejaculate of patients with chronic bacterial prostatitis (Riedasch et al, 1977). Similarly immunoglobulins, particularly IgA have been noted in patients with chronic prostatitis (Shah, 1976; Nishimura et al, 1977), but the specificity of this test has been questioned by the finding of elevated levels in patients with benign prostatic hyperplasia and early adenocarcinoma (Shah, 1976) in the absence of prostatitis. Elevated levels of complement C3 and caeruloplasmin have been found in patients with chronic prostatitis though the method of diagnosis was not clearly defined, (Blenk & Hofstetter, 1985).

The approach of using serological data to implicate possible aetiological organisms in chronic abacterial prostatitis has led to conflicting results. Initially the presence of a complement fixing antibody to chlamydiae at a titre of $\geq$ 1:5 in the serum of 33% of men with chronic prostatitis compared to 3% of controls (Màrdh et al, 1972) implicated Chlamydia trachomatis in the aetiology of this condition, despite reservations about it's sensitivity in localised infections (Tarizzo et al, 1968). These reservations were to prove well founded, when a later study (Màrdh et al, 1978), using a more sensitive micro-immunofluorescence method, failed to support their original claims. Although studies have used chlamydial serology to implicate C. trachomatis in the aetiology of chronic prostatitis (Grant et al, 1985; Weidner et al, 1988), they have not been supported by culture data. Equally, there are studies which have used chlamydial (Shortliffe et al, 1985) and ureaplasmal (Shortliffe et al, 1985) serology to support their negative culture data and argue against the role of these organisms in chronic abacterial prostatitis.

An autoimmune aetiology in chronic prostatitis was first mooted by Romanus (1953), who considered chronic prostatitis to be an initiator of ankylosing spondylitis. Similarly, a review of cases of acute anterior uveitis (Perkins, 1979) found a staggering 70% incidence of chronic prostatitis in the cohort of 200 patients; 34% of this group had accompanying joint abnormalities. However

in a study of patients with chronic prostatitis (Moller et al, 1980), none had ankylosing spondylitis, the prevalence of HLA B-27 was normal and there was no mutual asssociation between sacro-iliitis and HLA B-27. Later investigations (Moller et al, 1981) confirmed that there was no increase in prevalence of HLA B-27 or any evidence of a distorted distribution of phenotypes or allele frequencies (Moller, 1984), compared to the normal population.

TISSUE ANTIBODIES

The immunological properties of the prostate were first studied by two groups (Flocks et al, 1960; Barnes et al, 1963; 1965) who discovered that prostatic fluid contained unique antigenic properties. In particular, rabbit anti-human antibody raised against the fluid, stained tissue in the acinar cells, gland lumina and less strongly, the blood vessels of the stroma (Barnes et al, 1965). Ablin and associates (1970) in gel diffusion precipitation studies, observed specific antigenic differences between normal, benign and malignant prostatic tissue extracts. Later work by the same group (Ablin et al, 1971) characterised the classes and location of immunoglobulins within the prostate, in a study of normal cadaveric and benign hyperplastic prostatic tissue. IgA and IgG were found in granules within the duct lumen, IgG in the basal portion of the prostatic acini and IgA in the stroma, and it was concluded that these immunoglobulins were part of the normal prostatic secretion. Frozen sections of prostatic tissue from a group of patients with chronic prostatitis detected diffuse extra cellular IgA, IgG and IgM, and in some areas, immunoglobulin containing cells (Vinje et al, 1983). Although this latter information was drawn from a group of patients with chronic prostatitis, they were not diagnosed by standard localisation techniques, there was no control group and tissue was obtained by blind biopsy. Tissue antibodies have also been observed in

interstitial cystitis (Gordon et al, 1973; Mattila, 1982) and the painful bladder syndrome (Witherow et al, 1989). In the studies on interstitial cystitis, IgM was deposited in the vessel walls within the bladder and in one series (Mattila, 1982), was accompanied by complement C3. It was concluded that the antibodies formed part of a circulating immune complex or were raised in response to an exposed antigen within the vessel wall. The painful bladder syndrome, a possible variant of interstitial cystitis, has also been shown to be associated with IgM deposition in vessel walls as well as IgA deposition on the surface urothelium (Witherow et al, 1989). The possibility of circulating immune complexes, along with local IgA deposition in response to intravesical antigens, producing the inflammatory response in this condition, has been proposed. To date no such immunopathological pathways have been assessed in chronic prostatitis.

IMMUNOPATHOLOGICAL MECHANISMS

Little information is available on the mechanisms involved in the immunopathology of chronic prostatitis. The nature of the inflammatory infiltrate in this condition has been based on examination of the expressed prostatic secretion (EPS), (Anderson & Weller, 1979; Nishimura et al, 1980; Schaeffer et al, 1981). Although one study (Nishimura et al, 1980) examined prostatic tissue for the presence of macrophages from patients with a diagnosis of chronic prostatitis, standard localisation techniques were not employed, and the authors were only able to confirm the presence of these cells without deducing function.

The development of techniques using monoclonal antibodies to identify specific lymphocyte populations (Janossy et al, 1980; Thomas & Janossy, 1981) has enabled the immunopathology of chronic inflammatory diseases such as rheumatoid arthritis (Poulter et al, 1983), leprosy (Collings et al, 1985) and bronchiectasis (Lapa E Silva et al, 1989) to be studied.

The lymphocyte sub-populations within the normal male genital tract have been observed (El Demiry et al, 1985), with a predominantly T8 (suppressor/cytotoxic) T-lymphocyte presence noted in the epithelial areas of the prostate, compared to aggregates of T4 (helper/inducer) lymphocytes in the stroma. Aggregates of B-cells were also noted in the interstitium. The authors postulated

that the T-lymphocyte's function may be to minimise sensitisation to autoantigens in the sperm, with the consequent risk of an impaired response to opportunistic micro-organisms (Hargreaves et al, 1986).

In an attempt to explain recurrent infections in patients with chronic bacterial prostatitis due to gram-positive bacteria, a defect of phagocytosis and killing in the patient's peripheral blood leucocytes has been postulated (Wedren et al,1987). Knowledge of the discrete components of the inflammatory cell population and their function, in chronic prostatitis is, to say the least, sparse. In particular, there is no information regarding an immunological basis for the inflammatory reaction and thus any theory about pathogenesis must remain speculative.

SUMMARY

The histology of the normal prostate has been well defined. The inflammatory changes within the prostate have been categorised using routine staining techniques, but often from unselected biopsy material, such that the possible specific changes of chronic prostatitis may not be represented. Although few studies have analysed tissue from patients with chronic prostatitis, diagnosed by standard techniques, routine histology has provided little information on the possible pathogenesis of the disease. The focal nature of prostatitis has hampered the collection of representative tissue for analysis, from inflammatory lesions, using blind biopsy techniques.

Antibodies have been detected both in expressed prostatic secretion (EPS) and tissue and in the case of the former, reflect disease activitiy in chronic bacterial prostatitis. Although antibodies in EPS have been detected[1] in chronic abacterial prostatitis, levels are lower than in the chronic bacterial form and also lack organism specificity. The presence of tissue antibodies, have simply been noted to date, but lack of definition precludes any conclusions on their role in the pathogenesis of chronic prostatitis.

The nature of the immunocompetent cell population within the normal gland has been noted by one group of researchers but such information is completely lacking in

patients with chronic prostatitis and thus it is unknown whether an immunological basis for the inflammatory reaction exists in this condition.

SECTION 5 PROSTATE SPECIFIC ANTIGEN

The existence of antigens unique to the prostate has been known for nearly 30 years (Flocks et al, 1960; Ablin et al, 1970; 1972; Moncure et al, 1975), but it was not possible to purify the antigen, prostatic specific antigen (PSA), until comparatively recently (Wang et al, 1979). Immunohistochemical techniques have revealed that synthesis of this antigen is restricted to the epithelial cells of the prostate (Papsidero et al, 1980; Nadji et al, 1981). The development of enzyme immuno-assays (Kuriyama et al, 1980) and radioimmunoassays (Frankel et al, 1982) has enabled the levels of PSA to be estimated in serum and other body fluids.

The value of this antigen as a marker of prostatic carcinoma activity is well established (Kuriyama et al, 1981; Siddall et al, 1986; Ferro et al, 1987). Furthermore, it is known that PSA levels are dependent on patient's age, prostate gland size and any manipulation or instrumentation of the gland (Stamey et al, 1987). With particular reference to the latter, Stamey and associates (1987) noted a 1.5 to 2-fold increase in the serum value after prostatic massage, a 4-fold increase after massage and cystoscopy, and a greater than 50-fold increase following needle biopsy or transurethral resection of a benign gland. It is thought that inflammatory changes in the gland cause a rise in PSA levels (Collier and Pain, 1986; Stamey et al, 1987), especially in association with

urinary retention (Collier & Pain, 1986). However, there is little objective data on PSA levels in patients with chronic prostatitis or the effect of treatment on these levels.

SUMMARY

The role of prostate specific antigen (PSA) as a marker in prostatic carcinoma is well established. Furthermore certain factors that cause elevation of levels in benign prostatic disease are known: large gland, patient's age, manipulation and instrumentation of the prostate, in particular biopsy or transurethral resection. No objective data are available on PSA determination in chronic prostatitis.

SECTION 6 URODYNAMIC STUDIES IN PROSTATITIS - FREE FLOW RATES AND URINARY REFLUX

The value of free urinary flow rates as a basic, yet informative aspect of urodynamics is established (Abrams et al, 1983). At voided volumes of 150mls or greater, flow rate data are reproducible (Kaufman, 1961). Anatomical studies on the bladder neck and distal sphincter complex (Elbadawi & Schenk, 1974) have revealed a dual autonomic nerve supply, intercommunicating fibres between the two sphincters and a common distal sphincter composed of striated and smooth muscle containing ganglia which are interconnected. Thus, in normal circumstances the detrusor, bladder neck and distal sphincter act in a controlled, co- ordinated manner. In a study of patients with neuropathic bladders (Awad & Downie, 1977), excessive sympathetic activity at the distal sphincter resulted in a functional obstruction; treatment with alpha-blockers reduced the residual volumes and degree of upper tract dilatation.

Failure of relaxation of the distal sphincter complex has been observed in chronic prostatitis (Buck, 1975; Siroky et al, 1981) and also prostatodynia (Osborn et al, 1981; Barbalias et al, 1983; Meares, 1986; Hellstrom et al, 1987). In the study by Siroky and associates (1981), 36% of cases were described as having areflexic bladders with non-relaxing perineal floors and 41% had bladder hyperreflexia but normal distal sphincter function. The

work of Barbalias and colleagues (1983), discovered a reduced maximum flow rate in 85% of cases. This was due to a significantly raised maximum closing pressure, reaching its peak 4cm. from the bladder neck; features also observed by other groups (Osborn et al, 1981; Meares, 1986). Interestingly in both studies, electromyographic studies on the striated component of the distal sphincter were normal implying that the increased tone at this level was due to sympathetic nerve fibre mediated overactivity. Others have found increased distal sphincter tone on electromyography and demonstrated bladder-distal sphincter dyssynergia (Hellstrom et al, 1987). In a group of patients with chronic prostatitis, unfortunately diagnosed on clinical grounds alone, 24% had hyperactive distal sphincter mechanisms (Murnaghan & Millard, 1984). Sixty per cent of cases had urodynamic evidence of outflow obstruction, which was located at the bladder neck in 81% and prostatic urethra in 20% of cases. The concept of pelvic floor myalgia creating pain indistinguish- able from that of prostatitis has been postulated (Segura et al, 1979; Meares, 1986), but it does not meet with universal approval (Barbalias et al, 1983).

The consequence of abnormal distal sphincter function is intraprostatic reflux of urine (Buck, 1975; Hellstrom et al, 1987) and possible initiation or perpetuation of inflammatory changes in the prostate. Indirect evidence of this phenomenon is provided by chemical analysis of prostatic stones, 50% of which contain constituents

derived from the urine (Sutor & Wooley, 1974; Kim, 1982). The calculi are located most commonly in the peripheral zone (Huggins & Bear, 1944) in keeping with the orientation of the prostatic ducts (Blacklock, 1974). Furthermore, persistence of inflammatory changes in the prostate can result in fibrosis of the prostatic ducts which become patulous and more prone to further urinary reflux. Urinary reflux has been demonstrated (Kirby et al, 1982) in cadavers, patients with benign prostatic hyperplasia undergoing prostatectomy and patients with chronic abacterial prostatitis, and was felt to play an aetiological role in the latter group.

SUMMARY

Both chronic prostatitis and prostatodynia have been shown to be associated with distal sphincter abnormalities on urodynamic testing. The failure of the sphincter to relax fully, results in an elevated prostatic urethral pressure and the possibility of intraprostatic reflux of urine. The consequence of such reflux has yet to be evaluated fully, but may play an aetiological role in chronic prostatitis.

SECTION 7 PSYCHOLOGICAL FACTORS IN PROSTATITIS

The first description of an association between symptoms of prostatitis and psychological factors was provided by Bureau in 1792 with a case report of a young man with penile tip pain radiating to the groin and testes. All investigations proved negative and his symptoms persisted until he developed gonorrhoea; the subsequent urethral discharge was accompanied by symptomatic relief! A physical link between perigenital pain and prostatitis was proposed by Bangs (1886), who felt that the nerve supply to the genitalia was involved in a dual role - urinary and sexual - and thus prone to "misinterpretation". A "feeble nervous mechanism" and "perverted volition" were the root causes of perigenital pain in prostatodynia, according to Aronstam (1914). The same author also observed neuroses accompanying inflammatory conditions, which persisted after apparent cure of the inflammatory process.

A different perspective was supplied by Wolbarst (1921), who felt many of the functional voiding disturbances in prostatitis were due to abnormalities of the posterior urethra, verumontanum and bladder neck, though found no correlation between urethroscopic abnormalities and clinical features. Despite these findings the author concluded that urethroscopy was a mandatory investigation.

The suggestion that many of the "extragenital" symptoms in

chronic prostatitis were psychologically based was raised by De Keersmaecker (1931).

Although reports of the psychological component of prostatitis exist, (Bagge 1970; Mendlewicz et al, 1971; Nilsson et al, 1975), objective data are lacking. The general concensus of these studies is that a neurotic anxiety state is present in patients with chronic prostatitis, especially those who fail to respond, but details of the methods of both diagnosis and quantifying degrees of anxiety were not provided in these reports. In contrast, a study of patients with chronic prostatitis, using the General Health Questionnaire and the Eysenck Personality Inventory, found no greater tendency to neurotic disease in the study group over controls (Smart et al, 1976).

Some psychotherapeutic schools in their assessment of patients with chronic prostatitis, though again no details of diagnosis were supplied, felt that depressive and hypochondriacal features are neurotic symptoms and regard them as "disorders at the pre-genital level" (Keltikangas-Jarvinen et al, 1981; 1982). The voicing of psychoneurotic complaints has been shown to be more common in patients with chronic prostatitis than in those with prostatodynia (Brahler et al, 1986).

Finally, experimental studies in the rat, exposing the animals to standardised stress stimuli, have shown

decreased blood flow plus inflammatory changes within the prostates of the stressed animals compared to controls (Gatenbeck et al,1987). The role of stress in prostatitis has been further suggested on the basis of an improvement or cure rate of 86% in a group of patients with symptoms of chronic prostatitis, who were treated with stress management therapy only (Miller, 1988).

SUMMARY

Although awareness of the psychological influences in chronic prostatitis has been evident for nearly 200 years, objective data are lacking in patients with chronic prostatitis diagnosed by standard localisation procedures. There is a tendancy to make conclusions on groups of patients with a diagnosis of prostatitis based on symptoms alone, which inevitably includes a large number of patients with prostatodynia; there is a desperate need for an objective psychological assessment in patients with bona fide chronic prostatitis if myth and fact are to be distinguished. The role of psychological factors in prostatitis remains unquantified.

SECTION 8 EPIDIDYMITIS - AETIOLOGY AND IT'S RELATIONSHIP WITH THE PROSTATE

The importance of Neisseria gonorrhoeae, preceding urethritis and the vasal route of infection in the aetiology of epididymitis, were all noted by Keyes (1917). The problems of instrumentation in the presence of active infection or inadequate treatment of the gonococcus, with subsequent epididymitis were observed by the same author. Also a group of patients, in whom the gonococcus could not be isolated, who were particularly prone to abscess formation and recurrent epididymitis, was identified.

For many years the majority of cases of epididymitis were termed idiopathic, in view of the lack of isolation of an infecting organism (Handley, 1946; Gartman, 1961; Mittemeyer et al, 1966). Up to this period a number of infectious agents, in addition to the previously mentioned gonococcus, had been cited as playing a causative role: tuberculosis and undulant fever (Isaac, 1938), mumps (McElligot, 1943) and meningococcal septicaemia (Laird, 1944). However, it was becoming evident that organisms implicated in urinary tract infections, namely Escherichia coli and the other Enterobacteriaceae, were of increasing importance (Mittemeyer et al, 1966).

The aetiological influence of Chlamydia trachomatis was first noted on the basis of serological studies, (Heap, 1975; Harnisch et al, 1977). An active role played by

this organism was confirmed by the isolation of chlamydiae directly from the epididymis as well as the urethra (Berger et al, 1978; 1979; 1980). This conclusion has been further confirmed in recent studies (Grant et al, 1987; Melekos & Asbach, 1987; Mulcahy et al, 1987). A broad demarcation, based on age, has been observed with reference to aetiological organisms: in patients less than 35 years of age Chlamydia trachomatis is the most commonly isolated organism, in contrast to the Enterobacteriaceae organisms, in patients over this age (Berger et al, 1979; 1980; Mulcahy et al, 1987), although this demarcation is not absolute.

The mode of transmission of organisms has also been much debated. Vasal reflux of urine was felt by some (Abeshouse & Lerman, 1950; Graves & Engel, 1950) to be the major aetiological factor in acute epididymitis, yet refuted by others (Handley, 1946; Wesson, 1959; 1963). However, the studies of Berger and associates strongly supported an intracanalicular route of spread of organisms (Berger et al, 1978; 1979; 1980). Those authors who rebutted the vasal reflux theory (Handley, 1946; Wesson, 1959; 1963) believed that the underlying cause of epididymitis was prostatitis or seminal vesiculitis which in turn was secondary to urethritis. This view has been proposed more recently (Grant et al, 1987), though based on prostatic fluid studies where the risk of urethral contamination could not be discounted.

SUMMARY

In the early part of this century, Neisseria gonorrhoeae was the most common infectious agent implicated in acute epididymitis. Latterly, studies incorporating epididymal aspiration techniques have concluded that Chlamydia trachomatis is the most prevalent organism in young men (less than 35 years), whereas the Enterobacteriaceae assume increasing importance beyond this age. It appears that an intracanalicular route of spread of the organism from urethra or bladder exists, though the role of sterile urinary reflux via the vasa is uncertain. An associated or causative prostatitis in acute epididymitis has been postulated but to date no studies have been able to prove this theory as urethral contamination of prostatic fluid has complicated interpretation of bacteriological findings.

SECTION 9 ANIMAL MODELS OF PROSTATITIS

Initially, experiments on prostatitis involving animals were directed towards the effect of innoculating organisms, isolated from patients with prostatitis, into animals to observe the tendency for the organism to localise. Rosenow (1921) demonstrated that streptococci so obtained, localised in the prostate glands of rabbits. The findings were supported by similar studies (Nickel, 1926; Nickel & Stuhler, 1930) which also observed the development of arthritis in these animals.

Spontaneous prostatitis associated with ageing has been noted in Copenhagen and Wistar rats (Muntzing et al, 1979), in which the lateral lobes, the equivalent of the peripheral zone in man (Blacklock, 1977), were effected. In contrast, studies of bacterial prostatitis induced in rats, tended to produce inflammatory changes in the ventral lobes, which correspond to the central zone in man (Friedlaṅder & Braude, 1972; Weidner et al, 1981). This situation is not analagous to prostatitis in man. However, the intra-canalicular route of infection was demonstrated in the female Mastomys natalensis rat using Klebsiella pneumoniae (Weidner et al, 1981); the choice of female, rat, which surprisingly has a prostate, was due to urinary tract infections not being associated with bladder stone formation and pyelonephritis, as seen in the male. A dog model of bacterial prostatitis induced by injection of Escherichia coli into the prostatic artery (Baumueller

& Madsen, 1977a) has been described, chiefly to study the prostatic absorption and secretion of antimicrobials (Baumueller & Madsen, 1977b). A more artificial model was devised in dogs, by the innoculation of chlamydiae, at two different concentrations, directly into the prostate (Nielsen et al, 1982). Although a systemic antibody response was mounted, recovery of the organism from the prostatic fluid was only achieved at a higher concentation of innoculum, but could not be isolated from the prostatic biopsies despite there being an inflammatory infiltrate on histology.

The possible aetiology of chronic abacterial prostatitis has been addressed using Lewis and Wistar rats (Naslund et al, 1986), with reference to the effect of oestrogen administration. It was concluded that abacterial prostatitis has a multifactorial aetiology involving neonatal imprinting of the prostate with oestrogen, followed by age-related changes in the immune and endocrine systems in adulthood. Finally, the induction of autoimmune prostatitis by performing thymectomy on mice in the neonatal period, (Taguchi et al, 1985) has raised the possibility that prostatitis is an autoimmune phenomenon.

SUMMARY

Animal experiments were employed initially to assess the localising ability of certain organisms isolated from the prostate. Although the intra-canalicular route of infection has been observed, most animal models provide little insight into the aetiology of chronic prostatitis, especially the abacterial form. The model devised using direct innoculation of Chlamydia trachomatis into the prostate, a totally unnatural method of infection, failed to provide a definite link between inflammatory lesions in the prostate and infection by this organism.

SECTION 10 MEDICAL PHYSICS PERTINENT TO TRANSRECTAL PROSTATIC ULTRASOUND

The need to adopt the transrectal route to image the prostate arose from the inability of transabdominal ultrasound and computerised tomography (CT) to provide detailed information regarding the internal architecture of the prostate. Ultrasound is the high frequency transmission of a mechanical vibration through a medium, by means of an orderly oscillatory motion. The crystal, which provides the source of the wave, is caused to vibrate by an electrical current, which when placed in contact with a structure, alters the net force on the particles within that structure. The ability of a crystal to both emit and receive ultrasound waves, is a result of the piezoelectric effect. When a fluctuating voltage is applied across the crystal, an alteration in the charge on the crystal occurs, with resultant vibration and creation of an ultrasound wave. Conversely, when the mechanical vibrations strike the crystal, they cause small fluctuating electrical charges to appear on its surface, which can be constructed into an image. The ultrasound beam thus created can be continuous or pulsed. The frequency depends on crystal thickness, and in turn, beam pattern on frequency and crystal diameter.

The ability of ultrasound to provide information on structures deep to the transducer is dependent on the

reflection of incident ultrasound waves at tissue interfaces. Usually less than 1% of the ultrasound energy is reflected back at an interface, and the fraction reflected, depends on acoustic impedance, which in turn depends on the density and elasticity of the tissue. If the ultrasound wave strikes an interface at 90 to it's surface, it will be directed back to the transducer. However, this is rarely the case, and some of the reflected wave is scattered, and thus is not received by the transducer. A collection of closely patterned discontinuities that do not provide an interface, produce a marked scatter.

The interface can be localised by means of the beam direction and the "go-return time" (i.e. time of emission to the time of return). The velocity of sound in soft tissue is 1540 meters per second, and thus from the go-return time, a distance or depth value can be obtained. The magnitude of the received echo is dependent on the magnitude[1] of the emitted pulse and the degree of attenuation created by beam absorption, scatter and divergence. The received beam can be amplified such that echoes arising from deep interfaces are adequately represented. Conversley, weak signals can be rejected by a suppression device.

The most basic ultrasound device is an A-mode scanner, which provides a one dimensional scan. The received signal is represented by a dot on an oscillascope and the

height of any deflection is proportional to the size of the echo signal, and the distance it occurs along the Y axis, is a measure of the depth of the reflecting interface.

The B-scan was developed in an attempt to reduce the ambiguity of signals received. With this mode of scanning, the ultrasonic beam is made to sweep in a plane section through the body. For each beam position, interfaces are detected by determining the times of the returning echoes and the beam direction. In this way a two dimensional image is compiled. The interfaces are represented as bright spots along a scan line. The scan line is moved horizontally, such that a composite picture of a number of echo spots is produced. The brightness of each spot is related to the magnitude of the echo signal. This is referred to as grey scale representation, and the data is usually processed in a logarithmic fashion, such that more shades of grey are available to the weaker echoes, than the strong echoes from major tissue boundaries.

The third commonly used modality is real time scanning. Images of the same tissue section are generated rapidly and repetitively and thus enable motion to be observed. The rate of repetition is the frame rate, and the high velocity of ultrasound in the tissues, enables rapid production of images. Several thousand echo patterns are collected per second, from which 25 to 50 images are

generated in the same time period, each comprising approximately 150 lines of echo data. Thus an image is produced, that not only detects movement, but is flicker free and enables identification of genuine tissue structures by reducing the significance of spurious artefact shadows.

There are a number of real time probe designs, namely mechanical, electronic and compound. With the mechanical probe the transducer is rotated or rocked on a mounting, whereas the electronic type consists of rows of crystals that are fired in sequence to provide a sector scan (phased ray) or longitudinal scan (linear ray). The compound scanners transmit ultrasound to structures from several different directions at one time and thus, provide more echo information in one sweep.

There are two components to the resolution of images in real time ultrasound, namely axial and lateral resolution. Axial resolution is the ability of a device to separate structures lying one behind the another along a beam, and is dependent on the length of the ultrasound pulse; better resolution being achieved with a shorter pulse. Lateral resolution, the ability to separate structures that lie side by side across the ultrasonic beam, depends on the width of the ultrasound beam at the level of the structure. With increased frequency of the ultrasound wave, the pulse can be shortened and the beam width narrowed, however, with increased frequency comes

increased attenuation in the tissues, and thus, less penetration. Temporal resolution, i.e. the ability to separate events in time, is dependent on the frame rate.

The frequency of the transducers used in transrectal prostatic ultrasound ranges from 3.5 MHz to 7 MHz, with the most common device being 5.5 MHz. With such ultrasound ranges, especially in the 5.5 to 7 MHz range, a satisfactory compromise has been reached between tissue penetration and adequate resolution.

In all ultrasound examinations there needs to be a coupling agent between the tissue to be examined and the transducer. This enables the sound energy to pass through the tissue, rather than be reflected by an air interface which creates gross image distortion. Also the coupling agent provides a greater field of view, by creating a greater surface area of contact.

The distance of the prostate from the transducer in transabdominal scanning ensures that a compromise between tissue penetration and image quality must be reached. In order to achieve adequate penetration a transducer of low frequency has to be used, and thus the prostate falls into the divergent part of the image beam, and resolution is sacrificed. In contrast, the close proximity of the prostate to the transducer in transrectal scanning allows high frequency transducers to be employed with narrow beam patterns and better line density images, and therefore,

greater resolution and definition. A narrow transducer beam pattern results in a smaller volume of tissue from which echoes are received, and thus, a finer spatial image detail. A narrow beam pattern over a long range is the ideal combination.

The focal zone, the range of image depth along a beam pattern where the beam is sufficiently narrow to give good image detail, is dependent on the focal length and width of the transducer; the focal zone being directly proportional to focal length and inversely proportional to transducer width when all other factors are constant. The benefits of transrectal scanning become apparent: a small diameter transducer can be used, which thus has a long focal zone relative to its focal length, and as the received echo amplitude is independent of the transducer diameter, there is no decrease in the image signal. An increase in transducer frequency results in shorter ultrasound waves, which provide finer image detail, but increased tissue absorption (proportional to frequency). Again, the close proximity of the prostate to the transrectal transducer enables high frequencies to be employed.

Finally, image quality is governed by image line density, which in turn is governed by frame rate and velocity of sound. An image line represents the spatial direction of the beam pattern emitted and received from the tissues. The image detail along that line is in turn governed by

the transducer frequency and beam pattern. Increased line density is accompanied by increased image detail, yet the former is restricted by the velocity of the sound, as the next pulse emission of sound energy cannot be dispatched until the last echo is received. The small field of view used in transrectal scanning enables the magnified image to provide a greater line density. Also, the smaller image depth achieved, allows more frequent emission of signals. Thus, the close proximity of the prostate to the rectum allows it to be visualised by high frequency ultrasound, whose focusing ability and line density enable a high resolution image to be obtained.

SECTION 11 TECHNIQUE OF PROSTATIC LOCALISATION STUDIES AND DIAGNOSIS OF PROSTATITIS - DEFINITIONS.

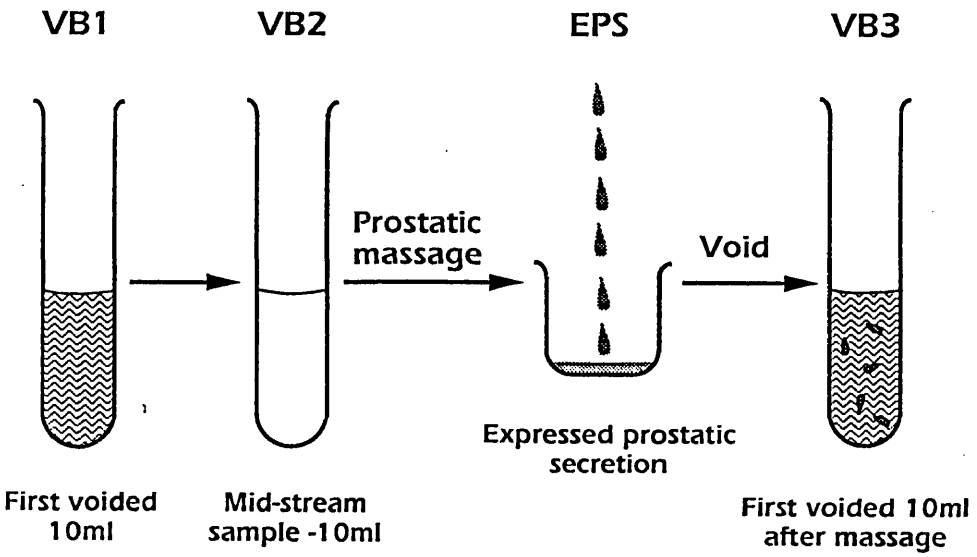
The importance of accurate localisation studies (Meares & Stamey, 1968) is paramount to any meaningful study of prostatitis and therefore the technique adopted in these studies is described (Fig. 11.1).

Prior to specimen collection a full bladder is essential. The patient retracts the foreskin, if uncircumcised, and cleans the glans penis with an antiseptic, Savlodil R (ICI). With the foreskin still retracted, as it should remain throughout the test, the first 10 mls of voided urine is collected in a sterile container and labelled VB1. The patient then passes 150 to 200 mls of urine, which is discarded prior to collecting a further 10 mls of urine, a mid-stream specimen, labelled VB2. The knee-elbow or standing position is adopted by the patient and prostatic massage performed; any free expressate from the external urethral meatus is collected in a sterile container and labelled expressed prostatic secretion (EPS). Finally, the patient voids again and the first 10 mls is collected in a sterile container, and labelled VB3.

In broad terms VB1 represents urethral and bladder urine, VB2 bladder urine and VB3 bladder urine and expressed prostatic secretion. Although passing urine prior to prostatic massage "cleanses" the urethra of possible contaminating organisms and leucocytes, these have to be

Fig. 11.1

Segmented Urine Localisation Technique (after Meares and Stamey)



quantified, such that the subsequent data in the EPS and VB3 can be objectively analysed with the "background" contaminants removed from the equation.

DIAGNOSIS

The expressed prostatic secretion (EPS), when obtained, was mixed by agitation with a pipette and a portion applied via a micro-pipette onto a Fuchs-Rosenthal counting chamber. The number of leucocytes were counted in sixteen fields under high power (X 1000) and represented as leucocytes/mm^3.

The leucocyte counts within the voided urine samples (VB1, VB2 and VB3) were performed on uncentrifuged urine. The whole sample was shaken vigorously, prior to withdrawing a 60 ul aliquot which was placed into a flat bottomed microtitre tray and leucocyte counts performed in ten random fields under high power (X 400); a mean value was calculated and recorded as a count of leucocytes /mm^3 . A diagnosis of prostatitis was made if 1) the leucocyte count in EPS was > 1000 cells/mm^3 , provided there were fewer leucocytes in the urethral (VB1) and bladder (VB2) urine and/or 2) the leucocyte count in the post massage urine (VB3) was greater than or equal to 50 cells/mm^3.

A 100 ul sample of either urine or EPS was innoculated onto each of the following plates: blood agar X 2 - one for aerobic and the other for anaerobic incubation, and MacConkey agar. The aerobic plates were incubated at 37^{o}C in 5% CO2 and examined for any bacterial growth at 24 and 48 hours post innoculation.

After this period, colony counts were performed and converted to the number of colony forming units per ml.

In order to localise an organism to the prostate, the colony count in VB3 and/or EPS had to be in excess of the colony counts in VB1 and VB2 by at least a factor of X10.

SECTION 12 TRANSRECTAL ULTRASOUND IN INFLAMMATORY PROSTATIC DISEASE

As outlined in the introduction, only tentative inroads have been made into the evaluation of transrectal ultrasound in inflammatory prostatic disease. No study has correlated ultrasound findings with the leucocyte count in expressed prostatic secretion (EPS) or post-massage urine (VB3), nor attempted to correlate these sonographic features with the histology of prostatic tissue obtained by needle biopsy.

This combination of studies aimed to confirm the ultrasound findings in normal individuals and then to examine groups of patients with chronic prostatitis and prostatodynia in an attempt to define any ultrasound abmormalities, and if present, to assess whether the two groups could be differentiated on ultrasound criteria alone. A number of patients from both study groups underwent follow-up examinations to observe any evolving patterns which may provide a clue to the natural history of chronic prostatitis. Finally, the chronic prostatitis group was subjected to ultrasound guided biopsy in order to correlate histology with the ultrasound abnormalities.

STUDY 12.I TRANSRECTAL PROSTATIC ULTRASOUND OF THE NORMAL GLAND

Patients and Methods

Thirty five patients aged 18 - 68 years, mean 37.7 years, with no history or symptoms of prostatitis, drawn from the sexually transmitted diseases and urology clinics, were studied. All patients underwent prostatic localisation studies and then transrectal prostatic ultrasound using a Bruel and Kjaer 1846 scanner with a 5.5 MHz transducer. The scan was performed in the left lateral position and any abnormalities recorded on X-ray plate.

The abnormalities within the gland parenchyma were categorised according to their zonal position, namely central, transitional and peripheral zone, and any abnormality of the capsule and periurethral zone also recorded.

Results

In all patients, no cytological or microbiological evidence of prostatitis was found. In only five patients (14%) were no abnormalities noted on ultrasound.

In all cases the gland was symmetrical, yet a homogeneous parenchyma (Fig. 12.1) occured in the minority. However, two signs were prominent, namely dense echoes (54%)

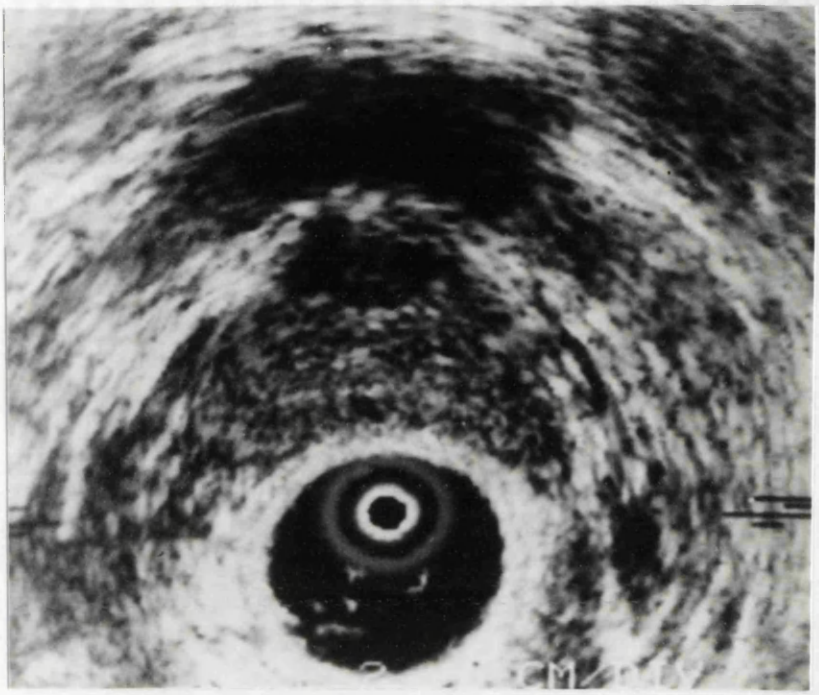


Fig. 12.1 Transrectal ultrasonograph displaying a homogeneous parenchyma

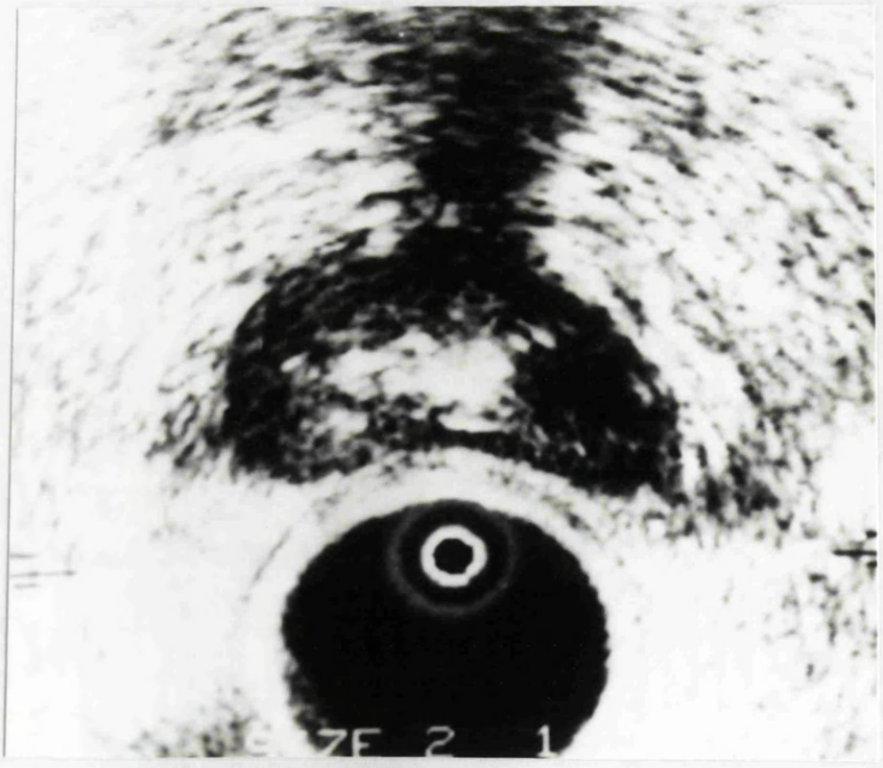


Fig. 12.2 High density echoes (HDE) in a prostatic ultrasonograph

generally within the central zone, termed high density echoes (HDE), (Fig 12.2), and softer more diffuse echoes (37%), termed mid-range echoes (MRE), (Fig 12.3). In one patient, dense echoes were observed in the region of the ejaculatory ducts, termed ejaculatory duct calcification (EDC) (Fig. 12.4). Two further abnormalities, an irregular periurethral zone (Fig. 12.5) in three patients (9%) and a hypoechoic halo in the region of the periurethral zone in five (14%), were detected. Full details of the ultrasound features of this group of normal controls appear in table Appendix III.

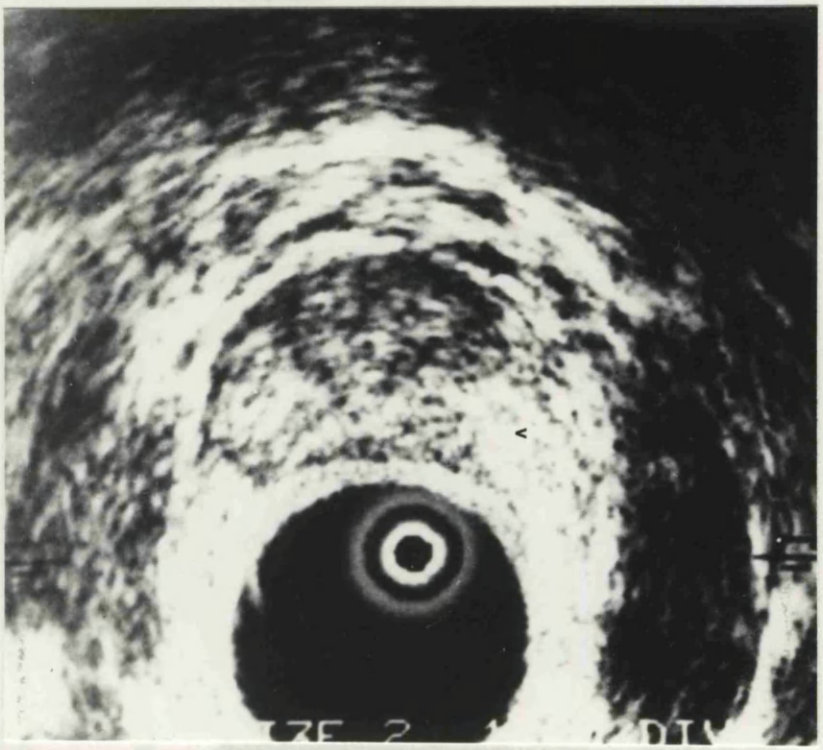


Fig. 12.3 A prostatic ultrasonograph displaying mid-range echoes (MRE)

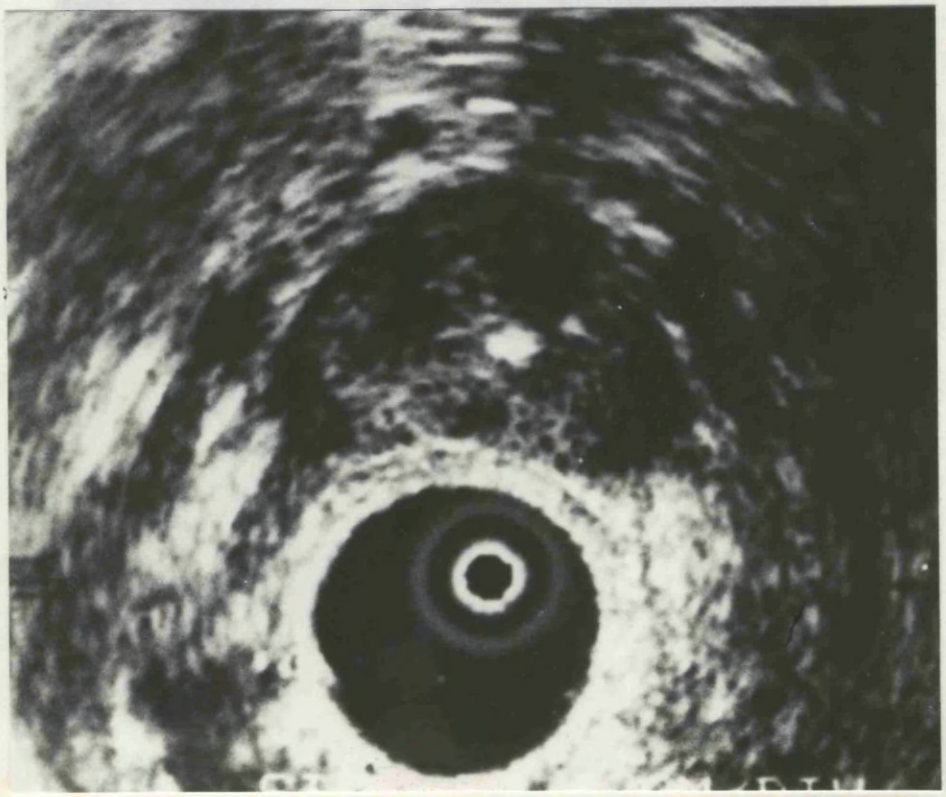


Fig. 12.4 Ejaculatory duct calcification (EDC) in a prostatic ultrasonograph

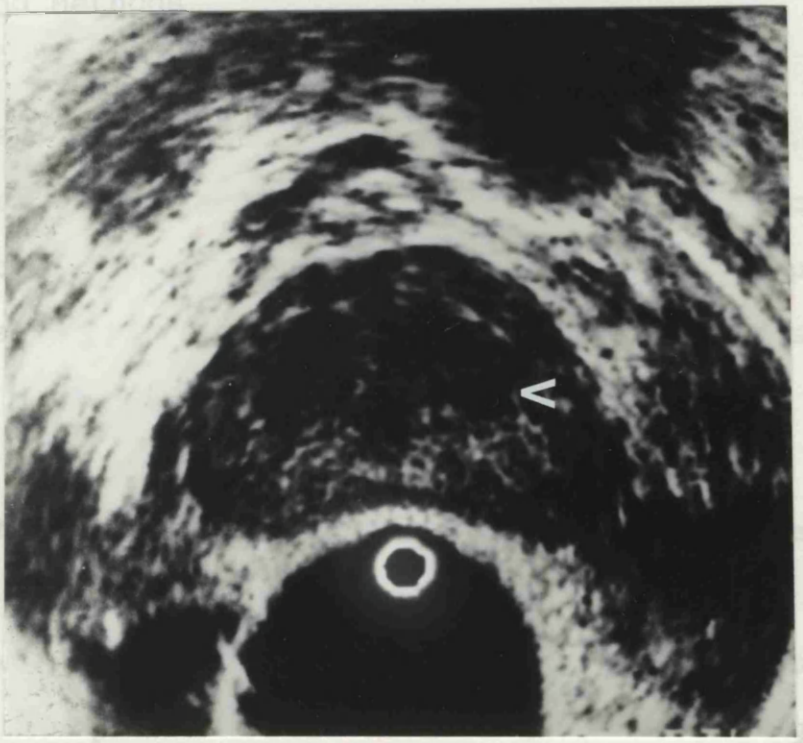


Fig. 12.5 A prostatic ultrasonograph displaying periurethral zone irregularity (PZI)

STUDY 12.II CORRELATION OF TRANSRECTAL PROSTATIC ULTRASOUND WITH THE LEUCOCYTE COUNT WITHIN THE EXPRESSED PROSTATIC SECRETION IN PATIENTS WITH SYMPTOMS OF CHRONIC PROSTATITIS

Patients and Methods

Sixty patients aged, 22-69 years, mean 35 years, drawn from the sexually transmitted diseases and urology clinics with symptoms of chronic prostatitis, in whom expressed prostatic secretion (EPS) was available for analysis, were studied.

All patients underwent prostatic localisation studies (see Section 11 and Appendix I) and the leucocyte count within the EPS was measured in a Fuchs-Rosenthal counting chamber. The patients were divided into three diagnostic groups, based on the leucocyte count: namely less than 100 leucocytes per mm^3 - prostatodynia, 100 to 600 leucocytes per mm^3 - borderline prostatitis and greater than 600 leucocytes per mm^3 - prostatitis.

Each patient underwent transrectal prostatic ultrasound using a Bruel and Kjaer 1846 scanner with a 5.5 MHz transducer.

On the basis of study 12.I, seven ultrasound signs (Table 12.1) were identified as worthy of consideration. With

each sonograph, the extent of each sign was graded on a scale of 1 to 3 (minimal, moderate and marked).

Table 12.1 Ultrasound Signs in Inflammatory Prostatic Disease

High Density Echoes	(HDE)
Mid-range Echoes	(MRE)
Echolucent Zones	(ELZ)
Capsular Irregularity	(CI)
Capsular Thickening	(CT)
Ejaculatory Duct Calcification	(EDC)
Periurethral Zone Irregularity	(PZI)

Examples of the capsular signs capsular irregularity and thickening (CI,CT) as well as echolucent zones (ELZ) are depicted in Figs. 12.6 - 12.8.

Results

The diversity of the presenting symptoms in the study group is depicted in Table 12.2.

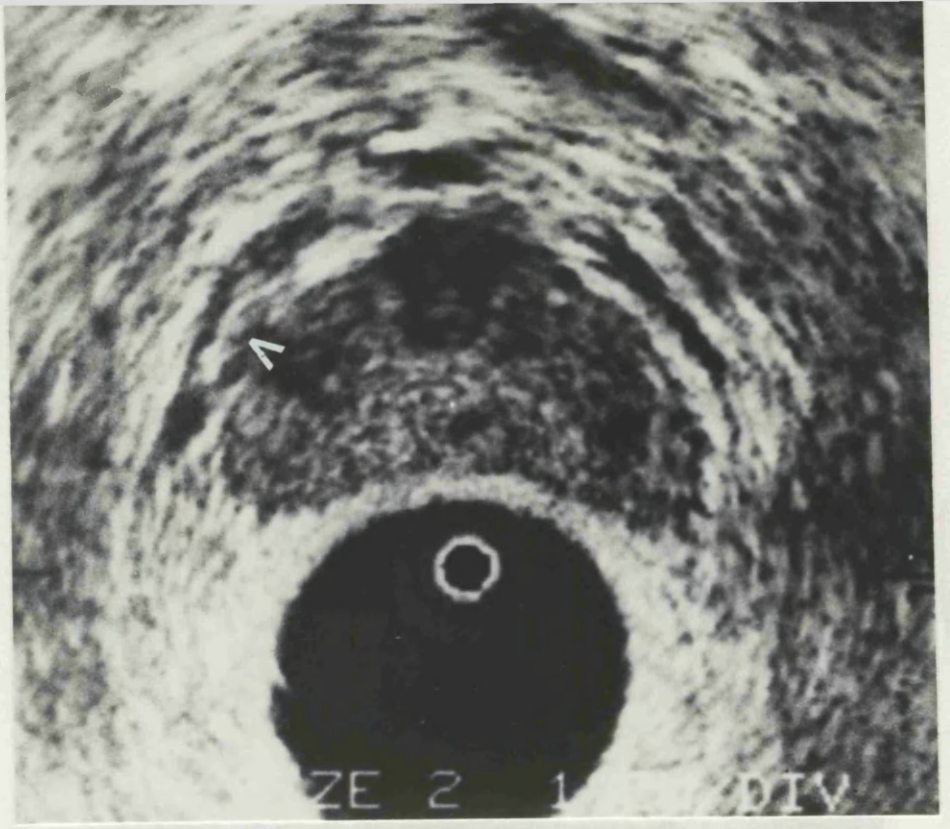


Fig. 12.6 Capsular irregularity (CI) in a prostatic ultrasonograph

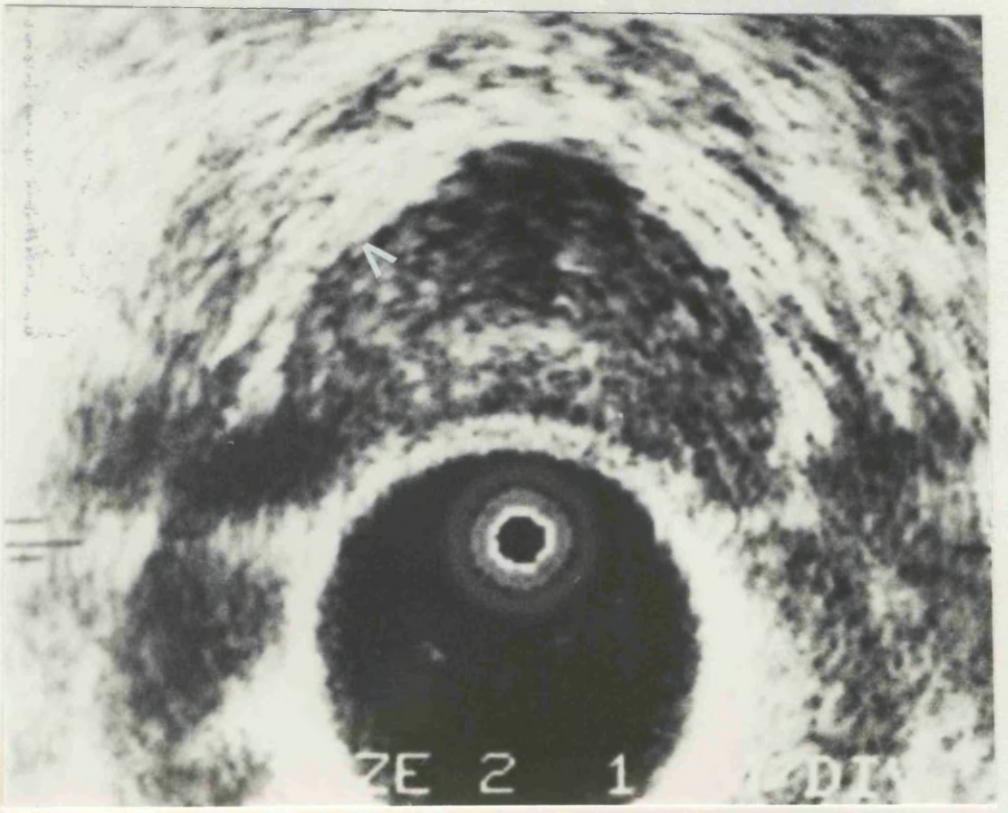


Fig. 12.7 Capsular thickening (CT) in a prostatic ultrasonograph

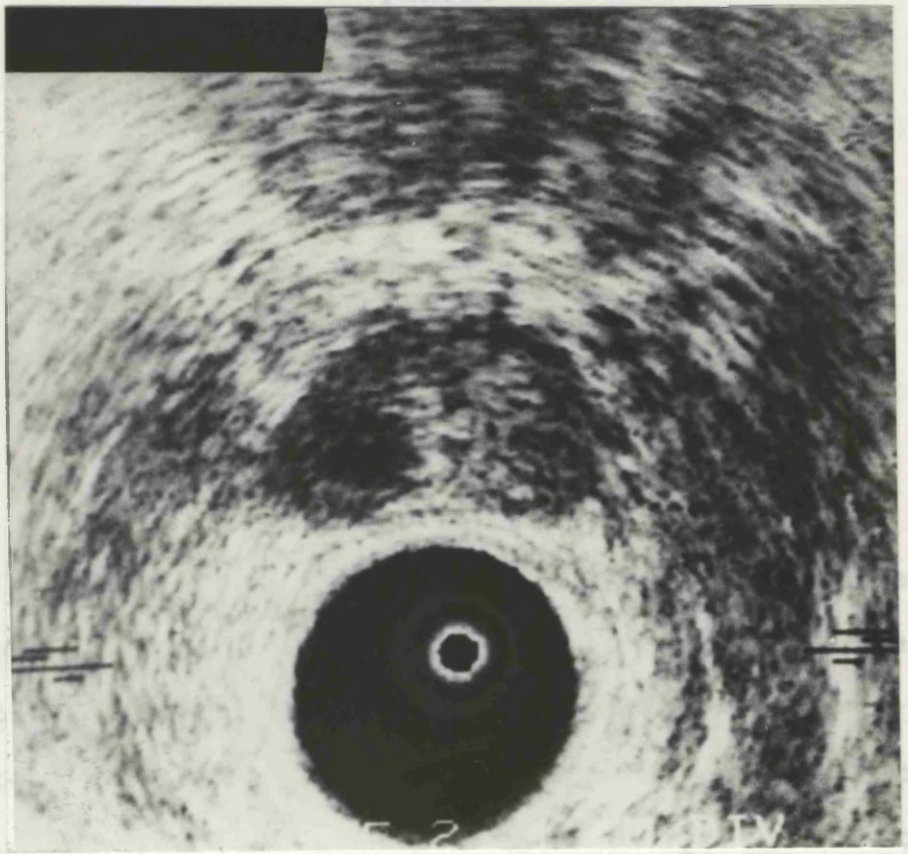


Fig. 12.8 An ultrasonograph of the prostate displaying an echolucent zone (ELZ)

Table 12.2 Presenting Symptoms in Patients with a Clinical Diagnosis of Chronic Prostatitis

Pain	Perineal	48%
	Testicular	40%
	Penile	37%
	Ejaculatory	33%
	Suprapubic	23%
	Rectal	13%
Dysuria		43%
Altered ejaculate		30%

Seven ultrasound features were identified as being associated with symptoms of chronic prostatitis, Table 12.1. Correlation of these ultrasound features, with the leucocyte count in the EPS, revealed that the sonographic abnormalities were most prevalent in those patients with a leucocyte count greater than 100mm , namely those with borderline prostatitis or prostatitis, compared to those with a leucocyte count less than $100mm^3$ and a diagnosis of prostatodynia (Table 12.3). Furthermore, this difference was more marked when the grade 2 and 3 abnormalities were considered (Table 12.4).

Table 12.3 Correlation Between EPS Leucocyte Count and the Number of Ultrasound Abnormalities

Number of Abnormalities	EPS Leucocytes per mm^3 0-99 (n=20)	100-600 (n=14)	>600 (n=26)
> 2	5 (25%)	11 (79%)	22 (85%)
> 3	1 (5%)	7 (50%)	18 (69%)
> 4	1 (5%)	5 (36%)	14 (54%)

Table 12.4 Correlation of EPS Leucocyte Count with Extent of Ultrasound Abnormalities

Extent of Abnormalities	EPS Leucocytes per mm^3 0-99 (n=20)	100-600 (n=14)	>600 (n=26)
All grades	12 (60%)	13 (93%)	25 (96%)
Grades 2 + 3	4 (20%)	11 (79%)	21 (81%)
Grade 3	0	6 (43%)	10 (38%)

Although the sensitivity and the specificity of each sign was not assessed in this pilot study, a normal scan was associated with a diagnosis of prostatodynia, i.e. EPS leucocyte count less than 100mm , in 80% of cases.

No correlation between ultrasound abnormalities and age (Table 12.5) or symptom duration (Table 12.6) was demonstrated.

Table 12.5 Correlation between Age and Extent of Ultrasound Abnormalities and a Diagnosis of Prostatitis

	Age		
Extent of Ultrasound Abnormalities	<30 yrs. (n=18)	30-40 yrs. (n=28)	>40 yrs. (n=14)
Grade 2 + 3	8 (44%)	17 (61%)	12 (86%)
EPS Leucocyte Count			
<100 per mm^3	9 (50%)	9 (32%)	2 (14%)

Table 12.6 Correlation between Symptom Duration and Extent of Ultrasound Abnormalities

Extent of Ultrasound Abnormalities	Symptom Duration <1 yr. (n=16)	1-5 yrs. (n=30)	>5 yrs. (n=14)
Grade 2 + 3	10 (63%)	17 (57%)	9 (64%)

STUDY 12.III CORRELATION OF TRANSRECTAL PROSTATIC ULTRASOUND WITH LEUCOCYTE COUNT WITHIN POST-PROSTATIC MASSAGE URINE (VB3) IN PATIENTS WITH SYMPTOMS OF CHRONIC PROSTATITIS

The pilot study (Study 12.II), correlating ultrasound abnormalities with the leucocyte count in the expressed prostatic secretion (EPS) of patients with a clinical diagnosis of chronic prostatitis, indicated that ultrasound abnormalities were more prevalent in patients with proven prostatitis (diagnosed on the basis of the leucocyte count in the EPS). Thus, a more detailed study, involving a larger group of patients with a clinical diagnosis of prostatitis, as well as a control group, was undertaken. In this study, the diagnosis of prostatitis was based on the leucocyte count in the post-prostatic massage urine (VB3), as EPS was not obtained from all cases.

Patients and Methods

Two hundred patients with a clinical diagnosis of chronic prostatitis along with thirty five normal controls (see Study 12.I) were investigated. This study group included the sixty patients comprising the chronic abacterial prostatitis cohort (see Section 13), and were drawn from the sexually transmitted diseases and urology clinics of St. Mary's Hospital, London.

All patients and controls underwent transrectal prostatic ultrasound in the left lateral position, using a Bruel and Kjaer 1846 scanner with a 5.5 MHz transducer. The sonograph was assessed for the presence of the ultrasound signs described in Study 12.II (Table 12.1), plus the presence of the "halo" sign, an area of decreased echogenicity in the periurethral zone, regarded by some authors (Griffiths et al, 1984; Wiegand & Weidner, 1986) as indicative of inflammatory prostatic disease. These signs are listed in Table 12.7.

Table 12.7 Ultrasound Signs Assessed in Patients with a Clinical Diagnosis of Prostatitis

High Density Echoes	(HDE)
Mid-range Echoes	(MRE)
Echolucent Zones	(ELZ)
Capsular Irregularity	(CI)
Capsular Thickening	(CT)
Ejaculatory Duct Calcification	(EDC)
Periurethral Zone Irregularity	(PZI)
"Halo" sign - Periurethral Zone	(Halo)

The location of each abnormality was recorded as being in the central, transitional or peripheral zones, in the case of parenchymal abnormalities, i.e. HDE, MRE and ELZ, and as being unilateral or bilateral in the case of capsular abnormalities (CI,CT) and ejaculatory duct calcification (EDC).

Each patient underwent prostatic localisation studies (see Section 11). The patients were then classified on the basis of the leucocyte count in the post prostatic massage urine (VB3) and assigned a score of 0-5 (see Appendix II). The results of the localisation studies were not available to the ultrasonographer when the prostatic scan was performed.

Results

The study group of two hundred patients was aged 16-91 years, mean 40, compared to the control group aged 18-68 years, mean 37.7. The duration of symptoms in the former group range from 2 months to 20 years, mean 2.4 years.

The patients were classified according to the Stamey score (Appendix II) as depicted in Table 12.8.

Table 12.8 Classification of Study Group in Terms of Stamey Score

Stamey Score	No. of Patients (% of Study Group)
0	40 (20%)
1	14 (7%)
2	41 (20.5%)
3	48 (24%)
4	31 (15.5%)
5	26 (13%)

Thus, the study group comprised 54 patients with prostatodynia, 41 with borderline prostatitis, 79 with prostatitis and 26 with a documented past history of prostatitis, although at the time of the analysis localisation studies showed no evidence of active inflammation.

Table 12.9 Ultrasound Findings in Normal Controls and Patients Compared with Stamey Score Based on VB3 Leucocyte Count

Ultrasound Signs	Normal Controls	Stamey Score			All Symptomatic Patients
		<2	2-4	5	
	n (%)	n (%)	n (%)	n (%)	n (%)
HDE	19 (54%)	34 (63%)	102 (85%)	24 (92%)	158 (79 %)
MRE	13 (37%)	25 (46%)	109 (91%)	16 (100%)	161 (80.5%)
ELZ	0	4 (7%)	82 (68%)	7 (27%)	92 (46 %)
CI	1 (3%)	7 (13%)	76 (63%)	15 (58%)	99 (49.5%)
CT	0	7 (13%)	52 (43%)	13 (50%)	71 (35.5%)
EDC	1 (3%)	17 (31%)	58 (57%)	14 (54%)	99 (49.5%)
PZI	3 (9%)	7 (13%)	41 (34%)	4 (15%)	53 (26.5%)
Halo	5 (14%)	7 (13%)	13 (11%)	1 (4%)	53 (11 %)

All the ultrasound signs, with the exception of the "halo" sign were significantly (chi-square test) associated with symptoms of chronic prostatitis compared to controls (Table 12.10). Furthermore, these ultrasound signs in patients with evidence of either borderline prostatitis or prostatitis, based on localisation studies, and more specifically, analysis of the leucocyte count in VB3, were significantly more prevalent than in those patients with a diagnosis of prostatodynia (Table 12.10).

Table 12.10 Significance of Ultrasound Abnormalities in Relation to Leucocyte Count in Post Prostatic Massage Urine (VB3) - Represented by Stamey Score

Significance - Chi-square Test

Ultrasound Signs	Normal Controls Vs All Patients	Stamey Score <2 VS Stamey Score ≥2
HDE	$p < 0.05$	$p < 0.001$
MRE	$p < 0.001$	$p < 0.001$
ELZ	$p < 0.001$	$p < 0.001$
CI	$p < 0.001$	$p < 0.001$
CT	$p < 0.001$	$p < 0.001$
EDC	$p < 0.001$	$p < 0.001$
PZI	$p < 0.02$	$p < 0.001$
Halo	Not Significant	Not Significant

From the data, the sensitivity and specificity of each sign was calculated for each category of patient based on the Stamey Score, i.e. leucocyte count in VB3 (Table 12.11).

Table 12.11 Sensitivity (Sen) and Specificity (Spec) For Each Ultrasound Sign Related to Stamey Score

	Stamey Score									
	≥1		≥2		≥3		≥4		5	
Ultra-sound signs	Sen	Spec	Sen	Spec	Sen	Spec	Sen	Spec	Sen	Spec
HDE	83.7	40.0	86.3	40.7	83.8	26.3	89.5	25.2	92.3	23.0
MRE	90.6	60.0	92.5	57.9	96.2	36.8	100.0	27.3	100.0	22.4
ELZ	57.5	100.0	61.0	94.4	57.1	66.3	54.4	57.3	26.9	57.1
CI	58.7	87.5	62.3	85.2	67.6	70.5	66.7	57.3	57.7	51.7
CT	41.9	90.0	44.5	88.9	48.6	78.9	47.4	69.2	50.0	66.7
EDC	54.4	70.0	56.2	68.5	57.1	58.9	56.1	53.1	53.8	57.1
PZI	30.0	87.5	30.8	85.2	29.5	76.8	26.3	73.8	15.4	71.8
Halo	10.0	85.0	9.6	85.2	10.5	88.4	8.8	88.1	3.8	87.9

The "best fit" correlation between Stamey score and ultrasound findings, with respect to sensitivity and specificity, is with a Stamey score of ≥ 2, i.e. a VB3 leucocyte count of ≥ 50 cells per mm^3. In order to make this association, a compromise has to be reached between sensitivity and specificity. With the majority of signs, the exceptions being echolucent zones (ELZ), ejaculatory duct calcification (EDC) and peripheral zone irregularity (PZI), the sensitivity of each sign as an indicator of prostatitis, tended to increase with higher Stamey scores, i.e. the level of confidence in the diagnosis of prostatitis increased. However, accompanying this increased sensitivity was a decrease in specificity.

In order to attain a satisfactory specificity for echolucent zones (ELZ), capsular irregularity (CI), capsular thickening (CT) and periurethral zone irregularity (PZI), a low sensitivity, ranging from 30.8 to 62.3 must be accepted, with the resultant risk of false-negative interpretations. The presence of high density echoes (HDE) and mid-range echoes (MRE) was predictive for a diagnois of prostatitis, with a sensitivity of 92.5% and 86.3% respectively, with a Stamey score of >2, but at a cost of low specificity, 51.9% and 40.7% respectively, and therefore a risk of false positive interpretations. Ejaculatory duct calcification (EDC) appeared to be a relatively poor indicator of inflammatory prostatic disease, with a sensitivity of 56.2% and specificity of 68.5% for a diagnostic Stamey score ≥ 2.

No single ultrasound sign, assessed in isolation, with the possible exception of echolucent zones (ELZ), was suggestive of a diagnosis of prostatitis. A combination of the signs of high density and mid-range echoes, echolucent zones, capsular irregularity or thickening, and periurethral zone irregularity was, however, suggestive of prostatitis. Multivariate analysis of the ultrasound signs in this study was undertaken, but the numbers of patients studied were too small to provide statistically significant information as to the diagnostic ability of any combination of signs.

The site of parenchymal ultrasound abnormalities, (Table 12.12), was distributed evenly between the zones, in the case of high density echoes (HDE), but showed a propensity for the peripheral zone in the case of mid-range echoes (MRE) and echolucent zones (ELZ). These data also indicated that in individual patients the abnormalities usually occupy more than one zone.

Table 12.12 Site of Parenchymal Ultrasound Abnormalities

	Abnormality (%)		
Site	HDE	MRE	ELZ
Central Zone	60.3	15.5	4.2
Transitional Zone	49.2	37.5	17.1
Peripheral Zone	44.7	83.7	87.1

There was no difference in symptom duration between patients with prostatodynia and prostatitis and furthermore, no ultrasound sign was associated with prolonged symptom duration.

Three of the ultrasound signs, echolucent zones (ELZ) ($p < 0.001$), ejaculatory duct calcification (EDC) ($p < 0.02$) and periurethral zone irregularity (PZI ($p < 0.01$), showed a significant increase in prevalence with increasing age. However, when the study group was sub-divided on the basis of age, an increase in the number of patients with prostatitis was also noted with increased age: < 31 years - 48%, 31 to 50 years - 62% amd > 50 years - 80%.

Study 12.IV SERIAL TRANSRECTAL ULTRASOUND SCANNING IN PATIENTS WITH SYMPTOMS OF CHORNIC PROSTATITIS

Patients and Methods

In order to assess the evolving pattern of ultrasound signs in patients with symptoms of chronic prostatitis, fifty of those in Study 12.III, comprising 30 patients with prostatitis, 11 with borderline prostatitis and 9 with prostatodynia, underwent repeat transrectal ultrasound scanning.

Results

The details of the alteration in ultrasound findings and Stamey score, plus duration of follow up are depicted in AppendixIV.

The mean overall follow up period was 10.4 months, being 10.4 months for the prostatitis group, 11.9 months for the borderline prostatitis group and 8.1 months in the case of the prostatodynia group.

In 36 patients (72%), the follow up ultrasound differed from the original study, respresenting a change in 87% of the prostatitis group, 73% of the borderline prostatitis group and 11% of the prostatodynia group.

Parenchymal signs, (high density echoes, mid-range echoes and echolucent zones), developed in 40% of chronic prostatitis patients, and 27% of borderline prostatitis patients; these parenchymal signs changed in 47% and 36% respectively, of chronic prostatitis and borderline prostatitis patients. In patients with chronic prostatitis, 30% of echolucent zones reverted to normal and 17% changed to mid-range echoes, compared to 9% and 27% respectively, in the borderline group. No patients with prostatodynia showed any change in parenchymal signs.

Changes in capsular signs were more variable, but developed de novo in 37% of the prostatitis and 55% of the

borderline group, and changed from existing abnormalities in 40% of patients with prostatitis and 18% of the borderline prostatitis group. The capsule reverted to normal in the prostatitis group and borderline prostatitis group in 20% and 9% of cases respectively.

These follow up data indicated that the development of new signs was more frequent than the resolution of existing ones in both prostatitis and borderline prostatitis, yet in prostatodynia, changes were minimal.

The loss of echolucent zones (ELZ) was mirrored by a reduction in the leucocyte count in VB3, and thus in Stamey score, whereas all other ultrasound changes occured irrespective of the inflammatory response within the prostatic secretion. Thus the echolucent zone may be an ultrasound sign worthy of follow up, as it alone appears to reflect inflammatory changes within the prostate in this study.

STUDY 12.V CORRELATION OF ULTRASOUND SIGNS WITH HISTOLOGY IN PATIENTS WITH CHRONIC PROSTATITIS

Unlike studies of prostatic cancer, in which the ultrasound features have been compared with the histology of whole prostates from either cadavers of patients undergoing radical prostatectomy, the correlation of ultrasound with pathology in patients with prostatitis is more difficult. However, the advent of transrectal prostatic ultrasound and subsequent ultrasound guided biopsy, has enabled this correlation to be attempted.

Patients and Methods

The sixty patients comprising the chronic abacterial prostatitis cohort (see Section 13), who were drawn from the larger group of patients with borderline prostatitis and prostatitis, investigated in this section, were subjected to ultrasound guided biopsy via the transperineal route; the technique is described in Appendix VI.

Any areas of abnormal echogenicity within the parenchyma, namely high density echoes (HDE), mid-range echoes (MRE) and echolucent zones (ELZ) were biopsied. In patients with more than one abnormality on their ultrasound scan, biopsies of each abnormality were performed.

The tissue obtained was placed immediately into 10% formal saline and in the case of multiple biopsies, each sample was placed in separate containers and a record made of the ultrasound abnormality corresponding to each biopsy site.

The prostatic tissue was submitted to routine histological analysis. In each case, the tissue was examined without any prior knowledge of the ultrasound findings. Once the histology was known the findings were then correlated with the ultrasound features.

Results

The sixty patients were aged 19-75 years, mean 39.8 years.

The tissue obtained revealed a chronic inflammatory infiltrate in 51 patients (85%) (see Appendix XVIII& Table 16.1). In 18 patients (30%) there was evidence of fibrosis. Histology was normal in 4 patients (7%) and revealed glandular proliferation, yet no inflammation, in a further 2 (3%), whilst insufficient tissue was obtained in 3 patients (5%).

The ultrasound features and corresponding histological findings are depicted in Appendix V . These findings are summarised in Tables 12.13, and 12.14, and indicate a close correlation between high density echoes (HDE) and corpora amylaceae, mid-range echoes (MRE) and fibrosis or inflammation, and echolucent zones (ELZ) and inflammation.

Table 12.13 The Correlation Between Histology and Ultrasound Features in Chronic Abacterial Prostatitis

		Ultrasound Features (%)		
Histology		HDE	MRE	ELZ
Inflammation	(n=53)	4	57	39
Corpora amylaceae	(n=20)	90	10	0
Fibrosis	(n=18)	0	89	11

Other findings on biopsy were benign nodular hyperplasia, glandular proliferation, dilated glands and telangiectasia.

Table 12.14 The Correlation Between Ultrasound Features and Histology in Chronic Abacterial Prostatitis

Ultrasound Features	Normal	Inflammation	Fibrosis	Corpora amylaceae	Others
HDE	4	8.5	0	75	12.5
MRE	3.5	55	28	3.5	10
ELZ	0	73	7	0	20

The pathological findings support the follow up ultrasound data and suggest that inflammation, as indicated by the presence of echolucent zones, heals by fibrosis, as manifest by mid-range echoes. High density echoes signifying corpora amylaceae tended to persist in follow up studies, as would be expected.

SUMMARY

Transrectal prostatic ultrasound is a mininally invasive, painless investigation that provides objective data on the topography of the prostate. Due to varied symptomatology and lack of physical signs, chronic prostatitis is associated with difficulties in diagnosis, which rests on the finding of excessive numbers of leucocytes in the prostatic secretion obtained during the Stamey localisation procedure. Seven ultrasound features, namely high density and mid-range echoes, echolucent zones, capsular irregularity and thickening, ejaculatory duct calcification and periurethral zone irregularity, displayed a significant correlation with a diagnosis of chronic prostatitis. The low specificity of high density and mid-range echoes (40.7% and 57.9% respectively) and the low sensitivity (range 30.8% - 62.3%) of the remaining five ultrasound features, preclude identification of any one feature as being diagnostic of chronic prostatitis. A possible exception is the echolucent zone which may also have a role in treatment monitoring. The detection of several ultrasound abnormalities favours a diagnosis of chronic prostatitis, whereas a normal scan in a symptomatic patient is highly suggestive of prostatodynia.

Ultrasonography confirmed the focal nature and frequent peripheral zone location of inflammatory prostatic disease. Follow up studies over a short period showed

that the echolucent zones may revert to normal, high density or mid-range echoes, in conjunction with a reduction in leucocyte count in the expressed prostatic secretion.

The ability to place a biopsy needle accurately under ultrasound control has enabled histological analysis of the parenchymal features. The high density echoes represent corpora amylaceae, the mid-range echoes inflammation, fibrosis or both, and the echolucent zones, inflammation.

SECTION 13 THE CONSTRUCTION OF A COHORT OF PATIENTS WITH CHRONIC ABACTERIAL PROSTATITIS

In order to elucidate the aetiological factors, particularly the role of micro-organisms, as well as the pathology and immunology, of chronic prostatitis, it is essential to ensure that a group of patients is constructed who have been diagnosed accurately by existing criterial. To make the diagnosis of prostatitis, the localisation studies described by Meares and Stamey (1968), are mandatory, and form the basis of the currently accepted classification of this complex of diseases (Drach et al, 1978).

Patients and Methods

Patients attending the sexually transmitted diseases and urology clinics of St. Mary's Hospital, London, with a clinical diagnosis of chronic prostatitis were studied. All patients, none of whom had received antibiotics for at least two months, underwent prostatic localisation procedures, and 60 of them were diagnosed as having chronic prostatitis. The demographic details of the cohort appear in Appendix VIII . In some cases, the diagnosis was not made at initial presentation, but following subsequent localisation studies.

The patients underwent repeat localisation procedures at 3 months following entry into the cohort. Thereafter, further localisation procedures were carried out as deemed clinically appropriate. Details of the findings of these investigations appear in Appendix VII .

The cohort was aged 19-75 years, (mean 39.8). The diagnosis of chronic prostatitis was made adopting the criteria detailed in Section 11. In 18 patients the diagnosis was made on the presence of leucocytes in both expressed prostatic secretion (EPS) and post-massage urine (VB3), and in the remaining 42 patients, on leucocytes in the VB3 sample alone.

The patients comprising this cohort were further subdivided on the basis of the VB3 leucocyte count, in

order to facilitate correlation of ultrasound features (see Section 12) and histological findings (see Section 16) with the degree of prostatic inflammation. A score of between 0-5 was devised, representing arbitrarily selected values of VB3 leucocyte count and correlated with diagnoistic categories suggested by Weidner and Ebner (1985) from their studies of post-massage urine see Appendix II and Table 13.1. This score was termed the Stamey score.

Table 13.1 Scoring System (Stamey Score) Based on the Post Prostatic Massage Urine (VB3) Leucocyte Count in Relation to Diagnosis

Prostatitis Score	VB3 Leucocyte Count/mm^3	Diagnosis
0	0	Prostatodynia
1	< 50	Prostatodynia
2	50- 99	Borderline Prostatitis
3	100-200	Prostatitis
4	> 200	Prostatitis
5	0 but documented history of prostatitis	Prostatitis

SECTION 14 A SEARCH FOR AETIOLOGICAL AGENTS IN CHRONIC ABACTERIAL PROSTATITIS

To elucidate the role of organisms, particularly Chlamydia trachomatis, mycoplasmas and ureaplasmas in chronic abacterial prostatitis, the cohort of patients with this condition, constructed by means of the localisation studies of Meares and Stamey (1968) (see Section 13), were studied. In an endeavour to overcome urethral contamination, a direct approach of ultrasound guided biopsy was adopted, to sample diseased areas of prostate and attempt to culture organisms directly from biospy samples.

Patients and Methods

The sixty patients comprising the chronic abacterial prostatitis (CABP) cohort, drawn from the sexually transmitted diseases and urology clinics of St. Mary's Hospital, London, were studied. Their demographic details appear in Appendix VIII .

Samples

Prior to performing the localisation studies, all patients had urethral swabs taken to be tested for Neisseria gonorrhoeae (by Gram-stain and culture) and Chlamydia trachomatis (by a direct immunofluorescence technique (see Appendix IX).

The urine samples collected as part of the localisation studies, ie VB1, VB2 and VB3, and the expressed prostatic secretion (EPS), following microscopy, was subdivided for:

i) aerobic culture
ii) anaerobic culture
iii) culture for Mycoplasma spp. and Ureaplasma spp.
iv) chlamydial detection - EPS only - culture and direct immunofluorescence technique.

Culture Techniques

For aerobic culture, 100 ul of each specimen was innoculated, with the aid of a sterile plastic spreader, onto blood agar and McConkey agar plates and incubated at 37 C in an atmosphere of 5% CO_2. The plates were observed for colony growth at 24 and 48 hours and the colonies counted and expressed as colony forming units per ml.

For anaerobic culture, the same method was adopted, except that innoculation of the sample was onto blood agar only, which was then incubated at 37°C in a gas packed anaerobic jar in 90% nitrogen and 10% carbon dioxide. The plates were examined for growth after 48 hours.

A portion of each urine specimen was centrifuged at 600g for 5 minutes before reconstitution in a small volume of supernatent fluid and placed in mycoplasma transport medium (see AppendixXII) and stored at -70°C for subsequent culture.[1] The EPS sample was placed directly into the transport medium and stored as for the urine specimens.

The frozen specimens were thawed in a water bath at 37°C and a 0.2ml sample transferred to 1.8mls of mycoplasma and ureaplasma culture medium; three ten fold dilutions were then performed for each sample. The specimens were then incubated at 37°C in atmospheric conditions and examined for any colour change (Fig 14.1). after 24 hours and then at 48 hour intervals for a total of 14 days. If a colour

change (from yellow to pink) occurred indicating the presence of mycoplasmas and/or ureaplasmas, aliquots of the medium were transferred to fresh growth medium and agar for the development of the colonies and their number expressed as the number of colour changing units per ml.

A portion of the EPS sample was spread onto a MicroTrak (Syva) slide for detection of Chlamydia trachomatis using a direct immunofluorescence technique (Appendix IX) Fig 14.2.

At the same time as the localisation studies, venous blood was drawn, for chlamydial serology, and then again six weeks later. An indirect immunofluorescence technique (Appendix XV) was used to detect IgG and IgM antibodies to serovars D-K of Chlamydia trachomatis.

In addition, during the study period, an isolated semen sample was collected and stored in liquid nitrogen until processed. The semen was thawed in a water bath at 37 ^{o} C and 1.5mls transferred into a Sarstedt vial and centrifuged for 5 minutes at 5000 rpm. The supernatant was drawn off and 50ul mixed with 50ul of phosphate buffered saline (PBS) in a microtitre plate. Doubling dilutions were carried out from 1:2 to 1:256 and IgA, IgG and IgM antibodies against serovars D-K of Chlamydia trachomatis sought by an indirect immunofluorescence technique, see Appendix XV.

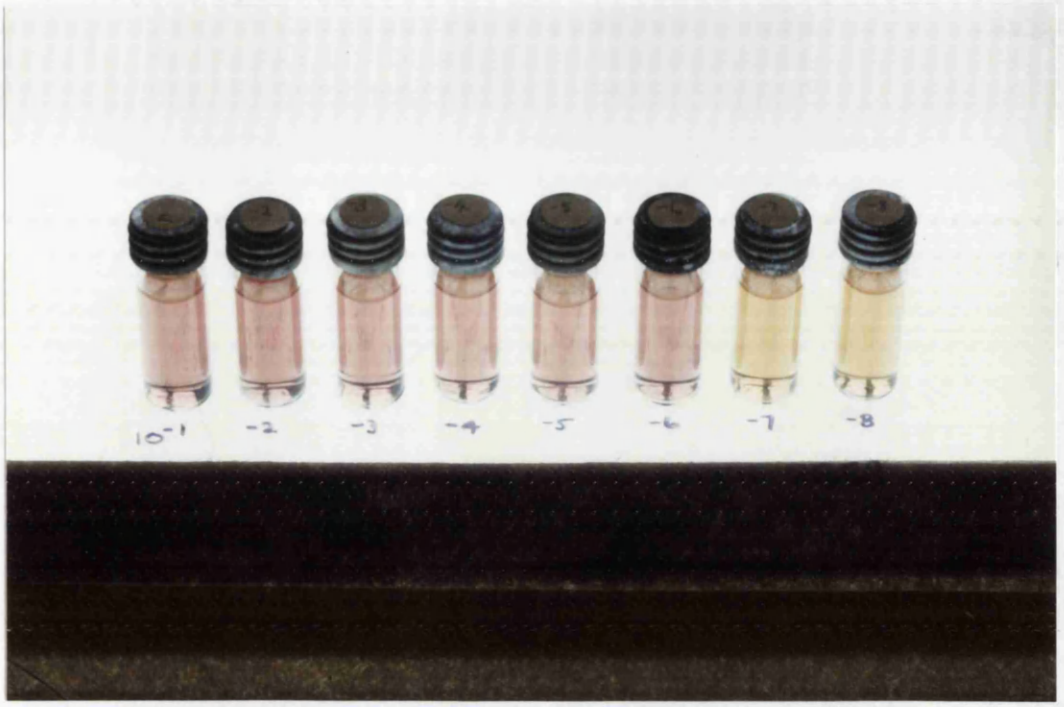


Fig. 14.1 The colour change of culture medium indicating the prescence of mycoplasmas

Fig. 14.2 Positive immunofluorescence (MicroTrak) indicating the presence of Chlamydia trachomatis

Prostatic Biopsy

Following these localisation studies, each patient underwent transrectal prostatic ultrasound, using a Bruel and Kjaer 1846 machine with a 5.5 MHz transducer. The examination was performed with the patient in the left lateral position. Under ultrasound guidance, prostatic tissue was obtained from areas of abnormal echogenicity via the transperineal route see AppendixVI.

The biopsy tissue was subdivided, one portion being placed into 10% formal saline for routine histological analysis, and one into 1ml of sucrose-phosphate (2SP) medium without antibiotics, which was stored in liquid nitrogen prior to chlamydial culture. A third portion was pressed onto a MicroTrak (Syva) slide to form an impression smear which was examined for chlamydiae by the MicroTrak (direct immunofluorescence) technique. A fourth portion was placed in mycoplasma transport medium and stored at $-70^{o}C$ prior to culture for mycoplasmas and ureaplasmas, and a fifth portion in modified Stuart's medium for the culture of anaerobic bacteria. Finally, a portion was placed in normal saline for routine aerobic bacteriological culture.

To the prostatic tissue in 2SP medium, 1ml of chlamydial growth medium CMGA (see Appendix X) was added and the tissue homogenised in a ground glass grinder. The homogenate was then made up to a volume of 8ml with CMGA and 1ml of the resultant sample added to each of 8 McCoy cell cultures (see Appendix XI).

These were incubated at 37°C for 48 hours, fixed with methanol, stained with Giemsa reagent and examined by dark ground microscopy for chlamydial inclusions.

The tissue to be cultured for mycoplasmas and ureaplasmas was teased between size 11 surgical blades in 0.4ml of mycoplasma culture medium (see Appendix XII). Then 0.2ml of the fragmented tissue was transferred to 1.8ml of mycoplasma culture medium containing arginine in a screw-capped glass vial of 2.5ml capacity and the other 0.2ml to 1.8ml of mycoplasma culture medium containing urea. Further ten-fold dilutions were made in the respective media up to a dilution of 10^{-4}. The vials were incubated at 37°C and examined as for the urine samples described previously.

The specimen for anaerobic bacteriological culture was homogenised and innoculated onto blood agar, placed in a gas packed anaerobic jar in 90% nitrogen and 10% carbon dioxide and incubated at 37°C for 5 days. The plates were examined for colonies and any growth subcultured and identified. The remaining tissue was homogenised in a ground glass grinder and innoculated onto blood and McConkey agar. The cultures were incubated aerobically in an atmosphere of 5% carbon dioxide at 37°C and examined for evidence of growth at 24 and 48 hours.

The patients in whom the Stamey localisation procedures suggested organisms may be arising from the prostate,

namely Mycoplasma hominis in patient 6 and Ureaplasma urealyticum in patient 51, Table 14.1 as well as those in whom organisms were recovered from prostatic biopsies (Table 14.2, Appendix) were all treated for a six week period with an antimicrobial to which the organisms were sensitive. Following treatment, the Stamey localisation procedure was repeated.

Results

The ultrasonic features have been described (see Section 12). The areas of abnormal echogenicity, namely high density (HDE) and mid-range echoes (MRE) and echolucent zones (ELZ) were biopsied. In 51 patients, a diagnosis of prostatitis indicated by the Stamey localisation procedure was confirmed by histological examination of prostatic tissue. Insufficient tissue was obtained from three patients, there were no inflammatory features in four and a further two displayed glandular proliferation yet no inflammation. The histological features (Appendix XVII) revealed a chronic inflammatory reaction, which in the majority of cases was of low grade. The degree of lymphocytic infiltration was graded (see Section 16) on a scale of 1-3: grade 1 being 5-10 cells per high powered field (hpf) (x400), 2 being 11-15 cells per hpf and 3 being >15 cells per hpf. The lymphocytes were most frequently located in the periglandular and stromal regions and were rarely intraglandular. Fibrosis was noted in 30% of the biopsies and vessel thickening in 10%.

Gonococci were not isolated from any of the patients. Chlamydiae were detected, by the direct immunofluorescence technique, in urethral specimens from only 2 patients.

Sufficient EPS for examination was obtained from 18 patients and in only 1 patient were chlamydiae detected by immunofluorescence; chlamydiae were also detected in the urethra from this case.

Chlamydiae were not isolated in McCoy cell culture from the prostatic tissue of any of the patients, nor were they detected by immunofluorescence in any of the impression smears of prostatic tissue.

No patient had evidence of prostatitis, as defined by Meares and Stamey (1968), caused by aerobic or anaerobic bacteria (see AppendixVII). In four patients, Mycoplasma hominis and in six, Ureaplasma urealyticum were found in the urine and/or EPS. The numbers of organisms isolated are depicted in Table 14.1 but only one patient, in whom Ureaplasma urealyticum was isolated, had a larger number of organisms in the EPS than in VB1 and VB2. However, in a further patient Mycoplasma hominis was isolated, albeit at a low titre, in VB3 alone, and thus localised to the prostate.

TABLE 14.1 ISOLATION OF MYCOPLASMAS AND UREAPLASMAS FROM URINE AND EXPRESSED PROSTATIC SECRETION

Patient's Study Number	No. of M. hominis organisms(*) in sample				No. of U. urealyticum organisms(*) in sample			
	VBI	VB2	EPS	VB3	VB1	VB2	EPS	VB3
6	-	-	-	10^2	-	-	-	-
26	-	-	-	-	10^3	-	-	-
34	10^2	-	-	-	-	-	-	-
47	-	-	-	-	10^2	10^2	-	-
49	-	-	-	-	10^2	-	-	10^2
50	10^2	-	-	-	10^3	-	-	-
51	-	-	-	-	10^2	10^2	10^3	10^2
52	10^2	-	-	-	10^3	-	-	10^2

* Expressed as colour-changing units /ml.

Mycoplasmas and ureaplasmas were not isolated from the prostatic tissue of any of the patients. In seven patients aerobic, and in three anaerobic, organisms were cultured from the prostatic tissue as detailed in Table 14.2.

TABLE 14.2 BACTERIA ISOLATED FROM PROSTATIC TISSUE

Number of Patients	Bacteria Isolated
3*	Escherichia coli
2*	Bacteroides fragilis
2	Staphylococcus epidermidis
1	Diphtheroids
1	Bacteroides urealyticus
1	Pasteurella spp.

* In one patient both these organisms were isolated.

In those patients in whom Mycoplasma hominis and Ureaplasma urealyticum were localised to the prostate and who received antimicrobial therapy, no alteration of either the EPS or VB3 leucocyte count, or symptoms, was observed following treatment, although the organisms were cleared from the prostatic fluid and were no longer isolated in any of the urine or EPS specimens, in subsequent localisation studies. Furthermore, there was no change in either EPS or VB3 leucocyte count or symptoms after the treatment period in those patients whose prostatic biopsies yielded bacterial growth.

The results of the serological examination of the "acute" samples (see Appendix XVI) revealed a chlamydial IgM

antibody titre of 1:8 in one patient, 1:4 in seven, 1:2 in three and 1:<2 in the remaining forty-nine patients. The IgG antichlamydial antibody was detected at the following titres: 1:32 in one patient, 1:8 in fifteen patients, 1:4 in six, 1:2 in five and 1:<2 in thirty-three patients. The patient with an IgG titre of 1:32 had an IgM titre of 1:4.

The convalescent serum sample (drawn six weeks after the "acute" specimen) revealed a chlamydial IgM antibody titre of 1:4 in three patients, 1:2 in four and 1:<2 in the remaining fifty- three patients. The IgG antichlamydial antibody was detected at the following titres: 1:32 in one patient, 1:16 in four patients, 1:8 in nine, 1:4 in six, 1:2 in five and 1:<2 in thirty-five patients (Appendix XVI)

In four patients there was a rise in IgM antibody titre between acute and convalescent samples: from 1:<2 to 1:2 in three patients and 1:<2 to 1:4 in one patient. In eleven patients a rise in IgG antibody titre was detected between acute and convalescent samples, though in only two cases was a four-fold increase (1:4 to 1:16 and 1:8 to 1:32) demonstrated. The acute and convalescent IgM titres were 1:8 and 1:4 respectively in the former patient and 1:<2 and 1:2 in the latter. Of the remainder, three patients increased their IgG antibody titres to 1:2, three to 1:4, one to 1:8 and two to 1:16, representing a two-fold rise in each case. Only one of these nine patients had a corresponding rise in IgM antibody titre,

from 1:<2 to 1:4, accompanying the 1:<2 to 1:2 rise in IgG antibody titre (Appendix XVI). Antichlamydial antibodies of the IgA, IgG and IgM class within the semen, were not detected in any of the patients studied.

SUMMARY

This study of 60 patients with chronic abacterial prostatitis, diagnosed by means of the Stamey localisation procedure, used transperineal ultrasound guided biopsy to obtain tissue from abnormal areas of the prostate to ascertain the role of micro-organisms in this condition.

Chlamydia trachomatis was not isolated from either prostatic fluid or tissue, save in one patient in whom the organism was detected in the EPS, yet also in the urethra at the time of sampling. Chlamydial serological data support these findings, with an IgG titre of 1:32 in only two cases, both with low IgM titres, implying absence of active infection; thirteen patients displayed a rise in either IgG or IgM antibody titre, but in only two cases was a four-fold rise noted, in one associated with a fall in IgM titre from 1:8 to 1:4 and the other with a rise in IgM titre from 1:<2 to 1:2, providing little supportive data of active infection.

Mycoplasma hominis and Ureaplasma urealyticum although localised to the prostate in one patient in each case, were not isolated from the prostatic tissue in any of the cases. Also, in view of the failure of antimicrobial therapy to alter symptoms or prostatic fluid leucocyte counts in the two patients in whom the organisms were localised to the prostate, it was concluded that these organisms were unlikely to play an active role in the

aetiology of chronic abacterial prostatitis. Furthermore, organisms were isolated from the prostatic tissue of only 9 (15%) and wer considered contaminants from the perineal skin as treatment with an appropriate antimicrobial agent failed to alter symptoms or the leucocyte count in the urine or prostatic fluid.

SECTION 15 A SEARCH FOR CHLAMYDIA TRACHOMATIS IN THE PROSTATE IN ACUTE NON-GONOCOCCAL URETHRITIS

Chlamydia trachomatis is undeniably an important cause of acute non-gonococcal urethritis (Schachter, 1978; Taylor-Robinson & Thomas, 1980). The inability to detect this organism from the prostate of patients with chronic abacterial prostatitis (see Section 14) drew the conclusion that Chlamydia trachomatis played no active role in the aetiology of this condition. However, the possibility of a role at an earlier stage in the disease process, for example at the initiation of the inflammatory response, could not be excluded with certainty.

This raised the question, as to whether C. trachomatis organisms enter the prostate during acute urethritis caused by this organism, and if so, can they be detected by ultrasound guided transperineal aspiration biopsy?

Patients and Methods

Patients attending the sexually transmitted diseases clinic at St. Mary's Hospital, London with a diagnosis of acute urethritis, based on the finding of > 5 leucocytes per high-power microscope field (x1000) on examination of an urethral smear, were studied. Seven patients in whom an urethral smear showed no evidence of Neisseria gonorrhoeae (Gram stain and culture), but in whom an urethral swab detected Chlamydia trachomatis by a micro-immunofluorescence technique (MicroTrak, Syva) (see Appendix IX), were studied. These patients agreed to further prostatic investigations. Each patient underwent full clinical examination including a rectal examination. Transrectal prostatic ultrasound in the left lateral position using a Bruel and Kjaer 1846 scanner with a 5.5 MHz transducer was then performed, and any abnormalities noted. Under ultrasound guidance, a 22 French aspiration cytology needle (Cook Urological) was placed into any areas of abnormal echogenicity and/or the periurethral zone. The sample was taken and placed on a MicroTrak (Syva) slide and subjected to a direct micro-immunofluorescence technique for the detection of C. trachomatis (see Appendix IX); a further sample was placed in mycoplasma transport medium and stored at -70°C for subsequent culture of Mycoplasma spp. and Ureaplasma urealyticum (see Appendix XIV).

At the same time, venous blood was drawn for chlamydial serology. The patients were then treated with Doxycycline 100mg bd for one week and reviewed two weeks after initial consultation. Three patients attended for review and had a further urethral swab taken, which was applied to a MicroTrak (Syva slide), for a test of cure, and also had a further venous blood sample drawn for chlamydial serology.

RESULTS

The patients studied were aged 21-40 years, mean 29, and had a mean symptom duration of 5.3 days, range 1-14 days. The demographic details of the patients appear in the Table 15.1.

TABLE 15.1 DEMOGRAPHIC DETAILS OF PATIENTS WITH ACUTE CHLAMYDIAL URETHRITIS

Patient study no.	Age	Symptom duration in days	Past History of urethritis	Other symptoms	Prostate on rectal examination
1	27	1	NGUx2	Nil	Normal
2	40	7	NGUx4	Dysuria	Normal
3	24	14	NGUx1	Dysuria	Normal
4	21	2	Nil	Dysuria	Normal
5	34	2	NGUx1	Nil	Normal
6	31	4	GUx1	Nil	Normal
7	26	7	NGUx1	Dysuria	Normal

Key: NGU - Non-Gonococcal Urethritis
GU - Gonococcal Urethritis

Three patients had abnormalities on transrectal prostatic ultrasound: high density and mid-range echoes in one patient, high density echoes in another and periurethral

zone irregularity in the third. All abnormalities were confined to the central zone of the prostate.

In all patients, both the direct immunofluorescence method for detection of Chlamydia trachomatis and the culture for Mycoplasma species and Ureaplasma urealyticum from the prostatic aspirate were negative. The serological data is detailed in the Table 15.2 below.

TABLE 15.2 ANTICHLAMYDIAL ANTIBODIES IN ACUTE CHLAMYDIAL URETHRITIS

Patient study No.	Antichlamydial IgG Titre		Antichlamydial IgM Titre	
	Acute	Convalescent	Acute	Convalescent
1	1:32	1:64	1:2	1:8
2	1:64	-	1:4	-
3	1:16	-	1:8	-
4	1:4	1:16	1:2	1:8
5	1:2	-	1:2	-
6	1:16	-	1:4	-
7	1:8	1:16	1:4	1:4

In all patients who attended for review, C. trachomatis was not detected in the urethral smear using the direct immunofluorescence technique (MicroTrak Syva), and thus demonstrated eradication of this organism following treatment.

SUMMARY

Seven patients with acute non-gonococcal urethritis were studied. All patients had Chlamydia trachomatis detected in their urethra by a direct micro-immunofluorescence technique, but using the same technique, the organism could not be detected in prostatic aspirates obtained by ultrasound guided prostatic biopsy. Neither were Mycoplasma spp. or Ureaplasma urealyticum isolated from prostatic aspirates similarly obtained. Therefore, it is proposed from this pilot study, that C. trachomatis does not enter the prostate at the time that it infects the anterior urethra in acute non-gonococcal urethritis.

SECTION 16 THE PATHOLOGY OF THE PROSTATE IN CHRONIC ABACTERIAL PROSTATITIS

Little data are available on the pathological changes within the prostate in patients with chronic abacterial prostatitis. The focal nature of chronic prostatitis (McNeal, 1968) is well established and accounts for the lack of meaningful data arising from blind biopsy studies (Schmidt & Patterson, 1966; Nielsen & Christensen, 1972). However, with the advent of transrectal ultrasound, parenchymal abnormalities have been identified in patients with chronic abacterial prostatitis (see Section 12), and the technique of ultrasound guided biopsy has enabled these areas to be studied in detail.

Patients and Methods

The sixty patients comprising the chronic abacterial prostatitis cohort (see Section 13) underwent ultrasound guided transperineal prostatic biopsy, of the areas of abnormal echogenicity detected on prostatic ultrasonography (see Section 12).

A control group of twenty patients with no history of chronic prostatitis, nor cytological or microbiological evidence of prostatitis, as judged by localisation studies, also underwent transperineal ultrasound guided biopsy. These patients were drawn from the urology clinic of St. Mary's Hospital, London and underwent prostatic biopsy, under general anaesthesia, at the same time as other surgical procedures, namely cystoscopy and scrotal surgery. Both patients and controls gave informed written consent to the procedure.

The ultrasound scan was performed using a Bruel and Kjaer 1846 scanner with a 5.5 MHz transducer and the biopsies sampled with a 14 G Tru-cut (Travenol) needle (see Appendix VI).

For this part of the study, the tissue was subdivided, one portion being placed immediately in 10% formal saline for routine histological analysis and another orientated on a cork disc, covered with OCT mounting compound (Miles Scientific Laboratories Limited) and snap frozen in

isopentane cooled in a bath of liquid nitrogen, and then stored at -180°C for subsequent cryostat sectioning. For each biopsy, the corresponding ultrasound abnormality was noted; in patients with more than one area of abnormal echogenicity of the parenchyma, biopsies were taken from each lesion and the tissue placed in separate containers.

Routine Histology

The tissue for routine histology was embedded in paraffin wax and 4u sections made which were stained with haematoxylin and eosin. The sections were analysed by a consultant histopathologist with a special interest in prostate pathology. Particular attention was paid to the presence of acute inflammatory cells, i.e. neutrophils, chronic inflammatory cells and mononuclear cells including lymphocytes and plasma cells, the presence of fibrosis and vessel abnormalities, such as telangiectasia or vessel thickening. Furthermore, the presence of benign prostatic hyperplasia and other pathological conditions of the prostate were also noted.

The location of the chronic inflammatory infiltrate within the gland, namely glandular, periglandular or stromal, was recorded. In addition, as an aid to classification, a grading system, reflecting the extent of the chronic inflammatory infiltrate, was devised. A grade of 1-3, based on the mean number of chronic inflammatory cells per high power field (hpf) (x400) on examination of the whole

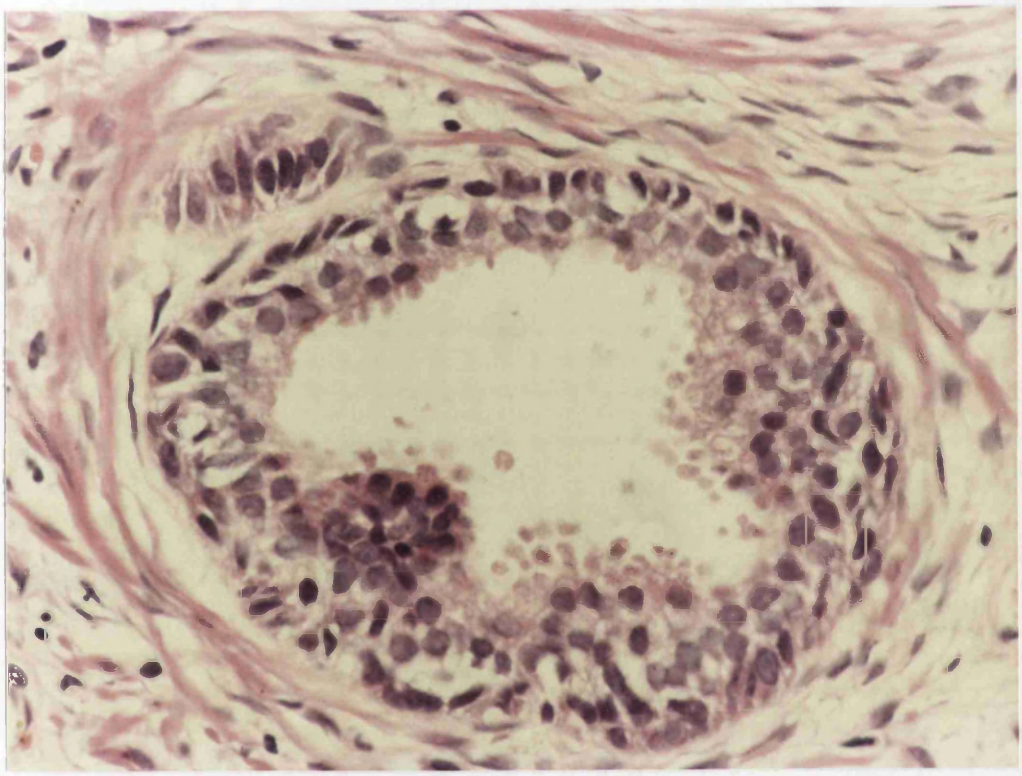


Fig. 16.1 Normal prostatic tissue

section or 20 random fields, which ever was the smaller, was assigned to each sample as follows: grade 1, 5-10 cells per hpf, grade 2, 11-15 cells per hpf and grade 3, >15 cells per hpf. A chronic inflammatory infiltrate of <5 cells per hpf was regarded as normal, Fig. 16.1. The patient's clinical details and ultrasound findings were not known to the pathologist at the time of examination of biopsy material.

Mast Cells

In thirty patients and ten controls, the biopsy material was also analysed for mast cell content.

Four micron sections of the paraffin embedded biopsies were cut and taken to water. The slides were then stained with 1% aqueous toluidine blue for 30 seconds before being washed in water for 30 seconds. The sections were transferred to acid alcohol and differentiated until only the nuclei remained coloured; this end point was assessed by viewing the section under a microscope. The differentiation was continued through graded alcohols, before clearing and then mounting the section in resin. Using this method, the cell nuclei appeared pale blue and the mast cell granules purple. By means of a graticule incorporated into the microscope eyepiece, the sections were studied and the mean number of mast cells in ten random high power fields (hpf) (x400) was measured, and a mast cell count per mm calculated.

Results

Routine Histology

The sixty patients with chronic abacterial prostatitis were aged 19-75 years, mean 39.8, compared to the control group 20-68 years, mean 39.2.

Within the cohort, insufficient tissue for analysis was obtained in three patients (5%), histology was regarded as normal in four patients (7%) and revealed glandular proliferation, yet no inflammation in a further two (3%). A chronic inflammatory infiltrate was identified in the remaining 51 patients (85%) (see Table 16.1 and Appendix XVIII). The corresponding ultrasound features of the lesions biopsied are depicted in Appendix V .

In the control group, sixteen patients (80%) had normal histology and 4 (20%) benign prostatic hyperplasia; in all cases, the biopsy was taken from parenchyma of normal echogenicity on prostatic ultrasound.

TABLE 16.1 HISTOLOGICAL FEATURES OF PROSTATIC BIOPSIES FROM THE COHORT OF PATIENTS WITH CHRONIC ABACTERIAL PROSTATITIS

Feature	No. of Patients (%)	
Inflammatory cells		
Acute and chronic	5	(8%)
Chronic - grade 1+ #	37	(62%)
Chronic - grade 2 #	10	(17%)
Chronic - grade 3+	5	(8%)
Granulomatous *	1	(2%)
Site of Inflammatory Cells		
Intraglandular	7	(12%)
Periglandular	34	(57%)
Stromal	27	(45%)
Other Features		
Corpora amylaceae	20	(33%)
Fibrosis	18	(30%)
Vessel hyaline thickening	6	(10%)
Glandular proliferation	2	(3%)
Telangiectasia	5	(8%)

Key:- +, # Patients with both grades of inflammation in the biopsies.

* Mycobacterium tuberculosis excluded.

The individual histological features of patients within the cohort is depicted in Appendix XVII.

The findings of acute inflammatory cells along with the chronic inflammatory infiltrate was uncommon. In the majority of cases the chronic inflammation was of low grade, Fig. 16.2 and occupied a periglandular and stromal site most commonly. Corpora amylaceae were found in 33% of the cohort, compared to 15% of controls though this difference failed to achieve statistical significance; the three patients within the control group in whom corpora amylaceae were found, had features of benign prostatic hyperplasia. In 30% of the cohort, fibrosis, Fig. 16.3 was noted and 18% had vessel changes, Fig. 16.4; neither of these features were observed in the control group.

The correlation between Stamey score, i.e. degree of inflammation based on localisation studies, and histological grade on biopsy is depicted in Table 16.2.

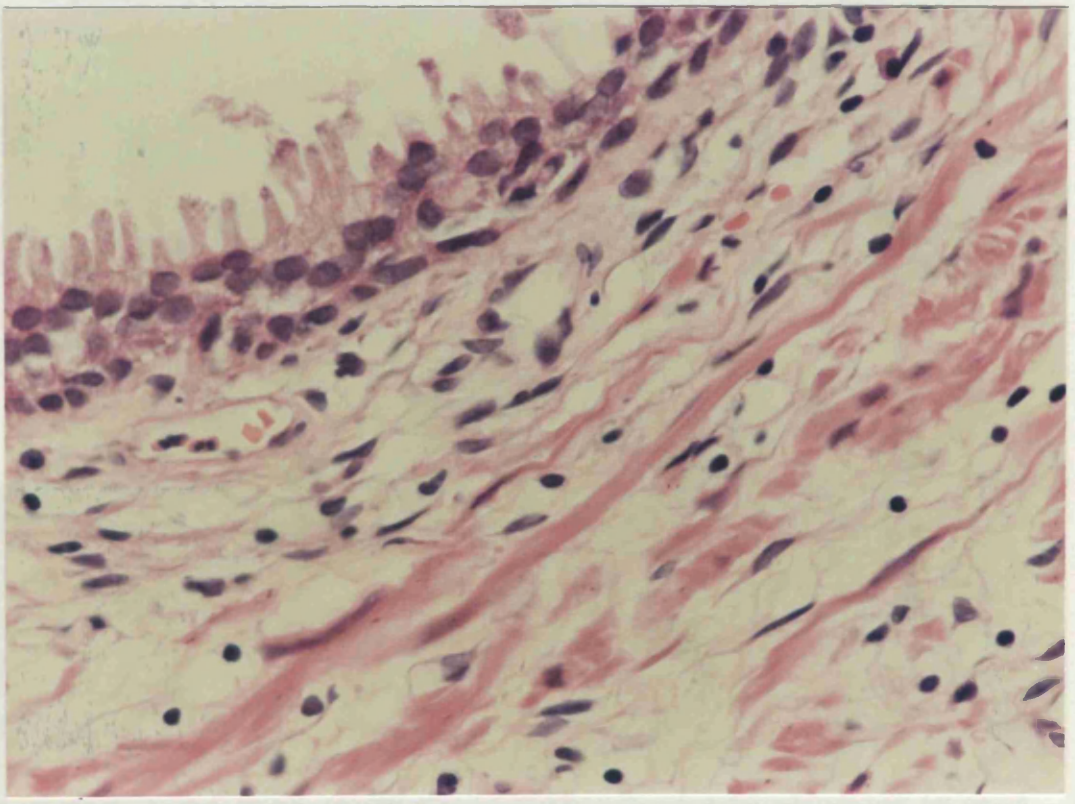


Fig. 16.2 A low grade (Grade 1) inflammatory lesion in a prostatic biopsy from the chronic abacterial prostatitis cohort

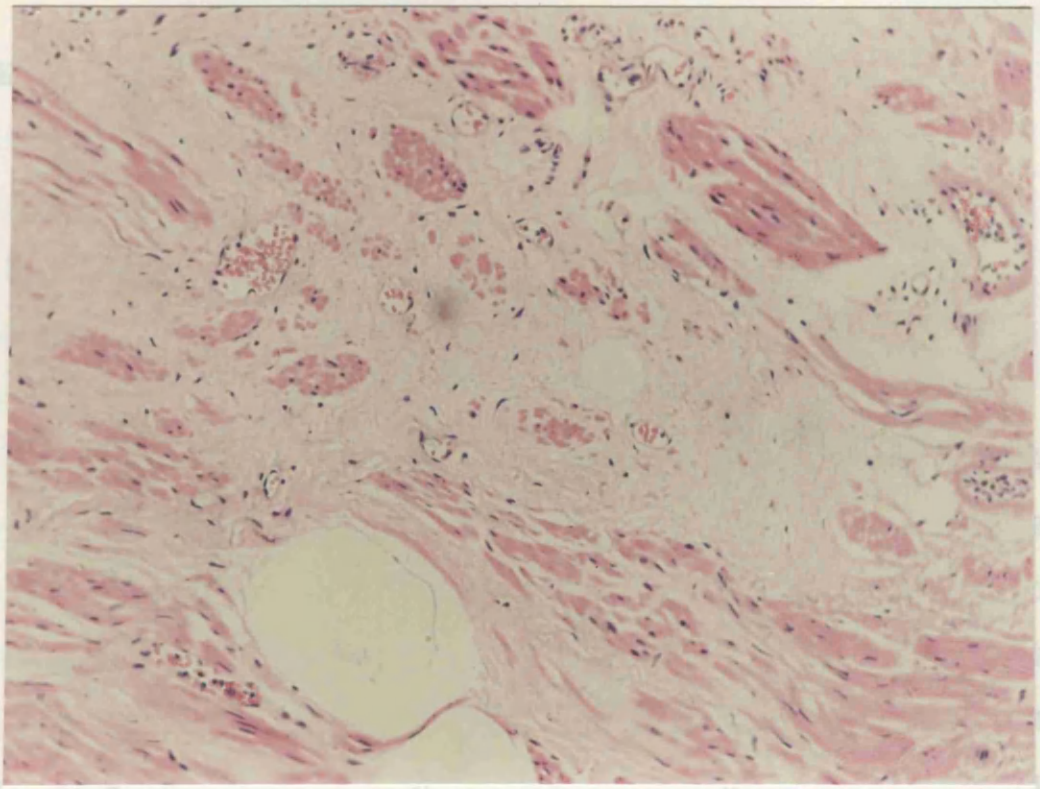


Fig. 16.3 Stromal fibrosis in chronic abacterial prostatitis

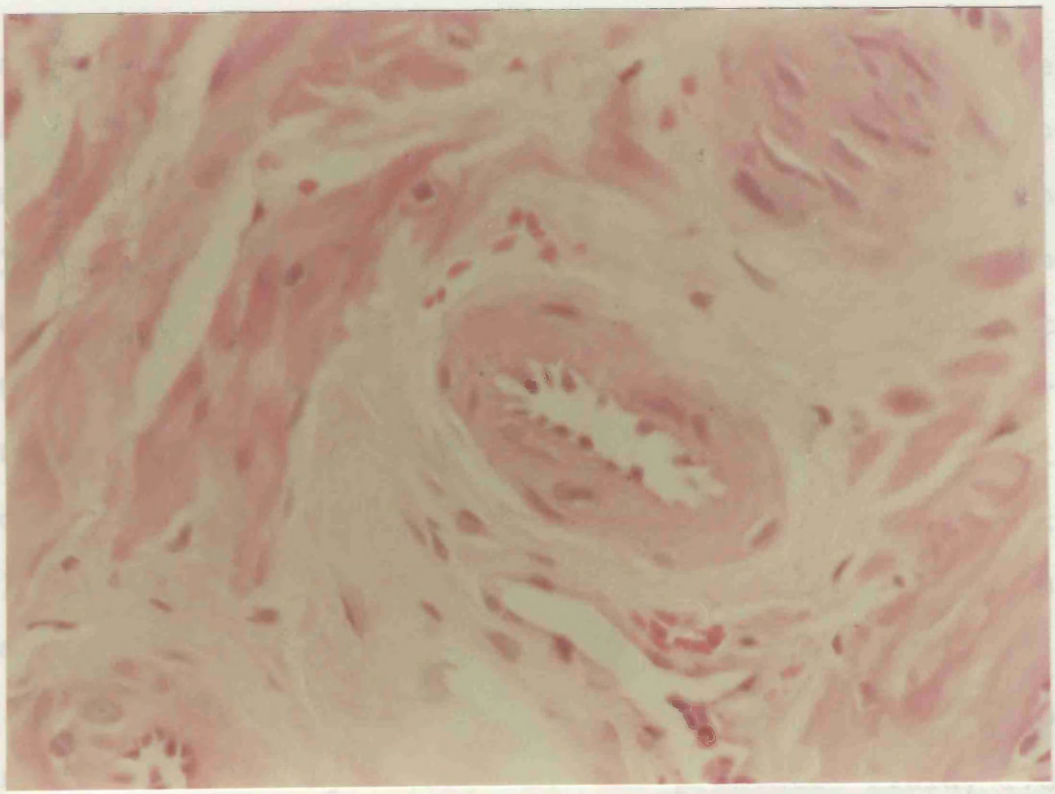


Fig. 16.4 Vessel thickening in a prostatic biopsy in chronic abacterial prostatitis

TABLE 16.2 CORRELATION BETWEEN STAMEY SCORE AND HISTOLOGICAL GRADE OF PROSTATIC BIOPSIES

Histological Grade	Stamey Score		
	2	3	4
0	1	1	2
1	16	11	10
2	1	7	2
3	1	0	4
4	0	0	1

N.b. Histological grade 4 = Granulomatous prostatitis

There was no clear correlation between Stamey score, i.e. the degree of inflammation in the prostatic fluid, and the grade of inflammation within the prostatic biopsy. The majority of inflammatory lesions were of low grade and although 16 grade 1 biopsies were obtained from patients with a Stamey score of 2, 21 biopsies of this grade were obtained from patients with Stamey scores of 3 and 4. In the case of grade 2 lesions, Fig 16.5 & 16.6, the majority (7) were obtained from patients with a Stamey score of 3. Similarly, all but one of the grade 3 biopsies, Fig 16.7 & 16.8, were taken from patients with the greatest number of chronic inflammatory cells in the prostatic fluid, i.e. Stamey score of 4. Thus, in the biopsies with more extensive inflammatory features, a trend existed towards a

positive correlation between the degree of inflammation within the prostatic fluid and that within the prostatic tissue.

Furthermore, although there was a difference in the mean symptom duration of the patients in each group of inflammatory grade: grade 1 (mean 3.75 years), grade 2 (mean 3.55 years) grade 3 (mean 1.96 years), this did not achieve statistical significance, chiefly due to the small numbers in the grade 3 inflammation group. No particular histological feature was associated with any grade of inflammation, nor any particular symptom or it's duration.

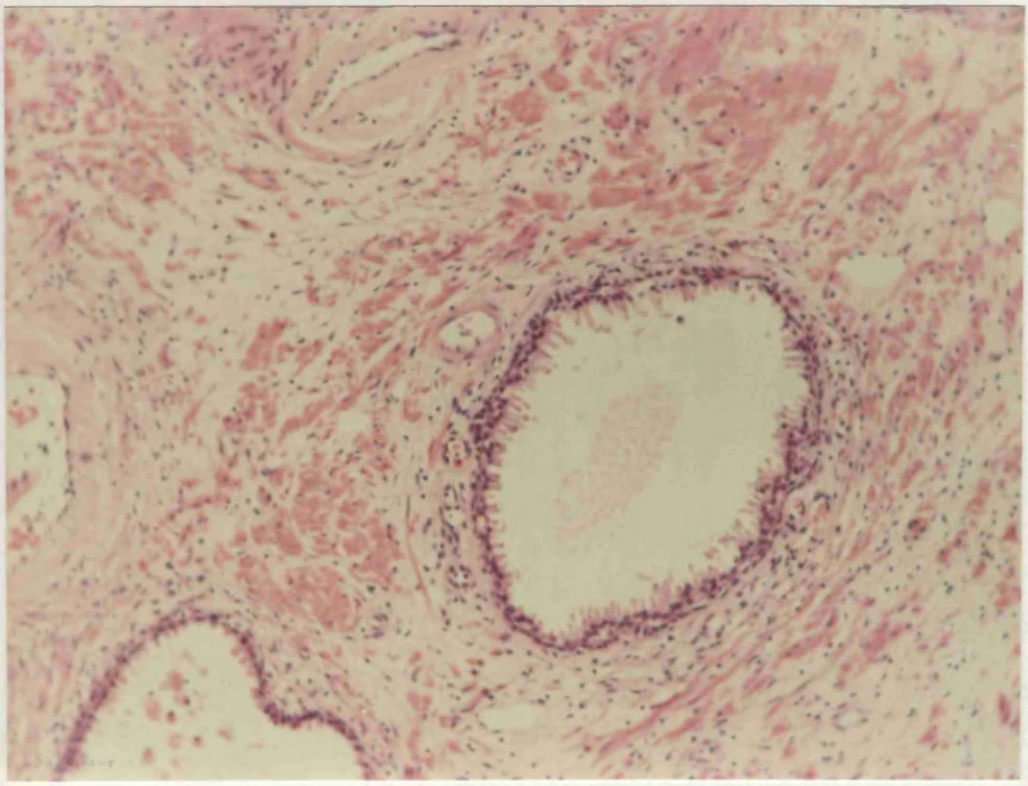


Fig. 16.5 Grade 2 inflammatory lesion in chronic abacterial prostatitis (x40)

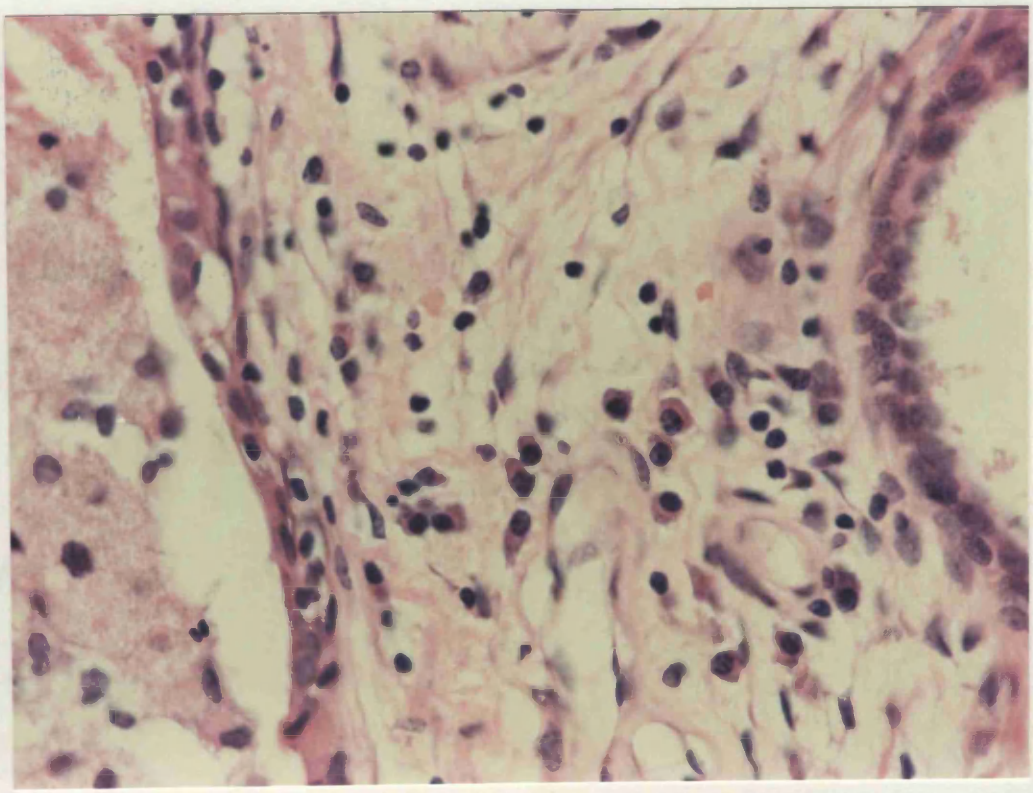


Fig. 16.6 A high power (x400) photomicrograph of a grade 2 lesion in chronic abacterial prostatitis

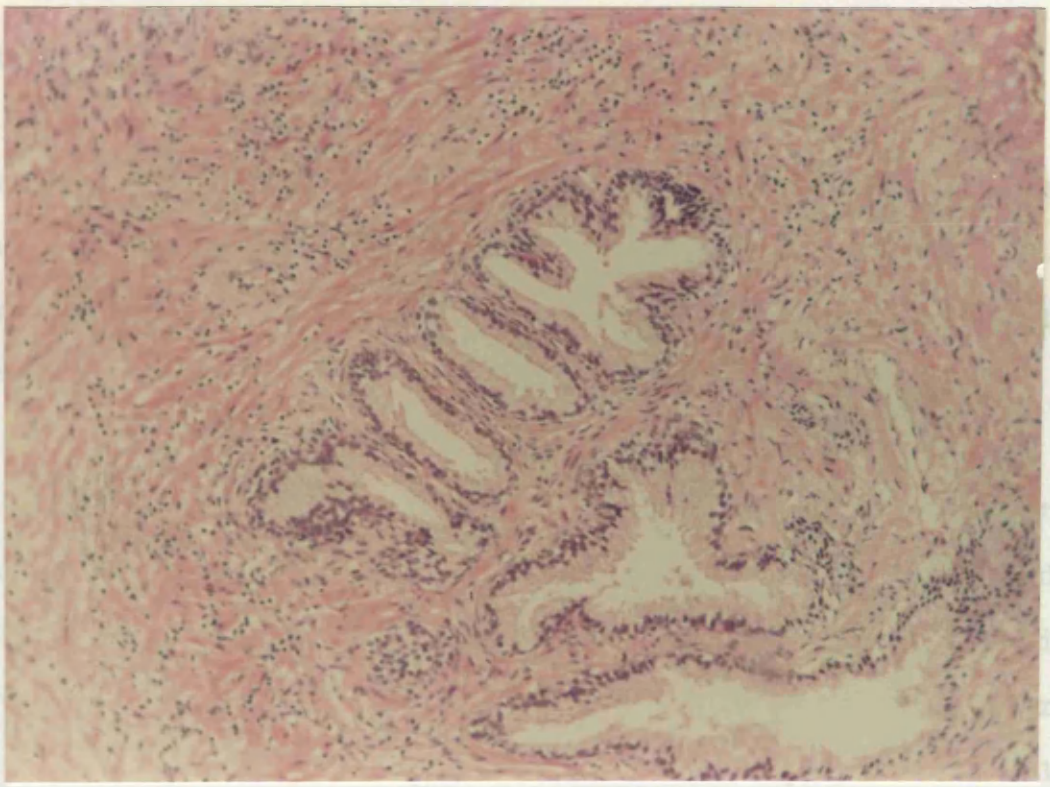


Fig. 16.7 Chronic abacterial prostatitis in a grade 3 lesion (x40)

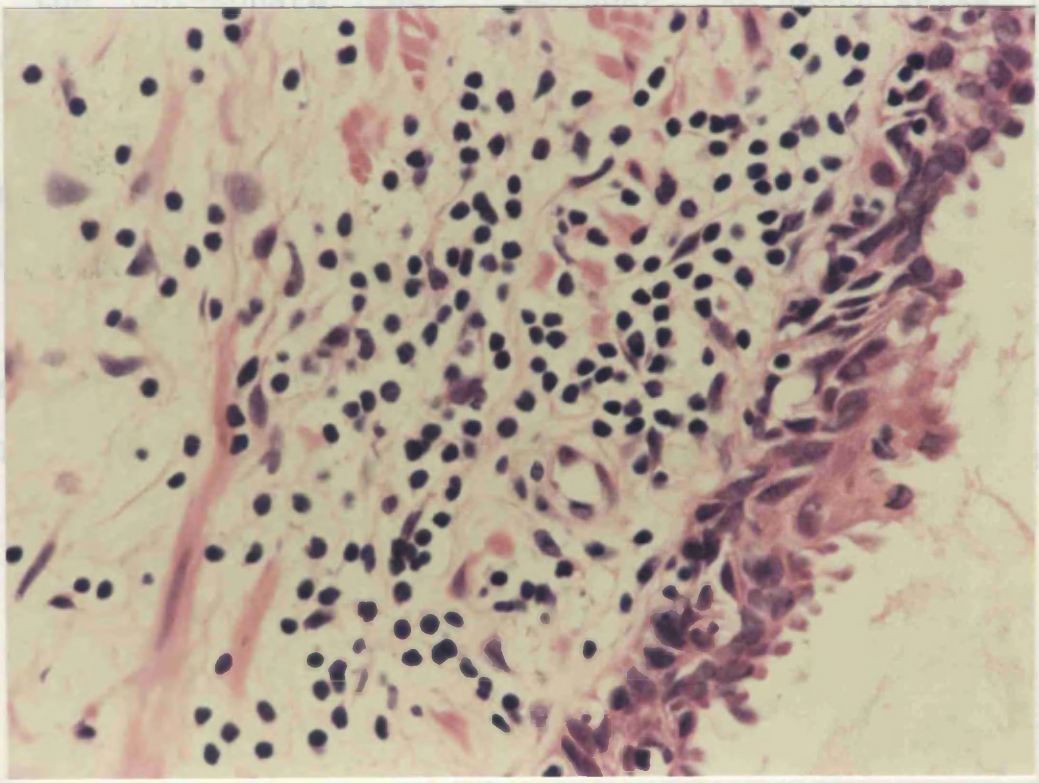


Fig. 16.8 Grade 3 inflammatory lesion in chronic abacterial prostatitis (x400)

Mast Cells

The sub-group of the chronic abacterial prostatitis cohort whose biopsies were analysed for mast cells, Fig 16.9, were aged 22-74 years, mean 41.8, compared to the control group of ten patients aged 20-68 years, mean 40.

The mast cell counts of the cohort patients are depicted in Appendices XIX & X X and the controls in Appendix XXI. The mast cell count per mm in the prostatic biopsies from the chronic abacterial prostatitis cohort ranged from 1-45, median 15, (S.d. + or - 9.14) compared to 1-10, median 3.2, (S.d. + or - 3.26) in the controls, which achieved statistical significance, $p < 0.001$, when analysed by the chi-square test. However, no correlation was detected between mast cell count and age, symptom duration or the severity of inflammation on routine histology (see Appendices XIX & X X). Furthermore, when the mast cell count in the prostatic tissue and the specific symptoms of pain, irritative voiding and voiding dysfunction were analysed, no statistically significant difference in mast cell content was observed between the symptom sub-groups.

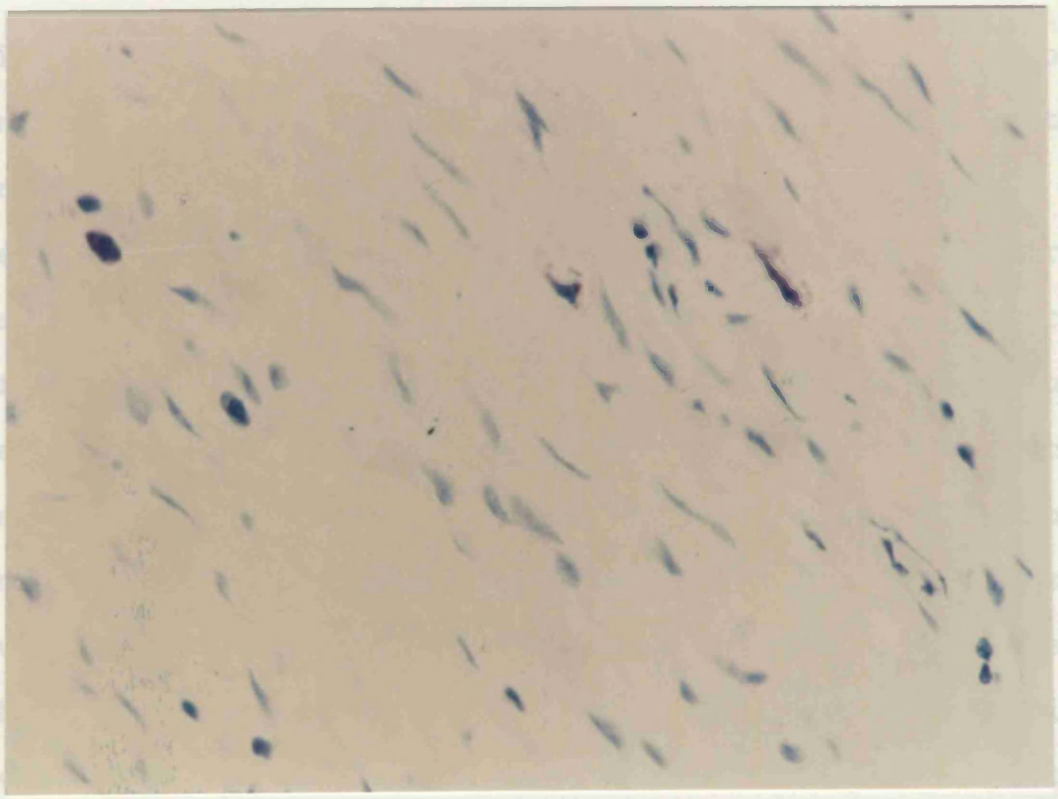


Fig. 16.9 Stromal mast cells in chronic abacterial prostatitis

SUMMARY

By means of ultrasound guided prostatic biopsy, tissue was analysed from a cohort of patients with chronic abacterial prostatitis, along with an age-matched control group with no evidence of prostatitis.

Within the study group, 85% of cases displayed a chronic inflammatory infiltrate, compared to none of the control group. A classification of inflammation , grades 1-3, was devised and in the majority of cases (62%), the infiltrate was of low grade, i.e. grade 1. The most common location of chronic inflammatory cells was in the periglandular region (57%), followed by the stroma (45%). Acute inflammatory changes, in addition to the chronic inflammatory cells, were observed in only 8% of cases. Furthermore, corpora amylaceae, fibrosis and vessel changes were noted in 33%, 30%, and 18% of biopsies, respectively. Within the control group, corpora amylaceae were noted in 15% of biopsies, in conjunction with benign prostatic hyperplasia; none of the other histological features identified in the prostatitis cohort were seen in the control group.

The correlation between the leucocyte count in post prostatic massage urine (VB3), as depicted in the Stamey score, and the histological grade of the prostatic biopsies, was poor in the grade 1 lesions, but displayed a closer correlation in the more severe grades. There was no correlation between any inflammatory grade and any particular symptom or it's duration.

The mast cell content of the prostatic biopsies was analysed and although there was a significantly greater number of mast cells ($p < 0.001$) in the biopsies from the chronic abacterial prostatitis patients, compared to controls, there was no correlation between this count and grade of inflammation, patient's age, symptom duration and specifically the individual symptoms of pain, irritative voiding and voiding dysfunction.

SECTION 17 THE IMMUNOPATHOLOGY OF CHRONIC PROSTATITIS - IMMUNOFLUORESCENCE STUDIES AND THE DETERMINATION OF THE PHENOTYPE OF THE CHRONIC INFLAMMATORY INFILTRATE

STUDY I - IMMUNOFLUORESCENCE STUDIES

INTRODUCTION

The immunological background to chronic abacterial prostatitis is poorly understood, although elevated levels of immunoglobulin have been found in the prostatic fluid of patients with this condition (Shortliffe & Wehner, 1986).

In normal subjects, local immunoglobulin of the IgA and IgG classes has been detected in prostate tissue obtained at necropsy (Ablin et al, 1971). Furthermore, in a poorly defined group of patients with chronic prostatitis, the presence of IgA, IgG and IgM was noted, but firm conclusions were not drawn as to their significance (Vinje et al, 1983).

Immunoglobulin deposits have been noted within the bladder of patients with interstitial cystitis (Gordon et al, 1973: Mattila, 1982) and were thought to be involved directly in the pathogenesis of this condition. Similarly, several dermatological (Harrist & Mihm, 1979) and renal (Albini et al, 1979) conditions have been characterised by immunoglobulin deposition.

Thus, a study was devised to examine prostatic tissue obtained by ultrasound guided biopsy from a cohort of patients, diagnosed accurately as having chronic abacterial prostatitis, to search for intraprostatic immunoglobulin deposition in an attempt to define the pathogenesis and possibly, further characterise this condition.

Materials And Methods

The patients studied were drawn from the sexually transmitted diseases and urology clinics of St. Mary's Hospital, London, and comprised the cohort of chronic abacterial prostatitis patients (see Sections 11 and 13). In addition, twenty patients with no history of chronic prostatitis and no cytological or microbiological evidence of prostatits as judged by localisation studies, acted as controls. This control group was drawn from the urology clinic of St. Mary's Hospital, London and underwent prostatic biopsy under general anaesthesia at the time of other surgical procedures, namely cystoscopy and scrotal surgery.

Each patient and control underwent transrectal prostatic ultrasound using a Bruel and Kjaer 1846 scanner with a 5.5 MHz transducer and subsequently transperineal biopsy using a 14G Tru-cut (Travenol) needle (see Appendix VI). Both the patient and control groups gave informed written consent to the procedure. In all patients, the biopsy was taken from abnormal areas of echogenicity (see Section 12), whereas in controls the tissue was obtained from gland parenchyma of normal echogenicity on ultrasound scanning.

The tissue obtained was subdivided, one portion being placed immediately into formal saline for routine histological examination, a further portion into transport

medium for subsequent immunofluorescence study and a third portion embedded in OCT (Miles Scientific Laboratory Limited) and frozen in isopentane, pre-cooled in a bath of liquid nitrogen; this tissue was stored in liquid nitrogen prior to further analysis (see Study II).

The transport medium consisted of 3.12M $(NH4)_2$ SO_4, 0.005M N-ethylmaleimide, 0.005M $MgSO_4$ and 0.025M potassium citrate, pH 7.0-7.2. The specimen was removed from the transport medium and washed in running water for ten minutes before being embedded in OCT (Miles Scientific Laboratory Limited) and frozen in isopentane, pre-cooled in a bath of liquid nitrogen and stored in liquid nitrogen. Cryostat sections, 6um thick, were cut at -25°C and allowed to air dry. Then they were washed for 30 minutes in 0.15M phosphate buffered saline (PBS), pH 7.2, and air dried prior to applying the fluorescein-conjugated polyclonal rabbit anti-human antibodies to IgA, IgG, IgM, complement C3 and fibrinogen (Dakopatts). The IgG antibody was diluted 1:100 and all other antibodies 1:50, and applied to the section for 30 minutes. (These dilutions were calculated following extensive preliminary studies to assess the degree of optimum staining). After a further wash in PBS for 30 minutes the section was counter-stained with Evans blue and mounted in a fluorescence free mountant of PBS and glycerol. All the steps in this staining process were carried out at room temperature. The slides were then examined for the presence of antibody, complement and fibrinogen under a

microscope with epifluorescent illumination (wavelength 497 nanometers) and the class of antibody and it's deposition site noted.

The results were analysed by means of an univariate system in which an assessment was made as to how age, symptoms and their duration, and histological features correlated with immunofluorescence positivity.

Results

The chronic abacterial prostatits cohort was aged 19 to 75 years, mean 39.8, compared to the controls, aged 20 to 68 years, mean 39.2.

Routine histolgy identified a chronic inflammatory infiltrate in 51 patients (85%), see Appendix XVIII and Section 16.

Thirty-four patients (57%) exhibited immunofluorescence staining within their prostatic biopsies, indicating the presence of immunoglobulin, complement or fibrinogen, compared to 1 (5%) of the controls ($p < 0.001$). In particular, the patient with granulomatous prostatitis had no antibody deposition. The individual immunofluorescence findings are depicted in Appendix XXII. Amongst those patients who showed immunofluorescence staining of the prostatic tissue, IgM (Figs. 17.1 and 17.2) was detected in 29 (85%), C3 (Fig.17.3) in 15 (44%), IgA (Fig.17.4) in 12 (35%) and fibrinogen in 8 (24%). The IgG subclass was not detected. The control patient with positive immunofluorescence exhibited IgA and IgM deposition. The sites of antibody deposition, Table 17.1, revealed periglandular, glandular and vessel wall deposition to be most prevalent.

The relationship between age, symptoms and histological features on the one hand and immunofluorescence staining

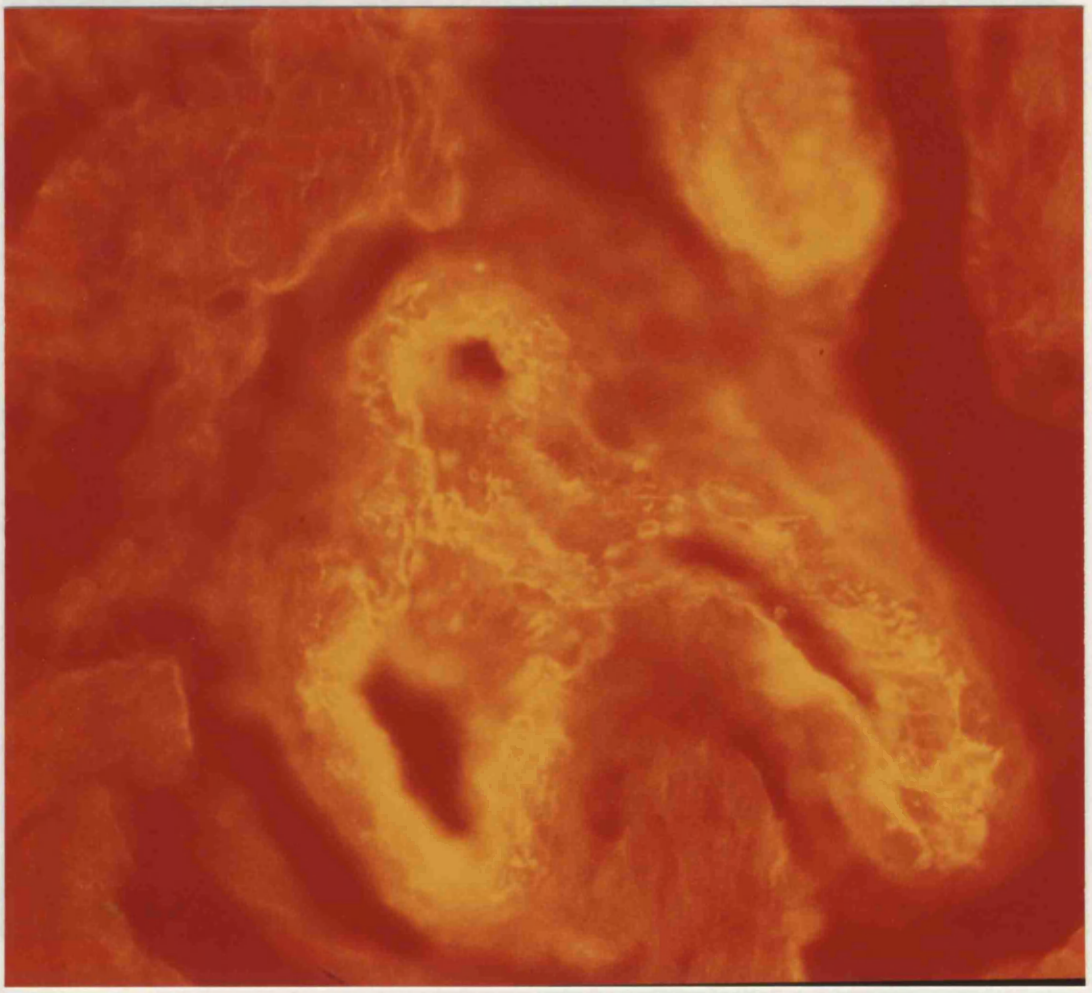


Fig. 17.1 Surface IgM deposition in the prostate of a patient with chronic abacterial prostatitis

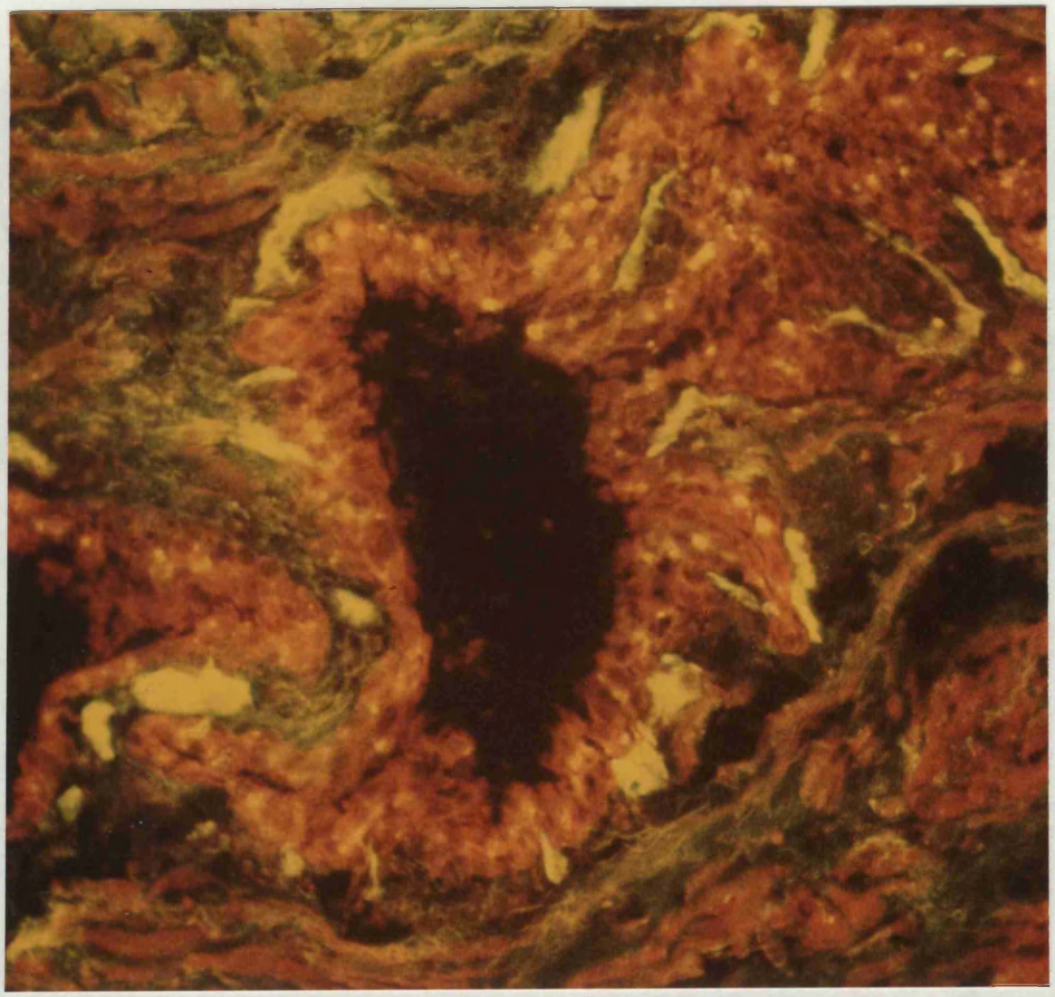


Fig. 17.2 A prostatic biopsy displaying periglandular IgM deposition in chronic abacterial prostatitis

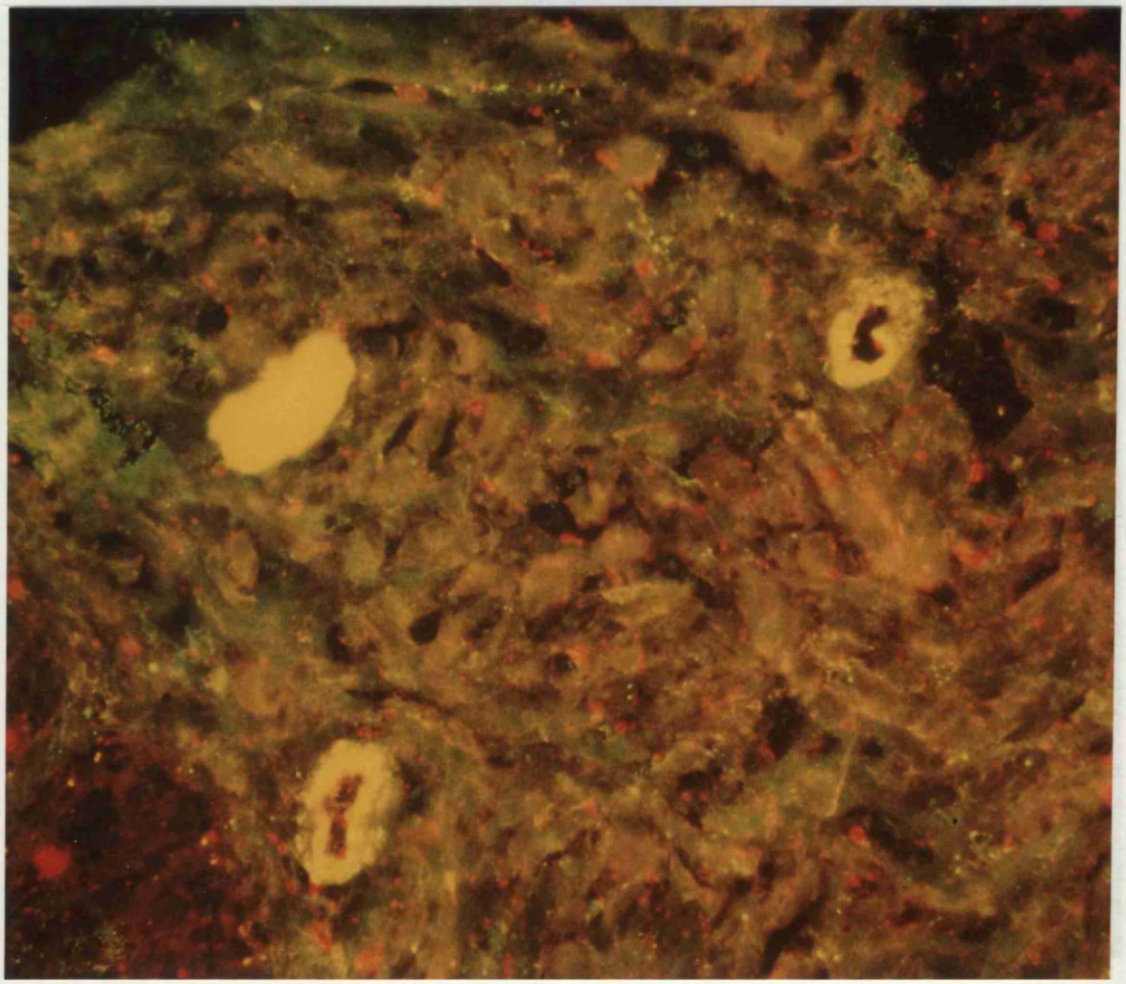


Fig. 17.3 C3 deposition within vessels in chronic abacterial prostatitis

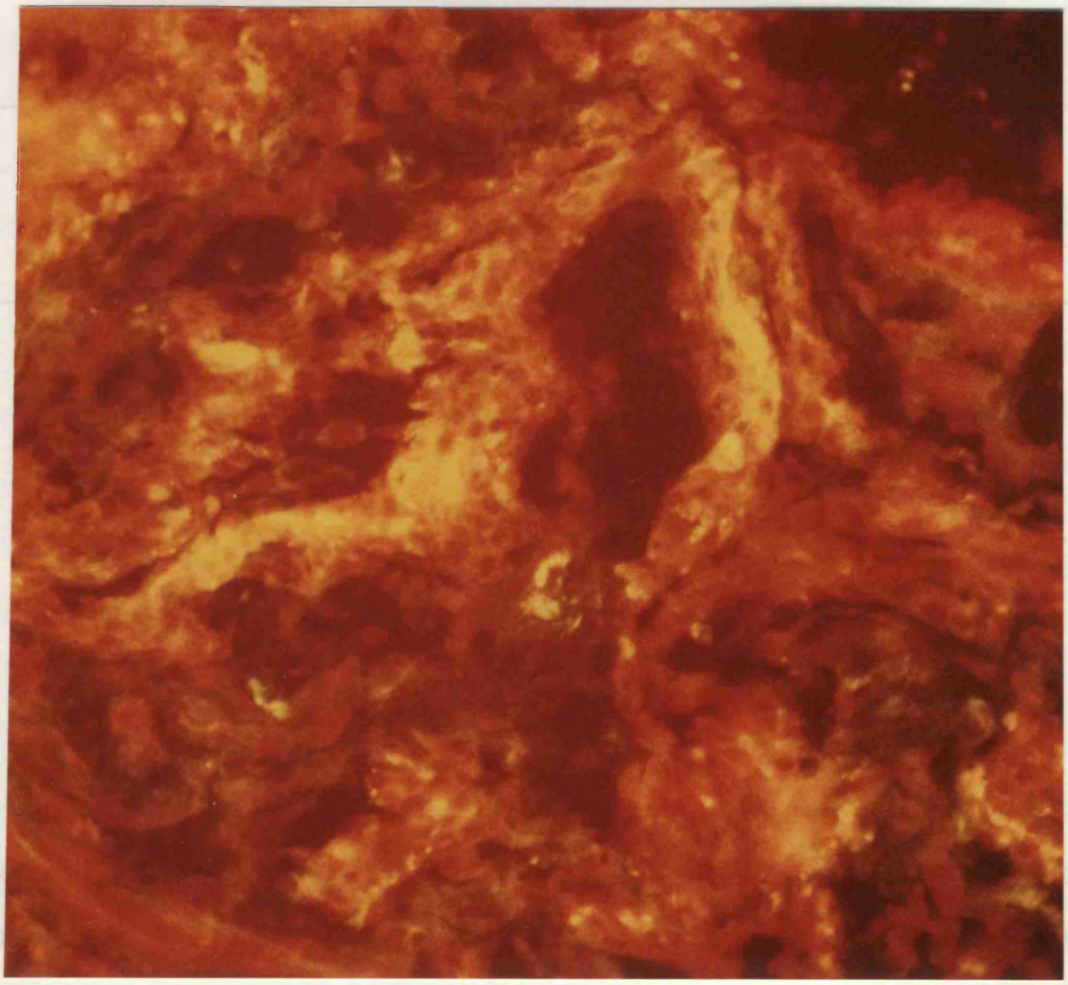


Fig. 17.4 Intraglandular IgA deposition in chronic abacterial prostatitis

on the other, was analysed and those correlations where statistical significance was achieved are listed in Table 17.2.

TABLE 17.1 SITE OF ANTIBODY, COMPLEMENT AND FIBRINOGEN DEPOSITION

Site of immunofluorescence deposition	% of patients with positive staining
Periglandular tissue	79
Gland	62
Vessel wall	62
Luminal cells	41
Stroma	29
Luminal surface	21

There was no correlation, however, between age, symptom duration, inflammatory grade and site, and positive immunofluorescence. Similarly no correlation between histological features and immunofluorescence findings was detected.

TABLE 17.2 SIGNIFICANT CORRELATIONS BETWEEN SYMPTOMS AND IMMUNOFLUORESCENCE FINDINGS

Symptoms	Findings	p value
Poor urinary flow	Immunofluorescence positivity	<0.01
	IgM deposition	<0.01
	IgM deposition in luminal cells	<0.02
	C3 deposition	<0.01
Irritative voiding	IgM deposition in stroma	<0.01
Urgency	IgM deposition in luminal cells	<0.01
Frequency	Fibrinogen deposition	<0.01
Nocturia	IF positivity	<0.05
Testicular pain	IF positivity	<0.05
Past history of NGU	C3 deposition	<0.02

STUDY II THE DETERMINATION OF THE PHENOTYPE OF THE INFLAMMATORY CELL POPULATION IN CHRONIC PROSTATITIS

The mechanisms involved in the immunopathology of chronic prostatitis have yet to be defined. Although, various studies have analysed the inflammatory cells within the expressed prostatic secretion (EPS) (Anderson & Weller, 1979; Nishimura et al, 1980; Schaeffer et al, 1981) and within biopsies taken from the diseased gland (Schmidt & Patterson, 1966; Nielsen et al, 1973; Kohnen & Drach, 1979), such work has only characterised broadly the inflammatory cell population, without attempting to define it's discrete components. Therefore, little information is available as to whether an active immunological process underlies the tissue reaction observed. In addition, many of the previous studies have used tissue removed at open surgery or obtained by "blind" biopsy techniques, which may be unrepresentative of the inflammatory lesions, and thus inappropriate samples for study. More recently, however, the ability to guide precisely a biopsy needle into the prostate under ultrasound control, has enabled tissue to be obtained from selected foci of prostatic inflammation.

Panels of monoclonal antibodies are now readily available to analyse tissue for the presence of lymphoid and non-lymphoid cells (Janossy et al, 1980; Thomas & Janossy, 1981), in particular macrophages and antigen-presenting cells (Poulter et al, 1986).

By combining the use of prostatic biopsy under ultrasound control with immunological techniques, the characteristics of the diverse populations of the inflammatory cells present within the tissue, of patients with chronic prostatitis, can be elucidated. In this way evidence may be sought for an immunological basis for the inflammatory reaction which may provide clues to the pathogenesis of the disease process.

Patients and Methods

The prostatic tissue analysed in this study was obtained from the chronic abacterial prostatitis cohort (see Section 13), all of whom underwent ultrasound guided transperineal prostatic biopsy, as described in Appendix VI .

Furthermore, twenty patients with no history of chronic prostatitis, nor cytological or microbiological evidence of prostatitis, as judged by localisation studies, acted as controls. These patients were drawn from the urology clinic of St. Mary's Hospital, London, and underwent prostatic biopsy under general anaesthetic at the time of other surgical procedures, namely cystoscopy and scrotal surgery. Both patients and controls gave informed written consent to the procedure.

The tissue obtained was handled and processed as described in Section 16, namely for subsequent routine histological examination and frozen section.

Routine Histology

The formal saline treated biopsies were processed in wax in routine fashion and sections stained with haematoxylin and eosin (see Section 16). Lymphocytic infiltration was graded on a scale of 1-3, grade 1 being 5-10 cells per high power field (hpf) (x400), 2 being 11-15 cells per hpf and 3 being greater than 15 cells per hpf (see Section 16).

The snap frozen prostatic tissue from 24 patients of the group comprising the chronic abacterial prostatitis cohort, and from 10 of the group of controls was then submitted for immunohistochemical analysis. From these biopsies 6u cryostat sections were cut at -35^{o}C, air dried and fixed for ten minutes in a one to one solution of chloroform and acetone. The sections were then wrapped in cling film and stored at -20^{o}C until used.

Immunoperoxidase Technique

This part of the study utilised mouse anti-human monoclonal antibodies (MoAbs) in an indirect immunoperoxidase technique. The antibodies used were RFDR, RFT Mix (comprising CD2, CD5 and CD8), CD4, CD8, RFB Mix (CD19 and CD20), RFD1, RFD7, RFD9 and CD14, see Table 17.3. The preparation and reactivity of these antibodies has been described previously (Janossy et al, 1986; Poulter et al, 1986).

The tissue sections were incubated with MoAbs used at a dilution of 1:5 in phosphate buffered saline (PBS), for a period of one hour. Sections of human palatine tonsil were run in parallel as positive controls for each MoAb used and the section from each patient incubated in PBS alone, as a negative control. After rinsing in PBS, all sections were incubated with a second layer of rabbit anti-mouse immunoglobulin/peroxidase conjugate for forty-five minutes. After rinsing for a further ten minutes, the sections were developed with a solution of di-amino benzidine and hydrogen peroxide, counterstained with Harris's haemotoxylin, dehydrated and mounted in DPX (BDH Chemicals Limited, Poole, Dorset), see Appendix XXIII. All tissue reactions were performed at room temperature.

TABLE 17.3 MONOCLONAL ANTIBODIES EMPLOYED IN IMMUNOHISTOCHEMICAL STAINING TECHNIQUES

Monoclonal Antibody (MoAb)	Cells Identified
RFDR	HLA-DR positive cell
RFT-mix (CD2, CD5, CD8)	Pan T-lymphocyte marker
CD4	Helper/inducer T-lymphocytes
CD8	Suppressor/cytotoxic T-lymphocytes
RFB-mix (CD19, CD20)	Pan B-lymphocyte marker
RFD1	Antigen presenting cells
RFD7	Tissue macrophages
RFD9	Epithelioid cells
CD14	Monocytes
CD7	Activated T-lymphocytes
CD45RO	T-lymphocyte subset including memory cells

The sections were examined under light microscopy and the number of positively stained cells counted in ten high power fields (hpf) (x400), and graded +/- to +++, based on the following scoring system: +/- <5 cells per hpf, + 5-10 cells, ++ 11-15 cells and +++ >15 cells per hpf. The site of positively stained cells, namely epithelium, gland, stroma and perivascular region was also noted.

Double Immunofluorescence Studies

A double immunofluorescence technique as previously described (Poulter et al, 1982a) was employed using the following antibody pairings: CD4 and CD8, CD45RO (T cell subset including memory cells (Akbar et al, 1988)) and CD4, CD45RO and CD8, CD7 and CD4, CD7 and CD8 and RFD1 and RFD7, see Table 17.3. The CD4, CD7, CD45RO and RFD7 antibodies were of the IgG class and CD8 and RFD1 of the IgM class. A CD4-FITC (fluorescein isothiocyanate) conjugate was used for the CD45RO/CD4 and CD7/CD4 pairings. The principle of the technique was the application of a primary layer, comprising a pair of monoclonal anitbodies, followed by a second layer of antibodies to the primary layer, with separate conjugated fluorescent components, in the form of anti IgG-TRITC (tetraethyl rhodamine isothiocyanate) and anti-IgM-FITC (fluorescein isothiocyanate). The exception to this method was the CD45RO/CD4 and CD7/CD4 pairings, as the CD4 antibody was already conjugated to FITC, only anti-IgG-TRITC and PBS were applied as the second layer. The details of the method appears in Appendix XXIV .

Sections of human palatine tonsil were run in parallel as positive controls for each MoAb pairing used and the section incubated with PBS alone as a first layer, in the case of a negative control.

The sections were viewed with a Zeiss microscope equipped with epifluorescent illumination and barrier filters for FITC and TRITC. The percentage of cells possessing one or both markers was calculated from the assessment of ten high power (x400) fields or at least 150 cells. When calculating the percentage of CD4 and CD8 cells that also expressed the CD7 and CD45RO epitopes, if no CD4 or CD8 cells were present within the section, the result was recorded as - , whereas when CD4 and CD8 cells were present, yet none possessed these epitopes, the result was recorded as 0%. Furthermore in calculating mean values of these data, results recorded as - were not included in the equation. Again the site of positive cells within the section was noted by examining the section under phase contrast illumination.

HLA DR - Glucose Oxidase (DR-GO) Histochemical Technique

An immunocytochemical technique employing glucose oxidase (GO) conjugated to RFDR (Poulter et al, 1982b) was adopted to obtain a quantitative evaluation of HLA-DR, in selected patients from each histological grade of inflammation (3 cases per grade) and normal controls. The RFDR conjugate was applied to sections of prostate and also to sections of human palatine tonsil as a positive control at a dilution of 1:10 in PBS for a period of 90 minutes. A negative control was run in parallel by applying PBS alone as a primary layer. Following washing in PBS, the sections were immersed in the developing medium of Tris-HCl, 0.05M pH 8.3,B-D glucose, nitroblue tetrazolium and phenazine methasulphate (PMS) for 30 minutes. The slides were then washed and mounted in PBS glycerol. Full details of the method appears in Appendix XXV .

The sections were examined using a scanning and integrating microdensitometer (Vickers M85 set at 585nm, slit width 20, spot size 1) and the relative absorption of reaction product in areas of gland, stroma and perivascular tissue measured in ten to thirty fields. Thereby the mean relative absorption per unit area for each tissue site was then calculated for each group of patients.

The background density was measured at five separate sites, a mean value calculated, and for each measurement of the tissue, the area under study measured.

Results

The twenty four patients drawn from the cohort of sixty patients with chronic abacterial prostatitis, whose prostatic biopsies underwent immunohistochemical studies were aged 19-68 year, mean 45, compared to the controls, aged 20-68 years, mean 37.3.

The routine histological features of the twenty four patients are depicted in Table 17.4.

Of the ten controls, eight were normal and two showed benign prostatic hyperplasia, and all were graded as normal with respect to the presence of a chronic inflammatory infiltrate, i.e. less than 5 inflammatory cells per hpf.

IMMUNOPEROXIDASE STUDIES

Normal Prostatic Tissue

HLA-DR expression was observed in the basal epithelial cells, the scanty mononuclear non-macrophage cells in the periglandular region, and the endothelial cells (Fig. 17.5). There were isolated T-mix positive cells distributed evenly throughout the section and with an equal prevalence of CD4 positive and CD8 positive cells. No RFB-mix positive cells were visualized. With regard to the monocyte/macrophage series, RFD1 , RFD9 , and CD14 cells were not detected. RFD7 cells were evident, most commonly in the periglandular and stromal regions.

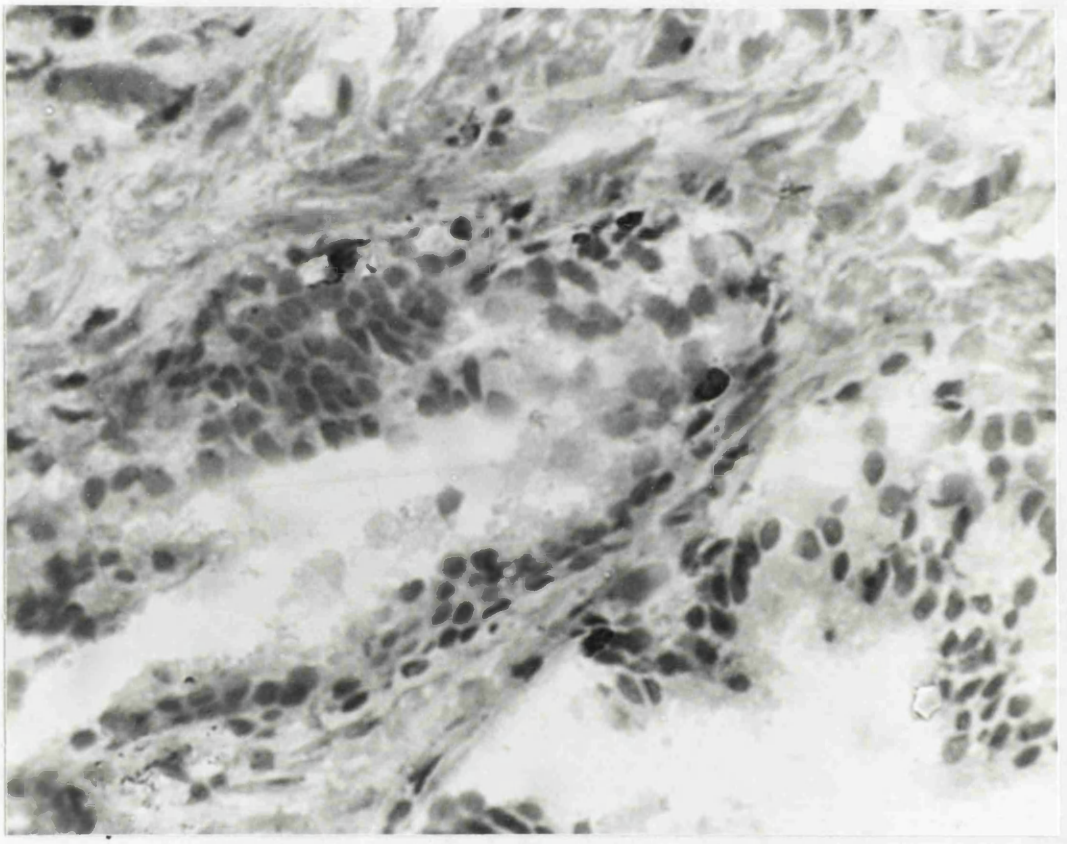


FIG. 17.5 HLA-DR expression in normal prostatic tissue

TABLE 17.4 HISTOLOGICAL FEATURES OF PROSTATIC BIOPSIES WHICH UNDERWENT IMMUNOHISTOCHEMICAL ANALYSIS FROM PATIENTS WITH CHRONIC ABACTERIAL PROSTATITIS

Feature	No. of patients (%)
Inflammatory cells	
Acute and chronic	4 (17%)
Chronic - grade 1	10 (42%)
grade 2	8 (33%)
grade 3	5 (21%)
Granulomatous	1 (4%)
Site of inflammatory cells	
Intraglandular	5 (21%)
Periglandular	18 (75%)
Stromal	10 (42%)
Corpora amylaceae	9 (37.5%)
Fibrosis	7 (29%)
Vessel thickening	3 (12.5%)
Telangiectasia	2 (8%)
Dilated glands	1 (4%)

Prostatitis Tissue

The individual results of the immunoperoxidase studies are depicted in Appendix XXV and summarised in Table 17.5.

TABLE 17.5 SUMMARY OF IMMUNOPEROXIDASE STAINING DATA IN PATIENTS WITH CHRONIC ABACTERIAL PROSTATITIS

Antibody	Grade of Inflammation in Prostatic Biopsies			
	G1	G2	G3	Gran.
HLA-DR	+	++	+	+++
RF T-mix	+	++	+++	+++
CD4	+	+/++	++	+/++
CD8	+	++	+++	+++
RF-B mix	-	-	-	-
RF D1	+/-	+/-	++	+
RF D7	+/-	+/++	+++	+++
RF D9	-	-	-	+
CD14	-	+/-	+	++

Key: +/- < 5 cells per high power field (hpf) (x400)
+ 5-10 cells per hpf
++ 11-15 cells per hpf
+++ > 15 cells per hpf

HLA-DR expression was present in all grades (Fig. 17.6) and increased in proportion to the severity of inflammation. The degree of expression was most marked in the glandular structures in all grades, yet, also in the stromal and perivascular tissues, in the more inflamed tissues.

As the severity of inflammation increased, so did the number of T-cells, forming local collections and follicles in grade 2 and 3 lesions, (Figs. 17.7 and 17.8). Although there was wide variation between the biopsies, the majority of T-cells were of the CD8 subset though parity between the CD4 and CD8 subsets was more likely in grade 1 lesions. In grade 3 cases and the granulomatous biopsy, CD8 cells displayed a marked predominance, most noticeably in the glandular and epithelial regions.

B-cells were not detected in any of the biopsies. With reference to the macrophage/monocyte series, increased numbers of both $RFD1^+$ and $RFD7^+$ cells occurred from grade 1 through to grade 3 lesions. In grade 1 samples, the positive cells were most noticeable in the glandular and perivascular regions and RFD7 cells (Fig. 17.9) were present in only marginally larger numbers than the RFD1 cells. In grade 2 lesions, although both phenotypes were present in increased numbers, there was a marked predominance of $RFD7^+$ cells, especially within glandular and stromal tissue. In the grade 3 and granulomatous prostatitis biopsies there was a relative re-emergence of

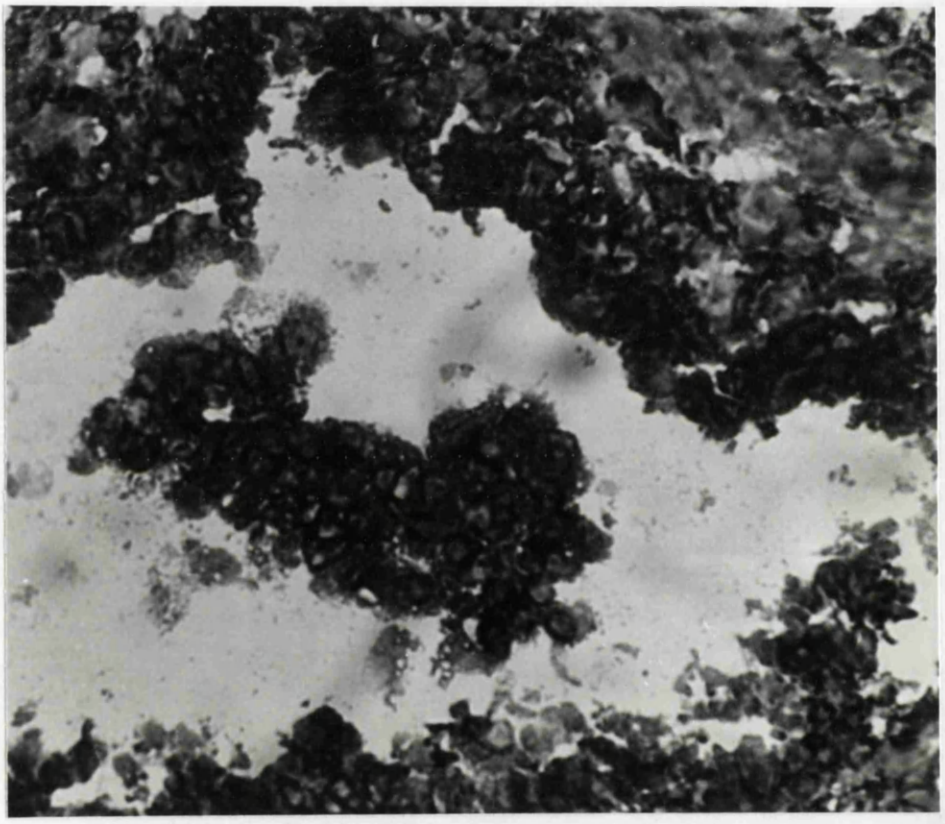


Fig. 17.6 HLA-DR expression in a grade 3 inflammatory lesion

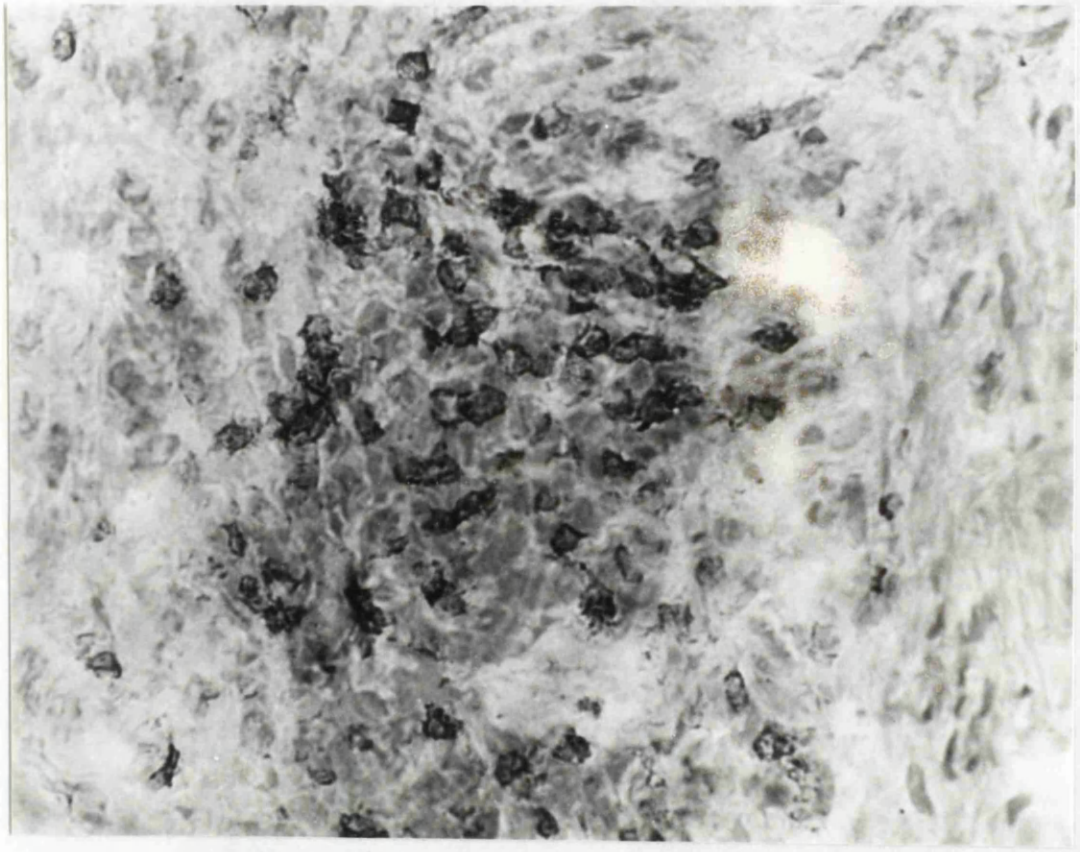


Fig. 17.7 T-lymphocytes in a grade 2 lesion of chronic abacterial prostatitis

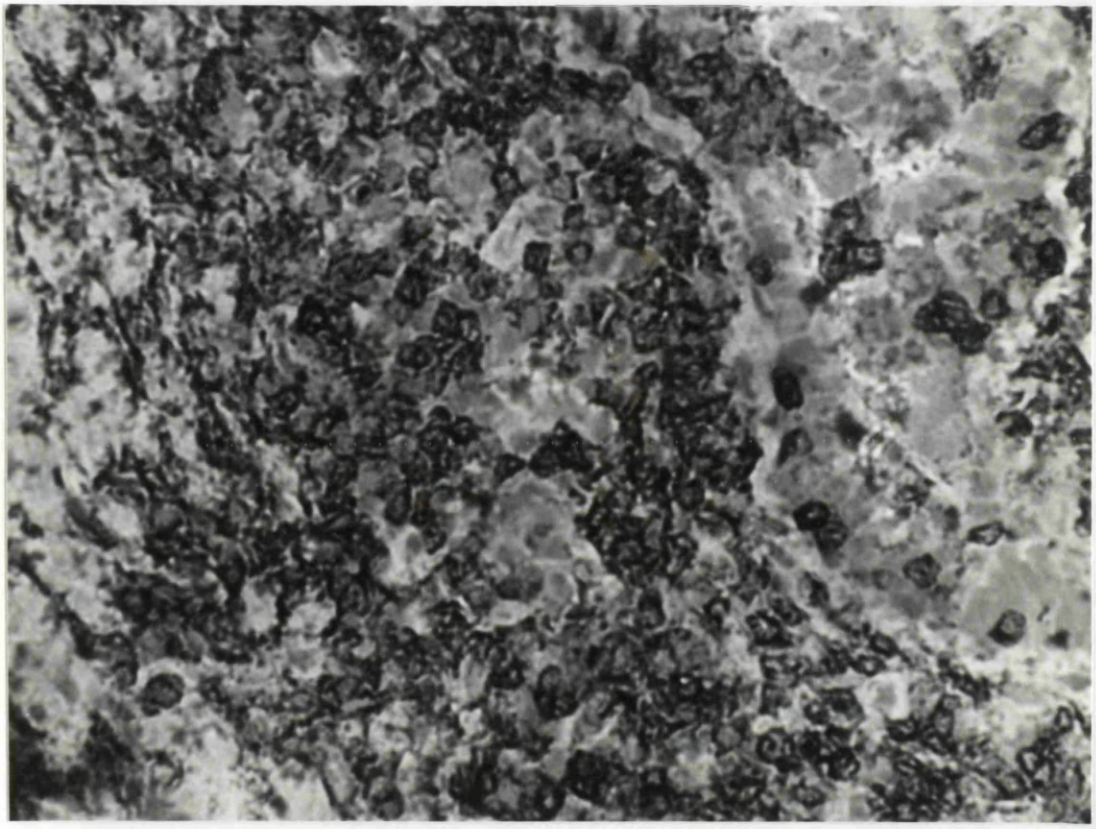


Fig. 17.8 T-lymphocytes forming a follicular aggregate in grade 3 chronic abacterial prostatitis

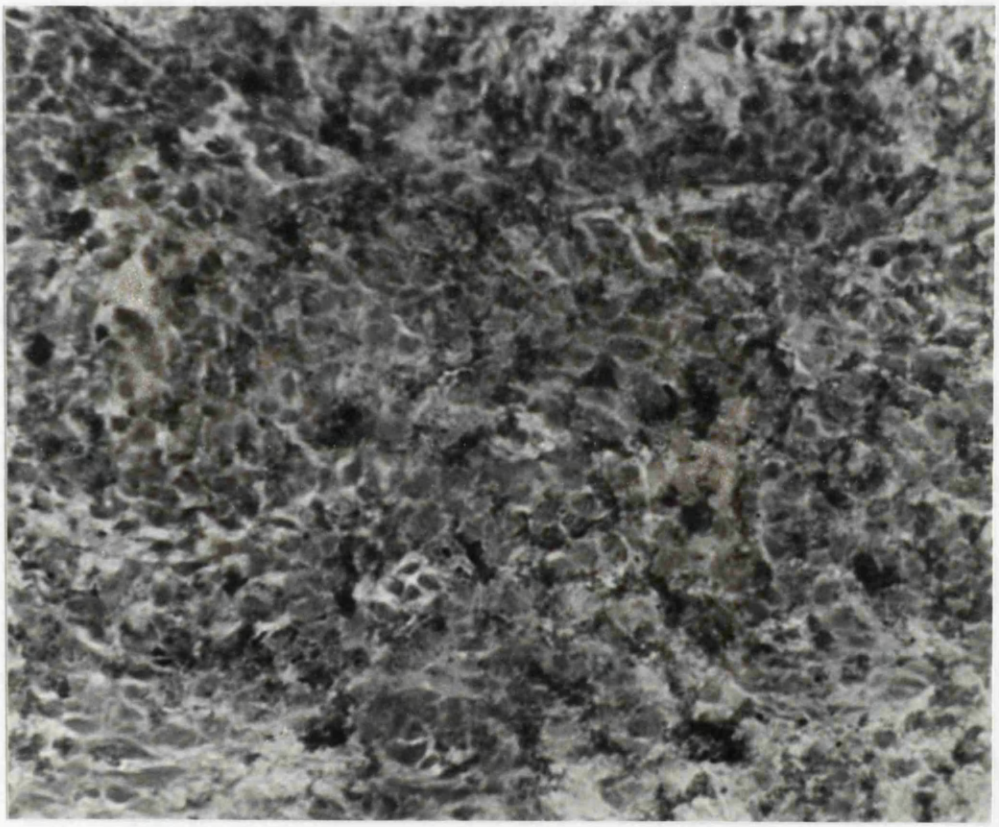


Fig. 17.9 Tissue macrophages (RFD7+cells) in grade 3 chronic abacterial prostatitis

the RFD1$^+$ cells, although both phenotypes (RFD1 and RFD7) were present in increased numbers compared to grade 2 lesions. RFD9$^+$ cells were only detected in the granulomata of the granulomatous prostatitis tissue.

No CD14$^+$ cells were detected in the grade 1 biopsies, and these cells occurred in only 50% of both grade 2 and grade 3 lesions, where they were most commonly located in the glandular regions, but were also prominent in stromal and periglandular tissues. The CD14$^+$ cells were most widespread in the granulomatous prostatitis biopsies.

Immunofluorescence Studies

The CD4 /CD8 ratio (Fig. 17.10, AppendixXXVII), confirmed the immunoperoxidase data on these cells. In grade 1 lesions CD4 and CD8 cells were present in roughly equal numbers yet with increasing inflammatory grade the progressive emergence of CD8 cells (Fig. 17.11) was evident.

The proportion of CD4 and CD8 cells that expressed the CD45RO epitope (Fig. 17.12 and 17.13) ranged from 0% to 100% in both subsets (see Appendix XXVIII), with mean values of 66.1% and 61.5% respectively.

In the case of the CD7 marker, there was an increase in the number of CD8 cells that expressed this antigen (Fig. 17.14) with increasing inflammatory grade (Fig. 17.15), (see Appendix XXIX), with the exception of the granulomatous prostatitis biopsy. In the CD4 subset no difference in the CD7 expression between the grades was observed (see Appendix XXIX).

The RFD1 /RFD7 cell ratio (Fig. 17.16), confirmed the immunoperoxidase findings of a slight RFD7 predominance over RFD1 cells in the grade 1 lesions. This was followed by a marked increase in the RFD7 cells (Fig. 17.17) and a relative diminution of RFD1 cells (Fig. 17.18) in the grade 2 lesions, before a relative re-emergence of RFD1 cells in the grade 3 lesions. There

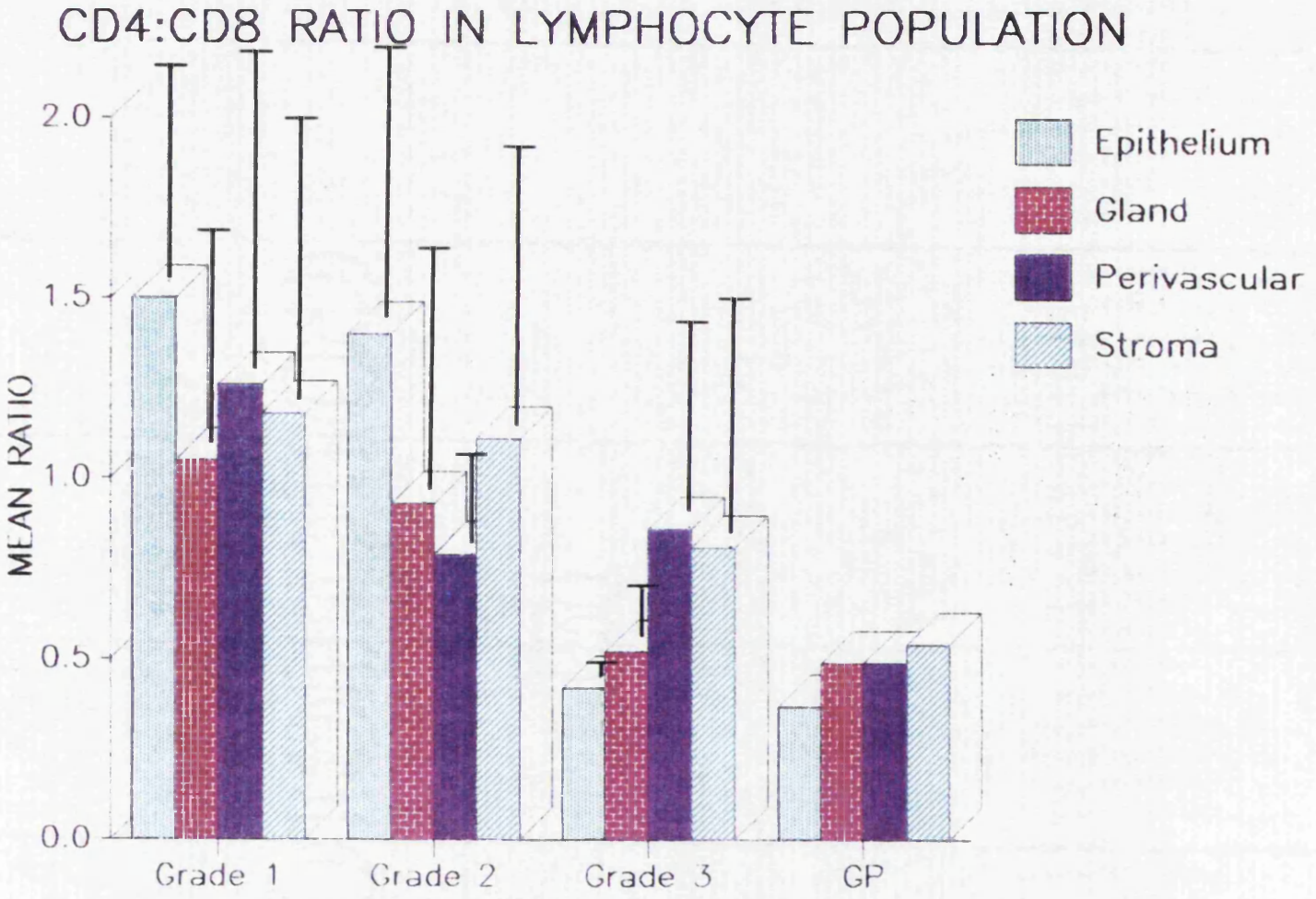

CD4:CD8 RATIO IN LYMPHOCYTE POPULATION
MEAN RATIO
2.0
1.5
1.0
0.5
0.0
Epithelium
Gland
Perivascular
Stroma
Grade 1
Grade 2
Grade 3
GP
INFLAMMATORY GRADE

Fig. 17.10

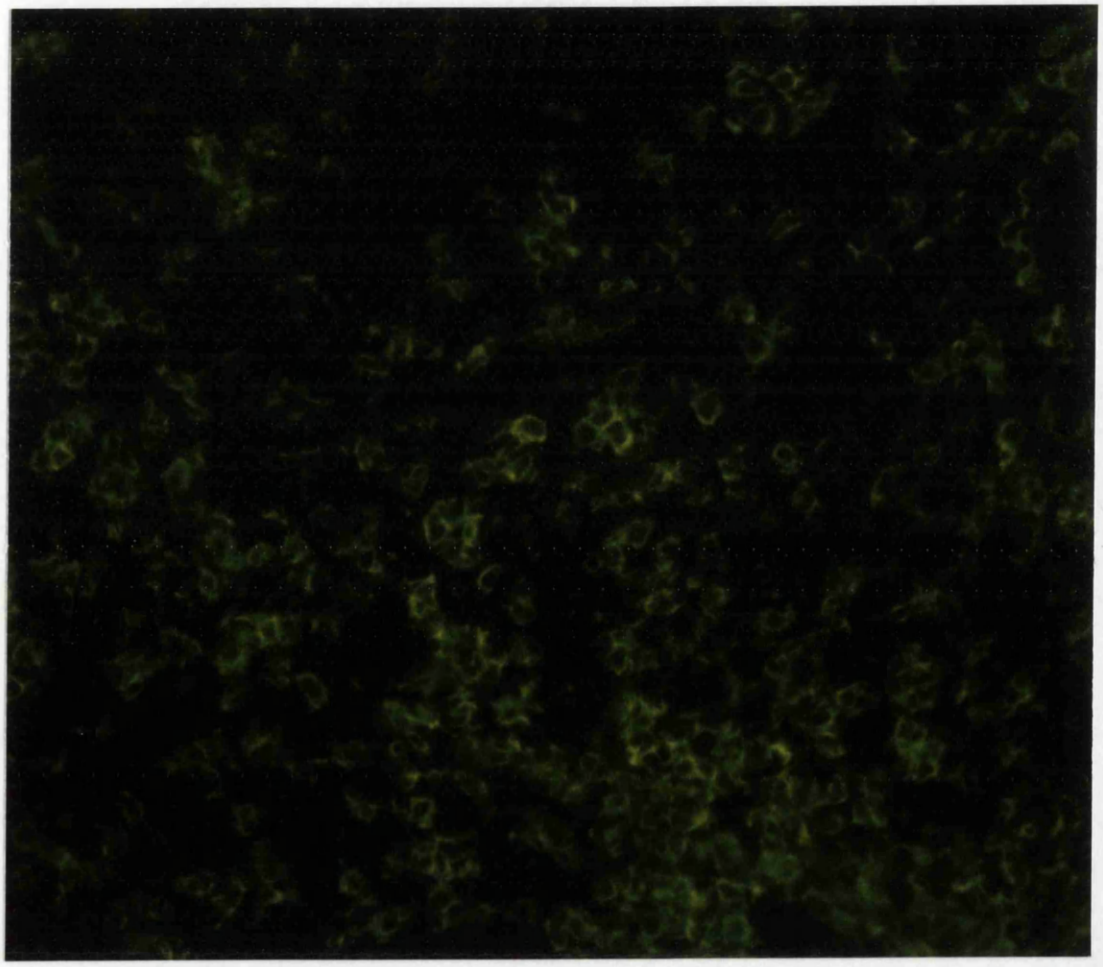


Fig. 17.11 CD8 + T-lymphocytes in a grade 3 lesion identified by an immunofluorescence technique

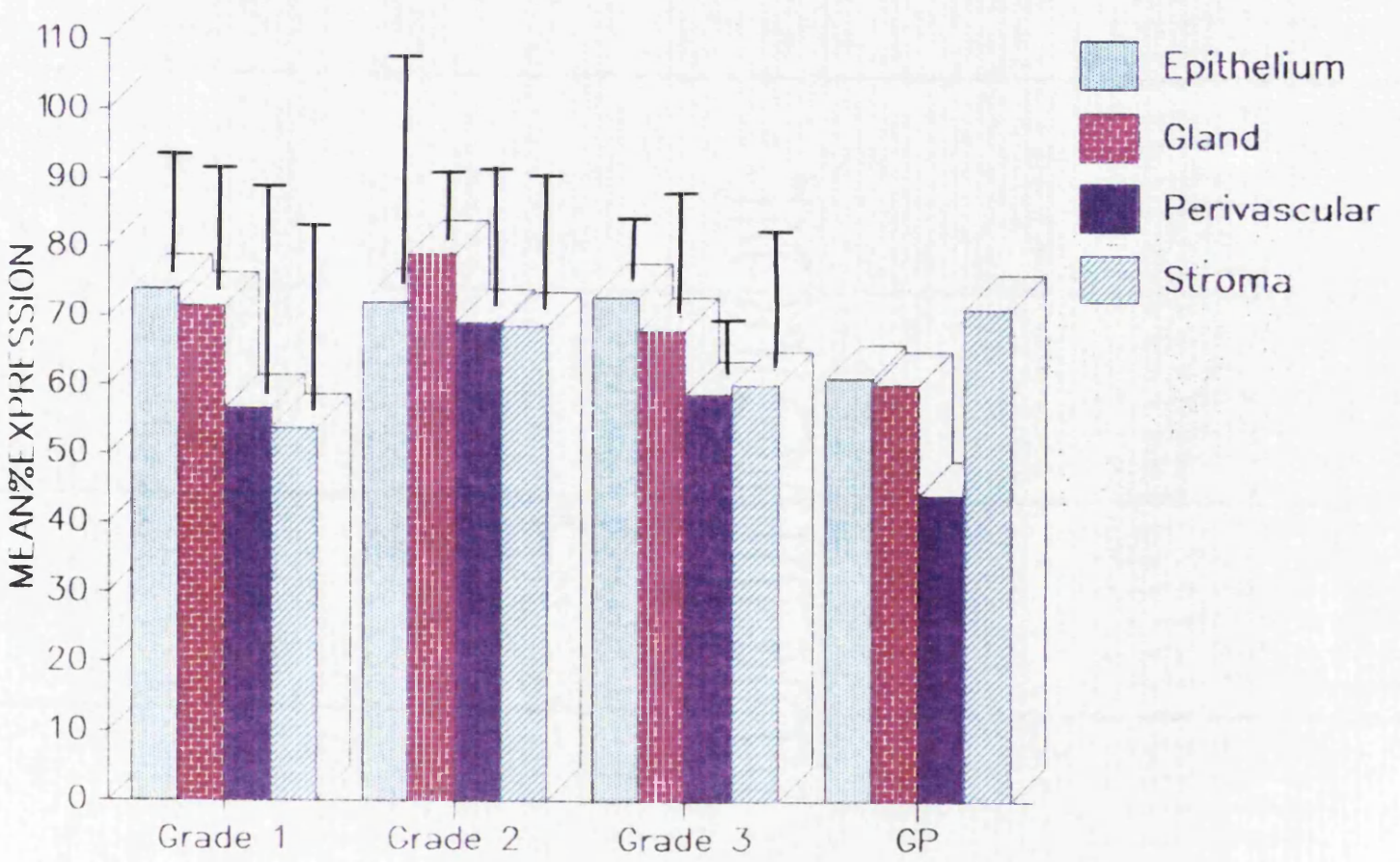

CD45RO EXPRESSION ON CD4 LYMPHOCYTES
MEAN%EXPRESSION
110
100
90
80
70
60
50
40
30
20
10
0
Grade 1
Grade 2
Grade 3
GP
INFLAMMATORY GRADE
Epithelium
Gland
Perivascular
Stroma

Fig. 17.12

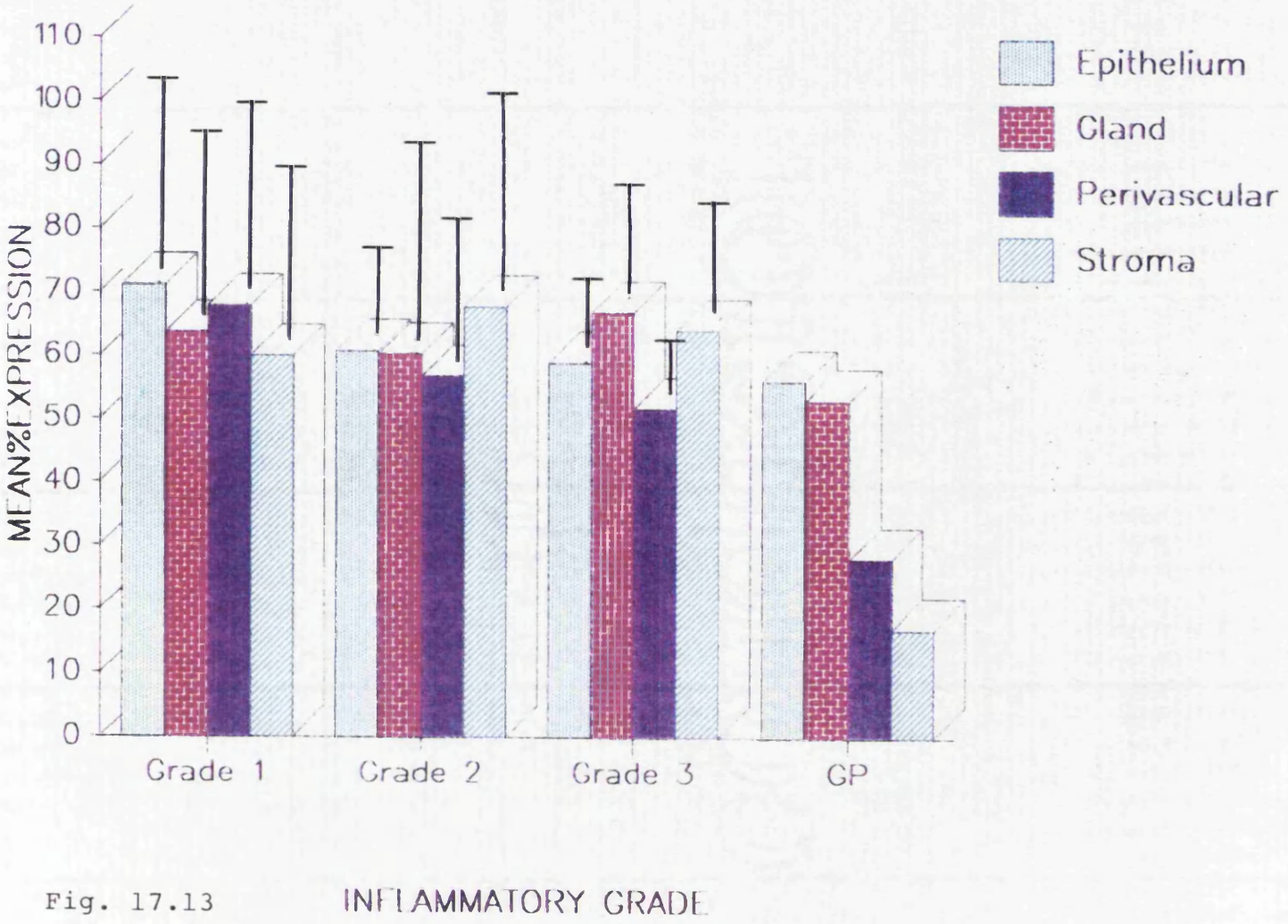

CD45RO EXPRESSION ON CD8 LYMPHOCYTES
MEAN%EXPRESSION
110
100
90
80
70
60
50
40
30
20
10
0
Grade 1
Grade 2
Grade 3
GP
INFLAMMATORY GRADE
Epithelium
Gland
Perivascular
Stroma

Fig. 17.13

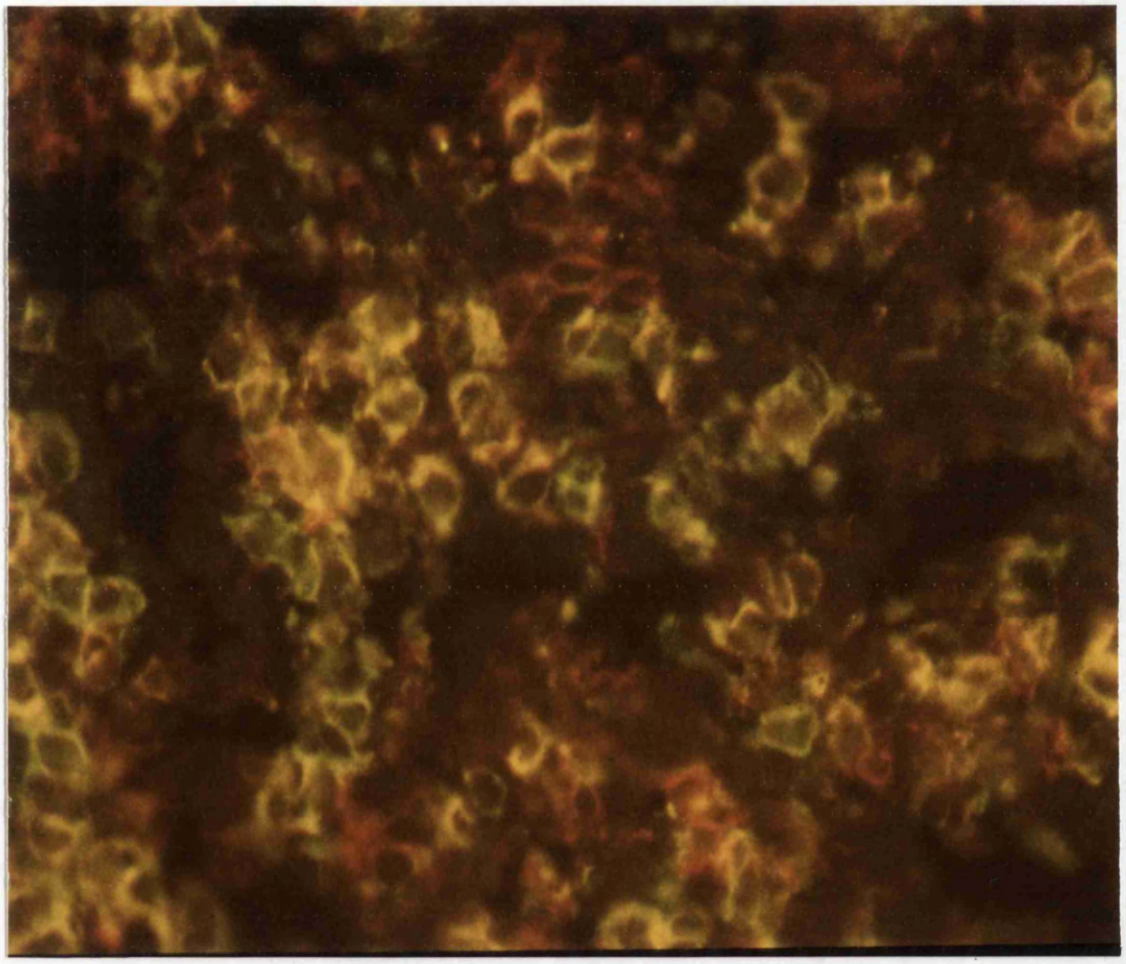


Fig. 17.14 The double immunofluorescence technique exhibiting CD7 expression on CD8 + T-lymphocytes

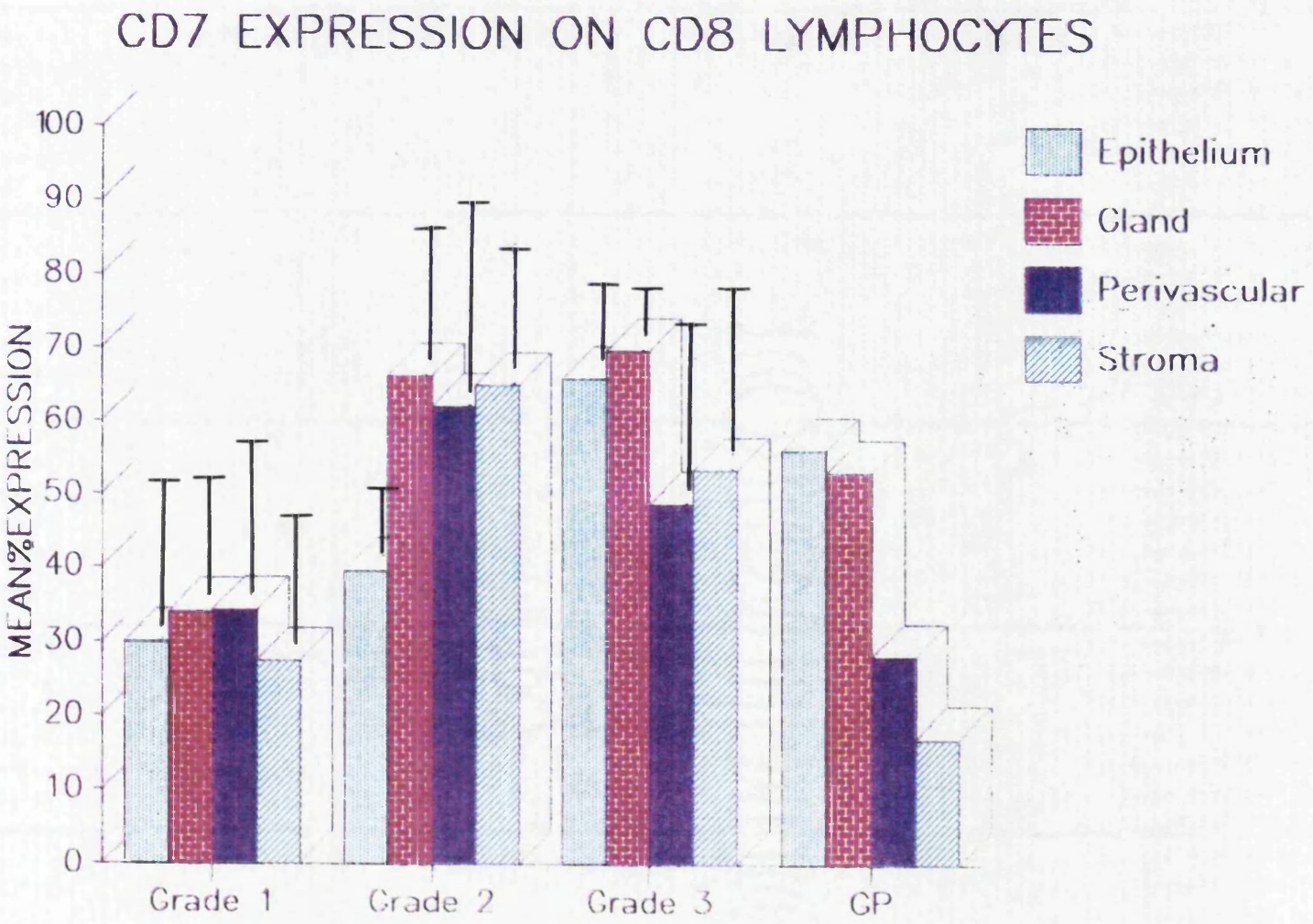

CD7 EXPRESSION ON CD8 LYMPHOCYTES
MEAN%EXPRESSION
100
90
80
70
60
50
40
30
20
10
0
Grade 1
Grade 2
Grade 3
GP
INFLAMMATORY GRADE
Epithelium
Gland
Perivascular
Stroma

Fig. 17.15

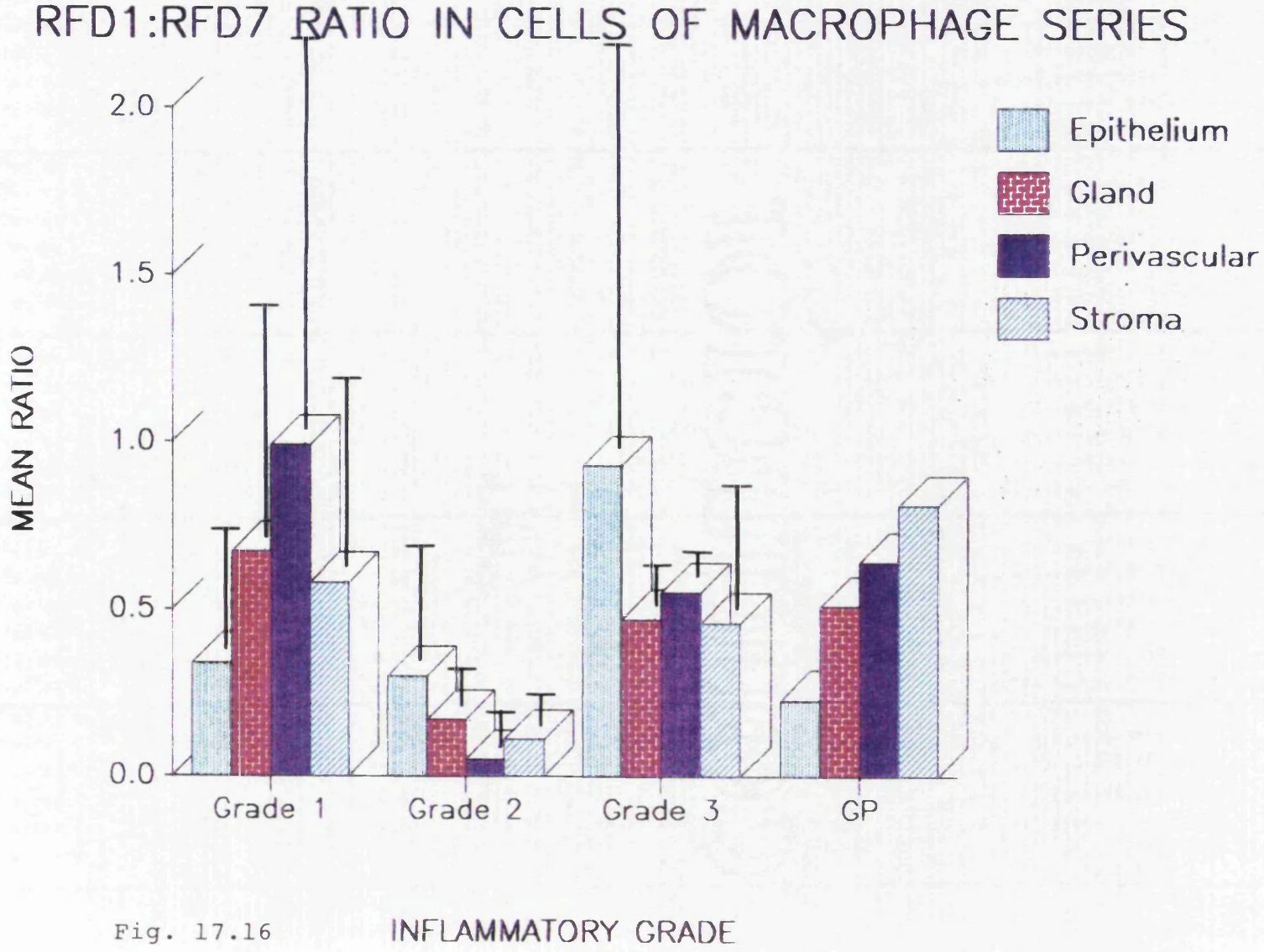

RFD1:RFD7 RATIO IN CELLS OF MACROPHAGE SERIES
MEAN RATIO
2.0
1.5
1.0
0.5
0.0
Epithelium
Gland
Perivascular
Stroma
Grade 1
Grade 2
Grade 3
GP
INFLAMMATORY GRADE

Fig. 17.16

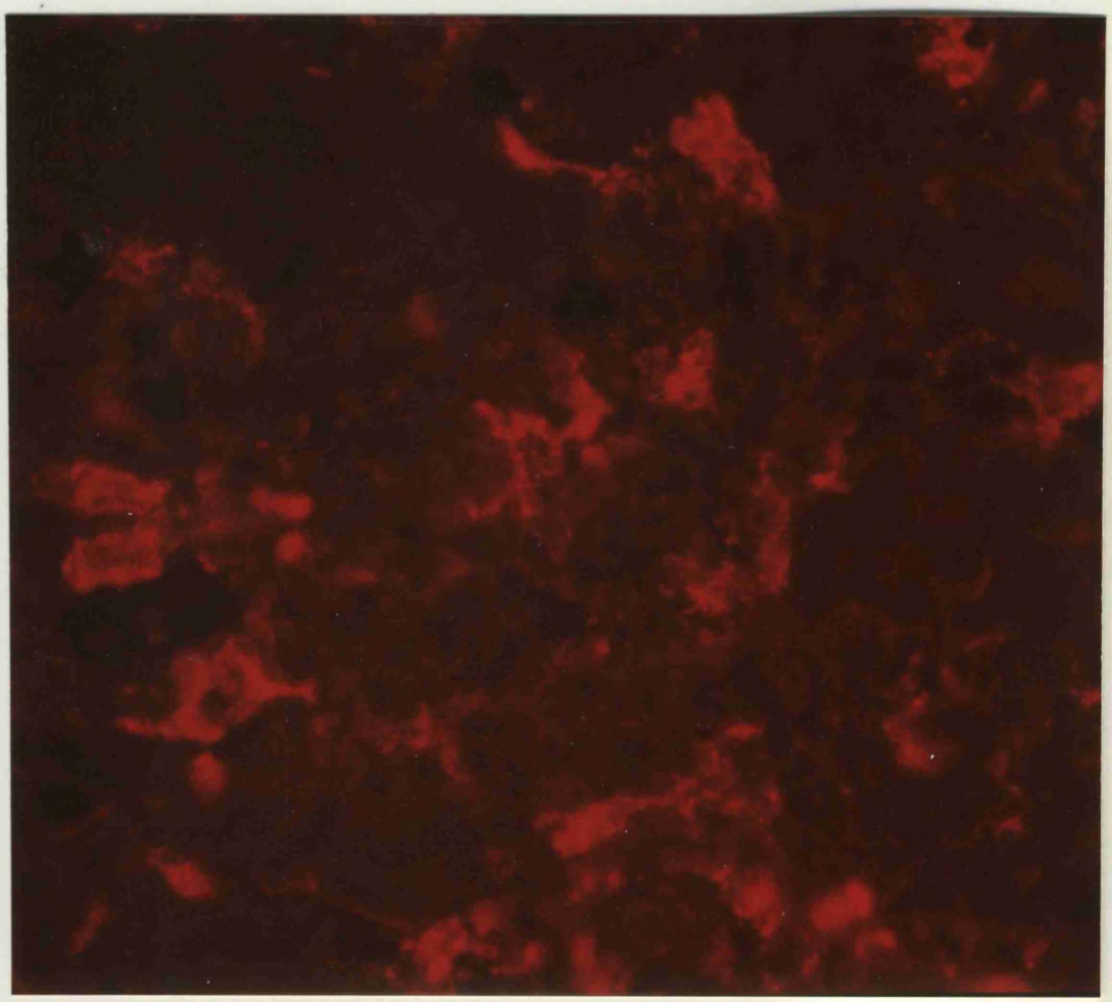


Fig. 17.17 RFD7 + cells in prostatic tissue identified by an immunofluorescence technique

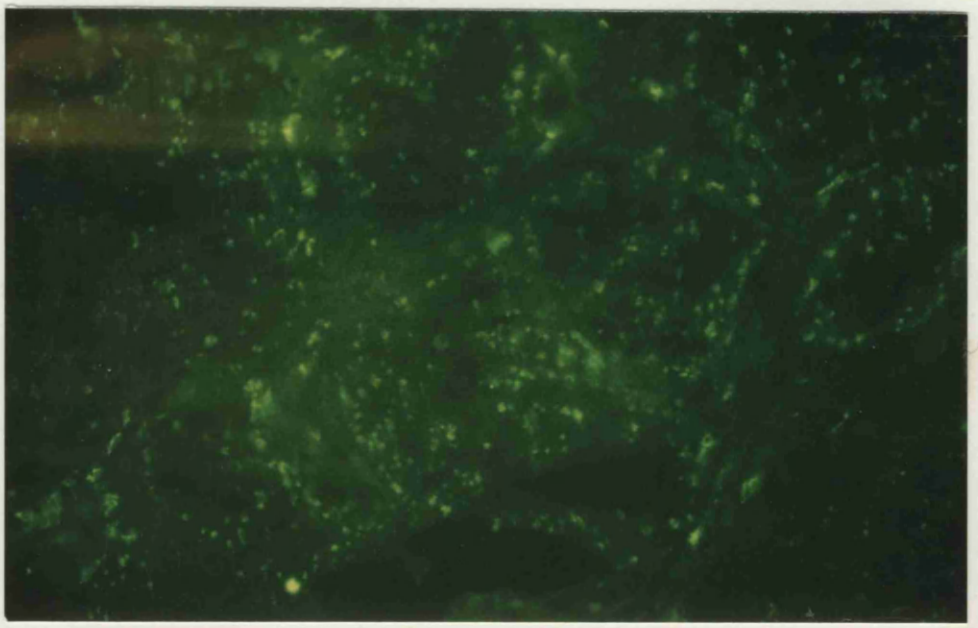


Fig. 17.18 RFD1 + cells in prostatic tissue identified by an immunofluorescence technique

was RFD7 dominance throughout all grades (see Appendices XX X & XXXI). In addition, in grade 1 biopsies, the most notable RFD1 presence was in the perivascular tissues, yet in the more severe inflammatory grades, the epithelial and glandular sites displayed the greatest RFD1 cell infiltration.

HLA-DR - Glucose Oxidase Conjugate Studies

The quantified HLA-DR expression assessed in ten of the study group and three controls (see Appendices XXXII & XXXIII), showed a progressive and significant increase in HLA-DR expression from grade 1 through to grade 3 lesions with the granulomatous tissue displaying the greatest HLA-DR expression, (Fig. 17.19), especially in the glandular areas. Interestingly, there was little difference in HLA-DR expression between normal and grade 1 biopsies and furthermore no difference in expression was evident between glandular, stromal and perivascular tissues.

SUMMARY

Utilising the technique of ultrasound guided biopsy, prostatic tissue was obtained from a group of patients diagnosed accurately as having chronic abacterial prostatitis.

The tissue obtained was examined for antibody, complement and fibrinogen deposition adopting a direct immunofluorescence (IF) technique. Within the group of chronic prostatitis patients, 34 (57%) had prostatic tissue that displayed immunofluorescence staining compared to only 1 patient (5%) from a control group ($p < 0.001$). IF staining for IgM was found in 85%, for C3 in 44%, for IgA in 35% and for fibrinogen in 24%, but the IgG subclass was not detected. Antibody deposition was mainly periglandular, glandular and in the vessel walls.

Five symptoms, particularly poor urinary flow, irritative voiding and urgency, were significantly correlated with IgM and C3 deposition and, to a lesser extent, fibrinogen deposition. There was no correlation between age, symptom duration, inflammatory grade and site, and positive immunofluorescence. The only correlation between histological features and immunofluorescence findings was a significant association between vessel thickening and positive immunofluorescence ($p < 0.05$).

In 24 patients from the chronic abacterial prostatitis cohort and 10 controls, the prostatic tissue obtained by ultrasound guided biopsy was subjected to immunohistochemical analysis.

Normal prostatic tissue displayed HLA-DR expression in the basal epithelial cells, endothelial cells and scanty mononuclear, non-macrophage cells. Isolated T-cells, with an equal prevalence of $CD4^+$ and $CD8^+$ cells were evenly distributed throughout the sections, yet no B-cells were evident. Scanty tissue macrophages ($RFD7^+$) were noted, but other members of the monocyte/macrophage population namely antigen-presenting cells ($RFD1^+$), monocytes ($CD14^+$) and epithelioid ($RFD9^+$) cells were not detected.

In patients with prostatitis, there was a progressive increase in the T-cell population, particularly the CD8 subset, and a greater expression of the CD7 antigen by these cells, with increasing severity of inflammation. Again B-cells were not detected. Both RFD1 and RFD7 cells were detected, with RFD7 predominance throughout. However, in grade 1 biopsies there was a prominent RFD1 cell presence, followed by a marked RFD7 cell influx in grade 2 biopsies and a relative re-emergence of RFD1 cells in grade 3 biopsies. CD14 cells were noted in only 50% of grade 2 and 3 lesions, but not in grade 1 biopsies, whereas RFD9 cells were confined to the granulomatous prostatitis tissue. HLA-DR expression increased both qualitatively and quantitatively with increasing severity on inflammation.

SECTION 18 THE MEASUREMENT OF PROSTATE SPECIFIC ANTIGEN IN CHRONIC PROSTATITIS

Since the purification of prostate specific antigen (PSA) (Wang et al, 1979), a number of studies have measured the the level of this glycoprotein in the serum of patients with a variety of prostatic diseases, particularly carcinoma (Kuriyama et al, 1981; Ferro et al, 1987; Stamey et al, 1987). Anecdotal data have indicated that inflammatory changes within the prostate gland may cause a rise in serum PSA (Collier & Pain, 1986; Stamey et al, 1987). However, no controlled studies have been undertaken to measure this marker specifically in patients with chronic prostatitis as diagnosed by standard criteria.

Patients and Methods

All members of the chronic abacterial prostatitis cohort (see Section 13), drawn from those patients attending the sexually transmitted diseases and urology clinics of St. Mary's Hospital, London, with a clinical diagnosis of chronic prostatitis were studied.

Venous blood was drawn from each patient at the start of the study, prior to any manipulation of the prostate by prostatic massage or endoluminal ultrasound and a further sample drawn three months later. The blood was allowed to clot and the serum separated by centrifugation within twenty-four hours of specimen collection and stored at $-20^{o}C$ prior to subsequent prostate specific antigen (PSA) quantification.

PSA estimations were made using the Tandem-R PSA ImmunoRadiometric Assay kit supplied by Hybritech. Each kit comprised the following:

Anti-PSA Tracer Antibody	-	mouse monoclonal anti-PSA antibody (IgG) labelled with I^{125}.
Anti-PSA Coated Beads	-	mouse monoclonal anti-PSA antibody (IgG) coated plastic beads.
Zero Calibrator	-	human serum containing no detectable PSA.

PSA Calibrators	- human serum containing PSA of known concentrations namely: 2, 10, 25, 50 and 100 ng/ml.
Wash Concentrate	- detergent solution containing 0.3% sodium azide as preservative.
Tandem-PSA Control Set	- human serum with an assigned concentration of PSA between 1-5 ng/ml.
	- human serum with an assigned concentration of PSA between 30-50 ng/ml.

The principle of the assay was a solid phase, two site immunoradiometric assay. Samples of test serum were incubated with a plastic bead coated with an anti-PSA monoclonal antibody (against site A on the PSA molecule) and a radiolabelled anti-PSA monoclonal antibody (against site B on the PSA molecule). As the two monoclonal antibodies bind to different sites on the PSA molecule, a sandwich is formed by the two antibodies with the PSA molecule forming the "filling", Fig. 18.1. The bead was then washed to remove excess unbound radiolabelled antibody and the radioactivity bound to the solid phase measured with a gamma counter. By incorporating serum of known PSA concentrations into the assay a standard curve of radioactivity versus PSA concentration was constructed and the radioactivity within the test samples read off from this curve to give the PSA concentration.

The details of the assay appear in Appendix XXXIV .

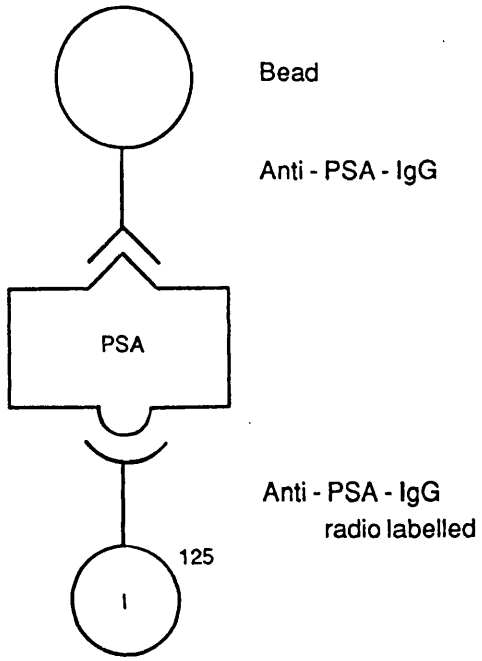


Fig. 18.1

SOLID PHASE - PSA - Labelled Antibody Sandwich

RESULTS

The patients comprising the chronic abacterial prostatitis cohort were aged 19-75 years, mean 39.8. The serum PSA values appear below in Table 18.1, along with the patients age and histological grade of the prostatic biopsy.

TABLE 18.1 SERUM PSA VALUES IN THE CHRONIC ABACTERIAL PROSTATITIS COHORT

Study No.	Age	Histological Grade	PSA ng/ml At presentation	At 3 months
1	53	2	5	<1
2	39	1	<1	<1
3	35	1	2	<1
4	28	-	<1	2
5	36	1	2	<1
6	37	-	1	1
7	35	2	<1	<1
8	25	2	<1	<1
9	38	1	<1	<1
10	65	3	<1	<1
11	63	3	2	<1
12	25	1	4	4
13	68	1	<1	2
14	34	1	<1	<1
15	34	1	<1	<1
16	42	0	1	<1

Study No.	Age	Histological Grade	PSA At presentation	ng/ml At 3 months
17	38	2	1	1
18	58	1	<1	<1
19	37	1	<1	5
20	39	1	1	<1
21	29	1	<1	<1
22	30	1	1	<1
23	32	1	<1	<1
24	29	1	3	2
25	25	1	1	1
26	41	0	<1	<1
27	50	3	1	<1
28	22	1	<1	<1
29	68	2	5	5
30	38	1	<1	<1
31	37	2	6	2
32	56	1	<1	<1
33	35	1	<1	<1
34	26	0	<1	<1
35	54	1	4	2
36	46	1	1	1
37	39	1	1	2
38	28	1	<1	<1
39	67	4	15	5
40	34	1	<1	<1
41	24	0	1	3

TABLE 18.1 SERUM PSA VALUES IN THE CHRONIC ABACTERIAL PROSTATITIS COHORT

Study No.	Age	Histological Grade	PSA ng/ml At presentation	At 3 months
42	55	3	5	6
43	44	-	1	<1
44	29	1	<1	<1
45	35	2	<1	<1
46	57	1	<1	<1
47	26	1	5	5
48	68	2	4	2
49	26	1	<1	<1
50	36	0	<1	<1
51	27	1	<1	1
52	35	2	1	<1
53	34	1	1	<1
54	19	2	8	5
55	34	0	<1	<1
56	58	1	<1	<1
57	34	1	5	2
58	34	3	4	5
59	24	1	<1	<1
60	75	1	<1	<1

In only ten cases were values greater than 4 ng/ml recorded, the upper limit of normal suggested by Lange and colleagues (1986) and in only one patient was such a value in excess of 10 ng/ml, an "operational" upper limit of normal (Siddall et al, 1986). There was no correlation between serum PSA levels and either age or histological grade on prostatic biopsy, in the cohort of patients with chronic abacterial prostatitis. The only patient with a PSA value greater than 10 ng/ml had granulomatous prostatitis, a florid inflammatory condition. However, at follow up, the value had fallen to 5 ng/ml from 15 ng/ml at presentation. Of the other nine cases with levels above the normal range, seven were elevated at presentation, one at 8 ng/ml, one at 6 ng/ml and five at 5 ng/ml. Of these seven, four patients had a lower PSA level at the three month follow up, with three entering the normal range. In the other three patients, two PSA values remained static at 5 ng/ml and the other increased to 6 ng/ml. Only 6 patients (10%) showed a rise in PSA concentration between the sample taken at presentation and that obtained three months later. Of these six cases, five had an initial value of either <1 or 1 ng/ml and in four of them the follow up value was still less than the accepted normal range of 4 ng/ml. In one patient a rise from <1 to 5 ng/ml was recorded and in the other a rise from 4 ng/ml to 5 ng/ml.

SUMMARY

The role of PSA in monitoring patients with prostatic carcinoma, although not defined fully, is well established. No objective data has previously been available on PSA values in chronic prostatitis. This study indicated that PSA levels were only slightly elevated in a small number of patients and in only one case out of sixty, was a value of greater than 10 ng/ml observed; that in a patient with granulomatous prostatitis. Thus, PSA appears to have little or no role to play in diagnosis and management of chronic abacterial prostatitis.

SECTION 19 FREE URINARY FLOW RATES IN CHRONIC PROSTATITIS AND PROSTATODYNIA

A functional outflow obstruction of the bladder has been observed in both chronic prostatitis (Buck, 1975; Siroky et al, 1981) and prostatodynia (Osborn et al, 1981; Hellstrom et al, 1987). The simplest form of urodynamic study is measurement of free urinary flow rates. A study was undertaken to measure these flow rates in the cohort of patients with chronic abacterial prostatitis to observe any changes in relation to the degree of inflammation in the prostate and any difference from a group of patients with prostatodynia.

Patients and Methods

The patients studied were drawn from the sexually transmitted diseases and urology clinics of St. Mary's Hospital, London, W2 and sixty of them comprised the cohort of patients with chronic abacterial prostatitis, see Section 13. Furthermore, sixty patients with a diagnosis of prostatodynia, drawn from the same source, were studied.

The patients were asked to attend with a full bladder and their free urinary flow rate was measured using a commercial flow meter supplied by Micromedics Ltd. Only flow rates with a voided volume in excess of 150 mls were included in the study. The voided volume and the maximum flow rate were recorded in each case and rounded up or down to the nearest whole number. A free urinary flow rate was measured at presentation and subsequently three and six months after entry into the cohort. The flow rate was measured within one week of prostatic localisation studies (see Section 11) in which the degree of inflammation within the prostate was measured.

The group of patients with a diagnosis of prostatodynia based on prostatic localisation studies underwent flow rate estimations at presentation and again three months later.

The maximum flow rates were plotted on one of two nomograms: the Bristol nomogram (Kadow et al, 1985) for patients aged over 50 years and that devised by Siroky and colleagues

(1979), for patients aged 50 years or less. Based on these nomograms the flow rate was assigned to one of five groups: + - supranormal (> 1 standard deviation about the mean), 0 - at or within 1 standard deviation above the mean, < 0 - within 1 standard deviation below the mean, 1 - 2 - between 1 and 2 standard deviations below the mean and > 2 - greater than 2 standard deviations below the mean.

Student's t test was applied to the crude data to identify any difference between the chronic abacterial prostatitis cohort and the prostatodynia patients. A chi-square analysis was performed on the grouped data, as categorised by the nomograms, to detect any difference between the two study groups with respect to the prevalence of: 1) significantly reduced (> 2 standard deviations below the mean) flow rates, 2) supranormal flow rates, and 3) flow rates below the mean. Finally, the correlation between flow rate and Stamey score (from localisation studies, see Section 11) and inflammatory grade (from histological analysis of prostatic tissue, see Section 16) was analysed.

RESULTS

The mean age of the chronic abacterial prostatitis cohort (CABP) was 39.8 years, (range 19-75) and that of the prostatodynia group (Pd) 36.05 years (range 19-66).

The flow rate data of the chronic abacterial prostatitis cohort appear in Table 19.1 and that of the prostatodynia group in Table 19.2.

The individual flow rates were plotted on the Bristol nomogram for patients aged over 50 years, Figures 19.1 and 19.2 and the Siroky nomogram for those aged 50 years or less, figures 19.3 and 19.4.

The percentage of cases within the CABP cohort which were assigned to each flow rate category were as follows: + : 8 - 12%; 0 : 17 - 22%; < 0 : 23 - 28%; 1 - 2 : 25 - 38%; > 2 : 8 - 18%. The percentages of the Pd group similarly assigned were: + : 13 - 15%; 0 : 30 - 37%; < 0 : 28 - 35%; 1 - 2 : 13 - 17%; > 2 : 5 - 7%.

The CABP cohort displayed a 42% change in flow rate category between the first and second series of recordings, 20% producing an improved and 22% a decreased flow rate. Between the second and third series a 27% change in category was observed, yet only 5% had improved values, compared to 22% with decreased figures. In the Pd

patients a 20% change was noted between the two series, consisting of 8% with an improvement at 12% with a decrease in flow rate category. In all cases the change in category reflected a less than one standard deviation variation.

The peak urinary flow rates of the CABP cohort were significantly ($p<0.001$) lower than those fo the Pd group.

The chi-square analysis produced conflicting data. If all the flow rate series were considered, six possible permutations for analysis were available. With respect to the > 2 category, a significant difference ($p < 0.05$) existed between the CABP cohort and Pd patients, in two analyses, yet no such difference was detected in the remaining four. In the case of the supranormal, +, category no difference existed between the two study groups. However, when flow rates below the mean (< 0) were taken in to consideration , a significant difference ($p <0.05$ in two, $p < 0.02$ in one and $p < 0.01$ in one) was noted in four analyses, whereas in the remaining two, no significant difference was observed.

Finally, there was no correlation between flow rate and Stamey score or inflammatory grade within the prostatic biopsies. This was observed on scatter diagrams and it was thus felt that calculation of correlation coefficients was unnecessary.

Peak Urinary Flow Rates in the Chronic Abacterial Prostatitis Cohort Aged Over 50 Years

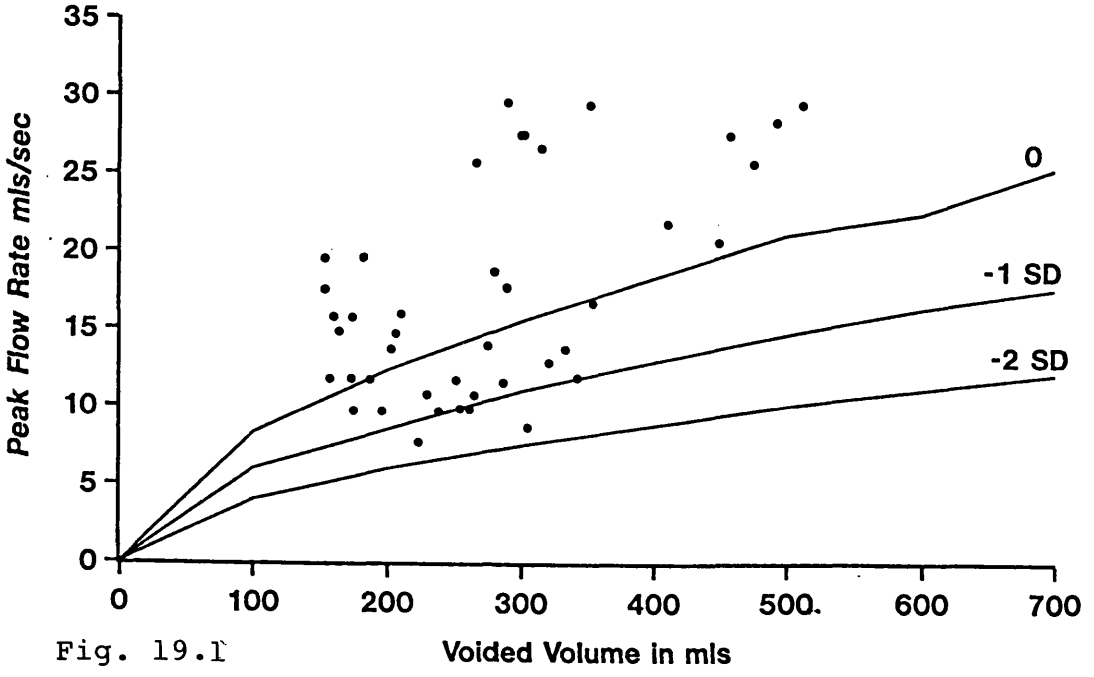


Fig. 19.1

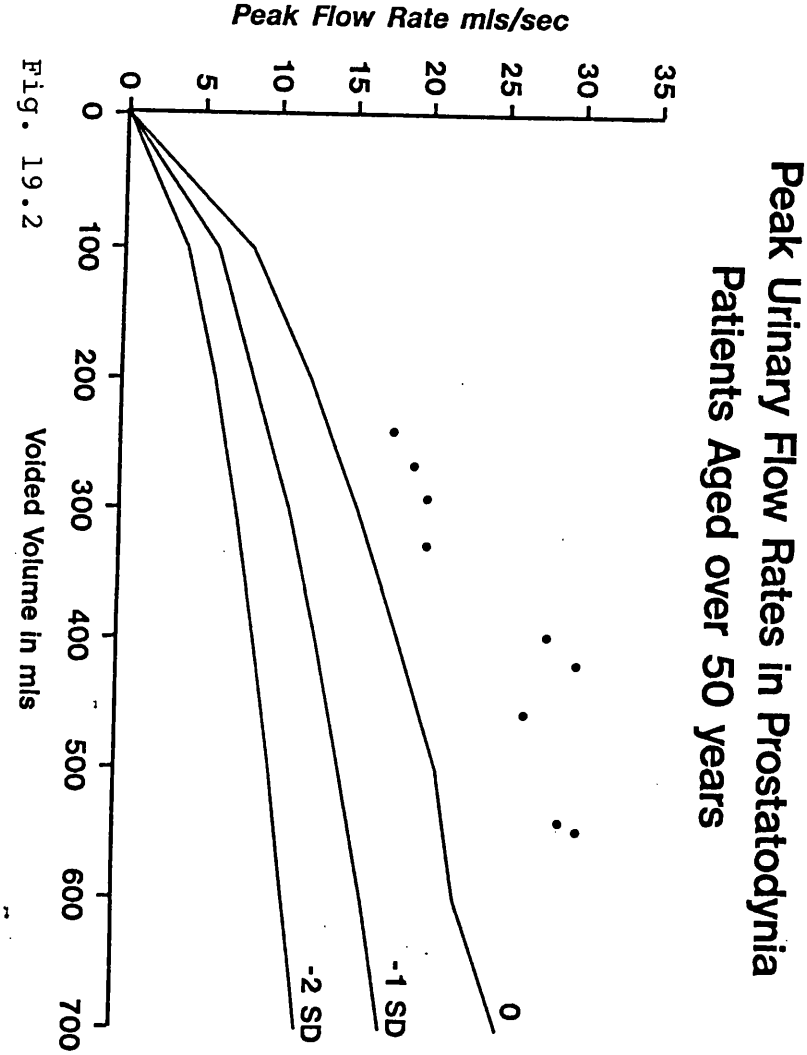

Peak Urinary Flow Rates in Prostatodynia
Patients Aged over 50 years
Peak Flow Rate mls/sec
0
5
10
15
20
25
30
35
0
100
200
300
400
500
600
700
Voided Volume in mls
0
-1 SD
-2 SD

Fig. 19.2

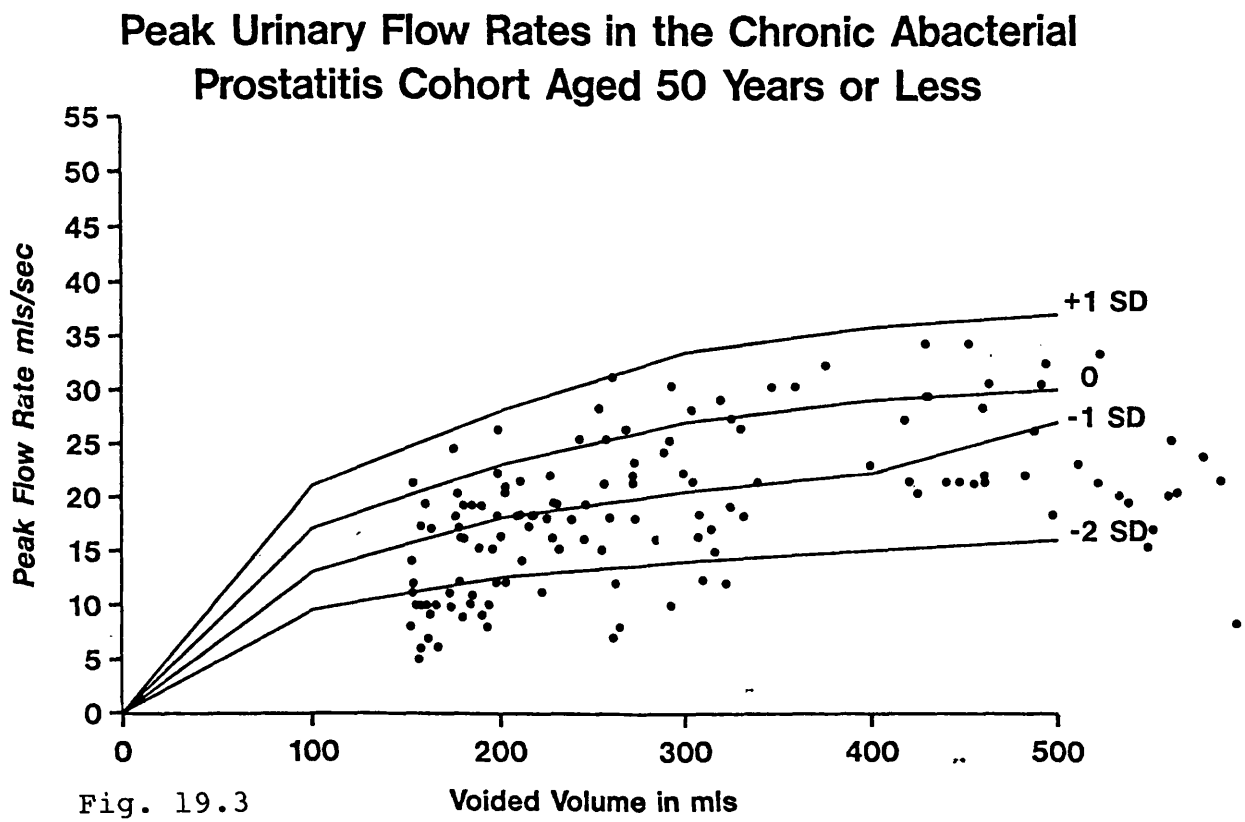

Peak Urinary Flow Rates in the Chronic Abacterial Prostatitis Cohort Aged 50 Years or Less
Peak Flow Rate mls/sec
55
50
45
40
35
30
25
20
15
10
5
0
0
100
200
300
400
500
Voided Volume in mls
+1 SD
0
-1 SD
-2 SD

Fig. 19.3

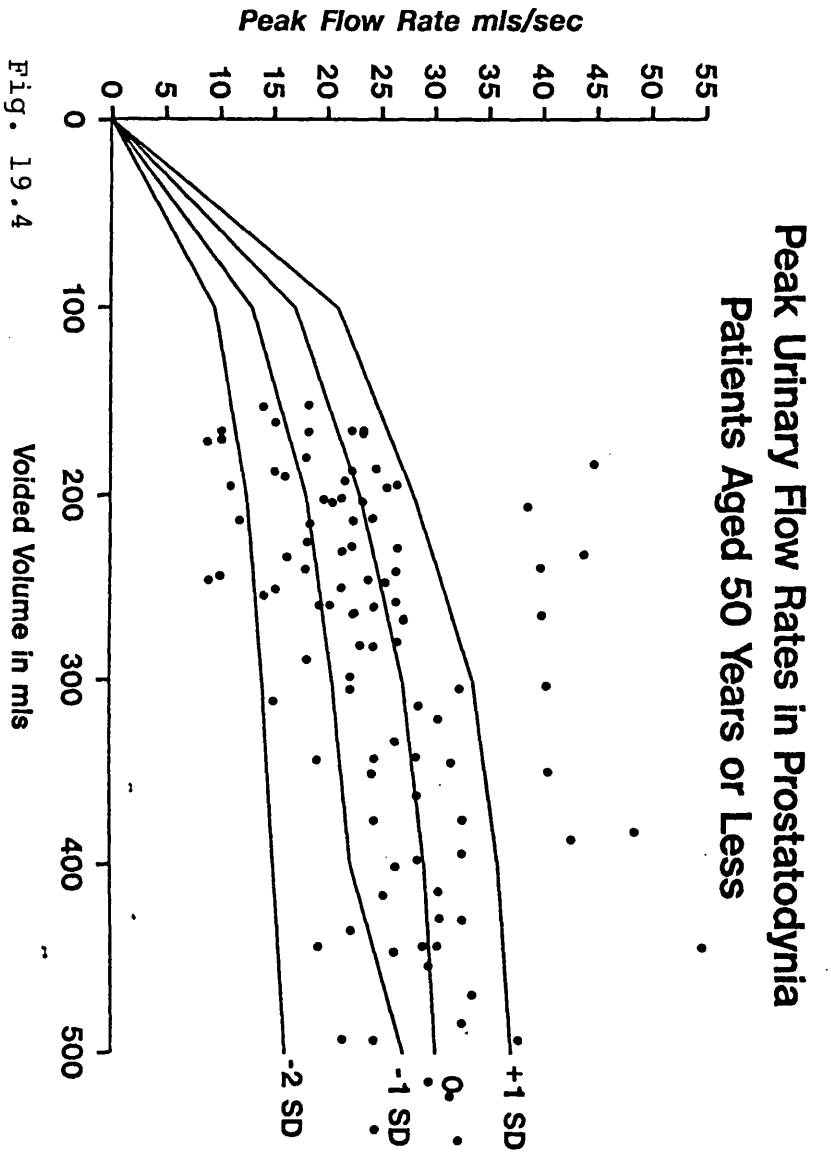

Peak Urinary Flow Rates in Prostatodynia
Patients Aged 50 Years or Less
Peak Flow Rate mls/sec
0
5
10
15
20
25
30
35
40
45
50
55
Voided Volume in mls
0
100
200
300
400
500
+1 SD
0
-1 SD
-2 SD

Fig. 19.4

TABLE 19.1 VOIDED VOLUMES AND PEAK URINARY FLOW RATES IN COHORT OF PATIENTS WITH CHRONIC ABACTERIAL PROSTATITIS

Study No.	Age in Years	Voided Volumes in Mls	Peak Flow Rate Mls/Sec
1	53	276	13
		240	10
		261	10
2	39	560	24
		480	22
		521	21
3	35	460	21
		432	20
		531	20
4	28	640	30
		329	26
		417	27
5	36	199	16
		224	18
		217	18
6	37	563	20
		722	22
		513	23
7	35	433	29
		491	30
		458	28
8	25	165	10
		153	12
		178	12
9	38	494	18
		252	15
		539	16
10	65	487	29
		449	21
		408	22

TABLE 19.1 VOIDED VOLUMES AND PEAK URINARY FLOW RATES IN COHORT OF PATIENTS WITH CHRONIC ABACTERIAL PROSTATITIS

Study No.	Age in Years	Voided Volumes in Mls	Peak Flow Rate Mls/Sec
11	63	290	18
		209	16
		280	19
12	25	344	30
		153	21
		321	28
13	68	254	10
		222	8
		302	9
14	34	182	11
		159	10
		156	6
15	34	258	7
		262	8
		192	8
16	42	316	15
		235	18
		278	16
17	38	170	10
		220	11
		191	10
18	58	201	14
		206	15
		253	12
19	37	525	33
		491	32
		462	30
20	39	155	5
		166	6
		161	7

TABLE 19.1 VOIDED VOLUMES AND PEAK URINARY FLOW RATES IN COHORT OF PATIENTS WITH CHRONIC ABACTERIAL PROSTATITIS

Study No.	Age in Years	Voided Volumes in Mls	Peak Flow Rate Mls/Sec
21	29	201	12
		560	20
		539	19
22	30	288	20
		254	18
		213	17
23	32	156	17
		456	22
		321	19
24	29	257	31
		196	26
		169	24
25	25	159	17
		196	18
		178	17
26	41	209	21
		337	21
		268	21
27	50	158	10
		225	16
		196	12
28	22	228	15
		242	16
		252	21
29	68	343	12
		196	10
		226	11
30	38	415	21
		310	17
		160	7

TABLE 19.1 VOIDED VOLUMES AND PEAK URINARY FLOW RATES IN COHORT OF PATIENTS WITH CHRONIC ABACTERIAL PROSTATITIS

Study No.	Age in Years	Voided Volumes in Mls	Peak Flow Rate in Mls/Sec
31	37	487	26
		180	16
		211	14
32	56	350	17
		156	12
		188	12
33	35	329	18
		207	18
		446	21
34	26	185	10
		305	16
		152	8
35	54	589	30
		560	28
		471	26
36	46	201	20
		229	19
		227	19
37	39	244	19
		307	18
		269	18
38	28	269	27
		256	25
		287	25
39	67	287	12
		173	10
		263	11
40	34	453	21
		587	21
		684	22

TABLE 19.1 VOIDED VOLUMES AND PEAK URINARY FLOW RATES IN COHORT OF PATIENTS WITH CHRONIC ABACTERIAL PROSTATITIS

Study No.	Age in Years	Voided Volumes in Mls	Peak Flow Rate Mls/Sec
41	24	177	16
		198	16
		216	18
42	55	155	20
		182	20
		151	18
43	44	189	19
		207	18
		174	18
44	29	151	14
		158	19
		180	19
45	35	180	9
		550	17
		320	12
46	57	288	30
		265	26
		302	28
47	26	155	10
		162	9
		173	11
48	68	351	30
		298	28
		314	27
49	26	677	38
		451	34
		429	34
50	36	270	22
		241	25
		285	24

TABLE 19.1 VOIDED VOLUMES AND PEAK URINARY FLOW RATES IN COHORT OF PATIENTS WITH CHRONIC ABACTERIAL PROSTATITIS

Study No.	Age in Years	Voided Volumes in Mls	Peak Flow Rate Mls/Sec
51	27	223	22
		268	23
		184	19
52	35	560	25
		421	21
		439	21
53	34	198	22
		201	21
		176	20
54	19	189	9
		596	18
		261	12
55	34	250	28
		374	32
		358	30
56	58	158	16
		176	16
		166	15
57	34	397	23
		176	17
		302	21
58	34	297	22
		187	15
		194	15
59	24	150	11
		549	15
		309	12
60	75	332	14
		171	12
		319	13

TABLE 19.2 VOIDED VOLUMES AND PEAK URINARY FLOW RATES IN PATIENTS WITH PROSTATODYNIA

Age in Years	Voided Volume in Mls	Peak Flow Rate in Mls/Sec
20	263	39
	304	40
34	171	9
	241	10
54	329	20
	284	20
31	602	27
	538	24
38	225	22
	279	24
34	434	22
	515	29
32	428	30
	361	28
49	446	26
	492	24
36	250	15
	360	28
24	240	30
	303	32
41	194	26
	689	30
38	330	26
	394	28
19	253	14
	308	15

TABLE 19.2 VOIDED VOLUMES AND PEAK URINARY FLOW RATES IN PATIENTS WITH PROSTATODYNIA

Age in Years	Voided Volume in Mls	Peak Flow Rate in Mls/Sec
50	339	19
	286	18
34	415	25
	373	24
38	304	22
	347	24
41	176	18
	202	19
48	685	35
	427	32
21	180	44
	229	43
46	602	35
	546	32
22	606	27
	521	26
29	165	22
	184	22
28	165	23
	186	24
26	214	22
	263	22
58	534	29
	452	26
35	415	30
	522	31

TABLE 19.2 VOIDED VOLUMES AND PEAK URINARY FLOW RATES IN PATIENTS WITH PROSTATODYNIA

Age in Years	Voided Volume in Mls	Peak Flow Rate in Mls/Sec
44	344	31
	256	26
56	783	34
	541	30
22	491	37
	467	33
21	392	32
	374	32
46	278	23
	249	21
28	160	15
	231	16
26	239	26
	246	25
42	170	10
	193	11
43	152	18
	187	16
45	165	10
	212	12
49	200	21
	258	20
24	228	26
	279	24
40	321	30
	374	32

TABLE 19.2 VOIDED VOLUMES AND PEAK URINARY FLOW RATES IN PATIENTS WITH PROSTATODYNIA

Age in Years	Voided Volume in Mls	Peak Flow Rate in Mls/Sec
32	224	18
	247	9
21	278	26
	260	24
44	194	21
	164	18
39	237	18
	263	22
28	695	24
	603	22
32	204	23
	246	24
20	385	42
	349	40
40	265	27
	315	28
38	443	30
	342	28
66	263	19
	240	17
32	215	18
	258	19
24	206	38
	237	39
34	443	19
	491	21

TABLE 19.2 VOIDED VOLUMES AND PEAK URINARY FLOW RATES IN PATIENTS WITH PROSTATODYNIA

Age in Years	Voided Volume in Mls	Peak Flow Rate in Mls/Sec
30	455	29
	401	26
28	197	25
	214	24
54	419	30
	394	28
44	442	29
	484	32
32	187	15
	154	14
41	297	22
	342	24
48	445	54
	380	48
24	204	20
	229	21

SUMMARY

The peak urinary flow rates were compared between the CABP cohort and an age matched group of Pd patients. There was a clearly significant difference ($p < 0.001$) between the crude rates in the two groups. However, when nomograms were employed, this difference was less marked, with respect to flow rates greater than 2 standard deviations below the mean ($p < 0.05$ in two of six analyses) and flow rates simply below the mean ($p < 0.05$ - $p < 0.01$ in flow of six analyses).

There was no correlation between the degree of inflammation, as judged by Stamey score or inflammatory grade in prostatic biopsies, and the serial flow rate data.

SECTION 20 INTRAPROSTATIC URINARY REFLUX IN CHRONIC ABACTERIAL PROSTATITIS

Indirect evidence of intraprostatic urinary reflux exists from the chemical analysis of prostatic stones (Sutor & Wooley, 1974; Kim, 1982), their location in the peripheral zone (Huggins & Bear, 1944) and the orientation of the prostatic ducts draining the peripheral zone (Blacklock, 1974). Furthermore, this phenomenon has been demonstrated directly in a small number of patients with chronic abacterial prostatitis (Kirby et al, 1982).

A pilot study was devised to investigate a small number of patients from the chronic abacterial prostatitis cohort (see Section 13), in an attempt to identify intraprostatic urinary reflux as a prelude to devising a quantitative estimation of this process.

Patients and Methods

Ten patients aged 19-68 years, mean 31.2, were randomly selected from the sixty patients comprising the chronic abacterial prostatitis cohort.

Each patient gave informed verbal consent to the procedure. The patients first emptied their bladder. Under strict aseptic conditions a 14 Fr silastic urethral catheter was inserted into
the bladder. A suspension of 10 mls of indian ink, which had been sterilised in an autoclave by the hospital pharmacy, was then made up to 500 mls with sterile normal saline. The mixture was vigorously shaken before being introduced into the patient's bladder in 50 ml aliquots until the patient noted a sensation of bladder fullness. The urethral catheter was removed and the patient was asked to void to completion. Once this had been achieved, the patient was allowed home and asked to re-attend three days later with a full bladder.

Three days after the instillation of the indian ink suspension into the bladder, the patient underwent prostatic localisation studies as described in Section 11. Each urine specimen (VB1, VB2 and VB3) and any expressed prostatic secretion (EPS) was examined for the presence of carbon particles from the indian ink suspension. In addition any side effects or complications as a result of the urethral catheterisation and the indian ink

instillation were sought. The VB2 sample (mid-stream urine specimen) was also cultured aerobically and anaerobically under standard conditions.

Results

All patients described mild dysuria for up to 24 hours following urethral catheterisation. However, none of the cultures of the mid-stream urine samples, yielded any organisms in significant numbers, (10^5/ml).

Seven of the patients studied had evidence of intraprostatic carbon particles on localisation studies, indicating that intraprostatic reflux of urine had taken place following instillation of the indian ink into the bladder, see Table 20.1.

SUMMARY

By means of a crude qualitative method, employing indian ink suspension instilled intravesically, intraprostatic urinary reflux was demonstrated in seven out of ten cases of a cohort of patients with chronic abacterial prostatitis. No controls were investigated in this pilot study.

TABLE 20.1 LOCATION OF CARBON PARTICLES WITHIN URINE AND EXPRESSED PROSTATIC SECRETION FOLLOWING INTRAVESICAL INSTILLATION OF INDIAN INK

Study No.	VB1	VB2	EPS	VB3
8	-	-	N/A	+
23	-	-	-	-
25	-	-	N/A	-
29	-	-	+	+
34	-	-	-	+
38	-	-	+	+
44	-	-	+	+
49	-	-	N/A	-
54	-	-	N/A	+
57	-	-	+	+

Key: carbon particles present :+.
carbon particles not detected :-+.
N/A - sample not obtained.

SECTION 21 PSYCHOLOGICAL ASSESSMENT OF PATIENTS WITH CHRONIC ABACTERIAL PROSTATITIS USING THE GENERAL HEALTH QUESTIONNAIRE

Although it is often stated that chronic prostatitis has a strong psychological component to it's aetiology and presentation, objective data to support this view are sparse. A study by Smart and colleagues, (1976) used the General Health Questionnaire to compare groups of patients with chronic prostatitis and controls, and were unable to find a greater tendency to neurotic disease in the former group.

In an attempt to quantify any functional psychiatric illness, the General Health Questionnaire (Goldberg, 1972) was employed in one group of patients with chronic abacterial prostatitis, one with prostatodynia and one comprising patients attending an urology outpatient clinic on a long term basis.

Patients and Methods

Three groups of patients were studied: (1) the chronic abacterial prostatitis (CABP) cohort (see Section 13), (2) a group of sixty patients with a diagnosis of prostatodynia (Pd) based on prostatic localisation studies, drawn from the sexually transmitted diseases and urology clinics of St. Mary's Hospital, London, W2 and (3) a group comprising thirty male patients (CUA), who attended the urology clinic on a chronic basis at St. Mary's Hospital, London, W2 with non-malignant disease.

Each patient was asked to complete a General Health Questionnaire, GHQ-60, comprising sixty questions which referred to the patient's health over the preceding few weeks. To each question, one of four possibilities was chosen by underlining the most appropriate response. The patients completed the questionnaires in their own homes without any researcher being present, at the time of entry to the cohort, in the case of patients with chronic abacterial prostatitis, at the time of diagnosis in patients with prostatodynia and at a random point in their illness in the group of chronic attenders of the urology clinic.

In accordance with the standard scoring system (Goldberg, 1978) patients were assigned a score of 0 if they gave an answer of "not at all" or "better than usual", and similarly 0 if a response of "same as" or "no more than usual" was given. Similarly if a response of "less" or "much less" to

positive questions and "rather more" or "more" to negative questions, was given, the score of 1 was assigned; a total score out of a maximum sixty was accorded to each patient. The interpretation of the General Health Questionnaire (Goldberg, 1978), in a number of previous validity studies, regarded a score of $\geq$ 12 as indicative of functional psychiatric illness, and this was the threshold adopted in this study.

RESULTS

The mean age of the chronic abacterial prostatitis (CABP) cohort was 39.8 years, range 19-75, that of the prostatodynia (Pd) group 36.05 years, range 19-66, and that of the chronic attenders of the urology clinic 43.7 years, range 34-62.

The mean symptom duration in the CABP cohort was 3.41 years, range 0.1 - 20, and for the Pd group 3.84 years, range 0.3 - 22.

The main diagnosis of the CUA group of patients is depicted in Table 21.1.

TABLE 21.1 MAIN DIAGNOSIS IN THE CHRONIC ATTENDERS OF AN UROLOGY CLINIC (CUA) GROUP

Urolithiasis	18
Detrusor instability	4
Urinary diversion	3
Interstitial cystitis	2
Peyronie's disease	2
Impotence	1

The scores in each group were subdivided into five categories, namely 0-2, 3-5, 6-8, 9-11 and $\geq$ 12. The percentage of patients in each scoring category from each of the study groups, CABP cohort, Pd group and the CUA group, are depicted in Table 21.2.

Although, there was no true control group, i.e. one without disease, the distribution in all the groups is skewed and non- parametric. Furthermore, the scores within both the CABP cohort and the Pd group are shifted to the right, a trend that is more marked in the prostatodynia (Pd) group.

TABLE 21.2 GHQ-60 SCORES IN CHRONIC ABACTERIAL PROSTATITIS COHORT (CABP), PROSTATODYNIA PATIENTS (Pd) AND CHRONIC ATTENDERS OF ANUROLOGY CLINIC (CUA)

	GHQ - 60 SCORE				
STUDY GROUP	NUMBERS AND PERCENTAGES ( ) WITH EACH SCORE				
	0 - 2	3 - 5	6 - 8	9 - 11	≥ 12
Chronic Abacterial Prostatitis (CABP)	23(38%)	9(15%)	8 (13%)	4 (7%)	16(27%)
Prostatodynia Pd	9 (15%)	8 (13%)	5 (8 %)	6 (10%)	32(54%)
Chronic Urology Attender (CUA)	13(43%)	6 (20%)	5 (17%)	1 (3%)	5 (17%)

The percentage of patients with score of ≤ 5 and ≥ 12 for each study group is depicted in Table 21.3.

TABLE 21.3 PERCENTAGE OF STUDY GROUPS WITH GHQ-60 SCORES OF < 5 AND > 12

Study Group	Percentage of Cases Score ⩽ 5 on GHQ-60	Score ⩾ 18 on GHQ-60
Chronic prostatitis cohort (CABP)	53%	27%
Prostatodynia group (Pd)	28%	54%
Chronic attenders urology clinic (CUA)	63%	17%

The standard error of the differences of the percentages of each study group with scores of ⩽ 5 and ⩾ 12, revealed a statistical difference between the CABP cohort and the Pd group, $p < 0.01$, and between the CUA group and the Pd group $p < 0.001$, in the case of scores ⩽ 5. A statistical difference was also achieved between the CABP cohort and Pd group, $p < 0.002$, and between the CUA group and the Pd group, $p < 0.001$, in the case of scores ⩾ 12. Specifically, there was no statistical difference between the CABP cohort and the CUA group in the percentage of patients with scores of either ⩽ 5 or ⩾ 12. Finally, there was no correlation between prolonged symptom duration and GHQ-60 score.

SUMMARY

Using the General Health Questionnaire, GHQ-60, as an indicator of functional psychiatric illness three groups of patients were studied, the cohort with chronic abacterial prostatitis (CABP), the group with prostatodynia (Pd) and a group of chronic attenders of a general urology clinic (CUA).

There was no statistical difference between the CABP and CUA groups with respect to a score indicative of psychiatric illness ($\geqslant$ 12). In contrast, statistically significant differences were observed in the percentage of patients who recorded such a score in the Pd group, compared to CABP patients, $p < 0.002$, and the CUA group, $p < 0.001$. Furthermore, the prevalence of a "normal" score ($\leqslant$ 5) was statistically greater in both the CABP, $p < 0.01$ and CUA, $p < 0.001$ groups, compared to the Pd patients. Prolonged symptom duration did not affect the GHQ-60 score in the CABP cohort.

SECTION 22 ACUTE EPIDIDYMITIS - A MICROBIOLOGICAL AND ULTRASONOGRAPHIC STUDY

As outlined in Section 8, an association between prostatic pathology and epididymitis has been postulated, but to date remains ill defined. Grant and co-workers (1987) performed prostatic localisation studies (Meares & Stamey, 1968) on a group of patients with acute epididymitis and from the expressed prostatic secretion, cultured Chlamydia trachomatis in approximately 9% and other organisms in 10% of patients.

The study below was undertaken to a) define the aetiological organisms in a prospective study of patients with acute epididymitis over an eighteen month period and b) assess the role of the prostate in this condition by means of transrectal ultrasound and attempt to correlate these observations with earlier studies, see Section 12.

Patients and Methods

Twenty-four patients aged 19-72 years, (mean 28.3) who presented to the sexually transmitted diseases and urology departments with a clinical diagnosis of acute epididymitis, were studied. All patients, except one, presented within five days of the onset of symptoms, the exception delayed consultation for 14 days, and none of the cases had received antibiotics prior to study.

The patients were investigated by means of urethral swabs, mid-stream urine analysis, chlamydial serology, epididymal aspiration and transrectal prostatic ultrasound.

From each patient two urethral swabs were taken, the first tested for Neisseria gonorrhoeae by Gram stain and culture and the second, for chlamydiae, by a direct immunofluorescence technique (MicroTrak, Syva), and mycoplasmas and ureaplasmas, by culture. In the case of the latter swab, a smear was first made onto the MicroTrak (Syva) slide before the swab was placed in mycoplasma and ureaplasma transport medium.

A mid-stream urine specimen was collected and tested for aerobic bacteria by routine microbiological methods.

Venous blood was drawn at presentation and two weeks later, for chlamydial serology. Serum IgG and IgM antibodies against Chlamydia trachomatis using serovas D-K were sought by an indirect immunofluorescence technique (Thomas et al, 1976).

Following these initial samples, epididymal aspiration was performed observing a strict aseptic technique under regional anaesthesia (Berger et al, 1978). The groin and scrotum were cleansed with Hibitane (ICI) and then 10 mls of 1% Lignocaine was instilled into the spermatic cord on the affected side. Once satisfactory anaesthesia had been achieved, as judged by applying gentle digital pressure to the epididymis, a further 2 mls of 1% Lignocaine was instilled into the scrotal skin overlying the most oedematous part of the epididymis. An 18G needle was then inserted into the epididymis and 2 mls of sterile normal saline was injected, before withdrawing approximately 1 ml of turbid fluid from the epididymis. The sample obtained was subdivided: one portion smeared onto a MicroTrak (Syva) slide for chlamydiae, another placed in mycoplasma and ureaplasma transport medium and stored at -70 C prior to culture, another placed in modified Stuart's medium for anaerobic culture and the fourth tested for aerobic bacteria by routine microbiological methods.

Each patient, also underwent transrectal prostatic ultrasound within three days of presentation, using a Bruel and Kjaer 1846 machine with a 5.5 MHz transducer. This was performed in the left lateral position and the findings were recorded on X-ray plate.

After the initial assessment and collection of specimens, the patients were treated with antibiotics, Doxycycline 100 mg once daily in the first instance, and then altered to an

appropriate antibiotic if bacteriological data deemed the initial medication to be unsuitable. The patients were reviewed two weeks after the initial consultation, were examined clinically and further mid-stream urine and venous blood samples taken; further studies to detect C. trachomatis, i.e. a test of cure were not performed.

RESULTS

In 15 patients (62.5%), organisms were detected at sufficient titres to imply infectivity. Chlamydia trachomatis accounted for 10 (42%) of the cases, being located in the urethra and epididymal aspirate in five patients, epididymal aspirate alone in two and urethra alone in three. In two patients (8%), Ureaplasma urealyticum was detected at titres $>10^4$, in the urethra alone in one patient and urethra and epididymis in another. A further two patients were found to have Ureaplasma urealyticum at a titre of 10^3 in the urethra alone and another two Mycoplasma hominis at a titre of 10^3, again in the urethra alone.

In five cases aerobic organisms were cultured from both the mid-stream urine and the epididymal aspirate: Escherichia coli in four patients and Streptococcus faecalis in one. Two patients displayed mixed infections of C. trachomatis and E. coli in one case and C. trachomatis and U. urealyticum in the other. Neisseria gonorrhoeae and anaerobes were not cultured from any patient.

The culture results of each patient are detailed in Table 22.1 and summarised in Table 22.2.

TABLE 22.1 ORGANISMS ISOLATED FROM URETHRAL SWABS, MID-STREAM URINE AND EPIDIDYMAL ASPIRATES

Study No.	Age	Urethral Swab	MSU	Epididymal Aspirate
1	34	C.trachomatis	Neg	C.trachomatis
2	66	Neg	E.coli	E.coli
3	34	U.urealyticum 10^3 M.hominis 10^3	Neg	C.trachomatis
4	23	U.urealyticum > 10^4	Neg	U.urealyticum >>10^4
5	21	Neg	Neg	Neg
6	27	C.trachomatis U.urealyticum >>10^4	Neg	C.trachomatis
7	20	Neg	Neg	Neg
8	22	C.trachomatis M.hominis 10^3	Neg	C.trachomatis
9	22	Neg	Neg	C.trachomatis
10	27	Neg	Neg	Neg
11	24	Neg	S.faecalis	S.faecalis
12	32	C.trachomatis	Neg	Neg
13	26	Neg	Neg	Neg

Study No.	Age	Urethral Swab	MSU	Epididymal Aspirate
14	23	Neg	Neg	Neg
15	21	C.trachomatis	Neg	C.trachomatis
16	34	C.trachomatis	E.coli	E.coli C.trachomatis
17	23	Neg	Neg	Neg
18	50	Neg	E.coli	E.coli
19	19	Neg	Neg	Neg
20	72	Neg	E.coli	E.coli
21	23	Neg	Neg	Neg
22	20	C.trachomatis U urealyticum 10^3	Neg	Neg
23	21	Neg	Neg	Neg
24	22	C.trachomatis	Neg	Neg

TABLE 22.2 SITE OF DETECTION OF ORGANISMS IN ACUTE EPIDIDYMITIS

Organism	Site of Detection		
	Urethra	Urine	Epididymis
Chlamydia trachomatis	8	-	7
Escherichia coli	-	4	4
Ureaplasma urealyticum	2	-	1
Streptococcus faecalis	-	1	1

The mean age of C. trachomatis positive patients was 26.8 years (range 20-34), those in whom cultures were negative, 22.6 years (range 19-27), and the E. coli positive patients 55.5 years (range 34-72). The difference in these mean ages between the two former groups and the lattermost one achieved statistical significance (Student's t test, $p < 0.001$).

The chlamydial serology data, Table 22.3, revealed a strong correlation between the IgG titres achieved in the C. trachomatis positive group compared to the other patients, with a demarcation level at a titre of 1:64, Table 22.4. In two patients with a titre of 1:64, this represented a four-fold rise in antibody, whereas the other patient with this titre failed to display any rise. Similarly, the two

patients with titres of 1:128 and five with a titre of 1:256, at presentation, failed to demonstrate any rise in titre in the convalescent serum samples.

TABLE 22.3 ANTI-CHLAMYDIAL IgG AND IgM TITRES (RECIPROCAL OF) IN PATIENTS WITH ACUTE EPIDIDYMITIS

Study No.	IgG Titres		IgM Titres	
	Acute	Convalescent	Acute	Convalescent
1	≥ 256	≥ 256	16	16
2	16	16	8	8
3	16	64	4	4
4	0	0	0	0
5	4	4	0	0
6	≥ 256	≥ 256	32	32
7	2	2	2	2
8	128	128	32	32
9	16	64	8	8
10	16	16	0	0
11	4	4	0	0
12	128	128	64	32
13	32	32	0	0
14	8	8	0	0
15	≥ 256	≥ 256	64	64
16	64	64	0	0
17	4	4	0	0
18	4	4	0	0
19	16	16	0	0
20	8	8	0	0
21	0	0	0	0
22	≥ 256	≥ 256	64	64
23	4	4	0	0
24	≥ 256	≥ 256	64	64

22.4 RELATION BETWEEN DETECTION OF CHLAMYDIAE AND CHLAMYDIAL IgG ANTIBODY

	No. of patients exhibiting an antibody titre (reciprocal of)					
Detection of chlamydiae	< 8	16	32	64	128	256
Positive	-	-	-	3	2	5
Negative	10	3	1	-	-	-

The values represent the highest titre detected.

Although, the data was less definitive in the case of IgM titres, Table 22.5, seven of the ten patients who were C. trachomatis positive, had titres of > 1:16, whereas none of the C. trachomatis negative group had titres of this magnitude, and twelve out of fourteen had absent titres. The patient with no detectable IgM antibody, but in whom chlamydiae were detected in the urethra and epididymal aspirate, presented at two weeks after the onset of symptoms. In all cases, bar one, there was no change in IgM titre between acute and convalescent samples; the exception recorded a two-fold decrease in titre from 1:64 to 1:32.

TABLE 22.5 RELATION BETWEEN DETECTION OF CHLAMYDIAE AND CHLAMYDIAL IgM ANTIBODY

	No. of Patients exhibiting an antibody titre (reciprocal of)						
Detection of chlamydiae	0	2	4	8	16	32	64
Positive	1	-	1	1	1	2	4
Negative	12	1	-	1	-	-	-

Twenty-two (92%) patients had abnormal prostatic ultrasound scans, Table 22.6. The most common features were mid-range echoes, Fig. 22.1, present in 18 (75%) of cases and located on the same side of the prostate as the affected epididymis in twelve (50%) and present bilaterally in six (25%). High density echoes, Fig. 22.2, were noted in 15 (62.5%) and when present in the region of the ejaculatory ducts, Fig. 22.3, termed ejaculatory duct calcification, occurred in fourteen (58%). Hypoechoic areas, termed echolucent zones, were present in ten (42%) of cases. The relationship between these ultrasonographic signs and the detection of organisms, Table 22.7, displayed a trend towards the presence of abnormal ultrasound features and positive culture, yet failed to achieve significance (chi-square, $0.5 > p > 0.1$).

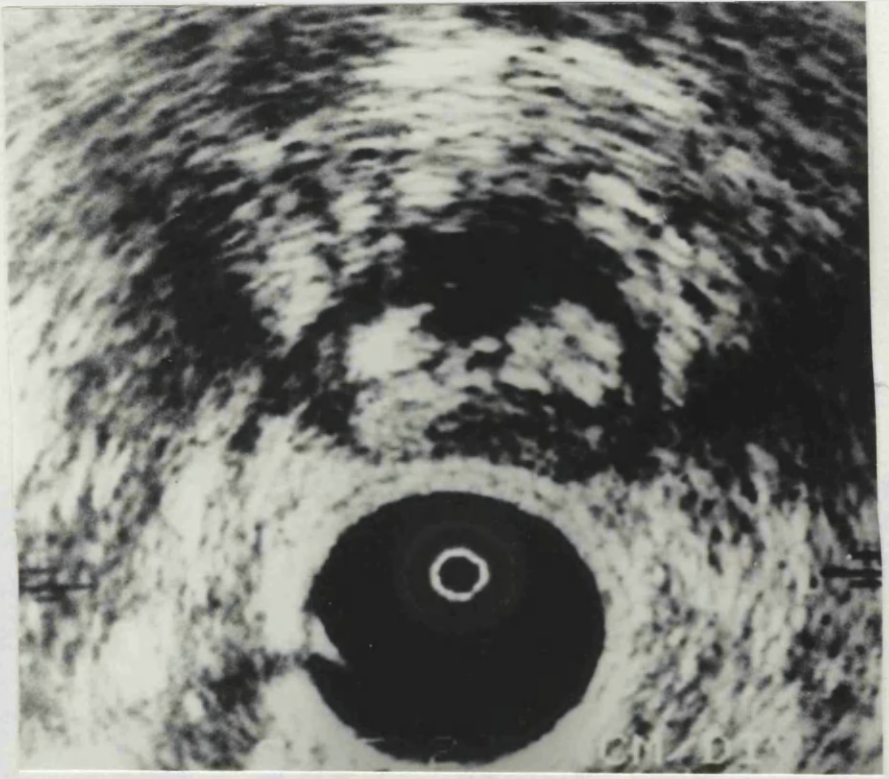


Fig. 22.1 Mid-range echoes (MRE) in the transrectal ultrasonograph of a patient with acute epididymitis

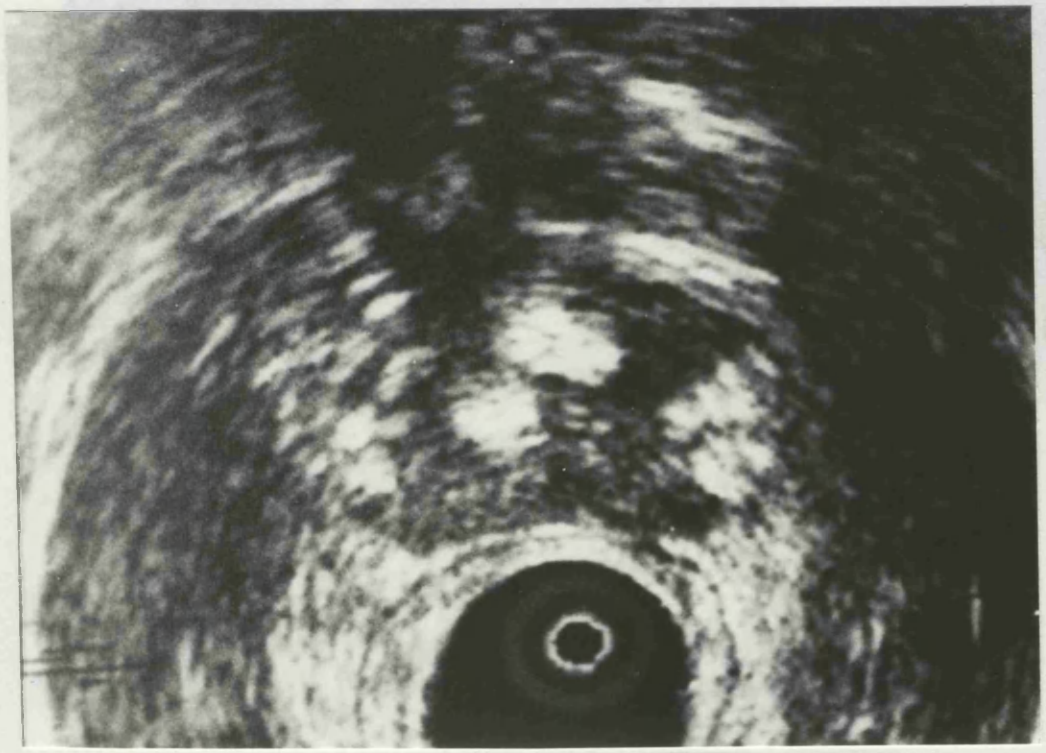


Fig. 22.2 A patient with acute epididymitis displaying high density echoes (HDE) in a transrectal prostatic ultrasonograph

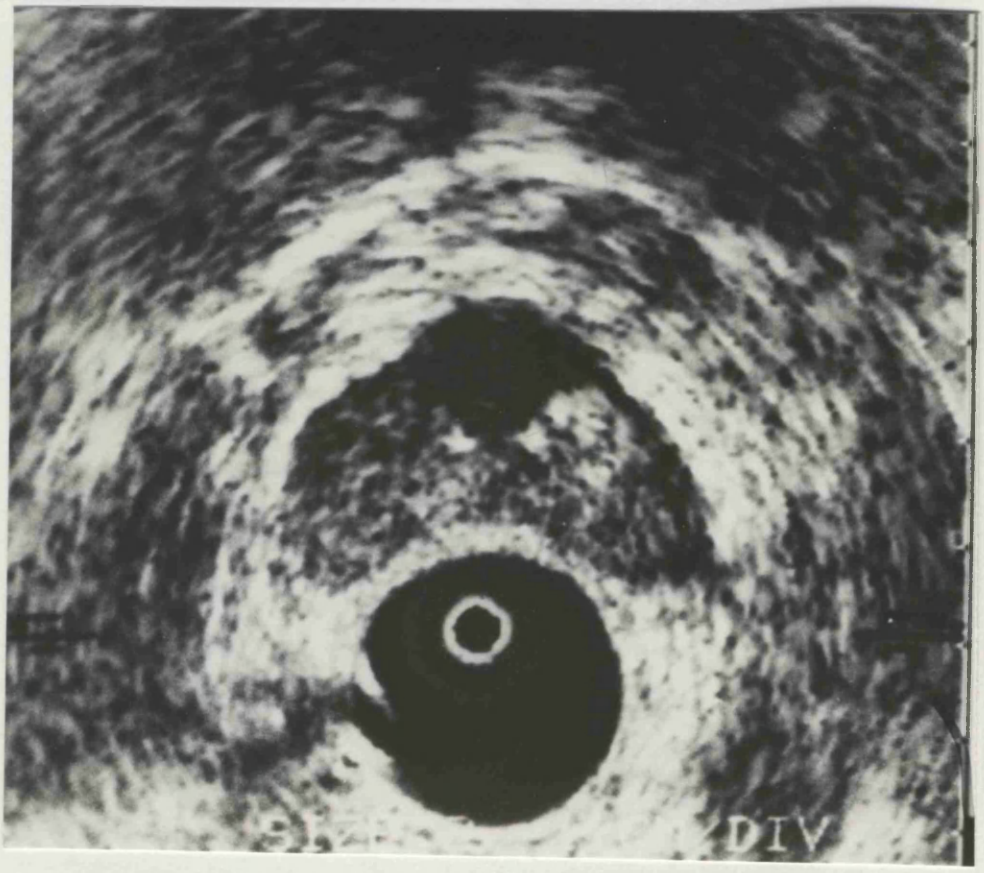


Fig. 22.3 Ejaculatory duct calcification (EDC) in a prostatic ultrasonograph of a patient with acute epididymitis

TABLE 22.6 PROSTATIC ULTRASOUND SIGNS IN PATIENTS WITH ACUTE EPIDIDYMITIS

Study	Ultrasound Signs						Organisms
No.	HDE	MRE	ELZ	CI	CT	EDC	Detected
1	-	2	1	L	-	L	+
2	3	3	-	-	B	B	+
3	1	1	1	B	-	R	+
4	-	3	2	R	-	-	+
5	2	-	-	-	-	-	-
6	2	-	1	R	B	R	+
7	-	1	-	-	-	-	-
8	1	1	-	-	B	B	+
9	1	2	1	L	-	L	+
10	-	-	-	-	-	-	-
11	2	3	1	R	-	B	+
12	1	2	1	-	B	B	+
13	-	1	-	-	-	-	-
14	-	1	-	-	-	L	-
15	2	2	-	L	-	-	+
16	1	2	-	-	B	L	+
17	1	-	-	-	-	-	-
18	1	3	-	L	-	L	+
19	-	-	-	-	R	R	-
20	1	2	1	R	B	R	+
21	-	-	-	-	-	-	-
22	2	1	1	-	B	L	+
23	-	2	-	L	-	-	-
24	3	2	2	R	-	-	+

KEY:

HDE	High density echoes
MRE	Mid-range echoes
ELZ	Echolucent zones
CI	Capsular irregularity
CT	Capsular thickening
EDC	Ejaculatory duct calcification
1-3	Extent of abnormality
L	Left
R	Right
B	Bilateral

TABLE 22.7 PROSTATIC ULTRASOUND FINDINGS IN RELATION TO THE DETECTION OF MICRO-ORGANISMS

Micro-organism status	No. (%) of patients with ultrasound signs			
	Mid-range echoes	High density echoes	Ejaculatory duct echoes	Echolucent zones
Positive	14 (58)	13 (54)	12 (50)	10 (42)
Negative	4 (17)	2 (8)	2 (8)	0

SUMMARY

In a prospective study of 24 men (mean age 28.3 years) with acute epididymitis who underwent epididymal aspiration, micro-organisms were detected in 15 (62.5%). Chlamydia trachomatis account for 10 (42%) of the cases, being located in both the urethra and epididymis in 5 individuals. Chlamydial serology supported the diagnosis of chlamydial infection, there being a strong correlation between the detection of C. trachomatis and elevated titres of both chlamydial IgG and IgM antibodies. In 4 patients (mean age 55.5 years), Escherichia coli was cultured from both mid-stream urine and epididymal aspirate. Transrectal ultrasound revealed abnormal prostatic scans in 22 patients (92%). These data confirm the aetiological role of C. trachomatis, support the notion that micro-organisms spread intra-canalicularly and suggest that the prostate is also involved in the inflammatory process in acute epididymitis.

SECTION 23 THE ATTEMPTED CONSTRUCTION OF AN ANIMAL MODEL OF CHLAMYDIAL PROSTATITIS USING THE MASTOMYS NATALENSIS RAT

To date chlamydial prostatitis has only been developed in the experimental animal by direct innoculation of the organism into the gland (Nielsen et al, 1982); a non-physiological situation in the extreme. However, an intra-canalicular route of infection by Klebsiella pneumoniae and subsequent prostatitis has been demonstrated in the female Mastomys natalensis rat (Weidner et al, 1981).

A study was thus devised using the Mastomys natalensis rat, whereby an attempt was made to induce a chlamydial prostatitis by intra-canalicular spread of organisms following innoculation of the lower urinary tract.

Materials and Methods

The basic plan of the experiment was to innoculate both male and female rats with NI1 p9 (Chlamydia trachomatis) either trans urethrally or intravesically, and then attempt to isolate these organisms from the bladder, by washouts, and the prostate, by excision of one lobe and subsequent culture and direct immunofluorescence techniques, at set time intervals following innoculation. The source of the organisms was a contact of a patient with non-gonococcal urethritis. The organism was purified, mycoplamsa species free, had undergone nine passages and was typed as a mixture of serovars E and F of C. trachomatis.

A systemic antibody response was also sought by analysing serum drawn from venous blood. The animals were sacrificed between twenty and twenty eight days post innoculation and the whole of the urogenital tract cultured and examined histologically.

Experiment 1

All Mastomys natalensis rats (60g), two female and two male, were innoculated with 0.1 ml of organism suspension (2x 10^7 inclusion forming units per ml) of NI1 p9. The innoculation was performed under intraperitoneal induced anaesthesia with 0.1 ml/30g body weight in the case of female rats and 0.25ml/30g body weight in the case of male rats, of the CRC anaesthetic cocktail (see Appendix XXXVI). The male rats were innoculated trans- urethrally with a 24G needle and the females intravesically by exposing the bladder and introducing the organisms directly into the bladder lumen with a 22G needle.

One female rat died under the anaesthetic for innoculation. The remaining three rats completed the study. On day 8, the three animals were anaesthetised as above and in each case bladder washouts performed with 0.1 ml of sugar phosphate (2SP) solution. Also on day 8, from male rat number 1, the lateral lobes of the prostate were exposed and one lobe excised.

On day 14, each animal underwent further bladder washouts as described above, and male rat number 2 along with the surviving female rat, underwent excision of one lobe of prostate. A venous blood sample was obtained from the tail vein of each animal for detection of anti-chlamydial antibodies.

The female rat and number 1 male rat were sacrificed on day 21 and the urogenital tract dissected out and samples obtained from the prostate, seminal vesicles, kidney and epididymis (in the case of the male rat). A further venous blood sample was obtained for chlamydial serology.

Finally, the number 2 male rat was sacrificed on day 28, and samples obtained as for the other two rats at day 21.

Experiment 2

Five Mastomys natalensis rats (60g), two males (Nos. 3 and 4) and three females (Nos 2, 3 and 4) were studied. As in experiment 1, the rats were innoculated transurethrally in the case of the male rats and intravesically in the female rats, under general anaesthesia induced by 0.1 ml/30g body weight in the case of the female rats and 0.25 ml/30g body weight in the male rats, of the CRC anaesthetic cocktail (see Appendix XXXV).

The rats were innoculated with 0.1 ml of NI1 p9 at a concentration of 6×10^6 inclusion forming units per ml.

Each subsequent procedure involving collection of samples was carried out under the same conditions of general anaesthesia.

On day 6 post innoculation, bladder washout and excision of one lateral lobe and prostate from number 3 male and number 2 female rat were performed. The excised tissue was subdivided for histology and chlamydial studies. Female rats numbers, 3 and 4, underwent excision of one lateral lobe of prostate on day 13 and again the tissue obtained was subdivided for histology and chlamydial studies. Male rat number 4 died on day 12, the prostate was excised and this tissue was also submitted for histology and chlamydial studies.

On day 20, male rat number 3 and female rats numbers 2 and 3 were sacrificed. A bladder washout was performed, venous blood drawn for chlamydial serology and the urogenital tract excised. After taking samples of prostate, bladder, epididymis, seminal vesicle, ovary and fallopian tubes for histology, the remaining tissue from these organs was homogenised in a ground glass grinder and submitted for chlamydial studies. In the case of number 3 male rat, all prostatic and urethral tissue was examined histologically.

Finally, female rat number 4 was sacrificed on day 28, and again, a bladder washout, collection of venous blood for chlamydial serology and excision of the urogenital tract was performed. The latter tissue was processed as for the other rats sacrificed at 20 days.

All material obtained for chlamydial studies in experiments 1 and 2 was first used to make an impression smear on a MicroTrak (Syva) slide and then submitted to a direct immunofluorescence technique, see Appendix , and then placed in 1 ml of sucrose phosphate (2SP) medium without antibiotics. This was then stored in liquid nitrogen prior to homogenization in a ground glass grinder and chlamydial culture on McCoy cell monolayers, see Appendix XI.

Tissue for histological analysis was placed into 10% formal saline prior to processing in routine fashion.

Venous blood was centrifuged and the serum stored at -40 C prior to estimation of anti-chlamydial IgG antibodies to serovars E and F. Positive and negative controls were run in parallel to the test sera. The technique adopted was the indirect micro-immunofluorescence technique described in Appendix XV.

Results

Experiment 1

Female rat number 1 failed to recover following the initial anaesthesia for innoculation of the animal.

In none of the bladder washouts were chlamydiae detected by direct immunofluorescence (MicroTrak, Syva) or isolated by culture techniques. Furthermore, none of the tissue homogenates of prostate or other urogenital organs yielded chlamydiae, either by direct immunofluorescence (MicroTrak, Syva) or culture techniques. There was no histological evidence of inflammation in any of the tissue excised from the animals studied. Finally, no anti-chlamydial antibodies were detected within the serum of any of the animals studied.

Experiment 2

As in Experiment 1, one of the animals studied died before the time of intended sacrifice, on this occasion unrelated to anaesthesia on day 12.

None of the bladder washouts yielded chlamydiae by either direct immunoflourescence or culture techniques. Neither were chlamydiae detected in any of the tissue homogenates of prostate or other organs excised during study. There was no histological evidence of inflammation in any of the tissues examined. In addition, none of the rats displayed an anti-chlamydial antibody response to innoculation by NI1 p9 during the period of study.

SUMMARY

In this pilot study, employing the Mastomys natalensis rat, it was not possible to infect the prostate or remainder of the urogenital tract, by innoculation of chlamydiae (NI1 p9) into the urethra of male, and bladder of female rats.

DISCUSSION

In the preceding sections, the clinical and experimental studies addressed specific questions and by necessity the aetiology, diagnosis and pathology of chronic abacterial prostatitis was investigated by means of a series of apparently unrelated studies. It is the aim of this section to analyse each study separately, before incorporating the conclusions into a global viewpoint of chronic abacterial prostatitis.

Section 24 TRANSRECTAL PROSTATIC ULTRASOUND FEATURES OF PROSTATITIS AND THEIR RELATION TO HISTOLOGY

The ultrasound features of the normal gland appear well defined as outlined in section 3. Indeed the circumferentially continuous capsule, so readily visualised, (Peeling et al, 1979; Brooman et al , 1981; Spirnak & Resnick, 1984), was present in this control group, with capsular abnormalities noted in only 3% of cases. However, the homogeneous echopattern (Brooman et al, 1981; Peeling & Griffiths, 1984; Spirnak & Resnick, 1984) was not the experience in this study, with high density echoes (HDE) present in 54% and mid-range echoes (MRE) in 37%. Thus, the presence of these parenchymal abnormalities does not necessarily imply a disease process within the prostate and these have to be taken into account when making a diagnosis of prostatitis, if an overwhelming number of false positive diagnoses are not to be encountered. As no details are given as to the source of the "normal controls" in the other studies cited, it is impossible to make direct comparisons or attempt to explain the differences between the findings in this thesis and those of other published reports. Save to say, however, the control group in this study were vetted for clinical, cytological and microbiological evidence of prostatitis, and all proved negative.

The second study attempted to correlate the presence of transrectal prostatic ultrasound abnormalities with the

degree of inflammation within the prostate, as judged by prostatic localisation studies (Meares & Stamey, 1968). This served to make a number of points: firstly, a number of ultrasound signs, namely high density (HDE) and mid-range echoes (MRE), echolucent zones (ELZ), capsular irregularity (CI) and thickening (CT), ejaculatory duct calcification (EDC) and periurethral zone irregularity (PZI), were compatible with a diagnosis of chronic prostatitis; secondly, in patients with prostatodynia, although some of the abnormalities were encountered, these signs tended to be less numerous and less extensive; thirdly, that age and symptom duration had little bearing on the prevalence of ultra sound abnormalities. Once identified, each ultrasound abnormality was then analysed in a larger study group to assess the sensitivity and specificity of each sign with respect to a diagnosis of prostatitis.

In the third study, an additional ultrasound sign, the periurethral halo was assessed as a diagnostic sign of chronic prostatitis. All seven signs previously listed, but not the halo sign, were found to be significantly more prevalent in patients with chronic prostatitis than those with prostatodynia. Furthermore, all signs, with the exception of the halo sign, were significantly more prevalent in all patients with a clinical diagnosis of chronic prostatitis and thus, some patients with prostatodynia, than in normal controls. This raises the possibility that some patients diagnosed as having

prostatodynia may in fact have chronic prostatitis, and the localisation studies have wrongly underestimated the degree of inflammatory disease within their prostates. Alternatively, factors that lead to the development of the ultrasound changes within the prostate may be active in both the prostatodynia and chronic prostatitis groups, which lie along a spectrum of disease, with the chronic prostatitis group displaying a more marked reaction. A further possibility is that the two sets of patients represent different responses to the same stimulus.

So many of the early reports of the ultrasound features of chronic prostatitis (Watanabe et al, 1974; Watanabe et al, 1975; Harada et al, 1979; Peeling et al, 1979; Brooman et al, 1981) made no reference to the criteria for diagnosis and thus it is impossible to compare the data in this thesis with their findings, as the groups are likely to be markedly heterogeneous and the ensuing conclusions misleading.

A further difficulty encountered in the comparison of studies is the lack of standardisation of the terminology adopted to describe the ultrasound signs. This rarely causes problems in the case of increased sound transmission, ie the hypoechoic lesion, but the more subtle changes, such as the mid-range echoes and capsular changes, may be referred to using alternative terminology. The difference in resolution of the equipment used in

various studies may result in some lesions being overlooked, again accounting for conflicting data.

Two studies (Griffiths et al, 1984; Wiegand & Weidner, 1986) to date have investigated groups of patients with an accurate diagnosis of chronic prostatitis based on prostatic localisation studies. Both these studies viewed the "halo sign" in the periurethral region as important; it was noted in 100% of cases of prostatitis in the former study and in 54.9% of prostatitis patients, but only 24.5% of prostatodynia cases, ($p < 0.05$), in the latter publication. In this thesis, no statistical difference was found in the prevalence of this sign, which ranged from 4-14% between normal controls, patients with prostatodynia and those with chronic prostatitis, and it was thus concluded, that the sign is probably a normal variant and certainly non-specific; a view supported by Harada and co-workers (1980).

Parenchymal changes, referred to as solitary calculi (type A) and diffuse calculi (type B), observed by Wiegand and Weidner (1986) to be more prevalent in patients with chronic prostatitis than in those with prostatodynia, $p < 0.05$ and $p < 0.001$, respectively, may be equivalent to the abnormalities termed high density echoes (HDE) in this work. Similarly, the heterogeneous echopatterns (Wiegand & Weidner, 1986) and multiple low amplitude regions in the peripheral zone of the prostate (Griffiths et al, 1984) may be equivalent to the mid-range echoes (MRE) and

echolucent zones (ELZ) respectively, encountered in this thesis. Furthermore, capsular changes were noted in between 20% and 80% of cases of chronic prostatitis by Griffiths and co-workers (1984) and in 43-63% in the present study. Thus, there do appear to be similarities between this work and the two previous "controlled" studies (Griffiths et al, 1984; Wiegand & Weidner, 1986), with regard to the ultrasonographic features of chronic prostatitis and prostatodynia. These other two studies were performed with transducers of 3.5 MHz, compared to the 5.5 MHz transducer used in this research, which is likely to account for some differences. However, these disparities apart, the consensus view supports the presence of identifiable lesions on prostatic ultrasound which enable normal individuals, patients with prostatodynia and those with chronic prostatitis to be differentiated.

No previous study to date, has attempted to calculate the sensitivity and specificity of the ultrasound signs with respect to a diagnosis of prostatitis. In order to assess these parameters, the patients were subdivided into six groups on the basis of the leucocyte count in the post-massage urine, VB3, and designated a score of 0-5, the Stamey score. This allowed simplification of analysis, albeit with a risk of artificial clustering. Simple calculation of the prevalance of ultrasound signs produced a separation of the two groups at a diagnostic level of Stamey score $\geqslant$ 2, i.e., a VB3 leucocyte count $\geqslant$

50 per mm^3. The calculated specificity and sensitivity of each ultrasound sign related to Stamey score (Table 12.11, Section 12) reveals that in order to achieve an acceptable sensitivity for high density (HDE) and mid-range echoes (MRE), a degree of specificity has to sacrificed, with the resultant risk of false positives. In the case of the signs of echolucent zone (ELZ), capsular irregularity (CI), capsular thickening (CT) and periurethral zone irregularity (PZI), the converse is true, namely in order to achieve acceptable specificity , sensitivity has to be sacrificed to some degree, with the resultant risk of false negatives. Ejaculatory duct calcification is a poor predictive sign for a diagnosis of prostatitis and the halo sign, not surprisingly, non-predictive.

In assigning a level of prostatic inflammation, as judged by localisation studies, and thus Stamey score, to a diagnosis of prostatitis in terms of ultrasound signs, a "best fit" compromise has to be reached of all the sensitivities and specificities for each sign. Taking all signs into account, with the exception of the "halo sign", the most acceptable combination of sensitivity and specificity is associated with the Stamey score of $\geqslant 2$, and thus a VB3 leucocyte count of $\geqslant 50$ per mm^3.

The debate concerning the number of leucocytes within the expressed prostatic secretion (EPS) and post-prostatic massage urine (VB3), over and above that in VB1 and VB2,

that is a diagnostic of prostatitis, has raged long and hard, yet a count of $\geqslant$ 10 leucocytes per high powered field (hpf) (x1000) in the EPS is now generally accepted as the upper limit of normal (Anderson & Weller, 1979). Furthermore, the studies of Weidner & Ebner (1985), comparing EPS and VB3 leucocyte counts, concluded that a VB3 leucocyte count of $\geqslant$ 4 per high power field (hpf) (x400), was highly suggestive of prostatitis and was associated with a leucocyte count of $\geqslant$ 10 (per hpf x 1000) in 85% of cases in their study. A VB3 leucocyte count of $\geqslant$ 10 per hpf (x400) was associated with an EPS leucocyte count of $\geqslant$ 10 per hpf (x1000) in 95% of cases. A leucocyte count of $\geqslant$ 4 per hpf (x400) is equivalent to 40 leucocytes per mm^3 and thus there is good correlation between the criteria for a diagnosis of chronic prostatitis on the basis of ultrasound signs and the leucocyte counts of post prostatic massage urine (VB3).

With the possible exception of echolucent zones (ELZ), no single ultrasound sign was suggestive of a diagnosis of chronic prostatitis. A combination of the following signs: high density (HDE) and mid-range echoes (MRE), echolucent zones (ELZ), capsular irregularity (CI) and thickening (CT) plus periurethral zone irregularity (PZI), is indicative of a diagnosis of prostatitis and the confidence of diagnosis increases with an increasing number of signs. However, the size of the sample was too small in this study, for multivariate analysis to provide any statistically significant information on particular combinations being more diagnostic than others.

The "halo sign" has been discounted as a relevant diagnostic sign. However, it is interesting to note that ejaculatory duct calcification (EDC) is unusual in normal subjects, but has a greater prevalence in prostatodynia (31%) and chronic prostatitis (54-57%), though it is statistically more prevalent in the latter group of patients, $p < 0.001$. This again raises the possibility of similar aetiological mechanisms in the two groups. Urodynamic abnormalities indicative of a failure to relax the distal sphincter complex have been noted in chronic prostatitis (Buck, 1975; Siroky et al, 1981) and prostatodynia (Osborn et al, 1981; Barbalius et al, 1983; Meares, 1986; Hellstrom et al, 1987) with consequent intraprostatic reflux of urine (Buck, 1975; Hellstrom et al, 1987). It is possible that repeated intraprostatic reflux and thus probably ejaculatory duct reflux, of urine, may result in the calcific deposits which represent the ultrasound feature of ejaculatory duct calcification (EDC). However, this proposal remains purely hypothetical at present.

It is well established that the pathological changes in prostatitis are both focal and reside predominantly in the peripheral zone (McNeal, 1968). This study adds weight to such a viewpoint in that the majority of parenchymal abnormalities (HDE, MRE and ELZ) were located in the peripheral and transition zones, although the high density echoes (HDE) were evenly distributed throughout the prostate. The ultrasound guided biopsy data confirmed

that inflammatory changes resided in these areas of abnormal echogenicity within the parenchyma, by virtue of 85% of biopsies revealing chronic inflammatory features on histological analysis.

The observation that no ultrasound sign was associated with prolonged symptom duration raises a number of points. If the aetiological processes behind the inflammatory changes were progressive, a change in ultrasound signs, if these indeed do reflect the underlying pathology, would be expected. The cause of the symptomatology is unknown and may reflect subtle changes at a cellular level, which extend far beyond the crude changes that the resolution of ultrasound can be expected to detect. Therefore, the symptoms may persist or change irrespective of the gross pathological features and thus, ultrasound appearances. Additionally, symptom duration, may be a rather crude yardstick of pathology; the underlying disease processes may have been present for some time without causing symptoms. In contrast, the follow up ultrasound data, Section 12.IV noted a very high incidence of ultrasound changes, 87% in the chronic prostatitis group and 73% in the borderline group, over a short mean follow up period of 10.4-11.9 months. Although, the gland in individual patients is ultrasonically in a state of flux, when each ultrasound sign is considered seperately, these changes "cancel out" to a degree, such that no one sign is associated with disease longevity. The observation that no patients with prostatodynia showed any changes in

parenchymal signs, suggests that the diagnosis is correct in these patients, and during the period of study at least, no inflammatory changes have developed within the gland parenchyma. Furthermore, the possibility that some patients with prostatodynia have been misrepresented by prostatic localisation studies, appears not to have been born out in those patients over this short period of follow up.

Although three ultrasound signs, echolucent zones (ELZ) ejaculatory duct calcification (EDC) and periurethral zone irregularity (PZI), appeared more prevalent with age, the increased prevalence of chronic prostatitis with age in this study group, probably accounts for these age related ultrasound changes. Alternatively, these sonographic changes may simply reflect other age related pathology, such as benign nodular hyperplasia and cyst formation, rather than inflammatory changes.

To date no study has examined the histology of patients with chronic abacterial prostatitis by ultrasound guided needle biopsy or attempted to correlate the ultrasound abnormalities with histology by means of this technique. The aim of this part of the study was simply to discover what pathological changes resided within each abnormality, such that a considered evaluation could be made from the ultrasonograph alone. Firstly, it was apparent that inflammatory lesions were present within the parenchymal abnormalities detected, as 85% of lesions contained a

chronic inflammatory infiltrate. Secondly, a close correlation existed between high density echoes (HDE) and corpora amylaceae, mid-range echoes (MRE) and fibrosis or inflammation, and echolucent zones (ELZ) and inflammation. Although the correlation was not absolute, a valid judgement can be made on the likely pathology that HDE and ELZ , at least, represent , when viewing an ultrasound in isolation. The follow up data adds weight to the validity of these pathological findings in that inflammatory lesions would be expected to heal by fibrosis. Areas of inflammation represented initially by echolucent zones (ELZ) on ultrasound, reverted to mid-range echoes (MRE) in 17% of chronic prostatitis and 27% of borderline prostatitis patients. This change in ultrasound image may reflect healing by fibrosis, or alternatively, persistence of inflammation, but with sufficient alteration in architecture to bring about the change in the ultrasonograph.

The ultrasound image is a compilation of reflected sound wave energy following it's impingement on a tissue interface. Thus , in order for an ultrasonograph to change, there must be modification of the tissue arcitecture. However, a change in the direction of the incidental sound energy and position of the probe in relation to the lesion, may also result in altered images. In this work the ultrasound studies were all performed by one operator, and as with any real time study, the final interpretation is a composite " still" of a number of

dynamic images; it would be expected that these sources of error should cancel out or be constant in any one examination.

The value of prostatic biopsy as routine practice in chronic prostatitis is questionable, but it does provide a means of defining a lesion. If doubt exists as to the precise nature of an ultrasound abnormality , the use of ultrasound guided biopsy enables accurate placement and thus characterisation of the lesion.

The lack of a clear pattern in the follow up ultrasound findings, Section 12. IV, draws into question it's role in the management of patients with chronic prostatitis. The only ultrasound feature which mirrored the findings of prostatic localisation studies, was the echolocent zone (ELZ), and thus it would be worth pursuing the natural history of this lesion, ideally including repeated biopsy, in future research into chronic prostatitis.

The only other consistent finding of value in the follow up work, was the lack of change in ultrasound features on serial scanning of patients with prostatodynia, and thus repeated prostatic ultrasound, if consistently normal, increases confidence in the diagnosis.

SUMMARY

Transrectal prostatic ultrasound provides objective data on the topography of the prostate, whilst being a painless , minimally invasive investigation. The diagnosis of prostatitis is hampered by varied symptomatology and lack of physical signs, and rests on the finding of excessive numbers of leucocytes in either the expressed prostatic secretion (EPS) or post-massage urine (VB3). Seven ultrasound signs possessing a significant correlation with a diagnosis of chronic prostatitis have been identified: high density (HDE) and mid-range echoes (MRE), echolucent zones (ELZ), capsular irregularity (CI) and thickening (CT) , ejaculatory duct calcification (EDC) and periurethral zone irregularity (PZI). The low specificity of HDE and MRE (40.7% & 57.9% respectively) and low sensitivity (range 30.8% to 62.3%) of the remaining five ultrasound features of chronic prostatitis, preclude any one feature as being diagnostic of this condition. A possible exception may be the finding of ELZ, which may also prove useful in the monitoring of response to treatment. The detection of several of these ultrasound signs within a patient's prostate suggests a diagnosis of chronic prostatitis, whereas a normal scan in a patient with symptoms of prostatitis is highly suggestive of prostatodynia.

Prostatic ultrasonography confirms the focal nature and frequent peripheral zone location of the chronic

prostatitis lesion. Follow up ultrasound studies over a short period indicated that the ELZ may resolve to normal, MRE or HDE in conjunction with a reduction in leucocyte count in the post-massage urine (VB3).

The ability to guide a biopsy needle accurately under ultrasound control has enabled histological analysis of the ultrasound features. The HDE represent corpora amylaceae, the MRE inflammation, fibrosis or both, and the ELZ, inflammation.

SECTION 25A A SEARCH FOR AETIOLOGICAL AGENTS IN CHRONIC ABACTERIAL PROSTATITIS

In any study attempting to define the role of infectious agents in chronic prostatitis, stringent criteria for diagnosis must be adopted if spurious data are not to be accumulated. At present the mandatory method of diagnosis involves the Stamey localisation procedure (Meares & Stamey, 1968), see Section 11, with leucocyte and colony counts being performed on all specimens obtained, such that urethritis, urinary tract infection and prostatitis can be differentiated. Furthermore, an increased colony count in either the expressed prostatic secretion (EPS) and or post-massage urine (VB3), by a factor of x 10, implies a prostatic location for the organims concerned; it seems reasonable to suppose that the organism when present, is responsible for an inflammatory reaction. Additional support for an infectious role can arise from serological data, especially if a rise in titre can be demonstrated. Treatment studies can also provide corrobarative evidence.

Thus, a cohort of patients with chronic abacterial prostatitis, see Section 13, was contructed using the standard localisation techniques, see Section 11, and the currently accepted classification of inflammatory prostatic disease (Drach et al, 1978). In this way the data obtained could be attributed to a group of patients

whose diagnosis of chronic abacterial prostatitis was genuine. Thereby, any conclusions would assume validity and enable comparison with other groups diagnosed similarly.

The problem with many studies of chronic prostatitis is that these standard diagnostic criteria are not adhered to, such that patients are either diagnosed by examination of expressed prostatic secretion (EPS) in isolation, with the inescapable result of misdiagnosis in a proportion of cases due to urethral contamination of the EPS, or worse still, purely on clinical grounds. Chronic prostatitis and prostatodynia have indistinguishable symptoms of pain, irritative voiding and voiding dysfunction, an with the absence of physical signs, the need for localisation studies (Meares & Stamey, 1968) and accurate classification (Drach et al, 1978), is obligatory.

Despite strict attention to detail in prostatic localisation studies, interpretation may be difficult. The prostatic secretion, a viscid fluid has to traverse the length of the urethra, and due to it's slow passage is likely to recruit contaminating organisms during it's journey, though "washing out" the urethra by sterile urine is likely to reduce the number of possible contaminants. However, if an organism is present in both the urethra and the prostate, having migrated from the former to the latter, prostatic location is difficult to confirm in view of it's urethral presence and the likelihood of

contamination. Furthermore, in the presence of an active urinary tract infection, all the urine specimens VB1, VB2, and VB3 are likely to contain similar number of organisms, and thus the study has to be repeated following treatment with an appropriate antimicrobial such as nitrofurantoin of penicillin, that has little penetration into the prostate (Meares & Stamey 1968).

In order to exclude the possibility of urethral contamination, a direct approach to the prostate should provide uncomtaminated material for analysis. However, the focal nature of prostatitis (Young et al, 1906; McNeal, 1968), ensures that "blind" prostatic biopsy studies are unlikely to yield meaningful data (Schmidt & Patterson, 1966; Nielsen & Justesen, 1974). Recently, with the advent of transrectal prostatic ultrasound, it has becomes possible to place accurately a biopsy needle, under ultrasound guidance, into the prostate (Holm & Gamelgaard, 1981). Therefore, provided ultrasound abnormalities can be identified (see Sections 12 and 24), they should be amenable to biopsy.

Thus, the framework of this part of the discussion is the attempted isolation of organisms directly from prostatic tissue, obtained by means of ultrasound guided biopsy of areas of abnormal ultrasound echogenicity, from patients with an accurate diagnois of chronic abacterial prostatitis.

In this study, two methods of detection of Chlamydia trachomatis were employed: culture in McCoy cell monolayers and a direct immunofluorescence technique using a mixture of monoclonal antibodies directed against the major outer membrane protein of the organism (MicroTrak, Syva). The use of cycloheximide McCoy cell monolayers is well established in our laboratories (Thomas et al, 1977; Evans & Woodland, 1983). However, chlamydiae are labile organisms, obligate intracellular bacteria and correct transport and storage of specimens is essential for maximal isolation of organisms. Also, many factors influence the sensitivity of the cell culture method, in particular, the correct identification of stained inclusions, which requires an experienced observer, and culture medium constituents.

Thus the ability to detect chlamydial elementary bodies, the infectious particle in the life cycle, within smears of exudates or host tissue by immunofluorescence, renders the associated problems of chlamydial isolation less relevant. Although, skill is required to interpret the slides in the direct immunofluorescence technique, the possitively stained elementary bodies, approximately 300 nm in diameter (Ward, 1983), can be distinguished from other organisms, which may bind the monoclonal antibodies (Krech et al, 1985), by their smaller size and more even staining characteristics. The positive control performed with each test also aids identification. In a study comparing the direct immunofluorescence technique and standard culture method (Thomas et al, 1984), in men with

gonnorrhoeal and non-gonorrhoeal urethritis, there was a 91% and 94% agreement respectively, between the two tests. Thus, by using both techniques in this thesis, any agreement between the tests provided greater validity to any result.

In this thesis, amongst the chronic abacterial prostatitis cohort, Chlamydia trachomatis was detected in the urethra of only two patients, one of whom also had evidence of the organism within the EPS, using the direct immunofluorescence technique. Thus, in view of the strong likelihood of urethral contamination of the prostatic fluid by urethral organisms in that one case, no patient was proven to have C. trachomatis localised to the prostate. Furthermore, none of the prostatic biopsies displayed evidence of C. trachomatis presence either by isolation in McCoy cell monolayers or by the direct immunofluorescence technique.

The prostate tissue and it's secretion are rich in enzymes, in particular glucosidases (Bostrom & Ockerman, 1971), lysozyme (Hirschauser & Kionke, 1971; Mardh & Colleen, 1974) and other proteolytic enzymes (Huggins & Neal, 1942; Rosenkrantz & Kirdani, 1961) which contribute to the constituents of seminal plasma. These enzymes are thought to impart an inhibitory effect on the formation of chlamydial inclusions in McCoy cell monolayers (Mardh et al, 1980). Homogenisation of prostatic tissue and

subsequent cell damage would be expected to cause release of these enzymes into the homogenate. Indeed earlier work (Thomas, personal communication) had noted the "toxic" effect of EPS and prostatic tissue on McCoy cell monolayers, resulting in stripping of the cell layers. This observation prompted the dilution of homogenised prostatic tissue used in this work (by a factor of X 8) prior to innoculation of the cell monolayers. As a result the monolayer remained intact , but it could be argued that this dilution may have reduced the likelihood of isolation of chlamydiae if only small numbers were present in the original tissue. However, the negative cell culture data were supported by negative findings on direct immunofluorescence.

A method of increasing the sensitivity of C. trachomatis isolation, namely blind passage (Schacter, 1985) has been shown to be more effective in increasing recovery rates in asymptomatic individuals, rather than symptomatic patients (Schacter & Martin, 1987). Although these authors only noted such benefit after one blind passage (Schacter & Martin, 1987), others (Jones et al, 1986) using multiple blind passages , observed a 77% increase in C. trachomatis isolation in a group of men with urethritis. The problems of such a technique are delay in diagnosis (up to 2 weeks)and it's labour intensiveness. Adoption of the technique in this work, may have yielded C. trachomatis from prostatic tissue or fluid , though again, the direct immunofluorescence results do not support this notion.

Our inability to detect C. trachomatis in chronic abacterial prostatitis is at variance with four recent studies (Bruce et al, 1981; Weidner et al, 1983, Poletti et al, 1985; Bruce & Reid, 1989). If each of these studies are analysed in turn the reasons for this disparity become manifest. Although the patients in this thesis were of similar age to those in the study of Bruce and collegues (1981), little else is comparable, and the greatest failing of the latter study is the diagnosis of patients on clinical grounds alone. Furthermore, C. trachomatis was apparently detected in 56% of cases, from early morning urine in 50% and prostatic fluid or semen in the remaining 6%. Urine samples, to date, have been regarded as poor sources of chlamydiae (Evans & Woodland, 1983), even after centrifugation (Smith & Weed,1976), chiefly due to the intracellular location of the organisms, though recently using DNA polymerase chain reaction (PCR) techniques, which increase the sensitivity of DNA detection, chlamydial DNA has been extracted from the urine of patients with non-gonococcal urethritis (Taylor-Robinson, personal communication). Bruce and co-workers (1981), attempted to add credibility to their findings by the isolation of organisms from expressed prostatic secretion (EPS), semen or post-prostatic massage urine in 36% of 39 patients studied, 71% of whom had positive cultures in urine and prostatic fluid, yet the lack of adequate patient selection, the use of inappropriate sampling techniques and the absence of corroborative serological data are serious deficiencies

in their study and overshadow their conclusions. In the work of Weidner and colleagues (1983), a large group of patients with chronic prostatitis, along with an aged matched control group, were studied and an apparent difference in the isolation rate of C. trachomatis between the groups was observed, namely 18.5% versus 7.7% on examination of EPS. Patients and controls underwent localisation studies, yet if the patients who fell short of a diagnosis of chronic prostatitis using currently accepted criteria (Anderson & Weller, 1979; Weidner & Ebner, 1985) are excluded, only 8.6% of patients with chronic prostatitis had C. trachomatis isolated. No data were available regarding the presence C. trachomatis within the urethra of these patients, and thus the risk of urethral contamination was unknown. The presence of C. trachomatis was supported by serological data with 87% of these patients having an antichlamydial IgG titre of one $1:\geq 8$, though serial estimations were not performed. Although, localisation studies were undertaken , patients who only had a clinical diagnosis of prostatitis were included in the figures,thereby distorting the data. These patients may indeed have had prostatitis, the EPS leucocyte count misrepresenting their pathology, yet if comparisons between studies are to be made, some form of standardisation has to be reached. The study which implicates C. trachomatis most strongly, (Poletti et al, 1985) had major design faults which may account for the findings. The study group was procured on the basis of symptoms alone and all patients had C. trachomatis

isolated from their urethra, which raises considerable doubt as to the accuracy of the diagnosis of chronic prostatitis. Furthermore, prostatic sampling consisted of blind transrectal aspiration biopsy. It is possible that C. trachomatis may have been sampled from the rectum (Munday et al, 1981) or conceivably from the urethra, or alternatively, false positive results may have arisen due to non-specific staining of other organisms and cells (Krech et al, 1985). Also, it was fortunate indeed to obtain such a high recovery rate of organisms (33%) by blind biopsy when the focal nature of prostatitis is considered (McNeal, 1968) and the total volume of gland affected by inflammatory changes may be less than 10% (Kohnen & Drach, 1979). Although interpretation of serological information would have been difficult in view of the urethal presence of organisms, it may have provided supportive data for an infective role by the organisms recovered.

The later study of Bruce and Reid (1989), was much more tightly controlled than their earlier work, with all patients undergoing urethral sampling, localisation studies and detection of C. trachomatis by both McCoy cell culture and direct immounfluorescence staining of smears. Six patients, whose mean age was very similar to the cohort studied in this thesis, were identified as having chronic abacterial prostatitis, as judged by standard criteria; all had negative urethral smears yet positive detection of C. trachomatis in the EPS by both techniques.

It would appear they have a water-tight case for C. trachomatis as an aetiological agent in chronic abacterial prostatitis. However, four of the group had sexual partners in whom the organism was isolated from the cervix, yet none of the six males had C. trachomatis detected in the anterior urethra, somewhat surprisingly. It could be conceivable that the patients had a posterior urethritis, which prevented C. trachomatis detection from urethral smears which sample only the first 3-5 cm of the urethra, yet resulted in the organism being present in the EPS. Urethral contamination of prostatic secretions is an ubiquitous possibility in prostatic localisation studies, and cannot be discounted unless, as in this thesis, the urethra is by-passed by a direct sampling technique. A further suspicion concering the work of Bruce & Reid (1989) is the universal cure rate after antibiotics; this is much more in keeping with the natural history of urethritis, rather than chronic prostatitis.

The findings in this thesis are much more atuned to those of Mardh and collegues (1978) and Berger's group (1989). Both these studies were unable to isolate C. trachomatis from groups of patients with chronic prostatitis, diagnosed on clinical grounds alone in the former study and on clinical grounds and localisation techniques, in the latter. The former group also backed up their conclusions with serological data. As in many cases, the patients studied had received antibiotics but not for at least six weeks in these two studies and two months in

the cohort analysed in this thesis. The demographic details of all the patients in these comparative studies discussed above were broadly similar, especially with respect to age, symptom duration and treatment, and thus are unlikely to account for any differences.

Once it had been established that transrectal prostatic ultrasound was able to identify areas of abnormality within the glands of patients with chronic abacterial prostatitis (see Sections 12 and 24), it became evident that ultrasound guided biopsy of these areas would not only circumnavigate the problem of urethral contamination, but may provide information on possible aetiological organisms. Routine histological analysis revealed a chronic inflammatory infiltrate in 85% of the chronic abacterial prostatitis cohort, yet in a control group of similar age, none of the twenty biopsies showed evidence of inflammatory features. In contrast to the lesions in the patient cohort, all biopsies from the control group were taken from areas of gland with normal echogenicity on ultrasound. These data clearly vindicate the technique of ultrasound guided biopsy in chronic prostatitis and suggest that the technique has sufficient sensitivity and specificity to provide valid information. By means of two methods, namely culture in McCoy cell monolayers and direct immunofluorescence staining, no evidence of C. trachomatis organisms was found in the prostatic biopsy of patients with chronic abacterial prostatitis, despite adaptations to the techniques to avoid the toxic effect of

prostatic tissue and fluid on the cell cultures, vide supra.

The ability to detect chlamydial antigen in prostatic tissue using monoclonal antibodies (Shurbaji et al, 1988) has resurrected an aetiological role for C. trachomatis in chronic prostatitis. Although a control group was also studied and no evidence of C. trachomatis was detected in them, the finding of chlamydial antigen in 31% of sixteen patients with histological evidence of prostatitis is surprising. The tissue was analysed retrospectively by recalling samples with a histological diagnosis of prostatitis, from a group with a mean age of 61 years, obtained by transurethral resection, open prostatectomy or needle biopsy. Only five out of the group of sixteen presented with symptoms of prostatitis, yet none of those with positive staining for chlamydial antigen were amongst them, though one had a past history of acute prostatitis. Of the five positive cases, three had histological features of acute prostatitis, two in conjunction with chronic prostatitis, and two displayed chronic inflammation with granulomata. In addition, the positive staining occured in areas of inflammation, but usually not the site of the most florid inflammatory features. Although the positive staining in the prostatitis patients is unlikely to be non-specific, as the control arm displayed no staining, the group studied are not patients with chronic abacterial prostatitis in the clinical sense. Firstly, none were symptomatic, their

mean age was in excess of those in most other studies by 20 years, and there was no supportive cytogical and microbiological data. Histological prostatitis is not uncommon (McNeal, 1968), especially in resection specimens of patients with bladder outflow obstruction (Attah, 1975; Kohnen & Drach; 1979), but it is inconceivable to assume that this process has the same immunopathology as that observed in clinical prostatitis. Thus, the findings of Shurbarji and colleagues (1988) although interesting, in isolation, are meaningless, but do prompt the need for a more controlled study in patients with a proven diagnosis of chronic prostatitis.

Although caution must be excercised in the interpretation of serological data (Treharne et al, 1983), especially in isolation, as was so clearly shown by the two studies of Mardh and colleagues (1972; 1978), such data may provide supportive evidence for culture findings. The initial report on the role of C. trachomatis in chronic prostatitis (Mardh et al, 1972) used a rather crude complement fixation test and noticed a greater incidence of antibodies at a titre of 1: $\geq$ 5 in patients with chronic prostatitis (33%),compared to controls (3%). However, in a similar study, using a highly specific micro-immunofluorescence technique, only 12% of cases had evidence of recent or current infection by C. trachomatis (Mardh et al, 1978). Furthermore, in the latter study the organism could only be isolated from the urethra of 2% of cases, and not at all from the prostatic fluid. In this

thesis, the modified micro-immunofluorescence technique (Thomas et al, 1976) was employed, which though simplified is no less sensitive or specific. However, only 2 patients had an antichlamydial IgG titre of 1:32, and that in association with a low (1: $\leq$ 4) IgM titre, and only two displayed a four - fold IgG antibody rise between acute and convalescence samples, (1:4 to 1: 16 and 1: 8 to 1: 32). These results appear to provide strong support to the negative culture findings, in keeping with other studies that reached the same conclusion (Mardh et al, 1978; Shortliffe et al, 1985).

Our inability to detect antibodies to chlamydiae in the semen of the cohort with chronic abacterial prostatitis is at variance with previous studies (Terho & Meurman, 1981; Suominen et al, 1983), though different techniques were employed. The method of diagnosis of chronic prostatitis was suspect in these other studies, relying solely on the presence of leucocytes within the semen. Furthermore, they studied patients who presented with infertility and although their findings may reflect previous infection of the lower genital tract by C. trachomatis, to postulate that C. trachomatis is responsible for chronic prostatitis from these data is misguided. Our failure to detect chlamydial antibodies in the semen may have been technical, although it was simply an adaptation of an accepted technique (Thomas et al, 1976), or sampling error as only one semen sample was examined. However, as a

screening test for chlamydial infection, past or present, we found semen antibody analysis unrewarding.

Defining a role for Mycoplasma hominis and Ureaplasma urealyticum in chronic prostatitis is plagued by the same problems as C. trachomatis. These organisms not only cause urethritis (Shepard, 1970; Taylor - Robinson & McCormack, 1980) but are also found in asymptomatic individuals (Furr & Taylor-Robinson, 1987), especially in blacks (Lee et al, 1976) and those with prior sexual exposure (McCormack et al, 1973). Quantification of organism numbers may help to localise them to a particular site (Shepard, 1974; Weidner et al, 1980). With the combination of the Stamey localisation procedure and quantification of colony counts, values of greater than 10^4 colony forming units (CFU) /ml in the EPS and greater than 10^3 CFU/ml in the post-massage urine and semen were regarded as significant; a possible aetiological role for Ureaplasma urealyticum was proposed in 19.3% of 187 patients with a diagnosis of chronic prostatitis (Weidner et al, 1980). However, after response to treatment with tetracyclines was taken into account, only 8.6% were considered as having U. urealyticum as the sole aetiological agent. However, in the control group, the organism was detected in 22.2% of cases, although only first void (VB1) urine samples were analysed in these subjects. A later and larger study, using the same method, by Brunner and colleagues (1983), implicated U. urealyticum in the aetiology of prostatitis in 13.7% of

patients and although, the organism was also isolated in 16.7% of controls, colony counts were too low to suggest a prostatic location. Eighty-seven per cent of patients responded to tetracycline therapy , supporting the proposal that the organisms were actually responsible for symptoms. However, the localisation studies were not substantiated either by estimation of the leucocyte count within the samples or serology, and thus attributing a definite infective role to the organisms is unjustified. In these two studies (Weidner et al, 1980; Brunner et al, 1983), Mycoplasma hominis was deemed not to be causative in chronic prostatitis, in contrast to the findings of others (Mardh & Colleen, 1975), who detected the organism in 10% of patients , but not in controls; the presence of organisms was qualitative and not localised to the prostate in this latter work, and the authors were guarded, justifiably, in their conclusions . The latter study also found no difference in the detection of U. urealyticum between patients and controls, which has been supported in later work (Berger et al, 1989).

In this thesis, all patients were accurately diagnosed as having chronic prostatitis , and in 6 (10%) U. urealyticum and in 4(7%) M. hominis were isolated. However, in only three of the patients with U. urealyticum were titres greater than 10^3 colour changing units (CCU) /ml achieved and in only one was the organism localised to the prostate; appropriate antibiotic therapy failed to alter symptoms. None of the patients in whom M. hominis was

isolated had titres greater that 10^2 CCU/ml, and in only one was the organism localised to the prostate and again, treatment failed to alter symptoms. These organisms were isolated at the time of inclusion into the cohort, i.e. at the time of diagnosis of chronic prostatitis , but in subsequent localisation studies, the recovery rates did not alter. Although the results were expressed as colour-changing units (CCU)/ml, all positive colour changes were verified by sub-culture on agar, though, colony counts were not performed on the subcultures, which contrasts with other studies in which quantification of cultures has been performed (Weidner et al, 1980; Brunner et al, 1983). The isolation methods adopted in this study are well established (Taylor-Robinson & Furr, 1981) and it is unlikely that this difference in technique accounts for the contrasting findings.

The biopsy data in this thesis fails to support a role for U. urealyticum and M. hominis in chronic prostatitis, which is contrary to the findings of Hofstetter (1977) who also adopted the transperineal route to sample the prostate, but did not have the benefit of prostatic ultrasound to guide the biopsy needle. The inability to detect these organisms in this thesis may have been technical, but there was no delay in sample handling and care was taken to tease the prostatic tissue in order to release organisms, rather than cause major disruption by grinding, in view of the problems encountered with isolation of mycoplasmas from other tissues (Kaklamanis

et al, 1969; Taylor-Robinson et al, 1972; Mardh & Taylor-Robinson, 1973). Although the lysolecithin responsible for mycoplasmacidal activity may have been released during processing of the biopsy it's effects would likely be counteracted by the dilution of material employed in isolation techniques. A search for antibodies against U. urealyticum and M. hominis was not made in the cohort, but Shortliffe and colleagues (1985) substantiated their negative culture results with similar serological data in a search for U. urealyticum in chronic abacterial prostatitis.

The cohort with chronic abacterial prostatitis provided a wealth of data on the organisms isolated by serial prostatic localisation studies (Appendix VII). Throughout the period of study no gram positive, gram negative , aerobic or anaerobic organisms were unequivocally isolated from the prostate, adopting the criteria of Meares and Stamey (1968).

However, the heavy colonisation of the anterior urethra by a large variety of commensals is readily apparent and highlights the risk of analysis of the expressed prostatic secretion in isolation, with the inevitable resultant, urethral contamination. Some authors (Drach 1975; Greenberg et al, 1985; Drach, 1986) regard the gram-positive organisms as pathogenic in prostatitis, but the data from this thesis fails to substantiate this theory in the case of patients diagnosed as having chronic

abacterial prostatitis. The possibility that this group of patients have an occult infection by gram-negative organisms, has also been refuted by Shortliffe and Wehner (1986), by measuring the levels of antigen specific immunoglobulins to the Enterobacteriaceae.

The biopsy material obtained by ultrasound guided sampling and subjected to microbiological analysis, detected aerobic organisms in seven patients and anaerobes in three. It was felt that these organisms were contaminants from the perineal skin, as in all cases treatment with an appropriate antimicrobial failed to alter symptoms or the inflammatory response within the prostate as judged by the EPS or VB3 leucocyte count. Of the aerobic organisms, skin commensals were detected in three cases: Staphylococcus epidermidis in two and diphtheroids in one, whilst in the remainder, Escherichia coli was isolated in three cases and Pasteurella in one. The latter organism is unusual and no satisfactory explanation can be forwarded to explain it's presence, yet in the case of the coliforms, these would be expected to contaminate the perineal skin. A failing of our technique was the lack of information on perineal skin commensals, and culture swabs of the skin before and after biopsy, as well as the needle track, may have supported the contamination hypothesis. Nielsen and Justesen (1974) routinely swabbed the biopsy puncture sites in their study, and detected organisms from over 66% of cases. In contrast, organisms were isolated from only 2 (11%) of 19 biopsies , yet in each case the

biopsy site organisms coincided with those cultured from the prostate tissue, thereby validating there contamination theory. In this previous study , anaerobes were not isolated from either the prostate or biopsy incision, in contrast to the three cases in this work.

As the findings in this thesis suggest, anaerobes are not implicated as occult infecting organisms in chronic abacterial prostatitis, in support of previous studies (Nielsen & Justesen, 1974, Mardh & Colleen, 1975), though Peptostreptococci have been isolated from semen (Mardh & Colleen, 1975) and VB3 (Meijer - Severs et al, 1981).

Finally, the paucity of positive bacteriological data in chronic abacterial prostatitis has led to a search for viral agents. This quest has proved as unrewarding as those for bacteria, with most studies failing to isolate viruses from semen (Mardh & Colleen, 1975), urine or prostatic secretions (Gordon et al, 1972; Nielsen & Vestergaard, 1973) and prostatic tissue (Nielsen & Vestergaard, 1973). No attempt was made to search for viruses in this thesis as it's specific aim was to define a role for chlamydiae, ureaplasmas and mycoplasmas in chronic abacterial prostatitis. It was felt that distribution of the clinical material in too many directions may have reduced the likelihood of obtaining representative and accurate data.

In any study where the results are consistently negative, the methodology has to be scrutinised to ensure that design error is not producing false negative results. In the case of chlamydiae, mycoplasmas and ureaplasmas, this source of possible error has been addressed above, and in essence, discounted. However, as eluded to earlier, the distribution of biopsy samples in particular, to a number of different specimen containers may have caused delay, with consequent loss of organisms; this is most likely to have affected anaerobic culture. The specimens were transported to the laboratory within one hour of sampling in the case of material for aerobic and anaerobic analysis, but those for chlamydial, mycoplasmal and ureaplasmal study were stored at the appropriate temperature within minutes of collection, and thus, organism numbers were unlikely to have suffered unduely. The culture techniques used for aerobic and anaerobic organisms were performed with standard, established methods which again would be unlikely to create a large source of error.

Although a control group was investigated in a number of sections in this thesis, prostatic biopsy samples were not submitted for bacteriological study. This is a valid source of criticism, but it was felt that the control group should be submitted to the minimum amount of biopsy and all the tissue obtained was submitted for histological and immunological analysis. Furthermore, as all controls had no evidence of inflammation within their prostates, it

was thought that a significant yield of organisms was unlikely. The presence of organisms within an asymptomatic population, with no evidence of prostatic inflammation, would however, have been an interesting conundrum.

SUMMARY

A cohort of patients classified as having chronic abacterial prostatitis, as judged by standard criteria, underwent transperineal ultrasound guided biopsy of abnormal areas detected by transrectal prostatic ultrasound. Despite histological analysis revealing a chronic inflammatory infiltrate in 51 patients (85%), organisms were only detected in 15% of cases. In particular, Chlamydia trachomatis, Mycoplasma hominis and Ureaplasma urealyticum could not be isolated from the prostate. Furthermore, in those patients in whom organisms were recovered, no change in symptoms or the number of inflammatory cells localised to the prostate , was observed following treatment with an appropriate antimicrobial agent. It was concluded that the organisms recovered were therefore contaminants from the perineal skin. In the case of C. trachomatis, the negative culture and direct immunofluorescence data were supported by low serum titres of anti-chlamydial antibody on serial sampling.

Although, viruses were not sought in the prostatic tissue, the inability to recover organisms from the prostate in patients with chronic abacterial prostatitis, despite sophisticated biopsy techniques, raises the possibility of this inflammatory process being non-organismal.

The weight of opinion from other studies in which similar strict diagnostic criteria have been employed, support this final conclusion. However, the literature is filled with reports where substandard methods of diagnosis have been adopted, such that the resultant spurious data serves only to cloud the aetiological picture further.

SECTION 25B A SEARCH FOR CHLAMYDIA TRACHOMATIS IN THE PROSTATE OF PATIENTS WITH ACUTE NON-GONOCOCCAL URETHRITIS

The role played by Chlamydia trachomatis in acute non-gonococcal urethritis is not in doubt (Schachter, 1978; Taylor-Robinson & Thomas, 1980). The part played by this organism in chronic prostatitis is however uncertain, with some groups supporting an active role (Bruce et al, 1981; Weidner et al, 1983; Poletti et al, 1985; Bruce & Reid, 1989) and other refuting such a claim (Mardh et al, 1978; Berger et al, 1989). The data from this thesis (Section 14) firmly supports the latter viewpoint.

If C. trachomatis has no active role in chronic abacterial prostatitis, is there a possibility, that at some time it was involved in the process, perhaps by initiating an inflammatory response? The low antichlamydial IgG titres in the serum of the chronic abacterial prostatitis cohort (see Section 14 & Appendix XVI) would suggest even this is not the case, unless, the initial insult occured some considerable time earlier, as titres often remain elevated in uncomplicated lower genital tract infections (Treharne, 1988).

C. trachomatis has been isolated from the prostatic fluid of patients with a diagnosis of acute non-gonococcal urethritis (Nilsson et al, 1981), and all positive cases

had excess numbers of leucocytes within the expressed prostatic secretion (EPS). After one week only 4% of patients with positive EPS cultures had cleared their organism spontaneously compared to 27% of the group with negative EPS cultures. None of these patients had symptoms of prostatitis and the failure to perform localisation studies precluded any analysis of the likelihood of urethral contamination, which probably occurred at least to some degree. However, this study does raise the possibility that C. trachomatis organisms may gain entry to the prostate at the time of an acute urethritis attributed to this organism. Indeed, such a canalicular route has been proven in acute epididymitis (Berger et al, 1979; 1980) and corroborated by the findings in sections 22 of this thesis. The information from Poletti's group (1985), in which all patients studied had C. trachomatis within their urethra, and thirty three percent of whom also had the organism detected within the prostate by aspiration biopsy, may support the notion of C. trachomatis entering the prostate at the time of an acute urethritis. Although, the latter study had a number of shortcomings (vide supra,) not least the claim that the patients had chronic prostatitis despite a hundred percent recovery of C. trachomatis from the urethra, it may in fact be describing inadvertently the findings of Nilsson and colleagues (1981). However the ultrasound guided aspiration of cells from the periurethral zone of the prostate in this thesis failed to detect C. trachomatis. The sampling was accurate and the technique for detection

well established, and although only a pilot group was studied, the early indications are that C. trachomatis does not enter the prostate at the time of an acute urethritis but a larger more comprehensive study is required.

SUMMARY

There is a paucity of information regarding the presence of Chlamydia trachomatis within the prostate during acute urethritis caused by this organism. The likelihood of urethral contamination is high, so the direct sampling technique provided by ultrasound guided aspiration biopsy offers an important diagnostic tool. Using this method, C. trachomatis could not be detected in the prostates of a small group of patients with active acute urethritis. Furthermore, this negative data was supported by low serum titers of antibody against this organism. In addition, Mycoplasma hominis and Ureaplasma urealyticum could not be isolated from these patients. The numbers studied however, were very small, and a more extensive study would be required before firm conclusions could be drawn regarding the inability of C. trachomatis to gain access to the prostate during an acute urethritis.

SECTION 26 THE PATHOLOGY OF CHRONIC ABACTERIAL PROSTATITIS

The technique of ultrasound guided biopsy (Holm & Gammelgaard, 1981; Saitoh et al, 1981) has provided a means of sampling specific areas of the prostate hidden from the examining finger, which may have been unsuspected as sites of pathology by clinical criteria alone. It has become possible to analyse specific foci within the gland and thereby provided an important breakthrough in the study of chronic prostatitis, a focal condition (Young et al, 1906; McNeal, 1968) par excellence. As stated on several occasions throughout this thesis the employment of "blind" biopsy techniques (Schmidt & Patterson, 1966; Nielsen & Justesen, 1974; Drach, 1974; Vinje et al, 1983) has no role in the study of chronic prostatitis as the likelihood of obtaining representative material is small. Thus, the comparison of such studies with data obtained by ultrasound guided biopsy must be approached with caution. The same must be said of studies where tissue is obtained either by open or endoscopic surgery as the amount of tissue available from the peripheral zone is unpredictable and likely to be modest.

In this thesis, an attempt was made to categorise the patients by histological criteria, based on the number of chronic inflammatory cells per high power field (hpf) (x 400). The patients were graded 1 to 3, (vide supra) using arbitrarily designated levels of classification. The

validity of this subdivision is questionable when the prostatic fluid inflammatory cell findings and the histological data are compared. Although there was a trend towards the higher grades of inflammation being associated with high Stamey scores (indicative of high prostatic fluid leucocyte counts), the patients with grade 1 inflammation were almost evenly distributed through the Stamey score range, with only 43% of this group having a Stamey score of 2, i.e. a low score yet still diagnostic of prostatitis. The reason for this lack of correlation may rest with the prostatic fluid being the secretion from a number of different inflammatory foci and thereby, a collective sample, whereas the tissue obtained by biopsy represents a solitary focus. Alternatively, the biopsied area may be non-representative of the inflammatory state of the patient's prostate gland, however, in each case, samples were taken from all abnormal areas detected on ultrasound. The region of abnormal echogenicity on the sonograph may not have been included in the biopsy, but this is less likely under ultrasound guidance. Normal histology from areas of normal echogenicity in the control group, also adds validity to the accuracy of the needle biopsy. Less common was "understaging" by Stamey score i.e. a high grade of inflammation on biopsy in association with a low prostatic fluid leucocyte count and thus low Stamey score.

The lack of correlation between inflammatory grade and symptom duration, intraprostatic antibody deposition

(Section 17) and serum PSA values (Section 18) implies that the sub-division of cases on the basis of inflammatory cell numbers alone may be an oversimplification and bear no direct relationship with the underlying pathophysiology. However, this classification makes for easier analysis of the data and in the section on immunopathology (Section 17, vide supra) was shown to correlate with the underlying immunological mechanisms and thereby enabled a hypothesis to be constructed as to possible aetiological sequences.

A better monitor of the natural history of chronic prostatitis may be the repeated analysis of tissue from discrete inflammatory foci by means of serial ultrasound guided biopsy. In the same way the effect of various therapeutic agents could be analysed. The estimation of leucocytes within the expressed prostatic secretion provides a "overall view" of the inflammatory status of the gland, but lacks detail on the situation at individual sites within the gland. A theoretical drawback of repeated biopsy of an area inflammatory change is that the trauma induced by the biopsy may in itself create an inflammatory reaction and thereby distort the underlying process.

The majority of biopsies from the cohort with chronic abacterial prostatitis were of low grade which raises the possibility of a proportion of these cases representing a "burnt out" state. In support of this concept is the lack

of fibrosis in any of the grade 3 lesions whereas 16 of the 18 biopsies in which fibrosis was noted displayed grade I inflammation. However, the lack of correlation between symptom duration and inflammatory grade fails to support this hypothesis, yet symptom duration may not be analagous with duration of inflammation. Indeed, the precise cause of the patient's symptoms is conjectural, though maybe produced by mediators released in the inflammatory process. The lack of correlation between symptoms and any histological feature adds weight to the complexity and lack of specificy of symptoms in chronic prostatitis.

Corpora amylaceae are undoubtedly a normal finding in prostate histology, yet they are believed to form a nidus for prostatic calculi (Vilches et al, 1982), which in turn may initiate a vicious circle of glandular distortion with resultant ductal obstruction, inflammation and fibrosis and further calculous formation (Klimas et al, 1985). Thus, in inflammatory prostatic disease, due to the likely distortion of gland architecture by inflammation and fibrosis, an increase in the presence of corpora amylaceae and prostatic calculi might be expected. Although , in this study, 33% of the chronic prostatitis cohort compared to 15% of the control group , possessed corpora amylaceae in their biopsies, this difference did not achieve statistical significance. Corpora amylaceae numbers increase with age and with the development of benign prostatic hypreplasia (Sondergaard et al, 1987),

and although 20% (4 patients) of controls displayed benign hyperplasia, this feature was seen in only 2% (1 patient) of the cohort , although a further 3% (2 patients) displayed glandular proliferation; furthermore the cohort and control groups were broadly age matched. The failure for the difference in prevelance of corpora amylaceae to achieve statistical significance may lie in the small numbers in each group. Alternatively, a true difference may not exist.

In a small proportion (18%) of the chronic abacterial prostatitis cohort, vessel changes in the form of hyaline thickening and telangiectasia, were observed. Such features were not seen in the control biopsies and thus reflect alterations within a pathological gland. The presence of telangiectasia has been noted in bladder biopsies from patients with interstitial cystitis (Mattila, 1982) and the painful-bladder syndrome (Witherow et al, 1989) and deposition of immune complexes producing a vascular injury and subsequent inflammation has been proposed as an underlying mechanism. A similar pathway may be present in chronic abacterial prostatitis and indeed these vessel changes did correlate with intraprostatic antibody and complement deposition (vide infra).

A large study of unselected prostates, harvested at autopsy by Moore (1937), found a 6.3% prevalence of inflammation, being highest in the 5th, 6th, 8th and 9th

decades. In the group of patients with ascending transluminal infection of the urinary tract, the inflammatory cells were most commonly located in the periductal and periacinar regions. A more recent survey of only twenty six patients (Bourne & Frishette, 1967), aged 62-74 years, who underwent expresssed prostatic secretion (EPS) analysis prior to TURP, found a focal chronic inflammatory infiltrate in 50% of cases, but no correlation with the EPS leucocyte count; a similar lack of correlation was concluded in this thesis - although the two groups were not comparable with respect to age, criteria for diagnosis and tissue sampling methods. The periductal and periglandular distribution of inflammatory cells was observed by Attah (1975), in a study of unselected retropubic prostatectomy specimens. In 36% of cases areas of inflammation were detected in the absence of infection, which were irregularly distributed and most commonly comprised a periductal and periacinar chronic inflammatory infiltrate with focal epithelial disruption. In some areas corpora amylaceae were felt to have eroded the epithelium and the resultant escape of prostatic fluid caused the subsequent inflammation. However, corpora amylaceae were also found without a concomitant inflammatory infiltrate and thus this mechanism was by no means universal throughout the gland. In this thesis, no correlation existed between the presence of corpora amylaceae and grade of inflammation, and it was felt the former were part of the normal prostate and unlikely to be anything more than a side product of the consequences of

an inflammatory process such as ductal distortion and obstruction.

The most comprehensive study of the pathology of the inflamed prostate (Kohnen & Drach, 1979) involved the analysis of 162 prostatectomy specimens. Thirty eight per cent of this group were diagnosed as having prostatitis by accepted criteria (Meares & Stamey 1968). Ninety eight per cent of cases demonstrated inflammatory features, which were sub-classified into six types: periglandular, segregated glandular, diffuse stromal, isolated stromal nodule, acute necrotising and localised granulomatous. The most prevalent inflammatory patterns were periglandular (88.8%), segregated glandular (85%) and diffuse stromal (77.5%). However, the periglandular and segregated glandular pattern was seen in combination in 80% and the segregated glandular and diffuse stromal was also associated commonly. Thus, these three types might in fact represent facets of the same inflammatory lesion. Indeed the only difference between segregated glandular and periglandular patterns was the presence of chronic inflammatory cells within the gland and ductal lumina in the former. The most common pattern of inflammatory infiltrate in this thesis was a periglandular (57%) and stromal (45%) distribution, often in combination, in agreement with the findings of Kohnen and Drach (1979), whilst only 12% possessed an intraglandular infiltrate. Eight per cent of chronic abacterial prostatitis cohort also possessed acute inflammatory cells and one case (2%),

granulomatous features, in their prostatic biopsies. Such features were noted in 3.7% and 3.5% of cases respectively, in the work of Kohnen and Drach (1979). The latter authors postulated ductal rupture as the possible initiator of the inflammatory reaction, but on routine histological analysis alone this must remain conjectual as the function of the chronic inflammatory cells is not known. Only by more accurate definition of this cell population by the use of monoclonal antibodies can a more precise hypothesis concerning pathophysiology be prosposed (vide infra). The volume of the specimen involved in the various inflammatory processes was estimated by Kohnen & Drach (1979), and interestingly, occupied only very small volumes: segregated glandular (4.7%) of gland volume, periglandular (3.8%), diffuse stromal (2.5%), isolated stromal module (1%). These low figures would explain the low yield of useful information from blind biopsy and further confirms the focal nature of these inflammatory lesions.

The inflammatory foci in patients with chronic bacterial prostatitis were more extensive, though no more prevalent, than those with chronic abacterial prostatitis, and the total volume of inflammation greater in patients with chronic bacterial prostatitis due to gram negative organisms and enterococci, compared to those with gram positive organisms (Kohnen & Drach, 1979). The major drawback of any attempt to compare studies is the disparity of patient populations. The work of Kohnen &

Drach (1979) is clearly detailed, but all patients had benign prostatic hyperplasia, the tissue was obtained by either open or endoscopic surgery and no information regarding the patients age was provided. In this thesis only one patient had evidence of benign prostatic hyperplasia in the biopsy tissue, although glandular proliferation was observed in 2 cases ; the biopsy material was harvested by ultrasound guided biopsy and the mean age of the study group was 39.8 years (19-75 years), the majority of whom would be unlikely to require surgery to relieve bladder outlet obstruction. Although similarities in the patterns of inflammation exist between this work and that of Kohnen and Drach (1979), this may only reflect a similar "end point" reached by differing pathways. In addition, more detailed comparisons are invalidated by the marked dissimilarity of clinical presentation in the two groups.

The studies cited above all analysed surgical specimens or autopsy material. The information available from tissue obtained by needle biopsy is much more restricted and prevents examination of the gland as a whole; yet this form of sampling enables tissue to be obtained from areas that may not be reached by even the most "radical" operator. However, with blind biopsy, the zone of the gland sampled can only be estimated. A study of twenty patients with a diagnosis of chronic prostatitis, based on symptoms alone, (Alfthan et al 1962), noted chronic inflammatory changes in 6% and a less pronounced

infiltrate in 24% of seventeen patients from whom tissue was obtained. A later study (Schmidt & Patterson, 1966), of 26 patients, half of whom had prostatitis or borderline prostatitis from examination of expressed prostatic secretion (EPS) alone, noted a chronic inflammatory infiltrate in 60% of the twenty successful biopsies. In agreement with this thesis, a periglandular, followed by stromal distribution of cells was encountered most commonly, though in contrast , fibrosis was rare and the inflammatory lesions most commonly occupied a central zone site.

The investigation of over 250 patients with benign prostatic hyperplasia from whom 308 blind transperineal biopsies were performed , noted a chronic inflammatory infiltrate in 62% of cases with 38% being normal. The site most commonly involved was the stroma, the periglandular region less so, and acini not at all (Nielsen & Christensen, 1972). Again, these latter patients did not have chronic prostatitis, were of an older age group than the chronic abacterial prostatitis cohort and were studied by blind biopsy. The prevalence of inflammation was less than in the studies of Kohnen & Drach (1979) and stromal inflammation predominated. There was no correlation between urinary tract infection and the presence of inflammation, prompting the authors to conclude that not all urinary tract infections result in prostatitis. Similarly, the conclusion that not all inflammation represents clinical prostatitis, should be

proposed. This study (Nielsen & Christensen, 1972) arbritrarily graded the degree of inflammation as either moderate or severe, the former being most prevalent; no correlation was observed between the higher grade and any clinical or pathological features, in agreement with this thesis. A later study by the same author (Nielsen et al, 1973) again classified the severity of inflammation on an arbritrary scale of 1-3, and found the less severe grades most prevalent, as was the case in this thesis, but on this occasion found the periglandular inflammatory infiltrate most commonly and stromal inflammation to a variable degree, but, never in isolation. At variance with the chronic abacterial prostatitis cohort, fibrosis and vessel changes were absent. The study (Nielsen et al, 1973) analysed tissue obtained by blind biopsy from two groups, one with clinical chronic prostatitis and another with clinical urethritis. The prevalence of inflammatory lesions was 50% and 45% respectively, with the grade of inflammation being most commonly grade I, and no difference existing between the chronic prostatitis and urethritis groups. The authors postulated that the inflammatory changes may reflect a previous infection or an auto-immune process. As standardised localisation procedures were not performed, an overlap of the two groups might be expected clinically and furthermore, the finding of inflammation in only half the biopsies may have indicated that some of the cases did not have prostatitis, although the blind biopsy technique may have been contributory to this low yield. Perhaps a more curious

aspect of this work, was the apparent lack of seperation of patients with chronic prostatitis and urethritis: the study concentrated on microbiological data, but did not find the very similar histological data within the two sub groups to be either worthy of comment or in conflict with expectations. One can only assume that the authors (Nielsen et al, 1973) felt that chronic prostatitis and urethritis were facets of the same disease spectrum. This view is strongly opposed in this thesis, feeling they are seperate conditions; indeed every effort was made to exclude patients with chronic urethritis in the construction of the chronic abacterial prostatitis cohort, as the former would likely "contaminate" the data from the chronic prostatitis group under study.

To date no study has utilised ultrasound guided biopsy to investigate the pathology of chronic abacterial prostatitis, and thus, no truly comparative data are available. The presence of a chronic inflammatory infiltrate in 85% of cases in the chronic abacterial prostatitis cohort, yet lack of inflammatory features in an aged matched control group, adds validity to the accuracy of this biopsy technique and the significance of the inflammatory cells. On the basis of the studies cited above , it is slightly surprising that none of the controls contained significant numbers of inflammatory cells in their prostatic biopsies, yet the tissue was obtained from ultrasonographically normal parenchyma and the control group was younger than the majority of other

studies. It is possible that the relatively small volume of prostatic tissue obtained by needle biopsy misrepresents the inflammatory activity in the gland, yet the focal nature and small volume of most inflammatory lesions, should lend itself to needle biopsy and analysis, provided the assumption that inflammatory lesions can be accurately identified by prostatic ultrasound, is correct. The initial conclusions of this thesis are that this assumption is sound, yet the study of a larger cohort of patients plus a larger control group, subjected to serial biopsies would help clarify the situation.

MAST CELLS

The discovery of the mast cell by Ehrlich identified a carrier of mediators which interact in inflammatory reactions, most commonly in response to antigen combining with IgE bound to surface receptors on the mast cell (Lewis & Austen, 1981; Schleimer et al, 1984).

Mast cells have been noted previously in prostatic tissue (Gupta, 1970), where an increase in numbers was noted with increasing age, particularly in the stroma as opposed to the acini. Furthermore, cases with benign prostatic hyperplasia with or without evidence of inflammation had the highest mast cell counts. Although details were not provided, the stromal position of these mast cells would suggest a proportion occupied a perivascular site. No clinical information was forthcoming, so the proportion of

patients with chronic prostatitis as compared to those whose biopsies simply displayed inflammatory cells, was unknown. In the patients from the chronic abacterial prostatitis cohort, random sections of the biopsy which included both glandular and stromal tissue were analysed with no differentiation between these areas being made. Although the total mast cell count was significantly greater ($p < 0.001$) in the cohort, compared to an age matched group of controls, there was widespread individual variation and no correlation with the severity of inflammation or any particular histological feature. It has been proposed that the positioning of mast cells at portals of entry, along with their role in mediator release, implies a physiological role for these cells in the regulation of the microenvironment (Austen, 1978). The increased numbers seen in chronic prostatitis may be a direct response to a noxious substance, representing an immediate hypersensitivity reaction (type I) to a previously encountered antigen, or alternatively an increased surveillance role, or an indirect effect as the result of other inflammatory mediators. No information regarding mast cell degranulation in the prostatic biopsy material is known, and thus no firm conclusions can be drawn as to mast cell function in these cases. A study of patients with interstitial cystitis observed mast cell degranulation in the patient group, but not controls (Larsen et al, 1982), suggesting an active role. It was perhaps an overoptimistic hope that the patient's symptoms might correlate with mast cell numbers in the prostatic

biopsies, such that the released mediators could be directly implicated in symptomatology. The lack of such a correlation ensured this hope was in vain, yet the complexity of inflammatory mediators (Schleimer et al, 1984) and their interrelationships is such that this hypothesis cannot be totally dismissed in such a small study where only the crude total mast cell numbers were assessed. The precise location of the mast cells within the gland and there relationship to other inflammatory cells, requires further elucidation. Furthermore, quantification of the mediators within the tissues may clarify their role.

SUMMARY

In this thesis the routine histology of chronic abacterial prostatitis was investigated utilising the technique of ultra- sound guided biopsy. In the less severe grades of inflammation, the most common lesion, there was a lack of correlation between the grade of inflammation and numbers of leucocytes in the expressed prostatic secretion (EPS) or post-massage urine (VB3); histologically low grade lesions were associated with high numbers of leucocytes in localisation studies, much more commonly than the converse situation. This disparity may reflect non-representative biopsy sampling or alternatively the fact that prostatic fluid leucocyte estimation represents pooling of a number of seperate inflammatory lesions.

There was no correlation between histological grade and any particular symptom or prolonged symptom duration. As little information regarding function can be gleaned from routine histological analysis, it's value may rest in simple identification of those patients with inflammatory prostatic disease. The low grade lesion may represent "burnt out" disease, as fibrosis was more common than in the high grade lesions. Furthermore, vessel changes were only identified in patients with chronic prostatitis and may be of significance in pathophysiology.

SECTION 27 THE IMMUNO PATHOLOGY OF CHRONIC ABACTERIAL PROSTATITIS

I INTRA-PROSTATIC ANTIBODY DEPOSITION

II DETERMINATION OF THE PHENOTYPE OF THE CHRONIC INFLAMMATORY INFILTRATE

The immunopathology of chronic abacterial prostatitis was explored from two different angles in this thesis, firstly, by a search for the presence of antibodies and complement deposited within the prostatic tissue, and secondly, by determination of the phenotype, and thereby the function, of the chronic inflammatory cell infiltrate. The findings of each line of investigation are discussed in turn.

The finding of IgM and IgA antibodies, complement and fibrinogen deposition within the prostate of chronic abacterial prostatitis patients, but not the controls, implies that these antibodies do not from part of the normal secretion of the prostate gland, but conversely, are produced in response to extrinsic antigen. These results contrast with those of a study of normal, cadaveric prostate and benign hyperplastic tissue (Ablin et al 1971), where IgA and IgG in granules within the ductal lumen, IgG in the basal part of the prostate acini and IgA in the stroma were found. The authors concluded that these antibodies comprised part of the normal secretion of the prostate. The discrepancy between these results and the findings of this thesis is difficult to explain, particularly as the method of production of the goat anti-human antibody in the former study (Ablin et al 1971), was not described. The antigens used to stimulate the rabbit anti-human antibodies in this work were IgA, IgG, IgM and complement C3 from pooled human serum and fibrinogen from pooled plasma. The IgA fraction in this study was not of the secretory type which accounts for most of the IgA normally secreted by the prostate (Fowler & Mariano, 1984), but the absence of IgG suggests that the antibodies used in this study were of different specificity to those used by Ablin and co-workers (1971).

A similar panel of antisera to those adopted in this work

were used by Vinje and colleagues (1983) to examine frozen sections of prostatic tissue from patients with chronic prostatitis. In most biopsies a diffuse extracellular fluorescence was observed, indicating IgA, IgG and IgM presence; all biopsies stained weakly positive for fibrinogen and some possessed immunoglobulin - containing cells, yet complement was not detected. The authors were loath to draw firm conclusions, save that the immunoglobulin present had probably diffused passively from the serum. The fact that the diagnosis of prostatitis in this study, based on symptoms, signs and the finding of $\geq$ 10 leucocytes per hpf in the expressed prostatic secretion (EPS), rather than the Stamey localisation method (Meares Stamey 1968), together with the adoption of a blind biopsy technique and failure to analyse prostatic tissue from normal controls, certainly throws doubt on the significance of the findings.

Since the detection of antibody-coated bacteria in the urine of patients with chronic bacterial prostatitis (Jones 1974), there has been increased interest in the immunoglobulins within prostatic fluid, noted previously in normal subjects (Chodirker & Tomasi, 1963). In a group of patients with a diagnosis of prostatitis, though localisation studies were again not detailed, significantly increased levels of IgA, IgG and IgM were found in the EPS compared with controls (Gray et al, 1974). Additional studies have noted that immunoglobulin levels, in particular IgA and IgG, were greater in chronic

bacterial prostatitis than in chronic abacterial prostatitis and remained elevated in cases that failed to resolve, yet returned to normal in those with a satisfactory response to treatment (Fowler & Mariano 1984; Shortliffe and Wehner, 1986). Shimamura (1979), found IgM as well as IgG levels, in the EPS, to follow a similar pattern.

The total and organism-specific immunoglobulin levels in EPS have now been measured by several investigators, (Shortliffe et al, 1981; Fowler et al, 1982, Wishnow et al 1982; Fowler & Mariano, 1984; Shortliffe & Wehner, 1986) and the general consensus is that the levels of IgA and IgG in prostatitis are elevated compared with those in controls. The IgA response was most marked and represented production within the prostate, rather than transudation from the serum. The elevated levels of these immunoglobulins in chronic abacterial prostatitis were less than those in chronic bacterial prostititis and were not specific for the Enterobacteriaceae family of organisms (Shortliffe & Wehner, 1986). Thus, chronic abacterial prostatitis does not appear to be an occult infection by these organisms that has escaped detection. Interestingly, elevated IgM levels in EPS were not detected in these later studies. This contrasts both with earlier reports (Gray et al,1974; Shimamura, 1979) and the findings of this thesis in which IgM was the most prevalent class immunoglobulin. However, it is possible that we are dealing with different types of immunoglobulin

since those detected in this thesis were tissue bound rather than free within the prostatic secretion.

Interstifial cystitis, possibly a condition akin to chronic prostatitis, yet affecting the bladder, has been studied by two groups of investigators (Gordon et al, 1973; Mattilla, 1982) seeking evidence of the immunoglobulins and complement within the tissues. IgM featured in both studies and was detected along with C3, in the vessel walls of the bladder in one (Mattila,1982). IgM was postulated to be either part of a ciurculated immune complex or produced in response to an exposed antigen within the vessel wall, which subsequently triggered the cascade (Mattila,1982). The results in this thesis are similar, with IgM (85%) and C3 (44%) being found most frequently, albeit most commonly in a periglandular site (79%), though vessel wall deposition (62%) was also prominent. The predominance of IgM in a chronic condition is curious, but may reflect repeated secretion in response to a new antigen, a viral aetiology or possibly an autoimmune process. Vessel wall deposition of immunoglobulin, together with complement, may imply the existence of circulating immune complexes.

The majority of symptoms that showed a significant correlation with immunofluorescence staining in this thesis were concerned with voiding dysfunction and irritative voiding. Symtoms of poor flow correlated with immunofluorescence positivity in general, but with IgM

deposition particularly, in the luminal cells, and also with C3 deposition. It is difficult to ascertain whether irritative voiding results in immunofluorescence positivity or whether prostatic inflammation exerts an irritative effect on the trigone. In a study of fifty patients with symptoms of prostatitis, 52% had a sensory problem on uro dynamic testing and 46% had detrusor instability (Murnaghan & Millard, 1984). Such abnormalities may be presant in our patients, though none had undergone formal urodynamic assessment. On the other hand, the link between symptomatology and immunology may rest with functional outflow obstruction such as occurs in incomplete relaxation of the distal sphincter complex (Buck, 1975; Hellstrom et al, 1987) where high pressure within the prostatic urethra is produced during voiding with resultant reflux of urine (Kirby et al, 1982). The urine within the prostatic ducts and acini may then incite an immunological response, either by acting as a carrier or antigen of possibly through it's chemical properties. The presence of IgM in the luminal cells may indicate an intracanalicular route for the inducing antigen.

Significant C3 deposition was linked with a history of non-gonococcal urethritis, which may indicate that potential causative organisms of this condition, i.e. Chlamydia trachomatis and Ureaplasma urealyticum, which have spread intracanalicularly, are implicated in chronic abacterial prostatitis. If the immunoglobulin and complement deposition reflect antibody formed in response

to these organisms, then it would be against non-viable antigens that remain tissue bound, since an active role for these organisms could not be found in this thesis (see Sections 14, 15 and 25). Indeed Shurbaji and colleagues (1988) detected clamidial antigen in 31% of cases in a retrospective review of surgical specimens with a "coding" of prostatitis. There was no correlation between symptoms or clinical presentation, and positive staining for chlamydial antigen, and thus, this organism may have played little active part in the inflammatory process. The possibilty of this antigen inciting an inflammatory response cannot be discounted. For that matter, nor can the proposal that these antigens just represent past history and though "trapped" in the prostate, were simply innocent bystanders in a totally separate process. More recently spermatazoa have been suggested as possible auto-antigens, producing inflammatory infiltrates in benign hyperplastic prostates (McClinton et al, 1990). It may be that similar particulate antigen is responsible for such a reaction in chronic abacterial prostatitis; it's identity, however, remains uncertain.

The absence of any correlation between the severity of inflammation and immunofluorescence positive in our patients is at variance with the results of a similar study of the painful bladder syndrome (Witherow et al, 1989), but suggests that the inflammatory response is not an entirely antibody driven process. In this thesis, B cells could not be detected in the prostatic biopsies of

either controls or patients with chronic abacterial prostatitis (see Section 17) and thus it was proposed that these immunoglobulins were not actively secreted by the prostate, but must diffuse from the serum.

The majority of patients (57%) within the chronic abacterial prostititis cohort possessed immunoglobulin, complement and fibrinogen deposition within the prostate, yet no clear relationship between immunofluorescence staining and aetiology was evident, save possibly for poor urinary flow. It remains to be seen whether the subgroup with immuno fluorescence staining represent a truly separate category in terms of disease natural history and response to treatment. This information can be gained only by longitudinal studies, possibly including follow up prostatic biopsy.

II DETERMINATION OF THE PHENOTYPE OF THE CHRONIC INFLAMMATORY INFILTRATE

The results of this study point to a cell-mediated response being the underlying process in chronic abacterial prostatitis. This conclusion is based on the consistent HLA-DR expression, which becomes more prominent with increasing inflammatory grade, the large number of T lymphocytes within the inflammatory infiltrate and absence of both plasma cells and B lymphocytes. Furthermore, the RFD1 interdigitating cells, which functionally have an antigen-presenting role (Sunshine et al, 1980; Knight et al, 1982; Poulter, 1983), were present in the chronic abacterial prostatitis lesions; this reflects the situation observed in the synovium in rheumatoid arthritis (Klareskog et al, 1981; Poulter et al, 1983), the skin in psoriasis (Poulter et al, 1984) and the bronchial wall in bronchiectasis (Lapa E Silva et al, 1989), all of which are examples of a well defined cell-mediated reaction. By contrast, in cryptogenic fibrosing alveolitis, thought to be due to an antibody mediated inflammatory response (Campbell et al, 1985), there are very few RFD1 antigen-presenting cells in the inflammatory cell population. In addition, the pattern of the RFD1 cells, namely prominence in grade 1 and less evidence in grade 2 prostatitis lesions, supports a cell-mediated response and is compatible with a type IV hypersensitivity reaction (Poulter et al, 1982c). A study of prostatic tissue from patients undergoing transurethral resection (TURP), which

revealed inflammatory changes in all cases, also concluded that a cell-mediated response, representing delayed hypersensitivity, possibly to spermatazoa, was the underlying process (McClinton et al, 1990). However, possible variation in sectioning and staining, the limited number of samples investigated in each case and the unknown kinetics of the disease process, inevitably place constraints on the immunopathology study in this thesis. Despite these limitations, however, the results presented here offer a firm base for the hypothesis that a T cell dominated acquired immune response is central to the pathogenesis of chronic abacterial prostatitis.

The absence of B lymphocytes and plasma cells is at variance with the observations of El Demiry and associates (1985), who found aggregates of B cells in the interstitium of normal cadaveric prostates. McClinton and colleagues (1990) observed isolated collections of B cells in the stroma in benign hyperplastic prostatic tissue harvested at TURP, but, as in this thesis, found plasma cells to be scanty or absent. Furthermore, studies of immunoglobulins within the prostatic fluid (Fowler & Mariano, 1984; Shortliffe & Wehner, 1986) found higher levels of IgG and IgA in chronic prostatitis patients than in controls, though these levels were lower and not specific for the Enterobacteriaceae antigens, in those with chronic abacterial prostatitis. The authors concluded, from studies in which antibodies in sera and prostatic fluid were compared, that the immunoglobins were

secreted actively within the prostate rather than being products of simple diffusion from the serum. The findings of this thesis, however, do not support this viewpoint. They are consistent with the notion that the immunoglobulins perfuse into the prostate rather than be produced locally, as neither B lymphocytes nor plasma cells were detected in either normal or inflamed prostatic tissue (see Section 17).

The mean CD4:CD8 T cell ration was less than 2 for all inflammatory grades and less than unity for the more severe in agreement with McClinton and co-workers (1990), in there investigations inflammatory infiltrate in benign hyperplastic prostatic tissue. This ratio for the peripheral blood of normal subjects is ine the order of two and, although it has not been measured in the patients in this study, it is likely that the presence of T cells in the prostate is due to an active process rather than passive migration, although the ratio in the peripheral circulation was not analysed specifically. The progessive increase in CD7 expression by the CD8 lymphocytes in grade 2 and 3 lesions of prostatitis, suggests that the inflammatory response is active. Further support for this hypothesis is the increase in HLA-DR expression, both qualitatively and quantitatively, with increasing degrees of inflammation, and the larger number of lymphocytes expressing the CD45RO antigen on T memory cells (Akbar et al, 1988).

The natural history of the delayed hypersensitivity inflammatory lesion is characterised by early infiltration of RFD1 cells (Poulter et al, 1982c). These, however, do not continue to accumulate, but are replaced by mature tissue macrophages (RFD7$^+$) (Poulter et al, 1982c). This is the pattern observed in the progression from grade 1 to grade 2 lesions in this study. The perivascular location of the RDF1 interdigitating cells in grade 1 lesions most likely reflects their early recruitment from the peripheral circulation. The progressive increase in HLA-DR expression through the inflammatory grades could be accounted for by recruitment of increasing numbers of both activated T lymphocytes and RDF1$^+$ cells, followed by the influx of mature tissue macrophages (RDF7$^+$ cells). This pattern of events suggests that chronic abacterial prostatisis is an active acquired cell-mediated reaction which appears to progress from low grade (grade 1) through to high grade (grade 3). However, this interpretation of a dynamic process is based on "static" observations and must, therefore, remain tentative.

There are few histological studies of prostatitis with which to make comparisons. In one study of unselected prostatectomy specimens (Kohnen & Drach, 1979), inflammatory changes were reported to have been detected in 98% of cases and six patterns of inflammation noted, namely segregated glandular, periglandular, diffuse stromal, isolated stromal nodule, acute necrotizing and localised granuloma. The first three patterns probably

represented degrees of the same inflammatory process and occured in approximately 80% of cases, an observation confirmed in the present study which goes further in revealing the distribution of lymphocyte and macrophage subsets.

The only other known estimation of the phenotype of inflammatory cells within the prostate (McClinton et al, 1990) examined tissue from patients with benign prostatic hyperplasia undergoing TURP. It was concluded that CD8 T lymphocytes were more prominent in the epithelium, where as CD4 cells occupied a stromal site. It was thus postulated that these CD8 cells had an immunosurveillance role against possibly harmful antigens, attempting to gain entry to the prostate via the ductal lumen. However, they failed to examine any "normal", non-inflamed prostate tissue, nor measure the proportion of CD8 cells that were cytotoxic. Despite classifying their biopsies into "light", "moderate" and "severe" inflammatory lesions, they failed to detail or discuss any differences in phenotype pattern between these grades of infiltrates. In this thesis, similar numbers of CD4 and CD8 lymphocytes were detected, with a homogeneous distribution, in both the normal and low grade (grade 1) inflammatory lesions. As the severity of inflammation increased, so did the numbers of all lymphocytes, but especially CD8 cells, initially in a perivascular and stromal location, in grade 2, and then a glandular postion, in grade 3 and granulomatous lesions. With regard the macrophage

population there are even less data with which to make comparisons. In hyperplastic prostatic tissue, (McClinton et al, 1990), macrophages have been noted in the stroma and, less commonly, the epithelium, though information was lacking regarding their possible role. In this thesis, both RDF1 (antigen-presenting cells) and RFD7 (tissue macrophage) populations were detected, and these occupied different sites and prominence within the differing infiltrates. The antigen-presenting cells were most commonly located in a perivascular position in the grade 1 lesions, before moving to a glandular and then even distribution in grade 2 and grade 3 lesions respectively. This pattern implied recruitment in the "early", grade 1 lesions, before a more even spread in "later" grades as the RFD1 cells moved to the presumed site of entry of antigen, namely the gland lumen. The tissue macrophages (RFD7) on the other hand appeared to be resident in the glandular and perivascular sites in the early stages, but occupied a stromal and glandular location in grade 2 lesions, as the cells surged into the inflammatory infiltrate to become the dominant macrophage phenotype. This again supports the theory of antigen gaining entry via an intracanalicular route and triggering this progressive cell-mediated response. In the grade 3 biopsies, the tissue macrophages were evenly distributed, having been previously primed, and thus ready to receive further antigen presented by a re-emergence of RFD1 cells.

The hypothesis outlined above proposes a progressive immune response from low (grade 1) to high (grade 3) lesions in chronic abacterial prostatitis. Is it possible that the grade 1 lesions actually reflect a fading, "burnt out" scenario? In support of this notion is the reduced HLA-DR and CD7 expression of the cells comprising the inflammatory infiltrate. Furthermore, fibrosis was not evident in any of the grade 3 biopsies, yet of the 18 cases displaying fibrosis, 16 had a low grade inflammatory infiltrate. In opposition to the "burnt out" theory, is the pattern of RFD1 and RFD7 cells, although caution must be exercised when small numbers of cells are analysed. RFD1 cells would not be expected to predominate, nor occupy a generally perivascular location in a reaction of declining activity; RFD7 cells would be expected to be in the majority. In addition, the number of cells expressing the CD45RO antigen might be expected to increase with longevity of reaction, which was not the case in grade 1 biopsies. Interestingly, there was little difference in the expression of this antigen throughout the grades, which might imply a stable population of T memory cells, an unexpected finding if persistent antigen is the basis for stimulation. However, misrepresentative staining, or an immune reaction of relatively short duration, such that memory cells were yet to be fully primed are possible explanations. The correlation of low grade inflammation with fibrosis, though not statisically significant, may just reflect the difficulties encountered in identifying fibrosis, when

"masked" by a very dense inflammatory infiltrate in lesions of high grade.

In conclusion, therefore, the data are more in keeping with a progressive immune mechanism from grade 1 through to grade 3 lesions, rather than grade 1 representing a "burnt out" state.

The results of this study raise the question as to whether the granulomatous lesion is the culmination of the progressive cell-mediated response discussed above, or whether it is a distinct disease. The former view is supported by an increase in the HLA-DR expression, the recruitment of RFD7 and in the lymphocyte infiltration, particularly the CD8 subset, compared with the grade 3 lesions. However, the finding that RFD1 cells occured in relatively large numbers in stromal and perivascular sites and that only 30% ofthe CD8 cells possessed the CD7 antigen, is more compatible with an early lesion. Together, therefore, these observations might suggest a separate disease process. Caution must be exercised, however, in interpreting histological observations, particulary when they are based on a solitary case.

The outstanding question concerns the nature of the driving force behind the immunological reaction. A defect of immunoregulation as occurs in rheumatoid arthritis (Janossy et al, 1981) seems unlikely. In the latter condition, interdigitating RFD1 cells are dominant

throughout the disease process in association with CD4 lymphocytes, whereas in chronic abacterial protatitis, the initial presence of RFD1 cells, followed by the RFD7 cell infiltration, accompanied by CD8 lymphocytes, are more in keeping with a persistent antigenic stimulus. This is especially likely in view of the re-emergence of RFD1 cells in grade 3 lesions. However, in a separate study (see Sections 14 and 25a), no organisms were isolated from the same biopsy materials as those analysed here, and the nature of any persistent antigen remains unknown.

Finally, the possibility of an auto-immune involvement in the immunopathological process deserves consideration. In favour, is the absence of an identifiable antigen, the possibility of HLA-DR-positive epithelial cells acting as presenters of antigen to the T lymphocytes and the occurrence of antibody deposition within the prostate (Ablin et al, 1971; Section 17). However, the excessive numbers of CD8 rather than CD4 lymphocytes and of RFD7 rather than RFD1 macrophages, together with the lack of B lymphocytes and plasma cells argues against an auto-immune aetiology, unless cell-mediated as in thyroiditis (Volpe, 1986). Assessment of the kinetics of the inflammatory reaction in repeated biopsies at different stages of the disease, and functional studies of lymphocytes extracted from prostatic fluid and tissue, need to be carried out in order to examine the question of an auto-immune aetiology more closely.

The two studies discussed in this Section provide two apparently separate viewpoints concerning the immunopathology of chronic abacterial prostatitis. On the one hand there was evidence of intraprostatic antibody deposition in over half (57%) of cases within the chronic abacterial prostatitis cohort. The predominant antibody was IgM (85%), with C3 (44%) also promient, and their deposition raised the possibility of immune complex formation. If such a reaction was taking place, one could postulate a type III hypersensitivity reaction, with local immune complex formation at the site of antigen entry. However, in such a chronic condition, one would expect IgG to be the dominant antibody and also polymorphs to be the major component of the inflammatory infiltrate. The correlation between antibody deposition and vessel thickening raised the possibility of circulating immune complexes; there wa also a significant correlation between symptoms of poor urinary flow as well a irritative voiding and immunofluorescence staining, drawing the conclusion of a possible intracanalicular route of entry for the inciting antigen. The stance adopted from the data concerning the phenotype of the inflammatory infiltrate population is of a progressive cell-mediated response compatible with a type IV hypersensitivity reaction. The pattern of distribution of cells of the macrophage series favours a persistent antigenic presence as the initiating stimulus. How can these two apparently separate conclusions be rationalised? As in Farmer's lung (types III and IV) may exist. Alternatively, the predominant

immune mechanism may be a type IV hypersensitivity reaction and the antibody and complement deposition are simply relics of previous antigen exposure, yet play no active role int he underlying immunopathological mechanism. This previous antigen may have been an organism which has long since been destroyed, yet leaves behind a mark of it's presence. Those patients with positive immunofluorescence may turn out to be a different group to those with negative staining and may pursue a different natural history and outcome. Although, only a theory, a prolonged follow up study may reveal such differences, not readily evident from the demographic data in this thesis. Finally, the two separate features of this study may reflect two entirely unlinked mechanisms or pathways whose interrelationships have yet to be ellucidated.

An analagous situation to chronic abacterial prostatitis may be present in bronchiectasis where a similar cell - mediated type IV hypersensitivity reaction is found in the bronchial mucosa (Lapa E Silva et al, 1989). It has been proposed that initial damage to the mucosa occurs, allowing microbial colonisation, which causes further damage and prevents clearance of the noxious agents responsible for the initial reaction (Cole, 1984; Lapa E Silva et al, 1989). Similarly, chronic abacterial prostatitis may result from damage to the prostatic ducts and acini, possibly caused by an organism, followed by a further antigen creating a chronic inflammatory response.

The sequelae of this reaction result indistortion of gland architecture, such that the antigen cannot be "cleared" from the prostate, creating yet another vicious circle. This explanation remains an hypothesis to date.

SUMMARY

The immunological studies on prostatic biopsy material from a cohort with chronic abacterial prostatitis explored two pathways. Firstly, the presence of antibody, complement and fibrinogen was investigated and detected in 57% of the cohort, yet only 5% of controls ($p < 0.001$). A positive correlation existed between such staining and symptoms of poor urinary flow ($p < 0.01$) and irritative voiding ($p < 0.01$). Although, no clear guidelines emerged as to the significance of this immunofluorescence staining, it was proposed that it may reflect previous antigenic presence, or possibly a type III hypersensitivity reaction, in response to antigen which gained entry to the prostate via it's ductal lumina.

The determination o the phenotype of the chronic inflammatory infiltrate proposed a cell-mediated, type IV hypersensitivity reaction as the underlying mechanism. The cellular pattern was more in keeping with the persistence of antigen that an auto-immune reaction. However, caution must be exercised with interpretation in view of the variation in sectioning and staining, the limited numbers of biopsies investigated and the unknown kinetics of the disease process. The latter requires further study, by repeated biopsy at different stages of the disease and by functional studies on cells extracted from the biopsy material.

SECTION 28 THE ROLE OF PROSTATE SPECIFIC ANTIGEN (PSA) ESTIMATION IN THE DIAGNOSIS OF CHRONIC ABACTERIAL PROSTATITIS.

The advent of commercial kits for the measurement of serum prostate specific antigen (PSA) has enabled readily reproducible results to be obtained using an immunoradiometric assay (Frankel et al, 1982).

The results from the cohort of patients with chronic a bacterial prostatitis revealed universally low values, with the exception of the case of granulomatous prostatitis. In contrast to the focal nature of chronic prostatitis (McNeal, 1968) involving a total volume of gland with florid necrotising inflammatory features (Stillwell et al, 1987). It is not surprising, therefore, that an elevated value of serum PSA may be observed in this condition; rather akin to the findings in acute retention with possible micro-infarction and acute inflammation (Collier & Pain, 1986; Armitage et al, 1988).

The data suggesteing elevated PSA values in prostatitis are largely anecdotal (Collier & Pain, 1986; Stamey, 1987) and probably represent cases of acute prostatitis, diagnosed by histological criteria of resected tissue.

Indeed a study, where resected prostate tissue was fully analysed histologically and compared with pre-operative PSA levels, concluded that values were raised in acute

(25%), but not chronic inflammation and that PSA was a poor differentiator of specific prostatic disease (Brawer et al, 1989); none of these patients had clinical evidence of chronic prostatitis.

Acute prostatitis is a completely different condition to chronic prostatitis, in that the inflammatory process affects the whole gland (Stamey, 1981) in an aggressive immune response that is likely to cause PSA release from damaged prostatic epethelium. It is impossible to make comparisons between two groups of patients, one diagnosed on histological grounds and the other selected on the basis of prostatic localisation studies (Meares & Stamey, 1968) and then classified according to universally accepted criteria (Drach et al, 1978), as there is no evidence to suggest that they are the same condition.

Although, the recognised upper limit of normal for PSA is 4ng/ml (Lange et al, 1986) a higher "operational" value of 10ng/ml as been suggested by Siddall and co workers (1986). If the latter value is adopted, only one patient in this thesis had an elevated level of PSA, the case of granulomatous prostatitis, and this value fell to within normal limits at three months follow up. The PSA level in serum has been shown to be related to age (Lange et al, 1986), though this may just reflect the degree of benign eerplasia, galnd volume (Stamey et al, 1987; Armitage et al, 1988; Vesey et al, 1988) and also to be elevated by manipulation, biopsy and resection (Stamey et al, 1987).

Thus, in the chronic abacterial prostatitis cohort with a mean age of 39.8 years, the low PSA values appear entirely compatible with this young population and the likelihood of small volume glands. As eluded to earlier, the inflammatory features in chronic prostatitis are both focal (McNeal, 1968) and involve a small volume of gland (Kohnen & Drach, 1979) and therefore, are likely to cause a low level of prostatic epithelial cell destruction and consequent release of PSA into the serum.

The only elevated value amongst the cohort, had a lower PSA value three months later, compatible with resolution of the inflammatory response. With such low values recorded in the other patients little meaningful information can be gleaned about the changes in PSA values in relation to the leucocyte count in the post prostatic massage urine (VB3). However, there was no correlation between histological grade and PSA level, save with the granulomatous case.

Thus, PSA has no definable role in the diagnosis and management of chronic prostatitis save for helping to separate those rare cases were there is difficulty in differentiating between inflammatory and neoplastic disease.

SUMMARY

The serial measurement of serum PSA in patients with chronic abacterial prostatis produced no significant deviation from normal values. This would appear to support the focal nature of the condition, plus the involvement of only a small volume of gland, in any process of prostatic epithelium destruction. In the granulomatous case, although the initial value was elevated, serum PSA returned to within the normal range as the inflammatory process resolved. Serum PSA therefore has no role in the diagnosis and management of patients with chronic prostatitis save to exclude a possbile carsinoma in those few cases with an abnormal gland on palpation.

SECTION 29 URINARY FLOW RATES AND PROSTATIC URINARY REFLUX IN CHRONIC ABACTERIAL PROSTATITIS

The flow rate nomograms (Siroky et al, 1979; Kadow et al, 1985) highlight the need to study normal subjects before assigning a tag of abnormality to any set of data. The analysis of the crude flow rate data implied a highly significant ($p < 0.001$) difference between the chronic abacterial prostatitis (CABP) cohort and the prostatodynia (Pd) patients. However, when plotted on their respective nomograms only 8-18% of the CABP cohort and 5-7% of the Pd cases had flow rates significantly ($p < 0.05$) less than the mean. Instead of using fixed criteria, such as a flow rate of 15mls/second (Stewart, 1960; Anikwe, 1976), as indicative of abnormality, the nomogram places the flow rate in the context of age and voided volume, and thus a more valid interpretation must ensue.

Although, there was variability in the flow rate data between the series for each patient, 27-42% of the CABP and 20% of the Pd group displaying altered flow rates compared to the previous measurement, each variation was by < 1 standard deviation. Thus, such changes could have occured by chance and support the validity of serial flow rate measurements.

The difference in prevalence of the abnormal (> 2 standard

deviations below the mean) flow rate between the CABP and Pd groups was borderline with $p < 0.05$ achieved in only two of the six analyses. The prevalence of supranormal flow rates was no different in the two groups, yet statistically significant differences were observed in four out of the six analyses when the prevalence of flow rates less than the mean was assessed. Any change in flow rate less than two standard deviations for the mean could occur by chance and thus observation of values simply below the mean probably over states the case for abnormality. In this context, values below the mean of 69 - 75% in the CABP cohort and 50 - 55% in the Pd group may not be as striking as first appears.

The flow rate data from other studies of patients with chronic prostatitis indicated abnormal flow rates in 54 - 60% (Siroky et al, 1981, Murnaghan & Millard, 1984), but in both groups the patients were selected on symptoms alone, consisted of a heterogenous group and used different criteria to define abnormal; Siroky and colleagues employed their nomograms (Siroky et al, 1981) but Murnaghan & Millard were content to use a peak flow rate of 15mls/second as the cut off point. The situation is no less confused in prostatodynia with abnormal peak flow rates of 85 - 96%, quoted (Barbalias et al, 1983; Meares, 1986), though again different criteria for abnormality were adopted. In this thesis, strict criteria have been employed and this may account for the lower abnormal rates achieved. Furthermore, in the other

chronic prostatitis studies quoted, diagnosis was made on symptoms alone, rather than standard localisation studies (Meares & Stamey, 1968).

There appears to be a consensus view on the cause of the abnormal flow rates in both chronic prostatitis and prostatodynia, namely a spastic or dyssynergic distal sphincter complex (Buck, 1975; Osborn et al, 1981; Siroky et al, 1981; Barbalius et al, 1983; Meares, 1986; Hellstrom et al, 1987). Murnaghan and Millard (1984) seem alone in attributing blame to the bladder neck, though a number of authors noted funnelling at this site on videocystometrograms (Barbalius et al, 1983; Meares, 1986). There is debate as to which component of the complex is responsible: several authors (Osborn et al, 1981; Barbalius et al, 1983; Meares, 1986) favour the smooth muscle component innervated by sympathetic nerves, whereas Siroky's group (1981) implicated the striated portion and still others apportion blame to both the smooth and striated components (Hellstrom et al, 1987). The outcome of a raised urethral closure pressure is intraprostatic urinary reflux, which can be demonstrated on voiding cystography (Hellstrom et al, 1987).

The underlying cause for the reduced flow rates encountered in this thesis, must remain conjectural as full urodynamic and electromyographic studies were not performed. However, the studies quoted above would imply that the distal sphincter complex is at fault. The

differences between the CABP cohort and Pd group were barely significant, which begs the question as to whether any difference in urodynamics exists between the two groups? There is no published data in which the urodynamic features of the two sets of patients have been compared with which to make a judgement. It is possible that distal sphincter abnormalities are more marked in chronic prostatitis, due in part to the inflammatory process (Hellstrom et al, 1987). This said, however, there was no correlation between flow rate and either Stamey score from localisation studies or inflammatory grade within the prostatic tissue. Furthermore, there was no correlation between change in Stamey score and flow rate on serial analysis. Therefore, there appears to be no direct cause and effect relationship between urinary flow rate and inflammatory changes in the prostate.

The aetiology of distal sphincter complex dysfunction remains uncertain, though abdominal straining (Hellstrom et al, 1987), behavioural disorders (Siroky et al, 1981) and adjacent prostatic inflammation (Meares, 1986; Hellstrom et al, 1987) have been postulated. Attempts to detect a neurological abnormality have been unrewarding.

A consequence of a raised urethral closure pressure is intraprostatic reflux of urine (Buck 1975; Osborn et al, 1981; Meares, 1986; Hellstrom et al, 1987), which was demonstrated by Kirby and colleagues (1982) in five patients with chronic abacterial prostatitis. In the

latter study the phenomenon was also exhibited in cadavers and patients undergoing prostatectomy for benign prostatic hypertrophy. Therefore, is intraprostatic urinary reflux ubiquitous? Biochemical analysis of prostatic calculi indicating that fifty per cent of these stones contain urinary constituents (Sutor & Wooley, 1974); Kim, 1982), supports this view but Fair and Sharer (1986) feel that such consistents only occur in calculi associated with infection. The pilot study in this thesis supported the findings of Kirby and colleagues (1982) with seven out of ten patients from the CABP cohort displaying intraprostatic reflux. Interestingly, the flow rate categories of the seven refluxers at the time of study were < 2 in three, 1 - 2 in one and 0 - 1 in three, compared to 0 - 1 in two and 0 in one of the patients not exhibiting intraprostatic reflux. The numbers are small and controls were not studied but an association between low peak urinary flow rate and intraprostatic urinary reflux may exist. The ductal anatomy, particularly of the peripheral zone (Blacklock, 1974), predisposes to urinary reflux and raised prostatic urethral pressure may be the sole trigger required.

There appears to be no clear cut difference between the urodynamic features encountered in chronic prostatitis and prostatodynia. The peak urinary flow rate data accumulated in this thesis supports this view, yet suggests that the two conditions lie along a spectrum of abnormality, with chronic prostatitis exhibiting more

striking variations. Whether prostatic urinary reflux per se is detrimental is unknown, but the prostatic ductal epithelium being cuboidal does not appear prepared for the insult. It is possible that such reflux occurs commonly, yet fails to cause symptoms until ductal damage is produced or a particular noxious agent or inciting antigen is washed in with the tide. Like so much in chronic prostatitis more research is required on this front, but at least there is a chink of light.

The use of urinary flow rate nomograms places the data in context, but most importantly, enables comparison of data. Unfortunately, there are few studies where this is possible due to the analysis of either unselected or poorly selected patients, or the use of non-standardised data.

However, the peak urinary flow rate appears reduced in both chronic prostatitis and prostatodynia caused by a failure of relaxation of the distal sphincter complex. No clear view exists as to whether the smooth or striated muscle component is at fault, or why, though behavioural factors, abdominal straining and reflex activity in response to adjacent prostatic inflammation have been proposed. The net effect appears to be a propensity for intraprostatic reflux of urine. However, there is no correlation between low peak urinary flow rates and the severity of inflammatory changes within the prostate as judged by analysis of either expressed prostatic secretion or prostatic tissue. Therefore, if low urinary flow rates are associated with intraprostatic urinary reflux as implied in this thesis, there is no direct cause and effect relationship between reflux and inflammatory disease.

The prevalence of such urinary reflux is unknown, but it alone may be insufficient to cause symptoms or initiate an inflammatory process. A noxious agent or inciting antigen may also be required.

SECTION 30 PSYCHOLOGICAL ASSESSMENT IN CHRONIC ABACTERIAL PROSTATITIS

The validity of the General Health Questionnarie (GHQ) a screening tool for functional psychiatric illness is established already (Goldberg, 1972, 1978; Goodchild & Duncan-Jones, 1985), though it has been criticised for failing to detect chronic neurotic disease (Finlay-Jones & Murphy, 1979).

The GHQ-60 was employed in this thesis as an indicator of the prevalence of neurotic psychiatric illness in the chronic abacterial prostatitis (CABP) cohort and compared with a group of patients with similar symptoms but no evidence of inflammatory prostatic disease ie. prostatodynia (Pd), plus a "control" group with established "chronic" conditions requiring repeated attendence to a hospital out patient clinic (CUA). As clinical experience often supports, those patients with prostodynia had a greater prevalence of neurotic psychiatric illness than both the CABP cohort and the CUA group, which achieved statistical significance at $p < 0.002$ and $p < 0.001$ respectively. Prevalence of low, that is "normal" scores was also significantly greater in the CABP, cohort, $p < 0.01$, and the CUA group, $p < 0.001$, compared to the Pd patients.

The threshold at which the GHQ-60 score implies psychiatric illness is somewhat arbitrary. The

questionnaire may be used to separate the patients along a spectrum of disease or, by selecting a definiate threshold, separating them into a "non-case" class and a "case" (neurotic psychiatric) class. The latter option was adopted in this thesis, albeit with reservations.

The GHQ-users guide (Goldberg & Williams, 1988), offers certain guidlines in selecting a threshold score for the GHQ-60 questionnaire. Twenty studies were analysed using a two stage validity technique, ie. comparing GHQ-60 with an alternative, recognised questionnaire that also detects neurotic psychiatric illness. Although the effect of chronicity in lowering thresholds and of physical illness, through the creation of somatic syptoms and social dysfunction, raising thresholds, was noted, 80% of studies accepted a threshold of $\geq$ 12 as indicative of neurotic psychiatric illness.

The chronic prostatitis patients were braodly polorised, having either no psychiatric illness or psychiatric illness, with few falling into the intervening grey area; there was a much more even distribution of scores between the ends of the spectrum in the prostatodynia group. In this small sample it is reasonable to conclude that there is a greater tendency to neurotic psychiatric illness in patients with prostatodynia, yet appreciable numbers with chronic abacterial prostatitis, and to a lesser degree, chronic physical illness are similarly afflicted.

The greater prevalence of neurotic psychiatric illness in the prostatodynia (Pd) patients, appears real and unrelated to symptom duration, as there was no statistical difference between the Pd and CABP groups with regard chronicity of symptoms. These two groups with comparable age and although no details of symptom duration were recorded in the CUA group, the latter patients would appear to be valied "chronic controls", despite their mean age being slightly greater. The lack of any statistical difference in scores between the CABP and the CUA patients, adds weight to the notion that the Pd patients belong to a separate class with respect to psychiatric illness.

The crux of the matter rests with whether these differences are cause or effect. From a single study, firm conclusions are impossible, yet a number of observations require expansion. The GHQ-60 has been shown to have gross deficiencies in follow up studies (Henderson et al, 1981), as scores fall with successive questionnaires. Thus, longitudinal studies, in conjunction with prostatic localisation studies, to assess changes in the degree of prostatic inflammation associated with changes in GHQ-60 score would be difficult to interpret. Furthermore, such a study, would have to assume that the physical inflammatory response was directly related to the severity of symptoms, which in turn were directly related to the degree of neurotic psychiatric illness. To date, there is no evidence to

support this premise, indeed, Sections 16 and 17 provide testimony to the contrary. The questionnaire invites the patient to answer questions on "how your health has been in general over the past few weeks" and thus, in a chronic disease provides an acute, up to date assessment. The GHQ-60 has also been criticised for failing to detect chronic neurotic illnesses (Finlay-Jones & Murphy, 1979). However, if the neurotic psychiatric illness was an effect of the prostatodynia, an increase in GHQ-60 score with prolonged symptom duration would be expected, which was not the case in this study. Furthermore, by studying a group with chronic non-prostatic disease has "controls", the chronicity effect may be expected to cancel out and any difference between the groups reflect other factors of the disease, unrelated to prolonged symptoms.

The only work with which direct comparisons can be made is that of Smart and colleagues (1979) who studied a group of 105 patients with symptoms and signs of chronic prostatitis, who were broken down into four groups: chronic bacterial prostatitis due to urinary pathogens, chronic due to Staphylococcus albus, patients with a distinct urological abnormality and those in whom all investigations were negative. It may be assumed that a number of the second group that had chronic abacterial prostatitis, whereas the lattermost category of patients had prostatodynia. Utilizing the General Health Questionnaire, the "no abnormality" group had a signigicantly greater prevalence of neurosis ($p < 0.01$),

compared to the other three categories. Furthermore, this research noted that patients with symptoms of chronic prostatitis who were symptomatic at 1.5 to 3 years from presentation, were significantly more neurotic (68% versus 20%). Does this finding indicate that due to the chronicity of the symptoms and lack of therapeutic progress the patients become more neurotic or alternatively, due to their functional psychiatric illness they remain symptomatic ? In this thesis, study of the chronic abacterial prostatitis cohort, produced the conflicting conclusion that chronic symptomatology was not associated with a greater prevalence of neurotic psychiatric illness as judged by the General Health Questionnaire, but agreed with the findings of Smart and co-workers (1976) in the prostatodynia group. The age of the patients in the chronic abacterial prostatitis cohort, mean 39.8 years, was less than the chronic prostatitis patients in Smart's group (1976), 56 years and 48.5 years. Otherwise there appears little difference between the patients, who were diagnosed by standard localisation of studies (Meares & Stamey, 1968), in both cases, though the precise diagnostic criteria were not eluded to in this comparative study (Smart et al, 1976).

A detailed psychiatric assessment by Nilsson and colleagues (1975) divided a group of patients with symptoms of chronic prostatitis into three on the basis of findings on rectal examination, leucocyte count in the EPS, antibacterial activity of semen, including lysozyme

concentration and serological and culture data. The patients were then assigned a low, intermediate or high score. Although these sub-divisions do not adhere to the classical diagnostic criteria for prostatitis, the likelihood is that those with the low score would be assigned a diognosis of prostatodynia and those with a high score, prostatitis in the acepted classification (Drach et al, 1978). The psychological assessment in this work was detailed and performed by a trained psychiotrist, and provided indepth analysis of personality. It concluded that patients with prostatodynia displayed a significantly ($p < 0.05$) greater prevalence of mental disturbance, particularly, anxiety and sexual identity, than those with higher scores, and thus a probable diagnosis of chronic prostatitis. These findings concur with the psychological data in this thesis, but direct comparisons are not possible, due to the failure of the latter study (Nilsson et al, 1975) to adopt accepted criteria for a diagnosis of chronic prostatitis (Meares & Stamey, 1968) plus their use of different instruments and standards for the assessment of psychological disease. This group (Nilsson et al, 1975) concluded that prostatodynia is essentially a psychiatric disturbance whereas chronic prostatitis is a physical one, although there is a level of mental disturbance that may lead the latter group to contract sexually transmitted disease and thus, chronic prostatitis. The assumption that chronic prostatitis is a sexually acquired infection has not been borne out in this thesis and thus this psycho-pathological pathway would not appear valid.

The differentiation between prostatodynia and chronic prostatitis is sometimes far from clear cut and some patients originally placed in the former group may indeed be found to have chronic prostatitis on subsequent testing. Thus, it would be surprising if patients fell neatly into the two diagnostic groups, adopting psychological criteria alone; some degree of overlap would appear inevitable.

Additional, conflicting data, eminates from a study (Brahler et al, 1986) of patients with chronic bacterial and abacterial prostatitis and those with prostatodynia, who although accurately classified using standard localisation techniques, underwent psychiatric assessment using a non-standard, "in house" questionnaire technique. The conclusions of this work were that patients with chronic prostatitis voiced more "neurotically-conditioned prostatitis" symptoms than patients with prostatodynia. It is impossible to make direct comparisons of these betweens these conclusions and the findings in this thesis, as the means of assessment were different and, in the foremost case, unstandardised. Introduction of such subjective data into the equation only serves to cause further confusion. The possibility that chronic prostatitis and prostadodynia have broadly similar aetiological mechanisms seems unlikely and the psychological make up of the study groups, in this work at least, is noticeably different. These changes may simply reflect a different response to the same symptoms, however.

SUMMARY

By means of the General Health Questionnaire, patients with prostatodynia were found to have a significantly prevalence of neurotic psychiatric disease than patients with chronic abacterial prostatitis, $p < 0.002$, and a "control" group of chronic attenders of a general urology clinic ($p < 0.001$). A breakdown of the scores below the diagnostic level for neurotic illness revealed that the chronic abacterial and chronic attenders groups were broadly similar, yet a more even distribution of scores was observed in the prostatodynia group; the prevalence of low, "normal" scores, was also significantly less in the latter patients, $p < 0.01$ and $p < 0.001$ respectively, than in the other two study groups.

Furthermore, there was no correlation between symptom duration and the prevalence of neurotic psychiatric illness in both the chronic abacterial and prostatodynia patients.

SECTION 31 DIAGNOSTIC CRITERIA IN CHRONIC ABACTERIAL PROSTATITIS

Unfortunately there is no consensus in the literature concerning the diagnostic criteria for chronic prostatitis. This results in the study of heterogeneous populations of patients with the consequent accumulation of data that are difficult to interpret. Furthermore, and most importantly, it prevents comparison of data and few studies have been corroborated by other researchers, because of this lack of concordance.

The symptomatology of both chronic prostatitis and prostatodynia is varied, non-specific and strikingly similar (Meares, 1980; Orland et al, 1985; Pfau, 1986; Meares, 1987). Furthermore, there are few or possibly no clinical signs to support the diagnosis and thus it is hardly surprising that patient selection, on clinical grounds alone, is steeped in error. In practical terms, any research where patients are selected on these grounds is meaningless.

Analysis of prostatic fluid is therefore mandatory, yet difficult to interpret unless meticulous techniques are pursued (Meares & Stamey, 1968). Examination of prostatic fluid alone fails to exclude urethral or bladder sources for inflammatory cells and organisms, and again cultivates misleading information. A small number, between 3% and 6%

(Blacklock, 1969; Anderson & Weller, 1979; Schaeffer et al, 1981), of normal men will possess a leucocytosis above the considered upper limit of normal (10 wbc/hpf) (Anderson & Weller, 1979), within the expressed prostatic secretion (EPS); this figure may rise to 25% when a group of patients with non-inflammatory urological disease are studied (Schaeffer et al, 1981). There has been considerable debate, as eluded to in Section 2, concerning the level of leucocytosis within the EPS, deemed as pathological. The consensus view, however, rests at a level of 10 wbc/hpf (Anderson & Weller, 1979).

Potential sources of error reside in the natural variation in counts between normal individuals (O'Shaughnessy et al, 1956; Colleen & Mardh, 1975) as well as the effect of recent ejaculation (Jameson, 1967). Thus, it is advisable to perform at least two Stamey localisation procedures before assigning a patient to a diagnostic group; a recommendation not relished by patient and clinician alike.

The detection and isolation of organisms from the prostate is similarly fraught with error. Colony counts of any organism isolated are mandatory and the diagnostic criteria of a tenfold increase in organism numbers within either EPS or post-massage urine (VB3), over and above that in first void (VB1) and mid-stream urine (VB2) (Meares & Stamey, 1968), is the preferred method over simple organism numbers (Drach, 1975). The need to

perform a somewhat unpleasant, complicated and time-consuming diagnostic test lays the whole process open to a perfunctory approach.

The Stamey localisation procedure (Meares & Stamey, 1968) enables patients with a clinical diagnosis of prostatitis to be classified correctly in the majority of cases, yet is by no means failsafe. Although urethral contamination is taken into consideration, the long course of urine, but particularly prostatic secretions, over a potentially contaminated field, creates a distinct source of error. Often, the results of inflammatory cell content and organism numbers are not so neatly packaged to clearly differentiate between urethritis, cystitis or prostatitis. Furthermore, if an urinary tract infection is present, thereby masking the contribution from the prostate, attempts to sterilise the urine using an antibiotic that doesn't "penetrate" the prostate, may inadvertently destroy organisms, resulting in erroneous classification. An additional drawback of the technique is the all too common experience of a dry expressate after prostatic massage. Often prostatic fluid resides in the urethra and is expelled in the post-massage urine (VB3), but it's precise contribution is debatable. Finally, due to the focal nature of chronic prostatitis (McNeal, 1968) and ductal obstruction, the inflammatory lesions may be underrepresented in the leucocytosis generated in the EPS.

Thus, a need exists for a diagnostic method which is

simple, reproducible and whereby the prostate can be viewed in isolation. Although a sampling catheter, which isolates the prostatic urethra enabling direct collection of EPS, has been devised (Jin & Guagi, 1987), it has yet to find it's way into routine practice and can be criticised for being over invasive.

Transrectal prostatic ultrasound appeared to have provided the potential for ready diagnosis, but unfortunately there are too many signs indicative of a diagnosis of prostatitis and these lack either specificity or sensitivity with the resultant risk of false positive or false negative diagnosis respectively (see Sections 12 and 24). A normal prostatic ultrasound, however, makes a diagnosis of chronic prostatitis unlikely.

Similarly, there is no biochemical marker that can differentiate the inflamed from the normal gland, nor reflect the severity of the inflammatory process within a group of patients with chronic abacterial prostatitis. Prostate specific antigen (PSA), an excellent marker of prostate carcinoma (Shearer, 1991), has failed to fulfil a similar role in inflammatory disease (see Section 18 & 28), though levels may be elevated in granulomatous prostatitis (see Section 18 & 28; Liu et al, 1992) and values above the normal range have been attributed to prostatitis in patients where there is no evidence of malignacy (Morote et al, 1988; Brawer et al, 1989).

The histological features of chronic prostatitis are non-specific, though some authors try to subdivide the lesions (Kohnen & Drach, 1979) although this provides little additional understanding of the underlying pathophysiology. Thus, prostatic biopsy has no clinical role in the diagnosis of chronic prostatitis yet is a useful research tool when ultrasound guided.

Finally, there are few immunological markers that have a role in the routine diagnosis of chronic prostatitis. Analysis of the immunoglobulins within the EPS enables the monitoring of response to treatment in chronic bacterial prostatitis (Fowler & Mariano, 1984; Shortliffe & Wehner, 1986) and the presence of organism specific antibodies indicates the offending organism (Fowler & Mariano, 1984; Shortliffe & Wehner, 1986). Similar patterns of IgA and IgG levels in the EPS have been observed in chronic abacterial prostatitis, yet the overall levels are lower in the abacterial form and not specific for any organisms. The development of an ELISA technique to measure total and specific immunoglobulin levels in pre and post prostatic massage urine has provided an ingenious method of diagnosing chronic bacterial prostatitis (Shortliffe et al, 1989). Except by means of exclusion this test has little role in chronic abacterial prostatitis, though may find a similar niche once the inciting antigen is identified. The analysis of prostatic tissue using immunological probes has helped define the immunopathology of chronic abacterial prostatitis (see Sections 17 & 27).

It may have a continuing role in the study of response to treatment if therapy is directed at immune manipulation, yet in the routine clinical setting, such analyses have no place.

The need to separate patients with a clinical diagnosis of chronic prostatitis into their classifying subsets is not only to apply appropriate treatment, but also to further define the underlying pathophysiology. The management of chronic bacterial prostatitis rests with antimicrobial therapy which, if not accompanied by cure, usually results in a subjective and objective response. Alas, the management of chronic abacterial prostatitis and prostatodynia is less clear.

There is no method of separating chronic abacterial prostatitis and prostatodynia on clinical grounds and the aetiology of both conditions remains unknown. This begs the question as to a common causation with the two conditions occupying different positions within a spectrum of disease.

In support of a common aetiology is the indistinguishable symptomatology and similar urodynamic data. These display a failure of relaxation of the distal sphicter complex in both groups. In this thesis the chronic abacterial prostatitis cohort possessed slower flow rates than the prostatodynia patients, yet the differences were barely significant (see Section 19 & 29). High prostatic

urethral pressures have been observed in chronic prostatitis (Buck, 1975; Siroky et al, 1981) and prostatodynia (Osborn et al, 1981, Barbalias et al, 1983; Meares, 1986; Hellstrom et al, 1987) due to failure of the smooth muscle fibres, innervated by the sympathetic nervous system to relax (Hellstrom et al, 1987). This abnormality may be behavioural. The outcome is a predilection for urinary reflux into the prostate. Why should such a process result in an inflammatory response in some patients yet an inert reaction in those with prostatodynia? It may be that the urine acts as a carrier of the initiating antigen in chronic prostatitis, yet patients with prostatodynia lack such an agent. Why then the similar symptoms? Perhaps it is the act of reflux that produces symptoms, not the inflammatory response. Indeed there was no correlation between degree of inflammation and symptoms or their duration in the examination of prostatic tissue in this thesis (see Sections 16 & 26).

The transrectal ultrasound data (see Sections 12 and 24) indicated a clear demarcation between the two groups and suggested that a normal ultrasound scan adds weight to a diagnosis of prostatodynia. However, patients in this latter category displayed more ultrasound signs the normal controls, possibly due to the effects of urinary reflux. Serial scanning though, indicated few changes with time in the sonographs of the prostatodynia patients in contrast to the chronic prostatitis cohort.

Finally, the psychological testing (see Sections 21 & 30) found a greater tendency towards neurotic psychiatric illness in the prostatodynia group. Furthermore, the chronic prostatitis cohort had no greater tendency to such illness than a "chronic" control group of regular long term attenders of an urology out patients; additional weight to the arguement that chronic abacterial prostatitis and prostatodynia are distinct conditions.

The issue of whether chronic abacterial prostatitis and prostatodynia are different entities or spectra of the same, remains unresolved, though there are sufficient pointers in this thesis to endorse a separate aetiology and pathophysiology.

SUMMARY

Twenty four years on from its inception, the Stamey localisation procedure (Meares & Stamey, 1968) remains the mandatory investigation and mainstay of diagnosis for chronic prostatitis. Furthermore, a solitary procedure may be insufficient. Significant inroads have been made towards defining the aetiology of chronic abacterial prostatitis, yet transrectal ultrasound guided biopsy, histological and immunological analysis of the prostatic tissue obtained, have little clinical value, but provide useful avenues for research.

Much is made of the need to differentiate chronic abacterial prostatitis from prostatodynia and indeed it is essential for the definition of aetiology and pathophysiology. Although this thesis has found the two conditions to be independent in the main, the possibility of common ground exists especially with urodynamic findings and the role of intraprostatic urinary reflux.

SECTION 32 THE ATTEMPTED CONSTRUCTION OF AN ANIMAL MODEL OF CHLAMYDIAL PROSTATITIS

The construction of animal models to study infertility and lower genital tract infections caused by Chlamydia trachomatis in female mice is well established, (Tuffrey et al, 1986a; 1986b).

A rather unconvincing, non-physiological model of chlaymdial prostatitis has been devised, (Nielsen et al, 1982), yet infection was achieved by direct innoculation and despite an inflammatory response on histology, organisms could not be recovered directly from the gland. This mechanism could be rationalised by a rapid clearance of viable organisms from the prostate, yet persistence of sufficient, (possibly "protected") antigen, to incite an inflammatory response, (Nickel et al, 1990). A similar pathophysiological pathway has been proposed in the human (Shurbaji et al, 1988) in a retrospective histological study.

The value of an animal model of chlaymdial prostatitis would be the study of the likely route of infection and the underlying pathological and immunological mechanisms, from which a rational management plan could be constructed. However, the failure to create such a situation merits scrutiny.

The failure to infect the Mastomys natelensis rats with Chlamydia trachomatis may rest with the animal itself, perhaps in possessing specific defence mechanisms against the organism. This species of rat has been employed in studies using Klebsiella organisms where prostatitis was satisfactorily produced (Weidner et al, 1981), by innoculation of the bacteria into the bladder. However, the successful chlamydial model of Nielsen and colleagues (1982) employed mongrel dogs.

The innoculum adopted in this study was 2×10^7 and 6×10^6 inclusion forming units in the two experiments, compared to 10^6 (Baumueller & Madsen, 1977a), 10^7 (Weidner et al, 1981) and 10^6 and 10^7 (Nielsen et al, 1982) organisms in other animal model of chronic prostatitis studies. It would seem unlikely that insufficient organisms were presented to the prostate, though if less virulent, the organism may be needed in greater numbers to induce an infection.

Well established methods of isolation and detection of chlamydial organisms where adopted (Thomas et al, 1977; Evans & Woodland, 1983; Thomas et al, 1984) and failure of these methods is an unlikely source of the negative results obtained. Furthermore, there was no histological evidence of an inflammatory response, providing added weight to the validity of these negative results.

Small numbers of rats were studied, but there appeared to

be no justification in persuing the investigation involving further rat sacrifice in the light of totally negative data.

Although the intra-canalicular route, ie. from urethra to prostatic acini via the prostatic ducts, has been successfully employed in studies with Enterobacteriaceae organisms, it may not be a valid route when attempting to incite a chlamydial infection in the prostate. In this thesis, no aetiological role in chronic abacterial prostatitis could be apportioned to Chlamydia trachomatis, and no baterological or serological evidence for the presence of this organism in the prostate could be found (see Sections 14 & 25a). Furthermore, in the case of acute non-gonococcal urethritis caused by Chlamydia trachomatis, in a small study, again the organism will not be isolated form the prostate (see Section 15 & 25b). Therefore, is the prostatic ductal route of speed of this organism, in the human, counteracted by local mechanical, biochemical or immunological mechanisms? If so, do they also exist in the Mastomys natalensis rat? An alternative view, may be that the organisms do in fact exist, but encased in a protective glycocalyx (Nickel et al, 1990) and thus, are difficult to identify and isolate. However, chlamydiae are obligate intra-cellular organisms and would be unlikely to survive for any length of time in an extra-cellular site particularly if clothed in a glycocalyx.

The previously quoted study of chronic bacterial prostatitis (Nickel et al, 1990), does however raise a number of discussion points pertinent to this thesis.

The histological feature in the animal model traversed an "acute phase" characterised by polymorphonuclear cells before entering the chronic state where the inflammatory infiltrate consisted chiefly of mononuclear cells with an acinar and periglandular distribution accompanied by variable degrees of fibrosis. In this thesis the infiltrate had a periglandular (57%) or stromal (45%) distribution most commonly, but fibrosis was present in only 30% of biopsies. Although there are broad similarities between the pathological findings in the two studies, the animal model is by no means analagous to the human situation, at least, of chronic abacterial prostatitis. This may be due to the latter having had a longer period to progress down a chronic pathway with regard to the distribution of inflammatory cells and also that the initiating antigen is non-organismal. The extensive fibrosis detected in the animal model may in part be artefactual due to the "forcible" entry of organisms created by high pressure reflux into the prostatic ducts during innoculation, with resultant ductal rupture.

A striking difference to human chronic prostatitis is that rarely is chronic bacterial prostatitis preceded by acute bacterial prostatitis, though perhaps an acute phase is

missed in the clinical setting when the prostate is first exposed to the organism. In chronic abacterial prostatitis, no acute phase was evident clinically, but 8% of biopsies contained acute inflammatory cells in conjunction with a chronic infiltrate, though their significance was not defined.

The animal model devised my Nickel and colleagues (1990) is undoubtedly well constructed and reproducible, but has a significant flaw in that it induced prostatitis in the ventral lobe of the prostate, analagous to the central zone in the human. However, an autopsy study of chronic prostatitis (McNeal, 1968), most commonly implicated the peripheral zone, with only 5% involving the central zone.

To date the aetiology of chronic abacterial prostatitis remains uncertain, but also non-organismal, a view put forward in this thesis. However, it remains conceivable that small numbers of organisms may be trapped in the prostate, exerting their influence on the immune system, yet escaping detection. The transmission electron microscopy data from Nickel and co-worker's (1990) study supported this notion and indeed cited the protective barrier, the glycocalyx, as the inducing agent of the immune response. However, this scenario may not be applicable to Chlamydia trachomatis, as discussed above. The hypothesis that substances other than viable organisms act as the inciting antigen is attractive and would be an avenue to explore in further studies into chronic abacterial prostatitis.

Finally, in this thesis, immunofluorescence studies detected IgM deposition within the prostate, most commonly at periglandular sites, though also within the gland and luminal cells, albeit less commonly. Although in the chronic bacterial prostatitis model (Nickel et al, 1990) IgG was detected in addition to IgM, their distribution was similar to the findings in humans. This raises the possibility of a similar route of entry of antigen, at least, namely the intracanalicular pathway.

SUMMARY

It was not possible to construct a rat model of chronic prostatitis using Mastomys natalensis despite adequate numbers of organisms (Chlamydia trachomatis) and proven methods of detection and isolation. This may have been due to the species of rat or alternatively, the attempted route of infection, namely the intra-canalicular pathway, being inappropriate. This pathway, however, would appear to be the valid portal of entry of the inducing antigen from the data accumulated thusfar in this thesis.

An animal model of chronic abacterial prostatitis would indeed be meritorious, yet only if it can be constructed so as to mirror closely the human situation. As yet the latter is still far from being unravelled and thus, such an animal model may be unachievable, at least for the present.

SECTION 33 ACUTE EPIDIDYMITIS - A MICROBIOLOGICAL AND ULTRASONOGRAPHIC STUDY

This study is the first to investigate acute epididymitis by the combined approach of epididymal aspiration, first employed by Furness and co-workers (1971), yet later described in a number of publications (Berger et al, 1978; 1979; 1980), and transrectal prostatic ultrasound. The aim was to define further the role played by the prostate in acute epididymitis, eluded to in previous studies (Grant et al, 1985; 1987; Melekos & Asbach, 1987; Weidner et al, 1987) in which microbiological studies alone of urethral swabs, fractionated urines and expressed prostatic secretion (EPS) were undertaken in acute epidiymitis. The importance of Chlamydia trachomatis as an aetiological agent was re-affirmed in this thesis with an overall prevalence of 42% in the group studied. This supports the findings of a number of studies which reported prevalence rates of C. trachomatis in acute epididymitis of 28% to 56% (Berger et al, 1979; Grant et al, 1985; 1987; Melekos & Asbach, 1987; Mulcahy et al, 1987; Weidner et al, 1987; De Jong et al, 1988. However, those patients with a chlamydial aetiology reside in the younger age groups (less than 35 to 40 years in the above studies) and if the C. trachomatis prevalence amongst these age groups is considered, values of 31% to 76% are achieved, compared to 48% observed in this thesis. The small difference between the prevalence rates for C.

trachomatis detection in the less than 35 years and all age groups, in this work, 48% versus 42%, is explained by 21 (87.5%) of the 24 patients studied being in the former age bracket.

Organisms regarded urinary tract pathogens, i.e. the Enterobacteriaceae family and Streptococcus faecalis, were isolated in 21% of cases. This is in keeping with the findings of a number of studies (Berger, et al, 1979; Grant et al, 1987; Mulcahy et al, 1987), where these organisms were isolatred in 15-26% of cases; 68-78% of the patients investigatedin the studies were in the "younger" group (less than 35 - 40 years) compared to 87.5% in this thesis. Those researchers with higher urinary tract pathogen isolation rates, 40% (De Jong et al, 1988) and 43% (Melekos & Asbach, 1987) had a lower proportion of patients in the "younger" category: 52% and 53% respectively. However, the recovery of E. coli, in one patient and Streptococcus faecalis in another, aged 34 and 24 years respectively, who on subsequent investigation had no anatomical or functional abnormality of their urinary tract were notable exceptions to the age rule. Therefore, neither bladder outlet obstruction nor seniority are obligatory aetiological factors in acute epididymitis caused by the common urinary tract pathogens, and these organisms must be sought in all age groups.

Two patients (8%) were noted to have Ureaplasma urealyticum at titres ($> 10^4$) deemed sufficently high to

imply infectivity. Weidner and co-workers (1987) isolated this organism in 3.5% of 114 patients, and Berger and colleagues (1979) in 56% of 34 patients, though no information or organism titres was provided. In the latter study ureaplasmas were isolated in conjunction with the organisms, as was observed in one of the patients in this work, in 58% of cases. However, the failure to provide information on organism numbers creates great difficulty in interpretation of these data, as these organisms have been found in the mid-stream urines of 22% of male patients aged less than 50 years attending a non-venereal out patient clinic and 20% of male volunteers of similar age, with geometric mean titres of $10^{2.5}$ and 10^{24}, respectively (Furr & Taylor-Robinson, 1987). With such high isolation rates, it would be reasonable to propose that an appreicable number were of low titre in the report of Berger and colleagues (1979).

The use of epididymal aspiration has been criticised for unneccessary interference with an inflamed epididymis (Grant et al, 1987) and for failing to provide information that cannot be obtained from urethral cultures (Melekos & Asbach, 1987; De Jong et al, 1988). However, there were no short-term sequelae as a direct result of epididymal aspiration in this thesis or the work of Berger and colleagues (1980), and no direct reference to complications was made in an earlier study (Berger et al, 1979).

In support of epididymal aspiration, two cases of C. trachomatis infection were diagnosed solely on the findings within the epidiymal aspirate, a similar occurrence being encountered by Scheibel and colleagues (1983). Furthermore, one case of epididymitis due to Ureaplasma urealyticum was diagnosed by finding a higher titre of organisms in the epdidymal aspirate, compared to the urethral swab; this was the first recorded case of Ureaplasma urealyticum from the epididymis of a patient with epididymitis and thus the first conclusive evidence of an intra-canalicular spread of this organism from the urethra to the epididymis. In an additional case, one of mixed infection, both organisms were recovered from the epididymis, thereby implicating them both in pathogenesis.

The detection of organisms within the epididymis as well as from mid-stream urine or the urethra provides direct evidence of an intra-canalicular route of infection, postulated by others (Rinker et al, 1970), presumably due to vasal reflux of urine (Gartman, 1961; Mittemeyer et al, 1966; Koff, 1976), in support of the experimental model of reflux of sterile urine (Graves & Engel, 1950).

In two patients, Ureaplasma urealyticum, and in a further two, Mycoplasma hominis, were isolated from the ureathra alone at titres of 10^3. As both these organisms have been found in asymptomatic individuals (patients attending non-venereal clinics and volunteers) in the order of 20-23% in the case of U. urealyticum and 4-6% in that of

M. hominis (Furr & Taylor-Robinson, 1987), caution has to be exercised in assigning a causal role to these organisms. It is generally regarded taht titres > 10^4 imply infectivity, and therefore titres of 10^3 within the urethra alone may just reflect asymptomatic colonisation. Thus, this work is in agreement with other studies (Berger et al, 1978; 1979; Weidner et al, 1987) which concluded that these organisms played a minor role in the aetioloy of acute epididymititis. In only two of the 15 patients in whom organisms were detected, did urethral swabs or mid-stream urine reveal organisms, yet epididymal aspirates prove sterile.

In 9 patients (37.5%), despite the extensive microbiological survey, micro-organisms were not detected. It appears that neither U. urealyticum nor M. hominis accounted for the shortfall in positive cultures. It is possible that organisms such as chlamydiae were actually present, but failed to be detected. However, the serological data, vide infra, does not support this proposal, where a clear demarcation existed that a titre of anti-chlamydial IgG antibody of 1:>64 and the detection of C. trachomatis. The mean age of the culture negative group, 22.6 years, would indicate that a search for sexually acquired organisms including viruses may be fruitful. However, no clear indicator to a likely organisms is at present available.

Clearly epdidymal aspiration is a useful research tool, and enables C. trachomatis in younger patients (less than 35 years) and Enterobacteriaceae in the older ones (greater than 35 years) to be implicated unequivocally in the aetiology of acute epididymitis (Berger et al, 1979; 1980; Scheibel et al, 1983). However, it does appear to be over invasive for routine practice. Thus, these data support the views of Berger and colleagues (1980) in advocating epidiymal aspiration for cases where mixed organisms are isolated from urethral and mid-stream urine specimens, patients who fail to respond to initial therapy and cases where sufficient diagnostic doubt exists to require surgical exploration. The long term complications of epididymal aspiration have not been assessed, but they are unlikely to outweigh those due to the inflammatory process itself.

Serological tests alone for the diagnosis of C trachomatis infections are of little value (Schacter & Dawson, 1978; Treharne et al, 1983), but may provide supportive data. The presence of IgM antibody is highly specific for active disease and a four-fold increase in the IgG antibody, again provides strong corroborative evidence of active infection (Treharne et al, 1983).

Rather unexpectedly a study provided a very close correlation between anti-chlamydial antibody titres and the recovery of organisms: when the highest titres achieved were analysed, no patient in whom C. trachomatis

was detected, had an anti-chlamydial IgG antibody titre less than 1:64, whereas all patients with whom C. trachomatis was not detected had titres of less than 1:64 and 10 (71%) of them had titres of 1:<8. Only two of the ten C. trachomatis cases however, demonstrated a four-fold increase, and five cases had titres at the upper range of presentation, ie, 1:≥256; all these five cases had IgM anti-chlamydial antibody titres of at least 1:16. Interestingly, the two cases that demonstrated the four-fold rise in anti-chlamydial IgG titres, failed to mount an appreciable IgM response, with titres of 1:4 and 1:8. The reason for the lack of this IgM response in the face of a rising IgG titre, may have been technical, as both these patients presented within five days the onset of symptoms and the IgM titres in the other C. trachomatis positive patients showed persistence of the IgM anti-chlamydial antibodies. Alternatively, the measurement of the anti-chlamydial antibodies may have fallen in the period between IgM and IgG curves of the primary response, Fig. 33.1, such that the IgM titres were low and the IgG titres rising. The high titres of both IgG and IgM anti-chlamydial antibody encountered in the five cases with maximal values, (ie, 1:>256) of anti-chlamydial IgG, are indicative of a secondary response to the chlamydial antigen, which is associated with a prolonged plateau of both IgG and IgM antibodies, Fig. 33.1. Similar data were recorded by De Jong and co-workers (1988), who also employed a micro immuno-fluorescence technique, and found titres of

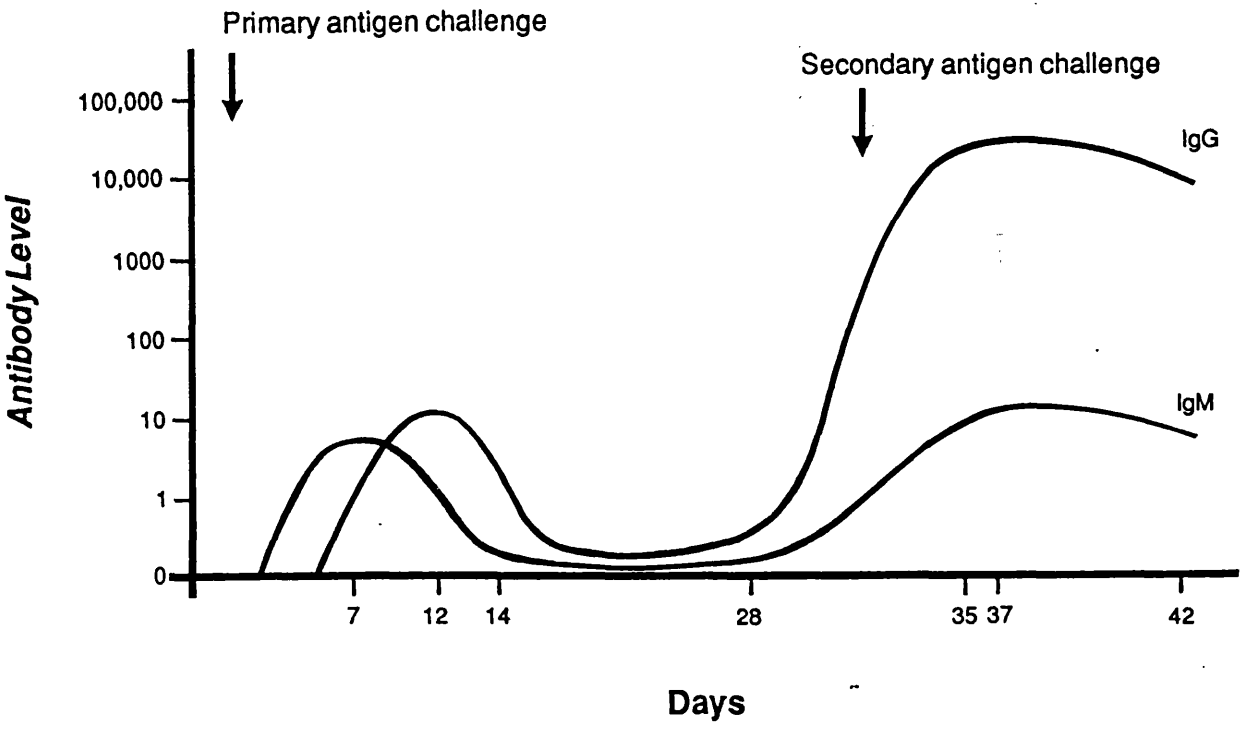


Fig. 33.1 **Primary and Secondary Antibody Responses**

After Roiff Bostoff & Male (1985)

anti-chlamydial IgG antibody of 1:>64 in eight out of ten cases in whom C. trachomatis was isolated; the remaining two had titres of 1:16. The same eight cases displayed an IgM response, though no details of titres were provided. Berger and colleagues (1979) observed significant differences in anti-chlamydial antibody titres, though the class of antibody was not stated, using the same micro-immuno-fluorescence technique: 94% of the C. trachomatis positive arm possessing antibodies compared to 40% in the non-chlamydial patients, $p<0.001$, and 62% versus 12.5%, $p<0.05$, with respect to a four-fold rise in titre in these groups. Both these studies, emphasise the support of role of serological analysis plays, though the latter study highlights the risks of relying on serological data alone. Some of the patients with negative chlamydial cultures may be wrongly categorised, but more likely, persistence of anti-chlamydial antibody may result in false positive diagnoses if serological information is not supported by attempts to detect the organism.

Transectal prostatic ultrasound provides objective data on the prostate, though the signs are not necessarily specific for any one disease (Rifkin, 1987), and as outlined in Sections 12 and 24, caution has to be exercised when analysing the signs attributed to a diagnosis of inflammatory prostatic disease. However, with 92% of cases possessing abnormal scans, most commonly mid-range echoes (MRE) (75%), indicative of either

inflammation and or fibrosis (see Section 12), the possibility of concomitant prostatic infection at the time of the epidiymitis is raised. Furthermore, the ultrasound sign which is most predictive, in isolation, of inflammation within the prostate, the echolucent zone (ELZ), (see Sections 12 & 24), was noted in 10 cases (42%), all of whom had organisms detected, whereas in the negative cultures group, no case displayed this sign. Although, C. trachomatis and other organisms, could not be isolated from such lesions, (see Sections 14 & 25), in patients with chronic abacterial prostatitis, this does not preclude their presence in such lesions in acute epididymitis.

The possibility of concomitant infection of the prostate or seminal vesicles in acute eididymitis was mooted by Handley (1946) in a study of young (average age 30 years) army personnel. Further support for this theory was proffered by Weidner and colleagues (1987) who detected evidence of prostatitis, on prostatic localisation studies (Meares & Stamey, 1968) in 22 (19%) of 114 patients with acute eididymitis. Further analysis of their data revealed that evidence of prostatitis occured in 18% of cases of infection by Enterobacteriaceae, 15% of C. trachomatis, 50% of U. urealyticum, and 20% of cases where no organisms were isolated. Examination of the expressed prostatic secretion in patients with epididymitis (Grant et al, 1987) isolated C. trachomatis in 5 out of 12 patients, all of whom had positive urethral cultures, and

other organisms (Escherichia coli, Neisseria gonorrhoeae, Streptococcus faecalis and Lancefield group B streptococcus) in 6 out 54 patients, though full details of the localisation techniques were not provided. Both these studies raised the possibility of the aetiological organism of the epididymitis also being responsible for a concomitant prostatitis, but in both cases it was impossible to rule out urethral contamination of the samples, especially in the case of C. trachomatis, where in no case was the organism detected in the prostatic fluid but not the urethra. Further information on this score is provided by Melekos and Asbach (1987) in their investigation of a group of patients with acute and chronic epididymitis, in whom they performed urethral swabs, mid-stream urine analysis, further urethral swabs following prostatic massage and also scrotal exploration and epididymal sampling. Of 20 patients who underwent all these studies, all had positive epididymal cultures, of whom 17 had positive urethral cultures following prostatic massage, but in only 1 case did the urethral swabs following prostatic massage reveal an organism that had not been detected in either the urethral swabs or the mid-stream urine. In only this 1 case can urethral contamination be deemed unlikely and a concomitant prostatitis be proposed. Although recurrent epididymitis is more likely to be due to and underlying prostatitis (Meares, 1985), the corroborative evidence for a concomitant prostatitis accompanying an acute eididymitis is more circumstantial than definitive, due to the

inescapable problem of urethral contamination of expressed prostatic secretion. However, the prostatic ultrasound data adds weight to the concomitant prostatitis hypothesis.

An alternative explanation of the prostatic ultrasound findings noted in this work, is that they reflect the underlying cause, namely intraprostatic reflux of urine, which has been demonstrated (see Section 20) in this thesis and others (Buck, 1975; Kirby et al, 1982), in patients with chronic abacterial prostatitis. This phenomenon, may also occur along the ejaculatory ducts as postulated earlier, (Gartman, 1961; Mittemeyer et al, 1966; Koff, 1976) and produced the ultrasound feature, of ejaculatory duct echoes. If organisms are residing in the urethra or urine at the time of such reflux, then they may be carried to the epididymis and produce an inflammatory response. This theoretical intracanalicular route of spread has been supported, not only by this study, but by other workers (Berger et al, 1979: Scheibel et al, 1983; Melekos & Asbach, 1987) and is presently undisputed.

The findings of this thesis have two major implications for the treatment of acute epididymits: a) in view of the possible concomitant prostatitis, treatment should be prolonged, up to six weeks, with an appropriate antimicrobial agent and b) in all patients in whom C. trachomatis is detected, a thorough assessment of consorts should be carried out.

SUMMARY

Acute epidiymitis is most commonly caused by Chlamydia trachomatis in patients less than 35 years and by Enterobacteriaceae organisms above this age. The mode of transmission is by intracanalicular spread of organisms. The prostatic ultrasound findings support the proposal that, in some cases at least, acute epididymitis is accompanied by a concomitant prostatitis. In such cases treatment should be prolonged for a six week period. The role of epididymal aspiration should be confined to cases of mixed infection, patients who fail to respond to therapy, cases in the whom surgical exploration is necessary in view of diognostic doubt and patients with recurrent infections. The causeative organism can generally be isolated from urethral swabs or mid-stream urine and additional supportive information may be gleaned from chlamydial seriology.

SUMMARY OF DISCUSSION

Transrectal ultrasound (TRUS) identified a number of abnormal signs which although present in a minority of normal subjects, provided support for a diagnosis of inflammatory prostatic disease. The drawback of high sensitivity yet low specifity with two of the signs and the resultant risks of false positives, yet the converse with the remaining five signs, and the consequent risk of false negatives, ensured that TRUS alone could not be used to make a diagnosis of chronic prostatitis; localisation studies (the Stamey procedure) were mandatory. The parenchymal signs represented certain histological features as determined by ultrasound guided biopsy; the greatest value of TRUS in the study of any prostatic disease.

A cohort with chronic abacterial prostatitis was created and a search made for any organisms which might play an aetiological role in the condition. The technique of TRUS guided biopsy was employed in an attempt to circumvent the perennial problem of urethral contamination encountered in standard localisation studies. However, no organism was identified either by standard localisation methods or from the biopsy material. In particular Chlamydia trachomatis, with the added endorsement of negative serological data, as well as Mycoplasma hominis and Ureaplasma urealyticum, could not be implicated in the aetiology of chronic

abacterial prostatitis. Furthermore, the migration of Chlamydia trachomatis into the prostate from an urethral location could not be identified in acute chlamydial urethritis.

The routine histological data yielded a high (85%) prevalence of inflammatory changes, which were generally of low grade and occupied a periglandular or stromal site. Although there was a close correlation between the parenchymal ultrasonograph abnormalities and inflammation, fibrosis and corpora amylaceae, no histological pattern pathognomic of chronic abacterial prostatitis could be identified. Possibly due to the majority of lesions being of low grade, no correlation existed between histology and Stamey score (degree of inflammation in prostatic fluid), although there was closer concordance with the more severe inflammatory lesions in the prostatic tissue. These data reflected the possible misrepresentation of individual inflammatory lesions by the localisation procedures, which analyse "pooled" material. Certain features, namely fibrosis, vessel thickening and telangiectasia, were found in the biopsies from the cohort, but not normal controls, however, no link between these findings and symptoms, their duration, or inflammatory grade could be identified. Thus standard histology of inflammatory lesions provided crude, non-specific data and could not be relied upon to make a diagnosis of any one type prostatitis, save for the granulomatous form; to make a diagnosis of prostatitis based on histology alone is invalid. Also mast cells,

although more prevalent in the prostatitis lesions, provided no additional enlightenment to aetiology.

The finding of intraprostatic antibody deposition in the majority of prostatitic lesions, but not controls, raised the possibility of local immunoglobulin production. Yet neither plasma cells nor B cells could be identified, suggesting that the antibodies were produced at a distant site, some possibly forming circulating immune complexes, before depositing in the prostate. The link with symptoms of poor flow and irritative voiding implied that voiding dysfunction and possibly intraprostatic urinary reflux may be involved.

The phenotype of the inflammatory cell population in the various biopsies indicated an active, cell-mediated immune reaction, more in keeping with persistent antigen presence and a type IV hypersensitivity reaction; an auto-immune process would appear unlikely. The possibility of the low grade lesions representing a "burnt out" scenario was not born out in this part of the study, though caution must be exercised in postulating the dynamics of a process when only static observations have been made.

Prostate specific antigen (PSA) appeared not to have any role either in the diagnosis or management of chronic abacterial prostatitis.

In both chronic abacterial prostatitis and prostatodynia, a proportion of patients had abnormally low free urinary flow rates, based on nomogram data, though more commonly in the cohort. Although, no studies on distal sphincter function or urethral pressure profiles were performed, it was postulated, on the basis of the research of others, that these low flow rates may be due to abnormal distal sphincter function, resulting in high intra-prostatic urethral pressures; intra-prostatic urinary reflux may then ensue. This phenomenon was identified in a proportion of the chronic abacterial prostatitis cohort. It was postulated that the persistent antigen responsible for the inflammatory reaction may thus be transported into the prostate.

Psychological assessment of the cohort, by means of the GHQ-60 questionnaire, failed to detect any higher prevalence of neurotic psychiatric illness than in a chronic illness control group, in contrast to a prostatodynia group. Thus, it was concluded that there was no clear evidence of a psychological aetiology in chronic abacterial prostatitis.

The inability to construct an animal model of intra-canalicular spead of Chlamydia trachomatis into the prostate, failed to provide any extrapolative information on such a phenomenon in the human. Until there is more understanding of the pathophysiology of chronic prostatitis, construction of animal models would appear profitless.

The role of Chlamydia trachomatis in acute epididymitis was confirmed, and of Ureaplasma urealyticum identified, in patients less than thirty five years, by means of epididymal aspiration. Furthermore, the high prevalence of abnormal prostatic ultrasonographs in this group, postulated either a concomitant prostatitis or a common aetiological factor in intra-prostatic urinary reflux; the latter common denominator is preferred. However, prolonged antibiotic treatment of cases of acute epididymitis with ultrasound abnormalities, would appear prudent.

CONCLUSIONS AND CLAIMS TO ORIGINALITY

1. The construction of a cohort of patients with chronic abacterial prostatitis enabled the study of an accurately diagnosed group as defined by standard criteria. The cohort, patients with prostatodynia and control groups were sub-classified by means of a scoring system based on the leucocyte count within the post-massage urine (VB3). Thus, the Stamey score system was devised, enabling correlation of data with the inflammatory changes detected in localisation studies.

2. Transrectal prostatic ultrasound provided a means of gaining objective data on the topography of the gland in chronic inflammatory prostatic disease. This thesis provided the first detailed study of a group of patients defined as normal controls, after undergoing prostatic localisation studies to exclude prostatitis, and described their ultrasound findings.

3. Seven ultrasound signs (high density and mid-range echoes, echolucent zones, capsular irregularity and thickening, ejaculatory duct calcifications and periurethral zone irregularity) were found to display a significant

correlation with a diagnosis of chronic prostatitis. This thesis provided the first report of the relationship between the leucocyte count in expressed prostatic secretion (EPS) and post-massage urine (VB3), and ultrasound signs in patients with an accurate diagnosis of chronic abacterial prostatitis (CABP) and prostatodynia (Pd).

4. The sensitivity and specificity for each sign with respect to a diagnosis of chronic prostatitis was calculated; the first time such an exercise has been performed. The ultrasound signs of high density and mid-range echoes have low specificity (40.7% and 57.9% respectively) yet high sensitivity (86.3% and 92.5%), for a diagnosis of chronic prostatitis, compared to the converse with the remainder of the signs, (sensitivity ranges 30.8% to 62.3%; specificity ranges 68.5% to 94.4%). Thus, no one ultrasound sign was diagnostic of chronic prostatitis, though the echolucent zone (sensitivity 61%, specificity 94.4%) held additional significance in reflecting the changing inflammatory pattern on serial scanning.

5. The "best fit" of sensitivity and specificity for the combination of signs rests at a Stamey score of >2. The finding of several ultrasound signs

in one patient favours a diagnosis of chronic prostatitis, whereas a normal scan in a symptomatic patient is highly suggestive of prostatodynia.

6. The follow up ultrasound data in the CABP cohort and prostatodynia patients concluded that in the former, the echolucent zone was most indicative of changes in the inflammatory status of the gland, whereas in the latter, parenchymal changes did not occur. This thesis provided the first report of follow up prostatic ultrasound data in relationship to prostatic localisation studies.

7. By means of ultrasound guided biopsy, the pathology of the ultrasound signs was defined: high density echoes (corpora amylaceae), mid-range echoes (inflammation and/or fibrosis) and echolucent zones (inflammation); the first such time this has been undertaken.

8. There was no correlation between the ultrasound signs and either age of symptom duration.

9. This thesis was the first study to utilise ultrasound guided biopsy in an attempt to detect organisms, in particular Chlamydia trachomatis, mycoplasmas and ureaplasmas, by means of culture and immunofluorescence techniques, in patients with chronic abacterial prostatitis.

10. The above biopsy technique prevented the perennial problem of urethral contamination of specimens, yet introduced the possibility of contamination by perineal organisms.

11. Chlamydia trachomatis was not detected in either the prostatic fluid or tissue by means of culture and immunofluorescence techniques. These data were supported by negative serological studies, with the resultant conclusion that Chlamydia trachomatis is not directly implicated in chronic abacterial prostatitis.

12. Similarly, no causative role for either Mycoplasma hominis or Ureaplasma urealyticum in chronic prostatitis, could be found.

13. There was no evidence of prostatic infection by Chlamydia trachomatis in patients with acute urethritis caused by that organism. Such a search utilising ultrasound guided aspiration biopsy, has not been undertaken previously.

14. The study of the histology of chronic abacterial prostatitis using ultrasound guided biopsy was performed for the first time in this thesis. Thus, by analysing an accurretly diagnosed group of patients and obtaining prostatic tissue from

areas deemed abnormal on prostatic ultrasound, as well as studying normal controls, an insight into the pathology of the condition has at least been achieved.

15. The pathology residing in the parenchymal ultrasound abnormalities was defined, (see 7). Furthermore, a grading system of inflammation in the tissue was devised, and indicated a lack of correlation between the findings in prostatic tissue and either prostatic fluid or post-massage urine.

16. There was no correlation between histological grade and any particular symptom or it's duration. Furthermore, no particular pathological abnormality showed any correlation with symptoms, their duration or patient's age.

17. The most prevalent inflammatory lesion was of low grade, occupying either a periglandular or stromal location.

18. Vessel changes and fibrosis were only detected in patients with chronic prostatitis, and not controls, and corpora amylaceae were more prevalent in the former, all implying a link with pathophysiology.

19. A quantified mast cell count in prostatic tissue from a chronic prostatitis cohort or a control group has not been reported previously. In this thesis there was no correlation between mast cell count and symptomatology or histology.

20. The search for antibody, complement and fibrinogen deposition in the prostatic biopsies, obtained by ultrasound guidance from a chronic prostatitis cohort and normal controls, had not been undertaken previously; neither had any correlation between the findings and either symptoms or routine histology.

21. A significant difference between controls and the cohort in antibody, complement and fibrinogen deposition, was observed. In addition, a significant correlation between this deposition and symptoms of poor flow, irritative voiding, a past history of non-specific urethritis.

22. This thesis provided the first information on the phenotype of the inflammatory cell infiltrate in the prostatic tissue in chronic abacterial prostatitis, and thereby the first accurate postulate on the underlying immunopathology.

23. An active immunological basis for the inflammatory changes was defined, consisting of a

type IV hypersensitivity reaction, in keeping with a persistent antigenic presence, rather than an auto-immune process.

24. The first study of serum prostate specific antigen (PSA) levels in chronic abacterial prostatitis was provided by this thesis. There was no significant deviation from normal except in one case of granulomatous prostatitis. Thus, PSA has no role in the diagnosis of chronic abacterial prostatitis.

25. This thesis confirmed the finding of abnormally low free urinary flow rates in both chronic abacterial prostatitis and prostatodynia.

26. The correlation between serial flow rates, analysed by nomograms, and the findings of serial localisation studies and histological data, has not been undertaken previously. Although the difference in flow rates between prostatodynia and chronic abacterial prostatitis patients just achieved significance, no such correlation existed between flow rates and symptoms, or inflammatory changes in either prostatic fluid or tissue.

27. The cause of the abnormal flow rate may indicate a similar aetiology in both chronic abacterial prostatitis and prostatodynia.

28. A proportion of patients within the chronic abacterial prostatitis cohort displayed intra-prostatic urinary reflux, a proposed aetiological mechanism.

29. Employment of the GHQ-60 questionnaire identified that patients with prostatodynia had a greater prevalence of functional psychiatric illness than either patients with chronic abacterial prostatitis or those attending an urology clinic on a chronic basis; this has not been identified previously.

30. There was no correlation between GHQ-60 score and prolonged symptom duration. Nor was there any statistical significance in score between the chronic prostatitis cohort and chronic attenders of the urology clinic.

31. The animal studies were the first to attempt to infect the prostate with Chlamydia trachomatis via the intra-canalicular (ie. transurethral) route. Furthermore, they were the first to employ the Mastomys natalensis rat in a model of chlamydial prostatitis. It was not possible to infect the prostates of this species of rat by innoculation of their bladders or urethrae with Chlamydia trachomatis.

32. The combination of epididymal aspiration and transrectal prostatic ultrasound was used to identify an aetiological link between acute epididymitis and chronic abacterial prostatitis, with particular reference to Chlamydia trachomatis; this was an original ploy.

33. A role for Chlamydia trachomatis in patients of thirty five years or less, and Enterobacteriaceae or Streptococcus faecalis in patients above this mark, supported the findings of previous studies. However, this cut off point was not absolute.

34. The intracanalicular route of organism spread was confirmed. Ureaplasma urealyticum was unequivocally isolated from the epididymis and thereby implicated in acute epididymitis, for the first time.

35. The high prevalence of ultrasound abnormalities in patients with acute epididymitis suggests a link, either in the form of a concomitant prostatic infection, or more likely, a common aetiological pathophysiological mechanism, such as urinary reflux.

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APPENDIX I PROSTATIC LOCALISATION (STAMEY) TECHNIQUE

The patient was advised to avoid any sexual activity for five days prior to the test. A full bladder was also requested.

If uncircumcised, the foreskin was retracted and remained so throughout the procedure, prior to cleansing the glans penis with an antiseptic, Savlodil R (ICI).

The first 10 mls of urine was voided into a sterile container and labelled VB1.

The patient was then asked to void a further 150-200 mls which was discarded, prior to the collection of a further 10mls into a sterile container and labelled VB2.

Prostatic massage was then performed in either the knee-elbow or standing position. Throughout the massage a sterile container was held over the external urethral meatus and any free expressed prostatic secretion (EPS) collected.

Prior to voiding, the patient was asked to milk the urethra to maximise the EPS yield.

A further 10 mls of urine was then voided into a sterile container and labelled VB3. Any voided urine in excess of this volume was discarded.

APPENDIX 1

Each urine sample was shaken vigorously prior to a 60 ul aliquot being withdrawn and placed onto a flat bottomed microtitre tray. Ten random fields were examined under high power (x400), from which a mean value was calculated and converted into a leucocyte count per mm^3.

The EPS was mixed by pipette agitation prior to applying a portion onto a Fuchs-Rosenthal counting chamber. Sixteen fields were examined under high power (x1000) and a value per mm^3 calculated.

A 100 ul sample of each of the urine specimens, along with the EPS, when obtained, was innoculated onto each of the following plates: blood agar - aerobic, blood agar - anaerobic and MacConkey agar - aerobic culture.

The plates for aerobic culture were incubated at $37^{\circ}C$ in an atmosphere of 5% CO2 and examined for any bacterial growth at 24 and 48 hours after innoculation.

The plates for anerobic culture were incubated at $37^{\circ}C$ in 90% nitrogen and 10% CO2 for five days prior to examination for any growth.

APPENDIX II SCORING SYSTEM (STAMEY SCORE) BASED ON THE POST PROSTATIC MASSAGE URINE (VB3) LEUCOCYTE COUNT IN RELATION TO DIAGNOSIS

STAMEY SCORE	VB3 LEUCOCYTE COUNT PER MM^3	DIAGNOSIS
0	0	Prostatodynia
1	< 50	Prostatodynia
2	50 - 99	Borderline Prostatitis
3	100 - 200	Prostatitis
4	> 200	Prostatitis
5	0 but documented past history of prostatitis	Prostatitis

APPENDIX III TRANSRECTAL PROSTATIC ULTRASOUND FINDINGS IN NORMAL CONTROLS

Age	ULTRASOUND FEATURES							
	HDE	MRE	ELZ	CI	CT	EDC	PZI	Halo
24	-	-	-	-	-	-	-	-
54	1-120	-	-	-	-	-	-	-
29	1-100	1-003	-	-	-	-	-	-
42	1-120	-	-	-	-	-	1	-
51	-	-	-	-	-	-	-	-
31	1-120	-	-	-	-	-	-	-
68	-	1-003	-	-	-	-	-	-
38	1-100	-	-	-	-	-	-	-
46	1-100	1-100	-	-	-	-	-	-
22	-	-	-	-	-	-	-	1
45	-	1-020	-	-	-	-	-	-
24	-	1-003	-	-	-	-	-	-
30	-	-	-	-	-	-	-	-
33	1-020	-	-	-	-	-	-	-
32	1-003	-	-	-	-	-	-	-
25	1-120	1-120	-	1-2	-	-	-	1
42	1-020	-	-	-	-	-	-	-
28	1-020	-	-	-	-	-	-	-
68	1-020	-	-	-	-	-	-	-
42	1-100	1-003	-	-	-	-	-	-
36	1-100	-	-	-	-	-	-	-
32	-	1-100	-	-	-	-	-	-
47	1-020	-	-	-	-	-	-	-
29	-	1-003	-	-	-	1-3	-	-
26	-	1-003	-	-	-	-	1	-
18	1-020	-	-	-	-	-	-	1
33	-	-	-	-	-	-	-	-
24	1-100	1-100	-	-	-	-	-	-
32	1-100	-	-	-	-	-	-	-
44	-	1-100	-	-	-	-	-	-
65	1-100	-	-	-	-	-	-	-
53	-	-	-	-	-	-	1	1
45	-	1-003	-	-	-	-	-	-
21	-	-	-	-	-	-	-	1

KEY: HDE - High density echoes
MRE - Mid-range echoes
ELZ - Echolucent zones
PZI - Periurethral zone irregularity
CI - Caps. irregularity
CT - Caps. thickening
EDC - Ejaculatory duct calcification
Halo - Periurethral halo

HDE, MRE, ELZ, - 1 - Present
- 100 - Central zone
- 020 - Transition zone
- 003 - Peripheral zone

CI, CT, EDC - 1 - Right, 2 - Left, 3 - Bilateral

PZI, Halo - 1 - Present

APPENDIX IV **SERIAL TRANSRECTAL ULTRASOUND FINDINGS IN PATIENTS WITH SYMPTOMS OF CHRONIC PROSTATITIS WITH REFERENCE TO STAMEY SCORE**

Stamey	ULTRASOUND FEATURES							
score	HDE	MRE	ELZ	CI	CT	EDC	PZI	Halo
4	1-020	1-023	1-003	1-3	1-1	1-3	2	1
1	1-120	1-023	2	1-1	1-2	1-3	2	1
4	1-103	1-103	2	1-2	2	2	2	2
2	1-103	2	2	2	2	2	2	2
4	2	1-023	1-003	1-2	1-3	2	2	2
0	2	1-023	2	1-2	1-3	2	2	2
4	1-100	1-003	1-003	2	2	1-1	1	1
1	1-100	1-003	2	2	2	1-1	2	2
3	1-023	2	1-023	1-1	2	2	1	2
1	1-023	1-023	1-003	2	1-2	2	2	2
4	1-100	1-003	1-003	1-1	1-3	1-1	2	2
1	1-100	1-003	1-003	2	1-3	1-1	2	2
5	1-100	1-003	1-023	1-2	1-3	1-3	2	2
5	1-100	1-023	2	1-2	1-3	1-3	2	2
3	1-100	1-003	2	1-2	1-2	1-3	2	2
2	1-100	1-003	2	1-2	1-2	1-3	2	2
3	1-103	1-003	1-003	2	2	1-3	2	2
1	1-103	1-003	2	2	2	1-3	2	2
3	2	1-020	1-003	1-3	2	2	2	1
2	1-003	1-023	1-003	1-3	2	2	2	2
4	1-023	1-020	1-003	2	2	1-3	2	2
2	1-023	1-023	2	2	1-3	1-3	2	2
4	1-120	1-023	1-003	1-1	1-3	1-1	2	2
2	1-120	1-023	1-003	1-1	1-3	1-1	2	2
3	1-003	1-003	1-003	1-2	2	2	1	2
3	1-103	1-003	1-003	1-3	2	2	1	2
5	1-020	1-020	1-003	1-3	2	1-3	2	2
5	1-020	1-020	2	2	1-2	1-3	2	2
5	2	1-003	1-003	2	2	1-3	1	2
5	1-003	1-003	2	1-2	2	1-3	1	2

APPENDIX IV

Stamey	ULTRASOUND FEATURES							
score	HDE	MRE	ELZ	CI	CT	EDC	PZI	Halo
3	2	1-003	1-003	1-2	1-3	2	2	2
2	1-003	1-003	1-003	2	1-3	2	2	2
4	1-120	1-023	1-003	2	2	1-3	2	2
1	1-120	1-023	1-003	1-1	2	1-3	2	2
5	1-023	1-023	2	2	1-3	1-3	2	2
5	1-023	1-023	2	2	1-3	1-3	2	2
4	1-003	1-023	1-003	1-2	2	2	1	2
4	1-023	1-023	1-003	2	2	2	2	2
3	1-120	1-023	1-003	1-1	1-2	1-1	1	2
1	1-120	1-023	1-003	1-1	1-2	1-1	1	2
4	1-020	1-023	1-003	1-1	2	1-3	1	2
2	1-123	1-023	2	1-1	2	1-3	2	2
3	2	1-003	2	2	2	2	1	2
1	1-003	1-003	2	2	2	2	1	2
3	1-020	1-003	2	1-2	2	1-3	1	2
0	1-123	1-003	2	1-2	2	1-3	1	2
4	1-023	1-023	1-020	1-2	2	2	1	2
4	1-023	1-023	1-023	2	1-2	2	1	2
4	1-123	1-123	2	1-2	1-1	1-3	2	1
1	1-123	1-123	2	1-3	1-3	1-3	2	2
3	1-120	1-020	2	1-1	2	1-2	2	2
2	1-120	1-023	1-003	1-2	2	1-2	2	2
3	2	2	1-003	2	2	2	2	2
2	2	2	1-003	1-1	2	2	2	2
4	1-100	1-023	1-023	1-2	2	1-3	1	2
1	1-100	1-023	1-003	2	1-2	1-3	2	2
3	1-120	2	1-003	1-1	2	1-1	2	2
3	1-120	1-003	1-003	1-1	2	1-1	2	2
3	1-103	2	1-003	2	2	1-3	2	2
2	1-103	1-003	1-003	1-1	2	1-3	2	2
2	1-103	2	1-003	1-2	2	1-2	2	2
2	1-103	2	1-003	2	2	1-2	2	2

APPENDIX IV

Stamey	ULTRASOUND FEATURES							
score	HDE	MRE	ELZ	CI	CT	EDC	PZI	Halo
2	1-120	1-120	1-003	1-1	2	1-3	1	2
1	1-120	1-123	2	1-2	1-1	1-3	2	2
2	1-003	1-003	1-003	2	2	2	2	1
1	1-003	1-003	2	2	2	2	1	2
1	1-020	2	1-003	2	2	1-3	1	2
2	1-020	1-003	1-003	2	2	1-3	1	2
2	1-020	1-003	1-003	1-1	1-3	1-3	2	2
2	1-020	1-003	1-003	1-1	1-3	1-3	2	2
2	1-020	2	1-023	2	2	1-3	1	2
2	1-020	1-023	1-003	2	2	1-3	1	2
2	1-120	1-023	1-100	2	2	1-3	2	2
1	1-120	1-020	1-100	1-1	2	1-3	2	2
2	1-103	1-003	1-003	1-2	2	2	1	2
1	1-103	1-003	1-003	1-2	2	2	1	2
2	1-023	1-023	2	2	2	1-3	2	2
2	1-123	1-023	2	1-1	2	1-3	1	2
2	1-023	1-023	2	2	1-3	1-3	2	2
2	1-023	1-023	1-003	1-2	1-3	1-3	2	2
2	1-103	2	1-003	1-2	2	1-3	2	2
2	1-103	2	1-020	2	2	1-3	2	2
1	1-120	2	2	2	2	2	2	2
1	1-120	2	2	2	1-1	2	2	1
0	1-020	2	2	2	2	1-3	2	2
0	1-020	2	2	2	2	1-3	2	2
0	2	1-100	2	2	2	2	2	2
0	2	1-100	2	2	2	2	2	1
0	2	1-100	2	2	2	2	2	2
0	2	1-100	2	2	2	2	2	2
1	1-020	1-020	2	1-2	2	2	2	2
1	1-020	1-020	2	2	2	2	2	2
0	2	1-003	2	1-1	2	1-3	2	2
0	2	1-003	2	1-2	2	1-3	2	2

APPENDIX IV

Stamey	ULTRASOUND FEATURES						
score	**HDE**	**MRE**	**ELZ**	**CI**	**CT**	**EDC**	**PZI**	**Halo**
0	1-100	2	2	2	2	2	2	2
0	1-100	2	2	2	2	2	2	2
0	1-100	2	2	2	2	2	2	2
0	1-100	2	2	2	1-2	2	2	2
0	1-100	2	2	2	2	2	2	2
0	1-100	2	2	2	2	2	1	2

KEY:

HDE - High density echoes
MRE - Mid-range echoes
ELZ - Echolucent zones
PZI - Periurethral zone irregularity
CI - Caps. irregularity
CT - Caps. thickening
EDC - Ejaculatory duct calcification
Halo - Periurethral halo

HDE, MRE, ELZ - 1 - Present
- 100 Central zone
- 020 Transition zone
- 003 Peripheral zone

CI, CT, EDC 1- Right, 2 - Left, 3 - Bilateral

PZI, Halo 1 - Present, 2 - Absent

APPENDIX V HISTOLOGICAL FINDINGS IN RELATION TO THE ULTRASOUND FEATURES IN THE CHRONIC ABACTERIAL PROSTATITIS (CABP) COHORT

Study no.	Histological Features	Parenchymal Ultrasound Features		
		HDE	MRE	ELZ
1	AI CI - G2 Vessel thickening	103	003	003
2	CI - G1 Corpora amylaceae	003	003	-
3	CI - G1 Vessel thickening	023	023	003
4	Insufficient tissue	100	-	-
5	CI - G1 Vessel thickening	020	003	003
6	Insufficient tissue	020	003	-
7	CI - G2 Telangiectasia	020	-	003
8	CI - G2 Telangiectasia	-	003	-
9	CI - G1 Vessel thickening Corpora amylaceae	120	-	003
10	CI - G1 G3 Telangiectasia	103	003	003
11	CI - G3	103	003	003
12	CI - G1 Telangiectasia	-	003	020
13	CI - G1 Corpora amylaceae	020	-	020
14	CI - G1 Fibrosis Corpora amylaceae	023	020	003

APPENDIX V

Study no.	Histological Features	Parenchymal Ultrasound Features		
		HDE	MRE	ELZ
15	CI - G1	020	023	003
16	Normal	020	020	003
17	CI - G2	003	003	003
18	CI - G1 Fibrosis	020	-	023
19	CI - G1 Corpora amylaceae	023	023	-
20	CI - G1 Corpora amylaceae	100	003	-
21	CI - G1 Fibrosis	100	100	003
22	CI - G1 Corpora amylaceae	100	003	003
23	CI - G1 Corpora amylaceae	023	-	023
24	CI - G1	003	003	003
25	CI - G1 Fibrosis	100	023	003
26	Normal	020	023	003
27	CI - G3 Corpora amylaceae	123	123	-
28	CI - G1 Fibrosis	103	003	-
29	CI - G2 Fibrosis Corpora amylaceae	023	003	003
30	AI CI - G1 Fibrosis	120	120	003

APPENDIX V

Study no.	Histological Features	Parenchymal Ultrasound Features		
		HDE	MRE	ELZ
31	CI - G2 Fibrosis	003	003	-
32	CI - G1 Fibrosis	023	023	020
33	CI - G1	100	100	120
34	Glandular proliferation	120	023	100
35	CI - G1 Corpora amylaceae	020	003	003
36	CI - G1 Corpora amylaceae	020	003	-
37	CI - G1 Corpora amylaceae	123	123	003
38	CI - G1 Fibrosis	-	-	003
39	Granulomatous prostatitis	023	123	003
40	CI - G1 Fibrosis Corpora amylaceae	100	003	-
41	Normal	120	123	003
42	CI - G3 Vessel thickening	100	003	003
43	Insufficient tissue	120	103	-
44	CI - G1 Fibrosis Corpora amylaceae	120	020	-
45	CI - G2 Vessel thickening	103	103	-
46	CI - G1 Fibrosis	003	003	003

APPENDIX V

Study no.	Histological Features	Parenchymal Ultrasound Features		
		HDE	MRE	ELZ
47	CI - G1 Fibrosis	103	003	003
48	CI - G2 Dilated glands	023	003	003
49	CI - G1 Telangiectasia	100	003	003
50	Normal	-	003	-
51	CI - G1 Corpora amylaceae Fibrosis	123	023	003
52	AI CI - G2 Corpora amylaceae	120	003	-
53	CI - G1 Corpora amylaceae	120	120	-
54	AI CI - G1 G2	003	023	003
55	Glandular proliferation	103	003	-
56	CI - G1 Fibrosis	123	003	-
57	AI CI - G1	103	103	-
58	CI - G3	120	023	003
59	CI - G1 Corpora amylaceae Fibrosis Benign nodular hyperplasia	123	003	-
60	CI - G1 Fibrosis	120	023	003

APPENDIX V

KEY:

AI - Acute inflammation

CI - Chronic inflammation

G - Inflammatory grade - 1 - 3

HDE - High density echoes

MRE - Mid-range echoes

ELZ - Echolucent zones

HDE, MRE, ELZ - 100 Central zone

- 020 Transition zone

- 003 Peripheral zone

APPENDIX VI TRANSPERINEAL PROSTATIC BIOPSY TECHNIQUE

All patients and controls gave informed written consent for the procedure, performed under local anaesthesia in the former and general anaesthesia in the latter group.

The individuals were placed in the lithotomy position. The transrectal ultrasound probe was inserted and mounted on a gantry, leaving the hands free for the biopsy procedure.

Then the perineal skin was cleaned with an aqueous antiseptic, Savlodil (ICI), and the perineum isolated by sterile drapes.

A proportion of the patients undergoing the procedure under local anaesthesia received intravenous Diazemuls (Dumex) to a maximum dose of 10 mg. All such cases were supervised by a nurse with the aid of continuous ECG monitoring.

In the local cases 1% Lignocaine plain was instilled into the perineal skin, subcutaneous tissues, perineal musculature and prostatic capsule.

A small stab incision was made in the perineal skin.

Under ultrasound guidance a 14G Tru-cut (Travenol) biopsy needle was then inserted into any abnormal areas and a sample taken. The position and the character of the

ultrasound abnormality was noted in each case and the tissue processed and stored as outlined in the individual sections.

Once the biopsies had been taken, gentle pressure was applied to the needle entry site. Prophylactic antibodies were not used.

The patients and controls were reviewed two weeks after the procedure and any morbidity recorded. A mid-stream urine sample was taken and processed in routine fashion.

APPENDIX VII **ORGANISMS ISOLATED FROM THE CHRONIC ABACTERIAL PROSTATITIS (CABP) COHORT DURING SERIAL SAMPLING**

Study no.	Stamey Score	Organism	Numbers isolated cfu/ml VB1	VB2	EPS	VB3
1	3	Staph. epiderm.	120	40	-	20
	2	Staph. epiderm.	10	-	-	-
		Diphtheroids	10	-	-	-
	2	Proteus spp.	10	630	-	30
		Coliform spp.	580	310	-	110
2	5	Staph. epiderm.	-	-	-	10
		Strep. viridans	-	70	-	-
	2	Alpha haem. streptococci	1900	600	-	110
3	3	Diphtheroids	$>10^5$	10^3	10^2	10^2
		Staph. epiderm.	1400	200	-	-
		Strep. faecalis	1400	-	-	200
	2	Nil	-	-	-	-
	2	Staph. epiderm.	1000	-	-	1000
	3	Nil	-	-	-	-
4	2	Diphtheroids	100	70	60	80
		Staph. epiderm.	80	35	35	25
	2	Diphtheroids	2000	300	-	80
		Staph. epiderm.	1500	150	-	50
	2	Alpha haem. streptococci	200	150	-	50
		Staph. aureus	15	4	-	3
5	3	Micrococci	-	-	-	50
		Staph. epiderm.	50	10	-	50
	0	Alpha haem. streptococci	220	150	-	60

APPENDIX VII

Study no.	Stamey Score	Organism	Numbers isolated cfu/ml VB1	VB2	EPS	VB3
5	2	Staph. epiderm.	10	10	-	-
6	2	Strep. faecalis	$2x10^4$	10^4	-	$2x10^3$
	2	Strep. faecalis	$6x10^3$	$4x10^3$	-	$5x10^2$
7	3	Beta haem. streptococci	100	-	-	-
		Staph. epiderm.	8	2	-	2
	2	Strep. faecalis	$>10^5$	$>10^5$	-	$>10^5$
	3	Strep. faecalis	10	900	300	260
8	3	Staph. epiderm.	40	30	-	40
	1	Alpha haem. streptococci	$3x10^3$	300	-	-
		Staph. epiderm.	1500	40	-	10
9	3	Diphtheroids	-	-	12	-
		Staph. epiderm.	-	-	10	5
	3	Nil	-	-	-	-
10	2	Nil	-	-	-	-
	1	Staph. epiderm.	400	-	-	1900
	1	Nil	-	-	-	-
11	4	Nil	-	-	-	-
	4	Staph.epiderm.	60	40	-	-
12	2	Diphtheroids	30	60	-	200
		Proteus	300	60	-	250
	2	Beta haem. streptococci	500	100	-	30
		Proteus spp.	$>10^4$	$>10^4$	-	$>10^4$

APPENDIX VII

Study no.	Stamey Score	Organism	Numbers isolated cfu/ml VB1	VB2	EPS	VB3
12	2	Mixed flora	500	200	-	100
13	2	Staph. epiderm.	$>10^3$	$>10^3$	-	170
	2	Staph. epiderm.	580	260	-	100
14	4	Beta haem. streptococci	140	30	-	10
	2	Coliform	40	500	-	60
	2	Staph. epiderm.	-	300	-	50
		Strep. faecalis	$>10^3$	500	-	-
15	4	Diphtheroids	500	150	40	70
	1	Diphtheroids	200	140	-	-
		Staph. epiderm.	$>10^3$	400	-	250
16	2	Alpha haem strep.	230	220	-	290
		Staph. epiderm.	40	-	-	30
	5	Nil	-	-	-	-
17	2	Nil	-	-	-	-
	2	Nil	-	-	-	-
18	2	Coliform spp.	3000	500	-	350
		Strep. faecalis	300	300	-	240
	2	Coliform spp.	2000	500	-	200
19	2	Nil	-	-	-	-
	2	Nil	-	-	-	-

APPENDIX VII

Study no.	Stamey Score	Organism	Numbers isolated cfu/ml VB1	VB2	EPS	VB3
20	3	Alpha haem. streptococci	60	-	-	-
		Coliform spp.	20	-	-	-
		Diphtheroids	50	10	-	-
		Staph. epiderm.	40	-	-	10
	2	Staph. epiderm.	5	2	-	30
	2	Nil	-	-	-	-
21	2	Strep. faecalis	800	-	-	-
	1	Alpha haem. streptococci	200	30	-	10
		Staph. epiderm.	5	-	-	1
	2	Mixed skin flora	350	40	-	240
22	4	Coliform spp.	-	-	-	2
	3	Mixed flora	$>10^3$	860	-	700
	4	Diphtheroids	2000	2000	-	-
		Staph. aureus	2000	2000	-	-
23	3	Alpha haem. streptococci	840	250	-	110
	1	Nil	-	-	-	-
24	2	NIl	-	-	-	-
	1	Alpha haem. streptococci	6	2	-	1
		Diphtheroids	25	25	-	30
		Staph. epiderm.	110	6	-	8
	2	Staph. epiderm.	2	22	-	80
25	2	Coliform spp.	$>10^5$	930	250	450
		Strep faecalis	$>10^5$	1060	-	480

APPENDIX VII

Study no.	Stamey Score	Organism	Numbers isolated cfu/ml VB1	VB2	EPS	VB3
25	2	Coliform spp.	$>10^3$	360	120	100
		Staph. epiderm.	360	110	-	-
		Strep. faecalis	940	400	90	40
26	4	Staph. epiderm.	20	50	-	-
	2	Mixed skin flora	10^3	400	-	30
27	4	Nil	-	-	-	-
	1	Nil	-	-	-	-
28	4	Nil	-	-	-	-
	4	Nil	-	-	-	-
29	3	Coliform spp.	640	480	-	400
	3	Coliform spp.	10^3	600	-	600
		Staph. epiderm.	-	20	-	-
30	2	Diphtheroids	$>10^4$	870	-	670
		Strep. faecalis	340	190	-	60
	1	Corynebacteria	1000	1400	-	1200
		Staph. epiderm.	400	1000	-	600
	2	Diphtheroids	140	340	-	-
		Staph. epiderm.	60	40	-	10
31	3	Beta haem. streptococci	690	580	100	40
	3	Beta haem. streptococci	400	500	140	300
32	4	Coliform spp.	10	10	-	-
		Staph. epiderm.	10	30	-	50
	4	Staph. epiderm.	10	10	200	500
		Strep. faecalis	2000	1500	800	600

APPENDIX VII

Study no.	Stamey Score	Organism	Numbers isolated cfu/ml VB1	VB2	EPS	VB3
33	3	Staph. epiderm.	20	6	-	2
	2	Staph. epiderm.	100	40	-	-
34	2	Nil	-	-	-	-
	1	Nil	-	-	-	-
35	3	Mixed growth	$>10^2$	540	-	84
	3	Mixed growth	$>10^2$	300	-	-
36	3	Anaerobes	3000	3000	3000	3000
		Staph. epiderm.	30	10	-	120
	3	Staph. epiderm.	400	200	-	80
37	3	Alpha haem. streptococci	800	-	-	30
		Staph. epiderm.	40	-	-	20
	2	Alpha haem. streptococci	300	100	-	20
		Staph. epiderm.	800	400	-	250
38	3	Coliform spp.	10^5	10^5	-	10^5
	3	Nil	-	-	-	-
39	4	Nil	-	-	-	-
	4	Nil	-	-	-	-
40	2	Staph. epiderm.	200	-	-	-
	2	Staph. epiderm.	400	240	-	10
41	3	Diphtheroids	220	150	-	30
		Staph. epiderm.	70	40	-	10

APPENDIX VII

Study no.	Stamey Score	Organism	Numbers isolated cfu/ml VB1	VB2	EPS	VB3
41	3	Nil	-	-	-	-
42	4	Coliform spp.	290	240	180	350
	1	Coliform spp.	880	400	-	60
43	3	Nil	-	-	-	-
	3	NIl	-	-	-	-
44	3	Staph. epiderm.	20	10	10	10
	2	Staph. epiderm.	10^3	10^3	-	10^2
45	4	Mixed skin flora	10^3	10^3	10^3	10^3
	2	Staph. epiderm.	200	40	-	10
46	2	Nil	-	-	-	-
	2	Coliform spp.	200	60	2	30
47	4	Mixed growth	10^3	540	20	84
	3	Diphtheroids Staph. epiderm.	140 200	40 80	- -	10 40
48	3	Mixed growth	2000	250	10	20
	3	Micrococci Staph. epiderm.	70 90	70 100	10 20	70 110
49	4	Staph. epiderm.	370	30	-	160
	1	Mixed skin flora	10^2	460	-	240
50	4	Staph. epiderm.	300	10	-	160
	4	E. coli	10^2	400	240	300

APPENDIX VII

Study no.	Stamey Score	Organism	Numbers isolated cfu/ml VB1	VB2	EPS	VB3
51	4	Diphtheroids	400	380	160	10
	4	Nil	-	-	-	-
52	3	Staph. epiderm.	30	24	-	10
	3	Acinetobacter	250	20	-	-
		Staph. epiderm.	40	-	-	-
53	2	Nil	-	-	-	-
	2	Nil	-	-	-	-
54	4	Staph. epiderm.	20	10	-	10
	4	Mixed skin flora	10^3	10^3	-	10^2
55	3	Beta haem. streptococci	10^4	10^4	-	10^2
	2	Beta haem. streptococci	100	100	-	60
56	5	Nil	-	-	-	-
	2	Staph. epiderm.	10^4	-	-	-
57	3	Mixed growth	$>10^3$	540	-	84
	3	Staph. epiderm.	600	430	-	200
58	4	E. coli	980	480	-	240
	2	Diphtheroids	800	540	-	290
		E. coli	400	140	-	10
59	5	Acinetobacter	10^5	-	-	10^4
		Staph. epiderm.	30	20	-	40
	3	Nil	-	-	-	-

APPENDIX VII

Study no.	Stamey Score	Organism	Numbers isolated cfu/ml VB1	VB2	EPS	VB3
60	4	Staph. epiderm.	800	250	210	180
	1	Diphtheroids	400	600	80	500
		Staph. epiderm.	600	800	300	700

APPENDIX VIII **DEMOGRAPHIC DETAILS OF THE CHRONIC ABACTERIAL PROSTATITIS (CABP) COHORT**

Study no.	Age	Symptom duration in yrs	Symptoms			Past History	
			Pain	I Void	Void D	NSU	UTI
1	53	1.5	S,E	D	Pf,Dr	N	N
2	39	6	Pl,R	-	-	N	Y
3	35	2	Pe	D	-	Y	N
4	28	5	R	D,N	D,Ie	Y	N
5	36	20	Pl,S,B	F,N	Pf	Y	Y
6	37	2	Pl,T,R,Pe	D,F,N	Pf	N	N
7	35	1.5	T,Pe	-	-	Y	N
8	25	4	T,Pe,G	D	-	N	N
9	38	0.3	Pl,B	-	Pf	Y	N
10	65	5	Pl,Pe	F,N	D	Y	N
11	63	1	S	U,F,N	Pf,Dr	N	N
12	25	2	-	D	-	N	N
13	68	0.3	T	N	Pf	N	N
14	34	2	Pl,T,S,E	U	-	Y	N
15	34	1	T,B,G	F	Pf	Y	N
16	42	3	Pe	F	D	Y	N
17	38	1	Pl,T,E	-	-	Y	N
18	58	0.3	Pl,T	D,F,N	Pf	N	Y
19	37	2	Pl,B	-	Pf	N	Y
20	39	12	T,R,Pe,B, E,G	D	-	Y	N
21	29	0.3	Pl,T,E	-	-	N	N
22	30	5	-	-	Pf	Y	N
23	32	0.8	T,S,Pe,E	D	H	Y	N
24	29	2	T,S	-	-	N	N
25	25	1.5	Pl,T,S,G	F	-	Y	N
26	41	0.3	Pl,B	D,F	-	Y	N
27	50	0.3	T,R	-	Pf	Y	N
28	22	1	Pl,T,S	-	Pf	Y	N
29	68	1	Pl,T,S	D	Pf	N	N
30	38	10	Pl,Pe,E	-	-	Y	N
31	37	1	Pl,B	U,F	-	Y	N
32	56	0.2	Pl,T	D	-	N	Y
33	35	10	Pl	-	-	N	N
34	26	0.5	S,E	-	-	N	N
35	54	10	Pl,T,B	-	Pf	N	N
36	46	2	T	-	-	Y	N
37	39	0.3	Pl,T,B	F,N	-	Y	N
38	28	1.5	S,R,Pe	-	-	N	Y
39	67	0.1	-	D,U, F,N	Pf	N	Y
40	34	1	Pl,E	F,N	Pf	N	N
41	24	0.5	T,Pe,E	D	-	Y	N

APPENDIX VIII **DEMOGRAPHIC DETAILS OF THE CHRONIC ABACTERIAL PROSTATITIS (CABP) COHORT**

Study no.	Age	Symptom Duration in yrs	Symptoms			Past History	
			Pain	I Void	Void D	NSU	UTI
42	55	3	Pe,G	D,U, F,N	Pf	N	Y
43	44	0.8	B,G	D,U,F, N,I	Pf	N	N
44	29	3	Pl,T,Pe, B,E	D,F	Pf	Y	N
45	35	2	Pl,T,B	-	Pf	Y	N
46	57	0.5	Pl,T,B	D	Pf	N	N
47	26	8	-	D,U, F,N	-	N	N
48	35	1.5	T,Pe	-	Dr	N	N
49	26	0.5	Pl,T,Pe	-	-	Y	N
50	36	10	Pl,S,B	F,N	Pf	Y	N
51	27	3.5	Pl,T,Pe,E	U	-	Y	N
52	35	1.5	T,Pe	-	Dr	N	N
53	34	2	E	D,U	-	Y	N
54	19	2	Pl,R,Pe,B	F,N	Pf	N	N
55	34	3	Pl,Pe	F	-	Y	N
56	58	13	S,P	D	Pf	N	N
57	34	3	Pl,T,S,B	D,F	-	Y	Y
58	34	0.5	Pl,T,B	D,F	-	Y	Y
59	24	2	Pl,T,B,E	-	Pf,Dr	N	N
60	75	1	Pl,T,Pe	-	Pf	N	Y

KEY:

I Void - Irritative voiding
Void D - Voiding dysfunction

Pl - Perineal
T - Testicular
S - Suprapubic
R - Rectal
Pe - Penile
B - Back
E - Ejaculatory
G - Groin

Y - Yes

D - Dysuria
U - Urgency
F - Frequency
N - Nocturia
I - Incontinence

Pf - Poor flow
Dr - Dribbling
Ie - Incomplete emptying
H - Hesitancy

N - No

APPENDIX IX DIRECT IMMUNOFLUORESCENCE TECHNIQUE (MicroTrak) FOR DETECTION OF Chlamydia trachomatis

The Chlamydia trachomatis reagent was reconstituted with reconstitution dilutent as directed by the manufacturers and allowed to equilibrate at room temperature for 30 minutes prior to use. Excess reagent was stored in the dark at 2 - 8°C.

The specimen slides were air dried after the smear had been applied and fixed in methanol prior to processing.

The specimen and control slides were allowed to equilibrate at room temperature for the same period.

30 ul of reagent was then placed on each of the specimen slides and onto the positive control slide, ensuring that the entire specimen was covered.

The slides were then incubated at room temperature for 15 minutes in a moist chamber and observed for drying out at least once during this period.

Excess reagent was then aspirated from the slide and discarded. The slides were then washed by agitation in distilled water for approximately 10 seconds.

Excess water was removed by blotting and the slides

allowed to air dry.

A drop of mounting fluid was applied to the slide well followed by a coverslip.

The specimen and control slides were viewed under an Olympus microscope with epifluorescence illumination under high power (x400; x1000), for elementary bodies (appearing as apple green pin-points).

Although a count of ten or more elementary bodies was recommended by the manufacturers, any case where they were observed in this thesis, was recorded as positive.

APPENDIX X CHLAMYDIAL GROWTH, MAINTENANCE AND TRANSPORT MEDIA

CMA - Complete medium with antibiotics

Maintenance :

Eagle minimal essential medium

5% heat inactivated foetal calf serum

1% 200 mM Glutamine

1% vitamins

Vancomycin - 100 ug/ml

Streptomycin - 50 ug/ml

Amphotericin B 2.5 ug/ml

4.4 mM bicarbonate to pH 7.0

CMGA - Complete medium with glucose and antibiotics

Culture :

CMA

5% extra heat inactivated foetal calf serum

0.5% glucose

TM - Transport medium

pH 7.0

0.2 M sucrose in 0.02 M phosphate buffer

(2SP)

10% heat inactivated foetal calf serum

Vancomycin - 100 ug/ml

Streptomycin - 50 ug/ml

Amphotericin B - 2.5 ug/ml

APPENDIX X

CY - Culture medium plus cycloheximide

CMGA + 1 ug/ml cycloheximide

APPENDIX XI Chlamydia trachomatis CULTURE TECHNIQUE

The specimen collection and handling has been described in the relevant sections.

The McCoy cell monolayers were grown in CMA in plastic culture flasks (Nunc) and subsequently used to seed coverslips in plastic flasks at 2×10^5 cells/tube.

Immediately prior to innoculation of the monolayers with the clinical specimen the CMA was removed from the cells and discarded. The sample was added to the cells, the tubes centrifuged at 4000g for 1 hour and then incubated at 37° C for 2 hours.

Positive (78 alpha) and negative controls were run in parallel with the clinical specimens.

The culture medium was then exchanged using 2 mls of CY and the cells were then incubated at 37° C for 72 hours in atmospheric conditions.

The medium was then removed from the tubes and the cells fixed with methanol for 10 minutes at 37° C.

The coverslips were then washed with buffer pH 6.8.

Freshly prepared 10% Giemsa stain was added and the tubes incubated at 37° C for 30 minutes.

APPENDIX XI

The Giemsa stain was removed from the tubes prior to washing the coverslips with 30% methanol in buffer (pH 6.8), before two further washes with buffer.

The coverslips were removed , dried and mounted (monolayer downwards) with Clearmount onto a slide.

The slides were then examined under dark ground illumination at a power of (x200).

APPENDIX XII MYCOPLASMA AND UREAPLASMA TRANSPORT MEDIUM (M/U TM)

PPLO broth (beef-heart infusion)

10% v/v fresh yeast extract (25% w/v)

20% v/v horse serum

Penicillin G 1000 IU/ml

APPENDIX XIII MYCOPLASMA AND UREAPLASMA CULTURE MEDIUM

M/U TM

Arginine 0.1% for mycoplasmas

Urea 0.1% for ureaplasmas

Thallium acetate - 0.5% for mycoplasmas

0.25% for ureaplasmas

Phenol red 0.002%

APPENDIX XIV MYCOPLASMA AND UREAPLASMA CULTURE TECHNIQUES

Firstly tenfold serial dilutions of the clinical sample were performed on three occasions for each case. 0.2 ml of the clinical sample (urine), homogenate (tissue) or elutant (swab) was innoculated into each of 1.8 mls of culture medium for mycoplasma and ureaplasma isolation. After mixing, further 0.2 ml aliquots were withdrawn and diluted in a further 1.8 mls of culture medium. This dilution was performed on three occasions (10^{-3}) with each sample.

The vials containing the medium and sample were then incubated at 37 ^{o} C in atmospheric conditions.

The vials were first examined for any colour change (yellow to pink) at 24 hours and subsequently at 48 hour intervals for 15 days.

Any vial displaying a colour change was then developed on agar at 37 ^{o} C in an atmosphere of 95% N2 and 5% CO2.

The agar for ureaplasmas contained 0.05 M Hepes buffer and ammonia manganous sulphate as an indicator.

Species were identified by colony characteristics and antisera discs and the organism numbers expressed as colour changing units (CCU) per ml.

APPENDIX XV MICROIMMUNOFLUORESCENCE TECHNIQUE FOR THE DETECTION OF ANTI-CHLAMYDIAL ANTIBODIES

The serum was thawed and two 25 ul aliquots, one for IgG and the other for IgM estimation, were withdrawn and each placed in a microtitre tray. The sera then underwent eight serial doubling dilutions with phosphate buffered saline (PBS), producing dilutions of 1:2 to 1:256.

Antigen plates had been prepared earlier on PTFE coated slides. At each well a group of Chlamydia trachomatis antigens, serotypes D through to K, had been applied with a mapping pen.

The antigens had been air dried and fixed in acetone.

10 ul aliquots of the diluted sera was applied in a sequential order on the antigen slide, along with a positive and negative control. The slides were then incubated at 37 ^{o} C in a moist chamber for one hour.

The serum was then removed with a preliminary PBS wash followed by two ten minute washes.

Sheep anti-human immunoglobulin (anti-IgG or anti-IgM) with a fluorescein isothiocyanate conjugate was applied to each well and the slides incubated at 37 ^{o} C in a moist chamber for thirty minutes.

APPENDIX XV

The anti-serum was removed with a preliminary PBS wash followed by two ten minute washes, with Trypan Blue added to the PBS of the second wash.

The excess PBS was blotted from the slide before air drying.

The slides were then viewed under epifluorescence illumination and the highest positive dilution recorded.

APPENDIX XVI ANTICHLAMYDIAL IgG AND IgM TITRES (RECIPROCAL OF) IN THE CHRONIC ABACTERIAL PROSTATITIS (CABP) COHORT

Study no.	Antichlamydial Antibody Titres (reciprocal of)			
	IgG		IgM	
	Acute	Conv.	Acute	Conv.
1	0	0	0	0
2	8	8	0	0
3	0	0	4	0
4	2	8	0	0
5	0	0	0	0
6	8	0	0	0
7	0	0	0	0
8	0	0	0	0
9	8	8	2	0
10	8	4	0	0
11	0	2	0	0
12	4	0	0	2
13	8	16	4	0
14	0	0	0	0
15	2	4	0	0
16	0	0	0	0
17	8	8	0	0
18	0	0	0	0
19	0	0	0	0
20	32	16	0	0
21	0	2	0	0
22	0	0	2	0
23	0	0	0	0
24	0	0	0	0
25	8	8	0	4
26	4	0	0	0
27	4	16	8	4
28	8	0	0	0
29	0	0	0	0
30	0	0	4	0
31	0	0	0	0
32	4	4	0	0
33	8	8	0	2
34	0	0	0	0
35	8	4	4	4
36	0	0	0	0
37	8	32	0	2
38	0	0	0	0
39	0	0	0	0
40	0	0	0	0
41	4	8	0	0
42	0	0	0	0

APPENDIX XVI

Study no.	Antichlamydial Antibody Titres (reciprocal of)			
	IgG		IgM	
	Acute	Conv.	Acute	Conv.
43	0	0	0	0
44	2	4	0	0
45	0	0	0	0
46	0	0	0	2
47	8	2	4	0
48	8	8	2	0
49	0	0	0	0
50	0	0	0	0
51	4	0	4	0
52	2	4	0	0
53	0	0	0	0
54	0	2	0	0
55	0	0	0	0
56	8	16	4	0
57	0	0	0	0
58	0	0	0	0
59	8	8	0	0
60	2	2	0	0

APPENDIX XVII HISTOLOGICAL FEATURES OF PROSTATIC BIOPSIES FROM THE CHRONIC ABACTERIAL PROSTATITIS (CABP) COHORT

Study no.	Stamey Score	Histological Features
1	3	AI - lumen CI - G2 - periglandular stromal Vessel thickening
2	2	CI - G1 - periglandular Corpora amylaceae
3	3	CI - G1 - periglandular Vessel thickening
4	2	Insufficient tissue
5	2	CI - G1 - periglandular Vessel thickening
6	3	Insufficient tissue
7	3	CI - G2 - intraglandular periglandular stromal Telangiectasia
8	3	CI - G2 - stromal Telangiectasia
9	3	CI - G1 - stromal Vessel thickening Corpora amylaceae
10	2	CI - G1 - stromal G3 - periglandular Telangiectasia
11	4	CI - G3 - periglandular stromal
12	2	CI - G1 - periglandular - stromal Telangiectasia
13	2	CI - G1 - periglandular Corpora amylaceae

APPENDIX XVII HISTOLOGICAL FEATURES OF PROSTATIC BIOPSIES FROM THE CHRONIC ABACTERIAL PROSTATITIS (CABP) COHORT

Study no.	Stamey Score	Histological Features
14	4	CI - G1 - stromal Fibrosis Corpora amylaceae
15	4	CI - G1 - periglandular stromal
16	2	Normal
17	2	CI - G2 - periglandular
18	2	CI - G1 - periglandular Fibrosis
19	2	CI - G1 - stromal Corpora amylaceae
20	3	CI - G1 - stromal Corpora amylaceae
21	2	CI - G1 - stromal Fibrosis
22	4	CI - G1 - periglandular Corpora amylaceae
23	3	CI - G1 - stromal Corpora amylaceae
24	2	CI - G1 - stromal
25	2	CI - G1 - periglandular Fibrosis
26	4	Normal
27	4	CI - G3 - periglandular Corpora amylaceae
28	4	CI - G1 - periglandular Fibrosis

APPENDIX XVII HISTOLOGICAL FEATURES OF PROSTATIC BIOPSIES FROM THE CHRONIC ABACTERIAL PROSTATITIS (CABP) COHORT

Study no.	Stamey Score	Histological Features
29	3	CI - G2 - periglandular Fibrosis Corpora amylaceae
30	2	AI - intraglandular CI - G1 - periglandular stromal Fibrosis
31	3	CI - G2 - periglandular stromal Fibrosis
32	4	CI - G1 - periglandular stromal Fibrosis
33	2	CI - G1 - stromal
34	2	Glandular proliferation
35	3	CI - G1 - periglandular Corpora amylaceae
36	3	CI - G1 - stromal Corpora amylaceae
37	3	CI - G1 - periglandular Corpora amylaceae
38	3	CI - G1 - stromal Fibrosis
39	4	Granulomatous prostatitis
40	2	CI - G1 - stromal Fibrosis Corpora amylaceae
41	3	Normal
42	4	CI - G3 - periglandular stromal Vessel thickening

APPENDIX XVII HISTOLOGICAL FEATURES OF PROSTATIC BIOPSIES FROM THE CHRONIC ABACTERIAL PROSTATITIS (CABP) COHORT

Study no.	Stamey Score	Histological Features
43	3	Insufficient tissue
44	3	CI - G1 - stromal Fibrosis Corpora amylaceae
45	4	CI - G2 - stromal Vessel thickening
46	2	CI - G1 - stromal Fibrosis
47	4	CI - G1 - periglandular Fibrosis
48	3	CI - G2 - periglandular Dilated glands
49	4	CI - G1 - periglandular Telangiectasia
50	4	Normal
51	4	CI - G1 - periglandular Fibrosis Corpora amylaceae
52	3	AI - intraglandular CI - G2 - intraglandular Corpora amylaceae
53	2	CI - G1 - periglandular Corpora amylaceae
54	4	AI - intraglandular CI - G1 - periglandular G2 - periglandular
55	3	Glandular proliferation
56	2	CI - G1 - periglandular Fibrosis

APPENDIX XVII HISTOLOGICAL FEATURES OF PROSTATIC BIOPSIES FROM THE CHRONIC ABACTERIAL PROSTATITIS (CABP) COHORT

Study no.	Stamey Score	Histological Features
57	3	AI - intraglandular CI - G1 - stromal
58	4	CI - G3 - periglandular
59	3	CI - G1 - stromal Fibrosis Corpora amylaceae Benign nodular hyperplasia
60	4	CI - G1 - periglandular Fibrosis

Key:

AI - Acute inflammation

CI - Chronic inflammation

G - Inflammatory grade

APPENDIX XVIII HISTOLOGICAL FEATURES OF PROSTATIC BIOPSIES FROM THE CHRONIC ABACTERIAL PROSTATITIS (CABP) COHORT

Feature	No. of patients (%)
Inflammatory cells	
Acute and chronic	5 (8%)
Chronic - grade 1 +,#	37 (62%)
- grade 2 #	10 (17%)
- grade 3 +	5 (8%)
Granulomatous *	1 (2%)
Site of Inflammatory Cells	
Intraglandular	7 (12%)
Periglandular	34 (57%)
Stromal	27 (45%)
Other Features	
Corpora amylaceae	20 (33%)
Fibrosis	18 (30%)
Vessel hyaline thickening	6 (10%)
Telangiectasia	5 (8%)
Glandular proliferation	2 (3%)

Key:

+,# - patients with both grades of inflammation present in the biopsies

* - Mycobacterium tuberculosis excluded

APPENDIX XIX MAST CELL COUNT IN PROSTATIC BIOPSIES FROM THE CHRONIC ABACTERIAL PROSTATITIS (CABP) COHORT WITH REFERENCE TO AGE AND SYMPTOM DURATION

Study no.	Age	Symptom duration in years	Mast cell count per sq mm
5	36	20	26
7	35	1.5	5
8	25	4	10
10	65	5	20
11	63	1	25
12	25	2	10
15	34	1	19
16	42	3	12
17	38	1	10
18	58	0.3	17
21	29	0.3	16
25	25	1.5	18
27	50	0.3	13
28	22	1	1
29	68	1	15
30	38	10	11
34	26	0.5	6
37	39	0.3	7
39	67	0.1	31
42	55	3	12
44	29	3	10
45	35	2	24
47	26	8	45
48	68	20	18
49	26	0.5	12
50	36	10	17
52	35	1.5	30
55	34	3	8
56	58	13	15
60	75	1	8

APPENDIX XX **MAST CELL COUNT IN PROSTATIC BIOPSIES FROM THE CHRONIC ABACTERIAL PROSTATITIS (CABP) COHORT WITH REFERENCE TO INFLAMMATORY GRADE**

Study no.	Inflammatory grade	Mast cell count per sq mm
5	1	26
7	2	5
8	2	10
10	3	20
11	3	25
12	1	10
15	1	19
16	0	12
17	2	10
18	1	17
21	1	16
25	1	18
27	3	13
28	1	1
29	2	15
30	1	11
34	0	6
37	1	7
39	4	31
42	3	12
44	1	10
45	2	24
47	1	45
48	2	18
49	1	12
50	0	17
52	1	30
55	0	8
56	1	15
60	1	8

APPENDIX XXI MAST CELL COUNT IN PROSTATIC BIOPSIES FROM NORMAL CONTROLS

Age	Mast cell count per sq mm
20	10
22	2
28	5
34	9
35	1
37	4
42	1
50	2
64	2
68	2

APPENDIX XXII IMMUNOFLUORESCENCE DATA FROM THE PROSTATIC BIOPSIES OF THE CHRONIC ABACTERIAL PROSTATITIS (CABP) COHORT

Study no.	Routine Histology			IF Results
	Inflammation	Corpora amylaceae	Vessel changes	
1	A - Lumen G2 - PG ST	N	VT	Neg
2	G1 - PG	Y	-	IgM - Cells F - PG ST
3	G1 - PG	N	VT	IgA - Cells IgM - Cells
4	I	-	-	-
5	G1 - PG	N	VT	Neg
6	I	-	-	-
7	G2 - G PG ST	N	T	Neg
8	G2 - ST	N	T	Neg
9	G1 - ST	Y	VT	Neg
10	G1 - ST G3 - PG	N	T	Neg
11	G3 - PG ST	Y	-	C3 - Lumen
12	G1 - PG ST	N	T	Neg
13	G1 - PG	Y	-	IgM - PG F - PG
14	G1 - ST	Y	-	IgM - Cells C3 - PG ST V F - PG
15	G1 - PG ST	N	-	C3 - Cells G
16	Normal	N	-	IgM - V C3 - V
17	G2 - PG	N	-	C3 - V
18	G1 - PG	N	-	Neg
19	G1 - ST	Y	-	IgA - ST IgM - G
20	G1 - ST	Y	-	C3 - G F - PG V

APPENDIX XXII IMMUNOFLUORESCENCE DATA FROM THE PROSTATIC BIOPSIES OF THE CHRONIC ABACTERIAL PROSTATITIS (CABP) COHORT

Study no.	Routine Histology			IF Results
	Inflammation	Corpora amylaceae	Vessel changes	
21	G1 - ST	N	-	IgM - G PG
22	G1 - ST	Y	-	Neg
23	G1 - ST	Y	-	IgM - Cells G
24	G1 - ST	N	-	IgA - PG IgM - PG ST V C3 - V
25	G1 - PG	N	-	Neg
26	Normal	N	-	IgM - PG V C3 - G V
27	G3 - PG	Y	-	IgA - G IgM - G PG
28	G1 - PG	N	-	IgM - PG ST F - V
29	G2 - PG	Y	-	Neg
30	A - G G1 - PG ST	N	-	Neg
31	G2 - PG ST	N	-	IgM - Cells G
32	G1 - PG ST	N	-	IgM - PG
33	G1 - ST	N	-	IgM - PG V F - Lumen V
34	Glandular proliferation	N	-	IgA - G PG IgM - PG
35	G1 - PG	Y	-	Neg

APPENDIX XXII IMMUNOFLUORESCENCE DATA FROM THE PROSTATIC BIOPSIES OF THE CHRONIC ABACTERIAL PROSTATITIS (CABP) COHORT

Study no.	Routine Histology			IF Results
	Inflammation	Corpora amylaceae	Vessel changes	
36	G1 - ST	Y	-	IgA - ST IgM - ST V F - ST
37	G1 - PG	Y	-	IgM - G C3 - G
38	G1 - ST	N	-	Neg
39	Granulomatous	N	-	Neg
40	G1 - ST	Y	-	IgM - PG V
41	Normal	N	-	IgA - Cells G IgM - Cells - G C3 - Cells
42	G3 - PG ST	N	VT	Neg
43	Insufficient	-	-	-
44	G1 - ST	Y	-	IgM - G
45	G2 - ST	N	VT	IgM - PG ST V
46	G1 - ST	N	-	IgA - PG
47	G1 - PG	N	-	IgA - Cells IgM - Cells G C3 - Cells
48	G2 - PG	N	-	Neg
49	G1 - PG	N	T	IgM - PG V C3 - G V
50	Normal	N	-	Neg
51	G1 - PG	Y	-	IgA - PG ST V IgM - PG C3 - G PG F - Lumen

APPENDIX XXII IMMUNOFLUORESCENCE DATA FROM THE PROSTATIC BIOPSIES OF THE CHRONIC ABACTERIAL PROSTATITIS (CABP) COHORT

Study no.	Routine Histology			IF Results
	Inflammation	Corpora amylaceae	Vessel changes	
52	A - G G2 - G	Y	-	IgA - PG IgM - G PG V
53	G1 - PG	Y	-	IgM - Cells G PG
54	A - G G1 - PG G2 - PG	N	-	Neg
55	Glandular proliferation	N	-	Neg
56	G1 - PG	N	-	Neg
57	A - G G1 - ST	N	-	IgM - PG C3 - Lumen G V
58	G3 - PG	N	-	IgA - Lumen IgM - V C3 - Lumen
59	G1 - ST	Y	-	Neg
60	G1 - PG	N	-	Neg

Key:

IF - Immunofluorescence

A - Acute inflammation
G1 - Grade 1 chronic inflammation
G2 - Grade 2 chronic inflammation
G3 - Grade 3 chronic inflammation

G - Gland
PG - Periglandular
ST - Stromal
V - Vessel
T - Telangiectasia
VT - Vessel thickening

APPENDIX XXIII INDIRECT IMMUNOPEROXIDASE TECHNIQUE FOR TISSUE SECTIONS

To each section 50 ul of substrate was applied.

6 micron sections were cut at -35°C.

Sections were then air dried for 20 minutes.

Sections were fixed in a 1:1 acetone-chloroform mixture for ten minutes.

Normal rabbit serum was applied to each section at a dilution of 1:100 in phosphate buffered saline (PBS) for 20 minutes.

A first layer of mouse antihuman monoclonal antibody at a dilution of 1:5 in PBS was applied to each section for 60 minutes in a moist chamber.

The sections were then washed twice in PBS for 5 minutes.

A second layer of rabbit anti-mouse antibody with a peroxidase conjugate, at a dilution of 1:100 in PBS along with normal human serum, at a dilution of 1:40 in PBS, was applied to each section for 45 minutes in a moist chamber.

The sections were then washed twice in PBS for 5 minutes.

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Diamino-benzidine and hydrogen peroxide in TRIS HCl (TRIS HCl, pH 7.5, 0.05M - 50mls, diamino-benzidine - 1.25mls and hydrogen peroxide - 50 ul) was applied to each section for 5-15 minutes. The end point of development was assessed by light microscopy.

Each section was washed in running tap water for 5 minutes.

Each section was then washed in distilled water for 2 minutes.

Each section was then counterstained with haematoxylin and eosin for 30-60 seconds.

Each section was then washed in running tap water for 5 minutes followed by distilled water for 2 minutes.

Finally the sections were dried in alcohol, cleared in xylene and mounted in PBS (90%) and glycerol (10%).

APPENDIX XXIV DOUBLE IMMUNOFLUORESCENCE TECHNIQUE FOR TISSUE SECTIONS

To each section 50 ul of substrate was applied.

6 micron sections were cut at -35°C.

Sections were then air dried for 20 minutes.

Sections were fixed in a 1:1 acetone - chloroform mixture for 10 minutes.

Normal rabbit serum was applied to each section at a dilution of 1:100 in phosphate buffered saline (PBS) for 20 minutes.

A first layer of a pair of mouse antihuman monoclonal antibodies at a dilution of 1:5, with the exception of CD45RO used at a dilution of 1:100 and RFD1 at a dilution of 1:2, were applied to the section for 60 minutes in a moist chamber. With the CD4 antibody this was applied already conjugated to FITC (fluorescein isothiocyanate).

Sections were washed in PBS for 5 minutes three times.

A second layer of rabbit antimouse antibodies, comprising anti-IgG-TRITC (tetraethyl rhodamine isothiocyanate) and anti-IgM-FITC (fluorescein isothiocyanate) at a dilution of 1:50 was applied to each section. In the case of the

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CD4/CD7 and CD4/CD45RO pairings in which CD4/FITC conjugate was employed as the primary layer, the second layer consisted of anti IgG-TRITC and PBS only. The second layer was incubated in a moist chamber for a period of 30 minutes.

The sections were then washed three times in PBS for 5 minutes on each occasion.

Excess moisture was dabbed from the slide prior to mounting in PBS (90%) and Glycerol (10%) containing p-phenylene diamine, an anti-bleaching agent.

Nail varnish was applied to the edge of the coverslip to reduce evaporation of mountant.

APPENDIX XXV QUANTITATIVE ASSESSMENT OF HLA-DR EXPRESSION USING GLUCOSE OXIDASE CONJUGATED RFDR ANTIBODY

To each section 50 ul of substrate was applied.

6 micron sections were cut at -35°C.

Sections were air dried for 20 minutes.

Sections were fixed in a 1:1 acetone-chloroform mixture for ten minutes.

The glucose oxidase conjugated anti HLA-DR antibody (GO-DR) at a dilution of 1:10 in phosphate buffered saline (PBS) was applied to each section and incubated in a moist chamber for 90 minutes.

During the preceding stage, incubation medium comprising 50 mls of Tris-HCl, 0.05M pH 8.3, 0.335g of B-D-glucose (Sigma) and 35mg of Nitroblue tetrazolium (NBT) (Sigma) was prepared, protected from the light and incubated at 37 C for 60 minutes to facilitate the stability of isomerisation of D-glucose.

The GO-DR was washed from the sections with PBS for 10 minutes.

0.1 ml of Phenazine methasulphate (PMS) (Sigma) was added

to the incubation medium prior to immersion of the slides in this medium, at 37°C, protected from the light for a period of 30 minutes.

After ensuring adequate development of the stain by light microscopy, the sections were washed in running tap water for ten minutes.

The slides were dabbed dry and the sections mounted in PBS (90%) and glycerol (10%).

The sections were then examined under a scanning and integrating microdensitometer.

APPENDIX XXVI SUMMARY OF THE RESULTS OF IMMUNOPEROXIDASE STAINING OF PROSTATIC TISSUE IN PATIENTS WITH CHRONIC ABACTERIAL PROSTATITIS

Study no.	Biopsy Grade	Monoclonal Antibodies RFDR	T-mix	B-mix	RFD1	RFD7	RFD9	CD14
13	1	+EPS ++G	+/-P +EGS	-	+/-G	+/-G	-	-
25	1	+EGPS	+GPS	-	+/-E GS +P	+/-P +EGS	-	-
30	1	+GPS +/-E	+GPS	-	-	+EGS	-	-
35	1	+ES ++G	+EGPS	-	+GPS	+/-GP +S	-	-
36	1	+EGPS	+EGPS	-	-	+S	-	-
37	1	+EGPS	+GPS	-	+/-E GPS	+EGPS	-	-
40	1	+/-E +GS	+EGPS	-	+/-E GPS	+EGPS	-	-
44	1	+EP ++GS	+EGPS	-	+/-GS	+GS	-	-
47	1	+/-P +EGS	+GPS	-	+/-GP S	+EGPS	-	-
54	1	+EGPS	+GPS	-	+/-GS	+GPS	-	-
1	2	+P ++EGS	+EP ++GS	-	+/-E GS	+EP ++GS	-	+GPS
7	2	+GPS	++GPS	-	+/-GS	+GS	-	+GS
17	2	+GPS ++E	+/++G ++PS	-	+/-GP S	+GPS +/++E	-	-
29	2	+GP ++S	+GP ++S	-	+/-GS	++GS	-	-
31	2	+EPS ++S	+EP ++GS	-	+/-GS	+GS	-	-

APPENDIX XXVI SUMMARY OF THE RESULTS OF IMMUNOPEROXIDASE STAINING OF PROSTATIC TISSUE IN PATIENTS WITH CHRONIC ABACTERIAL PROSTATITIS

Study no.	Biopsy Grade	Monoclonal Antibodies RFDR	T-mix	B-mix	RFD1	RFD7	RFD9	CD14
45	2	+E ++GS	+EP ++GS	-	-	++S	-	-
48	2	+E ++GPS	++GPS	-	+/-E	+GS	-	+S
52	2	+/-EP ++GS	+EGP ++S	-	+/-EG S	+EP ++GS	-	+/-P +G
10	3	+PS +++EG	++PS +++EG	-	+GPS ++E	+E +++G PS	-	-
11	3	+EPS +++G	+PS ++E +++G	-	+/-E +GPS	++GPS +++E	-	+/-P +G
27	3	++EP +++GS	++EP +++GS	-	+/-P +EGS	++P +++E GS	-	-
42	3	+P ++G +++S	++GP +++S	-	+G	++GS	-	-
58	3	++P +++E GS	++P +++E GS	-	+/-G +S	++S +++G	-	+G
39	4	+P ++S +++EG	++P +++E GS	-	+/-E +GPS	+++E GPS	+GS	+GP +++S

Key:

+/-	< 5 cells/hpf	E - Epithelium
+	5-10 cells/hpf	G - Glandular
++	11-15 cells/hpf	P - Perivascular
+++	>15 cells/hpf	S - Stromal

APPENDIX XXVII THE RATIO OF CD4+ TO CD8+ T-CELLS IN PROSTATIC TISSUE OF PATIENTS WITH CHRONIC ABACTERIAL PROSTATITIS

Study no.	Biopsy Grade	CD4+:CD8+ T-Cell Ratio			
		Epithelium	Gland	Perivascular	Stroma
13	1	3	1.2	0.82	0.82
25	1	-	0.47	1	0.67
30	1	-	0.4	1	0.6
35	1	2	0.89	4	2
36	1	1	1.43	1.28	2.5
37	1	-	2	1.1	2
40	1	1	0.6	1.05	0.82
44	1	0.5	0.41	1	0.33
47	1	-	1.7	1	1.63
54	1	-	1.38	0.33	0.43
Mean		**1.5**	**1.05**	**1.26**	**1.18**
SD +/-		**1**	**0.58**	**0.99**	**0.78**
1	2	0.8	0.63	0.75	0.63
7	2	-	0.59	0.59	0.5
17	2	-	0.45	0.5	0.27
29	2	-	1.5	1	1.6
31	2	1.8	1.3	1	1.8
45	2	2	2.13	1	1.94
48	2	-	0.2	1	0.25
52	2	0.51	0.61	0.49	1.86
Mean		**1.4**	**0.93**	**0.79**	**1.11**
SD +/-		**0.85**	**0.65**	**0.24**	**0.76**
10	3	0.37	0.37	0.5	0.5
11	3	0.47	0.47	1.7	1.94
27	3	0.42	0.59	0.6	0.71
42	3	-	0.45	0.5	0.27
58	3	0.43	0.72	1	0.64
Mean		**0.42**	**0.52**	**0.86**	**0.81**
SD +/-		**0.04**	**0.14**	**0.51**	**0.65**
39	4	0.37	0.49	0.49	0.54

APPENDIX XXVIII PERCENTAGE OF CD4+ AND CD8+ T-CELLS EXPRESSING THE CD45RO ANTIGEN

Study	Biopsy	CD4+ (%)				CD8+ (%)			
no.	Grade	E	G	PV	S	E	G	PV	S
13	1	78	57	100	50	74	75	80	52
25	1	50	78	67	37	44	84	43	46
30	1	78	87	39	78	-	30	-	46
35	1	80	78	84	59	100	50	34	20
36	1	50	46	19	13	-	25	-	25
37	1	100	100	100	100	100	100	100	100
40	1	100	79	35	82	-	100	-	90
44	1	66	73	25	50	46	50	50	67
47	1	76	71	50	39	100	88	100	86
54	1	61	45	45	28	34	34	-	68
Mean		**73.9**	**71.4**	**56.4**	**53.6**	**71.1**	**63.6**	**67.8**	**60**
SD +/-		**17.7**	**17.5**	**29.8**	**26.7**	**29.6**	**29.7**	**29.3**	**27**
1	2	100	81	100	33	50	40	-	63
7	2	0	88	68	85	-	64	64	64
17	2	50	75	68	69	50	41	32	39
29	2	87	93	50	47	-	100	-	100
31	2	93	83	39	83	78	92	-	100
45	2	100	68	-	67	-	10	-	10
48	2	71	77	74	79	-	85	-	90
52	2	74	67	84	85	65	50	74	76
Mean		**71.9**	**79**	**69**	**68.5**	**60.8**	**60.3**	**56.7**	**67.8**
SD +/-		**33.6**	**9.1**	**20.3**	**19.2**	**13.5**	**30.8**	**21.9**	**31.3**
10	3	82	84	54	59	63	63	84	79
11	3	67	77	66	68	48	77	-	100
27	3	63	68	68	84	64	64	28	25
42	3	67	38	50	29	-	69	-	75
58	3	85	72	55	60	61	62	43	41
Mean		**72.8**	**67.8**	**58.6**	**60**	**58.9**	**66.9**	**51.7**	**64**
SD +/-		**10**	**17.7**	**7.9**	**20**	**7.4**	**6.3**	**29**	**30.4**
39	4	61	60	44	71	56	53	28	17

Key:

E - Epithelium PV - Perivascular

G - Gland S - Stromal

APPENDIX XXIX PERCENTAGE OF CD4+ AND CD8+ T-CELLS EXPRESSING THE CD7 ANTIGEN

Study	Biopsy	CD4+ (%)				CD8+ (%)			
no.	Grade	E	G	PV	S	E	G	PV	S
13	1	63	30	25	67	40	38	50	39
25	1	0	27	68	27	0	51	33	0
30	1	100	89	26	59	-	39	-	10
35	1	33	80	72	69	30	34	60	20
36	1	66	62	16	71	-	30	-	30
37	1	-	100	50	100	-	17	-	17
40	1	100	43	-	55	-	10	-	20
44	1	66	73	68	69	-	68	0	69
47	1	34	31	22	16	-	35	-	41
54	1	50	75	50	34	50	18	28	28
Mean		**56.9**	**61**	**44.1**	**56.7**	**30**	**34**	**34.2**	**27.4**
SD +/-		**32.2**	**26.5**	**22.3**	**24.8**	**21.6**	**17.1**	**23.1**	**19.2**
1	2	88	65	67	86	35	57	49	49
7	2	100	100	80	96	32	77	63	52
17	2	75	50	33	65	-	78	-	73
29	2	100	83	50	56	-	50	-	61
31	2	69	53	61	91	53	50	-	68
45	2	78	94	75	81	-	75	100	75
48	2	59	64	92	91	-	100	-	100
52	2	86	76	71	78	38	42	36	41
Mean		**81.9**	**73.1**	**66.1**	**80.5**	**39.5**	**66.1**	**62**	**64.9**
SD +/-		**14.5**	**18.3**	**18.3**	**13.8**	**9.3**	**19.5**	**27.6**	**18.6**
10	3	73	75	83	88	82	78	-	82
11	3	71	79	50	79	56	75	75	44
27	3	63	68	68	84	64	64	28	25
42	3	67	38	50	29	-	69	-	75
58	3	85	72	55	60	61	62	43	41
Mean		**71.8**	**66.4**	**61.2**	**68**	**65.8**	**69.6**	**48.7**	**53.4**
SD +/-		**8.3**	**16.3**	**14.2**	**24.3**	**11.3**	**6.9**	**24**	**24.2**
39	4	61	60	44	71	56	53	28	17

Key:

E - Epithelium PV - Perivascular

G - Gland S - Stromal

APPENDIX XXX DISTRIBUTION OF CELLS EXPRESSING RFD1 AND RFD7 EPITOPES IN PROSTATIC BIOPSIES FROM PATIENTS WITH CHRONIC ABACTERIAL PROSTATITIS

		% OF CELLS EXPRESSING THE EPITOPE											
Study	Grade	Epithelium			Gland			Perivascular			Stroma		
no.		D1	D1/D7	D7	D1	D1/D7	D7	D1	D1/D7	D7	D1	D1/D7	D7
13	1	40	10	50	56	22	22	60	0	40	63	6	31
25	1	22	10	68	22	10	68	72	5	23	6	12	82
30	1	0	0	100	0	0	100	0	0	0	0	0	100
35	1	0	0	0	50	40	10	67	33	0	17	66	17
36	1	0	0	0	0	0	0	0	0	0	0	0	100
37	1	25	25	50	25	25	50	25	25	50	25	25	50
40	1	0	15	85	2	18	80	12	19	69	18	53	29
44	1	0	0	0	10	0	90	0	0	0	0	14	86
47	1	0	0	100	34	23	43	5	9	86	5	9	86
54	1	0	0	0	11	6	83	0	0	100	29	0	71
Mean		**14.5**	**10**	**75.5**	**23**	**16**	**61**	**34.4**	**13**	**53**	**16.3**	**18.5**	**65**
SD +/-		**17**	**9.5**	**23**	**20**	**13.2**	**31**	**31**	**13**	**35**	**19.6**	**23.2**	**31**
1	2	6	17	77	2	6	92	0	0	100	3	0	97
7	2	0	0	0	8	10	82	0	0	0	8	10	82
17	2	0	0	100	4	13	83	7	7	86	6	8	86
29	2	0	0	0	5	0	95	0	0	0	8	0	92
31	2	0	0	0	10	7.5	82.5	0	0	0	10	7	83
45	2	0	0	0	0	0	0	0	0	0	0	0	100
48	2	0	0	0	18	0	82	0	0	0	0	0	100
52	2	25	25	50	3	24	73	0	0	100	7	13	80
Mean		**10.3**	**14**	**75.7**	**7.2**	**8.6**	**84.2**	**2**	**2**	**96**	**5**	**5**	**90**
SD +/-		**13.1**	**13**	**25**	**5.6**	**8.3**	**7.3**	**4**	**4**	**8.1**	**4**	**5.4**	**8.4**
10	3	67	11	22	18	38	44	22	17	61	12	34	54
11	3	8	12	80	21	13	66	30	7	63	18	19	63
27	3	8	10	82	14	17	69	31	12	57	12	19	69
42	3	0	0	0	10	25	65	0	0	0	0	0	100
58	3	0	0	0	18	23	59	0	0	0	33	33	33
Mean		**27.7**	**11**	**61.3**	**16**	**23**	**61**	**28**	**12**	**60**	**15**	**21**	**64**
SD +/-		**34.1**	**1**	**34.1**	**4.3**	**9.5**	**10**	**4.9**	**5**	**3.1**	**12**	**13.8**	**24**
39	4	10	11	79	24	15	61	33	10	57	32	23	45

APPENDIX XXXI THE RFD1:RFD7 RATIO IN PROSTATIC TISSUE FROM PATIENTS WITH CHRONIC ABACTERIAL PROSTATITIS

Study no.	Biopsy Grade	RFD1 : RFD7 CELL RATIO Epithelium	Gland	Perivascular	Stroma
13	1	0.83	1.77	1.5	1.86
25	1	0.41	0.41	2.75	0.19
30	1	0	0	-	0
35	1	-	1.8	3.03	1.0
36	1	-	-	-	0
37	1	0.67	0.67	0.67	0.67
40	1	0.15	0.2	0.33	0.86
44	1	-	0.11	-	0.13
47	1	0	0.86	0.15	0.15
54	1	-	0.19	0	0.41
Mean		**0.34**	**0.67**	**0.99**	**0.58**
SD +/-		**0.35**	**0.69**	**1.27**	**0.56**
1	2	0.24	0.08	0	0.03
7	2	-	0.2	-	0.2
17	2	0	0.18	0.15	0.15
29	2	-	0.05	-	0.09
31	2	-	0.19	-	0.19
45	2	-	-	-	0
48	2	-	0.22	-	0
52		0.67	0.28	0	0.22
Mean		**0.30**	**0.17**	**0.05**	**0.11**
SD +/-		**0.34**	**0.08**	**0.09**	**0.09**
10	3	2.36	0.68	0.5	0.52
11	3	0.22	0.43	0.53	0.45
27	3	0.2	0.36	0.61	0.35
42	3	-	0.39	-	0
58	3	-	0.50	-	0.99
Mean		**0.93**	**0.47**	**0.55**	**0.46**
SD +/-		**1.24**	**0.13**	**0.06**	**0.36**
39	4	0.23	0.51	0.64	0.81

APPENDIX XXXII RELATIVE ABSORPTION OF REACTION PRODUCT FOR HLA-DR EXPRESSION IN NORMAL CONTROLS AND PATIENTS WITH CHRONIC ABACTERIAL PROSTATITIS

Study no.	Biopsy Grade	Site	D	MBG	CD	Area	RA
Cont.	0	EG	124.54	64.56	59.98	71.58	0.84
			94.53		29.97	43.26	0.70
			124.51		59.95	70.44	0.85
			88.11		23.55	35.12	0.67
			105.38		40.82	67.35	0.61
			82.93		18.37	25.95	0.71
			94.67		30.11	49.16	0.61
			81.83		17.27	27.25	0.63
			104.43		39.87	64.32	0.62
			103.83		39.27	59.38	0.66
			101.05		36.49	64.78	0.56
			94.16		29.60	50.59	0.59
			105.07		40.51	63.10	0.64
			99.10		34.54	59.56	0.58
			103.99		39.43	66.23	0.60
			101.72		37.16	66.12	0.56
			93.44		28.88	47.96	0.60
			91.52		26.96	49.46	0.55
			90.61		26.05	39.57	0.66
			87.40		22.84	33.22	0.69
Mean							**0.65**
SD +/-							**0.08**
		PV	87.99	64.54	23.45	32.35	0.72
			87.27		22.73	30.95	0.73
			91.40		26.86	42.50	0.63
			89.38		24.84	39.80	0.62
			87.76		23.22	35.56	0.65
			84.12		19.58	25.32	0.77
Mean							**0.66**
SD +/-							**0.08**
		ST	83.92	65.12	18.8	28.36	0.66
			89.50		24.38	38.65	0.63
			88.98		23.86	40.01	0.60
			83.48		18.36	24.97	0.74
			94.07		28.95	44.69	0.65
			84.06		18.94	28.92	0.65
			86.23		21.11	25.07	0.84
			75.21		10.09	17.17	0.59
			92.27		27.15	42.57	0.64
			83.22		18.1	25.88	0.70
Mean							**0.67**
SD +/-							**0.07**

APPENDIX XXXII

Study no.	Biopsy Grade	Site	D	MBG	CD	Area	RA
Cont.	0	EG	94.57	64.55	30.02	67.56	0.44
			75.26		10.71	34.52	0.31
			91.10		26.55	69.40	0.38
			80.0		15.45	43.43	0.36
			91.02		26.47	63.06	0.42
			93.47		28.92	60.87	0.48
			83.0		18.45	53.47	0.35
			78.52		13.97	48.19	0.29
			85.01		20.46	56.22	0.36
			88.52		23.97	67.09	0.36
			78.89		14.34	53.34	0.27
			92.95		28.40	70.36	0.40
			76.23		11.68	37.07	0.32
			80.59		16.04	54.10	0.30
			85.59		21.04	66.34	0.32
Mean							**0.36**
SD +/-							**0.06**
		PV	80.01	65.53	14.57	56.68	0.26
			87.34		21.81	49.83	0.44
			76.55		11.02	41.09	0.27
			79.63		14.1	53.77	0.26
			77.28		11.75	45.74	0.26
Mean							**0.30**
SD +/-							**0.08**
		ST	78.39	66.86	11.53	44.01	0.26
			74.97		8.11	35.19	0.23
			78.63		11.77	50.07	0.24
			76.27		9.41	36.64	0.26
			72.90		6.04	24.28	0.25
			75.69		8.83	37.72	0.23
			79.96		13.1	57.49	0.23
			74.96		8.1	36.17	0.22
			74.30		7.44	28.98	0.26
			76.27		9.41	40.46	0.23
Mean							**0.24**
SD +/-							**0.02**

APPENDIX XXXII

Study no.	Biopsy Grade	Site	D	MBG	CD	Area	RA
Cont.	0	EG	89.07	62.59	26.48	52.93	0.5
			85.64		23.05	46.64	0.49
			90.03		27.44	56.16	0.49
			86.53		23.94	55.41	0.43
			91.47		23.88	64.14	0.45
			89.17		26.58	63.13	0.42
			95.52		32.93	68.80	0.48
			86.97		24.38	56.27	0.43
			88.09		25.50	51.37	0.5
			81.51		18.92	44.61	0.42
Mean							**0.46**
SD +/-							**0.03**
		PV	78.38	62.59	15.79	23.42	0.67
			74.74		12.15	15.19	0.80
			94.36		31.77	60.95	0.52
			77.10		14.51	20.96	0.69
			88.60		26.20	52.41	0.50
Mean							**0.64**
SD +/-							**0.13**
		ST	83.9	63.04	20.86	42.93	0.49
			70.47		7.43	12.43	0.60
			81.21		18.17	28.69	0.63
			86.31		23.27	47.27	0.49
			100.71		37.67	68.17	0.55
			76.66		13.62	23.93	0.57
			100.02		36.98	59.01	0.63
			77.71		14.67	20.34	0.72
			81.31		18.27	35.61	0.51
			88.78		25.74	50.24	0.51
Mean							**0.58**
SD +/-							**0.07**

APPENDIX XXXII

Study no.	Biopsy Grade	Site	D	MB	CD	Area	RA
35	1	EG	99.36	61.06	38.30	68.49	0.56
			115.08		54.02	70.34	0.77
			90.76		29.70	58.56	0.51
			100.64		36.01	70.46	0.51
			86.84		25.58	64.59	0.40
			113.72		52.66	68.67	0.77
			96.82		35.76	54.98	0.65
			93.35		32.29	69.42	0.47
			92.96		31.90	70.42	0.45
			100.14		39.08	68.22	0.57
			119.49		58.43	72.09	0.81
			120.04		58.98	73.38	0.80
			120.97		59.91	61.24	0.98
			124.19		63.13	72.72	0.87
			102.35		41.29	64.73	0.64
			102.24		41.18	63.07	0.65
			115.16		54.10	69.46	0.78
			139.99		78.93	72.90	1.08
Mean							**0.68**
SD +/-							**0.19**
		PV	99.32	61.06	38.26	71.22	0.54
			87.07		26.01	63.64	0.41
			100.88		39.82	73.47	0.54
			80.06		19.00	52.79	0.36
			109.06		48.00	74.12	0.65
			85.25		24.19	62.18	0.39
			88.31		27.25	61.96	0.45
			88.16		27.10	51.40	0.53
			87.62		26.56	69.26	0.38
			90.85		29.79	64.19	0.46
Mean							**0.47**
SD +/-							**0.09**
		ST	104.33	61.06	43.27	64.86	0.67
			75.41		14.35	39.31	0.37
			79.68		18.62	51.39	0.36
			101.55		40.49	71.42	0.57
			102.34		41.28	72.58	0.59
			114.76		53.70	72.73	0.74
			73.05		11.99	35.53	0.34
			79.79		18.73	50.58	0.37
			102.50		41.44	65.45	0.63
			98.35		37.29	65.22	0.57
Mean							**0.52**
SD +/-							**0.15**

APPENDIX XXXII

Study no.	Biopsy Grade	Site	D	MB	CD	Area	RA
47	1	EG	130.27	64.63	65.64	66.19	0.99
			120.15		55.52	59.88	0.93
			103.15		38.52	51.36	0.75
			113.59		48.96	65.95	0.74
			110.24		45.61	65.95	0.69
			108.08		43.45	63.22	0.69
			114.12		49.49	65.44	0.76
			124.65		60.02	67.70	0.87
			96.98		32.35	59.06	0.56
			99.29		34.66	63.30	0.55
			108.29		43.66	72.49	0.60
			111.00		46.37	65.16	0.71
			89.36		24.73	52.47	0.47
			92.15		27.52	60.91	0.45
			125.21		60.58	65.97	0.92
			110.19		45.56	64.37	0.71
			104.60		39.97	67.66	0.59
			121.15		56.62	74.40	0.76
			99.16		34.53	60.29	0.57
Mean							**0.69**
SD +/-							**0.15**
		PV	155.85	64.43	91.12	74.15	1.23
			106.44		41.81	63.26	0.66
			86.31		21.68	43.36	0.50
			138.99		74.36	73.77	1.01
			111.95		47.32	65.09	0.73
			86.06		23.43	44.66	0.52
Mean							**0.78**
SD +/-							**0.29**
		ST	81.51	64.63	16.88	52.45	0.32
			76.61		11.98	32.85	0.36
			96.50		31.87	72.33	0.44
			103.28		38.65	74.12	0.52
			94.04		29.41	68.05	0.43
			85.29		20.66	62.10	0.33
			82.01		17.38	43.87	0.40
			88.37		23.74	62.78	0.38
			88.31		23.68	65.31	0.36
			87.76		23.13	67.50	0.34
Mean							**0.39**
							0.06

APPENDIX XXXII

Study no.	Biopsy Grade	Site	D	MB	CD	Area	RA
25	1	EG	95.74	67.75	29.99	57.79	0.52
			95.55		29.80	55.16	0.54
			90.45		24.70	34.40	0.72
			122.02		56.27	76.63	0.73
			108.82		43.07	73.29	0.59
			124.10		58.35	77.39	0.75
			107.23		41.48	76.33	0.54
			124.34		58.59	73.94	0.79
			83.72		17.97	32.66	0.55
			119.37		53.62	75.77	0.71
			124.28		58.53	73.27	0.80
			105.07		39.32	62.90	0.63
			122.85		57.10	77.23	0.74
Mean							**0.58**
SD +/-							**0.13**
		PV	99.89	65.76	34.13	60.57	0.56
			102.80		37.04	69.93	0.53
			92.50		26.74	53.30	0.50
			92.42		26.66	59.87	0.45
			105.75		39.99	78.36	0.51
Mean							**0.51**
SD +/-							**0.04**
		ST	99.44	65.76	33.68	76.95	0.44
			108.36		42.60	78.32	0.54
			103.99		38.23	77.28	0.50
			101.16		35.41	77.59	0.46
			103.22		37.46	76.82	0.49
			108.09		42.33	77.42	0.55
			105.06		39.30	77.60	0.51
			95.81		30.05	75.29	0.40
			90.82		25.06	73.61	0.34
			101.61		35.85	76.89	0.47
Mean							**0.47**
SD +/-							**0.06**

APPENDIX XXXII

Study no.	Biopsy Grade	Site	D	MB	CD	Area	RA
48	2	EG	186.59	59.00	127.59	76.15	1.68
			129.30		70.30	67.98	1.03
			135.18		76.18	71.57	1.06
			171.98		112.72	74.78	1.51
			121.98		62.98	62.60	1.01
			154.08		95.08	73.67	1.29
			163.74		104.74	71.87	1.46
			146.35		87.35	73.48	1.19
			124.31		65.31	68.46	0.95
			162.12		103.12	73.27	1.41
			123.13		64.13	58.67	1.09
			155.14		96.14	72.36	1.33
			155.27		96.27	72.76	1.32
			132.36		73.36	67.01	1.09
Mean							**1.24**
SD +/-							**0.22**
		PV	126.38	58.28	67.38	53.58	1.26
			140.80		87.52	71.43	1.16
			144.62		86.34	72.03	1.12
			159.62		101.34	72.12	1.41
			144.50		86.22	63.89	1.35
Mean							**1.26**
SD +/-							**0.12**
		ST	85.03	58.27	28.76	22.31	1.20
			86.74		28.47	24.31	1.17
			94.24		35.97	25.97	1.39
			98.53		40.26	27.33	1.47
			90.86		32.59	26.45	1.23
			81.61		23.34	21.63	1.08
			86.63		28.36	22.93	1.24
			89.56		31.29	25.06	1.25
			79.92		21.65	21.07	1.03
			99.88		41.61	22.86	1.82
Mean							**1.27**
SD +/-							**0.18**

APPENDIX XXXII

Study no.	Biopsy Grade	Site	D	MB	CD	Area	RA
7	2	EG	112.71	63.17	49.54	50.44	0.98
			92.60		28.89	27.61	1.05
			109.97		44.80	52.02	0.86
			124.28		61.11	60.50	1.01
			101.31		38.14	40.73	0.94
			108.17		45.00	42.65	1.06
			109.24		46.07	48.88	0.94
			104.13		40.96	40.46	1.01
			113.31		50.04	51.24	0.98
			108.04		44.87	51.56	0.87
			157.53		94.36	76.80	1.23
			153.89		90.72	77.29	1.17
			135.28		72.11	77.22	0.93
			159.08		95.91	78.99	1.21
			145.53		82.26	76.89	1.07
			123.70		60.53	57.57	1.05
			121.17		58.00	51.41	1.13
			110.61		47.44	45.29	1.05
			125.29		62.12	57.76	1.08
			111.14		47.97	47.59	1.01
Mean							**1.04**
SD +/-							**0.10**
		PV	139.78	63.17	76.61	78.10	0.98
			103.18		40.01	47.93	0.83
			98.65		35.48	44.91	0.79
			121.38		58.21	60.34	0.96
			126.03		62.86	72.23	0.87
			124.43		61.26	66.93	0.92
			145.74		82.57	77.52	1.07
			99.48		36.31	50.84	0.71
			155.77		92.60	78.02	1.19
			105.39		42.22	47.32	0.89
Mean							**0.92**
SD +/-							**0.14**
		ST	91.77	63.17	28.60	39.02	0.73
			106.31		43.14	57.57	0.75
			108.38		45.21	58.30	0.78
			87.16		23.99	32.34	0.74
			92.42		29.25	44.52	0.66
			96.74		33.57	41.98	0.80
			88.56		25.39	41.74	0.61
			90.87		27.70	46.79	0.59
			106.43		43.26	55.68	0.78
			88.24		25.07	30.82	0.81
Mean							**0.73**
SD +/-							**0.08**

APPENDIX XXXII

Study no.	Biopsy Grade	Site	D	MB	CD	Area	RA
11	3	EG	182.89	60.85	122.04	73.41	1.66
			221.75		160.90	73.46	2.19
			209.83		148.98	73.60	2.02
			145.62		84.77	62.81	1.35
			211.54		150.69	73.28	2.06
			163.22		102.37	74.00	1.38
			206.08		145.23	74.57	1.95
			228.19		167.34	73.99	2.26
			233.03		172.18	73.67	2.34
			229.41		168.56	73.30	2.30
			223.69		162.82	74.18	2.19
			229.66		168.81	73.97	2.28
			174.72		113.87	74.06	1.54
			216.62		155.77	73.72	2.11
			210.27		149.42	73.60	2.03
Mean							**1.98**
SD +/-							**0.33**
		PV	174.33	60.85	113.48	73.51	1.54
			150.55		89.70	72.42	1.24
			149.66		88.81	67.07	1.32
			146.30		85.45	63.16	1.35
			140.84		79.99	63.54	1.26
			169.26		108.41	73.57	1.47
			145.55		84.70	73.92	1.16
Mean							**1.33**
SD +/-							**0.13**
		ST	121.42	60.85	60.57	74.44	0.81
			163.29		102.44	74.80	1.37
			127.45		66.60	74.66	0.89
			163.27		102.42	73.39	1.40
			135.55		74.70	73.20	1.02
			125.73		64.88	73.52	0.88
			143.63		82.78	74.71	1.11
			170.63		109.78	74.00	1.48
			149.76		88.91	73.76	1.21
			136.63		75.18	74.02	1.02
Mean							**1.12**
SD +/-							**0.24**

APPENDIX XXXII

Study no.	Biopsy Grade	Site	D	MB	CD	Area	RA
58	3	EG	198.41	62.5	135.91	69.82	1.95
			195.23		132.73	71.36	1.86
			167.53		105.03	69.98	1.50
			168.49		105.99	62.69	1.69
			210.43		147.93	71.29	2.08
			167.88		105.38	71.28	1.48
			243.67		181.17	71.17	2.55
			139.93		77.43	57.26	1.48
			163.54		101.04	69.28	1.48
			171.44		108.94	71.73	1.52
			211.67		149.17	71.59	2.08
			214.34		151.84	69.35	2.19
			170.46		107.96	70.66	1.53
			111.47		48.97	32.92	1.49
			192.76		130.26	66.52	1.96
			157.87		95.37	63.73	1.50
			222.46		159.96	71.95	2.22
			146.61		84.11	53.00	1.59
			158.10		95.66	59.53	1.61
			121.78		59.28	36.09	1.64
Mean							**1.77**
SD +/-							**0.32**
		PV	156.57	63.07	93.50	68.05	1.37
			151.77		88.70	72.02	1.23
			164.70		101.63	72.31	1.41
			152.39		89.32	72.55	1.23
			150.26		87.76	72.29	1.21
Mean							**1.29**
SD +/-							**0.09**
		ST	122.45	63.07	59.38	72.83	0.82
			176.32		113.25	72.67	1.56
			193.57		130.50	72.30	1.80
			233.94		170.87	72.13	2.37
			200.72		138.22	71.92	1.92
			212.88		149.81	72.26	2.07
			178.69		115.12	72.38	1.59
			172.19		109.12	71.34	1.53
			164.73		101.66	72.09	1.41
			154.13		91.63	71.86	1.28
			104.92		41.85	54.95	0.76
			103.59		40.52	52.54	0.77
			109.30		46.23	62.93	0.73
			105.04		41.97	56.96	0.74
			102.17		39.10	51.93	0.75
Mean							**1.33**
SD +/-							**0.48**

APPENDIX XXXII

Study no.	Biopsy Grade	Site	D	MB	CD	Area	RA
39	4	EG	234.84	62.97	171.87	71.50	2.40
			221.34		158.37	71.40	2.22
			196.77		133.80	71.64	1.87
			255.43		197.46	70.60	2.72
			230.82		167.85	71.29	2.35
			222.82		159.85	70.89	2.54
			222.78		159.81	71.34	2.24
			172.30		109.40	62.43	1.76
			252.65		189.68	71.15	2.67
			219.10		156.13	71.07	2.20
Mean							**2.30**
SD +/-							**0.31**
		PV	132.91	63.72	69.19	68.65	1.01
			117.17		53.45	60.90	0.88
			126.15		62.43	61.63	1.01
			125.85		62.13	61.07	1.02
			145.62		81.90	70.13	1.17
			126.85		63.13	61.06	1.03
			122.11		58.39	60.53	0.96
			104.11		40.39	44.98	0.90
Mean							**1.00**
SD +/-							**0.09**
		ST	152.10	69.47	87.63	71.21	1.23
			153.61		89.14	70.91	1.26
			166.67		102.22	71.63	1.43
			139.54		75.07	71.50	1.05
			144.55		80.08	71.12	1.13
			146.43		82.96	71.90	1.15
			176.82		112.35	71.80	1.56
			189.02		124.55	72.06	1.73
			180.24		115.77	71.54	1.62
			158.87		94.40	73.76	1.28
Mean							**1.34**
SD +/-							**0.23**

KEY:

EG - Epithelium & gland
PV - Perivascular
ST - Stromal
Cont. - Control
D - Density
MB - Mean background
CD - Corrected density
RA - Relative absorption

APPENDIX XXXIII **QUANTITATIVE HLA-DR EXPRESSION - MEAN RELATIVE ABSORPTION PER UNIT AREA**

Biopsy grade	Mean Relative Absorption per Unit Area		
	Gland & Epithelium	Perivascular	Stroma
0 (n=3)	0.49 +/- 0.06	0.53 +/- 0.1	0.5 +/- 0.05
1 (n=3)	0.65 +/- 0.16	0.59 +/- 0.14	0.46 +/- 0.09
2 (n=2)	1.14 +/- 0.16	1.09 +/- 0.13	1.0 +/- 0.13
3 (n=2)	1.88 +/- 0.33	1.31 +/- 0.11	1.23 +/- 0.36
4 (n=1)	2.3	1.0	1.34

APPENDIX XXXIV IMMUNORADIOMETRIC ASSAY OF PROSTATE SPECIFIC ANTIGEN (PSA) IN SERUM

The serum for assay, stored at -20° C, and the kit components were allowed to reach room temperature.

After blotting, an anti-PSA coated plastic bead was placed in each test tube.

To each bead was added 50 ul of either calibrator, control or test serum and labelled appropriately.

To each tube was added 200 ul of anti-PSA tracer antibody and mixed well.

These substrates were then incubated for two hours at room temperature in a horizontal rotator at a speed of 160 rpm.

Following incubation the beads underwent two washes with wash solution containing 0.3% sodium azide.

Each tube, containing the beads, was then analysed with a gamma counter.

Using calibration samples of known PSA concentrations a curve was constructed and the PSA concentrations calculated from the radio-isotope count.

APPENDIX XXXIV

For each sample, calibrator and control two assays were performed and the mean value calculated.

APPENDIX XXXV GHQ-60 SCORE IN RELATION TO SYMPTOM DURATION IN THE CHRONIC ABACTERIAL PROSTATITIS COHORT

Study no.	Symptom duration - yrs	GHQ-60 Score
1	1.5	2
2	6	6
3	2	1
4	5	1
5	20	10
6	6	3
7	1.5	32
8	4	11
9	0.3	19
10	5	8
11	1	14
12	2	0
13	0.3	3
14	2	2
15	1	10
16	3	8
17	1	0
18	0.3	18
19	2	2
20	12	2
21	0.3	6
22	5	3
23	0.8	1
24	2	6
25	1.5	0
26	0.3	3
27	0.3	4
28	1	1
29	1	8
30	10	15
31	1	1
32	0.2	1
33	10	22
34	0.5	25
35	10	38
36	2	5
37	0.3	21
38	1.5	29
39	0.1	12
40	1	0
41	0.5	2
42	3	0
43	0.8	11
44	3	40
45	2	0
46	0.5	25

APPENDIX XXXV GHQ-60 SCORE IN RELATION TO SYMPTOM DURATION IN THE CHRONIC ABACTERIAL PROSTATITIS COHORT

Study no.	Symtom duration in years	GHQ-60 Score
47	8	6
48	20	0
49	0.5	0
50	10	27
51	3.5	3
52	1.5	0
53	2	7
54	2	22
55	3	1
56	13	2
57	3	3
58	0.5	4
59	2	12
60	1	0

APPENDIX XXXVI CRC - ANAESTHETIC COCKTAIL

This cocktail of anaesthetic agents was employed in the animal experiments detailed in section 23.

1 part Hypnorm (Janssen Pharmaceuticals)

2 parts sterile water

1 part Hypnoval (Roche Ltd)

Dosage - 0.1 ml/30g body weight - female rats

- 0.25 ml/30g body weight - male rats.

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