ASPECTS OF THE IMMUNE RESPONSE TO GONOCOCCAL INFECTION

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Detection of antibodies reactive with N. gonorrhoeae

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Detection of antibodies reactive with N. gonorrhoeae

- (a) Before treatment
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Saliva

Detection of antibodies reactive with N. gonorrhoeae

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INDIRECT IMMUNOFLUORESCENT ANTIBODY TEST IN THE DIAGNOSIS OF GONORRHOEA IN WOMEN

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INTRODUCTION

Since the discovery of Neisseria gonorrhoeae by Albert Neisser in 1879, considerable advances have been made in the understanding of the pathogenesis of and immune response to gonococcal infection. Although N. gonorrhoeae principally affects mucous membranes, most studies in the immunology of gonorrhoea have considered the serological response only. It was the purpose of the present study to examine the local humoral immune response of tissues infected with N. gonorrhoeae and to determine if the detection of antibodies in the secretion could facilitate the diagnosis of gonococcal infection.

The gonococcus may infect the urethra, oropharynx and anorectum of both sexes, the cervix of the adult female, the conjunctiva of infants and adults, and the vulvo-vaginal epithelium of prepubertal females. Genital infection in women may be complicated by the development of bartholinitis or salpingitis. Prostatitis and epididymitis may result from proximal spread of the gonococcus from the male urethra. Uncommonly, intravascular dissemination of the gonococcus occurs, producing the clinical features of arthritis, dermatitis, meningitis and endocarditis. Strains of N. gonorrhoeae associated with disseminated infection appear to have particular growth characteristics and are fully sensitive to the action of penicillin.

There follows a brief account of the transmission and infectivity of N. gonorrhoeae.

TRANSMISSION AND INFECTIVITY OF GONORRHOEA

Gonorrhoea in adults is transmitted almost exclusively by sexual intercourse. Gonococcal conjunctivitis in the new-born is acquired as the child passes through the birth canal of the infected mother. Although vulvovaginitis in prepubertal children may be transmitted accidentally, for example by the child sharing a bed with infected parents, the mode of transmission is usually sexual (Branch and Paxton, 1965).

The reported prevalence of urethral gonococcal infection amongst male contacts of infected women varies from series to series. Until the recognition of the asymptomatic infected male (see below), many workers assumed that lack of clinical symptoms or signs indicated absence of infection, and thereby underestimated the prevalence of gonorrhoea in male contacts. When cultural procedures have been used to detect gonococcal infection, the reported prevalence of the disease in male contacts of women with gonorrhoea has ranged from just over 25 per cent (Pariser, Farmer and Marino, 1964) to about 65 per cent (Brown and Pederson, 1974). Brown and Pederson (1974) also demonstrated that infection was more likely to be detected in men who had frequent sexual contact with an infected women than in those who had infrequent contact.

The reported prevalence of genital gonorrhoea in female sexual contacts of infected men ranges from about 35 per cent (Brown, 1973) to about 90 per cent (Willcox, 1973), although most workers report a prevalence of about 50 to 65 per cent (Marino, Pariser and Wise, 1972; Blount, 1972; Wallin, 1975). In the majority of reports, infection is excluded on the basis of microbiological examination

on only one occasion, and often only one site (most commonly the cervix) has been sampled. The data presented, therefore, must be considered as underestimates.

Pharyngeal gonorrhoea is more likely to be contracted through fellatio than cunnilingus. It has been estimated that about 30 per cent of women, and 25 per cent of homosexual men, who practise fellatio with an infected partner will become infected, but only 14 per cent of heterosexual men who practise cunnilingus will be infected (Bro-Jorgensen and Jensen, 1973).

The risk of transmission of the gonococcus from the oropharynx to the genitals has not been evaluated, but clinical experience suggests that this is low. Transfer of infection by kissing is rare (Willmott, 1974).

Although anorectal gonorrhoea in men is almost invariably the result of homosexual anal intercourse, the degree of infectivity of the gonococcus for this site is not known. Similarly, data are not available regarding the risk of transmission of gonorrhoea to the anorectum of the female either from anal coitus or contamination by vaginal secretions.

The epidemiology of gonorrhoea merits consideration in relation to the present work as follows.

EPIDEMIOLOGY OF GONORRHOEA

Prevalence

Although gonorrhoea is readily treatable with modern antibiotics, the worldwide prevalence of the disease is of almost epidemic proportions. Figure (1) illustrates the rise in incidence over the past decade in Scotland. Similar trends have been observed in almost every country, although, by reason of differing diagnostic and reporting criteria, it is difficult to compare data quantitatively from different geographical areas. Unlike other countries, the incidence of gonorrhoea in Sweden fell significantly during the period 1972 to 1974 (Kallings and Moberg, 1977). This decrease in incidence was related possibly to changes in the behaviour of patients, in social conditions, and to the increasing adoption of "epidemiological treatment" policies (that is, treatment of sexual contacts of infected patients before the results of laboratory investigations are available).

Age groups

Gonorrhoea is a disease chiefly of young adults, Figure (2). Although the peak incidence in Glasgow is in the 25 to 34 years age group, data from other clinics show a peak incidence in the 20 - 24 years group (Robertson and Hosie, 1970). Women tend to be infected at an earlier age than men; 57 per cent of patients in the 15 - 19 age group were women, compared with 42 per cent in the 20 - 24 group. The proportion of teenagers with gonorrhoea is increasing; in Scotland

Figure (1)

Gonorrhoea. Cases reported in Scotland 1945 - 1976.

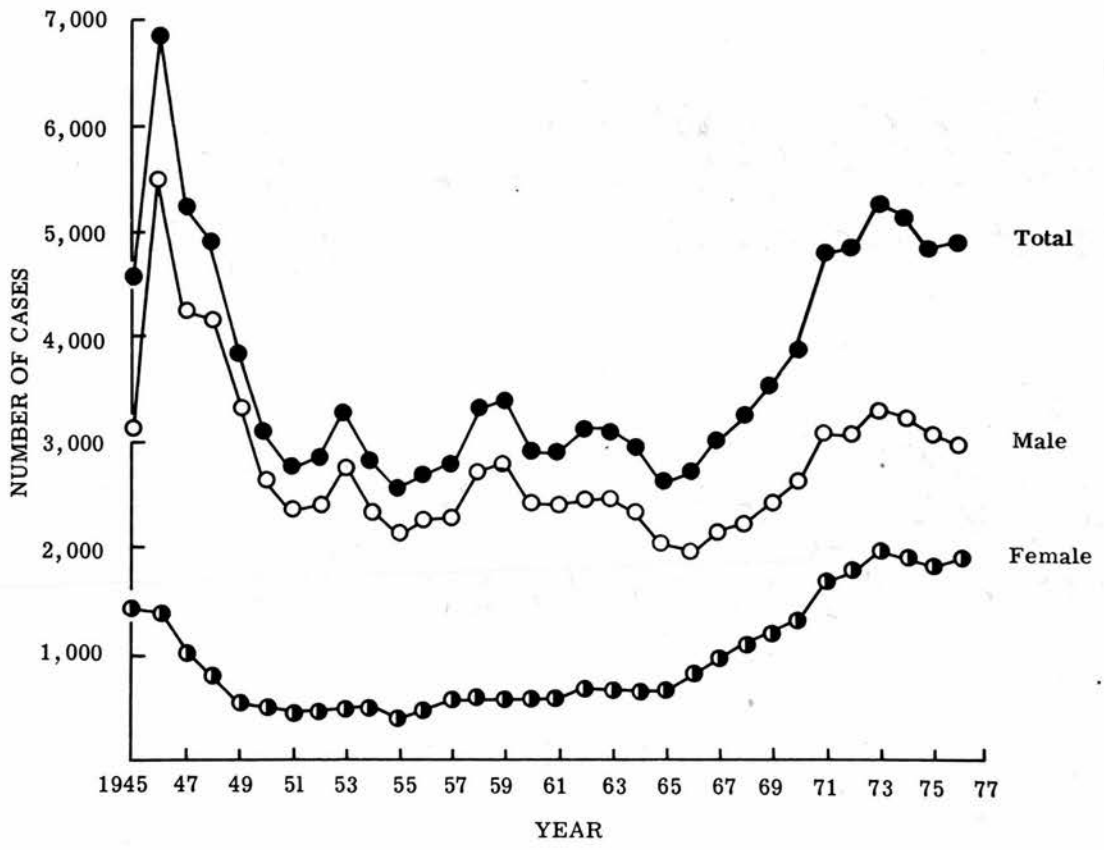
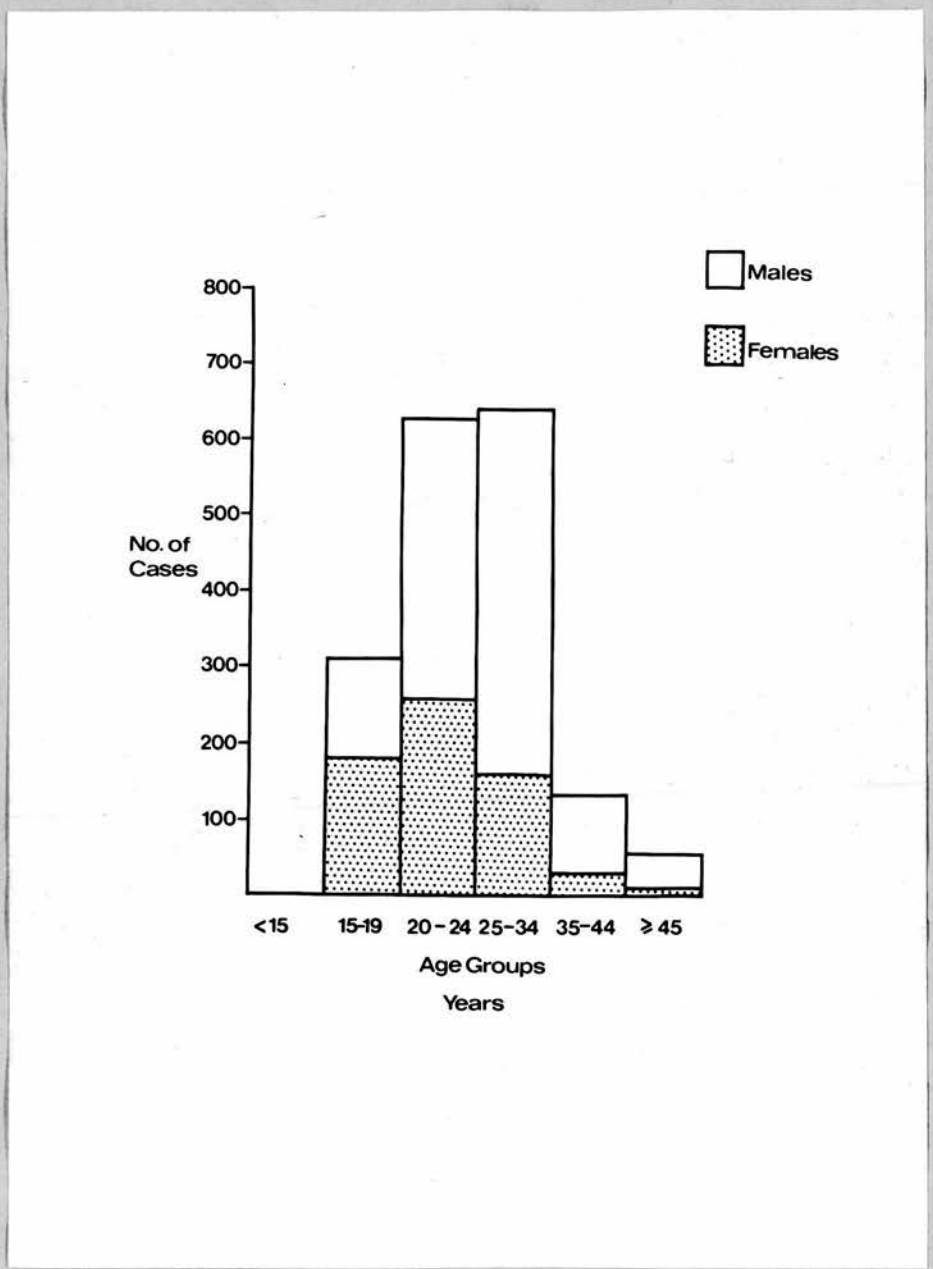


Figure (2)

Cases of gonorrhoea by sex and age group in Glasgow, 1976.



in 1966, just under 12 per cent of infections occurred in teenagers (15 - 19 years), but in 1976, 22 per cent of gonococcal infections were detected in this age group.

Sex ratios

The male to female ratio of patients with gonorrhoea in Scotland in 1945 was 2.12:1. This ratio had gradually decreased to 1:1.56 in 1976. A similar change has been observed in England and Wales (Morton, 1970), and it is clear that in areas where contact tracing is effectively employed, the ratio is consistently lower than elsewhere (Dunlop, Lamb and King, 1971).

Reasons for the increase in prevalence of gonorrhoea

The reasons for the increase in incidence of gonorrhoea observed over the past 15 or more years are multiple, and include the lack of immunity to infection, the short prepatent period making it difficult to interrupt the chain of infection, and variable standards of health care and decreased sensitivity of the organism to antibodies (Catterall, 1977).

Premarital sexual intercourse is becoming more prevalent, and men and women are having extramarital sex with more different partners than previously (Bell and Chaskes, 1970). Increased prosperity, particularly amongst the young has led to greater freedom and the rejection of moral standards laid down by religion and social custom (Morton, 1971).

An association between the use of oral contraceptives and the

incidence of gonorrhoea has been reported (Cohen, 1970), although this was not the finding of Juhlin and Liden, (1969). Although the sheath is an effective barrier against gonococcal infection, this form of contraception is used by less than 10 per cent of men who have frequent casual sexual intercourse (Hart, 1974).

Groups of individuals at particular risk

Although no social group is immune to infection, certain populations are at particular risk of acquiring gonorrhoea: A high prevalence of gonorrhoea is seen amongst long distance lorry drivers, seamen, soldiers, migrant workers, and tourists (Willcox, 1976).

Although prostitutes, of both sexes, have a higher prevalence rate of gonorrhoea than the general population, they play a lesser part in the epidemiology of the infection in most countries than previously (Dunlop, Lamb and King, 1971).

There is a high prevlalence rate of gonorrhoea in homosexual men (McMillan and Robertson, 1977), and it is sometimes difficult to persuade some of these men, fearing discrimination, to attend clinics for investigation.

The prevalence of gonorrhoea amongst immigrants in the United Kingdom had previously been shown to have been high (Willcox, Jefferiss and Naughten, 1966). The incidence of infection in families fell when this population were admitted also (Oller and Wood, 1970).

Patients with repeated episodes of gonorrhoea contribute significantly to the overall incidence of infection. In one study in the USA, gonorrhoea occurred more than once in more than 20 per cent of patients with gonorrhoea, and these patients accounted for

almost half of all cases (Noble et al. 1977).

One reason for the increasing prevalence of gonorrhoea is the development of resistance to antimicrobial agents, and this aspect merits brief discussion.

Antimicrobial resistance in N. gonorrhoeae

Resistance of N. gonorrhoeae to antimicrobial agents was first observed with sulphonamides shortly after their introduction into the therapy of gonorrhoea (Dunlop, 1949). Although there was little indication of reduced sensitivity of the gonococcus to penicillin for ten years after its introduction, a number of reports in the mid-1950's documented the isolation of strains relatively resistant to penicillin (Reyn, Korner and Bentzon, 1958; Curtis and Wilkinson, 1958), and this was associated with an increased failure rate with standard penicillin treatment schedules (Curtis and Wilkinson, 1958). The prevalence of strains of N. gonorrhoeae relatively-resistant to penicillin has continued to increase throughout the world. Although in Bristol in 1973 more than 80 per cent of strains had a minimal inhibitory concentration (MIC) of ≤ 0.09 units per ml (Shannon, Hedges and Edwards, 1975), in Bangkok, over a similar period, almost 90 per cent of strains isolated had an MIC of ≥ 0.1 units per ml (Brown and McMinn, 1974). Since the mid-1950's, the prevalence of relatively-resistant strains of the gonococcus MIC ≥ 0.06 µg per ml (0.1 units per ml) in the USA increased progressively until 1970 (Martin et al. 1970), when over the next four years, no further increase in prevalence of resistant strains was observed (Jaffe et al. 1976).

Such a trend has also been reported from Denmark (Nielsen, 1970); in Greenland there was a decrease in the proportion of resistant strains isolated, although the prevalence of infection with such strains has again increased (Olsen, 1973).

For the first time, in 1976, strains of N. gonorrhoeae were described which were capable of producing β-lactamase (Phillips, 1976; Ashford, Golash and Hemming, 1976). A small epidemic of gonorrhoea caused by these strains was reported in Liverpool (Percival et al. 1976); all of the known patients described had acquired the infection locally. Infections caused by β-lactamase-producing strains have now been recorded from several countries, but in most cases, the disease had been acquired in the Far East of West Africa. Perine et al. (1977) reported evidence for two types of β-lactamase-producing strains of N. gonorrhoeae. Strains from the Far East had relative resistance to tetracycline, depended on proline for growth and carried a plasmid of molecular weight 5.8×10^6 daltons which coded for β-lactamase production. Penicillinase-producing strains from West Africa were more susceptible to tetracycline, required arginine for growth, and had a smaller plasmid coding for β-lactamase (3.2×10^6 daltons). About half of the Far East strains, but none of those from West Africa, had an additional plasmid which transferred β-lactamase R factor(s) to other gonococci.

Strains of N. gonorrhoeae producing β-lactamase have not posed a major problem in the control of gonorrhoea, even although they are resistant to the most commonly employed antimicrobial agent, penicillin. To what extent this is related to instability of the β-lactamase-coding plasmid, as demonstrated by Percival et al. (1976) is uncertain.

Resistance to other antimicrobial agents has also occurred.

About 15 per cent of strains of N. gonorrhoeae have absolute resistance to streptomycin (Snell, Morris and Strong, 1963; Lomholt and Berg, 1966). Reduced sensitivity to tetracyclines also occurred over the same period as that described for penicillin, but the number of strains sensitive to tetracyclines has increased slightly in the USA (Jaffe et al. 1976).

There is a correlation between the sensitivities of gonococci to penicillin, tetracycline, streptomycin, erythromycin and other antimicrobial agents (Reyn, 1961; Reyn and Bentzon, 1969; Stolz et al. 1975).

It is clear that treatment failure is more likely when patients are infected with strains of N. gonorrhoeae relatively-resistant to penicillin. Holmes et al. (1973) showed that there was a progressively increasing failure rate with a standard dose of penicillin as the sensitivities of the strains decreased; similar results were detailed by Jaffe et al. (1976). Treatment failure with single-dose tetracycline treatment is also directly related to the sensitivity of the infecting strain of the gonococcus (Wiesner et al. 1973a).

The development of reduced sensitivity of strains of the gonococcus to antimicrobial agents is probably related to the indiscriminate use of these drugs in ineffective doses (Willcox, 1970). A prolonged, low plasma level will favour emergence of relatively-resistant strains. In some areas, an increase in prevalence of these strains may be due to importation by travellers, such as seamen, as described in Rotterdam by Stolz, Zwart and Michel, (1975).

As is the case with other bacteria, N. gonorrhoeae is not a homogeneous group of organisms, and the host response to infection

is not identical in individuals. An account of the pathogenicity of N. gonorrhoeae and the pathology of gonorrhoea now follows.

PATHOGENICITY

Kellogg and co-workers (1963) identified by consideration of the variability of the morphology, four colonial types (types 1, 2, 3 and 4) of N. gonorrhoeae isolated from men with gonorrhoea. In primary isolates, 90 per cent of the clones were of type 1, and 10 per cent were of types 2 and 3. If the growth on the medium was transferred without selection, types 3 and 4 became predominant. Based on the results of inoculation experiments in human volunteers, it was shown that type 1 was associated with virulence, but that type 4 was avirulent.

Further studies (Kellogg et al. 1968) showed that types 1 and 2 were the predominant clones isolated from infected men and women, and that types 3 and 4 were associated with non-selective subculture. All strains studied under conditions of non-selective growth transfer converted from types 1 and 2 to 3 or 4. These workers confirmed the association of types 1 and 2 clones with virulence and types 3 and 4 with non-virulence. A fifth colonial type (type 5) was identified in the growth of old laboratory strains by Jephcott and Reyn (1971), and a sixth type was described by Chan and Wiseman (1975).

Jephcott and others (1971) demonstrated the presence of fibrils or pili projecting from the surface of organisms of colony types 1 and 2; only rarely could these fibrils be seen in gonococci of other colony types.

Pili were subsequently shown to enhance attachment of gonococci to tissue culture cells (Heckels et al. 1976). Piliated gonococci were found to be more resistant to phagocytosis by polymorphonuclear leucocytes than non-piliated organisms (Ofek, Beachey and Bisno, 1974).

However the role of pili in the pathogenesis of gonorrhoea is uncertain; these structures are rarely seen on electron micrographs of urethral exudate (Novotny, Short and Walker, 1975) and Watt and Ward (1977) have shown that pre-incubation with anti-pilus antiserum does not prevent invasion. Other factors which may play a role in the virulence of N. gonorrhoeae include the production of toxin (Johnson, Taylor-Robinson and McGee, 1977), an IgA protease (Plaut et al. 1975) or a phospholipase (Senff et al. 1976). Ward, Watt and Glynn (1970) showed that gonococci in urethral exudates were resistant to the bactericidal action of serum, but that this resistance was lost on subculture. Virulent colonial types are more resistant to the activity of serum than the avirulent types (McCutchan, Levine and Braude, 1976).

The production of an extracellular toxin has been suggested from the results of infection of fallopian tube tissue cultures (Johnson and co-workers, 1977), and might explain why epithelial cells are damaged before invasion by gonococci (Ward and Watt, 1975).

Neisseria gonorrhoeae has been shown to produce an IgA1 protease and this aspect will be discussed later.

Strains of the gonococcus isolated from patients with disseminated gonococcal infection have been shown to be more susceptible to penicillin than those associated with uncomplicated gonorrhoea (Wiesner, Handsfield and Holmes, 1973 b). It was further shown that the auxotype* of these strains was significantly different from that of strains isolated from uncomplicated infections (Knapp and Holmes, 1975).

*Footnote:- The existence of strains of N. gonorrhoeae which were unable to synthesise one or more amino-acids, nucleic acid bases or vitamins from available precursors was established by Catlin (1973).

cont'd

These authors suggested that these strains, which required arginine, hypoxanthine and uracil $A^- H^- U^-$ for growth were more virulent than other strains, and that the low incidence of disseminated infection in some areas of the world was associated with absence of these strains. The strains producing disseminated gonococcal infection have also been shown to be more resistant to the bactericidal action of normal human serum than those not associated with complications (Schoolnik, Buchanan and Holmes, 1976). McCutchan et al. (1978) have suggested that normal human serum contains an IgG blocking antibody which competes with natural bactericidal antibody. They further postulated that natural IgG binds to certain gonococci and by protecting them from the action of antibody and complement, enables dissemination to occur.

Such strains were called auxotrophs, and a system of typing gonococci - auxotyping - based on nutritional requirements was described.

PATHOLOGY AND PATHOGENESIS

The pathology of gonorrhoea has been ably reviewed by Harkness (1948). Gonococci are found in the subepithelial tissues of the male urethra by the third day of infection and stimulate an acute inflammatory reaction. The capillaries are dilated and there is a dense infiltration with polymorphonuclear leucocytes and, to a lesser extent, lymphocytes, plasma and mast cells. These changes are initially most marked in the region of the intramucosal glands and ducts. There is patchy involvement of the epithelium of the urethra; the surface cells desquamate exposing subepithelial connective tissue. Obstruction of the ducts of the glands results in the formation of retention cysts and abscesses in the lamina propria. The squamous epithelium of the fossa navicularis appears resistant to infection.

The histopathological appearance of gonorrhoea of the cervix and rectum is similar to that described for the male urethra (Harkness, 1948) although Evans (1977) considers that organisms attach to the squamous epithelium and that the endocervix is resistant to infection.

Attachment to epithelial cells is the first stage in the invasion of tissues by gonococci, and this aspect of the pathogenesis of gonorrhoea has been studied during natural infection and in laboratory models. Organisms may be demonstrated by electron microscopy of exfoliated urethral cells closely adherent to these cells (Ward and Watt, 1972). The method of attachment is, however, not clearly understood. Gonococci from Type 1 colonies of N. gonorrhoeae possess pili which enhance but are not essential for attachment of organisms

to tissue culture cells (Heckels et al. 1976).

Watt and co-workers (1976) have shown that gonococci invade epithelial cells by phagocytosis. Although organisms may be found in the intercellular spaces, it is thought that the tight junctions and desmosomes offer a barrier to intercellular penetration from the surface, and that gonococci in these spaces have been derived from reversed phagocytosis by epithelial cells (Ward et al. 1975).

In fallopian tube tissue cultures, gonococci multiply intracellularly and then rupture into the submucosa, intercellular spaces and mucosal surfaces. Multiplication of the organisms results in the lateral spread of the infection (Watt and Ward, 1977).

Novotny and colleagues (1975) showed that in smears of urethral exudate gonococci appeared in clusters, either free or in association with epithelial cells. Electron microscopic examination showed that these clusters were covered by a sheet-like structure and that remnants of cell organelles surrounded the gonococci. It was postulated that these clusters were formed within phagocytic cells, probably macrophages. As it was suggested that these aggregates surrounded by granules of host cell remnants, were loci of multiplication the term infectious units was applied to the clusters seen in exudates.

As it is known that polymorphs are capable of killing gonococci (Watt, Ward and Robertson, 1976), it is likely that many organisms phagocytosed by these cells are destroyed. In the theory advanced by Novotny and others (1977), these organisms taken up by macrophages multiply and infectious units are formed. Epithelial cells may then by capable of resorbing the remnants of phagocytic cells, and with them the clusters of gonococci.

This theory however, although attractive, lacks experimental verification

Some aspects of the clinical spectrum of gonorrhoea require special consideration and these will now be detailed.

ASPECTS OF GONORRHOEA REQUIRING SPECIAL CONSIDERATION

Asymptomatic urethral gonorrhoea in men

Although the majority of men with urethral gonorrhoea have symptoms of urethritis, it has become evident that a substantial number have no symptoms. The prevalence of asymptomatic infection varies according to the population sampled. In consideration of male contacts of women with gonorrhoea, asymptomatic infection may be found in between about 20 and 60 per cent of infected individuals (Wallin, 1975; Marino, Pariser and Wise, 1972; Portnoy et al. 1974; Pariser, Farmer and Marino, 1964). The prevalence of asymptomatic urethral gonorrhoea in men who were not known contacts has been much lower. Carpenter and Westphal (1940) found that about one per cent of prisoners had asymptomatic urethral gonorrhoea. Thatcher et al. (1969) failed to isolate the gonococcus from the urethra of servicemen undergoing routine physical examination, and Handsfield et al. (1974) found asymptomatic urethral gonorrhoea in 1.5 per cent of military personnel. McMillan and Pattman (1979) found a prevalence rate of asymptomatic urethral gonorrhoea of four per cent in men attending a sexually-transmitted diseases clinic.

Asymptomatic urethral infection is important from both the public health and the individual's standpoint. The patient is infectious (Marino, Pariser and Wise, 1972) and himself at risk from the development of disseminated infection (Holmes, Counts and Beaty, 1971).

It has recently been shown (Crawford et al. 1977) that there is a significant difference in the nutritional requirements between

strains of the gonococcus isolated from patients with symptomatic and those from men with asymptomatic urethral infection. They demonstrated that strains isolated from 24 of 25 men with asymptomatic gonorrhoea required arginine, hypoxanthine and uracil for growth; strains from only 10 of 25 men with symptomatic infection required these amino acids for growth ($P<0.0001$).

Asymptomatic gonorrhoea in females

Women with gonorrhoea are often asymptomatic, and thus constitute a reservoir of infection (Nielsen, Sondergaard and Ullman, 1975). The true incidence of asymptomatic infection in females is difficult to determine. Women with symptoms of vaginal discharge and dysuria will attend physicians for treatment, and, as the majority of women attending sexually transmitted diseases clinics as a result of contact tracing are asymptomatic, bias is encountered in determining the true incidence of symptomatic infection.

Oropharyngeal gonorrhoea

Although the possibility that the oropharynx could be infected and serve as a reservoir for gonorrhoea has been known for half a century (Janet, 1929), only recently has detailed investigation of this aspect of the disease been undertaken. Fiumara, Wise and Many (1967) found pharyngeal infection in three homosexual men, and Iqbal (1971) described a case on gonococcal tonsillitis.

The prevalence of pharyngeal gonorrhoea has varied from series to series, reflecting differences in sexual habits between various population groups. Bro-Jórgensen and Jensen (1973) working in

Denmark, found that of patients with anogenital gonorrhoea, 10 per cent of women and six per cent of heterosexual men had concomitant oropharyngeal infection. Similar findings were reported by Ödegaard and Gundersen (1973) from Norway and Stolz and Schuller (1974) from Holland. These former workers also showed that the pharynx was the only site of infection in about one per cent of patients with gonorrhoea.

A high prevalence (39 per cent) of pharyngeal infection was noted by Corman et al. (1974) in obstetric patients with gonorrhoea.

Orogenital intercourse is common amongst homosexual men. Although Bro-Jørgensen and Jensen (1973) found that 25 per cent of homosexual men with gonorrhoea had oropharyngeal infection, the prevalence of infection was lower in the series reported from the United Kingdom by Shahidullah (1976) and by McMillan and Young (1978), being six and eight per cent respectively.

Most patients with oropharyngeal infection have no symptoms, but a few may complain of having a sore throat (Owen and Hill, 1972).

Rarely, disseminated infection may occur from the oropharynx (Metzger, 1970).

Pharyngeal gonorrhoea is more difficult to treat than anogenital infection, single-dose therapy being ineffective (Ödegaard and Gundersen, 1973).

Anorectal gonorrhoea

In the male, anorectal gonorrhoea is invariably acquired through homosexual anal intercourse. The reported incidence of male rectal infections has markedly increased in many clinics (Fluker, 1976)

probably as a result of improved reception at clinics of individuals who are homosexually-orientated, the increasing awareness by clinicians of this aspect of gonorrhoea, and the greater acceptance by society of homosexuality.

Although gonococcal proctitis may produce symptoms of anal discharge, perianal pain, rectal bleeding and tenesmus, at least two-thirds of infected patients have no symptoms (Catterall, 1962). Perianal abscesses and gonococcal disseminated infection may rarely complicate anorectal infection (Catterall, 1962).

The prevalence of rectal gonorrhoea depends on the population sampled, the method of sampling, and the culture medium used. In homosexual patients attending sexually-transmitted diseases clinics this has varied between five and 40 per cent (Merino and Richards, 1977; Owen and Hill, 1972). The anorectum may be the only site infected with the gonococcus, being the sole site of infection in over 30 per cent of infected men in one series (McMillan and Young, 1978).

In general, homosexual men tend to have more sexual partners than heterosexual men (Kinsey, Pomeroy and Martin, 1948). It is therefore important to be able to identify and treat, infected individuals as quickly as possible.

In women with genital gonorrhoea, the anorectum is infected in between 25 and 65 per cent of patients (Keith, Moss and Berger, 1975; Schroeter and Reynolds, 1972) although most series record a prevalence of about 45 per cent (Phillips et al. 1972). The anorectum may be the sole site of infection in about five per cent of infected women (Schroeter and Reynolds, 1972). Anorectal infection may be acquired by anal intercourse, or, probably more commonly, by auto-inoculation by vaginal secretions. As in the male, symptoms

of rectal infection in the female are uncommon. Single-dose treatment, as usually employed in the management of uncomplicated genital gonorrhoea, may not be curative in the case of anorectal infection, and persisting rectal infection may be an important cause of treatment failure (Schroeter and Reynolds, 1972); in about 30 per cent of women who were considered treatment failures, the rectum only was found to be infected.

Pelvic inflammatory disease

Acute pelvic inflammatory disease in patients attending sexually-transmitted diseases clinics is usually caused by organisms ascending from the uterine cervix (Rees and Annels, 1969). Although the uterine tubes and adnexal tissues share in the inflammatory reaction, tubal inflammation is most obvious clinically, and the terms "acute salpingitis" and "acute pelvic inflammatory disease" are used synonymously.

Acute salpingitis is a complication in about 10 per cent of women with untreated gonorrhoea (Rees and Annels, 1969). The onset of symptoms occurs most commonly during or immediately after menstruation. Neisseria gonorrhoeae can be isolated from the uterine tubes in only about 12 per cent of women with salpingitis associated with gonococcal infection of the lower genital tract (Mardh and Westrom, 1970). This discrepancy may be explained by the difficulty in isolating organisms present in small numbers, by the antimicrobial activity of tubal fluid, or by the possibility that other organisms may be responsible.

Gonococci in the lumen of the tube die or move to an intracellular situation within a few days of tubal infection (Studdiford,

Caspar and Scadron, 1938), and it has been shown that the isolation of organisms from the pouch of Douglas is maximal within the first three days of symptoms (Lip and Burgoyne, 1966).

Other organisms are capable of causing acute salpingitis and recently attention has been focused on the role of Chlamydia trachomatis in the aetiology of this condition. Rees et al. (1977) isolated C. trachomatis from the cervix of seven of nine women with postpartum salpingitis, and showed an antibody response in the serum of three of these patients. Chlamydia trachomatis has been cultured from tubal exudate collected at laparoscopy in two women with acute salpingitis, and an IgM serum response demonstrated by a micro-immunofluorescence test in each case (Mardh et al. 1977). Serological studies (Treharne et al. 1979) suggest that C. trachomatis is the probable aetiological agent in about two-thirds of women with acute pelvic inflammatory disease.

It is clear that N. gonorrhoeae is a pathogen capable of producing damage to tubal epithelium. Ward, Watt and Robertson (1974) showed that in organ culture, gonococci infected and invaded tubal epithelial cells. However, it is possible that other organisms, acquired at the same time as N. gonorrhoeae, may produce acute salpingitis, and it is of interest that C. trachomatis may be isolated from the cervix of about 45 per cent of women with uncomplicated gonorrhoea (Burns et al. 1975).

Disseminated gonococcal infection

Although N. gonorrhoeae usually infects mucous surfaces only, dissemination throughout the body may occur and produce the syndrome

of disseminated gonococcal infection. Barr and Danielsson (1975), working in Sweden, reported an incidence rate of disseminated infection of 2.5 per cent, but Catterall (1975) from London, noted that only 6 (0.2 per cent) of 2,500 patients with gonorrhoea had features of dissemination. The most common manifestations are arthritis and dermatitis; other features are rare and include meningitis, pericarditis and endocarditis (Holmes, Counts and Beaty, 1971).

In pre-antibiotic days, disseminated gonococcal infection was found most commonly in men with gonococcal urethritis, but now the prevalence in women is some three times that in men (Graber, Sanford and Ziff, 1960). It is suggested that the sex difference reflects the prompt treatment of the majority of men with urethral gonorrhoea, whereas many infected women have few symptoms, and the organism has a greater opportunity for dissemination. Systemic spread of the gonococcus may occur from any infected mucous surface (Holmes, Counts and Beaty, 1971), including the pharynx which may be the only site infected (Metzger, 1970).

Pregnancy is a recognised precipitating factor in the development of disseminated infection (Taylor, Bradford and Patterson, 1966). In non-pregnant women, dissemination occurs most commonly during menstruation (Holmes, Counts and Beaty, 1971). Complement deficiency favours the sytemic spread of the organism (Peterson, Graham and Brooks, 1976, Lee et al. 1978).

There is a broad spectrum of clinical features of disseminated gonococcal infection. In the bacteriaemic phase, patients are febrile and may complain of polyarticular pain. At this stage of the syndrome, skin lesions are common, and N. gonorrhoeae may be

isolated from the blood in up to 50 per cent of early cases (Holmes, Counts and Beaty, 1971). Most workers report a much lower rate of isolation from the blood, perhaps because patients are investigated somewhat later than those seen by former clinicians who showed that as the duration of symptoms increased, the chances of isolating the gonococcus from the blood decreased.

The skin lesions of disseminated gonorrhoea are maculopapules, pustules or haemorrhagic vesicles (Seifert, Warin and Miller, 1974) found principally on the extremities. Histological examination shows the basic lesion to be a small vessel vasculitis; conventional staining techniques usually fail to demonstrate gonococci, but these may be demonstrated in sections by immunofluorescent-antibody techniques (Kahn and Danielsson, 1969). Gonococci have rarely been cultured from skin lesions and it has been postulated that at least some of these lesions result from complement activation, possibly via the alternative pathway, by gonococcal endotoxin (Scherer and Braun-Falco, 1976).

The joint manifestations of disseminated gonococcal infection vary from a mild polyarticular arthritis to a typical "septic joint". Some articular features, particularly the migratory arthralgia of the bacteriaemic phase of disseminated gonorrhoea, may result from vasculitis as described above; circulating immune complexes have been demonstrated in some patients (Danielsson, Norberg and Svanbom, 1975). Gonococci have been demonstrated in the synovial membrane of infected joints by electron microscopy (Garcia-Kutzbach et al. 1974), but N. gonorrhoeae may be isolated on culture of synovial fluid from only about 15 per cent of patients with disseminated gonorrhoea (Handsfield, Wiesner and Holmes, 1976).

Having described the sites which may be infected with the gonococcus, and some aspects of the clinical manifestations, the laboratory diagnosis of gonorrhoea will now be discussed.

LABORATORY DIAGNOSIS OF GONORRHOEA

Basically, two laboratory techniques are used in the diagnosis of gonorrhoea: microscopic examination of smears and culture of material obtained from sites which may possibly be infected.

IDENTIFICATION OF N. GONORRHOEAE IN SMEARS

Microscopy of Gram-stained smears

Men

Microscopic examination of Gram-stained smears of urethral exudate is widely used in the diagnosis of gonococcal urethritis in men. Although some workers have reported a sensitivity (that is, the number of cases diagnosed by microscopy compared with those diagnosed by culture) of as high as 99 per cent (Fiumara, 1972), in most clinics this is somewhat lower, usually being reported as between 90 and 95 per cent (Phillips et al. 1972; Dans and Judson, 1975). When smears are examined by experienced observers, the specificity, when typical Gram-negative diplococci are seen within the cytoplasm of polymorphonuclear leucocytes, is about 98 per cent (Jacobs and Kraus, 1975).

Smears from about 15 per cent of men with urethritis, either gonococcal or non-gonococcal, contain a mixture of organisms; some are pleomorphic Gram-negative cocci and the identification of gonococci in these smears may be impossible (Jacobs and Kraus, 1975). Urethral culture is essential for accurate diagnosis in such cases. Organisms of the genus Mimeae, and Gram-positive cocci overdecolorised by acetone may be a source of confusion, especially to inexperienced observers (Wilkinson, 1962). In the urethra, species of Neisseria other than N. gonorrhoeae are uncommonly found, although N. meningitidis may be isolated with unusual frequency from men who have had homosexual contact (Givan, Thomas and Johnston, 1977).

When patients have passed urine several minutes before examination, the interpretation of a Gram-stained smear is difficult (Judson, Wright and Mann, 1977), but the value of taking urethral smears when the patient has held his urine for at least eight hours has recently been reconfirmed (Simmons, 1978).

Although examination of a Gram-stained smear is a useful and rapid diagnostic procedure in the investigation of men with urethritis, it is unreliable in the diagnosis of asymptomatic urethral gonorrhoea. Handsfield et al. (1974) showed that gonococci could be found in urethral smears from less than 70 per cent of infected men who had no symptoms or signs of urethritis.

Examination of Gram-stained smears of material obtained from the anorectum lacks both sensitivity and specificity. Organisms morphologically-resembling gonococci are found in the rectal exudate of only between 30 and 60 per cent of men from whom N. gonorrhoeae may be isolated on culture (Deheragoda, 1977; Fluker, 1976; McMillan and Young, 1978). Meningococci are found in the anorectum of about two per cent of homosexual men (Judson, Ehret and Eickhoff, 1978) making interpretation of smears difficult.

The presence of commensal Neisseria, and the large numbers of other Gram-negative organisms renders Gram-smear microscopy a useless diagnostic test for oropharyngeal gonorrhoea.

Women

Although some 90 per cent of men with gonococcal urethritis may be diagnosed correctly by microscopic examination of a Gram-stained smear of exudate, this technique is much less sensitive in the

diagnosis of gonorrhoea in women. Only about 60 per cent of infected women will be identified by microscopy of urethral and cervical exudate (Barlow et al. 1976). This lack of sensitivity is due to the small number of gonococci in the secretions as compared with the male, and to the presence of large numbers of other organisms.

The specificity, however, as defined above, is at least 97 per cent (Center for Disease Control, Atlanta, Georgia).

As judged from the results of culture, the uterine cervix and the urethra are infected in about 90 and 75 per cent respectively of women with gonorrhoea (Wilkinson, 1977; Barlow and Phillips, 1978).

Although several sites in the body are usually found to be infected concomitantly, the cervix and the urethra may be the sole sites of infection in about 20 and 5 per cent of cases respectively; in these individuals, the sensitivity of Gram-smear examination is about 50 and 8 per cent respectively (Barlow and Phillips, 1978). In women, as in men, gonorrhoea of the anorectum and oropharynx cannot be diagnosed with confidence by microscopy.

Having shown the inadequacy of Gram-smear examination in diagnosing gonorrhoea, it should be stressed that, at present, this is the only test which can be performed in a clinic and produce results in an acceptable time. Barlow and Phillips (1978) emphasise that more than half of infected women can be diagnosed at their initial attendance.

The low sensitivity of Gram-smear examination in the diagnosis of gonorrhoea in women prompted an investigation into the value of fluorescent-antibody tests to determine if these were sufficiently sensitive, specific and rapid to be of value to the clinician. A review of the experience with the direct fluorescent-antibody test

follows.

Fluorescent-Antibody Tests

The use of fluorescein-conjugated antigonococcal serum in the identification of N. gonorrhoeae was first described by Deacon et al. (1959). This paper detailed a "direct" fluorescent antibody test in which urethral smears from men with gonococcal urethritis were stained immediately with conjugated antiserum. In a later paper (Deacon et al. 1960), the use of the "delayed" immunofluorescent test was reported. Instead of directly testing material from the patient, smears were prepared from culture media which had previously been inoculated with genital secretions, and incubated for 16 to 20 hours.

Numerous problems however, were encountered when the direct test was evaluated as a diagnostic procedure. Non-specific fluorescence, particularly of polymorphonuclear leucocytes, as a result of adsorption by electrostatic forces of gamma globulins to the surface of cells, was an initial difficulty; this was, however, reduced by using protein-bound counterstains, such as Naphthalene black (Sommerville, 1968; Thin, 1970).

Cross-reactivity of antisera was found with N. meningitidis, and to a lesser degree with N. catarrhalis, N. flava and N. subflava (Danielsson, 1963). Although this cross-reactivity could be removed by absorption with meningococci much of the specific reactivity was also lost (Peacock, 1970). In practice, as N. meningitidis is uncommonly found in the genital tract, absorption of antisera with meningococci is not routinely undertaken.

Some strains of Staphylococcus aureus were found to react with

the conjugate (Danielsson, 1965) as a result of non-specific binding of the Fc part of the immunoglobulin molecule to the surface antigens - protein A - of the bacterial cell (Lind, 1972). Although prior absorption of antisera with cross-reacting strains of Staph aureus (Danielsson, 1965) this cross-reactivity was reduced but specific staining was also markedly reduced. By using a mixture of conjugate and normal rabbit or human serum, staining of Staph aureus was, however, successfully inhibited (Lind, 1967; Thin, 1970).

Although the difficulties encountered with non-specific fluorescence were overcome, Lind (1967) demonstrated that the sensitivity of the direct immunofluorescent-antibody method was comparable to that of Gram-smear examination of genital material. Other workers however, reported higher sensitivity as compared with conventional staining techniques and culture on non-selective media (Gallwey, Nicol and Ridley, 1967; Thin, 1970; Henderson et al. 1970).

The sensitivity of the direct fluorescent-antibody test depends on the sample size, distribution and depth on the slide. As the sample size and the number of organisms per unit volume is small, particularly in the female, examination of stained smears is time-consuming and negative results are meaningless (Kellogg, 1977).

Cross-reactivity of antisera with N. meningitidis (Reyn, 1969) precludes the use of the direct test in the diagnosis of oropharyngeal gonorrhoea, as meningococci colonise this site in some 15 per cent of patients attending sexually-transmitted diseases clinics (Wiesner et al. 1973c).

With the increasing use of selective media, allowing identification of gonococci even in heavily contaminated sites, such as the rectum, the need for the direct fluorescent-antibody test for the diagnosis of

gonorrhoea is difficult to justify.

The direct immunofluorescent test has been used to identify gonococci in tissue sections and scrapes from the skin lesions of disseminated gonococcal infection (Kahn and Danielsson, 1969; Barr and Danielsson, 1975). Danielsson and Molin (1971) have claimed to have demonstrated N. gonorrhoeae in prostatic fluid of patients previously treated with antibiotics. However, it has been shown that gonococci which have been exposed to a lethal concentration of penicillin retain their fluorescence for up to 24 days (Lucas et al. 1967); it is impossible to conclude that the fluorescing organism noted by Danielsson and Molin were viable.

IDENTIFICATION OF NEISSERIA GONORRHOEAE BY CULTURE TECHNIQUES

Sampling sites

Men

Where laboratory facilities are optimal, urethral gonorrhoea in men should be diagnosed by examination of a Gram-stained smear and culture on selective medium (World Health Organisation, 1978). Culture is necessary in the identification of men with asymptomatic urethral infection (Handsfield et al. 1974). Moore et al. (1973) have shown that culture of urinary sediment from the first 10 to 15 ml of urine passed by the patient was only slightly (six per cent) less sensitive than culture of material obtained from the urethra by direct sampling. By its simplicity, and lack of trauma to the patient, this technique was proposed as a useful tool in screening for gonorrhoea. More experience is required, however, before this examination can be introduced as a routine.

In areas where facilities are minimal, microscopy of a Gram-stained smear of exudate from men with gonococcal urethritis is sufficiently sensitive for routine use (World Health Organisation, 1978).

A diagnosis of oropharyngeal or rectal gonorrhoea can only be made with confidence by culture investigation.

A diagnosis of anorectal gonorrhoea in the male may be made in 90 to 95 per cent of men infected at this site by a single culture examination (Deheragoda, 1977; McMillan and Young, 1978). At least two and preferably three negative cultures, taken at weekly intervals,

should be obtained before excluding gonococcal infection of the rectum (McMillan and Young, 1978).

Only about 75 per cent of pharyngeal infections can be diagnosed by a single culture examination (Hallqvist and Lindgren, 1975; McMillan and Young, 1978); repetitive testing is required to exclude infection in this area.

Women

Ideally, in the investigation of women for gonococcal infection, Gram-stained smears of urethral and cervical secretions, and cultures from the urethra, cervix, anorectum and oropharynx should be examined.

However, these microbiological investigations are expensive, and in situations where economic resources are limited, the sampling only of those sites giving the highest yield of positive results is justifiable.

As the cervix is affected in about 90 per cent of women with gonorrhoea (Barlow and Phillips, 1978), Gram-stained smears and cultures from this site should be examined in every case. The value of microscopy of material from the urethra has recently been questioned (Barlow and Phillips, 1978); only one of 607 cases of gonorrhoea would have been missed if this examination had been omitted. Although Dans and Judson (1975) recommend a combination of cervical and anorectal cultures for the diagnosis of gonorrhoea in women attending a venereal diseases clinic, about six per cent of patients have urethral infection only (Barlow and Phillips, 1978), and would be missed. In the routine screening of a group of women with a low prevalence rate of gonorrhoea, for example, those attending a family planning clinic, a cervical

culture has been reported as being the most appropriate test, based on cost-benefit analysis (Keith, Moss and Berger, 1975); about 15 per cent of these infected patients had anorectal or oropharyngeal gonorrhoea.

Catterall (1970) demonstrated that by relying on the results of only one set of tests (that is microscopy of Gram-stained smears and culture on non-selective medium) taken from women with gonorrhoea, 34 per cent of infected patients would have been missed. A second and third set of tests, taken two and three weeks after the patients' initial attendance, identified a further 20 and 10 per cent of infected women respectively; four per cent of women with gonorrhoea were diagnosed at a fourth attendance.

A possible reason for the failure of growth on the surface of solid medium may be the inoculation of too small a number of organisms; it has been shown that selective enrichment in broth increases the recovery of bacteria from clinic specimens when compared with culture on solid medium (Taylor and Schelhart, 1969). It is recognised that there is marked variation in the numbers of organisms obtained on sampling the cervical secretions; in one study the number of N. gonorrhoeae recovered ranged from 4.0×10^2 to 1.8×10^7 colony forming units (Lowe and Kraus, 1976).

Chipperfield and Catterall (1976) showed that by improving both isolation media and diagnostic methods, just over 90 per cent of women with gonorrhoea could be diagnosed by examination of one set of tests. Inhibition of growth of gonococci may be the result of the proliferation of saprophytic organisms (Kaye and Levison, 1977) and theoretically, the use of selective media should enhance the detection of N. gonorrhoeae in specimens taken at the patients' initial clinic attendance. This

was clearly shown to be so by Barlow et al.(1976) who, using selective media for the isolation of the gonococcus, identified more than 97 per cent of infected women by a single set of tests.

Before the introduction into general use of selective media and improved diagnostic laboratory methods, three or four sets of tests were recommended before gonococcal infection in a woman could be excluded (Catterall, 1970). Provided that clinic and laboratory techniques are constantly under review, in centres where facilities are good, two sets of tests are sufficient (Jephcott and Rashid, 1978).

As in men with oropharyngeal gonorrhoea, multiple testing of this site is required to exclude infection (Hallqvist and Lindgren, 1975).

The organismal diagnosis of acute salpingitis is not simple. Even in cases where N. gonorrhoeae may be isolated from the endocervix, material taken at laparoscopy from the uterine tubes often fails to grow gonococci (Mardh and Westrom, 1970). Laparoscopy is potentially hazardous investigation and, unless the diagnosis of salpingitis is in doubt, is not routinely performed. Gonococcal salpingitis is diagnosed clinically and by the finding of N. gonorrhoeae in the urethra, cervix and/or anorectum.

Pelvic examination is not always acceptable to patients, and alternative methods for the diagnosis of gonorrhoea have been investigated. The use of self-inserted tampons has been described (McCormack et al. 1973); plates of selective media are inoculated with material collected on the surface of the tampon. One group of workers, (Haughie, Ames and Madsen, 1975) found only a five per cent

difference in the isolation rate of gonococci between directly-obtained cervical and tampon specimens.

Chapel and Smeltzer (1975) demonstrated that, although culture of urinary sediment from women with gonorrhoea was about 20 per cent less sensitive than cervical culture, the method was acceptable for routine screening.

Culture media for Neisseria gonorrhoeae

Neisseria gonorrhoeae grows well on nutrient agar supplemented with whole blood (which may be untreated or heated at 75°C for about 10 minutes - "chocolate" agar), serum, or hydrocoele fluid (Reymann, 1944). Until some fifteen years ago this medium with minor modifications, was routinely employed in the diagnosis of gonorrhoea in patients. Other organisms, however, including commensals, also grow on this medium, and may inhibit the growth of gonococci (Kaye and Levison, 1977).

Although some strains of N. gonorrhoeae will grow on media incubated in air, a carbon dioxide-enriched atmosphere (about 8 per cent) is beneficial for the growth of most strains (McLeod et al. 1934).

Although there had previously been unsuccessful attempts to suppress the growth of contaminating organisms, without inhibition of the growth of gonococci, by incorporation of chemicals into the culture medium it was not until anti-microbial agents were used that "selective" media became established in the diagnostic laboratory. Following on the experiments by Crookes and Stuart (1959) on the inhibition by polymyxin B sulphate of growth of Escherichia coli, but not of N. gonorrhoeae, Thayer and Martin (1964) introduced a medium

of a "chocolate" agar containing polymyxin and ristocetin, for the culture of the gonococcus. With this combination of antibiotics, the growth of many Gram-positive and Gram-negative organisms, including saprophytic neisseriae, but with the notable exception of N. meningitidis, was inhibited. As experience with this medium accumulated, it became evident that significantly more infected patients could be identified, when compared with non-selective media (Wilkinson, 1965; Martin, Peacock and Thayer, 1965) and that gonococcal infection in sites, such as the anorectum, where saprophytes are abundant, could be easily diagnosed (Scott and Stone, 1966).

In 1965, ristocetin became unavailable and Thayer and Martin (1966) modified their original medium, by substituting vancomycin sulphate and colistimethate sodium for polymyxin and ristocetin. They also added nystatin to inhibit growth of fungi. The modified medium was found to give even greater inhibition of staphylococci and saprophytic neisseriae.

Amies and Garabedian (1967) noted that a medium consisting of a peptone-starch base with the addition of yeast extract, serum and the antibiotics sodium colistimethate and vancomycin, made identification of gonococcal colonies easier; a luxuriant growth of gonococci occurred on this medium.

Proteus spp, which often colonise the anorectum, are not inhibited by the antimicrobial agents used in the Thayer-Martin medium, and their characteristic spreading may make identification of gonococci in the anogenital region impossible. Proteus is inhibited by trimethoprim and at a concentration inhibitory to Proteus, the growth of most strains of the gonococcus is not affected. Seth (1970) showed that trimethoprim at a concentration in the medium of 8 μg per ml

suppressed the growth of Proteus in samples from patients with gonorrhoea, and allowed identification of gonococcal infection in cultures previously overgrown with Proteus.

Phillips et al (1972) reported that the use of a selective medium, containing vancomycin, colistin, nystatin and trimethoprim increased the isolation rate of gonococci by about 10 per cent in women, and by 24 per cent in men, when compared with isolation on non-selective media.

Growth of gonococci on Thayer-Martin medium is slow, and other media have been developed to isolate the organism more rapidly. A medium New York City (NYC) medium, described by Faur et al. (1973, a and b) and modified by Young (1978), (modified NYC medium) consisted of a commercially-available base (Difco GC base) enriched with lysed human blood, yeast dialysate, glucose, lincomycin, colistin, amphotericin B and trimethoprim. Lincomycin was used in favour of vancomycin as about three per cent of strains of gonococci are sensitive to that agent (Reyn, 1969). The isolation rate of gonococci in this medium was significantly higher than on Thayer-Martin medium.

The isolation of N. gonorrhoeae on culture is the standard against which other tests are usually judged, but it is clear that organisms may not invariably grow on the medium used. Reasons for the failure of growth include faulty sampling techniques, a small inoculum of organisms, organismal death on transport to the laboratory and sensitivity of gonococci to the antibiotics in the medium. Ideally, to detect strains susceptible to the action of antibiotics in the medium used, a non-selective medium, such as "chocolate" agar should be inoculated in addition to a selective medium (Wilkinson, 1977). It is unlikely that any further modification in culture media will increase

the sensitivity of diagnosis. What is required, however, is a means of rapidly identifying infection at the patients' initial clinic attendance.

Confirmation of isolates

Sugar utilisation tests

The identity of colonies of organisms grown on the surface of media requires to be confirmed by sugar utilisation or fluorescent-antibody tests. In conventional testing, colonies giving a positive oxidase* reaction are subcultured and used in the sugar utilisation reactions. Species of Neisseria differ in their ability to ferment various sugars; for example, N. gonorrhoeae ferments glucose but not maltose, and N. meningitidis ferments both of these sugars. Fermentation reactions are usually complete within 24 hours, but occasionally the reaction may be obvious only after prolonged incubation. Results may, therefore, not be available to the clinician until four days after the specimens have been collected.

The use of a rapid carbohydrate utilisation test, based on the detection of enzymes, preformed by the gonococcus, has been described by Young, Paterson and McDonald (1976), and shown to give results comparable to those obtained by conventional sugar testing. The majority of strains gave positive reactions within 60 minutes of incubation, enabling results to be reported within 72 hours of specimen collection. Wilkinson, (1977), however, failed to obtain satisfactory results with this rapid test.

* The oxidase reaction depends on the presence in bacteria of

enzymes which catalyse the transfer of electrons between electron donors in the bacteria and a dye, tetramethyl-paraphenylenediamine, which is reduced to a deep purple colour. A positive oxidase reaction is not diagnostic of the gonococcus; other species of Neisseria. including saprophytes, Pseudomonas, Vibrio, Alcaligenes and Aeromonas, also give a positive reaction (Cruickshank, Duguid, Marmion and Swain, 1975).

Fluorescent-antibody methods

In contrast to the "direct" fluorescent antibody test, the "delayed" test is widely used to confirm the identity of suspected colonies of N. gonorrhoeae growing on culture media.

Lind (1967) showed the superiority of the "delayed" over the "direct"method in diagnosis, and although the sensitivity was only slightly greater than that of culture on a non-selective medium, results were more available sooner. In a subsequent report (Lind, 1969) she demonstrated that the percentage of positive results obtained by inoculation on to selective medium was the same, whether identified by sugar utilisation tests, or the fluorescent-antibody test; using the latter test results were, however, available 24 to 48 hours earlier.

In a comparison of data reported on the delayed fluorescent test, it is important to take into consideration the type of medium and the techniques used and the specificity of the antiserum (Reyn, 1969). It is not surprising that different conclusions, regarding the sensitivity of the delayed technique, have been reached by various workers. As mentioned above, Lind (1969) reported results comparable with those of culture. Thin, Williams and Nicol (1971), found that

the immunofluorescent test was more sensitive, and McGill et al. (1969) that it was less sensitive than culture on non-selective media. Phillips et al. (1972) reported that as experience was gained in interpretation of the test, the results obtained approached those of culture on selective medium, and identification by sugar utilisation tests.

The use of the "delayed" test enables identification of gonococci in primary culture from 95 per cent of male, and from 93 per cent of female patients, and provides considerable saving of media and time (Lind, 1975). Young, Paterson and McDonald (1976) found that gonococci from primary culture on MNYC medium could be identified in every case within 48 hours of taking the specimen from the patient, and that almost two-thirds of cases could be diagnosed within 24 hours.

Cross-reactivity of antisera with N. meningitidis is well known (Reyn, 1969), and, particularly in the case of pharyngeal specimens, care should be exercised in the interpretation of positive results.

Co-agglutination methods

There have been recent reports on the identification of cultured gonococci by the technique of co-agglutination (Danielsson and Kronvall, 1974; Lue and Ellner, 1978). This test is based on the known ability of protein-A containing staphylococci to absorb the Fc piece of IgG molecules to the surface of the cell. Strains of these organisms were coated with anti-gonococcal antiserum and incubated with suspected gonococci. Agglutination was observed only with N. gonorrhoeae, and not with saprophytic neisseriae (Danielsson, Olcen and Sandstrom, 1977). In view of the many strains of the organism, commercially-prepared

antigonococcal antiserum may not react with strains in every locality. This test requires further evaluation before it can be introduced as a diagnostic technique.

Differentiation by inhibition by manganous chloride and Congo red

A simple and reliable method for the differentiation of N. gonorrhoeae and N. meningitidis has recently been reported (Odugbemi, McEntegart and Hafiz, 1978). It was shown that the growth of gonococci, but not meningococci, was inhibited by a mixture of Congo red and manganous chloride. These agents were incorporated into a blotting paper disc and this disc was placed on the surface of a culture medium inoculated with the organisms under test. After incubation, a zone of inhibition of growth around the disc was observed if the organisms cultured were gonococci.

Identification of N. gonorrhoeae by genetic transformation

Bacterial transformation is a process whereby homologous DNA is taken up by "competent" strains of bacteria and recombined with the DNA of the recipient cell chromosome. Only DNA from homologous or closely related strains will recombine with the recipient cell chromosome. Janik and co-workers (1976) described the application of bacterial transformation to the detection of N. gonorrhoeae. This method was based on the ability of DNA from "wild" strains of the organism to transform nutritional mutants of the gonococcus. Specificity was limited, however, by the observation that DNA from N. meningitidis and other species of Neisseria could also transform these strains of gonococci

Although some workers have confirmed the presence of gonococci in genital isolates on the basis of the colonial morphology, oxidase reactivity and Gram-staining of organisms growing on selective culture medium (Dans and Judson, 1975), this procedure is not acceptable when medico-legal issues arise. The transmission of gonorrhoea from a husband to his wife or vice versa may constitute grounds for the award of a matrimonial order, leading to divorce (Grant and Levin, 1973). It is essential, particularly in these circumstances, that the identification of an isolate should be as accurate as possible; sugar utilisation reactions are therefore necessary. Rectal and pharyngeal isolates must be confirmed similarly, as saprophytic Neisseria are common in these sites.

Transport media

It is clear that, at present, cultural methods for the detection of gonorrhoea are superior to other techniques, particularly with respect to infection in women. However, it is not always possible to have a diagnostic laboratory in the immediate vicinity of the clinic. In some countries, laboratory and clinic may be hundreds of miles apart, and transport of specimens may take several days. There are two problems associated with the transport of gonococci (1) the maintenance of viability so that subsequent culture is possible (2) the suppression of other organisms which might overgrow the gonococci.

Although gonococci may be inoculated on to growth medium such as "chocolate" agar and transported on this to the laboratory organisms may not be isolated from about 40 per cent of infected cases

when the transit time is greater than 12 hours (Reymann, 1944). Stuart (1946) introduced a transport medium of semi-solid agar containing thioglycollic acid neutralised by sodium hydroxide, sodium glycerophosphate, calcium chloride and methylene blue as redox indicator. It was reasoned that by the incorporation into the medium of a reducing agent, sodium thioglycollate, organismal death by oxidation, considered an important factor (Rahn, 1945) was reduced; sodium glycerophosphate acted as a buffer. As the medium did not contain nutrients, theoretically, the growth of contaminating organisms should be minimal.

Early experience with Stuart's medium showed that gonococci could be subcultured for up to fifteen days, after inoculation, and that contaminating organisms grew poorly in the medium (Stuart, 1946). The incorporation of charcoal into the cotton-wool swabs used to collect samples neutralised the bacteriostatic properties of some batches of agar (Moffett, Young and Stuart, 1948).

The usefulness of Stuart's medium was confirmed by other workers. Wilkinson (1951) showed that even when specimens had to be held in transport medium for 48 hours, the chances of isolating gonococci were not greatly diminished. In a review of five years experience with his transport medium, Stuart (1956) demonstrated that, provided proper sampling techniques were used by the clinician, 80 per cent of infected cases could be identified even when there was a delay of 72 to 96 hours in transport to the laboratory.

Reyn, Korner and Bentzon (1960) reported on the superiority of Stuart's medium to their previous routinely-used transport medium.

It became clear that Stuart's medium, although lacking nutrients, would sustain the growth of organisms such as Escherichia coli and

Aerobacter spp (Amies, 1967). Some bacteria produce a glycerophosphatase which hydrolyses glycerophosphate, liberating glycerol as a source of energy for growth. On the basis of this observation, Amies (1967) modified Stuart's original medium by substituting an inorganic phosphate buffer for glycerophosphate; he also incorporated a low concentration (0.3 per cent w/v) of sodium chloride favourable to the gonococcus and added charcoal to the medium. He demonstrated that this modification maintained the viability of the gonococci better than the original preparation, and that, with the exception of pharyngeal specimens, bacterial overgrowth was greatly diminished. Swabs taken from the throat are heavily impregnated with mucus and cell debris, and probably it is the presence of this nutrient material that allows growth of commensal organisms to take place in transit.

Amies' medium has not however been found to be satisfactory for use in tropical countries (Khandari, Prakash and Singh, 1972).

When selective media for the growth of N. gonorrhoeae were developed, it seemed reasonable to employ these also as transport media. The system described by Reymann (1944) failed because contaminants grew in the non-selective medium. Martin and Lester (1971) described first the use of a combined transport and culture system, Transgrow, consisting of a flat-faced bottle containing a modified Thayer-Martin medium, and having a carbon dioxide enriched atmosphere. The isolation rates of gonococci were found by Toshach, Kadis and Diadio (1972) to be similar, whether Stuart's medium of Transgrow was used for transport, but that Transgrow had the advantage of speed and elimination of subculturing procedures. When combined with "delayed" immunofluorescent identification of gonococci, however, this system has been shown to be more rapid and more sensitive than

conventional culture techniques, results being obtained within 24 hours of sampling (Jephcott, Morton and Turner, 1974).

The Transgrow system suffers from the disadvantage that condensation on the glass may render visualisation of the surface of the medium impossible and necessitate "blind" sampling for identification of growth of gonococci. Carbon dioxide may also be lost from the bottle. These difficulties were overcome by the use of a flat, plastic tray containing selective medium with a recess for a tablet generating carbon dioxide on contact with moisture; the tray and contents are sealed, after inoculation, in a plastic bag (Martin, Armstrong and Smith, 1974). The use of this system with modifications, has facilitated the identification of gonococci (Martin and Jackson, 1975; Jephcott, Bhattacharyya and Jackson, 1976).

Reports on the value of directly plating material on to culture media in the clinic over the use of transport media have been conflicting. Danielsson and Johanisson (1973) found the use of transport media only slightly inferior to direct plating, and similar results were reported by Thin and Shaw (1979). Other workers (Hosty et al. 1974; Symington, 1975) found that direct plating was greatly superior.

It is clear that a considerable time must elapse before results are available to the clinician. When he is dealing with a reliable patient this may be of little importance, but many patients fail to realise the importance of re-attendance at the clinic and default after their first visit. If the results of Gram-smear microscopy are negative in the clinic, but gonococci are subsequently grown on culture

medium, it is essential that the patient be contacted; sometimes this is impossible if he or she has given false personal details. A test which is more sensitive than Gram-smear examination, and which gives results whilst the patient waits would clearly be desirable.

Although culture of material obtained from the genital and extragenital sites is the most sensitive procedure available for the diagnosis of gonorrhoea it has the disadvantage that screening of populations for asymptomatic infections, particularly those with a low prevalence rate of infection, is impracticable. To many individuals genital examination is psychologically unacceptable, and laboratories could not handle the volume of work involved in such screening programmes. Tests based on the detection of gonococcal antibodies in serum were therefore introduced and these are now discussed.

SEROLOGY OF GONORRHOEA

From the early 1900's until the mid 1930's, considerable work was undertaken into the development of a serological test for gonorrhoea. Unfortunately, with the introduction of anti-microbial agents, and their effective application, this research activity declined until the mid-1960's when the incidence of gonorrhoea began to increase world-wide.

The available serological tests must now be considered. However, it is first necessary to relate the serological possibilities to the

antigenic heterogeneity of the gonococcus.

The antigenic heterogeneity of N. gonorrhoeae

That N. gonorrhoeae is not an antigenically-homogeneous group of organisms has been known for more than half a century. Using agglutination techniques, Hermanies (1921 a, b) distinguished six subgroups of gonococci, and Casper (1937) recorded the existence of two types of N. gonorrhoeae by the demonstration of a type-specific carbohydrate moiety. Wilson (1954) in an extensive study, confirmed previous observations (Reyn, 1949) that N. gonorrhoeae contained both group-specific and type-specific antigens.

A close antigenic relationship between N. gonorrhoeae and N. meningitidis may be shown by agglutination (Boor and Miller, 1944), precipitation (Miller and Boor, 1933), complement fixation (Wilson, 1954) and immunofluorescent antibody techniques (Deacon et al. 1959).

There have been many attempts to develop a typing system for the gonococcus for epidemiological purposes, but, although typing is possible, it is still at the experimental stage. Some of the methods used recently are described below.

Glynn and Ward (1970) defined four groups of gonococci according to their resistance to killing by complement with normal human or immune rabbit serum. They suggested that the antigens involved in the bactericidal reaction were lipopolysaccharides of several distinct specificities.

Neisseria gonorrhoeae has recently been shown by microimmunofluorescence testing to be divisible into at least three major immunotypes and eight subtypes (Wang et al. 1977). Mark and Wang

(1978) subsequently showed that the results of the microimmunofluorescent test and the serum bactericidal assay were comparable, but that the latter was simpler, more rapid and reproducible.

Using the Lancefield hydrochloric acid extraction procedure, Hutchison (1970) identified five serogroups of N. gonorrhoeae.

Maeland (1968) prepared lipopolysaccharide from gonococci by extraction with phenol-water, and showed that an antigenic determinant of lipopolysaccharide "a" determinant included several factors (Maeland and Kristoffersen, 1971). Apicella (1974) extracted with alkali and phenol, an acidic polysaccharide from N. gonorrhoeae and showed that two antigenically-distinct populations of organisms could be identified by the demonstration of this polysaccharide.

In a consideration of the reactivity of antisera against outer membrane protein from N. gonorrhoeae, Johnston and co-workers (1976) were able to identify 16 types of the organism, and showed further (Johnston, 1977) that patients may harbour two or more strains of the gonococcus possessing several different serotype antigens.

A further method used to type the gonococcus was based on a study of the nutritional requirements of different strains of the organism, the auxotype (Catlin, 1973; Carifo and Catlin, 1973). At least 35 auxotypes of N. gonorrhoeae exist (Catlin, 1977), and certain types are clearly associated with disseminated infection (see above). Auxotyping is however laborious and not suitable for routine use in a diagnostic laboratory.

A possible means of gonococcal typing was described by Geizer (1968) and was based on the different susceptibility of different strains of gonococci to bacteriocins produced by other organisms. Sidberry and Sadoff (1977) examined the bactericidal action of a

variety of bacteriocins, produced by Pseudomonas aeruginosa against over a hundred isolates of N. gonorrhoeae and observed thirty different patterns of inhibition.

It is clear then, that although different types of gonococci exist, no single system of typing is of value at present. The above observations clearly indicate that serological tests based on the detection of antibodies against a single antigenic determinant are of little value.

Serological tests for gonorrhoea

Many tests have been described to detect gonococcal antibodies in serum, but only the more important are detailed below.

Tests based on the detection of complement-fixing antibodies

Following rapidly upon the description of the Wasserman test, Mueller and Oppenheimer (1906) described first the detection, by complement fixation, of gonococcal antibodies in the serum of a patient with gonococcal arthritis. Not all sera from patients with gonorrhoea gave positive results and Teague and Torrey (1907) postulated that negative results in the complement fixation test may be due to infecting strains different to those employed as antigen. Although Price (1931) maintained that a positive result was definite evidence of gonococcal infection, false positive results were, however, frequently reported (Carpenter, 1937).

The test is frequently negative in patients in whom N. gonorrhoeae may be isolated on culture from the anogenital tracts (Nabarro, 1938);

Danielsson et al. (1972) showed since that the test may take several weeks to become positive. Once formed, the antibodies detected persist for weeks or months even after effective treatment (Harkness, 1944).

With the increase in incidence of gonorrhoea during the 1960's interest in the complement fixation test was re-kindled. Magnusson and Kjellander (1965) reported their experience, using a suspension of 20 strains of heat-treated gonococci as antigen. They found positive tests in 20 per cent of men, and in 50 per cent of women with uncomplicated gonorrhoea, and positive results in 70 per cent of men and 60 per cent of women with complicated infection. Slightly more than one per cent of non-infected control patients gave a positive reaction.

Watt, Ward and Glynn (1971), in a comparative study of serological tests, reported identical results to those previously described by Magnusson and Kjellander (1965). Broadly comparable results with the complement fixation test have been reported by other workers (Ratnatunga, 1971; Danielsson et al. 1972), using different antigenic preparations. Positive results are obtained about twice as frequently in women with uncomplicated gonorrhoea than in men. The finding of positive tests in non-infected patients has varied from about two to seven per cent.

Antigen preparations other than whole cell gonococci, have been used in the complement fixation test. Reising et al. (1969) used two protoplasmic antigens designated FI and SA, prepared from F62 strain of N. gonorrhoeae. Cells were disrupted in a Ribi cell fractionator, and following centrifugation to remove insoluble cell material, the supernate (SA) was used. Gel-filtration of the gonococcal SA yielded FI in the first peak eluted. About four per cent of blood donors gave

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a positive reaction with SA, but none with FI. Twenty-eight per cent of men, and 88 per cent of women with gonorrhoea had serum complement-fixing antibodies against SA; somewhat fewer gave a positive result when FI was used as antigen. Similar results were obtained when Watt, Ward and Glynn (1971) examined this test system, but 12 per cent of their non-infected patients gave a positive result.

An automated complement fixation test was described by Peacock (1971), using a protoplasmic antigen. About 80 per cent of women, and 50 per cent of men had detectable antibody; four per cent of non-infected individuals also gave positive results.

Danielsson et al. (1972) showed that by using a single strain of N. gonorrhoeae as the source of antigen for the complement fixation test, there was high specificity, but low sensitivity. It was further shown (Sandstrom and Danielsson, 1977) that various strain antigens participate in the immune response. Although sensitivity could be increased using a polyvalent antigen, this was at the expense of specificity. They found that superior results were obtained when whole cell antigens were used instead of protoplasmic preparations. These workers also demonstrated the importance of examining paired-sera in diagnosing complicated gonococcal infection. Although less than half of the sera taken at the time of presentation from patients with cutaneous gonorrhoea had complement-fixing antibodies, these antibodies were found in 95 per cent of convalescent sera (Danielsson et al. 1972).

The interpretation of a positive result in the complement fixation test may be difficult. As mentioned above, complement-fixing antibodies may be detectable many months after successful treatment. It is therefore impossible to tell whether a patient, previously infected and treated, has a current infection. The gonococcus shares antigenic

features with other species of Neisseria (Reyn, 1943; Wilson, 1956) and positive results in the complement fixation test may be obtained in patients harbouring these organisms (Oliver, 1929; Rodas and Ronald, 1974).

From a consideration of the above data, it is clear that the gonococcal complement fixation test is of little value in the diagnosis of complicated gonorrhoea. It may, on occasion, be useful in confirming a clinical diagnosis of disseminated infection.

Detection of gonococcal antibody using indirect immunofluorescent techniques

By using an indirect immunofluorescent-antibody technique, Hess, Hunter and Ziff (1965) demonstrated that gonococcal antibodies could be detected in the serum of almost 90 per cent of patients with gonococcal arthritis. Although these workers demonstrated only minimal cross-reactivity with meningococci, Peacock et al. (1965) reported that reactivity, at a titre of $\geqslant 20$, could be obtained in this test with 64 per cent of sera from meningococcal carriers.

Using fluorescent antibody techniques, with monospecific antisera, Cohen (1967) investigated the immunoglobulin classes of serum anti-gonococcal antibody. Using as antigen a heat- or formalin-treated strain (F26) of N. gonorrhoeae, he showed that whilst antibodies of the IgM, IgA and IgG classes were present in serum from infected and non-infected patients, immune IgG antibodies were distinguishable from natural IgG antibodies by their ability to react with heat-labile surface antigens. No distinct differences could be detected in the reactivity of IgA or IgM antibodies in serum from infected, and from

non-infected patients. In a subsequent study (Cohen, Kellogg and Norins, 1969), it was demonstrated, in the experimental situation, that during infection, there was a significant increase in IgG reactivity to heat-labile antigens, and in IgA reactivity to somatic antigens.

Welch and O'Reilly (1973) and O'Reilly, Welch and Kellogg (1973) reported an intensive study of the immunofluorescent antibody method on sera from patients with uncomplicated gonorrhoea. They selected, as antigen, a strain of the gonococcus (strain 9), which was demonstrated to possess antigenic features in common with other gonococcal strains, but not shared significantly with other species of Neisseria. A polyvalent fluorescein-conjugated antiserum was used in the test. Only four per cent of non-infected females had serum antigonococcal antibody at a titre of $\geqslant$ 16, but almost 80 per cent of women, and 60 per cent of men with untreated gonorrhoea, had antibody at this titre.

In view of these encouraging results, Reddick (1975), investigated an automated fluorescent antibody test, using the strain of the gonococcus described by O'Reilly, Welch and Kellogg (1973), but found an apparent false positive rate in pregnant women, attending an ante-natal public health clinic, of 15 per cent. The sensitivity could not be assessed, as only seven of 600 women were found to be infected with gonorrhoea. She considered the test of little value in screening low-risk population.

Wilkinson (1975), using a subculture of a primary isolate of N. gonorrhoeae, found a lower sensitivity than that described by O'Reilly and co-workers. Only 60 per cent of women, and 20 per cent of men with gonorrhoea had gonococcal antibody at a titre of $\geqslant$ 16 in

the sera. There was a presumed false-positive rate of just under four per cent. In addition in place of a polyvalent conjugated antiserum, he used a monospecific anti-human IgM conjugate in a small series of patients, and found that antibody of this class was detectable in the serum of just over 30 per cent of men and 43 per cent of women with gonorrhoea.

Encouraging results were described by Gaafar and D'Arcangelis (1976) who used as antigens, strains of N. gonorrhoeae said to possess heat-labile surface antigen, and an anti-human IgG conjugate. In this study serum from 95 per cent of infected women, and 87 per cent of infected men, but from only one per cent of normal individuals, were reactive. When the test was evaluated further, (Gaafar, 1976) in screening high and low risk groups of patients, however, the sensitivity was reduced to 81 per cent in low risk, and 75 per cent in high risk groups of women. The specificity in the low-risk group was just over 90 per cent.

Flocculation tests

Wallace and his colleagues (1970) reported on the use of a flocculation test in the serodiagnosis of gonorrhoea. In the test, bentonite particles were sensitised with soluble gonococcal antigen and incubated with the patient's serum. Gonococcal antibody was detected in the sera of about three-quarters of men and women with untreated gonorrhoea, but on only four per cent of control sera. However, sera from about 20 per cent of patients attending the sexually-transmitted diseases clinic gave positive results even although N. gonorrhoeae could not be isolated from any of these patients. Watt,

Ward and Glynn (1971) used as antigen in the bentonite flocculation test a soluble component extracted with aqueous phenol from disrupted organisms, and showed that high titres of antibody were present in both infected patients and in controls. Although these observed differences may be due to the use of different methods of antigen preparation, it was emphasised that proteins extracted from gonococci contain group reactive antigens common to both gonococci and meningococci (Maeland, 1969).

A flocculation test using a crude protoplasmic antigen was described by Lee and Schmale (1970). Sera from almost 70 per cent of men and 85 per cent of women with gonorrhoea contained antigonococcal antibody, as did sera from about 12 per cent of control subjects.

Using a microflocculation assay in which the antigen consisted of cholesterol-lecithin particles sensitized with a soluble antigen prepared by disruption of gonococci, Reising (1971) found that serum from about 80 per cent of infected women, but from only 50 per cent of men with gonorrhoea gave positive results. The specificity of this test was superior to those described above in that antibody activity was found in less than seven per cent of sera from non-infected patients. Similar results were reported by Rodas and Ronald (1974) who used the same antigen system as that described by Reising (1971).

Latex particles may be sensitised with lipopolysaccharides and used in serological tests (Watt, Ward and Glynn, 1971); the sensitivity and specificity of the test described by Watt and his colleagues were, however, low.

Precipitin tests

A precipitin test based on the detection of antibodies against an aqueous-phenol extract of type 1 N. gonorrhoeae was described by Reising and Kellogg (1965). Antibodies could only be detected in the sera of about 60 per cent of infected patients, but in none of the control subjects.

Chacko and Nair (1969) used a lipopolysaccharide as antigen in a precipitin test and found gonococcal antibodies in the serum of 88 per cent of men with gonorrhoea. It was shown that the detection of serum antibody increased directly with the duration of infection. The specificity in the male was not clearly defined. Unfortunately, in this study the numbers of women with gonorrhoea were too small to draw conclusions regarding the sensitivity of the test in women.

Haemagglutination tests

Using red blood cells sensitised with an alkaline extract of N. gonorrhoeae, Logan and co-workers (1970) detected haemagglutinating antibodies in the sera from about half of the women and a fifth of the men with gonorrhoea. When the test was modified by using a soluble antigen and absorbing the sera with antigen of N. sicca, the sensitivity in women was increased to 88 per cent, with a specificity of between 82 and 94 per cent.

Maeland and Larsen (1971) described an indirect haemagglutination test in which erythrocytes were coated with endotoxin prepared by aqueous ether extraction of N. gonorrhoeae. Although serum from both infected and non-infected patients contained haemagglutinating antibody,

this antibody activity was destroyed in the control, but not in the infected group by pre-treatment of the serum with 2-mercaptoethanol.

Haemagglutinating antibodies were found in the sera from 84 per cent of women and 46 per cent of men with gonorrhoea, using red cells coated with lipopolysaccharide (Ward and Glynn, 1972); only two per cent of controls gave a positive reaction.

With lipopolysaccharide as antigen in an indirect haemagglutination test, Fletcher, Miller and Nicol (1973) found gonococcal antibodies in the serum of 82 per cent of women and 50 per cent of men with gonorrhoea, and in 20 per cent of control subjects.

Haemagglutination tests, then, have a high degree of sensitivity, but low specificity, probably on account of cross-reactivity of antibodies against other Neisseria.

Radioimmunoassay

This highly sensitive test system has been used to detect gonococcal antibodies in serum. Buchanan and others (1973) found significant levels of antibody against pilus antigen in the serum of about half of the men and 90 per cent of the women with gonorrhoea. Although Luoma, Cross and Rudbach (1975) found gonococcal antibodies in sera from each infected female studied, sera from 22 per cent of non-infected females also had detectable antibody; these workers used a biosynthetically-labelled antigen. Using an I^{125}-labelled pilus antigen, Oates and her colleagues (1977) detected antibody in the serum of 86 per cent of women with gonorrhoea, but in only 13 per cent of non-infected female patients.

More data are required before conclusions can be drawn about the value of radioimmunoassay in the serodiagnosis of gonorrhoea.

Enzyme-linked immunosorbent assay (Elisa)

The first report on the use of the Elisa test in the diagnosis of gonorrhoea was made by Glynn and Ison (1978). These workers used as antigen in the test an outer membrane protein prepared from a clinical isolate of N. gonorrhoeae. Antibody of the IgG class was present in significantly higher levels in the serum of infected patients than in controls. Just over 70 per cent of men and women with untreated gonorrhoea had abnormal levels of IgG antibody (>15 extinction units), such levels being found in only 16 and 11 per cent of non-infected men and women respectively.

In the rabbit, Brodeur and co-workers (1978) have shown that the detection of gonococci by Elisa is both specific and highly sensitive.

Clearly this test requires further evaluation.

Counterimmunoelectrophoretic demonstration of gonococcal antibodies

Kwapinski and colleagues (1978) have recently shown that gonococcal antibodies against cytoplasmic antigens could be detected in the sera of 92 per cent of patients with gonorrhoea. The necessity of concentrating the serum fourfold before use was clearly shown; only seven per cent of non-concentrated sera were reactive. Although it was suggested that this procedure might be applied to the serological diagnosis of gonorrhoea, further evaluation is required; in particular the persistence of antibody after treatment must be defined.

It is clear that no serological test has been discovered which

combines satisfactory sensitivity with specificity. It has been well demonstrated that a serum antibody response takes time to develop (Chacko and Nair, 1969; Glynn and Ison, 1978) and therefore very early infections will be missed. With the exception of the precipitin tests, serological tests appear more sensitive in the female than in the male, possibly on account of the longer duration of infection in women before clinic attendance. The specificity of the various tests varies greatly. Although there may be some cross-reactivity of antibody with other species of Neisseria not all so called "false positive" results can be explained in this way. Antibody may remain detectable for weeks after successful treatment of gonorrhoea, whether this was primarily or inadvertently treated, and the finding of serum antibody does not indicate active infection. As different population groups were often compared in the evaluation of serological tests, it is sometimes difficult to determine the significance of "false positive" results. In many series, for example, sera obtained from patients attending ante-natal clinics or from blood donors were studied to determine specificity. Although it was assumed that these patients did not have gonorrhoea, culture investigation was not performed.

Gonorrhoea is essentially an infection of mucosal surfaces. If there is a local immune response to the gonococcus this will antedate a serum antibody response. Accordingly, it was decided to define the dynamics of local humoral antibody responses and to determine whether the detection of antibodies in secretions could facilitate the diagnosis of infection, particularly in women.

The concept of the secretory immune system is of basic importance

to the work of this thesis and it is outlined below.

THE SECRETORY IMMUNE SYSTEM

In continuity with the external medium from which antigenic stimulation regularly occurs, are the mucous membranes and glands of the ocular, respiratory, gastro-intestinal and genito-urinary systems, and the glands of the mammary gland. It is at these sites that secretory immune responses occur. The typical secretory site consists of a single layer of ciliated or non-ciliated columnar, or cuboidal epithelial cells, possibly interspersed with goblet cells. These cells rest on a basement membrane which separates them anatomically from the underlying lamina propria. A narrow intercellular space separates the lateral margins of adjacent cells, except distally where there is a firm complete junction between each cell - zonula occludens - which represents the barrier between the internal and external environment of the body (Farquhar and Palade, 1963). The lamina propria consists of connective tissue, blood and lymphatic vessels, and a variety of cells, including plasma cells, mast cells, fibroblasts, polymorphonuclear leucocytes and eosinophils.

Besredka (1927), from observations on experimental oral infections with toxin-producing enterobacteria, and skin infections with Bacillus anthracis, suggested the existence of a local immune system independent of circulating antibodies. Further experimental work by Walsh and Cannon (1938) showed that antibody in the nasal cavity was manufactured locally. Burrows and Havens (1948), demonstrated that, in guinea pigs, resistance to infection with cholera was mediated by antibody - coproantibody - in the gastrointestinal secretions. They demonstrated

that the peak titre of agglutinins in the secretions occurred significantly earlier than the peak serum titre (Koshland and Burrows, 1950). Fazekas de St Groth and Donnelley (1950) showed that antibody to influenza virus was produced when antigen was applied directly to mucosal surfaces, and that protection against disease correlated better with the presence of antibody in the bronchial secretions than the titre of serum antibody.

Chodirker and Tomasi (1963) studied the gamma globulin composition of human parotid saliva, colostrum and lacrimal secretions, and demonstrated that γ_1A globulin was the predominant globulin. Although the ratio of γ_2/γ_1A in normal human serum is about 6, in the secretions studied this ratio was less than 1 (Tomasi and Zigelbaum, 1963). It was this observation which suggested that there was preferential secretion of γ_1A globulin into these fluids.

Tomasi et al. (1965) isolated γ_1A globulin from human colostrum and parotid saliva by ion-exchange chromatography followed by gel filtration and/or density gradient ultracentrifugation. They demonstrated that about 60 per cent of the γ_1A in colostrum had a sedimentation coefficient of 11.5, 20 per cent 18.5, and 20 per cent 7.5. Saliva contained about 90 to 95 per cent of the 11S γ_1A and five to 10 per cent of 7S γ_1A. More than 90 per cent of the γ_1A in ascitic fluid (serum) was of the 7S form. Although no difference was found between the chemical properties of salivary and colostral γ_1A, significant differences were found between the γ_1A in these fluids and serum with respect to sialic acid and hexose content. Immunological studies, using the Ouchterlony immunodiffusion technique, demonstrated the presence of 11S γ_1A of antigenic determinants not present in the 7S form. No difference was demonstrated between 7S colostral and 7S serum γ_1A.

The 11S γ_1A globulin (subsequently termed 11S immunoglobulin A or secretory IgA) was shown to be the principal form of γ_1A in various secretions, including bronchial secretions (Masson and Heremans, 1966), lacrimal secretions (Josephson and Weiner, 1968), nasal fluid (Butler, Rossen and Waldman, 1967), duodenal fluid (Girard and de Kalbertmatten, 1970), and cervicovaginal secretions (Waldman, Cruz and Rose, 1971).

Evidence that serum immunoglobulin was not utilised in the production of 11S IgA came from the studies of Butler, Rossen and Waldman (1967) and Tomasi and Bienenstock (1968) who administered parenterally radioactive-labelled serum IgA to experimental animals and noted that no radioactivity became incorporated into the 11S IgA molecule.

Immunochemical analysis demonstrated that 11S IgA (secretory IgA, SIgA) was a dimer, composed of two 7S IgA monomeric subunits bonded, through the α-chains, by disulphide bridges to a single glycoprotein chain (secretory component) (Cebra and Small, 1967). Secretory IgA contains an additional polypeptide chain, the J-chain (Halpern and Koshland, 1970) produced by the same cells as IgA and IgM.

Secretory component may protect the immunoglobulin against intracellular proteolysis by lysosomal enzymes and extracellular degradation in the external secretions. It has been shown that attachment of secretory component to the dimeric IgA molecule is accompanied by increased resistance to trypsin and pepsin proteolysis (Lindh, 1975). The function of the J-chain is uncertain.

Two subclasses of IgA have been described in serum, based on the capacity of certain antisera against IgA_1 proteins to demonstrate

an antigenic deficiency in IgA_2 proteins (Vaerman and Heremans, 1966). The hinge peptide, that is, the segment between the Fab and Fc segments of the molecule containing the inter-H chain disulphides, of IgA_2 is shorter by nine amino-acid residues than that of IgA_1 (Frangione and Wolfenstien-Todel, 1972). The light and heavy chains of IgA_2 are not linked by disulphide bridges, as they are in IgA_1 (Grey et al. 1968). Although data for other secretions are lacking, IgA_2 is the dominant subclass of IgA in human colostrum (Grey et al. 1968).

Alpha chain determinants are found, using immunofluorescent antibody techniques, in plasma cells in the lamina propria of many secretory sites, spread linearly along the basement membrane, in the intercellular spaces, within epithelial cells, particularly at the apex, and within the lumen of the organ, (Tomasi et al. 1965; Crabbe and Heremans, 1966; Tourville et al. 1969; Soutar, 1976). Secretory component is found within the cytoplasm particularly in the area of the Golgi apparatus of the epithelial cells (Tourville et al. 1969).

A model, based on the above experimental observations, for the route of transport of IgA across mucous membranes has been proposed by Tourville et al. (1969). Dimeric IgA produced by plasma cells in the lamina propria diffuses towards the epithelial surface, and, after a short delay, traverse the basement membrane, collecting in the intercellular spaces. The molecules then enter the epithelial cells. It is possible that secretory component, localised on the plasma membrane of the lateral margins of the cells, acts as receptor for dimeric or polymeric IgA (and IgM) (Tomasi, 1976a). From the apical regions of the epithelial cells, the secretory IgA molecules are transported, possibly by reversed pinocytosis, into the lumen.

Serum IgA monomers originate mainly in the bone marrow, spleen and lymph nodes, but polymers, with J-chain, probably arise from the lamina propria of the mucous membranes, entering the circulation through blood or lymphatic vessels (Heremans, 1974). The small quantity (0.00003 to 0.00004 g per l) of secretory IgA in serum probably represents reabsorption from secretory sites, the concentration in the serum increasing during a local inflammatory reaction, and during lactation (Waldman et al. 1970).

Although secretory IgA is the predominant form of IgA in the secretions, during mucosal inflammation, considerable quantities of 7S IgA may escape into the secretions from the plasma (Heremans, 1974).

Other immunoglobulin classes are detectable in secretions, but comparatively few studies have been undertaken on these. The majority of IgM found in the secretions is probably produced at the secretory surface. Plasma cells containing μ determinants are demonstrable in the lamina propria of various mucous membranes and glands (Tourville et al. 1969), although they are less numerous than α-chain-containing cells. Fluorescent-antibody studies demonstrate a distribution of IgM similar to that of IgA, and it is likely that the mechanism of secretion is similar to that of secretory IgA. It is of interest that in patients with selective IgA deficiency, local synthesis and secretion of IgM is enhanced (Brandtzaeg, Fjellanger and Gjeruldsen, 1968).

Immunoglobulin G is detectable in most secretions, although no chemical or immunological difference has been demonstrated between serum and secretory IgG (Brandtzaeg, 1971). That the majority of IgG in secretions is derived from transudation through epithelium, and is not locally produced is suggested by several observations.

(1) There is, generally, a scarcity of plasma cells containing γ -chain

determinants in the mucous membranes (2) Immunofluorescent studies show that leakage of IgG into the oral cavity takes place through the epithelium surrounding the teeth (Brandtzaeg, 1966), and into the intestinal secretions through the epithelium covering the villi (Brandtzaeg and Baklien, 1972) (3) The concentration of IgG in whole saliva correlates well with the degree of inflammation of the oral mucosa (Brandtzaeg, Fjellanger and Gjeruldsen, 1970) (4) Direct passage of I^{131}-labelled IgG from serum into human nasal fluid has been demonstrated (Tomasi, 1976b).

During nasal infection with rhinovirus and coxsackie virus, the changes in IgG concentration in the nasal secretions follow those of albumin, suggesting that at least some of the IgG is derived from serum; the rise in IgG concentration usually occurs during the middle of the first week of infection (Butler et al. 1970).

In some tissues however, at least some of the IgG is locally-produced. Gingival tissues contain large numbers of IgG-containing cells, as does the nasal mucosa immediately below the epithelium (Brantzaeg, 1966; Brandtzaeg, Fjellanger and Gjeruldsen, 1967).

Much less information is available with respect to IgD and IgE. The ratio of salivary to serum IgE is high, and IgE-containing plasma cells may be demonstrated in the lamina propria of mucous membranes, suggesting local production (Tada and Ishizaka, 1970).

Present knowledge of the immune responses at mucosal surfaces to gonococcal infection is scanty, and it was the purpose of this thesis to examine some aspects of these responses.

The first aim was to establish by an immunofluorescent method whether local antibodies were produced during infection with N. gonorrhoeae and to determine to which immunoglobulin class(es) these antibodies belonged. If antibodies could be detected in the secretions, it was intended to study the dynamics of the immune responses both before and after treatment of gonorrhoea.

As the gonococcus may infect the mucous membranes of the urethra, uterine cervix, anorectum, oropharynx and conjunctiva, it was proposed to examine for antibodies secretions from each of these surfaces, and to correlate the findings with those from blood serum.

By consideration of the results of this preliminary study the possibility of developing a diagnostic test based on the detection of local antibodies would be investigated.

MATERIALS AND METHODS

ROUTINE INVESTIGATION OF PATIENTS

Men and women who attended the Department of Sexually Transmitted Diseases, Black Street, Glasgow were investigated. As fewer women than men attended this centre, the physicians working in the female sexually transmitted diseases clinic in Edinburgh Royal Infirmary agreed to participate in the study to augment the number of female cases. Male patients who attended the Edinburgh clinic were not investigated.

A standard history was taken from all patients participating in the study and details were entered on a cyclostyled sheet (Appendix 1).

Particular attention was paid to any relevant past history, the number of sexual partners within the preceding three months and, if infected with gonorrhoea, the probable duration of infection. Wherever possible, the case records of the patient's sexual contact(s) were examined to ascertain, as accurately as possible, when the patient had become infected.

After careful clinical examination of the entire skin surface, buccal cavity, pharynx and external genitalia, specimens for microbiological examination were obtained as follows.

Males

Urethral specimens

After gently massaging the penile urethra, the external meatus

was examined for the presence of urethral discharge and, if present, a sample of exudate was obtained using a plastic disposable inoculating loop (Nunc Products, Kamstrup, Denmark) and a Gram-stained smear was prepared. Gram-stained smears were not prepared from the urethra of men who had no evidence of urethritis.

Regardless of symptoms, specimens for culture for N. gonorrhoeae were taken from the urethra of each patient by gently inserting a charcoal-impregnated bacteriological swab into the anterior urethra; immediately after withdrawal, the swab was broken off into a bijou bottle filled with Stuart's transport medium. The specimen was sent to the laboratory for culture, the mean interval between specimen collection and inoculation on to culture medium being 9 hours (range 4 to 16 hours).

Specimens for culture for Chlamydia trachomatis were taken from men with recurrent non-gonococcal urethritis and from those with epididymitis or arthritis.

When a patient had symptoms of urethral discharge and/or dysuria, or was a contact of a person known or suspected to have gonorrhoea but had no obvious discharge on inspection, he was asked to return to the clinic on the following morning so that he could be examined before he had passed the first urine of the day. This simple procedure facilitates the early detection of urethritis (Simmons, 1978).

Pharyngeal specimens

Material for culture for N. gonorrhoeae was obtained from the tonsillar area and pharynx of all men with gonorrhoea, from known contacts of a case of gonorrhoea, from all patients admitting to

homosexual contact, and from men, not infected with gonorrhoea, whose saliva was to be investigated for antibody to the gonococcus.

Anorectal specimens

Cultures were taken from the anorectum of all homosexual patients, by inserting a charcoal-impregnated swab about 4 cm into the anal canal.

To exclude gonococcal infection in males who had had homosexual contact, if the first set of cultures from the oropharynx and anorectum was negative, cultures from these sites were repeated twice at weekly intervals. Similarly in men who had been in contact with an infected female, urethral and oropharyngeal cultures were repeated twice if the initial tests had not shown evidence of gonococcal infection.

Females

Urethral specimens

A Gram-stained smear of secretions obtained by gently massaging the urethra was prepared. Culture of urethral material for *N. gonorrhoeae* was also undertaken.

Vaginal specimens

Secretions sampled from the posterior fornix of the vagina were

examined for Trichomonas vaginalis, by phase-contrast microscopy of a suspension in normal saline, and for Candida spp. by examination of a Gram-stained smear.

Cervical specimens

Cervical secretions, obtained under direct vision, were examined by microsocpy of a Gram-stained smear, and by culture. Specimens from women with cervicitis and salpingitis were taken for culture for Chlamydia trachomatis.

Oropharyngeal specimens

Oropharyngeal cultures for N. gonorrhoeae were taken from all female patients.

Anorectal specimens

Anorectal cultures were taken from each female patient by inserting a bacteriological swab into the anal canal to a distance of about 4 cm. Proctoscopy was performed only if there were symptoms referrable to the anal region.

Material for culture for N. gonorrhoeae was taken on charcoal-impregnated swabs as described above from women attending the Glasgow clinic. In Edinburgh, plates of culture medium (see below) were inoculated directly, and held at 36°C until transfer to the laboratory (mean time interval 2.1 hours, range 30 minutes to 3 hours).

Serological Tests for Syphilis

Serum samples for serological tests for syphilis were taken from each male and female patient at the initial visit, at 28 days and 3 months later.

In Glasgow, these tests were the automated reagin test (ART) and the Reiter protein complement fixation test (RPCFT). If one or both of these tests were positive, the presence of treponemal antibody was confirmed by the fluorescent treponemal antibody-absorbed test (FTA-ABS).

In Edinburgh, the Treponema pallidum haemagglutination (TPHA) and the Venereal Diseases Research Laboratory (VDRL) tests were used, and the FTA-ABS when indicated.

COLLECTION OF SPECIMENS FOR ANTIBODY STUDIES

Urethral secretions in the male

When a urethral discharge was present, about 30 µl of exudate were collected with a sterile disposable plastic inoculating loop (Nunc Products, Kamstrup, Denmark) and suspended in 1 ml of sterile physiological saline (Appendix 2).

When a patient had no obvious urethritis, secretions were obtained by instilling 0.5 ml of saline into the anterior urethra by means of a soft polythene capillary tube sterilised by ethylene oxide (chromatography tubing, internal diameter 1.0 mm, obtained from Pharmacia Fine Chemicals, Uppsala, Sweden); the tube was attached by a 19 SWG needle to a 2 ml syringe. After allowing 90 seconds for equilibration, the saline was collected directly by allowing the fluid to drain into a wide-mouthed bottle. After centrifuging at 1,500 *g* for 10 minutes the supernate was taken; one drop (0.05 ml) of 0.1 per cent sodium azide in physiological saline was added as a bacteriostatic agent, and the supernate was held at -20°C until required.

Cervical secretions in the female

The apparatus used to collect cervical secretions consisted of a 10 cm length of sterile polythene capillary tubing (Pharmacia Fine Chemicals, Uppsala, Sweden) attached to a 5 ml syringe by a disposable hypodermic needle, SWG19.

Immediately before use, 2 ml of sterile physiological saline was drawn into the syringe. Under direct vision the capillary tube was placed just inside the endocervical canal, and the secretions were gently aspirated. Material collected in the capillary tube was discharged into a sterile bijou bottle by flushing with 1 ml of saline from the syringe.

In an attempt to avoid contamination of the secretions with whole blood introduced by trauma to the cervical tissues, obviously blood-stained secretions, or those giving a positive result when tested with Hemastix strips (Ames Co Ltd, Slough, Bucks) were discarded.

Subsequent treatment was as described for urethral secretions.

Whole saliva

Patients were instructed to rinse their mouths with 10 ml of physiological saline, and the diluted saliva was collected in a sterile plastic Universal Container (Sterilin Ltd, Teddington, Middlesex).

After centrifugation at 1,500 g for 15 minutes, the supernate was concentrated to 1 ml by positive pressure dialysis, with an Amicon Microfiltration System, Model 8 MC, and a Diaflo Membrane, PM 30 (Amicon, Lexington, Massachussetts). A drop of 0.1 per cent sodium azide in saline was added, and the concentrated saliva was stored as above.

Anorectal secretions

A proctoscope, the exterior of which had been lightly lubricated with K-Y jelly (Johnson-Johnson, Slough, Bucks), was gently inserted into the anal canal; after removing the obturator, excess jelly was removed with a cotton wool-tipped applicator stick. Secretions were collected by rolling a sterile bacteriological swab (Exogen Ltd, Glasgow) over the mucous membrane, and eluting in 1 ml of saline in a bijou bottle. The diluted secretions, after centrifugation and addition of sodium azide, were stored as above.

Lacrimal secretions

Lacrimal secretions were collected by placing a strip of filter paper (No 1, Whatman Ltd, Maidstone, Kent), 20 mm x 5 mm along the

conjunctival surface of the lower eyelid. When the filter paper was saturated, the secretions were eluted in 1 ml sterile saline, and after adding sodium azide, stored at -20°C.

Serum

A volume of 5 ml of blood was obtained from each patient and after allowing to clot, serum was collected by centrifugation at 1,500 g for 30 minutes. Before use, in the immunofluorescent antibody test, serum was heated at 56°C for 30 minutes.

LABORATORY DIAGNOSIS OF GONORRHOEA

IN GLASGOW

The culture medium used throughout the period of the study was Columbia agar base (Oxoid Ltd, Basingstoke, UK) enriched with 7 per cent (v/v) defibrinated horse blood, and containing vancomycin 2.5 µg per ml, trimethoprim 3.0 µg per ml and polymyxin 15 units per ml. After incubation at 36°C for 48 to 72 hours in a carbon dioxide-enriched atmosphere (candle extinction jar), the identity of suspected colonies of N. gonorrhoeae was confirmed by the oxidase reaction and sugar utilisation tests.

IN EDINBURGH

During the period of the study, a modified New York City (MNYC) medium was used for the isolation of the organism (Young, 1978). This medium consists of a commercial (Difco) gonococcal base supplemented with 10 per cent (v/v) human blood lysed by saponin 0.5 per cent (v/v), yeast dialysate 2.5 per cent (v/v), glucose 0.1 per cent (v/v), lincomycin 1.0 µg per ml, colistin 6 µg per ml, amphotericin B 1.0 µg per ml, and trimethoprim lactate 6.5 µg per ml.

After incubation at 36°C for 24 hours in a CO_2-enriched atmosphere (10 per cent) the identity of suspected colonies was confirmed by the oxidase reaction, the rapid carbohydrate utilisation test and by delayed immunofluorescence (Young, Paterson and McDonald, 1976).

TREATMENT OF GONORRHOEA

In Glasgow, two regimens were used in the treatment of uncomplicated genital gonorrhoea in men and women.

(1) Minocycline, a tetracycline, was given in a single oral dose of 300 mg. This drug may produce vertigo some hours after ingestion; if the patient was likely to use complicated machinery, or to drive within eight hours of treatment, the following regimen was used.

(2) Ampicillin was given in a single oral dose of 2 g; 1 g of probenecid was given at the same time to delay renal excretion of the penicillin.

In all, 61 men and 39 women were treated successfully with minocycline and 66 men and one woman were treated with ampicillin.

Spectinomycin hydrochloride in a single dose of 2 g was given by intramuscular injection to one man whose infection was not cured by minocycline or ampicillin.

Single-dose therapy is less effective in the management of oropharyngeal gonorrhoea in both sexes and anorectal gonorrhoea in males (Ödegaard and Gundersen, 1973; Scott and Stone, 1966); 27 men with rectal or pharyngeal gonorrhoea, and six women with throat infection were treated with cotrimoxazole (each tablet containing 400 mg of sulphamethoxazole and 80 mg of trimethoprim), given in a dosage of two tablets every 12 hours for seven days.

The patient with acute epididymitis and the three women with salpingitis were treated with doxycycline hydrochloride in an oral dosage of 200 mg eight-hourly for 7 days and thereafter 100 mg eight-hourly for a further 7 days. Both women with bartholinitis were treated with ampicillin, given orally in a dosage of 500 mg

six-hourly for seven days.

Two men were treated for disseminated gonococcal infection: one received benzyl penicillin intramuscularly, 600 mg six-hourly for 10 days; the other received oral ampicillin, 500 mg six-hourly for 10 days.

In Edinburgh, women with gonorrhoea were treated with ampicillin/probenecid, 24 women, each of whom had uncomplicated gonorrhoea, were treated with this regimen.

FOLLOW-UP ASSESSMENT

To assess the efficacy of therapy of urethral gonorrhoea in men, patients were examined 7, 14 and 28 days after treatment; regardless of symptoms, a Gram-stained smear of urethral material was prepared and a specimen taken for culture at the first post-treatment visit. Smears and cultures were only repeated if the patient had symptoms or signs of urethritis.

The efficacy of treatment of oropharyngeal and anorectal gonorrhoea was determined by culturing the affected sites at weekly intervals, until three consecutive cultures for N. gonorrhoeae were negative.

In both Glasgow and Edinburgh, women who had been treated for gonorrhoea were examined at weekly intervals for three weeks after treatment. At each visit, specimens for microbiological examination were taken from the urethra and cervix, and from the anorectum and oropharynx if these sites had been infected initially.

PATIENTS INVESTIGATED

The method of presentation of details of the patients investigated is outlined below.

MALES

MEN WITH GONORRHOEA

Uncomplicated Infection

Complicated Infection

(a) Acute epididymitis

(b) Disseminated gonococcal infection

MEN NOT INFECTED WITH GONORRHOEA

Known sexual contacts of individuals with gonorrhoea

Men not known to have been sexual contacts of individuals with gonorrhoea

FEMALES

There were two parts to the investigation of women with respect to the local immune response to gonococcal infection.

I The detection of antibodies reactive with N. gonorrhoeae in the serum and secretions, the determination of the immunoglobulin classes of these antibodies, and the examination of the changes in antibody classes following treatment

WOMEN WITH GONORRHOEA

Uncomplicated Infection

Complicated Infection

(a) Acute bartholinitis

(b) Acute salpingitis

WOMEN NOT INFECTED WITH GONORRHOEA

Known sexual contacts of men with proven gonorrhoea

Women not known to have been contacts of men with gonorrhoea

II Evaluation of the indirect immunofluorescent-antibody test in the diagnosis of gonorrhoea in the female

MALES

Male patients attending consecutively the Department of Sexually Transmitted Diseases, Black Street, Glasgow, on two mornings per week between January 1977 and March 1978, were studied.

MEN WITH GONORRHOEA

Uncomplicated Infection

A diagnosis of gonorrhoea was made only if N. gonorrhoeae was isolated on culture.

One hundred and fifty five men had uncomplicated gonorrhoea, 38 having been infected through homosexual contact.

The mean age of the infected group of patients was 24.9 (range 15 to 48 years).

One hundred and fourteen (74 per cent) were unmarried, 26 (17 per cent) married, 11 (7 per cent) separated from their wives and four (2 per cent) divorced.

The mean number of sexual partners in the preceding 3 months amongst the 117 men admitting to only heterosexual contact was 2.1, and 3.8 amongst the 38 homosexual men.

Thirty four men had previously been infected with gonorrhoea; 27 had been infected once, four twice, two three times and one six times previously. The mean interval between the most recent and present infections was 2.6 years (range 3 months to 6 years). During the period of the project three patients became re-infected once with N. gonorrhoeae, and one man was re-infected twice.

In the present study, three men, each heterosexual, were considered minocycline treatment failures. When seen for review seven days after initial treatment, urethritis was still present, and the gonococcus was isolated on culture. Two of these men were successfully treated with the ampicillin/probenecid regimen, and one man, whose infection did not respond either to minocycline or to ampicillin was cured with a single intramuscular injection of 2 *g* of spectinomycin hydrochloride.

Complicated Infection

(a) Acute epididymitis occurred in one heterosexual man. He had had symptoms of urethritis for about two weeks and painful swelling in the scrotum for two days prior to attendance. A Gram-stained smear of urethral exudate showed Gram-negative intracellular diplococci, and the diagnosis of gonorrhoea was confirmed by culture. Examination of the urine by standard microbiological tests showed no evidence of infection with eubacteria, but Chlamydia trachomatis was isolated on culture of urethral material.

(b) Disseminated gonococcal infection occurred in two men with urethral gonorrhoea.

The first man, aged 17 years, had had untreated urethral gonorrhoea of at least eight weeks duration, and developed a septic arthritis of the right knee joint. Although N. gonorrhoeae was not isolated from the fluid aspirated from the joint, the arthritis improved dramatically within 24 hours of commencing treatment with benzylpenicillin. In addition to N. gonorrhoeae, Chlamydia trachomatis

was isolated from the urethra.

The other man, aged 23, developed fever, arthralgia of the shoulder, elbow, wrist, metacarpophalangeal and interphalangeal joints, and an erythematous maculopapular rash on the dorsa of both hands, four days after noticing a urethral discharge. Neisseria gonorrhoeae was isolated from the urethral exudate but not from the blood; Chlamydia trachomatis could not be cultured from the urethra. There was no clinically obvious arthritis, and joint aspiration was not attempted. The patient's symptoms improved within 36 hours of commencing therapy with ampicillin.

Table 1 indicates the sites infected with N. gonorrhoeae in men with uncomplicated and complicated gonorrhoea at the time of entry into the present study.

TABLE 1M

Distribution of sites infected with N. gonorrhoeae in 158 men with gonorrhoea

Site(s) infected	Number of patients with positive cultures for N. gonorrhoeae from that (these) site(s)	
	Heterosexual patients	Homosexual patients
Urethra only	120	11
Oropharynx only	0	9
Anorectum only	ND*	13
Anorectum and oropharynx	ND	2
Anorectum and urethra	ND	2
Oropharynx and urethra	0	0
Urethra, oropharynx and anorectum	ND	1
Total	120	38

* ND = Culture from site or combination of sites not performed.

NON-INFECTED MEN

Known sexual contacts of individuals with gonorrhoea

Three heterosexual men attended as named contacts of women with culturally-proven gonorrhoea. Each was asymptomatic, had not recently had anti-microbial therapy, and three sets of cultures from the urethra and oropharynx failed to show infection with N. gonorrhoeae.

Men not known to have been sexual contacts of individuals with gonorrhoea

To be included in this group of patients it was required that gonococcal infection had been excluded (see above); that there was no known past history of gonorrhoea; that they were not known contacts of patients with gonorrhoea; that they had not received anti-microbial therapy within the preceding three months.

One hundred and eighty three men, attending the clinic during the period of the study, satisfied the above criteria. Twenty nine (16 per cent) had had homosexual contact.

The mean age of this group was 22.8 years (range 16 to 54 years). One hundred and forty seven (80 per cent) were single, 25 (14 per cent) married, four (2 per cent) separated and seven (4 per cent) divorced. The mean number of sexual partners was 2.8 in the heterosexual and 3.7 in the homosexual group. Table 2 shows the diagnoses made in this group of patients.

TABLE 2 M

Diagnosis made in 183 men with no evidence of infection with N. gonorrhoeae

Diagnosis	Number of patients with each condition	
	Heterosexual patients	Homosexual patients
Non-gonococcal urethritis	50	4
Genital candidiasis	7	0
Sarcoptes scabiei infestation	3	2
Pthirus pubis infestation	12	4
Genital herpes	1	1
Condylomata acuminata	25	4
Molluscum contagiosum	1	0
Non-specific balanitis	11	1
Tinea cruris	2	0
No disease present	42	13
Total	154	29

FEMALES

I The detection of antibodies reactive with N. gonorrhoeae in the secretions, the determination of the immunoglobulin classes of these antibodies, and the examination of the changes in antibody classes following treatment.

Female patients consecutively attending the Department of Sexually Transmitted Diseases, Black Street, Glasgow on two mornings per week between January 1977 and March 1978 were studied. Women consecutively attending one morning session per week at the Department of Venereology, Royal Infirmary, Edinburgh, were also included in the investigation. Pregnant women, for ethical reasons, were not investigated.

WOMEN WITH GONORRHOEA

To be included in this group, it was required that N. gonorrhoeae had been isolated on culture from at least one site examined.

Uncomplicated Infections

Seventy women with uncomplicated gonorrhoea were investigated, 46 in Glasgow and 24 in Edinburgh.

The mean age was 23.4 years (range 16 - 48).

Forty five were unmarried, 14 married, six separated from their husbands and five were divorcees.

The mean number of sexual partners within the preceding three

months was 1.6.

Eight women had previously been infected with N. gonorrhoeae, the mean interval between the present and previous infection being 2.7 years (range 3 months to 6 years).

As contraception, 54 used oral oestrogen-progestogen preparations, three had had an intra-uterine device fitted; the male partner of one woman used a sheath. Three women had been sterilised by tubal ligation, and nine used no contraceptive methods at all.

In 37 untreated women the menstrual period had begun within the preceding 14 days. Thirty three women were in the second half of the menstrual cycle at the time of the initial attendance.

Each patient was cured by treatment given at the initial visit and no cases of re-infection with gonorrhoea occurred during the period of the study.

Complicated Infection

(a) Acute bilateral bartholinitis with abscess formation was diagnosed in two women who had been infected for at least 3 weeks.

(b) Acute salpingitis was diagnosed in three women each of whom had been infected for about 3 weeks. Chlamydia trachomatis was not isolated from the cervix of any of these women.

Table 3 indicates the sites from which N. gonorrhoeae was isolated on culture from women with uncomplicated and complicated gonorrhoea. In each patient studied, the organism was isolated from at least one site cultured at the patient's initial clinic visit.

TABLE 3

Sites from which N. gonorrhoeae was isolated on culture of 75 women with gonorrhoea

Site(s) infected	Number of patients with positive cultures from that (these) site(s)
Urethra only	1
Cervix only	12
Anorectum only	0
Urethra and cervix	32
Urethra, cervix and anorectum	23
Urethra, cervix, anorectum, oropharynx	4
Cervix and anorectum	1
Urethra, cervix and oropharynx	2
Oropharynx only	0
Total	75

WOMEN NOT INFECTED WITH GONORRHOEA

Known sexual contacts of men with proven gonorrhoea

Sixty-three women attended as named sexual contacts of men with culturally-proven gonorrhoea. Twelve of this group had no cultural evidence of infection in any site, three sets of tests having been taken at weekly intervals as previously described; there was no history of recent antibiotic therapy.

Women not known to have been contacts of men with gonorrhoea

To be included in this control group, infection had to be excluded as described above; there had to be no history of sexual contact with a man known or suspected to be infected; the patient should not have received anti-microbial therapy within the preceding three months; there had to be no past history of treatment for gonorrhoea.

Seventy women satisfied these criteria, 36 attending the Edinburgh clinic, and 34 the Glasgow clinic.

The mean age was 23.6 (range 16 to 41 years).

Fifty two of the women were unmarried, 11 married, 4 separated and three were divorced from their husbands.

The mean number of sexual partners within the preceding three months was 1.8.

As contraceptive, 52 women used an oral oestrogen-progestogen preparation; two had had an intrauterine device fitted; the partners of four women used a sheath; one woman had been sterilised, and 11

used no contraceptive methods.

At their initial attendance 37 women were within the first half of the menstrual cycle, and 33 in the second half.

Table 4 indicates the conditions diagnosed in this group of patients.

Cervicitis was diagnosed when the cervix was oedematous and reddened and mucopus exuded from the external os.

TABLE 4

Conditions diagnosed in 70 women who had no evidence of gonococcal infection, and who were not known contacts of men with gonorrhoea

Condition diagnosed	Number of patients
Late latent syphilis	1
*Non-gonococcal cervicitis	13
Trichomoniasis	12
Candidiasis	11
Pthirus pubis infestation	1
Condylomata acuminata	14
Molluscum contagiosum	1
No abnormal findings	33
Total	86

* *Chlamydia trachomatis* was isolated from the cervix of eight of these women.

II Evaluation of the indirect immunofluorescent-antibody test in the diagnosis of gonorrhoea in the female

Each new female patient with the exception of pregnant women, attending the Black Street Clinic, Glasgow, between May 1st and September 30th 1978 was studied.

Two hundred and ninety four women were examined. Table 5[M] shows the diagnoses made in these women.

TABLE 5[M]

Diagnosis(es) made in 294 women attending consecutively during a 5-month period

Diagnosis	Number of patients
Gonorrhoea	115
Trichomoniasis	109
Candidiasis	101
*Non-specific cervicitis	38
Pthirus pubis infestation	6
Condylomata acuminata	18
Genital herpes	2
Non-specific vaginitis	37
Early latent syphilis	2
No abnormalities detected	76
Total	504

* *Chlamydia trachomatis* was isolated from the cervix of 14 of these women

SPECIMENS COLLECTED FROM PATIENTS

MALES

From each man with gonorrhoea, serum, and secretions from the infected site(s) were obtained before initial treatment.

Sera from 158 infected men were available for study, as were urethral secretions from 134 men with urethral gonorrhoea, anorectal secretions from 18 men with gonococcal proctitis, and saliva from 12 men with oropharyngeal infection.

Serum and urethral secretions were obtained from 183 non-infected patients attending consecutively during the period of the study. Anorectal secretions and saliva were also collected from each of the 29 men who had had homosexual contact.

To investigate possible antigenic stimulation of the local mucosal immune system at sites remote from the actual site of infection, samples of saliva were collected from each of 70 men attending during an 11-month period with untreated urethral and/or anorectal gonorrhoea, but with no evidence of oropharyngeal infection. From non-infected men attending during the period of this investigation, saliva in addition to serum and urethral secretions (and where indicated rectal secretions) were obtained. Lacrimal secretions from 23 infected and 15 non-infected men attending consecutively were also examined.

It was aimed to obtain samples of serum and urethral secretions from patients with gonorrhoea, seven, 14 and 28 days after successful treatment. There was a considerable default rate, and specimens were available from only 70 men, seven days post-treatment, 45 men 14 days post-treatment, and 48 men 28 days after treatment of urethral gonorrhoea.

Urethral secretions and sera were collected before and after successful treatment of three men considered to be treatment failures, and from four men who became re-infected during the course of their follow-up.

Secretions and sera were obtained twice, with an interval of seven days, from three men who had no evidence of infection but were named sexual contacts of infected women.

FEMALES

Part I of project

From each woman studied, cervical secretions and serum were obtained at the initial attendance.

Specimens of cervical secretions and sera from 75 untreated infected and 70 non-infected women were available for study. Cervical secretions and sera were obtained also from 31 of the non-infected patients who attended 14 days after their initial visit. Repeat testing of the other 39 women was not possible because these patients defaulted.

In addition to the above specimens, anorectal secretions were collected from each female patient studied during the period October 1st, 1977 to April 30th, 1978. Forty such specimens were examined, 14 from women with anorectal gonorrhoea, 11 from women with genital infection only, and 15 from women with no evidence of infection at any site.

To examine possible antigenic stimulation of the secretory immune system at sites remote from those infected, samples of saliva were collected from 12 women with anogenital gonorrhoea and from 15 non-infected women attending consecutively over a two-month period (May 1st, 1978 to June 30th, 1978). Lacrimal secretions were examined from each infected patient, and from 10 of the non-infected women.

Two hundred and fourteen samples of sera and cervical secretions were examined after treatment (54 from women attending seven days post-treatment, 47 from patients attending 14 days after treatment, and 43 from women examined 28 days after treatment).

Cervical secretions and sera from 12 women who, although named contacts, were not found to have gonorrhoea, were examined at weekly intervals for three weeks.

Part II of project

Cervical secretions only were obtained from 294 women attending during a four-month period.

LABORATORY METHODS

The first part of the study was to determine the classes of immunoglobulins in the secretions and for this purpose, thin-layer gel filtration was used.

THIN-LAYER GEL FILTRATION OF URINOGENITAL SECRETIONS

The method used to determine the classes of immunoglobulins in the urethral, cervical, rectal and lacrimal secretions was a modification of that used by Hanson, Holmgren and Wadsworth (1971).

A 3.5 (v/v) suspension of Sephadex G-200, superfine grade (Pharmacia Fine Chemicals, Uppsala, Sweden), in 0.02 M Tris-HCl buffer, pH 8.05 (Appendix 4), containing 28.38 g of sodium chloride per litre (0.4 M with respect to NaCl), was prepared, and spread uniformly at a thickness of 0.6 mm on a chemically-clean rectangular glass plate, 30 cm x 25 cm. The gel was connected by filter paper (Whatman 3 MM) to two buffer reservoirs, and covered by a "perspex" lid. A downward flow of buffer was set up by elevating one buffer vessel thereby inclining the plate at an angle of about 10° from the horizontal. At the start of the experiment, a difference of 10 cm was maintained between the surfaces of each buffer solution.

After allowing the system to stabilise overnight, 3 µl of (a) Naphthalene black-labelled human albumin (Appendix 5) (b) serum (c) secretions (d) secretory IgA were placed on the gel, at 2.0 cm intervals, on a line 3 cm from the top of the plate. During the application of the samples, the plate was in a horizontal position, and care was taken to avoid disturbing the gel surface.

When the labelled albumin had migrated about 10 cm from its original position, a filter paper replica of half of the plate was

prepared by carefully layering on a sheet of filter paper (3 MM, Whatman), and allowing it to remain in contact with the gel for two minutes. The locations of the separated proteins were determined by staining of the filter paper with Coomassie brilliant blue (Appendix 6).

A block of agarose containing 5 per cent (v/v) sheep antiserum against human IgA, IgG or IgM (Wellcome Reagents Ltd, Beckneham, Kent) was carefully placed over the area on the undisturbed gel thought from inspection of the filter paper replica, to contain these immunoglobulins. After allowing four days incubation at room temperature in a moist chamber, to permit diffusion of immunoglobulins to take place, the agarose slabs were fixed and stained with thiazine red (Appendix 7).

The results showed that IgG and IgA, the majority of which was of the secretory IgA type, could be detected readily in secretions other than lacrimal which contained only IgA. Immunoglobulin M could not be demonstrated by this method.

Having shown that IgA and IgG were present in the secretions tested, the next aim was to quantitate the total IgA and IgG to serve as a source of reference when considering specific antibody activity.

DETERMINATION OF IMMUNOGLOBULIN CONCENTRATIONS IN THE SECRETIONS

Preliminary experiments demonstrated the inability to determine IgM concentrations in the secretions by radial immunodiffusion. More sensitive methods, such as the methods of single radioactive radial immunoprecipitation (Rowe, 1969), were not, however, accessible. Data on IgM concentrations are not, therefore presented.

Radial immunodiffusion was used in the estimation of IgG and IgA. A stabilised serum IgG standard (Hoescht Pharmaceuticals, Hounslow, Middlesex) was used in the determination of IgG in the secretions.

The use of a serum IgA standard in the measurement of secretory IgA by immunodiffusion underestimates the concentration by about tenfold (Samson, McClelland and Shearman, 1973). Where there may be a mixture of 7S IgA, 11S IgA and perhaps higher polymers, the use of a single standard would lead to considerable inaccuracy in the determination of the total IgA concentration. As may be seen from the results of thin-layer gel filtration, the majority of the IgA in each secretion examined existed as the secretory 11S IgA molecule. In view of the small quantity of monomeric 7S IgA in the secretions, it was felt justifiable to employ, throughout the study, a secretory IgA standard, the error in estimating total IgA being small.

Secretory IgA standard was prepared from colostrum (Newcomb, Normansell and Stanworth, 1968) and standardised by measurement of the extinction coefficient (Tomasi, 1970). Aliquots of diluted standard, containing 0.042 g per l, were stored at -20°C until required.

Commercially prepared low-level immunodiffusion plates (Hoescht Pharmaceuticals, Hounslow, Middlesex) were employed. Wells in the agar were filled twice with 20 µl of secretions, and precipitation lines were measured 72 hours later. Faint areas of precipitation were itensified using DOPA reagent (Appendix 8) (Sieber and Becker, 1974).

To investigate the reproductibility of results, secretions were examined in duplicate at random intervals. Variation in immunoglobulin concentrations was no greater than ± 0.1 mg per dl.

In view of the high cost of the commercially-available immunodiffusion plates, it was not possible to estimate the immunoglobulin concentration of every secretion. Concentrations of IgA and IgG in genital secretions from infected patients attending consecutively between March 1st, 1977 and January 31st, 1978 were determined, as were the immunoglobulin concentrations in secretions from non-infected patients attending consecutively between March 1st, 1977 and August 1st, 1977.

Estimation of immunoglobulin in anorectal secretions was performed on five men and five women attending consecutively with anorectal gonorrhoea. Anal secretions were collected from patients (four men and five women) with no evidence of infection at any site, who attended during the same week as those who had been found to have anorectal gonorrhoea.

The immunoglobulin concentrations in lacrimal secretions were estimated in five patients attending consecutively during the course of a clinic.

Having demonstrated the presence of IgA and IgG in various secretions, and measured the total concentrations of these immunoglobulins, the next step was to examine the secretions for antibody against N. gonorrhoeae and, if present, to determine with which class(es) of immunoglobulins the antibody was associated. For this purpose, and for the investigation of serum obtained on the same day as the secretions, an indirect immunofluorescent-antibody test was used.

INDIRECT IMMUNOFLUORESCENT-ANTIBODY TEST USING FLUORESCEIN-CONJUGATED ANTISERUM AGAINST IgA (α-chain), IgG (γ-chain) and IgM (μ-chain)

Antigen preparations

Neisseria gonorrhoeae

Strain 9 of N. gonorrhoeae as described by O'Reilly, Welch and Kellogg (1973) and kindly supplied by Dr D S Kellogg, Centre for Disease Control, Atlanta, Georgia, USA, was used as standard gonococcal antigen in the indirect immunofluorescent-antibody test. Freeze-dried organisms were reconstituted in sterile distilled water, and cultured on modified New York City medium. After incubation for 16 to 20 hours at 37°C in a carbon dioxide-enriched atmosphere, colonies were selected using an inoculating loop, and dispersed in distilled water until the solution was faintly turbid. Aliquots of 200 μl were stored at -20°C until required.

Before use, the identity of the organism was confirmed by Gram-staining, the oxidase reaction and by the direct immunofluorescent-antibody test. In the latter test, organisms dried on a slide, were incubated at 37°C in a moist chamber with fluorescein-labelled antigonococcal conjugate (Difco Laboratories, West Molesey, Surrey, UK) for 10 minutes. After washing in phosphate buffered saline (Bacto FA, Difco) slides were examined under ultraviolet-light (see below).

In addition to using Strain 9 in the immunofluorescent-antibody test, the homologous isolate of N. gonorrhoeae from each of 23

patients (10 men and 13 women) was used in parallel when their secretions were examined.

N. meningitidis

Strains of N. meningitidis representing groups A, B, C, D, E, 29E, W-135, X and Z were obtained from the stock of cultures held in the Department of Bacteriology, University of Edinburgh. These strains had initially been obtained from the Neisseria Repository, Berkeley, California.

Freeze-dried cultures were reconstituted in distilled water, cultured on MNYC and dispensed in aliquots of 200 ul as above.

N. lactamica, N. perflava and N. catarrhalis

Cultures of these species of neisseriae were obtained from the University Department of Bacteriology, Royal Infirmary, Edinburgh. These organisms had been isolated on blood agar from clinical specimens and their identity confirmed by sugar utilisation reactions. Aliquots were prepared as before.

Staphylococcus aureus, Staphylococcus albus, Streptococcus faecalis and Escherichia coli

Cultures on blood agar were provided by the University Department of Bacteriology, Edinburgh Royal Infirmary. Suspensions of these organisms were prepared as above.

In the preparation of slides for the indirect immunofluorescent-antibody test, the above antigen preparations were thawed, and 20 µl of suspension used to fill each well of a multi-spot slide (Hendley, Essex). After drying at 37°C for 15 minutes, the slides were ready for use.

Antisera used in indirect immunofluorescent-antibody test

Fluorescein-conjugated sheep anti-human IgM (µ-chain), IgA (α-chain) and IgG (γ-chain) sera were obtained from commercial sources (Wellcome Reagents Ltd, Beckneham, Kent). Before use, the absence of unconjugated fluorescein iso-thiocyanate was confirmed by thin-layer gel filtration (Johnson, Holborow and Dorling, 1978). Specificity was confirmed by double diffusion in gel against purified immunoglobulin preparations.

Antisera were used at a dilution of 1 in 16 in the immunofluorescent test.

Standard indirect immunofluorescent-antibody test (IFA test)

One drop (approximately 10 µl) of diluted secretions or sera was layered on to the dried bacterial preparation, and the slide incubated in a moist chamber at 37°C for 30 minutes. After washing for 10 minutes in two changes of PBS, pH 7.4, 10 µl of fluorescein-conjugated antiserum was added and the slide maintained at 37°C for 30 minutes. The preparation was then washed in two changes of PBS, each of 5 minutes duration, rinsed in distilled water and mounted in buffered glycerol (Difco Labs, West Molesey, Surrey).

Optical system

A Zeiss microscope (Large Universal) was used, equipped with an HBO-200 mercury vapour lamp for excitation of fluorescence by transmitted light through a thin (3 mm) BG-12 primary filter at a wavelength of 475 nm within the blue-violet range, with secondary filters Nos 53 and 44 with a darkfield ultra-condenser, 1.2/1.4 N.A. Smears were scanned with a low power objective and finally examined with a x 100 oil immersion objective.

Grading of fluorescence

Slides were examined by an observer who was unaware of the source of the specimen, and of the antiserum used. Each set of slides contained secretions or serum from know infected and uninfected subjects, and whose reactivity had previously been assessed.

At least six microscope fields of uniformly-distributed organisms were examined. Fluorescence of clumps of bacteria was ignored.

The method of grading was that described by Welch and O'Reilly (1973) viz. 4+ = brilliant fluorescence of all organisms in the field; 3+ = well defined fluorescence of all organisms; 2+ = low-intensity but definite fluorescence of 75 per cent or more of the organisms in the field; 1+ = occasional organisms with low-intensity fluorescence.

Only preparations showing 2+ or greater fluorescence were scored as positive. In determining the titre of secretory antibody,

the end point was taken as the final dilution giving 2+ fluorescence. The titre of serum antibody was the reciprocal of the final dilution showing 3+ fluorescence.

During the initial six months of the project, the immunofluorescence test was repeated on 50 specimens. The results of both tests agreed within the limits of ± one dilution.

Antibody of the IgA class reactive with N. gonorrhoeae was demonstrated in the urethral secretions of 97 per cent of men with gonococcal urethritis and in the cervical secretions of 95 per cent of infected women. To determine the molecular type of IgA involved in this reaction, secretions were absorbed with anti-human secretory component and the immunofluorescence test repeated.

ABSORPTION OF SECRETIONS WITH ANTI-HUMAN SECRETORY COMPONENT

To determine the nature of the IgA molecules detected in the antibody reaction with N. gonorrhoeae, urethral secretions from seven male and cervical secretions from nine female patients with gonorrhoea were absorbed with rabbit anti-serum against human secretory component (anti-SC).

To 0.25 ml of secretion, an equal volume of anti-SC serum (Hoescht Pharmaceuticals, Hounslow, Middlesex) was added, and incubated at 37°C for one hour, after which any reaction was allowed to proceed at 4°C for 12 hours. The mixture was centrifuged at 2,000 g for 30 minutes, and the supernate used in the immuno-fluorescent-antibody test.

As a control experiment, secretions were treated with normal

rabbit serum in place of the anti-SC serum. Similarly secretions were reacted with sheep anti-human IgA.

The results of these experiments showed that the majority of IgA antibody activity in the genital secretions was associated with secretory IgA molecules. It appeared logical to use an antiserum against secretory component rather than against serum IgA in the immunofluorescence test.

INDIRECT IMMUNOFLUORESCENT-ANTIBODY TEST USING FLUORESCEIN-CONJUGATED ANTISERUM AGAINST SECRETORY COMPONENT

Fluorescein-conjugated rabbit antiserum against human secretory component was prepared as described in Appendix 9. Cervical secretions from 15 women with gonorrhoea, and urethral exudate from 16 infected men were examined by the fluorescent-antibody test using this conjugate in place on anti-humanα-chain serum.

Fluorescence was not observed when anti-SC serum was used; all the secretions tested gave marked fluorescence with anti- -chain serum.

Antigonococcal IgA was demonstrated in rectal secretions of only 16 per cent of men and nine per cent of women with anorectal gonorrhoea. A possible explanation was that anorectal secretions had greater proteolytic activity than the other secretions examined.

DETERMINATION OF THE PROTEOLYTIC ACTIVITY OF SECRETIONS

Urethral and rectal secretions, were obtained as described from 10 male patients.

Four men had urethral gonorrhoea, four anorectal infection, and two had non-gonococcal urethritis. In all cases, infection in other possibly infected sites, had been exluded by culturing secretions from these areas (oropharynx, urethra, anorectum).

Secretions from the cervical canal and anorectum, were obtained from five women, two of whom had anorectal gonorrhoea.

Into each of four test tubes, 76 mm x 10 mm, labelled "urethral", "rectal", "cervical", was pipetted 100 µl of a solution of secretory IgA obtained from colostrum, and containing 0.042 g of SIgA per l. Fifty microlitres of secretion was added to the appropriately-labelled tube, mixed well with the antibody-containing solution, and incubated at 37°C. At intervals of 0, 20 and 30 minutes, and 1, 2, 3, and 4 hours after the start of incubation, 5 µl quantities were removed from each tube, mixed with an equal volume of soya bean-trypsin inhibitor (British Drug Houses, Poole, Dorset) 0.01 g per l, and the SIgA concentration determined by single radial immunodiffusion.

Antibody of the IgM class reactive with N. gonorrhoeae was detected in the serum of about 50 per cent of men and 40 per cent of women with uncomplicated gonorrhoea.

Shirodaria, Fraser and Stanford (1973) demonstrated that the presence of rheumatoid factor in sera regularly produced misleading

results when such sera were examined for viral-specific IgM by fluorescent antibody techniques. This non-specific staining resulted from adsorption of rheumatoid factor to complexes of virus and specific IgG.

ABSORPTION OF ANTIGLOBULIN ACTIVITY IN SERUM BY AGGREGATED GAMMA GLOBULIN

In the present study to demonstrate that the fluorescent staining of gonococcal antigen, following treatment with patients' sera and fluorescein-conjugated anti-human IgM, resulted from primary IgM-antigen reaction, and was not secondary to binding of antiglobulin to a complex of IgG and antigen, serum antiglobulin activity was absorbed with aggregated gamma globulin.

Although the majority of patients with rheumatoid arthritis have IgM antiglobulins demonstrable in the serum by classical agglutination tests, others, seronegative for IgM rheumatoid factor, may have IgG antiglobulins in the serum (Zvaifler, 1974). The technique of absorption of these proteins was therefore preferred to that of testing for IgM rheumatoid factor.

The method of absorption was that described by Shirodaria, Fraser and Stanford (1973). Human gamma globulin, Cohn fraction II (Serva, Heidelberg, Federal Republic of Germany), was aggregated by heating a 2 per cent solution in PBS, pH 7.2, at 73°C for 10 minutes. After centrifuging at 1,500 *g* and washing once in PBS, the aggregated protein was suspended in four times its volume of PBS.

Three volumes of serum, diluted 1 in 3, with PBS, were mixed with 2 volumes of the suspension of gamma globulin. Following

incubation for 12 hours at 4°C, the solution was centrifuged at 2,000 g for 15 minutes, and the supernate, equivalent to a serum dilution of 1 in 5, used, at doubling dilutions, in the immunofluorescent-antibody test, using fluorescein-conjugated anti-human IgM.

As control preparations, sera were incubated with PBS in place of aggregated gamma globulin.

Sera from 39 patients who had serum IgM antibody reactive with N. gonorrhoeae at a dilution of 1 in 32 or greater were examined.

It was shown that the IgM detected in the immunofluorescence test was directed against the gonococcus and not IgG adsorbed to the surface of the organism.

Antibody reactive with N. gonorrhoeae could be detected in the cervical secretions of 97 per cent of women with untreated gonorrhoea, and to assess the value of the indirect fluorescent-antibody test, as applied to secretions, in rapid diagnosis, the standard test was modified to reduce the time required to produce results.

MODIFICATION OF THE FLUORESCENT-ANTIBODY TEST

Secretions were incubated at 37°C with the bacterial cell preparation for 20 minutes, and the slide washed in three changes of PBS for 5 minutes. This was followed by incubation at 37°C for 15 minutes with fluorescein-conjugated antiserum, and after washing in three changes of PBS, each of 2 minutes duration, the preparations were mounted and examined as before.

This modified test was used in the examination of cervical secretions taken between June and August 1978. These specimens were examined without further dilution, and were graded according to the above scoring system. A previous study on 45 preparations showed no difference in the results between those treated as in the standard test and those as in the modification.

RESULTS

The results of the study are presented as follows:

I

STUDIES ON MALES

URETHRAL SECRETIONS

1 Quantitation of immunoglobulins

2 Detection of antibodies reactive with N. gonorrhoeae

RECTAL SECRETIONS

1 Quantitation of immunoglobulins

2 Detection of antibodies reactive with N. gonorrhoeae

SALIVA

Detection of antibodies reactive with N. gonorrhoeae

LACRIMAL SECRETIONS

SERUM

Detection of antibodies reactive with N. gonorrhoeae

STUDIES ON FEMALES

CERVICAL SECRETIONS

1 Quantitation of immunoglobulins

2 Detection of antibodies reactive with N. gonorrhoeae

RECTAL SECRETIONS

1 Quantitation of immunoglobulins

2 Detection of antibodies reactive with N. gonorrhoeae

SALIVA

LACRIMAL SECRETIONS

SERUM

Detection of antibodies reactive with N. gonorrhoeae

II

EVALUATION OF THE INDIRECT IMMUNOFLUORESCENT-ANTIBODY TEST IN THE DIAGNOSIS OF GONORRHOEA

I

The first part of the study was to determine if antibodies against N. gonorrhoeae could be detected in secretions and sera from patients with gonorrhoea; which immunoglobulin classes were involved; and what happened to these antibodies after treatment.

MALES

URETHRAL SECRETIONS

QUANTITATION OF IMMUNOGLOBULINS

Using the method of thin-layer gel filtration, IgG and IgA were detected in each of eight men, four of whom had untreated gonococcal urethritis; the other four men had non-gonococcal urethritis.

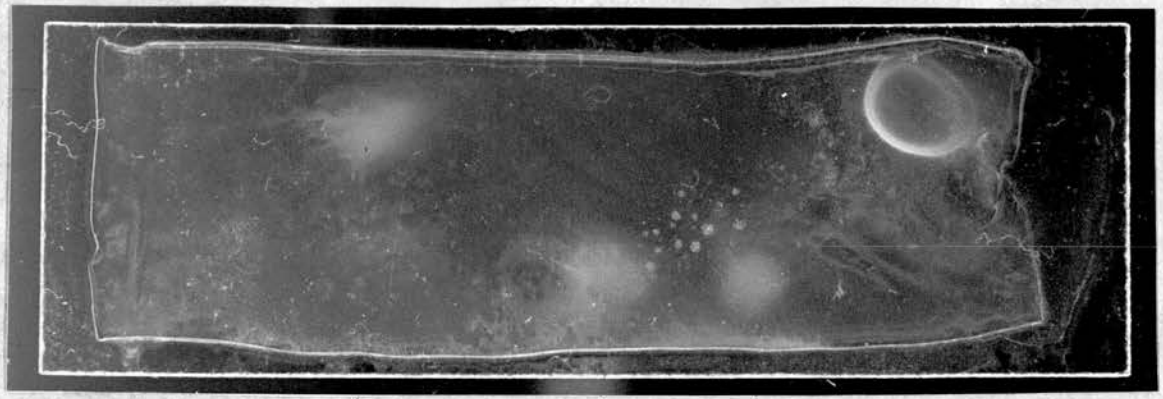
The majority of IgA in the secretions was of the secretory IgA class, as determined by the position relative to secretory and serum IgA standards in the gel (Figure 3). Immunoglobulin G appeared as a single band in each gel separation. Immunoglobulin M was not detectable with this test; this probably reflects the low concentration of this immunoglobulin class in these secretions.

Table 1R shows the concentrations of IgA and IgG in the diluted urethral secretions of men with untreated or treated gonorrhoea, with non-gonococcal urethritis, and with no clinical evidence of urethritis.

There was considerable variation in the concentrations of each immunoglobulin from patient to patient as would be expected with the

Figure 3

Thin layer gel filtration of urethral exudate from a man with untreated gonococcal urethritis.



sampling method employed. The distribution of immunoglobulin concentrations was log normal, and by applying the student t test, no significant difference in the mean log concentration of IgA was found between the individual groups of patients. During the month after successful treatment, the IgA concentration in the urethral secretions from each individual patient did not vary by more than $\pm$ 0.002 g per l; the IgG concentration varied however, by $\pm$ 0.011 g per l.

There was no significant difference in the mean log IgG concentration between the group of men with untreated gonorrhoea and those with non-gonococcal urethritis ($t = 0.009$; 89 degrees of freedom). A significant difference in mean log IgG concentration was, however, found between men with untreated gonorrhoea and those who had no urethritis ($t = 2.947$; 93 degrees of freedom); similarly, the mean log IgG concentration in the secretions seven days after treatment of gonorrhoea was significantly lower than before treatment ($t = 3.017$; 83 degrees of freedom).

Table 1[R]

Immunoglobulin concentrations in the urethral secretions of men with gonorrhoea, non-gonococcal urethritis and with no evidence of urethritis

Groups of patients	Number of patients in each group	Mean (range) IgA concentration g per l	Mean log IgA concentration	Mean (range) IgG concentration g per l	Mean log IgG concentration
Patients with gonorrhoea					
Untreated	60	0.030(0.012–0.044)	$\bar{2}$.463	0.028(0.005–0.048)	$\bar{2}$.409
7 days after treatment	25	0.027(0.011–0.039)	$\bar{2}$.423	0.017(0.005–0.021)	$\bar{2}$.256
14 days after treatment	22	0.028(0.013–0.037)	$\bar{2}$.463	0.016(0.005–0.020)	$\bar{2}$.200
28 days after treatment	18	0.026(0.010–0.033)	$\bar{2}$.397	0.012(0.005–0.020)	$\bar{2}$.068
Patients with non-gonococcal urethritis	31	0.029(0.009–0.042)	$\bar{2}$.451	0.027(0.011–0.040)	$\bar{2}$.402
Patients with no clinical evidence of urethritis (and whose cultures for N. gonorrhoeae were negative)	35	0.024(0.005–0.031)	$\bar{2}$.363	0.017(0.005–0.035)	$\bar{2}$.222

DETECTION OF ANTIBODIES REACTIVE WITH N. GONORRHOEAE

Before Treatment

Figure (4) illustrates the immunoglobulin classes of antibody reactive with N. gonorrhoeae as detected by the immunofluorescent-antibody test in the urethral secretions of 134 men with untreated urethral gonorrhoea and 54 men with non-gonococcal urethritis.

IgA

Antibody of the IgA class was detected in 130 (97.0 per cent) of the 134 infected men, but in only one (0.6 per cent) of 183 patients who had no evidence of gonococcal infection, and who were not known contacts. Antigonococcal IgA was the only antibody detected in seven (5.2 per cent of the 134 men), all of whom had been infected for less than seven days; IgA was associated with IgM and IgG classes of antibody in 55 (41.0 per cent) and 120 (89.6 per cent) men respectively. The mean log titre of IgA in the secretions was 0.657 (SD 0.227).

To determine the nature of the antigonococcal IgA molecules, urethral secretions from seven infected patients were absorbed with anti-human secretory component; Table 2 illustrates the results obtained and indicates that the majority of antibody activity was associated with secretory IgA molecules.

Urethral secretions from 16 patients with untreated gonorrhoea and in which antibody reactive with the gonococcus was detected using sheep fluorescein-conjugated anti-human IgA (α - chain), were

Figure 4

Immunoglobulin classes of gonococcal antibodies detected in male urethral secretions.

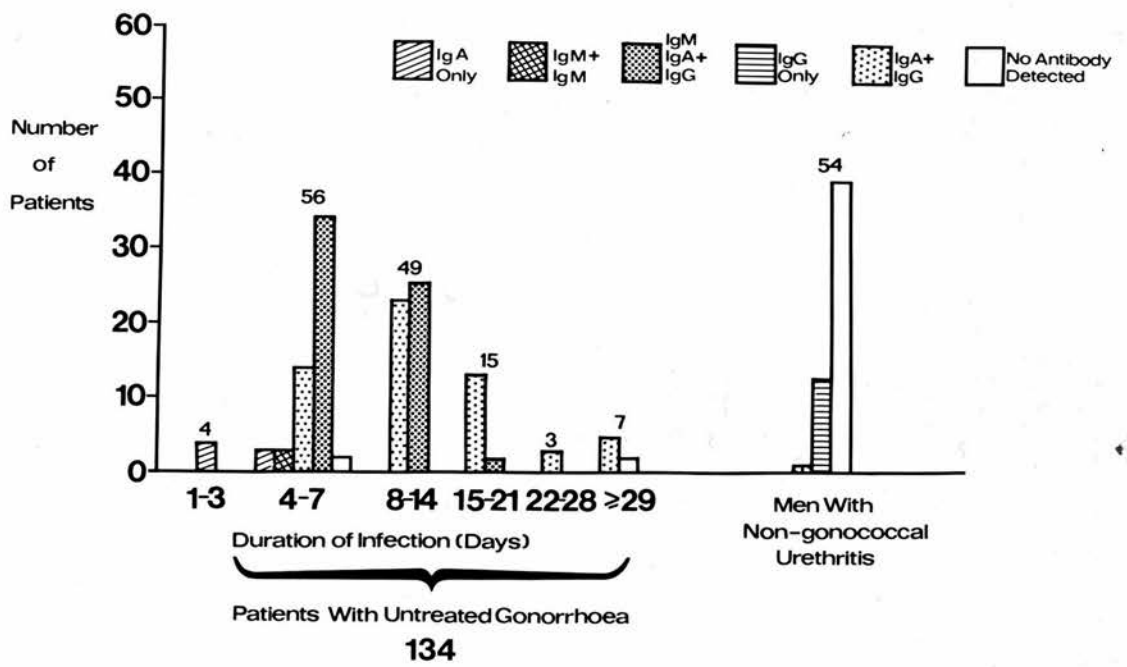


Table 2

Results of absorption with rabbit antiserum against human secretory component of urethral secretions from men with gonorrhoea

Case number	Duration of infection (days)	Titre of antigonococcal IgA after incubation with normal rabbit serum	Titre of antigonococcal IgA after incubation with rabbit anti-human secretory component serum	Change in titre of antigonococcal IgA
11	4	16	2	-3x
12	7	8	1	-3x
13	7	8	0	-4x
14	8	4	0	-3x
15	24	16	1	-4x
16	28	16	1	-4x
17	19	8	1	-3x

examined using rabbit fluorescein-conjugated antiserum against human secretory component. Fluorescence was not observed in any of these secretions.

IgG

Antibody of the IgG class against the gonococcus was found in 121 (90.3 per cent) of 134 infected men, being associated in each case with antigonococcal IgA, and in 62 (51.2 per cent) with antigonococcal IgM. In 14 (25.9 per cent) of 54 patients with non-gonococcal urethritis, IgG antibody was demonstrated, being associated with IgA in only one case; examination of urethral secretions from nine of these men who attended 14 days after treatment with tetracyclines failed to show antigonococcal antibody. Antigonococcal IgG was found in two (1.6 per cent) of 129 men who had no evidence of urethritis, and who were not infected with gonorrhoea.

As there was considerable variation in the concentration of IgG in the secretions from patient to patient, it was thought necessary to relate the titre (T) of antigonococcal antibody to total concentration (C) of IgG in the secretions to compare infected and non-infected groups of patients (Waldman, Mann and Kasel, 1968). Table 3 shows the results obtained by calculating the T/C ratio with respect to IgG; the ratio T/C was significantly lower in patients with non-gonococcal urethritis than in those with untreated gonorrhoea ($P<0.001$; $t = 51.6$ with 66 degrees of freedom).

The mean T/C ratio with respect to IgG in the secretions of 21 men infected for seven days or less was 1.18 and 2.16 in the 39 men infected longer. This difference is highly significant

($P < 0.01$; $t = 5.797$ with 58 degrees of freedom).

IgM

Antigonococcal IgM was detected in the urethral secretions of 65 (49.5 per cent) of 134 men with urethral gonorrhoea, but in none of 183 non-infected men; the mean log titre of IgM was 0.258 (SD 0.091). This antibody was found in 63 (57.8 per cent) of 109 men infected for less than 15 days, but in only two (8.0 per cent) of 25 infected for a longer period. This difference is statistically significant ($P < 0.05$ by the method of binomial probabilities).

Secretions for seven men with uncomplicated urethral gonorrhoea, selected at random, were examined for antibody against strain 9 and the infecting strain of the gonococcus. In each case, the antibodies detected against both strains were identical with respect to immunoglobulin class and titre.

Three men (patients X, Y and Z) attended as sexual contacts of women with proven gonococcal infections; none of these men was found to be infected with N. gonorrhoeae. In each case, antibody of the IgA and IgG classes reactive with N. gonorrhoeae was demonstrated in the urethral secretions; IgM antibody was not detectable.

The ratio IgA T/C was 3.64, 2.67 and 2.13 in patients X, Y and Z respectively. Similarly the IgG T/C ratio in these men was 2.00, 1.25 and 1.91.

Similar results were obtained when urethral secretions from these men, obtained seven days after their initial attendance, were examined.

Table 3 R

Ratio of titre of antigonococcal IgG to concentration of IgG (T/C) in the urethral secretions of men before and after successful treatment of gonorrhoea, and in men with untreated non-gonococcal urethritis (NGU)

	Number of patients	Mean T/C of IgG	Standard deviation
Men with urethral gonorrhoea			
Before treatment	60	1.79	0.63
7 days after treatment	25	1.89	0.33
14 days after treatment	22	1.13	0.87
28 days after treatment	18	1.27	0.70
Men with non-gonococcal urethritis	31	0.47	0.19

Men with complications of gonorrhoea

The patient who had acute epididymitis had antigonococcal IgM, IgA and IgG in the urethral secretions; the ratio T/C was 2.12 and 1.95 with respect to IgA and IgG respectively.

Both men with disseminated infection were found to have antibody of the IgA and IgG classes reactive with the infecting strain and strain 9 of N. gonorrhoeae in the urethral secretions; the IgA T/C ratio was 1.82 and 2.00 in each patient, and the IgG T/C 1.44 and 2.22 respectively.

Proteolytic activity of urethral secretions

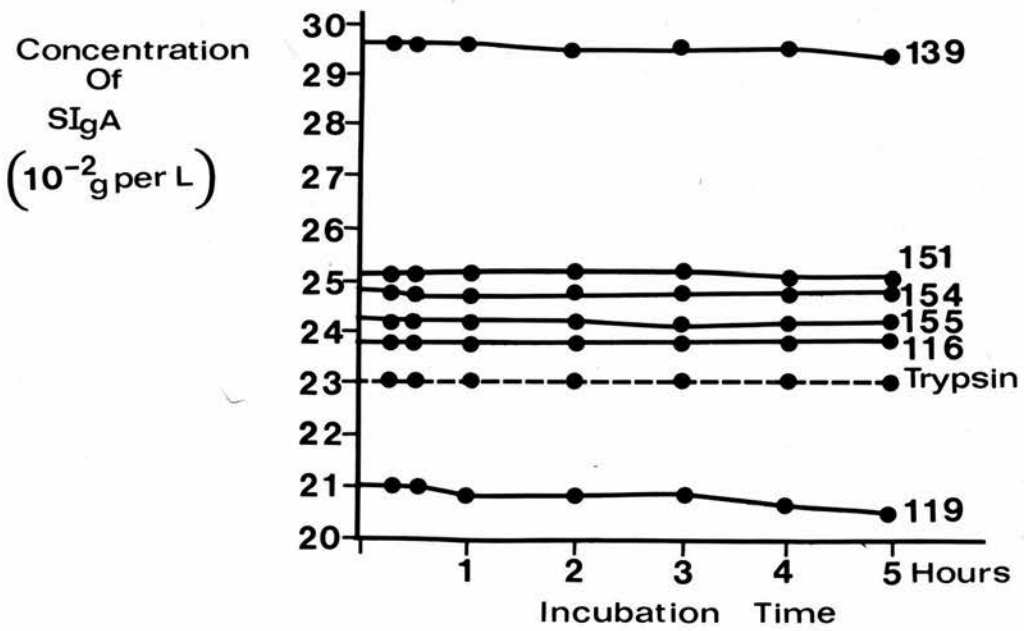
The absence of antigonococcal IgA in the urethral secretions of four men with gonococcal urethritis prompted an investigation to determine if proteolytic activity accounted for this observation.

Proteolytic activity was investigated in the urethral exudate from two men with untreated gonococcal urethritis in whose secretions antigonococcal IgA was not demonstrable (case numbers 151 and 154). Exudate from two infected men shown to have produced this secretion antibody (case numbers 155 and 139) were also tested. Secretions from two men with non-gonococcal urethritis (case numbers 116 and 119) were also examined.

As may be seen from Figure 5 there was no appreciable enzymatic degradation of the secretory IgA used as substrate. Particularly, there was no difference in proteolytic activity between the secretions found to contain antigonococcal IgA, and those which did not. Resistance of secretory IgA to tryptic digestion is demonstrated.

Figure 5

Concentration of secretory IgA during incubation with urethral secretions from four men (Nos 151, 154, 155 and 139) with gonorrhoea and from two men (Nos 116 and 119) with non-gonococcal urethritis.



Immunoglobulin A may exist in monomeric and polymeric forms, with differing diffusion rates. In the determination of secretory IgA it was assumed that lower molecular weight immunoglobulin was not produced by proteolysis of secretory IgA with resultant overestimation of the secretory IgA concentration at the end of incubation (Brandtzaeg, Fjellanger and Gjeruldsen, 1970). To determine the validity of this assumption, 3 µl of the reaction mixture at the end of five hours incubation was applied to a thin-layer gel filtration system. Only secretory IgA could be detected; in particular, there was no evidence of monomeric IgA.

Reactivity of immunoglobulins in urethral secretions with antigens other than N. gonorrhoeae

IgA

Table 4R shows the results obtained when urethral secretions from men with gonococcal urethritis were examined for IgA antibody against various species of Neisseria. Antibody against N. lactamica was detected in five of the six patients investigated, but IgA reactive with N. meningitidis was found in the secretions from only two men.

Secretions from 12 men with non-gonococcal urethritis did not contain IgA antibody against any of the species of Neisseria tested.

IgG

Urethral secretions from six men with gonococcal urethritis were tested for IgG antibody against various Neisseria spp. Table 5R

shows the results obtained; the activity of this class of secretion antibody (IgG) was wider than that of IgA.

Antibody against N. gonorrhoeae was found in four of 12 secretions from men with non-gonococcal urethritis; in each of these secretions, IgG against N. meningitidis (groups B, C, D, 28E and W135), N. catarrhalis, N. lactamica and N. perflava was also detected. The secretions from each of the other eight men with non-gonococcal urethritis contained IgG against N. perflava; in addition, in two men antibody against N. catarrhalis was found.

IgM

Table 6R shows the findings when secretions from six infected men were examined for IgM antibody against N. gonorrhoeae and other Neisseria species. In addition to the detection of IgM reactive with the gonococcus, secretions from each patient contained antibody against N. meningitidis and N. catarrhalis; IgM against N. lactamica and N. perflava was found in the secretions from four and two patients respectively.

Urethral specimens from three of 12 men with non-gonococcal urethritis contained IgM reactive with N. meningitidis (groups B and E) but IgM antibody against the other species of Neisseria could not be detected.

Antibody of any class against Staphylococcus aureus, Staphylococcus albus, Streptococcus pyogenes, Streptococcus faecalis and Escherichia coli was not detected in the secretions of any patient studied.

Table 4[R]

Reactivity in the immunofluorescent-antibody test with *N. gonorrhoeae* and other species of Neisseria of IgA in urethral secretions from six men, Nos. 1 - 6, with gonorrhoea

Species of *Neisseria* used as antigen	Reaction* obtained in test with stated antigen against specimen from patient :					
	1	2	3	4	5	6
N. gonorrhoeae	3+	3+	3+	3+	3+	3+
N. meningitidis						
Group A	-	-	-	-	-	-
B	-	-	-	-	-	-
C	-	-	-	-	-	-
D	-	-	-	-	-	-
E	-	-	2+	-	-	-
29 E	-	2+	-	-	-	-
W135	-	-	-	-	-	-
X	-	-	-	-	-	-
Z	-	-	-	-	-	-
N. catarrhalis	-	-	-	-	-	-
N. lactamica	2+	2+	2+	2+	-	2+
N. perflava	-	-	-	-	-	-

* see Methods p 103

Table 6 R

Reactivity in the immunofluorescent-antibody test with *N. gonorrhoeae* and other species of Neisseria of IgM in urethral secretions from six men, Nos. 1 - 6, with gonorrhoea

Species of *Neisseria* used as antigen	Reaction obtained in test with stated antigen against specimen from patient :					
	1	2	3	4	5	6
N. gonorrhoeae	3+	3+	3+	3+	3+	4+
N. meningitidis						
Group A	-	-	-	-	-	-
B	2+	-	-	-	2+	-
C	2+	+	2+	-	-	-
D	-	-	-	-	-	-
E	-	2+	+	-	-	-
29 E	-	-	+	-	+	-
W135	-	-	-	2+	-	-
X	-	-	-	-	-	-
Z	-	+	2+	2+	-	2+
N. catarrhalis	2+	2+	2+	2+	2+	2+
N. lactamica	2+	2+	3+	-	2+	+
N. perflava	3+	-	-	2+	-	-

Table 5 R

Reactivity in the immunofluorescent-antibody test with N. gonorrhoeae and other species of Neisseria of IgG in urethral secretions from six men, Nos. 1 - 6, with gonorrhoea

Species of Neisseria used as antigen	Reaction obtained in test with stated antigen against specimen from patients :					
	1	2	3	4	5	6
N. gonorrhoeae	3+	3+	3+	3+	+	2+
N. meningitidis						
Group A	-	2+	-	-	-	-
B	-	2+	-	+	-	-
C	-	2+	+	+	2+	+
D	2+	-	2+	2+	2+	-
E	2+	+	2+	-	2+	2+
29 E	-	+	-	-	-	-
W135	-	-	+	-	-	-
X	+	+	2+	-	+	-
Z	-	2+	-	2+	-	-
N. catarrhalis	-	2+	-	2+	-	2+
N. lactamica	2+	+	2+	2+	+	2+
N. perflava	+	-	2+	2+	-	2+

Detection of antibodies in urethral secretions from men after treatment

IgA

Although there was considerable variation in IgA concentration in the secretions from patient to patient, the concentration of IgA in the diluted secretions from each individual remained constant ($\pm$ 0.002 g per l) during the period of the study; it was felt justifiable to report mean log titres of antigonococcal IgA during consideration of the effect of successful treatment on secretion antibody. Table 7^{R} shows the rapid decline in mean log titre of IgA following successful treatment; this antibody was no longer detectable 28 days after treatment.

IgG

In view of the marked variation in IgG concentration from patient to patient, and within individual patients, it was felt desirable to examine the ratio of titre of antibody to concentration of IgG (T/C), rather than to draw conclusions from titre values only (Table 3^{R}).

Although, as noted previously (Table 1^{R}) there was a significant decrease in the mean concentration of total IgG in the urethral secretions seven days following treatment, there was no significant difference in the mean T/C ratio between secretions examined before and seven days after treatment ($P > 0.05$; $t = 3.416$ with 45 degrees of freedom).

IgM

Of the 70 patients who were available for review seven days after successful treatment, antigonococcal IgM was not detectable in the urethral exudate; 33 of these patient had had this antibody in the secretions before treatment.

In each of the three patients whose infection was not cured at the initial clinic visit, antigonococcal IgA and IgG were still detectable seven days later. The ratio of antibody titre to IgA and IgG concentration (T/C) was calculated and the results are shown in Figure (6); the constancy of the ratio T/C with respect to both IgA and IgG before successful treatment is evident. IgM antibody against the gonococcus although found in the secretions of each man at the initial attendance was not subsequently detected.

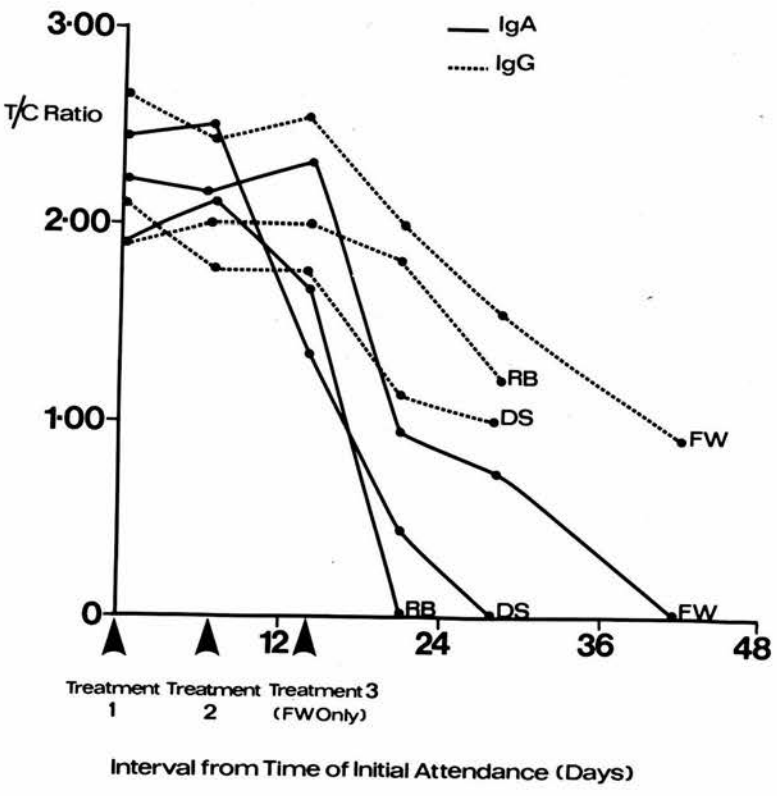
Influence of re-infection on the secretion antibody (IgM, IgA and IgG) response

Thirtyfour men had suffered previously from urethral gonorrhoea. Urethral secretions from 24 of these men were available for determination of IgA and IgG concentrations. The secretion antibody response in these men was compared with that in groups of infected men who had had no previous gonococcal infections, and who were matched with respect to age, marital status, the number of sexual partners within the preceding three months, and the duration of infection.

Antigonococcal antibody was detected in each patient in each group. The mean ratio of antibody titre to immunoglobulin

Figure 6

Ratio of titre of antigonococcal IgA and IgG to concentration (c) of these immunoglobulins in urethral secretions of three men before and after successful treatment.



concentration (T/C) with respect to IgA was 1.89 (range 1.21 to 2.86) in the secretions of those who had not been infected previously, and 2.27 (range 1.48 to 2.96) in men who had a past history of urethral gonorrhoea; the difference in T/C ratios between these groups is not statistically significant ($P > 0.1$ by applying the student t test; $t = 0.763$ with 51 degrees of freedom).

Similarly there was no significant difference in the IgG T/C ratios between the two groups of patients; the mean ratio T/C in the secretions of men with an initial infection was 1.74 (range 1.32 to 2.07) and 1.81 (range 1.27 to 2.15) in those who had previously been infected; ($P > 0.2$, $t = 0.544$, 51 degrees of freedom).

Antibody of the IgM class was found in nine (37.5 per cent) of the 24 previously infected men, and in 12 (41.4 per cent) of the 29 men who gave no history of infection.

Figure (7) shows the antibody response in the urethral secretions from three men who became reinfected during the period of the study. The rapid decline in IgA T/C ratio following treatment, and abrupt rise on reinfection is clearly shown; a more gradual change in IgG T/C ratio is noted.

It is of interest that following treatment in case PR, the IgG T/C ratio was rising as the IgA T/C was decreasing.

Figure (8) illustrates the findings in the urethral secretions of a 24-year old homosexual man who was reinfected twice during a four-month period. Data on IgA concentration were available for each specimen of urethral exudate (0.033 ± 0.004 g per l), but IgG concentration could only be determined at the patient's initial and second visit (0.008 and 0.012 g per l respectively). For this reason only titres of antigonococcal antibody are presented. As in the

Figure 7

Urethral antigonococcal antibodies in three men who were re-infected during the period of the study.

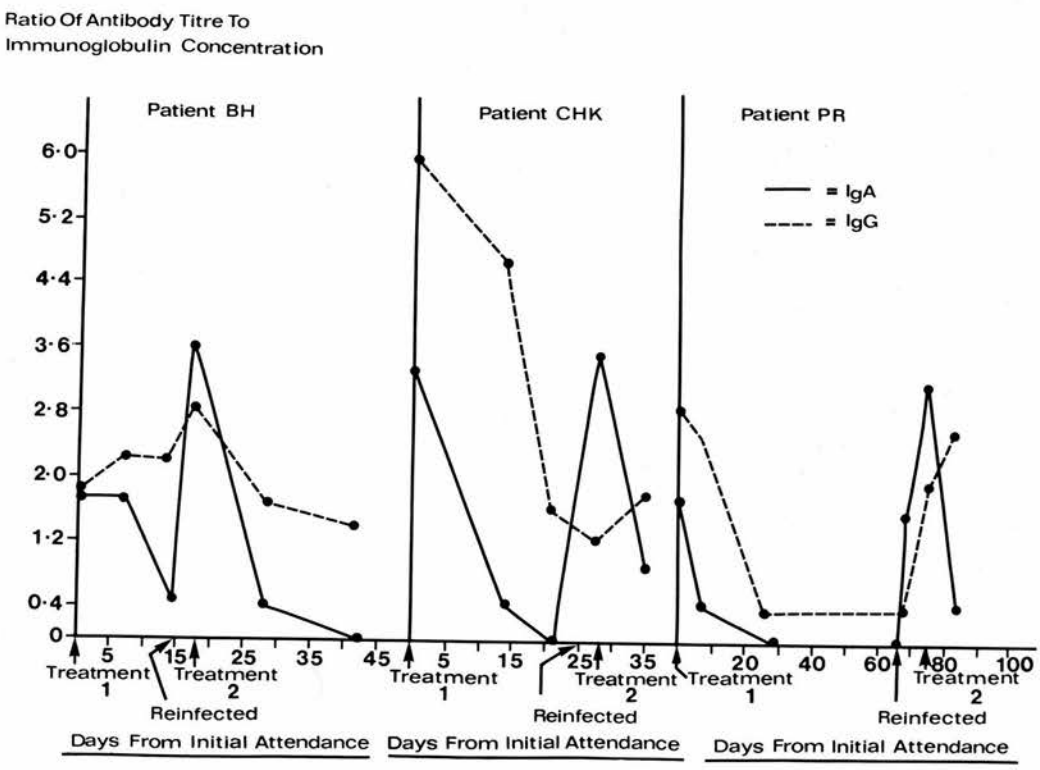
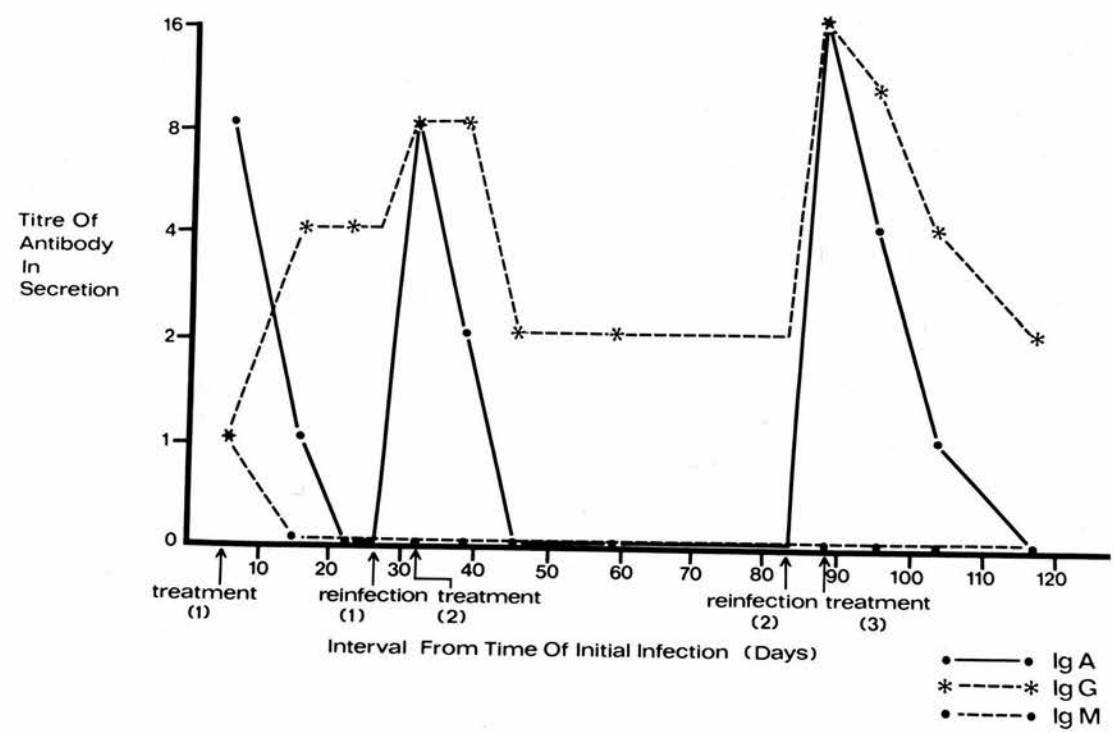


Figure 8

Urethral antibodies in a homosexual man who was re-infected twice during the period of the study.



previous three cases, the titre of IgA antibody rapidly rose after reinfection, and decreased promptly after treatment; the persistence of IgG antibody activity for weeks after successful treatment is also shown.

DETECTION OF ANTIBODIES IN RECTAL SECRETIONS FROM MALE PATIENTS

QUANTITATION OF IMMUNOGLOBULINS

Using the thin-layer gel filtration technique, secretory IgA and IgG could be detected in the rectal secretions from eight men, four of whom had anorectal gonorrhoea.

The IgA and IgG concentrations in the rectal secretions from five infected and five non-infected men were determined; the mean IgA concentrations was 0.023 g per l (range 0.021 to 0.026 g per l) and the mean IgG concentration was 0.017 g per l (range 0.008 to 0.023 g per l).

DETECTION OF ANTIBODIES REACTIVE WITH N. GONORRHOEAE

Secretions were collected from 18 men with anorectal gonorrhoea and from 29 men who had had anal intercourse, but had no evidence of gonococcal infection; none of the control group was a sexual contact of a patient known to have gonorrhoea.

Using the immunofluorescent-antibody test, antibody of the IgA class reactive with strain 9 of N. gonorrhoeae was detected in the secretions from three of the 18 infected men, and in one of the 29 non-infected patients. Antigonococcal IgG was found in six of the 18 infected, and in eight of the 29 non-infected individuals.

Antibody of the IgM class against N. gonorrhoeae could not be detected.

Proteolytic activity of rectal secretions

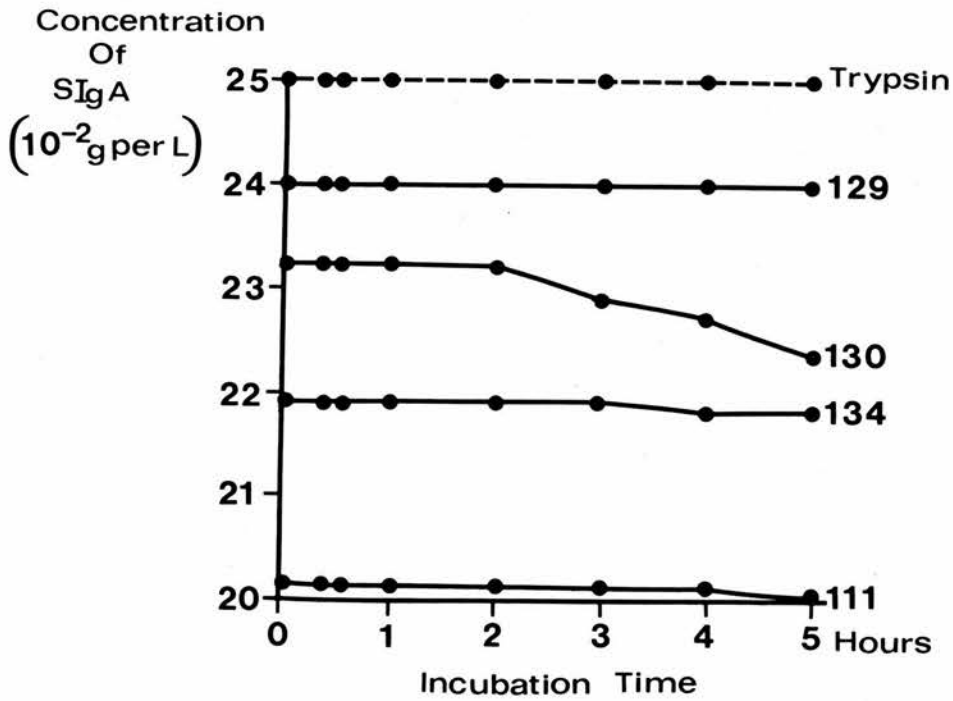
The absence of gonococcal antibody in the rectal secretions of the majority of men with anorectal gonorrhoea, prompted an investigation into the proteolytic activity of these secretions to determine if this activity accounted for the apparent lack of antibody.

Rectal secretions from four men (case numbers 111, 129, 130, 134) with anorectal gonorrhoea were incubated with secretory IgA; secretions from only one man contained antigonococcal IgA (111).

As may be seen from Figure (9), no appreciable loss of secretory IgA occurred over an incubation period of five hours; only secretory IgA was detected by thin-layer gel filtration in the reaction mixture after incubation.

Figure 9

Concentration of secretory IgA during incubation with anorectal secretions from four men with rectal gonorrhoea.



DETECTION OF ANTIBODIES IN SALIVA FROM MALE PATIENTS

QUANTITATION OF IMMUNOGLOBULINS

As there are ample data available on the immunoglobulin concentrations in saliva (Brantzaeg, Fjellanger and Gjeruldsen, 1970), this estimation was not performed.

DETECTION OF ANTIBODIES AGAINST N. gonorrhoeae IN SALIVA

Before treatment

For the investigation of saliva for antibody against the gonococcus, the patients were divided into five groups (Table 9).

IgA

Antibody of the IgA class reactive with N. gonorrhoeae was detected by immunofluorescence, in each of 12 homosexual men with oropharyngeal gonorrhoea, but in only two (6.9 per cent) of 29 homosexual men who were not found to be infected at any site. This antibody was found in only three (7.7 per cent) of 39 non-infected heterosexual patients.

Fourteen (56.0 per cent) of 25 homosexual, and five (14.3 per cent) of 35 heterosexual men with gonococcal infection of a site other than the oropharynx, had demonstrable antibody in the saliva. The difference between these groups of patients is significant ($P = 0.005$ with Yates' correction $X^2 = 9.879$).

IgG

Antigonococcal IgG was found in nine (75.0 per cent) of 12 men with oropharyngeal gonorrhoea, in only four (13.8 per cent) of 29 homosexual and in five (12.8 per cent) of 39 heterosexual men with no evidence of gonococcal infection at any site.

In eight (32.0 per cent) of 25 homosexual and in three (8.6 per cent) of 35 heterosexual men who had anogenital gonorrhoea, but no apparent pharyngeal infection, IgG antibody was detected in the saliva. The difference between these two groups is significant ($P < 0.02$; $X^2 = 6.267$ applying Yates' correction).

IgM

Immunoglobulin M reactive with the gonococcus was detected in three (25.0 per cent) of 12 men with pharyngeal infection, and in one homosexual man who had rectal, but not apparently, pharyngeal gonorrhoea.

Reactivity of immunoglobulins in saliva with other species of Neisseria

Table 10 shows the results obtained when saliva from each group of patients was examined for antibody against various species of Neisseria. Saliva which contained antibody against N. lactamica always showed reactivity with N. gonorrhoeae, but the converse was not always the case.

Table 9R

Groups of male patients whose saliva was investigated for anti-gonococcal antibody

Group of patients	Number of men in each group
I Homosexual men with oropharyngeal gonorrhoea	12
II Homosexual men with urethral and/or rectal gonorrhoea but no evidence of oropharyngeal infection	25
III Homosexual men with no evidence of gonococcal infection	29
IV Heterosexual men with urethral gonorrhoea but no evidence of oropharyngeal infection	35
V Heterosexual men not infected with *N. gonorrhoeae*	39

Table 10R

The detection of antibodies in the saliva of patients in Groups I to V*

Species of Neisseria	Number of patients in each group with detectable antibody against the stated antigen				
	Group I	Group II	Group III	Group IV	Group V
N. meningitidis Group B					
IgA	1	0	0	1	1
IgG	1	1	1	0	2
IgM	0	0	0	1	1
N. lactamica					
IgA	10	8	1	3	2
IgG	8	5	2	1	3
IgM	1	0	0	0	0
N. catarrhalis					
IgA	1	2	1	0	1
IgG	0	1	2	2	1
IgM	0	0	1	0	0
N. perflava					
IgA	0	1	0	0	1
IgG	0	0	1	1	0
IgM	0	1	0	0	0
N. gonorrhoeae					
IgA	12	14	2	5	3
IgG	9	8	4	3	5
IgM	3	1	0	0	0

* see Table 9R

Detection of antigonococcal antibodies in saliva after treatment

Only nine of the 12 men who were treated for oropharyngeal gonorrhoea were available for study 28 days after their initial attendance. Saliva obtained at this time from these men; IgG antibody was detected in four cases but antigonococcal IgA, was not detectable.

DETECTION OF ANTIBODIES IN LACRIMAL SECRETIONS FROM MALE PATIENTS

Using the thin-layer gel filtration technique, only IgA could be detected in the lacrimal secretions; the mean concentration in these secretions from five men was 0.010 g per l (range 0.005 to 0.014 g per l).

Antigonococcal antibody of any immunoglobulin class could not be detected by the immunofluorescent antibody test in any of the 38 men investigated.

DETECTION OF ANTIBODIES IN SERUM FROM MALE PATIENTS

Some of the data presented here have already been published (McMillan et al. 1979).

Before treatment

Table 11 shows the results obtained when sera was examined by

Table 11 R

Reactivity with N. gonorrhoeae in the fluorescent-antibody test of serum from infected and non-infected men.

Immunoglobulin classes of antibody against N. gonorrhoeae in sera from men with untreated gonorrhoea and from non-infected patients

	No of sera	IgM titre					IgA titre					IgG titre				
		⩽ 8	16	32	64	128	⩽ 8	16	32	64	128	⩽ 8	16	32	64	128
(a) Infected Patients																
Duration of Infection (days)																
1 - 7	61	19	23	15	4	0	24	16	14	6	1	0	6	17	19	19
8 - 14	62	25	17	18	2	0	17	21	19	5	0	0	0	0	17	45
15 - 21	18	13	5	0	0	0	14	2	2	0	0	0	0	0	4	14
22 - 28	3	3	0	0	0	0	1	1	1	0	0	0	0	0	1	2
⩾ 29	14	14	0	0	0	0	14	0	0	0	0	0	0	0	4	10
(b) Non-infected Patients	183	177	6	0	0	0	181	2	0	0	0	159	24	0	0	0

the test for antibody against the gonococcus.

IgM

Antibody of the IgM class reactive with N. gonorrhoeae was detected at a titre of $\geqslant$ 16 in 84 (53.2 per cent) of 158 men with untreated gonorrhoea, but in only six (3.3 per cent) of 183 non-infected men; the titre of IgM antibody was $\leqslant$ 8 in three men who were named contacts.

When the duration of infection was 14 days or less, this antibody was found at a titre of $\geqslant$ 16 in 79 (64.2 per cent) of 123 men, but in only 5 (14.3 per cent) of 35 men who had been infected longer. This difference is statistically significant ($P < 0.05$ by the method of binomial probabilities).

Sera from the 90 patients who had antigonococcal IgM detectable at a titre of $\geqslant$ 16 were treated with heat-aggregated Cohn fraction II to absorb antiglobulin. In each case, identical titres were obtained before and after such absorption, clearly indicating that the IgM was directed towards the bacterial cell and not to a coating of host protein.

IgA

Antibody of this class reactive with the gonococcus was detected at a titre of $\geqslant$ 16 in the serum of 88 (55.7 per cent) of the 158 men with gonorrhoea, but in only two (1.1 per cent) of the 183 non-infected men; in the sera from three men who were named contacts, the IgA antibody titre was $\leqslant$ 8.

Although antigonococcal IgA was found in 75 (70.0 per cent) of 123 men who had been infected for 14 days or less, this class of antibody was detected in only five (14.3 per cent) of 35 men who had been infected longer. This difference is highly significant ($P < 0.05$ by the method of binomial probabilities).

IgG

This class of antibody was found at a titre of $\geqslant 16$ in the serum of each of 158 men with gonorrhoea, but in only 24 (13.1 per cent) of 183 non-infected men; in each of three men who were named contacts, the IgG antibody titre was $\geqslant 128$.

There was a statistically significant difference in the mean log titre between men infected for 7 days or less (mean log titre 1.794) and those infected longer (mean log titre 2.027) ($P < 0.001$ using student's t test $t = 5.685$, 156 degrees of freedom).

The titre of antigonococcal IgG was $\geqslant 512$ in each of the two men with disseminated gonococcal infection; the titre was 128 in the patient with acute epididymitis.

Reactivity of serum immunoglobulins with other species of Neisseria

Sera diluted 1 in 16, from six men with uncomplicated gonorrhoea were examined for antibody against various species of Neisseria. Tables 12[R], 13[R] and 14[R] show the results obtained.

IgM

Sera from six non-infected men were also tested; IgM against N. meningitidis was found in each serum, but in each case, the reactivity was with a single and different serogroup (B, C, E, 29E, X and Z). Antibody of this class against N. catarrhalis (all six sera), N. perflava (two sera) and N. lactamica (two sera) was also demonstrated.

IgA

In sera from non-infected men, IgA could be detected against N. meningitidis (four sera, each reactive with a different serogroup - D, W135, X and Z), N. lactamica (one serum), N. perflava (six sera) and N. catarrhalis (two sera).

IgG

The reactivity of serum IgG from non-infected patients was broad: in addition to IgG antibody against N. gonorrhoeae (two cases), serum contained IgG reactive with various groups of N. meningitidis (six sera), N. lactamica (four sera), N. perflava (six sera) and N. catarrhalis (six sera).

Table 12R

Reactivity in the immunofluorescent antibody test with anti human IgM and N. gonorrhoeae and other species of Neisseria of serum from six men Nos. 1 - 6 with uncomplicated gonorrhoea

Antigen	Reaction* obtained in test with stated antigen against specimen from patient:					
	1	2	3	4	5	6
N. gonorrhoeae	3+	3+	3+	3+	3+	3+
N. meningitidis						
group A	-	+	+	2+	-	-
B	2+	+	+	2+	2+	2+
C	2+	2+	2+	-	+	2+
D	-	-	2+	-	-	-
E	-	2+	2+	-	-	-
29E	-	-	2+	2+	-	-
W135	2+	-	-	-	-	-
X	+	+	-	+	-	+
Z	+	2+	2+	3+	2+	2+
N. lactamica	2+	2+	3+	3+	2+	2+
N. perflava	2+	-	-	3+	1+	-
N. catarrhalis	2+	2+	2+	2+	2+	2+

* see Methods p 103

Table 13R

Reactivity in the immunofluorescent antibody test with anti human IgA and N. gonorrhoeae and other species of Neisseria of serum from six men Nos 1 - 6 with uncomplicated gonorrhoea

Antigen	Reaction obtained in test with stated antigen against specimen from patient:					
	1	2	3	4	5	6
N. gonorrhoeae	4+	4+	4+	4+	4+	4+
N. meningitidis						
group A	-	-	-	-	-	-
B	-	-	-	-	-	-
C	-	-	-	-	-	-
D	2+	2+	2+	-	2+	1+
E	-	-	-	-	-	-
29E	-	-	-	-	-	-
W135	-	-	-	-	-	-
X	-	1+	1+	1+	1+	-
Z	-	-	-	-	-	-
N. lactamica	2+	-	2+	-	-	1+
N. perflava	2+	1+	1+	1+	2+	3+
N. catarrhalis	1+	1+	1+	1+	2+	2+

Table 14 R

Reactivity in the immunofluorescent antibody test with anti human IgG and N. gonorrhoeae and other species of Neisseria of serum from six men Nos. 1 - 6, with uncomplicated gonorrhoea

Antigen	Reaction obtained in test with stated antigen against specimen from patient:					
	1	2	3	4	5	6
N. gonorrhoeae	4+	4+	4+	4+	4+	4+
N. meningitidis						
group A	-	3+	-	-	-	1+
B	2+	2+	2+	3+	1+	1+
C	2+	2+	2+	2+	1+	2+
D	2+	1+	2+	2+	-	1+
E	2+	2+	2+	1+	1+	2+
29E	-	-	-	3+	-	1+
W135	-	-	-	3+	2+	2+
X	2+	1+	3+	2+	2+	3+
Z	-	2+	-	3+	2+	2+
N. lactamica	3+	3+	3+	3+	3+	3+
N. perflava	3+	2+	3+	2+	2+	2+
N. catarrhalis	2+	2+	3+	2+	3+	3+

Detection of antigonococcal antibodies in serum from male patients after treatment of gonorrhoea

Table 15R illustrates the rapid decline in the mean log titre of IgM and IgA antigonococcal antibodies in the serum following successful treatment of gonorrhoea; a more gradual decline in the mean log titre of the IgG class of antibody is also shown.

Table 15 R

Arithmetic mean of log titres of antibodies reactive with N. gonorrhoeae in the serum of men with gonorrhoea before and after successful treatment

	Number of patients	Mean log titre		
		IgM	IgA	IgG
Before treatment	158	1.149	1.042	1.922
7 days after treatment	74	1.019	1.136	1.882
14 days after treatment	53	0.950	1.023	2.081
28 days after treatment	45	0.903	0.917	1.772

FEMALES

DETECTION OF ANTIBODIES IN CERVICAL SECRETIONS

QUANTITATION OF IMMUNOGLOBULINS

Immunoglobulin G and secretory IgA were demonstrated by thin layer gel filtration in the cervical secretions of eight women, four of whom had untreated gonorrhoea; IgM could not be detected.

Table 16R shows the concentrations of IgA and IgG in cervical aspirates from patients with treated and untreated gonorrhoea, women with non-gonococcal cervicitis, and women with clinically normal cervices.

As we found in our studies of urethral secretions of the male, there was a considerable variation in immunoglobulin concentrations from female patient to patient. The distribution of concentrations of IgA and IgG was log normal, and the student t test was used to compare mean concentrations. There was no statistically-significant difference in the mean secretion IgA concentrations between women with gonorrhoea and non-gonococcal cervicitis ($\pm$ = 0.017; 45 degrees of freedom); nor between women with gonorrhoea and those with apparently healthy cervices (t = 1.003; 45 degrees of freedom).

Similarly there was no significant difference in the mean secretion IgG concentration between women with gonorrhoea and those with non-gonococcal cervicitis (t = 0.294; 45 degrees of freedom), nor between patients with gonorrhoea and those who had no evidence of cervicitis (t = 0.748; 45 degrees of freedom).

Although the IgA concentration in the cervical aspirate did not

Table 16 R

Concentration of secretory IgA and IgG in the cervical secretions of women with untreated and treated gonorrhoea, non-gonococcal cervicitis and women with no evidence of cervicitis

Group of patients	Number of patients	Mean concentration (range) of IgA g per l	Mean log concentration of IgA	Mean concentration (range) of IgG g per l	Mean log concentration of IgG
Gonorrhoea					
Before treatment	28	0.024(0.011–0.048)	$\bar{2}$.362	0.020(0.010–0.036)	$\bar{2}$.278
7 days after treatment	29	0.020(0.011–0.037)	$\bar{2}$.280	0.023(0.010–0.041)	$\bar{2}$.343
14 days after treatment	30	0.023(0.010–0.038)	$\bar{2}$.342	0.023(0.010–0.039)	$\bar{2}$.300
28 days after treatment	28	0.022(0.010–0.038)	$\bar{2}$.320	0.025(0.013–0.039)	$\bar{2}$.387
Non-gonococcal cervicitis	19	0.023(0.012–0.044)	$\bar{2}$.339	0.022(0.011–0.040)	$\bar{2}$.317
No clinical evidence of cervicitis	29	0.020(0.010–0.041)	$\bar{2}$.285	0.018(0.012–0.037)	$\bar{2}$.248

vary by more than ± 0.002 g per l within individual patients during the period of the study, the IgG concentration varied considerably by more than ± 0.012 g per l.

DETECTION OF ANTIBODIES REACTIVE WITH N. GONORRHOEAE

Before treatment

Figure (10) illustrates the results obtained in the immunofluorescent-antibody test using strain 9 of N. gonorrhoeae as antigen.

IgA

Antibody reactive with N. gonorrhoeae was detected in the cervical secretions of 73 (97.3 per cent) of 75 women with gonorrhoea. In one woman the urethra only was found to be infected; she had, however, antibody in the cervical fluid.

In 71 (94.7 per cent) of these patients the antibody was of the IgA class.

Antigonococcal IgA was not detected in the cervical secretions of any of 70 non-infected women; repeat testing of secretions from 31 of these women obtained 14 days after their initial attendance again failed to detect IgA antibody.

Cervical secretions from eight women with untreated gonorrhoea were absorbed with anti-human secretory component. In each case, this resulted in a three-fold, or greater, reduction in titre of IgA antibody activity as detected in the immunofluorescent test (Table 17).

When the immunofluorescent-antibody test was repeated on cervical

Figure 10

Immunoglobulin classes of gonococcal antibodies in the cervical secretions from infected and non-infected women.

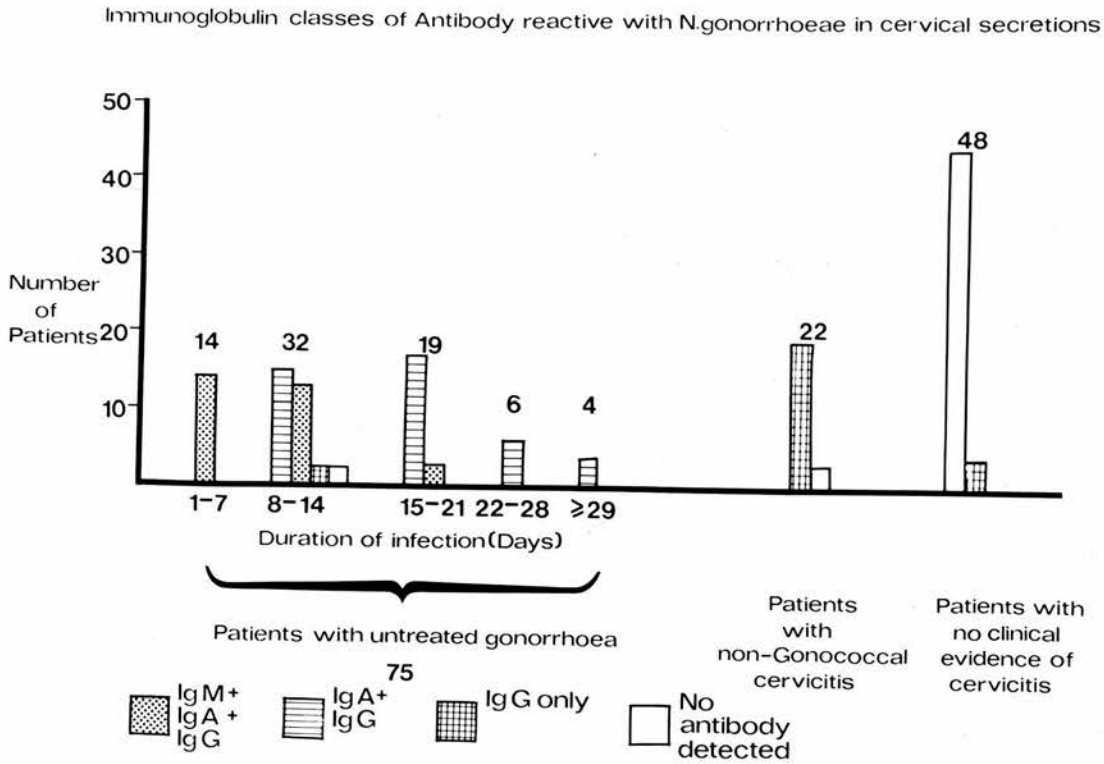


Table 17

Results of absorption with rabbit anti-human secretory component of cervical secretions from women with gonorrhoea

Case number	Duration of infection (days)	Titre of antigonococcal IgA after incubation with normal rabbit serum	Titre of antigonococcal IgA after incubation with rabbit anti-human secretory component	Change in titre of antigonococcal IgA
18	10	8	0	-4x
19	6	4	0	-3x
20	7	8	0	-4x
21	14	8	0	-4x
22	21	8	1	-3x
23	10	16	2	-3x
24	10	8	1	-3x
25	6	8	0	-4x

secretions from 15 women in whom positive results were obtained using anti-human IgA (α-chain) but, using fluorescein-conjugated rabbit anti-human secretory component serum, instead, fluorescence was not observed.

Antibody of the IgA class was found in association with IgM in the secretions of 29 (39.7 per cent) of the 73 women in whom anti-gonococcal antibody was detected, and in association with IgG in 71 (97.3 per cent) women.

IgG

Immunoglobulin G reactive with strain 9 of N. gonorrhoeae was detected in the cervical secretions of 73 (97.3 per cent) of the 75 infected women, and was the only class of antibody found in two women.

Antigonococcal IgG was demonstrated in the cervical fluid of 19 (86.4 per cent) of 22 women with non-gonococcal cervicitis, but in only four (8.3 per cent) of 48 women who had no clinical evidence of cervicitis. Fourteen days after treatment with tetracyclines, secretions from only three of fourteen women available for re-investigation had anti-gonococcal IgG antibody.

The ratio of the titre of antigonococcal IgG to the concentration of IgG in the aspirated fluid (T/C) was calculated; Table 18 shows the results obtained in patients with treated and untreated gonorrhoea and with non-gonococcal cervicitis and patients with normal cervices. The difference in the mean T/C ratio between patients with untreated gonorrhoea and non-gonococcal cervicitis is statistically significant ($P < 0.05$ using student t test, $t = 2.235$ with 45 degrees of freedom).

Table 18R

Ratio of titre of antigonococcal IgG (T) to concentration of IgG (C) in the cervical secretions

	Number of patients	Mean T/C	Standard deviation
Patients with gonorrhoea			
Before treatment	28	2.30	0.69
7 days after treatment	29	1.80	0.71
14 days after treatment	30	1.51	0.60
28 days after treatment	28	0.86	0.81
Patients with non-gonococcal cervicitis	19	0.64	0.36
Patients whose cervix was clinically normal	3	0.87	0.28

The small number of women whose cervix appeared normal precluded a statistical analysis of the T/C ratio compared with women with gonorrhoea.

Data on IgG concentration in the cervical fluid were only available in four of the 14 women infected for 7 days or less. It was, therefore, not possible, as it was in the male patients to investigate any change in T/C ratio with duration of infection.

IgM

Antigonococcal IgM was found in 29 (38.7 per cent) of the 75 infected women. This antibody was detected in the cervical secretions of 27 (58.7 per cent) of the 46 women who had been infected for 14 days or less, but in only two (6.9 per cent) of 29 women who had been infected for a longer period; this difference is statistically significant ($P < 0.05$ using the method of binomial probabilities).

When cervical secretions from eight infected women were examined for antibody against both strain 9, and the infecting strain of the organism, in each case the pattern of immunoglobulin classes, and their titres, were identical with each antigen.

Twelve women had no microbiological evidence of gonococcal infection, but were known sexual contacts of men with culturally-proven gonorrhoea; antibody of the IgA and IgG classes reactive with N. gonorrhoeae was detectable in the cervical secretions of each of these women. These antibodies were again found when the fluorescent-antibody test was repeated on secretions obtained on two further occasions within a period of three weeks.

Data for the T/C ratio in the cervical fluid were available in nine of these women. The mean T/C ratio was 2.70 and 1.71 for IgA and IgG respectively.

Antigonococcal IgM was not found in the cervical secretions from these patients.

In each of the three women with acute salpingitis and two with acute bartholinitis, IgA and IgG reactive with both strain 9 and the infecting strain of the gonococcus were detected in the cervical secretions; IgM antibody could not be demonstrated.

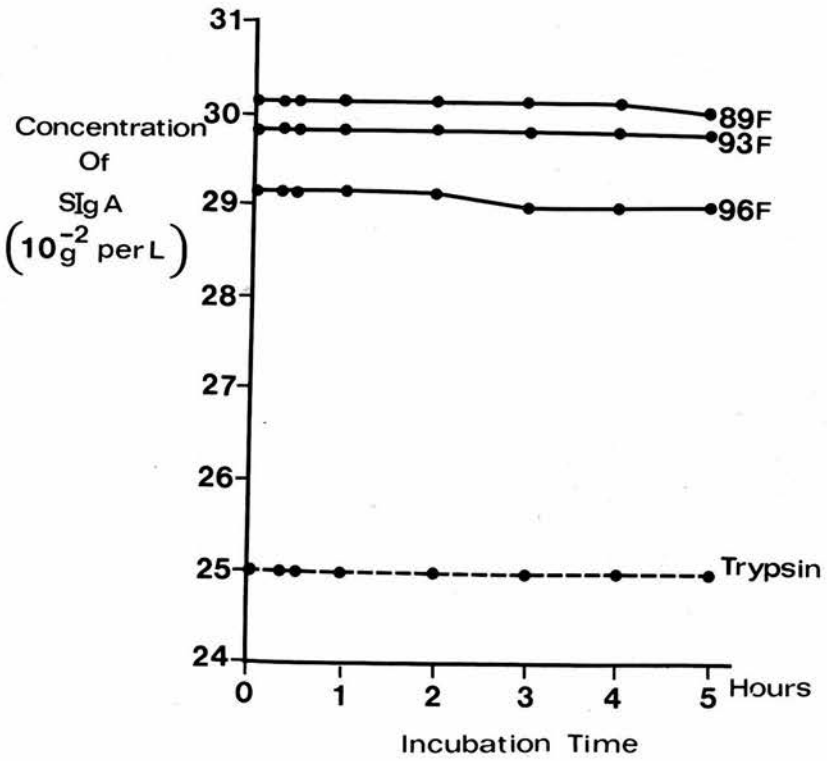
Proteolytic activity of cervical secretions

Cervical secretions from three women (89F, 93F and 96F) were investigated for proteolytic activity to determine if this accounted for the apparent absence of antigonococcal IgA in the secretions from some women; secretion IgA antibody could not be detected in cases 93F and 96F.

Figure (11) shows the results obtained, and indicates the lack of proteolytic activity of the cervical secretions. At the end of incubation, secretory IgA molecules were the only molecular types detectable by thin-layer gel filtration.

Figure 11

Concentration of secretory IgA during incubation with cervical secretions from three women with gonorrhoea.



Reactivity of antibodies in the cervical secretions with various antigens

Tables 19R, 20L and 21R show the results obtained when cervical secretions from six women with untreated gonorrhoea were examined for antibody against various species of Neisseria.

IgA

In addition to having IgA reactive with the gonococcus, cervical secretions from infected women contained IgA antibody against N. meningitidis (two cases), and N. lactamica (three cases).

Cervical fluid from six non-infected women was not found to contain IgA antibody against any of the species of Neisseria examined.

IgG

As in the urethral secretions from men with gonorrhoea, there was a broader spectrum of IgG reactivity in cervical secretion of infected women, antibody of this class being found against N. meningitidis (five cases), N. lactamica (three cases), N. catarrhalis (four cases) and N. perflava (two cases).

Immunoglobulin G reactive with N. meningitidis (six cases), N. catarrhalis (six cases), N. lactamica (five cases), N. perflava (three cases) and N. gonorrhoeae (four cases) was demonstrated in the cervical fluid from six non-infected women, five of whom had cervicitis.

Table 19R

Reactivity in the immunofluorescent antibody test with *N. gonorrhoeae* and other species of Neisseria of IgA in cervical secretions from six women, Nos. 1F - 6F, with gonorrhoea

Species of Neisseria	Reaction* obtained in test with stated antigen against specimen from patient:					
	1F	2F	3F	4F	5F	6F
N. gonorrhoeae	4+	4+	3+	4+	4+	4+
N. meningitidis						
Group A	-	-	-	-	-	-
B	-	-	-	-	-	+
C	-	-	-	-	-	-
D	-	-	-	-	-	-
E	-	-	-	-	-	-
29E	-	-	2+	-	-	-
W135	-	-	-	-	-	-
X	-	-	-	-	-	-
Z	+	2+	-	-	-	-
N. lactamica	2+	2+	-	-	2+	+
N. catarrhalis	-	-	-	-	-	-
N. perflava	-	-	-	-	-	-

* see Method p 103

Table 20[R]

Reactivity in the immunofluorescent antibody test with N. gonorrhoeae and other species of Neisseria of IgG in the cervical secretions from six women, Nos 1F - 6F, with gonorrhoea

Species of Neisseria	Reaction obtained in test with stated antigen against specimen from patient:					
	1F	2F	3F	4F	5F	6F
N. gonorrhoeae	4+	4+	3+	3+	4+	4+
N. meningitidis						
Group A	-	-	-	-	-	-
B	2+	-	2+	-	2+	-
C	2+	+	+	+	2+	+
D	-	-	-	-	-	-
E	-	-	2+	2+	-	-
29E	2+	-	-	-	-	-
W135	2+	2+	+	2+	-	-
X	2+	+	2+	+	-	+
Z	2+	2+	2+	-	2+	+
N. lactamica	2+	-	-	2+	-	2+
N. catarrhalis	2+	2+	-	2+	-	2+
N. perflava	2+	-	+	-	2+	-

Table 21[R]

Reactivity in the immunofluorescent antibody test with N. gonorrhoeae and other species of Neisseria of IgM in the cervical secretions from six women, Nos 1F - 6F, with gonorrhoea

Species of Neisseria	Reaction obtained in test with stated antigen against specimen from patient:					
	1F	2F	3F	4F	5F	6F
N. gonorrhoeae	3+	3+	4+	4+	3+	4+
N. meningitidis						
Group A	-	-	-	-	-	-
B	2+	-	2+	-	-	-
C	-	-	2+	-	+	-
D	-	-	-	-	-	-
E	-	-	2+	-	-	-
29E	-	-	-	-	-	-
W135	-	-	-	-	-	-
X	-	-	-	-	-	-
Z	2+	+	2+	2+	-	2+
N. lactamica	2+	-	3+	-	-	2+
N. catarrhalis	2+	2+	2+	2+	2+	2+
N. perflava	2+	-	-	-	+	2+

IgM

Immunoglobulin M reactive with N. meningitidis (four cases), N. lactamica (three cases), N. catarrhalis (six cases) and N. perflava (two cases) was found in the cervical fluid of six infected women.

This class of antibody was demonstrated against N. meningitidis (four cases), but not against N. gonorrhoeae, N. lactamica, N. catarrhalis or N. perflava, in the secretions from six non-infected women.

Antibody of any class against Staph aureus, Staph albus, Strept pyogenes, Strept faecalis or Esch coli could not be detected in the cervical secretion from infected and non-infected women.

Detection of antibodies in cervical secretion after treatment

IgA

As in the male, the concentration of IgA in the diluted cervical fluid did not vary by more than ± 0.002 g per l within the individual patient during the period of the study; titres of IgA antibody are, therefore, presented. Table 22[R] indicates the rapid decline in the mean log titre of this antibody following successful treatment; of the 43 patients who attended 28 days post-treatment, antigonococcal IgA was only detectable in two women.

Table 22R

Mean log titre of IgA antibody reactive with N. gonorrhoeae in the cervical secretions before and after successful treatment

	Number of patients	Mean log titre of antigonococcal IgA antibody	Standard deviation
Before treatment	75	0.683	0.228
7 days after treatment	54	0.273	0.270
14 days after treatment	47	0.064	0.124
28 days after treatment	43	0.000	0.000

IgG

Immunoglobulin G concentrations in the cervical aspirate fluctuated by more than ± 0.012 g per l within the same patient; the T/C ratio was therefore calculated to investigate any change in IgG antibody activity. Table 18R demonstrates the gradual decline in this antibody activity following treatment. Of the 43 women examined 28 days after treatment, antigonococcal IgG was detectable in all but four women.

IgM

Within seven days of successful treatment, antigonococcal IgM could not be detected in the cervical secretions of any of the 54 women who attended at this time; this antibody had been detected in 26 of these patients before treatment.

Influence of re-infection and secretion antibody response

Eight women with gonorrhoea had previously been infected; antibody reactive with N. gonorrhoeae was detected in the cervical secretions of each of these women, being of the IgA and IgG class in each case. Two women (of three) both of whom had had gonorrhoea for less than 14 days had in addition, antigonococcal IgM in the cervical fluid.

There was no qualitative difference in the antibodies detected in the secretions between women who had previously been infected, and those who had not, but the small number of patients precluded a

quantitative comparison.

No patients in the study group became re-infected during the study period.

DETECTION OF ANTIBODIES IN RECTAL SECRETIONS FROM FEMALE PATIENTS

QUANTITATION OF IMMUNOGLOBULINS

Secretory IgA and IgG were demonstrated by thin-layer gel filtration in the rectal secretions from eight women, four of whom had anorectal gonorrhoea. The concentrations of IgA and IgG were determined in rectal secretion from each of these women; the mean concentrations of IgA and IgG were 0.016 *g* per l (range 0.013 to 0.021 *g* per l) and 0.013 (range 0.008 to 0.017 *g* per l) respectively.

DETECTION OF ANTIBODIES REACTIVE WITH N. GONORRHOEAE

Material from the anorectum was collected from 14 women with anorectal gonorrhoea, 11 women with urethral and/or cervical gonococcal infection only, and from 15 women who had no cultural evidence of infection, and who were not known contacts of men with gonorrhoea.

Antibody of the IgA class reactive with *N. gonorrhoeae* was detected using the immunofluorescent-antibody test in one woman with anorectal gonorrhoea, but in none of the women with genital infection only.

Antigonococcal IgG was detected in the rectal secretions of two women who had anal infection, but in none of the patients who were not infected at that site.

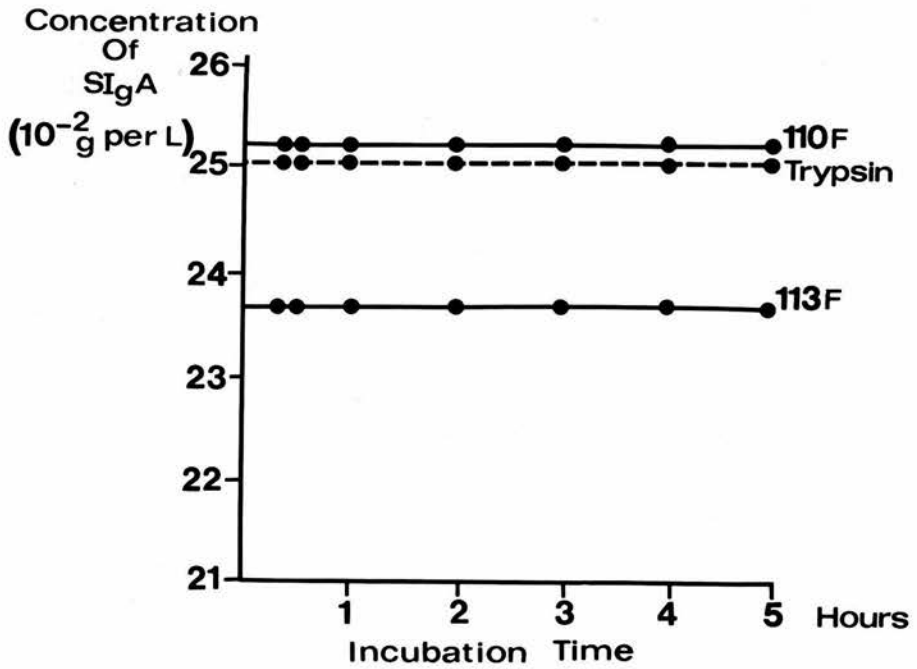
Antibody of the IgM class reactive with the gonococcus was not detectable.

Proteolytic activity of rectal secretions

As in the male, antigonococcal antibody was only occasionally found in rectal secretions from infected patients. That this finding was not the consequence of proteolysis of antibody as shown by the results of incubation of secretions with secretory IgA (Figure 12). Although the rectum was infected in both cases 110F and 113F, antigonococcal antibody could not be demonstrated in the immunofluorescent test

Figure 12

Concentration of secretory IgA during incubation with rectal secretions from two women with anorectal gonorrhoea.



DETECTION OF ANTIBODIES IN THE SALIVA OF FEMALE PATIENTS

As in the male, the concentrations of IgA and IgG in the saliva were not determined.

Before treatment

Using the immunofluorescent-antibody test, antigonococcal IgA was detected in four of six women with oropharyngeal gonorrhoea, but in only one of 15 non-infected patients. Antibody of the IgG class was found in the saliva of three infected and in two non-infected women; antigonococcal IgM was not detectable.

Saliva from two of 12 women with anogenital gonorrhoea but no cultural evidence of oropharyngeal infection, was found to contain antigonococcal IgA and IgG.

Reactivity of antibodies in saliva with other species of Neisseria

IgA

The saliva from each of the five women in whom IgA antibody against *N. gonorrhoeae* was found, also contained IgA reactive with *N. lactamica* (five cases), *N. meningitidis* group B (two cases) and *N. catarrhalis* (two cases). Saliva from 15 non-infected women contained IgA reactive with *N. meningitidis* (three cases from two of whom *N. meningitidis* was cultured from the pharynx), *N. catarrhalis* (seven cases) and *N. perflava* (one case).

IgG

Immunoglobulin G in the saliva of four women with pharyngeal infection reacted with N. meningitidis (three cases), N. lactamica (four cases) and N. catarrhalis (four cases). Similar antibody against N. meningitidis (nine cases), N. lactamica (two cases), N. catarrhalis (11 cases) and N. perflava (one case) was detected in saliva from 15 non-infected women.

Detection of antigonococcal antibody in saliva after treatment

Saliva from three of the six women who had been treated for oropharyngeal gonorrhoea was obtained 28 days after the completion of therapy. Antigonococcal IgA could not be detected, but IgG antibody was found in all three specimens of saliva.

DETECTION OF ANTIBODIES IN LACRIMAL SECRETIONS FROM FEMALE PATIENTS

Lacrimal secretions from two women were shown by thin-layer gel filtration to contain secretory IgA; neither IgG nor IgM could be detected.

The concentrations of IgA in the secretions from two women were 0.009 g per l and 0.011 g per l respectively.

Lacrimal secretions from 12 women with anogenital gonorrhoea, and from ten non-infected women were examined for antigonococcal antibody; neither IgM, IgA nor IgG classes of antibody could be demonstrated.

DETECTION OF ANTIBODIES IN SERUM FROM FEMALE PATIENTS

Before treatment

Table 23R shows the results obtained when sera were examined for antibody against the gonococcus.

IgM

Antigonococcal antibody of the IgM class was found using the fluorescent antibody test at a titre of $\geqslant$ 16 in 32 (42.7 per cent) of 75 women with untreated gonorrhoea, but in only two (2.9 per cent) of 70 non-infected women; the titre was $\leqslant$ 8 in each of 12 women who were named contacts. As in the male, there was a significant difference in the rate of detection of this antibody between patients infected for $\leqslant$ 14 days (28/39) and those infected longer (4/36) ($P < 0.005$ by method of binomial probabilities).

That the IgM detected was not reacting with IgG attached to the surface of the cell, was confirmed by the failure of absorption of sera with heat-aggregated Cohn fraction II to alter the titre of IgM antibody.

IgA

Antigonococcal IgA was found at a titre of $\geqslant$ 16 in the serum of 39 (52.0 per cent) of 75 women with gonorrhoea, but in none (0 per cent) of 70 non-infected women; the titre was $\leqslant$ 8 in each of 12 women who were

Table 23R

Reactivity with N. gonorrhoeae in the immunofluorescent-antibody test of serum from infected and non-infected women.

Immunoglobulin classes of antibody against N. gonorrhoeae in sera from women with untreated gonorrhoea and from non-infected patients

	No of sera	IgM titre					IgA titre					IgG titre				
		⩽ 8	16	32	64	128	⩽ 8	16	32	64	128	⩽ 8	16	32	64	128
(a) Infected Patients																
Duration of Infection (days)																
1 - 7	15	4	5	6	0	0	7	3	4	1	0	1	4	8	2	0
8 - 14	24	7	7	8	2	0	3	9	8	3	1	0	0	0	6	18
15 - 21	19	15	4	0	0	0	10	7	1	1	0	0	0	0	4	15
22 - 28	9	9	0	0	0	0	8	1	0	0	0	0	0	0	2	7
⩾ 29	8	8	0	0	0	0	8	0	0	0	0	0	0	0	2	6
(b) Non-infected Patients	70	68	2	0	0	0	70	0	0	0	0	66	4	0	0	0

named contacts. This antibody was found in 29 (74.4 per cent) of 39 women infected for 14 days or less, but in only ten (27.8 per cent) of 36 infected for longer, a significant difference ($P < 0.05$ using method of binomial probabilities).

IgG

This class of antibody was detected at a titre of $\geqslant 16$ in the serum of 74 (98.9 per cent) of 75 women with gonorrhoea; IgG antibody was found in only four (5.7 per cent) of 70 non-infected women; the titre was $\geqslant 128$ in each of 12 women who were named contacts. There was a significant difference in the mean log titre between women infected for less than eight days (mean log titre 1.425) and those infected longer (mean log titre 2.037) ($P < 0.05$ using student's t test $t = 7.313$ with 73 degrees of freedom).

In each of the five women with acute bartholinitis or salpingitis, the IgG titre was 128.

Serum from five women who were found to have N. meningitidis in the pharynx was examined; the titres of IgM, IgA and IgG reactive with N. gonorrhoeae were, in each case $\leqslant 8$, $\leqslant 8$ and 16 respectively.

Reactivity of serum immunoglobulins with other species of Neisseria

The results obtained when sera diluted 1 in 16 from six women with untreated gonorrhoea were tested against various species of Neisseria are shown in Tables 24, 25 and 26.

IgM

Immunoglobulin M reactive with N. meningitidis (six cases in each of which reactivity was with only one serogroup of meningococcus), N. lactamica (two cases), N. perflava (two cases) and N. catarrhalis (six cases) was found in the sera of six non-infected women.

IgA

Sera from non-infected women contained IgA antibody against N. meningitidis (three cases), N. lactamica (two cases), N. perflava (six cases) and N. catarrhalis (three cases).

IgG

Immunoglobulin G in the sera from all the non-infected women reacted with many of the serogroups of N. meningitidis, N. lactamica, N. perflava, N. catarrhalis and N. gonorrhoeae.

Detection of antigonococcal antibody in serum of women after treatment of gonorrhoea

Table 27R shows the prompt decline in the mean log titre of IgM and IgA antibodies against the gonococcus, and the more gradual fall in mean log titre of IgG antibody.

Table 24

Reactivity in the immunofluorescent-antibody test with anti human IgM and *N. gonorrhoeae* and other species of Neisseria of serum from six women, Nos 1F - 6F, with uncomplicated gonorrhoea

Antigen	Reaction* obtained in test with stated antigen against serum from patient:					
	1F	2F	3F	4F	5F	6F
N. gonorrhoeae	3+	3+	3+	3+	3+	3+
N. meningitidis						
Group A	-	-	2+	-	-	-
B	2+	2+	+	2+	-	+
C	2+	2+	+	2+	-	+
D	-	-	2+	-	-	-
E	-	2+	-	-	2+	-
29E	-	-	2+	-	-	-
W135	-	-	-	-	-	-
X	+	+	+	-	-	+
Z	2+	2+	+	3+	+	+
N. lactamica	2+	2+	2+	2+	2+	2+
N. perflava	-	2+	-	3+	-	+
N. catarrhalis	2+	2+	2+	2+	2+	2+

* see Methods p 103

Table 25 R

Reactivity in the immunofluorescent-antibody test with anti human IgA and N. gonorrhoeae and other species of Neisseria of serum from six women, Nos 1F - 6F, with uncomplicated gonorrhoea

Antigen	Reaction obtained in test with stated antigen against serum from patient:					
	1F	2F	3F	4F	5F	6F
N. gonorrhoeae	4+	4+	4+	4+	4+	4+
N. meningitidis						
Group A	-	-	-	-	-	-
B	-	-	-	-	-	-
C	-	-	-	-	-	-
D	-	2+	-	-	+	-
E	-	-	-	-	-	-
29E	-	-	-	-	-	-
W135	-	-	-	-	-	-
X	-	+	-	+	+	+
Z	-	-	-	-	-	-
N. lactamica	-	-	2+	-	-	2+
N. perflava	-	-	+	-	2+	3+
N. catarrhalis	+	+	+	+	2+	+

Table 26 R

Reactivity in the immunofluorescent-antibody test with anti human IgG and N. gonorrhoeae and other species of Neisseria of serum from six women, Nos 1F - 6F, with uncomplicated gonorrhoea

Antigen	Reaction obtained in test with stated antigen against serum from patient:					
	1F	2F	3F	4F	5F	6F
N. gonorrhoeae	4+	4+	4+	4+	4+	4+
N. meningitidis						
Group A	-	2+	-	-	-	+
B	2+	2+	2+	+	2+	3+
C	2+	+	2+	2+	2+	2+
D	2+	-	2+	-	-	+
E	+	+	+	-	-	3+
29E	-	-	+	-	-	3+
W135	2+	+	2+	-	+	3+
X	+	2+	3+	+	2+	2+
Z	3+	2+	2+	+	+	3+
N. lactamica	3+	3+	3+	3+	3+	3+
N. perflava	3+	2+	2+	2+	3+	3+
N. catarrhalis	3+	3+	2+	3+	3+	2+

Table 27[R]

Arithmetic mean log titres of antibodies reactive with N. gonorrhoeae in the serum of women with gonorrhoea before and after successful treatment

Time of test	Number of patients	Mean log titre		
		IgM	IgA	IgG
Before treatment	75	1.104	1.164	1.915
7 days after treatment	54	1.120	1.071	1.904
14 days after treatment	47	0.977	1.057	1.894
28 days after treatment	43	0.903	0.911	1.860

II

EVALUATION OF THE INDIRECT IMMUNOFLUORESCENT-ANTIBODY TEST IN THE DIAGNOSIS OF GONORRHOEA IN WOMEN

The rapid diagnosis of gonorrhoea in women is limited, and it was felt desirable to evaluate the indirect immunofluorescent-antibody test to determine if this test could be used as a routine diagnostic procedure.

The indirect immunofluorescent-antibody test was used in the examination of cervical secretions from 294 women who attended Black Street Clinic consecutively during a five-month period.

Gonorrhoea was diagnosed by culture in 106 women; a further nine women, all contacts of infected men, were diagnosed by microscopy of a Gram-stained smear of cervical material.

As may be seen from Table 28, antigonococcal IgA and/or IgG was detected in secretions from 95 (89.6 per cent) of 106 women with gonorrhoea. Antibody of the IgG class was, however, detected in 80 (49.4 per cent) of 162 women who had no microbiological evidence of infection, and who were not named contacts.

When the results of the fluorescent-antibody test are examined by consideration of the IgA response only, 97 (84.3 per cent) of 115 infected women were identified; IgA reactivity was found in only 19 (10.6 per cent) of 179 non-infected women.

Table 28

Results of the indirect immunofluorescent-antibody compared with those of Gram-smear microscopy and culture

Immunofluorescent-antibody test results	Gram-smear positive		Gram-smear negative		Total
	Culture positive	Culture negative	Culture positive	Culture negative	
IFA-test positive					
- IgA only	3	0	0	0	3
- IgG only	5	0	0	61	66
- IgA and IgG	72	7	15	36*	130
IFA-test negative	9	2	2	82	95
Total	89	9	17	179	294

* 17 of these women were named sexual contacts of men with culturally-proven gonorrhoea

Discussion

The antibody response to N. gonorrhoeae detected in the secretions and serum is considered, then the conclusions regarding the application of the indirect immunofluorescent-antibody test in diagnosis are presented.

The results obtained in each part of the study are discussed separately.

I

Urethral secretions in the male

In the present study, antibody against the gonococcus was demonstrated in the urethral secretions of 97 per cent of 134 men with gonococcal urethritis. The prevalence of IgA antibody was higher than that reported by Kearns et al. (1973) who detected antigonococcal IgA in 83 per cent of urethral secretions from 35 infected men who attended a clinic in Atlanta. These workers employed an indirect immunofluorescent antibody test using the same strain (designated strain 9) of organism as that used in the present study. The lower prevalence in their series of secretion (IgA) antibody may be the result of there being less cross-reactivity with strain 9 of antibody produced against the infecting strains in Atlanta compared with cross-reactivity of antibody against strains acquired in Glasgow with strain 9.

Tapchaisri and Sirisinha (1976) using the techniques of immunofluorescence, haemagglutination-inhibition and opsonization examined seminal fluid for an antibody response to N. gonorrhoeae. They were unable to demonstrate antibody in each of 11 patients who

had been treated for "acute" urethral gonorrhoea (that is, infection acquired within a week of clinic attendances) several days previously; specimens were not obtained before treatment. Antigonococcal antibody was, however, detected by immunofluorescence in the semen of about a third of men who had been treated within the preceding three to five days for "subacute" gonorrhoea (that is, those who had been infected more than one week before attendance at the clinic). The finding that this antibody was principally of the IgG class is not surprising: Rümke (1974) showed that the predominant immunoglobulin in seminal fluid was IgG, only small concentrations of secretory IgA being detected. Rümke (1974) further demonstrated that the majority of the seminal IgG was derived from transudation of serum immunoglobulin. A serum IgG primary response to antigen does not usually become detectable until several days after exposure (Newcomb and Ishizaka, 1967) and this was also shown to be true of gonococcal infection by Cohen and co-workers (1969). They demonstrated a tenfold increase of IgG antibody reactivity to a heat-labile surface antigen within five days of infection. It is probable that most of the antigonococcal IgG detected in the semen by Tapchaisri and Sirisinha (1976) was derived from transudation of serum IgG. This could explain why antibody was not detectable in the semen from patients with "acute" infection but was found in men who had been infected for long enough to allow a detectable serum antibody response. Tapchaisri and Sirisinha (1976) found that IgA reactive with N. gonorrhoeae could be demonstrated in only two of 37 samples of seminal fluid from men treated for "subacute" gonorrhoea; absorption with antiserum to secretory component inhibited the IgA antibody activity, indicating that this immunoglobulin was in the secretory IgA form. The difference

in prevalence of IgA antibody between their series and the present study probably reflects the use of semen, known to contain little IgA (Rumke, 1974) and not urethral secretions as used above.

It has been shown that normal urethral secretions do not contain appreciable amounts of IgG (Burdon, 1971), but antigonococcal IgG was found in the present study in the urethral exudate from 90 per cent of men with gonococcal urethritis. The question arises: what is the origin of this immunoglobulin? Titres of IgG antibody in the urethral secretions were significantly higher in men who had been infected for more than seven days, than in those whose infection was of a shorter duration. This finding, which parallels the changes in serum antibody, suggests that at least some of the secretion IgG is derived from transudation from plasma. During microbial infection at other mucosal surfaces, for example the nasal mucosa, the changes in the total IgG concentration follow those of albumin, a finding which strongly supports the hypothesis that urethral IgG is mainly derived from plasma. The mean IgG antibody titre in the diluted urethral secretions was 3.2. About 30 µl of exudate were diluted to 1 ml, that is some thirty times; the IgG titre in the actual exudate would, therefore, be about 95. The mean serum IgG antibody titre was 84; that is, there was no significant difference between serum and secretion antibody titres as would be expected if antibody was mainly produced in the mucous membrane.

Antigonococcal IgG was found in about a quarter of men with non-gonococcal urethritis; the titre was significantly lower than in the secretions from men with urethral gonorrhoea. Normal human serum contains natural antibody of the IgG class with reactivity for N. gonorrhoeae (Cohen, 1967), and it is probable that the low titres

of IgG antibody in men with non-gonococcal urethritis resulted from transudation of natural antibody from the plasma through the inflamed urethral mucosa. When the inflammatory process resolved, usually within two weeks of commencing treatment with tetracyclines, IgG antibody activity against the gonococcus became undetectable.

Microbial infections of mucous surfaces often elicit a secretory immune response, IgA being the predominant antibody class produced (Ogra et al. 1968). Is there evidence for a secretory immune system in the urethra?

Although stratified columnar epithelium lines the cavernous urethra, which comprises the greatest part of the male urethra, the epithelium of the numerous intramucosal glands is simple columnar or cuboidal (Ham, 1965). Plasma cells are sparsely distributed in the lamina propria, but during gonococcal infection, these become prominent and numerous, particularly around the intramucosal glands and ducts (Harkness, 1948); however, the classes of immunoglobulins produced by these plasma cells are not known. The urethra then has all the anatomical features of a typical secretory site.

The data presented here strongly suggest that the antigonococcal IgA found in the urethral secretions is locally produced. Presumptive evidence for this is the higher titre of IgA antibody in the secretions and than in the sera of men with gonococcal urethritis. The mean titre of secretion IgA was 135 and that of serum IgA antibody was ten; this twelve-fold difference may be accounted for by either local production or selective transport of immunoglobulin from the serum. Following absorption of secretions with antiserum against secretory

component, there was a three- or four-fold reduction in IgA antibody activity, demonstrating that the majority of this activity was associated with secretory IgA.

The association of antibody activity with secretory IgA suggested the possibility of replacing anti-human α-chain serum in the immunofluorescent antibody test with fluorescein-conjugated anti-human secretory component serum. By employing the latter serum, difficulties associated with the interpretation of results from secretions contaminated with blood should be obviated. However, when secretions shown to contain antigonococcal IgA by the use of antiserum against α-chains, were tested, fluorescence could not be detected. The anti-secretory component serum had been obtained from commercial sources, and may not have been sufficiently potent for use in the fluorescent-antibody test; Tramont (1977) was able to demonstrate antigonococcal secretory IgA antibody in genital secretions by using fluorescein-conjugated anti-human secretory IgA serum which had been absorbed with monomeric IgA.

Secretion IgA reactive with N. gonorrhoeae appeared early in infection and persisted throughout the duration of the disease. Butler and co-workers (1970) showed that the IgA concentrations in nasal secretions rose within 24 hours of infection with Coxsackie B virus, and following a second rise six to nine days later, reached a peak during the second week after inoculation of virus on to the nasal mucosa. The later changes in IgA concentration were considered the result of local synthesis of antibody, but the earlier rise was thought to result from the release of IgA stored within secretory tissues. This biphasic IgA response was not investigated in the present study, as it was felt unjustifiable to withhold treatment when a man had

urethritis.

Immunoglobulin M antibody was found in the urethral exudate of 48 per cent of men with gonococcal urethritis and appeared within 14 days of infection but could not be detected when the gonococcus had been established for a longer period. The high molecular weight of IgM (900,000) impairs the passage of this immunoglobulin from serum into the exocrine secretions (as compared with IgG with a molecular weight of 150,000). Although natural antibody of the IgM class is present in most sera (Cohen, 1967), this antibody could not be detected in the secretions from men with non-gonococcal urethritis, further suggesting that transudation from serum does not occur readily.

Assuming a dilution factor of about 30 (see above), the mean concentration of antigonococcal IgM (55) in urethral exudate was four times that in serum (15); this suggests either selective transport of serum IgM, or, more likely, local synthesis of antibody. Although it is not known whether IgM-producing plasma cells are present in the urethral mucosa, these cells have been demonstrated in other secretory surfaces (Baklien et al. 1972; Brandtzaeg, 1973).

Immunoglobulin A reactive with the gonococcus became undetectable in the urethral secretions within two weeks of successful treatment, but IgG antibody activity declined more slowly. This latter finding contrasts with the data described by Tapchaisri and Sirisinha (1976) who found that IgG reactivity in the seminal fluid became undetectable within seven days of treatment. It has been shown that following resolution of viral infection, the total IgG concentration in nasal secretions decreases within seven days to pre-infection concentrations,

these changes parelleling those of albumin concentrations in the secretions (Butler et al. 1970). Assuming that most of the IgG in the seminal fluid is derived from serum (Rumke, 1974), it would be expected that IgG antibody in this fluid would decrease within a few days of treatment of gonorrhoea. This would explain the findings of Tapchaisri and Sirisinha (1976). Persistence of IgG antibody in urethral secretions after successful treatment may reflect some local synthesis of antibody by the urethral mucosa. The rapid decline in IgA antibody titre compared with that of IgG may reflect differences in the half-life of the different immunoglobulin-producing plasma cells in the lamina propria. Data to support this hypothesis are, however, lacking, although Mattioli and Tomasi (1973) have shown that the half-life of intestinal IgA-producing plasma cells is about five days.

In men whose infection was not cured by antibiotic treatment, IgA antibody persisted until treatment had been successful. The rapid increase in antigonococcal IgA activity following re-infection was clearly shown in the secretions from four men who became re-infected during the period of the study.

Cervical secretions in the female

Antibody reactive with N. gonorrhoeae was detected in the cervical secretions from 97 per cent of 75 women with gonorrhoea. In women in whom secretion antibody was demonstrated, this was of the IgG class in each, of the IgA class in 97 per cent, and of the IgM class in 40 per cent. Antibody of the IgM class against the gonococcus was found significantly more frequently in the secretions from women infected for less than 14 days, than in those infected longer. An insufficient

number of women were studied to determine if the IgG antibodies titres in the secretions increased directly with the duration of infection.

The IgA antibody results agree with those reported by O'Reilly, Lee and Welch (1976) who made a detailed study of six women with gonorrhoea. Using an immunofluorescent-antibody method, these workers demonstrated antigonococcal IgA in the cervicovaginal secretions from each woman. Significantly different results were obtained by Tapchaisri and Sirisinha (1976) who investigated secretion antibody reactivity with strains F62T1 and F62T4 of N. gonorrhoeae. They detected antibody in the cervical secretions of only 60 per cent of infected women, and showed that most of this antibody was of the IgG class; IgA reactivity was found in only ten per cent of these women. The higher prevalence of secretion antibody in the present study may be the result of using an antigen with wider cross-reactivity with local strains of N. gonorrhoeae (O'Reilly, Welch and Kellogg, 1973).

The source of the cervical antibodies will now be considered:

The uterine endocervix has the anatomical features of a typical secretory site. Tall, mucus-secreting columnar and occasional ciliated cells line the cervical canal. Long, wide glands which extend from the surface deeply into the lamina propria are also lined by such cells (Ham, 1965). Plasma cells containing IgA, and less commonly IgM and IgG are found in the lamina propria (Tourville et al. 1970; Rebello, Green and Fox, 1975), and secretory component may be demonstrated in the epithelial cells of these glands (Tourville et al. 1970). Lymphoid cells staining for IgM, IgA and IgG are very sparsely distributed in the stroma of the endometrium and uterine tubes,

although secretory component is found at the apices of the epithelial cells lining these structures. Consideration of the immunohistological structure of the female genital tract suggests that the endocervix is the principal secretory site (Rebello, Green and Fox, 1975).

As the dilution factor of the cervical secretions could not be estimated, it was not possible to compare serum and secretory antibody concentrations.

In an investigation of the antibody response of the female genital tract to immunization with poliovaccine, Ogra and Ogra (1973) showed that after parenteral administration of vaccine, the antibody response detected in the cervicovaginal secretions was limited to IgG and that the highest titres were detected three to four weeks later, when the serum antibody titres were at their peak. They attributed secretion IgG antibody activity to transudation of plasma immunoglobulins. It was, however, further shown that intrauterine immunization resulted in the appearance in the uterine secretions of IgG antibody only, and that this response was independent of that detected in the serum. Although in the present study, cervical secretions were collected for antibody studies, these represent the secretory products of the uterine tubes and endometrium in addition to those of the cervix. The total response of the cervix, endometrium and uterine tubes was therefore measured. By consideration of the work described above by Ogra and Ogra (1973) and by the finding of an increased number of IgG-containing plasma cells in the lamina propria of the endocervix of women with gonorrhoea (Chipperfield and Evans, 1972), it is likely that the antigonococcal IgG found in the secretions was derived from both local production and transudation from plasma.

It was shown that IgG antibody activity against the gonococcus

could be demonstrated in the secretion from the majority of women with non-gonococcal cervicitis; similar findings were reported by Tapchaisri and Sirisinha (1976). Following resolution of the inflammatory process, usually within two to three weeks of treatment with tetracyclines IgG antibody became undetectable; this observation appears to be analagous to that of non-gonococcal urethritis in the male.

Chipperfield and Evans (1975) showed that the mean total IgA concentrations in cervical secretions from women with gonorrhoea was significantly higher than that from women who had no detectable microbial, trichomonal or fungal infection. On the basis of the ratio of IgG to IgA (2.5:1) in the secretions, they postulated that a considerable proportion of the IgA was synthesised locally. These workers had previously shown that during gonococcal infection, IgA-containing plasma cells in the lamina propria of the cervix were significantly more prevalent than when the cervix was not infected (Chipperfield and Evans, 1972). In the present study, the significant reduction in IgA antibody activity following absorption with anti-serum to secretory component confirms the findings of other workers that this reactivity is associated with secretory IgA (O'Reilly, Lee and Welch, 1976; Tapchaisri and Sirisinha, 1976). The finding that absorption with secretory component antiserum reduced, but did not abolish IgA antibody reactivity suggests that some monomeric or polymeric IgA molecules, not associated with secretory component, were also present in the cervical secretions. Although monomeric IgA could not be demonstrated in the cervical fluid by thin-layer gel filtration in the present study, Tjokronegro and Sirisinha (1975) found traces

of this immunoglobulin in the secretions. Secretory IgA in cervical fluid has been demonstrated against other micro-organisms.

In experiments on the local antibody response to poliovaccine, Ogra and Ogra (1973) inoculated the vagina with inactivated vaccine (Salk) and observed an IgA antibody response in the cervicovaginal secretions from each of five women. Antibody activity became detectable within a week of inoculation and was still demonstrable in two women 16 weeks later.

Coughlan and Skinner (1977) found IgA against Herpes virus hominis in the cervical mucus of more than three-quarters of women attending a gynaecological clinic. Neutralizing antibody activity to type 1 herpes virus was found in 24 of 28 patients and to type 2 herpes virus in 18 of 24 women; most of these women were from social classes III and IV.

IgA antibody against Candida albicans was found in the cervicovaginal secretions from nine of ten women attending a gynaecological clinic (Waldman, Cruz and Rowe, 1971). An IgA response was elicited in seven of ten women given intravaginal immunization by instillation of C. albicans antigen. The duration of the antibody response was not, however, demonstrated. Milne and Warnock (1977), however, found IgA antibody in only six per cent of women with candidiasis.

Ackers and his co-workers (1975) by radio-immunoassay detected IgA against Trichomonas vaginalis in the vaginal secretions of 22 of 29 women with trichomoniasis. The effect of treatment on the antibody response was not examined.

Antibody of the IgA class found in the cervical fluid of 20 of 35 women with infection with Chlamydia trachomatis (Treharne et al. 1978); again no data were presented as to what effect treatment had

on antibody levels.

The increase in IgM-containing plasma cells found in the cervix of women with gonorrhoea (Chipperfield and Evans, 1972), suggests that most of the IgM in the cervical fluid is locally produced.

As in the urethral secretions of infected men, the IgA antibody activity in the cervical fluid from women with gonorrhoea declined rapidly after treatment, becoming undetectable in most cases, within two weeks. In two women secretion IgA antibody persisted at low titre for more than four weeks. Similar results were reported by O'Reilly, Lee and Welch (1976). In the absence of continued antigenic stimulation in these women the persistence of IgA is difficult to explain.

Antigonococcal IgG activity in the cervical secretions declined slowly after treatment, and was still detectable in 90 per cent of women who had been treated a month previously. This further suggests that at least some of the secretion IgG is synthesised locally, as this antibody would not be demonstrable so long after treatment if it was solely derived by transudation from plasma (Butler et al. 1970).

Immunoglobulin M against N. gonorrhoeae could not be detected in the secretions from any treated woman.

Specificity of antibodies in genital secretions

In the majority of genital secretions from men and women with gonorrhoea, IgA antibody against the gonococcus cross-reacted with N. lactamica. This is probably due to sharing of antigenic

determinants; Johnson and co-workers (1977) have shown that the core lipopolysaccharide produced by N. lactamica contains the same constituent sugars as those found in N. gonorrhoeae. Although IgA antibody activity in respiratory secretions appears to be less specific than that of IgG (Waldman, Wigley and Small, 1970), this was not the case in the present series; secretions from infected and non-infected patients contained IgG which reacted with N. gonorrhoeae, various groups of N. meningitidis, N. catarrhalis, N. lactamica and N. perflava. Genital secretions from men and women with gonorrhoea contained IgM antibody against N. lactamica and N. catarrhalis in addition to reactivity for N. gonorrhoeae; secretions from non-infected patients did not contain this antibody activity, suggesting that IgM antibody against the gonococcus cross-reacted with the other species of Neisseria.

Non-infected sexual contacts of individuals with gonorrhoea

Antigonococcal antibody was found in the urethral or cervical secretions from three men and 12 women, who, although named sexual contacts of infected partners, had no evidence of gonococcal infection. A possible explanation may be that these patients were infected, but that the organisms failed to grow on culture. In Glasgow, specimens were transported to the laboratory in Stuart's transport medium and it is known that this medium will support the growth of organisms which may interfere with the subsequent culture of N. gonorrhoeae (Amies, 1967). Vancomycin was used in the selective culture medium in Glasgow, and if these patients had been infected with vancomycin-sensitive strains of the gonococcus, the organisms may not have

grown; Reyn (1969) reported that three per cent of strains of N. gonorrhoeae are susceptible to the action of vancomycin at the concentration used in the medium (3 µg per ml).

A second possible explanation for the failure to isolate the organism may be that the patient had taken antimicrobial drugs before attendance at the clinic. Although careful enquiry was made regarding antibiotic use or abuse within the preceding three months, some patients may have been reluctant to disclose this information.

The third possibility is that these men and women had become infected, but that an immune reaction or non-specific factors had led to elimination of the organism. Chipperfield and Evans (1972) showed that there was an increased number of immunoglobulin-containing plasma cells in the lamina propria of the endocervix of women, who, although named contacts of men with gonorrhoea, were not infected. Growth of N. gonorrhoeae may be inhibitied in vitro by other micro-organisms which may be present in the genital tract; these include Staph aureus, Staph epidermidis and "Gaffkya anaerobia" (Kaye and Levison, 1977). However, the mechanism of inhibition of N. gonorrhoeae in vivo by these organisms is not clear. Staphylococcus epidermidis has been isolated from the urethra of men, who although named contacts were not infected (Kraus and Ellison, 1974).

Secretion antibodies (IgM, IgA and IgG) in patients with complicated gonorrhoea

Tramont (1977) observed that two women with acute pelvic inflammatory disease did not have specific IgA antibody against N. gonorrhoeae in the cervical secretions, although one did have IgA

against the test strain (strain 9) of the organism. He suggested that there was a lack of strain-specific response to infection in these women, and local spread of the gonococcus through lack of inhibition of attachment of the organisms to the epithelial cells. These findings were not confirmed in the present study. The cervical secretions from each of three women with acute pelvic inflammatory disease had IgA antibody against both strain 9 and the infecting strain of the organism. It may have been that the salpingitis in the women in the present series was not due to the gonococcus. Although N. gonorrhoeae is certainly capable of producing tubal damage (Ward, Watt and Robertson, 1974), other organisms, such as Chlamydia trachomatis are also thought to play a part in the aetiology of pelvic inflammatory disease (Treharne et al. 1979). Chlamydia trachomatis may be isolated from the cervix of up to 45 per cent of women with gonorrhoea (Burns et al. 1975) and it is possible that this agent was responsible for the salpingitis in the cases presented here. Although the organism could not be cultured from the cervix in any of the three women, cell culture appears to be less effective for demonstrating cervical than for demonstrating urethral infection (Wentworth, 1977).

Similarly IgA antibody against both strain 9 and the infecting strain of the gonococcus was found in the urethral secretions of one man with acute epididymitis, and two men with disseminated gonococcal infection. Although N. gonorrhoeae is an aetiological agent in epididymitis, C. trachomatis also appears to be pathogenic (Harnisch et al. 1977) and this organism was cultured from the urethra of the patient with epididymitis.

Rectal secretions in men and women

The finding that antigonococcal antibody could not be detected in the rectal secretions from men and women with anorectal gonorrhoea was surprising. Histologically, the rectum has the structure of a typical secretory surface: columnar epithelium lines the rectal mucosa, and plasma cells containing IgA and to a lesser extent IgM and IgG are found in the lamina propria (Crabbe and Heremans, 1966). During gonococcal infection these plasma cells increase greatly in number (Harkness, 1948). By immunodiffusion and gel filtration techniques, IgA and IgG were demonstrated in the rectal secretions. It was initially considered that the lack of antibody activity could be accounted for by the proteolytic action of the rectal secretions. There was, however, no experimental evidence for this hypothesis.

Salivary antibodies

Although the number of patients available for investigation in the present study is small, an antibody response to infection in the oropharynx was found in most patients with infection of these surfaces. An interesting observation was the high proportion (56 per cent) of homosexual patients with anogenital gonorrhoea but no evidence of pharyngeal infection, who had antibody against the gonococcus in the saliva. Several factors may explain this situation. These men may have had pharyngeal gonorrhoea, which was not detected; the diagnosis of pharyngeal infection is not always simple, multiple testing being required to exclude infection (McMillan and Young, 1978). Clearly it would have been undesirable to withhold treatment for anogenital

gonorrhoea in order to perform repetitive pharyngeal cultures. Another possibility may have been that the patient had been infected, and that by immune or non-immune mechanisms, the infection had been eradicated. Bacterial interference in which gonococci are inhibited by meningococci has been suggested as a possible mechanism for the patient's resistance to infection (Volk and Kraus, 1974). Normal saliva contains an amylase capable of inhibiting the growth of N. gonorrhoeae (Mellersh, Clark and Hafiz, 1979). A third hypothesis is that antigenic material may have been absorbed from the anorectum or urethra and carried to the oropharyngeal mucosal surface. Ogra and Karzon (1969), however, have shown that a local immune response is elicited at surfaces only to which antigen is applied. They immunized one lumen of a double-barrelled colostomy against poliovirus and showed that antibody was produced only in that side of the colostomy.

Analysis of lacrimal secretions from 38 men with anogenital gonorrhoea failed to reveal any antigonococcal antibody, suggesting that absorbed antigen did not elicit an immune response at sites distant from those infected.

Serum antibody response to gonorrhoea

Although indirect immunofluorescent-antibody techniques, using various antigen preparations, have been evaluated as diagnostic tests (Welch and O'Reilly, 1973; Rodas and Ronald, 1974), there have been few studies correlating the distribution of antigonococcal antibodies within the immunoglobulin classes, and duration of infection.

Scott and Rasbridge (1972) demonstrated a small increase in the mean concentration of IgG, IgA and IgM in the serum of patients with

gonorrhoea as compared with non-infected contacts.

Antibody of the IgM class, reactive with N. gonorrhoeae was detected, in the present study, at a titre of $\geqslant$ 16 in 53 per cent of infected men, and in 43 per cent of infected women before treatment. The results obtained in women were similar to those recorded by Wilkinson (1975) who also found this class of antigonococcal antibody at a titre of $\geqslant$ 16 in 43 per cent of infected patients. He found similar antibody in only 32 per cent of infected males; the duration of infection was not recorded.

IgM antibody was detectable in the serum of about two-thirds of the men, and two-thirds of the women with untreated gonorrhoea who had been infected for less than two weeks. When infection was of greater duration, this antibody was less commonly found at the lowest dilution of serum examined. This early IgM response was observed, under experimental conditions, by Cohen, Kellogg and Norins (1969) who also showed that even without treatment, in those subjects who developed antigonococcal IgM, the titres of this antibody gradually fell over a period of about four months. Few patients in the present study who had been infected for more than 14 days had titres of antibody of $\geqslant$ 16. Cohen and others (1969), however, used inocula of organisms much in excess of what would be acquired in natural infection, and this may explain partly the more gradual decline in IgM activity observed by them. Natural antibody of the IgM class reactive with N. gonorrhoeae was not detected in sera from non-infected patients, but antibody of this class was demonstrated against N. meningitidis, N. catarrhalis and less commonly against N. lactamica and N. perflava. Sera from infected patients which reacted with N. gonorrhoeae also reacted with N. lactamica; this observation is

similar to that found with secretion antibody.

The antigonococcal IgA response to infection was similar to that of IgM. Antibody of this class reactive with N. gonorrhoeae, was found in the serum of 70 per cent of men, and 74 per cent of women who had been infected for less that 14 days. When the infection was of greater duration, only 14 per cent of men, and 28 per cent of women had this antibody in the serum at a diluation of 1 in 16 or greater. Similar findings of a rapid decline in serum IgA antibody activity within two to three weeks of acquisition of infection were reported by Cohen and co-workers (1969). In general, the IgA response to microbial infection appears after a latent period of about seven days and peaks 10 to 14 days after infection; thereafter there is a rapid decline in IgA antibody activity (Newcomb and Ishizaka, 1967).

Naturally-occurring IgA reactive with N. gonorrhoeae was not detected although antibody against N. perflava, N. catarrhalis, N. meningitidis and N. lactamica was found in infected and non-infected patients.

Antigonococcal antibody of the IgG class was found in the serum at a dilution of 1 in 16 or greater in all infected males, and almost all infected females. The false-positive rates were 13 per cent and six per cent respectively for males and females; some of these patients may, however, have previously been infected and inadvertently treated. In one study, where heat-labile antigen and anti-human IgG were used in a fluorescent-antibody test, the false-positive rate was lower, as was the sensitivity (Gaafar and D'Arcangelis, 1976).

IgG antibody activity increased throughout the first week of infection, so that by the second week, the majority of patients had antibody at high titre ($\geqslant 64$). No obvious decline in antibody activity

occurred in the subsequent two to three weeks. These results agree with those obtained by Cohen and co-workers (1969), and those obtained by Glynn and Ison (1978) with an Elisa procedure. Chacko and Nair (1969) used a precipitin test to demonstrate an increase in antibody activity in the serum, during the first week of gonococcal infection.

Antibody of the IgG class was demonstrated at a dilution of 1 in 16 in sera from non-infected patients against various species of Neisseria, including against N. gonorrhoeae in two cases. It was of interest to note that the titre of IgG antibody against N. gonorrhoeae in each of five women from whose pharynx N. meningitidis was cultured, was not greater than 16.

Successful treatment of infection in both sexes resulted in a rapid decline in serum antigonococcal IgM and IgA activity. A much more gradual decline in IgG antibody activity occurred. This presumably reflects differences in the half-life of the antibody classes (IgM 5 days; IgA 6 days; IgG 22 days) (Tomasi, 1976).

Immunity to gonococcal infection

Although N. gonorrhoeae provokes an immune response, as measured by the demonstration of humoral antibody against the organism in secretions from infected mucosal surfaces, it is paradoxical that immunity to re-infection does not occur. This is strikingly shown in the case of AR (Figure 8) a young homosexual man who was reinfected twice over a four-month period. Such cases are not unusual, and it is well recognised that repeated infections in individuals contribute significantly to the overall prevalence of gonorrhoea (Noble et al. 1977).

Immunoglobulin A in secretions has been shown to protect experimental animals from infection with Vibrio cholerae, an organism which is usually localised to the gastro-intestinal tract and exerts its pathogenic effects through an exotoxin (Fubara and Fretar, 1973; Pierce, Sack and Sircar, 1977). Although the mode of action of IgA in protecting against infection is not completely understood, it has been shown that this immunoglobulin is capable, in vitro, of fixing complement via the alternative pathway (Ellman, Green and Frank, 1971); of having bactericidal properties in the presence of lysozyme (Adinolfi et al. 1966); and possibly of having opsonizing properties (Kaplan, Dalmasso and Woodson, 1972). The role of complement in immunity in secretions is uncertain, as there are no data detailing the existence of components of the alternative pathway in genital secretions. It has been shown that purified preparations of IgA show little opsonizing activity but impure preparations have this activity; trace amounts of IgM may act synergistically with secretory IgA and explain these findings (Rowley, 1973). Secretory IgA inhibits the adherence of commensal strains of Streptococcus spp to buccal epithelial cells (Williams and Gibbons, 1972), and, as attachment of N. gonorrhoeae to epithelial cells appears to be a prerequisite for the invasion of mucous membranes, it might be expected that secretory IgA would interfere with this process, resulting in protection against disease. Indeed the inhibition of attachment of gonococci to epithelial cells by secretory IgA has been shown by Tramont (1977).

It is accordingly surprising that immunity to re-infection is not established. Several theories have been advanced to explain this.

Firstly, it may be that symptoms appear and the patients treated before an immune response of sufficient magnitude is elicited. The

ability to synthesise IgA varies from person to person as has been shown in nasal secretions: individuals with a high baseline IgA concentration mobilize an antibody response more quickly and appear less likely to develop influenza viral infections than those who have a low-baseline IgA concentration (Rossen et al. 1970). The significance of this finding in relation to genital infections is uncertain.

A second explanation is that re-infection by a different strain of the organism occurs. Thirdly, the secretory system does not have an immunological memory: this suggestion lacks experimental proof, although Mattioli and Tomasi (1973) have shown in the mouse that the mean half-life of intestinal IgA-producing plasma cells is just under five days; this suggests that significant numbers of long-lived plasma cells do not exist.

A fourth explanation may be that cell-mediated immunity plays a greater part than humoral antibodies in protection from infection. Evidence for a local cellular immunity system in the respiratory tract was presented by Waldman and Henney (1971). Using lymphocyte transformation techniques, cell mediated immunity to gonococcal antigen has been shown not to correlate with resistance to re-infection (Grimble and McIllmurray, 1973). Although weak in first episodes of infection, lymphocyte responsiveness to gonococcal antigens is significantly greater after repeated infections (Kearns et al. 1973). The role of cell-mediated immunity in protection from infection remains to be more fully evaluated.

A fifth possibility is that antibody in the secretions is destroyed by the gonococcus. Although secretory IgA is reported as being resistant to the proteolytic action of enzymes such as trypsin and

pepsin (Lindh, 1975), a protease produced by Streptococcus sanguis has been shown to cleave specifically IgA_1 heavy chains at the "hinge" region; IgA_2 subclass molecules are resistant to the action of this enzyme (Plaut, Genco and Tomasi, 1974). In an extension of this work, Plaut and co-workers (1975) demonstrated that N. gonorrhoeae and N. meningitidis produced a similar protease capable of cleaving IgA_1 molecules. The proteolytic activity of this enzyme has been shown to interfere with the antibody activity of the IgA molecule (Plaut, Gilbert and Wistar, 1977). It has been demonstrated that this protease is produced by both pilated and non-pilated gonococci throughout their growth cycles, and that it is detectable in the genital secretions from infected patients (Blake and Swanson, 1978; Blake, Holmes and Swanson, 1979). The significance of this protease in overcoming local humoral antibodies is uncertain. As the enzyme is only active against the IgA_1 subclass, it would be of interest to know what subclass of IgA is present in the genital secretions; IgA_2 molecules predominate in colostrum (Grey et al. 1968), but whether this is the predominant subclass in genital secretions is unknown.

The part played by IgG in protection against gonococcal infection is uncertain. It has been shown that opsonization of gonococci occurs with serum IgG in the absence of complement (Bisno et al. 1975). In the goat mammary gland it has been demonstrated that attachment of the homologous strain to the tissues is inhibited by anti-gonococcal IgG in the mammary secretions, but that there is no interference with adherence of heterologous strains (Ashton et al. 1977).

It is likely that both humoral and cell-mediated immunity play a part in the local response to gonococcal infection, but more studies are required to establish the mechanism of the resistance of the gonococcus to these responses.

II

INDIRECT IMMUNOFLUORESCENT ANTIBODY TEST IN THE DIAGNOSIS OF GONORRHOEA IN WOMEN

The finding that a secretory immune response could be regularly detected in the cervical secretions of women with gonorrhoea prompted an investigation into the feasibility of using the indirect immunofluorescent-antibody test in the diagnosis of gonococcal infection in the female.

At present the diagnosis of gonorrhoea in women is not always simple. The only test available to the clinician which gives results whilst the patients wait in the clinic is microscopic examination of a Gram-stained smear of material from the urethra and cervix. This technique has a sensitivity of only about 60 per cent (Barlow et al. 1976). Although the specificity of Gram-smear examination of genital material is about 98 per cent, it is much lower when anorectal or pharyngeal secretions are examined. Direct immunofluorescent-antibody techniques have a sensitivity and specificity similar to that of Gram-smear examination (Lind, 1967) and are much more laborious.

The most valuable diagnostic procedure is to culture material from the anogenital and pharyngeal sites. However, culture may not be reliable. Organisms may die on transport to the laboratory or may fail to grow on culture medium; about three per cent of strains of gonococci are susceptible to the action of vancomycin, an antibiotic widely used in the preparation of selective media (Reyn, 1969). Serial sampling of infected sites may be necessary before infection can be identified (Catterall, 1970), and this is particularly so in

the case of pharyngeal gonorrhoea (McMillan and Young, 1978). Demonstrable growth of gonococci on culture medium requires incubation for at least 18 to 20 hours; even by using "delayed" immunofluorescent or rapid carbohydrate utilisation tests, results are not usually available to the physician until about 24 hours after the patient's attendance at the clinic. Clearly a test which is both sensitive and specific, and gives results in a reasonable time would be desirable.

Serological tests lack sensitivity in early gonorrhoea; the infection must have been established for several days before a serological response can be detected (Kellogg and Ballows, 1976). As shown in the present study, significant titres of IgG antibody can be detected in the serum weeks after successful treatment, and their detection cannot be used to diagnose active infection. Immunoglobulin M antibody is found in the serum of only about half of infected men and women, and its detection therefore is of little value.

Gonorrhoea is an infection of the mucosal surfaces of the body, and in the first part of the present study, it was shown that IgA and IgG are detectable in the cervical secretions from most infected women. The IgA response appears early, and becomes undetectable within a few weeks of successful treatment. Secretion IgG, however, appears somewhat later, is apparently a less specific marker of infection, and persists much longer after treatment than IgA.

When the indirect immunofluorescent-antibody test was applied to secretions from women consecutively attending the Glasgow clinic, the sensitivity was found to be only 84 per cent; the specificity however, was about 90 per cent. Although the results were better than those obtained by microscopy of a Gram-stained smear, and were available within an hour of specimen collection, the test is too

insensitive and laborious to consider for use in routine clinic practice. The sensitivity might, however, be improved by using a different antigen, for example, a pool of various strains of gonococci isolated in the locality.

A possible application of the test may be in the investigation of women who have taken antimicrobial agents within a week of clinic attendance. The finding of IgA antibody against the gonococcus would prompt an examination of their sexual contacts.

The immunofluorescent test described has the disadvantage in that genital secretions must be examined. In the screening of populations, genital examination is unacceptable. Clearly a serological test would be of value in this situation and it is of interest that Glynn and Ison (1979) have reported the detection by an enzyme-linked immunosorbent assay of specific secretory component antibody in serum from patients with gonorrhoea. It was suggested that this antibody was absorbed from the local sites of production. Further experience is required before conclusions can be drawn regarding the sensitivity of this test.

Although serological tests, performed on serum or secretions may help to identify infected individuals, these tests cannot replace standard microbiological investigations. Only be examining the characteristics of isolated gonococci can the emergence of strains of the organism resistant to antimicrobial agents be monitored, and existing regimens of treatment modified rationally and effectively.

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APPENDICES

APPENDICES

Appendix 1

Standard history and examination sheets.

MALE

Case No..... Date of Attendance..... Marital Status.....

Date of Birth..... Homosexual.....

Past History of S.T.D.....

Antibiotic treatment within preceeding 6 months

Presenting Complaint

Sexual History withing preceding 3 months

(1)
(2)
(3)
(4)
(5)
(6)

Urethral discharge - Mucoid - Mucopurulent - Purulent

Complications -

Inguinal lymph node enlargement - YES/NO Tender YES/NO

Urethral Film

Urethral culture for N. gonorrhoeae
Pharyngeal " " " "
Rectal " " " "

Urethral culture for Herpes simplex virus

Urethral culture for Chlamydia sp.

Comments

FEMALE

Case No. ..... Date of Attendance..... Marital Status.....

Age..... L.M.P. .....

Past History of STD

Obstetric History

Contraception Sanitary Protection.....

Antibiotic Therapy within Preceding 6 Months

Present Complaint

Sexual History within preceding 3 months

Urethral film Urethral culture .....

Cervical film Cervical " .....

*Bartholin's gland film (R)..... Rectal " .....

(L)..... Pharyngeal " .....

Vaginal film

Complications noted

Comments

* If applicable

Appendix 2

Physiological saline

Sodium chloride ("Analar")	8.50 g
Distilled water	to 1 l

Appendix 3

Phosphate-buffered saline, pH 7.4 (PBS)

Solutions required:-

A 0.2 M NaH_2PO_4

Sodium di-hydrogen orthophosphate, dihydrate ("Analar")	31.2 g
Distilled water	to 1 l

B 0.2 M Na_2HPO_4

di-Sodium hydrogen orthophosphate, anhydrous ("Analar")	28.39 g
Distilled water	to 1 l

To prepare PBS, 8.5 g of Na Cl are dissolved in 100 ml of 0.1 M phosphate buffer, pH 7.4, prepared by mixing 9.5 ml of A with 40.5 ml of B and diluting to 100 ml. The resulting solution is made up to 1 l with distilled water.

Appendix 4

Tris-HCl buffer solution for thin-layer gel filtration

Solutions required:-

(i) 0.2 M Tris

Tris (hydroxymethyl) methylamine ("Analar")	24.228 g
Distilled water	to 1 l

(ii) 0.1 M Hydrochloric Acid

To prepare a 0.02 M buffer solution at pH 8.05, 25 ml of solution (i) was mixed with 27.5 ml of solution (ii), and diluted with distilled water to 1 l.

Appendix 5

Preparation of Naphthalene black-labelled albumin

A 4 per cent solution in PBS of human serum albumin was prepared and 100 mg of Naphthalene black 10 B (Searle and Co Ltd, High Wycombe, Bucks) added to each 10 ml. After allowing the solution to stand at 4°C for one hour, unlabelled dye was removed by gel-filtration on a Sephadex G - 25 column, 20 cm x 1 cm. The labelled albumin was eluted with PBS and the fraction concentrated by dialysis against polyethylene glycol 20 M (British Drug Houses, Poole, Dorset).

Appendix 6

Staining of paper replicas of gel filtrates

To fix the separated proteins the filter paper strip was immersed in a bath of sulphosalicylic acid (20 per cent w/v in distilled water) for one minute. Papers were then stained with Coomassie brilliant blue (Raymond Lamb, London) 0.25 per cent w/v in distilled water, for five minutes. After washing in distilled water for five minutes, the papers were dried at room temperature, and the positions of the proteins noted.

Appendix 7

Staining of agarose slabs

The agarose slabs were removed from the surface of the Sephadex, rinsed in distilled water and floated on to a sheet of glass. After drying overnight at 37°C, the agarose was washed in several changes of PBS for 5 hours. Blocks, after washing in distilled water for 10 minutes, were then stained in Thiazine red R (Raymond Lamb, London) for 10 minutes. The slides were then decolorized in acetic acid (1 per cent v/v) for about 20 minutes, and then dried.

Appendix 8

Intensification of precipitation areas on immunodiffusion plates

DOPA reagent

3- (3, 4-dihydroxyphenyl)-L-alanine (British Drug Houses) 4.903 g

Phosphate buffer, pH 7.4, 0.1 M (Appendix 3) to 250 ml

After incubation, the immunodiffusion plates were flooded with DOPA reagent and kept overnight at room temperature. Following a wash in distilled water, plates were ready for reading.

Appendix 9

Conjugation of anti-human secretory component with fluorescein iso-thiocyanate

The method of conjugation was a modification of that described by Nairn (1976).

A solution of fluorescein iso-thiocyanate, (FITC) isomer 1, (British Drug Houses, Poole, Dorset) was prepared by dissolving 1 mg in 2 ml of 0.1 M Na_2HPO_4, and the solution maintained at 25°C in a water bath.

To 2 ml of rabbit antiserum against human secretory component containing 8 mg of globulin, and warmed to a temperature of 25°C, 0.5 ml of 0.1 M Na_2HPO_4 preheated to 25°C, was added with gentle stirring. The solution of FITC was now added dropwise, and the pH of the mixture determined (Whatman-BDH Narrow Range Indicator Paper, pH range 8-10). A 0.1 M Na_3PO_4 solution was used to adjust the pH to 9.5, and the volume of the mixture was made up to 5 ml with 0.145 M NaCl.

Reaction was allowed to proceed at 25°C for 12 hours, and after cooling to 0°C, the solution was centrifuged at 1,500 g for 10 minutes.

Unconjugated FITC was removed by gel-filtration through a column 100 mm x 9 mm of Sephadex G-25 (Pharmacia Fine Chemicals, Uppsala, Sweden), using phosphate buffered saline, pH 7.4, as eluent. The diluted conjugated globulin was concentrated by positive pressure dialysis (Amicon Microfiltration System, Amicon, Lexington, Massachussetts, USA).

The specificity of the conjugated antiserum was confirmed by

applying it to cryostat sections of human small intestine. Only the apical portion of the epithelial cells, known to contain secretory component, and luminal contents gave marked fluorescence.

Serum immunoglobulin response in uncomplicated gonorrhoea

BY

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Serum immunoglobulin response in uncomplicated gonorrhoea

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SUMMARY Sera from 225 men and 140 women were examined by an indirect immunofluorescent antibody technique for antibody reactive with *Neisseria gonorrhoeae*. Antigonococcal IgM was demonstrated at a titre of $\geqslant 16$ in about 45% of infected, but in only 3% of non-infected, patients. Most of this antibody occurred in sera of patients who had been infected for less than 14 days. Antibody of the IgA class was found at a titre of $\geqslant 16$ in over half the infected, but in none of the non-infected, patients. IgG antibody reactive with the gonococcus was found in each infected patient at a titre of $\geqslant 16$ but in only 8% of controls. The mean log titre of this antibody was significantly higher in patients who had been infected for more than seven days than in those whose infection was of shorter duration.

Introduction

Although gonorrhoea is an infection usually confined to the mucosal surfaces of the body, a systemic antibody response may be demonstrated by various methods such as complement-fixation tests, haemagglutination reactions, and indirect immunofluorescent antibody techniques (Ratnatunga, 1971; Ward and Glynn, 1972; Welch and O'Reilly, 1973). The classes of antibody involved in this response have not, however, been clearly defined other than under experimental conditions (Cohen *et al.*, 1969).

It is the purpose of this paper to record the classes of immunoglobulins reactive with *Neisseria gonorrhoeae* which were found in the serum of patients with naturally acquired uncomplicated infection and to examine the effect of treatment.

Material and method

PATIENTS

Sera were obtained from patients (225 men and 140 women) attending the department of venereology at the Black Street Clinic, Glasgow.

Urethral gonorrhoea in men was diagnosed by microscopical examination of a Gram-stained smear and confirmed by culture on selective medium The identity of suspected colonies was confirmed by direct immunofluorescence and sugar fermentation reactions. Material from the urethra was taken for culture from all the male patients and where there had been homosexual contact specimens from the oropharynx and anorectal area were also taken for culture.

Gram-stained smears of urethral and cervical secretions were examined and culture specimens taken from these sites and from the oropharynx and anorectum in all female patients. Gonococcal infection was excluded only if three sets of cultures taken at weekly intervals from these sites gave negative results.

Neither those patients who had had antimicrobial treatment within the six weeks preceding attendance at the clinic nor those with complicated infection were included in the study.

ANTIGEN PREPARATIONS

As gonococcal antigen, strain 9 of *N. gonorrhoeae* as described by O'Reilly *et al.* (1973) was used (kindly supplied by Dr D. S. Kellog, US Center for Disease Control, Atlanta), as it had been shown to contain antigenic characteristics which were common to a variety of gonococcal strains but not shared by other neisseriae. The organism was cultured on a selective medium (Young, 1978). Cultures of *Neisseria meningitidis*, *Neisseria perflava*, *Neisseria lactamica*, and *Neisseria catarrhalis* for use in

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control studies were identified by fermentation reactions. They were obtained from stock cultures held in the Department of Microbiology at the University of Edinburgh.

Colonies were suspended in phosphate-buffered saline (PBS), pH 7·4, and the suspensions diluted until the fluid was faintly turbid. Aliquots were stored at −20°C until required.

A drop of suspension was placed in each well of a Multidot slide (Hendley, Essex, UK) and dried by incubation at 37°C for 10 minutes.

INDIRECT IMMUNOFLUORESCENT TEST

A standard indirect immunofluorescent technique (IF test) was used (Johnson *et al.*, 1978). Fluorescein-conjugated sheep anti-human IgA, IgG, and IgM sera were obtained from commercial sources (Wellcome Reagents, UK) and their specificity confirmed by gel immunodiffusion.

Doubling dilutions of sera, previously inactivated by heating at 56°C for 30 minutes, were prepared and layered on to the prepared slides. After the slides had been incubated at 37°C for 30 minutes and washed in PBS, conjugated antiserum at a dilution of 1/16 was added, and the slides maintained at 37°C for 30 minutes. The slides were then thoroughly washed in PBS and mounted in buffered glycerol (Difco).

Preparations were examined with a Zeiss microscope (Large Universal). After being scanned with a low power objective detail was examined with a ×100 oil immersion lens.

Fluorescence was graded according to the system used by Welch and O'Reilly (1973) as follows: 4+ indicated brilliant fluorescence of all organisms; 3+, well-defined fluorescence of all organisms in the field; 2+, low density fluorescence of at least 75% of organisms; and 1+, occasional fluorescing organisms. Only a 2+ fluorescence or higher reading was recorded as a positive result.

Statistical comparisons were made by the method of binomial probabilities and Student's *t* test.

Results

UNTREATED PATIENTS

Tables 1 and 2 show the results obtained in the indirect immunofluorescent antibody test applied to the serum from infected and non-infected men and women.

IgM

A titre of ⩾16 of IgM reactive with *N. gonorrhoeae* was found in 44·8% (56/125) of men with untreated gonorrhoea but in only 3% (3/100) of non-infected men ($P<0.001$ by the method of binomial probabilities). Similarly in 45·7% (32/70) of infected women and in 2·9% (2/70) of non-infected women antigonococcal antibody of this class was found at a titre of ⩾16 ($P<0.001$).

Table 1 *Immunoglobulin classes of antibody against* Neisseria gonorrhoeae *in sera from men with untreated gonorrhoea and from non-infected patients*

		IgM titre					*IgA titre*					*IgG titre*				
Patients	*No. of sera*	⩽8	16	32	64	128	⩽8	16	32	64	128	⩽8	16	32	64	128
Infected																
For 1–7 days	52	19	19	9	5	0	19	14	12	6	1	0	4	13	18	17
8–14 days	45	25	11	8	1	0	17	12	13	3	0	0	0	0	10	35
15–21 days	14	11	3	0	0	0	12	1	1	0	0	0	0	0	4	10
22–28 days	2	2	0	0	0	0	1	1	0	0	0	0	0	0	1	1
⩾29 days	12	12	0	0	0	0	12	0	0	0	0	0	0	0	4	8
Non-infected	100	97	3	0	0	0	100	0	0	0	0	92	8	0	0	0

Table 2 *Immunoglobulin classes of antibody against* Neisseria gonorrhoeae *in sera from women with untreated gonorrhoea and from non-infected patients*

		IgM titre					*IgA titre*					*IgG titre*				
Patients	*No. of sera*	⩽8	16	32	64	128	⩽8	16	32	64	128	⩽8	16	32	64	128
Infected																
For 1–7 days	15	4	5	6	0	0	7	3	4	1	0	1	4	8	2	0
8–14 days	24	7	7	8	2	0	3	9	8	3	1	0	0	0	6	18
15–21 days	19	15	4	0	0	0	10	7	1	1	0	0	0	0	4	15
22–28 days	6	6	0	0	0	0	5	1	0	0	0	0	0	0	2	4
⩾29 days	6	6	0	0	0	0	6	0	0	0	0	0	0	0	2	4
Non-infected	70	68	2	0	0	0	70	0	0	0	0	66	4	0	0	0

In 54·6% (53/97) of men who had been infected for 14 days or less but in 10·7% (3/28) who had been infected for longer the titre of antigonococcal IgM exceeded 8 ($P<0·05$). The titre of IgM antibody was $\geqslant 16$ in 71·8% (28/39) of women infected for less than 14 days but in only 12·9% (4/31) of women whose infection was of a longer duration.

IgA

Antibody of the IgA class reactive with the gonococcus was found at a titre of $\geqslant 16$ in 51·2% (64/125) of men and in 55·7% (39/70) of women. Patients who were not infected (100 men and 70 women) had serum titres of $\leqslant 8$ ($P<0·001$).

When the infection had been present for 14 days or less antigonococcal IgA was found (at a titre of $\geqslant 16$) in the serum of 62·9% (61/97) of men but in only 10·7% (3/28) of men who had been infected for longer ($P<0·05$). Similarly, IgA antibody was found at a titre of >16 ($P<0·05$) in 74·3% (29/39) of women who had had gonorrhoea for 14 days or less but in only 32·3% (10/31) of women whose infection was of longer duration.

IgG

Antibody of the IgG class reactive with the gonococcus was found at a titre of >16 in the serum of all the 125 (100%) men with untreated gonorrhoea but in only 8% (8/100) of men who were not infected ($P<0·001$). In women, this antibody was detected at a titre of >16 in the serum of 98·6% (69/70) of infected patients and in 5·7% (4/70) of non-infected patients ($P<0·001$).

The arithmetic mean of the log titre of IgG reactive with *N. gonorrhoeae* in the serum of men infected for seven days or less was 1·7830 and in patients who had been infected longer it was 2·0289. This is a statistically significant difference ($P<0·001$ by Student's t test). Similarly, the arithmetic mean log titre of IgG antibody in women was 1·4249 and 2·4903 in the serum of women infected for seven days or less and for eight days or more respectively ($P<0·001$).

Naturally occurring IgA reactive with other neisseriae

Table 3 shows the results obtained in the indirect immunofluorescent antibody test with monospecific IgA when applied to sera diluted to 1/16 from patients with gonorrhoea and from non-infected controls.

EFFECT OF TREATMENT ON CLASSES OF ANTIBODY

Table 4 shows the arithmetic mean log titre of antigonococcal antibodies before and at intervals after treatment. The decline in titre of antibodies of the IgM and IgA classes is shown as well as the much more gradual fall in IgG titre.

Discussion

Although indirect immunofluorescent antibody techniques using various antigen preparations have been evaluated as diagnostic tests (Welch and O'Reilly, 1973; Rodas and Ronald, 1974), there have been few studies correlating the distribution

Table 3 *Results of the indirect immunofluorescent antibody test with monospecific anti-human IgA on sera of patients with gonorrhoea and of non-infected controls*

Antigen	*Patients' case no. (and sex)*											
	Infected						*Non-infected*					
	1 (M)	2(M)	3(M)	4(F)	5(F)	6(F)	7(M)	8(M)	9(M)	10(F)	11(F)	12(F)
N. gonorrhoeae	4+	4+	4+	4+	4+	4+	—	—	—	—	—	—
N. meningitidis												
group A	—	—	—	—	—	—	—	—	—	—	—	—
group B	—	—	—	—	—	—	—	—	—	—	—	—
group C	—	—	—	—	—	—	—	—	—	—	—	—
group D	2+	2+	2+	—	2+	1+	—	—	2+	1+	—	—
group E	—	—	—	—	—	—	—	—	—	—	—	—
group 29E	—	—	—	—	—	—	—	—	—	—	—	—
group W135	—	—	—	—	—	—	2+	—	—	—	1+	2+
group X	—	1+	1+	1+	1+	—	1+	1+	1+	2+	—	2+
group Z	—	—	—	—	—	—	—	—	1+	—	—	—
N. lactamica	2+	—	2+	—	—	1+	1+	—	2+	1+	1+	1+
N. perflava	2+	1+	1+	1+	2+	3+	2+	2+	2+	2+	2+	2+
N. catarrhalis	1+	1+	1+	1+	2+	2+	1+	1+	1+	2+	1+	2+

1+ Occasional fluorescing organisms
2+ Low intensity fluorescence of all organisms
3+ Well-defined fluorescence of all organisms
4+ Brilliant fluorescence of all organisms

Table 4 *Arithmetic mean of log titres of antibodies reactive with* Neisseria gonorrhoeae *in the serum of infected patients before and after successful treatment*

	No. of patients	Immunoglobulin class		
		IgM	IgA	IgG
Before treatment				
Men	125	1·108	1·170	1·927
Women	70	1·118	1·183	1·901
7 days after treatment				
Men	62	1·039	1·131	1·869
Women	43	1·120	1·071	1·904
14 days after treatment				
Men	48	0·947	1·029	2·157
Women	41	0·977	1·057	1·894
28 days after treatment				
Men	45	0·903	0·917	1·772
Women	39	0·903	0·911	1·860

of antigonococcal antibodies within the immunoglobulin classes with the duration of infection.

In the present study antibody of the IgM class reactive with *N. gonorrhoeae* was detected at a titre of $\geqslant$16 in 45% of infected men and in 46% of infected women before treatment. The results obtained in women were similar to those recorded by Wilkinson (1975), who found this class of antigonococcal antibody at a titre of $\geqslant$16 in 43% of infected patients. He found similar antibody in only 32% of infected men; the duration of infection was not recorded.

IgM antibody was detectable in the serum of more than half of the men and of two-thirds of the women with untreated gonorrhoea who had been infected for less than two weeks. When infection was of longer duration this antibody was less commonly found at the lowest dilution of serum examined. This early IgM response was observed under experimental conditions by Cohen *et al.* (1969), who also showed that even without treatment those subjects who developed antigonococcal IgM had titres of this antibody which gradually fell over a period of about four months. Few patients in the present study who had been infected for more than 14 days had titres of antibody of $\geqslant$16. Cohen *et al.* (1969), however, used inocula of organisms which were much larger than those which would be acquired in natural infection, and this may partly explain the more gradual decline in IgM activity which they had observed.

The antigonococcal IgA response to infection was similar to that of IgM. Antibody of this class reactive with *N. gonorrhoeae* was found in the serum of about two-thirds of men and of three-quarters of women who had been infected for less than 14 days. When the infection was of longer duration only about 11% of men and 32% of women had this antibody in the serum at a dilution of 1/16 or greater. Similar findings of a rapid decline in serum IgA antibody activity within two to three weeks of acquisition of infection were reported by Cohen *et al.* (1969). The relationship between production of antigonococcal secretory IgA in the mucous membranes and the detection of serum IgA antibody will be dealt with elsewhere.

No naturally occurring IgA reactive with *N. gonorrhoeae* was detected, although natural antibody against *N. perflava* and *N. catarrhalis* was found in infected and non-infected patients as was, less commonly, antibody against *N. lactamica* and *N. meningitidis*, groups D, W135, and X.

Antigonococcal antibody of the IgG class was found in the serum at a dilution of 1/16 or greater of all infected men and of almost all infected women. The presumed false-positive rates were 8% and 6% respectively for men and women. In one study, where heat-labile antigen and antihuman IgG were used in a fluorescent antibody test, the false-positive rate was lower as was the sensitivity (Gaafar and D'Arcangelis, 1976).

IgG antibody activity increased throughout the first week of infection, so that by the second week most patients had antibody at high titres ($\geqslant$64). No obvious decline in antibody activity occurred in the subsequent two to three weeks. These results agree with those obtained by Cohen *et al.* (1969).

Successful treatment of infection in both sexes resulted in a rapid decline in antigonococcal IgM and IgA activity. A much more gradual decline in IgG antibody activity occurred. This presumably reflects differences in the half-life of the antibody classes (IgM, five days; IgA, six days; and IgG, 22 days [Tomasi, 1976]). This gradual decline in IgG antibody activity makes interpretation of diagnostic immunofluorescent test results difficult if the patient has recently been treated for gonorrhoea.

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