IMMUNOFLUORESCENCE IN THE DIAGNOSIS OF GONORRHOEA

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SUMMARY: A direct immunofluorescent (IF) staining method was used to demonstrate *Neisseria gonorrhoeae* in smears of patients' secretions. This method, Gram stained smears and cultures were used to investigate soldiers presenting with urethritis and 106 out of 184 men were diagnosed by one or more methods as suffering from gonorrhoea. Results of IF tests alone were positive in 2 cases but they were negative in 3 cases who had positive results by Gram stained smears and cultures. It is concluded that in men direct IF stained smears are less valuable in the diagnosis of gonorrhoea than in women. The IF technique was also used to investigate prostatic secretion three months after treatment for gonococcal urethritis.

Immunofluorescent tests were helpful in the investigation of female contacts of servicemen who had recently had gonorrhoea. Five out of 14 such women had positive results by IF stained smears alone.

There were no difficulties in starting the technique in Singapore and it is considered that it was as satisfactory there as it had been in London.

Introduction

Deacon and his colleagues (Deacon et al 1959, Deacon et al 1960) were the first to demonstrate that *Neisseria gonorrhoeae* can be stained by the immunofluorescent method. Since then there have been many reports on this technique as noted by Reyn in her review published in 1969.

Thin (1970) reported a direct (or immediate) IF staining technique used to investigate women who had recently had sexual contact with a man suffering from gonorrhoea. This technique gave more positive results than Gram stained smears or cultures and others have reported similar results (Fry and Wilkinson 1964, Mouton 1966, Gallwey, Nicol and Ridley 1967). We applied the same IF technique to investigate soldiers presenting with urethritis in Singapore. In some cases prostatic secretion obtained during follow-up after treatment was examined. Female contacts of a few men were also investigated. The object of this paper is to report the findings obtained.

Materials and methods

Optical equipment and staining

The microscope used for this work was the Gillet and Sibert Conference Research instrument previously described (Thin 1970). Staining materials and methods were also identical.

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Specimens

Specimens of secretion for IF staining were collected from the urethra in men and from urethra and cervix in women, and thin smears were made on clean glass slides. Material for Gram stained smears and cultures was taken from the same sites in all cases. Material for culture was collected on charcoal swabs which were placed in Stuart's transport medium; they were plated on to McLeod's chocolate agar (without antibiotic supplements) the same day. Positive cultures were identified by the typical colonial appearance, positive oxidase reaction, and sugar fermentation tests.

Initially, 48 consecutive female patients attending the clinic were studied but positive results were only obtained in women known to be gonorrhoea contacts. Thereafter, IF tests were reserved for these women.

In all cases serum tests for syphilis were done. In men urethral secretion was examined for *Trichomonas vaginalis* and in women vaginal secretion was examined for *T. vaginalis* and *Candida albicans*.

In men who had full prostatic investigations secretion was obtained by careful massage three months after treatment; material was collected for wet smear, IF stained smear, Gram stained smear, and culture. The number of pus cells in the fresh wet smear was counted using a x40 objective. Smears containing clumps of pus and/or 10 cells per field were regarded as abnormal.

Treatment

The routine treatment was probenecid 1 g orally followed after 15 to 30 minutes by benzyl penicillin 5 mega units intramuscularly. Patients with penicillin hypersensitivity were given kanamycin 2 g intramuscularly.

Laboratory results

Immunofluorescent staining had not previously been used at Singapore. Preliminary studies indicated that *N. gonorrhoeae* stained as brightly there as in London (Thin 1970, Thin, Williams and Nicol 1971). No cross reactions were observed with strains of *Staphylococcus aureus*, *Staphylococcus albus*, *B. haemolytic streptococcus*, *Streptococcus viridans* and *Escherichia coli* isolated from the genito-urinary tract, or with *Neisseria catarrhalis* from the throat. Two strains of *Neisseria meningitidis* fluoresced faintly but were readily distinguished from *N. gonorrhoeae*.

Clinical results

Urethritis in men

One hundred and eighty-four consecutive cases of urethritis in soldiers were studied and gonorrhoea was diagnosed by one or more methods in 106. The results of three methods were positive in 91 cases. Two methods gave positive results in 13 cases; IF stained smears and Gram stained smears in 7, IF stained smears and cultures in 3 and Gram stained smears and cultures in 3 cases. The results of IF stained smears alone were positive in 2 cases (Table 1).

Two smears were taken from each of the first 30 cases and stained by the IF technique. They were read 'blind' and the results agreed in each case.

Prostatic investigation

Prostatic investigations were carried out in the cases of 55 patients with gonorrhoea.

Table I
Results in 184 men at Singapore

Result	Numbers	IF smear	Gram stained smear	Culture
Negative	78	—	—	—
Positive	91	+	+	+
	7	+	+	—
	3	+	—	+
	3	—	+	+
	2	+	—	—
Total positive	106	103	101	97

These were all the patients who completed three months follow-up after routine treatment and who had not received any other chemotherapy. None of the prostates were enlarged or tender but 12 with abnormal wet smears and 15 with normal wet smears were soft or irregular. In 22 cases fresh wet smears of prostatic secretion were considered to be abnormal. Results were:—Abnormal wet smear 22, normal wet smear 33, a total of 55. In all cases IF stained smears and Gram stained smears were negative. In 54 cases cultures were negative. In the case of one man with a normal wet smear, normal Gram stained smear and negative IF stained smear, one colony of *N. gonorrhoeae* was cultured. Two days later this patient presented with acute urethritis and investigation showed the presence of gonococci. He admitted to a sexual contact two days before prostatic examination.

Investigations in women

Fifty-five women were investigated. Fourteen had recently had contact with a man suffering from gonococcal urethritis and gonorrhoea was diagnosed at the first visit by one or more methods in each case. The results of IF stained smears, Gram stained smears and cultures were all positive in 5 cases. Immunofluorescent stained smears and Gram stained smears gave positive results in 1 case and IF stained smears and cultures were positive in 3 cases. The results of IF tests alone were positive in 5 cases (Table II). Among urethral specimens, results of 10 IF stained smears, 5 Gram stained

Table II
Positive results in 14 women at Singapore

Numbers	IF smear	Gram stained smear	Culture
5	+	+	+
1	+	+	—
3	+	—	+
5	+	—	—
Total 14	14	6	8

smears and 5 cultures were positive. Among cervical specimens, results of 13 IF stained smears, 7 Gram stained smears and 8 cultures were positive. None of the patients with negative results to all tests was a gonorrhoea contact.

Discussion

Preliminary tests indicated that in Singapore the IF staining technique was as sensitive and specific as it had been in London (Thin 1970, Thin, Williams and Nicol 1971). Reproducibility using duplicate smears was good and no difficulties were encountered starting the method in Singapore.

Among men, there was good correlation between results obtained by IF stained smears, Gram stained smears and cultures. In 2 cases results of IF tests alone were positive and in 3 cases IF tests gave negative results but Gram stained smears and cultures were positive. Among men, Danielsson (1965) found that the direct IF method gave a few more positive results than cultures. On the other hand, Danielsson (1963) and Lind (1967) observed that cultures gave a few more positive findings than IF tests. Lind (1967) also reported that Gram stained smears gave considerably fewer positive results than culture or IF staining. These observations and the findings presented here indicate that direct IF staining is less valuable for investigation in men than in women; in several reported series of women IF tests gave more positive results than culture (Fry and Wilkinson 1965; Gallwey, Nicol and Ridley 1967, Thin 1970).

In this small series of women 5 of 14 patients who had recently had contact with a man suffering from gonorrhoea had positive results by IF tests alone. These patients were saved the trouble of repeated examination to establish the diagnosis and in this respect the method was most useful. No case of gonorrhoea was found among the other women examined by this method.

Abnormal wet prostatic smears were present in 22 of 55 men examined. The only other abnormal prostatic finding was a single positive culture which was ascribed to incubating gonococcal urethritis. These findings contrast with those of Danielsson and Molin (1971) who studied prostatic secretion from 33 men two to six weeks after treatment for gonococcal urethritis. They reported that a number of cases had positive results by IF tests and Gram stained smears, though none had positive culture results. Danielsson and Molin (1971) considered that their findings indicated invasion by gonococci. After describing other cases the same authors (Molin and Danielsson 1970) suggested that gonococci may exist for long periods in the prostate causing asymptomatic prostatitis and leading to the spread of gonorrhoea. Davis (1965) also suggested that gonococci may persist in the prostate for several months after treatment for gonococcal urethritis. However, our findings indicate that gonococci do not remain in the prostate for three months after treatment for gonococcal urethritis.

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