ISOLATION AND SEROTYPING OF *CHLAMYDIA TRACHOMATIS* IN PATIENTS WITH GENITAL INFECTION

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ISOLATION AND SEROTYPING OF *CHLAMYDIA TRACHOMATIS* IN PATIENTS WITH GENITAL INFECTION.

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A dissertation submitted to the Faculty of Graduate Studies of the University of Manitoba in partial fulfillment of the requirements of the degree of

Master of Science

To my husband, Allen.

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ABSTRACT

McCoy cells pretreated with 5-iodo-2-deoxyuridine, an established tissue culture technique, were utilized to isolate *Chlamydia trachomatis* from the genital tracts of men and women attending clinics at the Health Science Centre, Winnipeg. The chlamydial recovery rate was 33.3% in men with nongonococcal urethritis. This was significantly higher than that obtained in men with gonococcal urethritis (7.0%), or in men with no urethritis (4.2%). The chlamydial recovery rate was 18.9% in women specifically requesting a venereal disease check. This was significant when compared with the chlamydial recovery rate of 1.4% obtained in women who had no known contact with a venereal disease or no clinical evidence of a genital infection. Immunotyping of 33 of the 66 chlamydial isolates revealed that types D and F were the most common immunotypes. Immunotypes E, G, I, and J were also present. The results of this study implicate *Chlamydia trachomatis* as an important agent in genital infections in both men and women.

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INTRODUCTION

Chlamydia trachomatis has been established as a pathogen of the human eye and genital tract. It is known to cause ocular diseases such as trachoma, inclusion conjunctivitis, and opthalmia neonatorum, and the venereal disease lymphogranuloma venereum. These agents have biological and antigenic differences. They have been separated into 15 different immunotypes by the micro-immunofluorescence technique (Wang and Grayston, 1975). Types A, B, Ba, and C were associated with trachoma and types D, E, F, G, H, I, J, and K were associated with inclusion conjunctivitis. The lymphogranuloma venereum serotypes were L_1, L_2, L_3.

Early studies showed that parents of affected babies, and adults with inclusion conjunctivitis and their consorts often had chlamydiae in their genital tract and often suffered from genital infections 'non-specific' in nature (Dunlop *et al*, 1966: Schachter *et al*, 1967). These workers postulated that as well as being pathogenic in the eye these chlamydial strains may cause genital infections. Researchers in Great Britain and the United States have isolated chlamydiae in 23-44% of men with nongonococcal urethritis and in 0-7% of sexually active men with no urethritis (Grayston and Wang, 1975). Chlamydiae have been isolated in 18-31% of women attending venereal disease clinics in the United States and Great Britain (Richmond and

Sparling, 1976). In these studies the relationship of factors such as age, clinical symptoms and signs, a previous history of genital infections, the use of oral contraceptives, promiscuity, and the presence of other potentially pathogenic organisms, to the presence of chlamydiae have been investigated. (Grayston and Wang, 1975: Richmond and Sparling, 1976).

The only Canadian studies on isolating chlamydiae from the human genital tract were done by Ford and McCandlish, (1969:1971). Their results did not support the concept that chlamydiae cause genital infections.

The objective of this research was to isolate chlamydiae from the genital tract of males and females and to determine if chlamydiae were associated with genital infections in our study population in Winnipeg. The chlamydial isolates obtained were serotyped by microimmunofluorescence to determine the frequency distribution of the immunotypes isolated in our population.

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LITERATURE REVIEW

A. INTRODUCTION TO LITERATURE

Similarities of morphology, growth cycle, chemical structure, metabolic properties, susceptibility to antibiotics, and antigenic relationships serve to establish the essential characteristics of the genus Chlamydia and separate it from other organisms. The genus Chlamydia has been divided into two subgroups based on metabolic differences and DNA heterogenicity, Chlamydia trachomatis and Chlamydia psittaci (Page, 1968).

Chlamydia psittaci includes the agents of psittacosis and ornithosis and most of the animal pathogens. Chlamydia trachomatis includes the primary human pathogens of the eye and genital tract and the agent of mouse pneumonitis.

The agents of Chlamydia trachomatis are known to cause such diseases as trachoma, inclusion conjunctivitis, and lymphogranuloma venereum in man. A micro-immunofluorescence technique has been devised which allows for the immunotyping of these agents. Using this method, fifteen separate immunotypes have been described (Wang and Grayston, 1975). Serotypes A, B, Ba, C, D, E, F, G, H, I, J and K are the trachoma - inclusion conjunctivitis immunotypes and L, L_2 and L_3 are the lymphogranuloma venereum immunotypes. Complex immunological relationships exist between these serotypes (Grayston and Wang, 1975). The agents causing lymphogranuloma venereum are known to be biologically as well as antigenically different from the trachoma - inclusion conjunctivitis agents (Kuo et al, 1972).

Under natural conditions the trachoma agents have a restricted host and tissue range. In nature, they have been shown to infect the epithelial cells of the conjunctiva, genital tract and rectum in humans.

Trachoma is an ocular disease that is endemic in areas with low standards of personal hygiene and general living conditions. It is usually acquired in childhood and if untreated, it tends to run a progressive course which may lead to a total loss of vision. The major diagnostic features include upper tarsal follicular hypertrophy, pannus, and conjunctival scars. Secondary bacterial infection is frequent and may play a role in the protraction of the clinical disease. Trachoma is known to be the greatest single cause of blindness in the world (Dawson, 1975). Inclusion conjunctivitis is a self-limiting disease. Follicles develop principally on the lower tarsal conjunctiva and there is no formation of scars or pannus (Dawson, 1975).

Biologically the agents which cause these two diseases are indistinguishable. It has been suggested that the host response to the chlamydial infection is important in pannus formation (Wang and Grayston, 1967). Trachoma would be the disease form developing in an individual sensitized by previous contact(s) with a chlamydial agent, while classical inclusion conjunctivitis would be the consequence of a single exposure (Tarizzo et al, 1967).

B. RELATIONSHIP OF OCULAR TO GENITAL CHLAMYDIAL INFECTIONS

The association of infection of the conjunctiva and the genital tract has been known since 1879, when Neisser demonstrated the gonococcus in conjunctival material from newborn babies suffering from conjunctivitis and in genital material from adults. The venereal nature of N. gonorrohoeae ocular infections is now well established.

The first demonstration of the nature of genital to eye transmission with trachoma agents occurred in 1909 when Halbersataedter and Von Prowazek described the presence of identical cytoplasmic inclusions in conjunctival scrapings of neonates with abacterial conjunctivitis and in the cervical scrapings from their mothers. At the time of these findings, Halbersataedter and Von Prowazek erroneously suggested that the agents in trachoma and inclusion conjunctivitis were probably biologically distinct agents because of the extreme differences in the courses of the two diseases.

The venereal nature of the organism came under more intensive investigation relatively recently when isolation techniques for the trachoma agent became available (1957).

C trachomatis has been isolated from babies suffering from abacterial conjunctivitis, from their mothers suffering from genital tract infections, and from their fathers suffering from urethritis (Dunlop et al, 1966). Therefore, it was postulated that the organism was probably acquired at the time of passage through the birth canal. At this time it was suspected that the genital tract infections of the parents might have been due to the presence of chlamydial agents.

Further research into the relationship between ocular chlamydial infections and genital infections was done by studying the relationship between adult conjunctivitis and genital isolations of chlamydiae. Both Vaughan-Jackson et al, (1972) and Schachter et al, (1967) described the venereal nature of inclusion conjunctivitis. Not only was a correlation found between isolations of chlamydiae from the genital tract of adults suffering from ocular chlamydial infections, but an association between abnormalities of the genital tract and the presence of chlamydial agents was described.

Utilizing the microimmunofluorescent technique, the immunotypes have been described for chlamydial isolates from both ocular and genital sources (Grayston and Wang, 1975). Immunotypes A, B, Ba, and C are the predominant types found in populations of endemic trachoma. The types D through H are considered the genital strains. They have been isolated from genital sources in both areas in which trachoma is endemic and sporadic.

Table I summarizes the results of immunotyping trachoma - lymphogranuloma venereum isolates from the eyes and genital tracts of 453 persons around the world (Grayston and Wang, 1975). The ocular serotypes found in areas where trachoma is sporadic show a distribution similar to that of the genital isolates. This was found to be true regardless of the clinical diagnosis of the ocular infection. This is not surprising considering the relationship between ocular infections and genital infections previously described.

TABLE I

IMMUNOTYPING OF TRACHOMA - LYMPHOGRANULOMA VENEREUM ISOLATES

Isolates	A	B	Ba	CJ	ED	GF	H	I	K	L_1	L_2	L_3
Ocular strains												
Trachoma endemic	10	25	12	79	4	1						
Trachoma nonendemic		1	3	3	26	11	1	4	1			
Genital Strains		11		9	113	76	16	12	17	3	12	3

(Grayston and Wang, (1975))

Two cases of chlamydial ocular infections associated with genital tract transmission of trachoma organisms were studied using micro-immunofluorescence (Grayston and Wang, 1975). One serotype was isolated from a male with adult onset trachoma. He had had an episode of urethritis prior to the onset of the ocular disease. His wife had a history of increased vaginal discharge. Cervical follicular erosion was seen upon examination, and chlamydiae were isolated from her genital tract. The isolates from the patient's ocular infection and from his wife's genital tract were found to be the same serotype, type D. In the other case, a woman gave birth to a child who had neonatorum inclusion conjunctivitis. Chlamydiae of serotype E were isolated from the ocular infection. The same serotype was isolated from the genital tract of the child's mother, even though on examination there was no detection of genital abnormalities. This finding of identical serotypes from genital infections and from the associated ocular infections strengthened the suggestion that the genital tract was the reservoir for ocular infections.

Research with the agent of guinea pig inclusion conjunctivitis (a member of the species Chlamydia psittaci) has shown a similar oculo-genital relationship. It has been shown that this agent can be sexually transmitted (Mount et al, 1973) and that chlamydial ocular infections are a risk to newborns of females with genital infections (Mount et al, 1972).

C. NONGONOCOCCAL GENITAL INFECTIONS IN MALES

Venereal diseases are becoming an increasing health problem. Table II shows the statistics for venereal diseases of males in Great Britain for 1974. The most prevalent diseases are non-specific genital infections, often referred to as nongonococcal urethritis. Since 1967 statistics from Great Britain have shown that nongonococcal urethritis is more common than gonococcal urethritis.

The diagnosis of nongonococcal urethritis is made by a process of elimination. Urethritis is diagnosed as nongonococcal urethritis if no N gonorrhoeae can be demonstrated by gram stain or conventional isolation techniques.

In the United States, several studies showed that approximately one-half of the men with urethritis attending venereal disease clinics had nongonococcal urethritis (Jacobs et al, 1975: Volk et al, 1974).

In Canada current epidemiological tabulations of reportable infectious diseases do not include nongonococcal urethritis. Therefore, the problem has not been well defined. But through personal communications with clinicians in Winnipeg, it has been learned that nongonococcal urethritis constitutes a substantial proportion of the venereal disease cases.

Throughout the years many different agents have been implicated as possible pathogens in the etiology of nongonococcal urethritis. T-mycoplasma and Chlamydia trachomatis are two organisms which have been intensively studied. The role of T-mycoplasma has come into dispute since some studies have shown similar percentages of isolations

TABLE II

STATISTICS FROM THE DEPARTMENT OF

HEALTH AND SOCIAL SECURITY, 1974

Diagnosis	Total	Males
Gonorrhoea	58,139	37,361
Syphilis	2,278	1,906
Chancroid	48	38
Lymphogranuloma venereum	46	38
Granuloma inguinale	11	6
Non-specific genital infections	84,213	69,307
NSGI with arthritis	414	392
Trichomoniasis	19,011	1,420
Candidiasis	32,457	5,236
Scabies	2,742	2,239
Pediculosis pubis	4,936	3,582
Genital herpes	5,245	3,516
Genital warts	18,733	12,451
Genital molluscum	666	461
Other conditions requiring treatment	35,672	24,732
Other conditions not requiring treatment	83,547	52,422

(Br. J. Vener. Dis., 1976 52:351)

for T-mycoplasma in both sexually active men with no urethritis and men with nongonococcal urethritis (McCormack et al, 1973).

Holmes et al, (1975) studied men attending a venereal disease clinic in an attempt to define the etiology of nongonococcal urethritis. Isolation attempts were made for several organisms: N gonorrhoeae, C trachomatis, M. hominis, T-mycoplasma, H. hominis and Cytomegalovirus. Of these organisms, only C trachomatis was isolated significantly more often from men with nongonococcal urethritis than from men with no urethritis.

There have been a considerable number of studies on C trachomatis infections of the male genital tract. Table III summarizes the results of different investigations for the presence of C trachomatis in urethral specimens obtained from men with nongonococcal urethritis, from those with gonococcal urethritis, and from the control group with no urethritis.

The study by Ford and McCandlish (1971) done in Vancouver, British Columbia is the only Canadian work published. The reason for the very low isolation of Chlamydia (5.3%) found in men with nongonococcal urethritis is not readily apparent. In other studies (Table III) the percentage of chlamydial infections varied between 23-57% in men with nongonococcal urethritis, 11-23% in men with gonococcal urethritis, and 0-7% in men with no urethritis.

The differences in successful isolations of chlamydiae may be due to differences in the selection of the study population. They may also be due to differences in handling and processing the specimen.

The recovery of C trachomatis from urethral specimens may depend

TABLE III

SUMMARY OF RESULTS FROM STUDIES

OF THE ASSOCIATION OF URETHRITIS IN MALES

WITH ISOLATION OF *CHLAMYDIA TRACHOMATIS*

Location of Study	Diagnostic Category			Reference
	NGU[1]	GU[2]	NU[3]	
Vancouver (Canada)	151 (5.3%)	---	---	Ford *et al*, (1971)
Washington (USA)	35 (23%)	32 (16%)	---	Philip *et al*, (1971)
London (England)	135 (36.3%)	---	31 (0)	Oriel *et al*, (1972)
London (England)	99 (45%)	---	---	Dunlop *et al*, (1972)
Bristol (England)	103 (39%)	99 (32%)	92 (5%)	Richmond *et al*, (1972
London (England)	---	44 (25%)	---	Oriel *et al*, (1975)
Seattle (USA)	113 (42%)	69 (19%)	58 (7%)	Holmes *et al*, (1975)
San Fransisco (USA)	76 (35%)	18 (11%)	57 (0)	Schachter *et al*, (1975)
London (England)	240 (52%)	141 (25%)	74 (4%)	Oriel *et al*, (1976)

1. nongonococcal urethritis
2. gonococcal urethritis
3. no urethritis
4. number cultured (% chlamydial isolations)

on the technique used for specimen collection, on conditions under which and duration of time that the specimens were stored before inoculation of the cell monolayers, and on the sensitivity of the system utilized for isolation C trachomatis. Dunlop et al, (1972) have stated that an endourethral curette or an endourethral cotton-wool tipped wire swab yields a greater percentage of C trachomatis isolates than a urethral meatal cotton-wool swab. Yet Oriel et al, (1972) found no significant difference in the percentage of isolations obtained using an endourethral curette or a cotton-wool swab of the urethral meatus. No studies have been conducted on the efficiency of the different transport media used in various studies. One factor which may have affected the isolation of C trachomatis in these investigations is the difference in handling the specimens prior to testing. Schachter et al, (1975) and Oriel et al, (1976) routinely processed the specimens the same day they were obtained. In the other studies, the specimens were frozen at -70° C until they were tested. Reeve et al, (1975) suggested that the immediate freezing of the specimen at -70° C or storage of the specimen at $+4^{\circ}$ C for over forty-eight hours may reduce the isolation of C trachomatis by as much as 20%.

The technique for chlamydial isolation used in these studies was based on the McCoy tissue culture method that was first described by Gordon and Quan in 1965. This technique required irradiating the cells before inoculating them with the specimen. Two alternative methods of pretreating the cells have been described: 5 - iodo - deoxyuridine (Wentworth and Alexander, 1974), and Cytochalasin B (Sompolinsky and Richmond, 1974). Both these methods have been shown to be as sensitive as irradiation. Hobson et al, (1974) suggested that the use of irradiated McCoy cells was not necessary as non-irradiated McCoy cells were found just as sensitive. Nayyar et al, (1976) using non-treated McCoy cells in their study of chlamydial genital infections in women, obtained results comparable to those obtained in studies using treated McCoy cells. Johnson and Harper, (1975) claimed the contrary was true.

Even with the differences in these studies, the majority of investigations support the proposition that C trachomatis is an important agent in nongonococcal urethritis. It has been demonstrated that the agent exists in a significant proportion of men with nongonococcal urethritis, (23 - 57%). In comparable groups of men with no urethritis the percentage of isolation was very low, 0 - 7%.

C trachomatis has been isolated in 11% to 32% of men suffering from gonococcal urethritis. Several investigators have noted that men who had gonococcal urethritis and were positive for isolation of chlamydiae are more liable to develop post-gonococcal urethritis than men who did not have evidence of chlamydial infections (Richmond et al, 1972: Oriel et al, 1975: Holmes et al, 1975). This association of the development of post-gonococcal urethritis with the presence of C trachomatis is further support for the suggestion that chlamydiae are pathogenic in the urethra of males.

The pathology of C trachomatis in the male urethra has been studied by Dunlop et al, (1972) in men with nongonococcal urethritis. In only three nongonococcal urethritis cases follicles resembling those seen in the conjunctiva of chlamydial ocular infections were found. All three patients had chlamydial genital isolates. Papillary congestion was seen in 40 cases. Chlamydiae was isolated in 30 of these 40 cases. In two other cases with chlamydial isolates the urethra meatus appeared normal although purulent urethral secretions were present.

Experimental chlamydial genital infection has not been performed in humans. Urethritis has been established experimentally in the urethra of a male baboon. Digiacomo et al, (1975) described the experimental infection of two baboons with a chlamydial agent (serotype D) initially isolated from an urethral specimen of a man suffering from nongonococcal urethritis. All subsequent isolates from these baboons were of the original immunotype. In one of the baboons lesions in the urethral mucosa were present, however no evidence of urethral discharge or pyuria was observed.

D. CHLAMYDIAL GENITAL INFECTIONS IN FEMALES

In 1909 Halbersataedter and Von Prowazek described cytoplasmic inclusions in smears of cervical scrapings in women who had infants with abacterial conjunctivitis. This was the first suggestion that the agents now recognized as chlamydiae could be demonstrated in the genital tract of women.

Hilton et al, (1972) isolated chlamydiae in 3% of women attending a family planning clinic. These women were sexually active but had only one consort. This low incidence of chlamydiae infections has been confirmed by other workers. Schachter et al, (1975) isolated chlamydiae in 3.5% of 665 asymptomatic women. Burns et al, (1975) reported the isolation of chlamydiae in 4%, Oriel et al, (1974) 2% and Nayyar et al, (1976) 2% of women who had no known contact with a sexually transmitted disease and no clinical or microbiological evidence of disease. Therefore, chlamydiae were not considered part of the normal flora of the genital tract of sexually active women.

Table IV summarizes the chlamydial isolation results in women from six recent studies. Chlamydiae were isolated in 11.9% to 31% of the women attending venereal disease clinics.

Chlamydiae were isolated in 21.6% (Burns et al, 1975) to 34% (Hilton et al, 1974: Oriel et al, 1974: Nayyar et al, 1976) of women who were contacts of males with nongonococcal urethritis.

Oriel et al, (1972) studied the isolation of chlamydiae in women who were contacts of known chlamydiae-positive (by isolation) nongonococcal urethritis

TABLE IV

SUMMARY OF CHLAMYDIA ISOLATION

IN WOMEN IN SELECTED STUDIES

Location	Study population	*Chlamydia* isolation
Seattle (USA) Wentworth *et al*, (1973)	V. D. Clinic	385(21.5%)*
Bristol (England) Hilton *et al*, (1974)	V. D. Clinic	279(31%)
London (England) Oriel *et al*, (1974)	V. D. Clinic	247(18%)
San Fransisco (USA) Schachter *et al*, (1975)	Symptomatic females	604(15.6%)
London (England) Burns *et al*, (1975)	V. D. Clinic	638(11.9%)
Leeds (England) Nayyar *et al*, (1976)	V. D. Clinic	300(20%)

* number of women studied (% chlamydial isolations)

males. Twelve out of 18 of these women had chlamydial isolates. Holmes et al, (1975) obtained chlamydial isolates from 15 out of 24 similarly described women. This was significant when compared to chlamydial isolation rates of one out of 23 (Oriel et al, 1972) and two out of 24 contacts of chlamydial isolation negative males with non-gonococcal urethritis (Holmes et al, 1975).

Holmes et al, (1975) found that the immunotype of the strains isolated from both sexual partners was identical in 11 out of 13 cases.

Oriel et al, (1972) attempted to define the contacts of chlamydial isolation positive nongonococcal urethritis males as either primary or secondary contacts. Chlamydiae were isolated from seven women who were thought to be primary contacts and four who were secondary contacts. There were two cases where the primary contacts were positive and the secondary contacts were negative. Burns et al, (1975) also found that significantly more primary contacts (68%) as compared to secondary contacts (28%) of nongonococcal urethritis males had chlamydial isolates.

Thus it was shown that chlamydiae are sexually transmitted. The difference found between the percentage of chlamydial isolations in primary and secondary contacts of men with a chlamydial genital infection reflects upon transmission of the organism.

In females attending venereal disease clinics there exists a high proportion of mixed genital infections. Wentworth et al, (1973) isolated more than one potential pathogen simultaneously from the cultures of 65% of the female patients attending a venereal disease clinic.

Table V summarizes the data from three studies on the simultaneous

TABLE V

ASSOCIATION OF CHLAMYDIAL ISOLATES

WITH OTHER LABORATORY FINDINGS

Lab. findings Associated with Chlamydiae isolates	Burns *et al*, (1975)	Hilton *et al*, (1974)	Oriel *et al*, (1974)
N. gonorrhoeae	14(18.4%)*	25(29%)	9(20%)
Candida	8(10.5%)	7(8.1%)	1(2.2%)
Trichomonas	3(4%)	6(7.0%)	1(2.2%)
Mixed infection	7(9%)	13(15.1%)	7(15.6%)
Herpesvirus type 2	---	1(1.2%)	1(2.2%)
Genital warts	---	---	2(4.4%)
No other findings	44(57.9%)	34(39.5%)	24(53.3%)
TOTAL	76	86	45

* number of chlamydial isolates (% of total chlamydial isolates)

isolation of chlamydiae with other selected pathogens.

To explain the high association of gonococcal and chlamydial genital infections, Hilton et al, (1974) postulated that the gonorrhoea infection may reactivate a latent chlamydial infection. In their study, 63% of the women who had gonococcal infections also had a chlamydial infection. Other researchers have found slightly lower chlamydiae percentage of isolation in women with gonococcal infections. Oriel et al, (1974) and Burns et al, (1975) obtained chlamydial isolates from 33% and 48% respectively in women with gonococcal infections. No association between chlamydial genital infections and other pathogens (such as trichomonas) has been described. Therefore, the association between N gonorrhoeae and C trachomatis could also be due to the chance occurence of two sexually acquired organisms being found simultaneously.

It has been suggested by Hilton et al, (1974) that hormonal influences may affect the isolation of Chlamydia from women. They found an association between the use of oral contraceptives and an increased isolation of Chlamydia, particularly during weeks one and four of the menstrual cycle. Investigations by Oriel et al, (1974), Burns et al, (1975), and Nayyar et al, (1976) do not provide support for this finding.

It is of some interest that Hilton et al, (1974) found that increased promiscuity in the three months prior to attending the clinic did not significantly increase the percentage of isolation of chlamydiae from women. This was surprising in view of the evidence for the sexual transmission of the organism.

Different researchers have attempted to correlate cervical pathology with the presence of C trachomatis. Schachter et al, (1975b) described a relationship of chlamydial infections to cervical dysplasia that was similar to that found for Herpes Simplex type 2 infections. Hilton et al, (1974) found that chlamydial genital infections were associated with an increased incidence of cervical cytological changes even though the infection was not associated with other symptoms. Oriel et al, (1974) also found that cervical abnormalities were increased in women with chlamydial genital infections. Nayyar et al, (1976) did not find an increase in cervical cytological changes among women with chlamydial genital infections; only 3 of 60 patients were found to have macrofollicles of the cervix. The problem arising in comparative studies of the association of chlamydiae with cervical abnormalities is due to the fact that different investigators used different criteria.

MATERIALS AND METHODS

CLINICAL SPECIMENS

A. Patients

1. Men: Men included in this study were chosen from men attending the Primary Health Care Unit of the Health Sciences Centre. There were two major groups. The first group consisted of 182 men who complained of urethral discharge. The second group of 24 men attended the clinic for a venereal disease check even though they were asymptomatic. Any men who were known to have taken antibiotics in the month preceding their examination were excluded from the study.

2. Women: There were 419 women included in this study. They were women who were attending either the Women's Centre or the Primary Health Care Unit of the Health Sciences Centre. Initially specimens were collected from women attending both the urinary tract infection clinic and the "gyne" clinic at the Women's Centre. After collecting specimens at six of the urinary tract infection clinics, it was decided to discontinue obtaining specimens from this clinic. This decision was based on the finding that the same women attended this clinic each week. There were very few new patients attending. From the "gyne" clinic, women who were having a pelvic examination were included in this

study. Women attending Primary Health Care Unit included in this study were women who came to the clinic for a venereal disease check and/or complaints of vaginal discharge.

B. Specimens for Clinical Tests

Following the examination, the urethral swabs were taken from men providing the patient had not voided in the preceding two hours. If the patient had voided in the preceding two hours, he was asked to return after two hours had elapsed.

1. *C trachomatis*: A calcium alginate swab (Inolex, Ingram and Bell) was used for taking the urethral swab from men. In women a cotton swab was used for taking the endocervical swab. After the taking of the specimen the swab was placed in 1.5 ml of sucrose phosphate transport media as previously described by Gordon *et al*, (1969). This transport media contained 0.2 M sucrose in 0.02 M phosphate. The antibiotics were gentamicin (25 µg/ml) and mycostatin (50 units/ml). The swab was left in the transport media.
2. *N gonorrohoeae*: Cotton swabs were used for collecting specimens from both men and women. All the men had two swabs taken for testing for *N gonorrohoeae*.

The first urethral swab was used to make a smear on a microscope slide for a gram stain. The second was planted directly on a Thayer-Martin chocolate blood split plate. Endocervical swabs were obtained from women and planted on Thayer-Martin chocolate blood split plates. These swabs were taken from any women whom the attending physician suspected might have N gonorrhoeae and also from all women who had not had a swab taken for testing for N gonorrhoeae in the past year.

3. Syphilis: Blood was obtained to serologically test for syphilis. All the men included in the study were tested. Blood was taken from women who were under 65 years of age and were attending for a venereal disease check or a routine check-up.

4. Other specimens: In the male patients a urine specimen was occasionally indicated to confirm sub-clinical urethritis. A urine specimen was obtained from female patients if the woman was attending for a medical check-up or the physician felt it was indicated.

Hanging drops to test for the presence of trichomonas and yeast were done at the discretion of the attending physician.

Pap smears were done on all women who had no record of a Pap smear having been taken in the preceding year, and on women who had atypical findings in a previous smear.

C. Handling the Clinical Specimens

1. C trachomatis: The transport media with the swab was immediately placed at 4°C. Within six hours it was transferred to a - 70°C freezer where it was stored. The specimen was thawed in a 37°C water-bath immediately prior to testing.

2. Other specimens: The slides and plates for testing for N gonorrohoeae, urine specimens, and Pap smears were sent to laboratories in the Health Science Centre. The blood for testing for syphilis was sent to the Cadham Provincial Laboratory, Winnipeg. The hanging drops were read immediately by a hospital technician.

D. Patients' Histories

Histories of all the patients were obtained from their hospital charts. This information was obtained after at least two weeks had elapsed from the date of the original examination to ensure that all pertinent information, such as the bacteriological report, had been filed.

II. LABORATORY METHODS FOR ISOLATION AND IDENTIFICATION OF CHLAMYDIAE

The method of isolation and propagation of chlamydiae utilized was that described by Wentworth and Alexander (1974). For passing the positive isolates into HeLa 229 cells the method of Kuo et al, (1972) was used.

A. Cells and media:

McCoy cells were obtained from E. R. Alexander, University of Washington, Seattle. HeLa 229 cells were obtained from S. P. Wang, University of Washington, Seattle. Both cell lines were routinely subcultured in Autopow minimum essential media containing 10% heat inactivated fetal calf serum, 2 mM l-glutamine, 0.75% sodium bicarbonate (Flow Laboratories), 5 μg/ml gentamicin (Cidomycin), and 25 units/ml mycostatin (Nystatin).

The McCoy cells were split using 0.02% EDTA (disodium ethylenediamine tetracetate dehydrate) in phosphate buffered saline (PBS). The HeLa 229 cells were split with PBS containing 0.02% EDTA and 0.25% trypsin (Grand Island Biological Company).

The media for seeding dram vials with HeLa 229 cells was the same as used in routine subculturing. The media for seeding the dram vials with McCoy cells contained 30 mM glucose and 10 μg/ml 5-iodo-2-deoxyuridine (Stoxil, Smith Kline and French Laboratories).

Inoculated McCoy cells were overlayed with media containing 30 mM glucose, 10 µg rather than 5 µg gentamicin per ml, 50 units rather than 25 units mycostatin per ml, and 20 mM HEPES (B-2-hydroxyethylpiperazine-N-2-ethanesulphonic acid) buffer, pH 7.5. For serial passes of chlamydial isolates in McCoy cells the concentration of gentamicin was increased by 5 µg/ml to a maximum concentration of 50 µg/ml (Wentworth, 1973). This media (minus glucose) was used to overlay infected HeLa 229 cells.

Tissue culture for specimen inoculation was prepared in 1-dram glass vials with polyethylene snap caps (Titeseal, Lab Apparatus Company). A 12 mm circular glass coverslip was placed in the bottom of each vial (Bellco Glass). The McCoy cells were seeded in the dram vials at a concentration of 1.5×10^5 cells/ml/vial. The HeLa 229 cells were seeded in the dram vials at a concentration of 2×10^5 cells/ml/vial.

The HeLa 229 cells were incubated 24 hours and the McCoy cells were incubated 72 hours at 36°C to form monolayers.

B. Pretreatment of cell cultures:

The McCoy cells were pretreated, at the time of seeding the dram vials, with 5-iodo-2-deoxyuridine at a concentration of 10 μg/ml (Wentworth and Alexander, 1974), to enhance the susceptibility of the cells to chlamydial infection.

Both McCoy cells and HeLa 229 cells were pretreated with DEAE-dextran (diethylaminoethyl dextran). After the incubation to form monolayers the growth media was removed from the vials. The media was replaced with 1.0 ml of DEAE-dextran solution (30 μg/ml in saline). This solution was allowed to remain in each vial for 30-45 minutes for the McCoy cells and 45-60 minutes for the HeLa 229 cells. The DEAE-dextran solution was removed before inoculation of the cell monolayers.

C. Inoculation of tissue culture

After removing the DEAE-dextran 0.1 ml of the specimen was added to each of four vials.

The vials were centrifuged at 1500 x g for 1 hour at 25°C. After centrifugation the specimen was removed and 1.5 ml of the overlay media was added to each vial.

Positive and negative controls were processed with each group of specimens. The positive control was a genital isolate (serotype G) obtained from E. R. Alexander, University of Washington, Seattle. The negative controls were cells inoculated with transport media and cells inoculated with growth media.

The infected cell cultures were incubated at 36°C for 72 hours.

D. Staining and examination of tissue culture:

After incubation the media was removed from the vials to be stained: two vials for the initial isolation of chlamydiae in McCoy cells; one vial for the propagation of chlamydial isolates in McCoy cells and one vial for the passage of the chlamydial isolates in the HeLa 229 cells.

The McCoy cells were fixed in methanol-formalin and methanol and stained with iodine as described by Gordon *et al*, (1969). The cells were mounted cell side down on a microscope slide with equal amounts of iodine solution and glycerin.

The HeLa 229 cells were fixed in methanol and stained 10 minutes with each of May Gruenwald and 10% Giemsa. The coverslip was mounted, cell side down, on a microscope slide with perimount.

The coverslips were scanned under 100 x magnification and suspected inclusion bodies were then viewed at 400 x magnification to confirm the morphological criteria characteristic of chlamydial inclusions.

The number of inclusions were counted per coverslip. For coverslips on which there were many inclusions the number was calculated by counting the number of inclusions in ten random microscopic fields taking into consideration there were approximately 55 fields per coverslip at 100 x magnification and 650 fields per coverslip at 400 x magnification.

E. Propagation of Chlamydial Isolates:

Initially all the specimens were passed twice in McCoy cells whether or not inclusions were observed. Seventy specimens which had no chlamydial inclusions on the initial examination were passed. After not finding any inclusions in the second passage of any of these, this policy was discontinued. From this point only specimens in which chlamydial inclusions (or questionable inclusion-like bodies) were observed were passed.

The media was removed from the vials and 0.5 ml transport media was added to each vial. These were frozen at - 70°C and thawed at 37°C to release the chlamydiae. This suspension was used as the inoculum for four vials of McCoy cells. When the inclusion count exceeded 15 inclusions per 400 x magnification the isolate was passed into the HeLa 229 cells.

III. IMMUNOTYPING THE CHLAMYDIAL ISOLATES

The method of immunotyping the chlamydial isolates has been described by Wang et al, (1973). This method requires the production of mouse antisera, directed against the chlamydial isolate, which is tested against prototype trachoma antigens in the micro-immunofluorescence test.

1. Mice: The mice used were five to eight week old out-breed albino mice (#CD-1, Canadian Breeding Farm).
2. Antigen: The pass of the chlamydial isolates in the HeLa 229 cells was used for inoculating the mice. The HeLa 229 cells were harvested by alternate freezing at - 70°C and thawing at 37°C. The vials of each chlamydial isolate in HeLa 229 cells were pooled in a vial containing sterile glass beads, and vortexed for three minutes at medium speed. This suspension was used as the antigenic material for inoculating mice.
3. Production of Antisera: A 27 gauge 1/2 inch needle was used to inoculate 0.5 ml of the chlamydial suspension into the tail vein of each mouse. There were two to four mice inoculated per isolate. Negative controls consisted of:

    (1) uninfected HeLa 229 cell suspension

    (2) sucrose phosphate media control.

After four days the mice were bled by an adaptation of the orbital bleeding technique (Riley, 1960). Large bore capillary tubes (1.5 to 2.0 mm) were cut into lengths of one to two cm. The tip of this short tube was placed into the orbital cavity and the blood was collected in a sterile tube. Using this technique, approximately 1.0 ml of blood per mouse was obtained.

The blood was left at room temperature for one to two hours. The clot was then rimmed using an applicator stick. The tubes of blood were left overnight at 4°C. The next day, the blood was spun twice at 800 x g. to obtain the maximum amount of sera. The sera was then stored at - 20°C.

4. Immunotyping the antisera: The antisera was shipped in dry ice to Dr. S. Wang, University of Washington for immunotyping by the micro-immunofluorescence technique. This micro-immunofluorescence method allows multiple antigens to be tested simultaneously against the same serum. The test antigens were prepared from infected yolk sacs. The test antigens used were C, CJ, J, I, K, E, D, G, and F. Nine groups of antigenic dots were fixed on microscope slides.

Twofold serial dilutions of antiserum (using 0.1 ml) were made in PBS and tested against the groups of antigens. The highest dilution of serum which showed definite fluorescence to each of the antigen dots was considered the end point of the serum to that antigen. Reactions at 1:8 or lower were non-specific.

Using the micro-immunofluorescence test a random sample consisting of 33 of the 66 chlamydial isolates were immunotyped.

RESULTS

A. Laboratory Isolation of Chlamydiae

Initially 3 out of 17 clinical specimens obtained from women were grossly contaminated. Because of this, the antibiotics in the transport media were changed. The streptomycin was replaced with 25 μg per ml of gentamicin and the concentration of mycostatin was increased from 25 units to 50 units per ml. With this change in the antibiotics only two of 402 clinical specimens from females were grossly contaminated on initial testing. One of these contaminated clinical specimens yielded chlamydial inclusions. The four contaminated clinical specimens which did not yield chlamydial isolates from female patients were excluded from the study. None of the clinical specimens from men were lost to contamination.

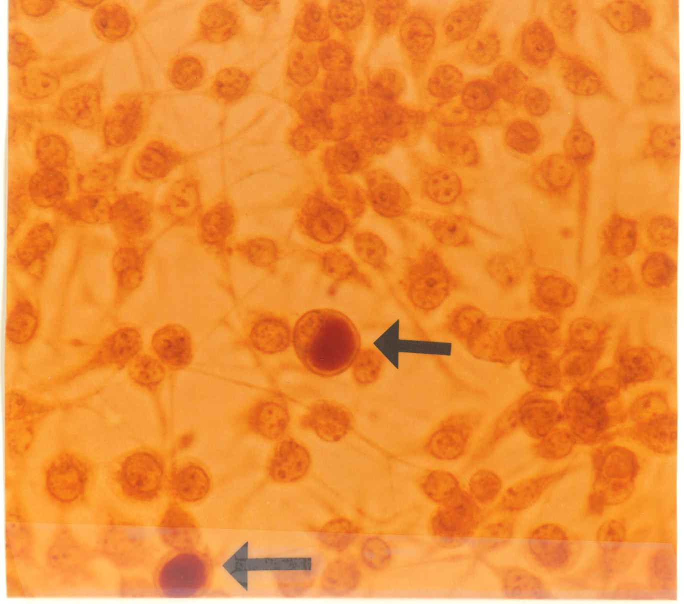
Chlamydia trachomatis inclusions were always found in the cytoplasm of the cell and were usually surrounded by a halo which could be seen by focusing up and down at 400 times magnification. In the iodine stained McCoy cells the chlamydial inclusion stained brown and the cell stained yellow as illustrated in the photograph in Figure 1. In May Gruenwald Giemsa stained HeLa 229 cells the chlamydial inclusions stained blue and the nucleus of the cell stained purple. The photograph in Figure 2 illustrates chlamydial inclusions in HeLa 229 cells. The blue of the cell's cytoplasm was not accurately reproduced in the photograph.

Figure 1

McCoy cells stained with iodine.

→ marks a chlamydial inclusion.

Note the halo that surrounds the inclusion. (magnification 256X)



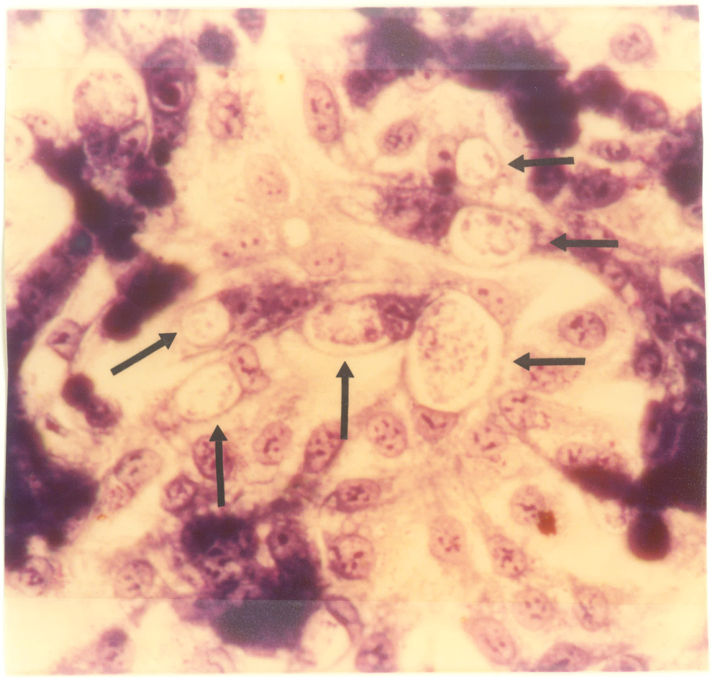


Figure 2

HeLa 229 cells stained with
May Gruenwald Giemsa.
There are six chlamydial inclusions
in the centre of the photograph.
(magnification 256X)

Table VI summarizes the chlamydial inclusion counts obtained on initial isolation in McCoy cells. The initial inclusion count is also related to the immunotype for the 33 isolates immunotyped. For the inclusion count an average of the number of inclusions observed on two coverslips was obtained. There was always close agreement between the two coverslips with respect to the inclusion count. In only one case was one coverslip read as positive for chlamydial inclusions and the duplicate coverslip recorded as negative for chlamydial inclusions. The number of isolates in each immunotype was too small to analyze differences between immunotypes with respect to the inclusion counts on initial isolation.

On the initial isolation of the chlamydiae there was one specimen which was grossly contaminated and cytopathic effect was observed from the contamination. There were two clinical specimens from females which yielded chlamydial isolates in which yeast was visible. The presence of yeast did not appear to affect the McCoy cells and did not hinder the observation of chlamydial inclusions. There were two clinical specimens from women that yielded chlamydial isolates in which a slight cytopathic effect upon the cells was observed.

The chlamydial isolates required between zero to seven passes in McCoy cells to obtain average inclusion

TABLE VI

CHLAMYDIAL INCLUSION COUNT ON

INITIAL ISOLATION

Inclusion count per coverslip*	Total Isolates	Immunotypes D	E	F	G	I	J
1-5	13(19.7%)+	2	1	1	-	-	1
5-15	15(22.7%)	4	1	1	1	1	-
15-50	16(24.2%)	3	-	3	-	1	-
50-300	11(16.7%)	2	2	1	-	-	2
300-650	7(10.6%)	2	-	-	-	1	1
> 650	4(6.1%)	-	-	1	1	-	-
TOTAL	66	13	4	7	2	3	4

* the average of 2 coverslips

\+ No of isolates (% of total)

counts in excess of 15 inclusions per microscopic field at 400 times magnification. The average number of passes required were three to four passes. All the isolates required only one pass in the HeLa 229 cells. On an average the inclusion count obtained in HeLa 229 cells was in the range of 20-30 inclusions per microscopic field at 400 times magnification.

B. Male Data and Analysis

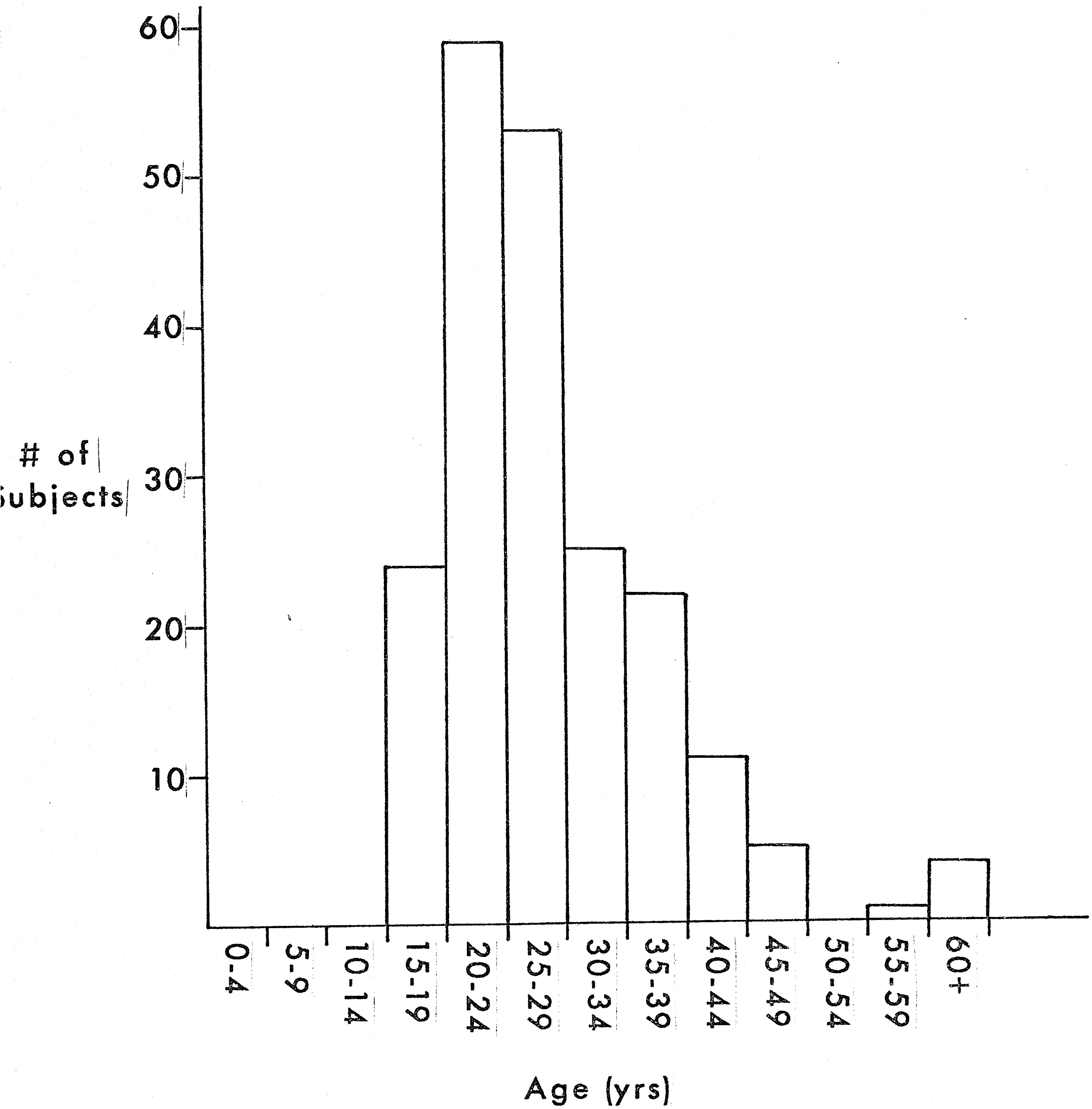
There were 206 men who attended the Primary Health Care Unit included in this study. Twenty-four of these men requested a venereal disease check even though they had no symptoms. The remaining 182 men had complaints of urethral discharge frequently associated with urinary tract symptoms, such as increased frequency, burning sensation on micturition and nocturia.

The age distribution of these men is shown in Figure 3. The range of the age is from 16 to 69 years, with a mean age of 27.9 years (SD 9.1).

Male patients could be separated into three diagnostic categories. The men who attended the clinic with urethritis and subsequently had growth of N gonorrhoeae from a urethral swab were classified as having gonococcal urethritis (GU). There were 86 men in this group. There were 96 men who had urethritis but in whom no growth of N gonorrhoeae was demonstrated. These men were diagnosed as having non-gonococcal urethritis (NGU). The third category consisted of the 24 men who had no clinical urethritis (NU), (regardless of the bacteriological results.) N gonorrohoeae was subsequently isolated from three of the men who had no clinical urethritis.

<u>Figure 3</u>

Age distribution of men in study.


60
50
40
30
20
10
of
Subjects
0-4
5-9
10-14
15-19
20-24
25-29
30-34
35-39
40-44
45-49
50-54
55-59
60+
Age (yrs)

Chlamydia trachomatis (Table VII) was isolated in 33.3% of the patients with nongonococcal urethritis, in 7.0% in men with gonococcal urethritis, and in 4.2% in men attending with no urethritis.

The Chi square test was used to test for significant differences between these isolation rates. The isolation rate of chlamydiae in men with non-gonococcal urethritis (33.3%) is highly significant when compared to that in men with no urethritis (4.2%) ($X^2 = 6.79$, df = 1, $\alpha = 0.01$) and also highly significant when compared to the isolation rate in men with gonococcal urethritis (7.0%) ($X^2 = 17.51$, df = 1, $\alpha = 0.005$). The difference in chlamydial isolation rates found in men with gonococcal urethritis (7.0%) and men with no urethritis (4.2%) is not significant ($X^2 = 0$).

Figure 4 shows the frequency distribution of the ages of the men in the three diagnostic categories. The mean age of the men with nongonococcal urethritis was 28.2 years (SD 9.3), of the men with gonococcal urethritis was 27.7 years (SD 9.1) and of the men with no urethritis, 25.7 years (SD 6.6). Two-tailed t-tests were used to test for significant differences between the mean ages of the diagnostic categories. Comparing the mean age of the group of men with nongonococcal urethritis with that of the group of men with no urethritis ($t = -1.158$, df= 118) no significant difference was found at $\alpha = 0.05$. Also comparing the mean age

TABLE VII

ISOLATION OF C TRACHOMATIS FROM MALES IN DIFFERENT DIAGNOSTIC CATEGORIES

Diagnostic Category	No. of men	Chlamydia trachomatis isolates number	%
nongonococcal urethritis	96	32	33.3
gonococcal urethritis	86	6	7.0
no urethritis	24	1	4.2

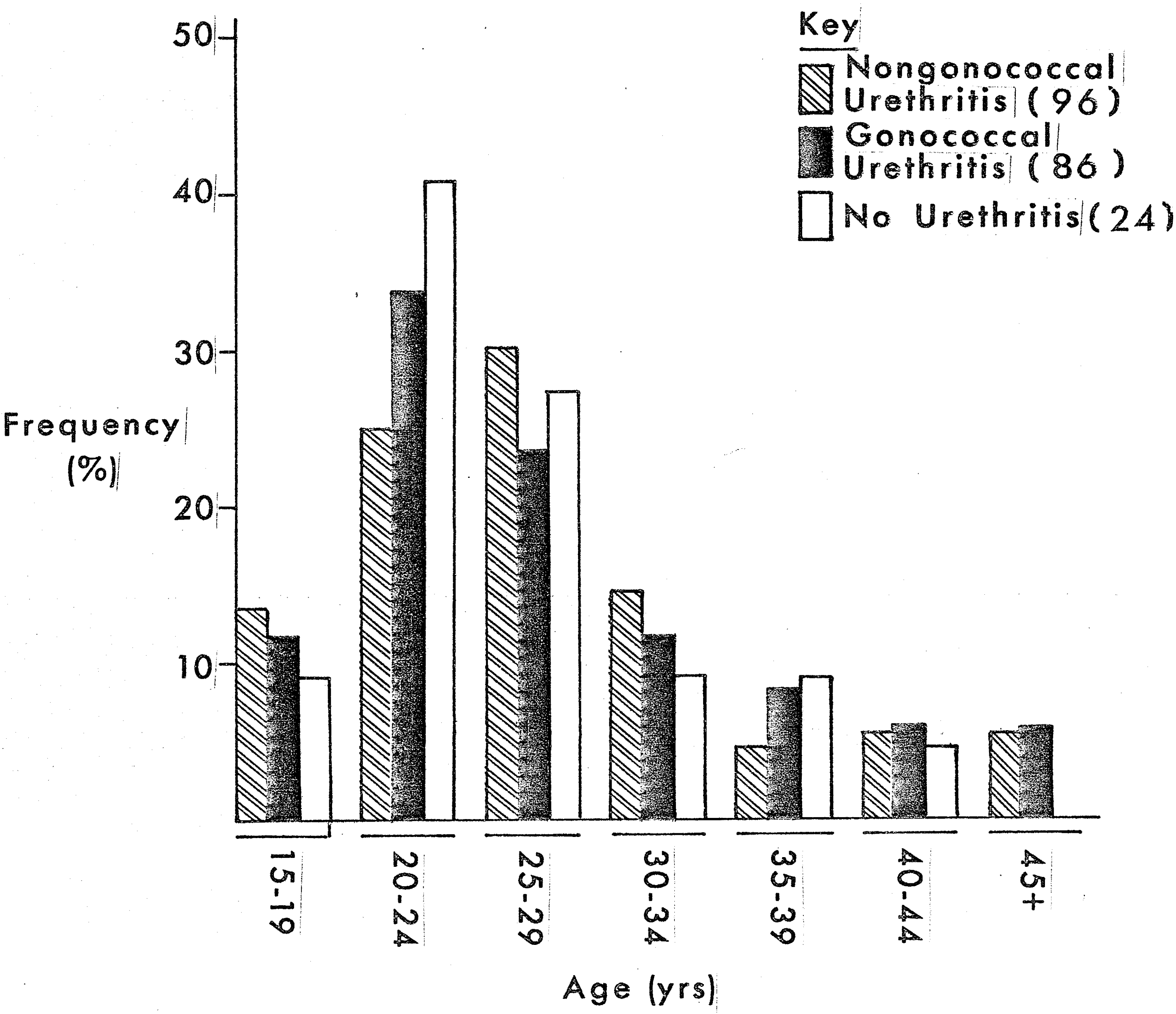
<u>Figure 4</u>

The frequency distribution of the ages of men in the three diagnostic categories:

Nongonococcal urethritis,

Gonococcal urethritis, and

No urethritis.



Key
Nongonococcal Urethritis (96)
Gonococcal Urethritis (86)
No Urethritis (24)
Frequency (%)
50
40
30
20
10
15-19
20-24
25-29
30-34
35-39
40-44
45+
Age (yrs)

of the group of men with nongonococcal urethritis with the mean age of the group of men with gonococcal urethritis ($t = 0.319$, $df = 180$) no significant difference was found at $\alpha = 0.05$. There was no significant difference in the mean ages of the men with gonococcal urethritis and the men with no urethritis at $\alpha = 0.05$ ($t = 0.946$, $df = 108$).

Figure 5 shows the age distributions of the men with urethritis who had chlamydiae isolated and men with urethritis who had no chlamydiae isolated. A two-tailed t-test established no significant difference between the mean ages of these two groups at $\alpha = 0.05$ ($t = - 1.76$, $df = 180$). Figure 6 shows the age distributions of both men with chlamydial isolates and those in whom chlamydiae was not isolated in the nongonococcal urethritis category. A two-tailed t-test was used to test for a significant difference between the mean ages of these two groups ($t = 2.48$, $df = 94$). A significant difference was found at $= 0.05$ but not at $= 0.01$. Therefore the isolation of chlamydiae does not seem to be strongly influenced by the patient's age. This holds true when all the men with urethritis were analyzed. When only the men with nongonococcal urethritis were considered, the effect of the patients' age upon the isolation of chlamydiae was not highly significant.

Figure 5

The age distribution of men with nongonococcal urethritis with positive and negative isolations of chlamydiae.

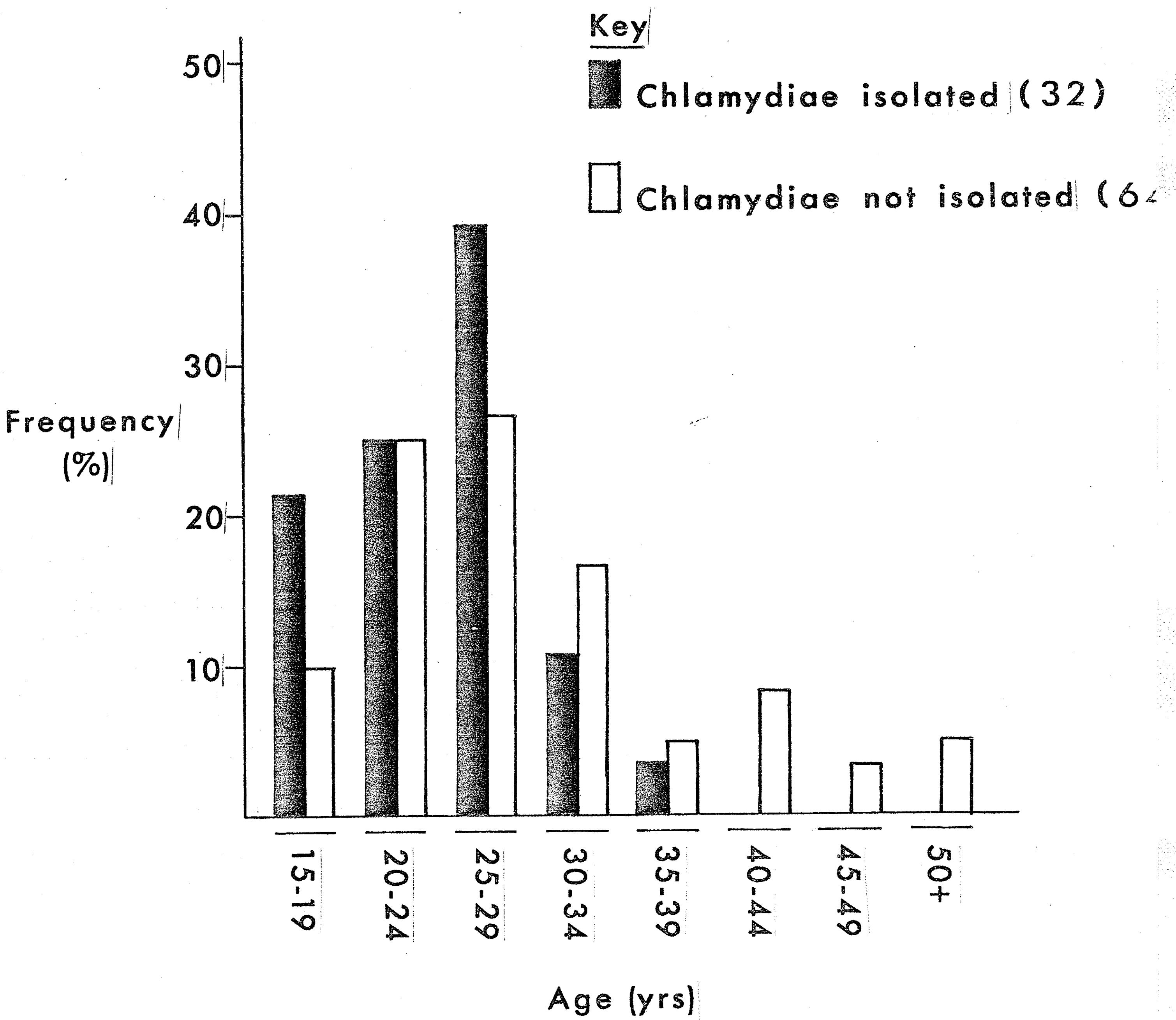

Key
Chlamydiae isolated (32)
Chlamydiae not isolated (6
50
40
30
20
10
Frequency
(%)
15-19
20-24
25-29
30-34
35-39
40-44
45-49
50+
Age (yrs)

Figure 6

The age distributions of men with chlamydial isolates and men without chlamydial isolates in all men with urethritis.

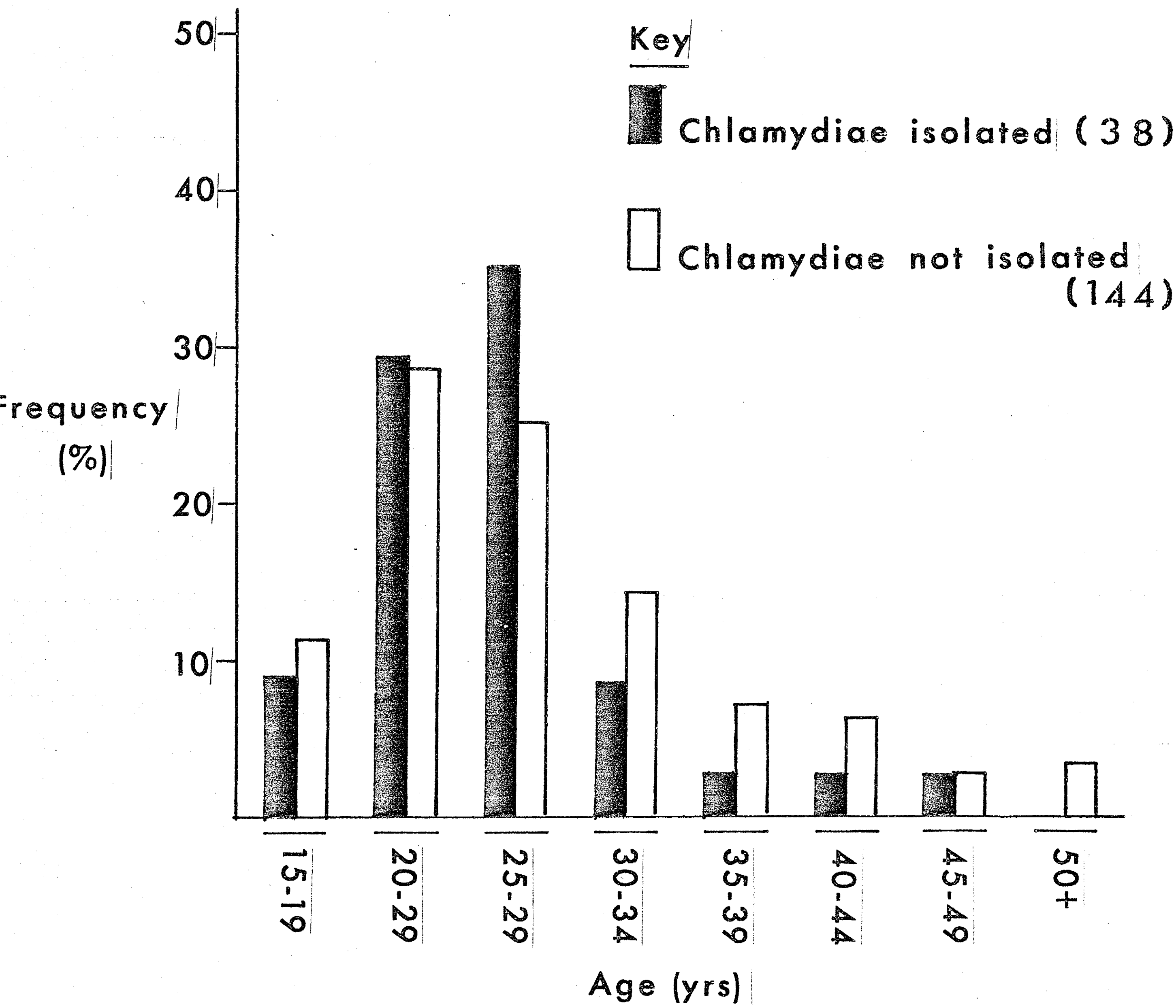

Key
Chlamydiae isolated (38)
Chlamydiae not isolated (144)
Frequency (%)
50
40
30
20
10
15-19
20-29
25-29
30-34
35-39
40-44
45-49
50+
Age (yrs)

Table VIII summarizes the complaints of male patients when they attended the clinic. All the men with nongonococcal urethritis and gonococcal urethritis had complaints of urethral discharge and between 72-77% of these men had urinary tract symptoms such as increased frequency, nocturia, and burning sensation on micturition.

On examination, not all men who complained of urethral discharge had visible discharge (Table IX). All the men with gonococcal urethritis had visible discharge upon examination. Only 89.6% of the men with nongonococcal urethritis had visible discharge at the time of examination. In the remaining 10.4% of these men the urethritis was confirmed by a urinalysis. Urethritis was diagnosed when the urine specimen contained greater than 20 pus cells per high power field.

A Mantel-Haenszel test was used to test for a significant difference between the number of men with visible discharge with nongonococcal urethritis and with gonococcal urethritis. The difference is highly significant at $\alpha = 0.05$ ($X^2 = 8.49$, df = 1). The difference in the number of men with visible discharge who had chlamydial isolates and men from whom no chlamydiae was isolated within the group of men with nongonococcal urethritis was not significant at $\alpha = 0.05$ (Chi square test, $X^2 = 0.35$, df = 1).

TABLE VIII

THE COMPLAINTS OF MALE PATIENTS

ON ATTENDING AT THE CLINIC

Diagnostic Category	No. of Men	Urethral Discharge		Urinary Tract symptoms*	
		No. of men	%	No. of men	%
nongonococcal urethritis	96	96	100%	69	71.9%
gonococcal urethritis	82	86	100%	66	76.7%
no urethritis	24	0	0	0	

* includes symptoms such as increased frequency, burning sensation on micturition, and nocturia

TABLE IX

VISIBLE DISCHARGE IN MEN

ON EXAMINATION

Diagnostic Categories	No. with visible discharge
Nongonococcal urethritis	
Chlamydiae isolated	30/32 (93.8%)*
Chlamydiae not isolated	56/64 (87.5%)
TOTAL	86/96 (89.6%)
Gonococcal urethritis	
Chlamydiae isolated	6/6 (100%)
Chlamydiae not isolated	80/80 (100%)
TOTAL	86/86 (100%)
No urethritis	
Chlamydiae isolated	0/1
Chlamydiae not isolated	0/23
TOTAL	0/24

* Number of men with visible discharge/ total men in that category (%)

Differences have been observed when comparing men with nongonococcal urethritis and gonococcal urethritis with respect to the colour and amount of discharge visible at the time of examination (Figures 7 and 8, respectively). White, clear or yellow was characteristic of discharge seen in most patients with nongonococcal urethritis. Yellow, green, white, or bloody was characteristic of discharge seen from patients with gonococcal urethritis. Thus, it seems that men with nongonococcal urethritis had discharge that was lighter in colour (Figure 7) and less in amount (Figure 8) than men with gonococcal urethritis. The discharge seen in men with nongonococcal urethritis does not seem to differ between the patients who had chlamydial isolates and those with no chlamydiae isolated with respect to the colour (Figure 9) and the amount (Figure 10).

The past histories of the men in the study were evaluated to determine if there existed any differences among men with nongonococcal urethritis, gonococcal urethritis, and no urethritis with respect to having a prior episode(s) of urethritis. Table X summarizes the men with respect to a previous treated episode(s) of urethritis. To differentiate the past urethritis as either gonococcal or nongonococcal was not possible.

Figure 7

The colour of discharge in men with nongonococcal urethritis and gonococcal urethritis.

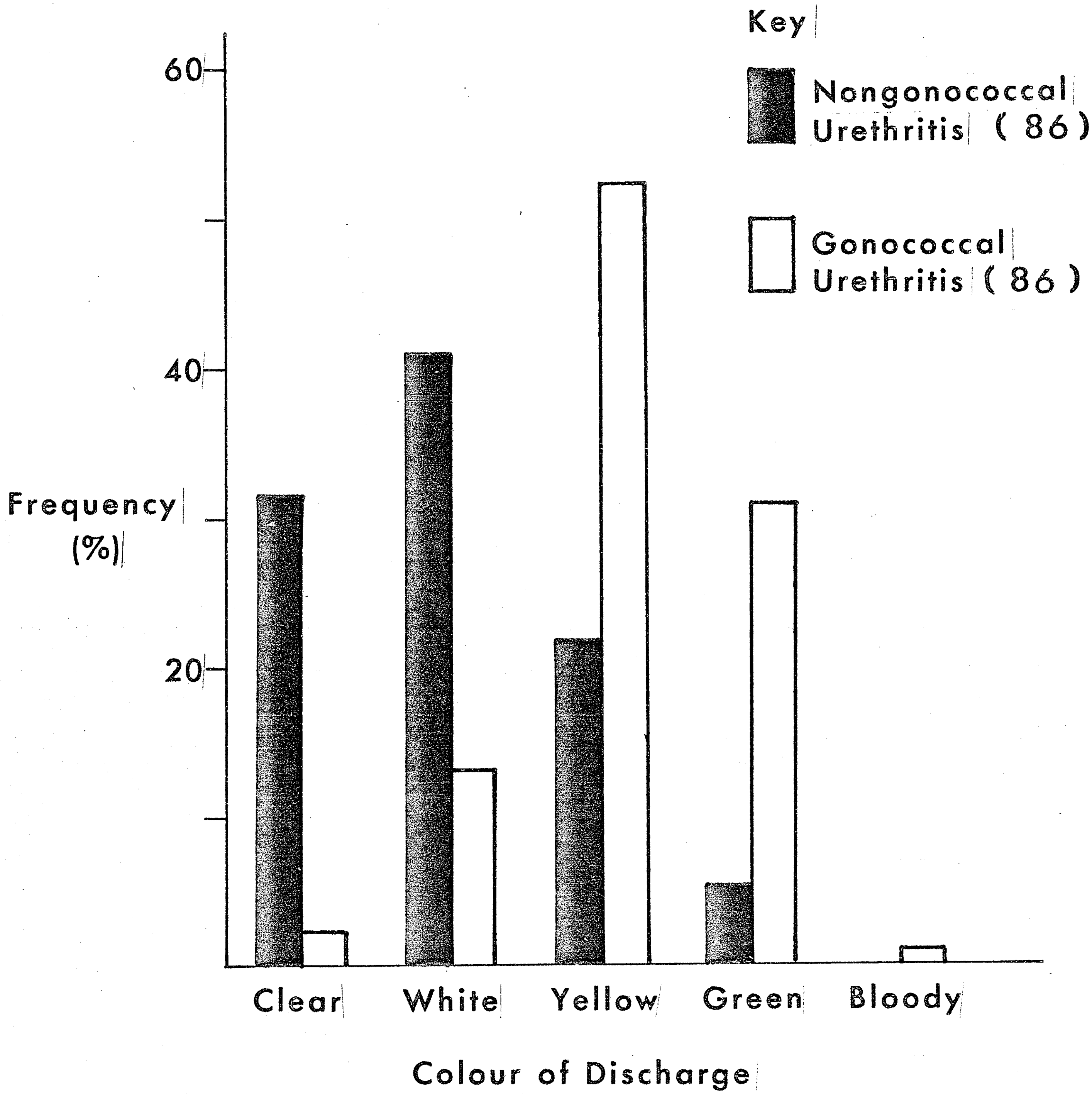

Key
Nongonococcal Urethritis (86)
Gonococcal Urethritis (86)
60
40
20
Frequency (%)
Clear
White
Yellow
Green
Bloody
Colour of Discharge

Figure 8

The amount of discharge in men with nongonococcal urethritis and gonococcal urethritis.

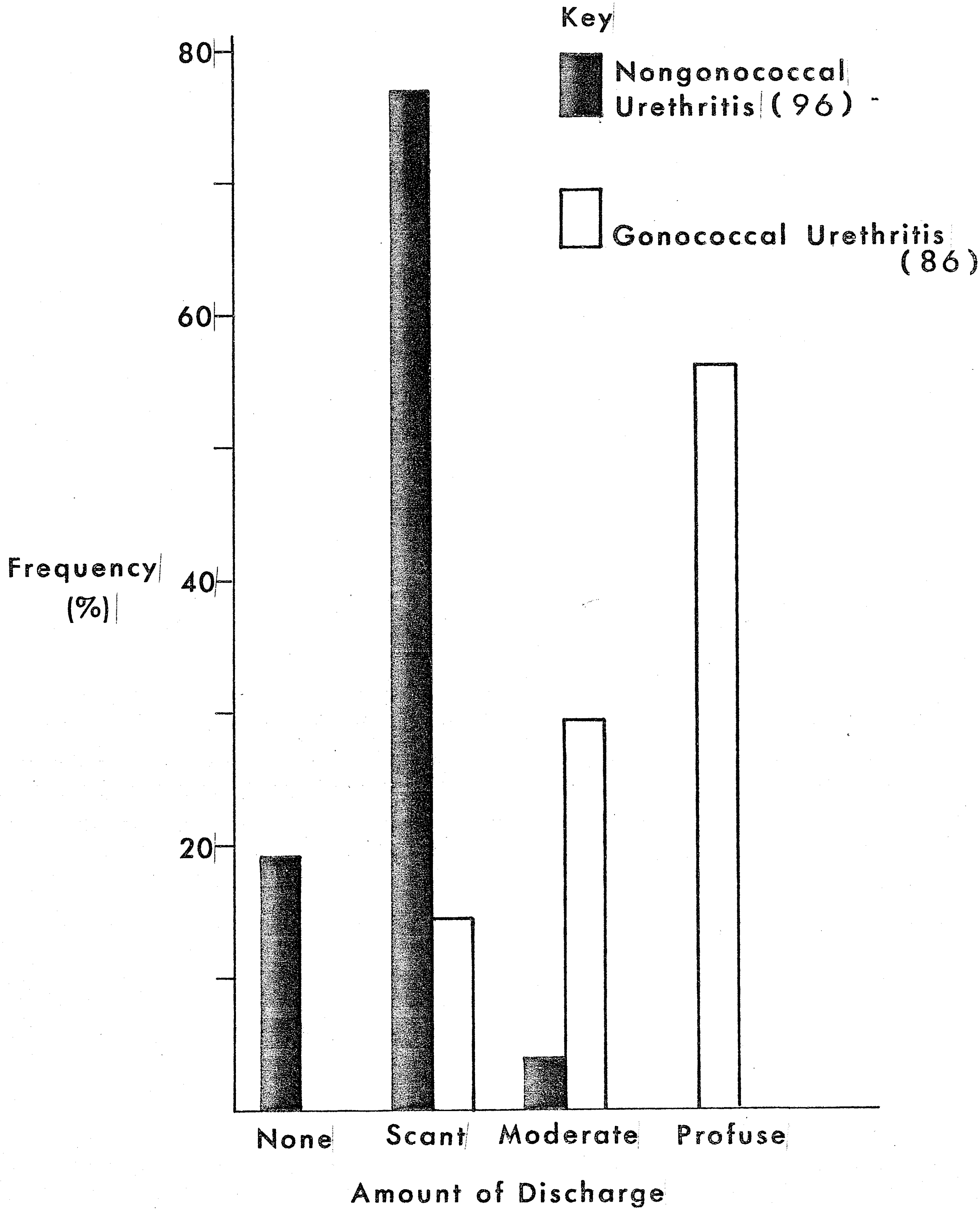

Key
Nongonococcal Urethritis (96)
Gonococcal Urethritis (86)
80
60
40
20
Frequency (%)
None
Scant
Moderate
Profuse
Amount of Discharge

Figure 9

The colour of discharge in men with nongonococcal urethritis with positive and negative chlamydial isolations.

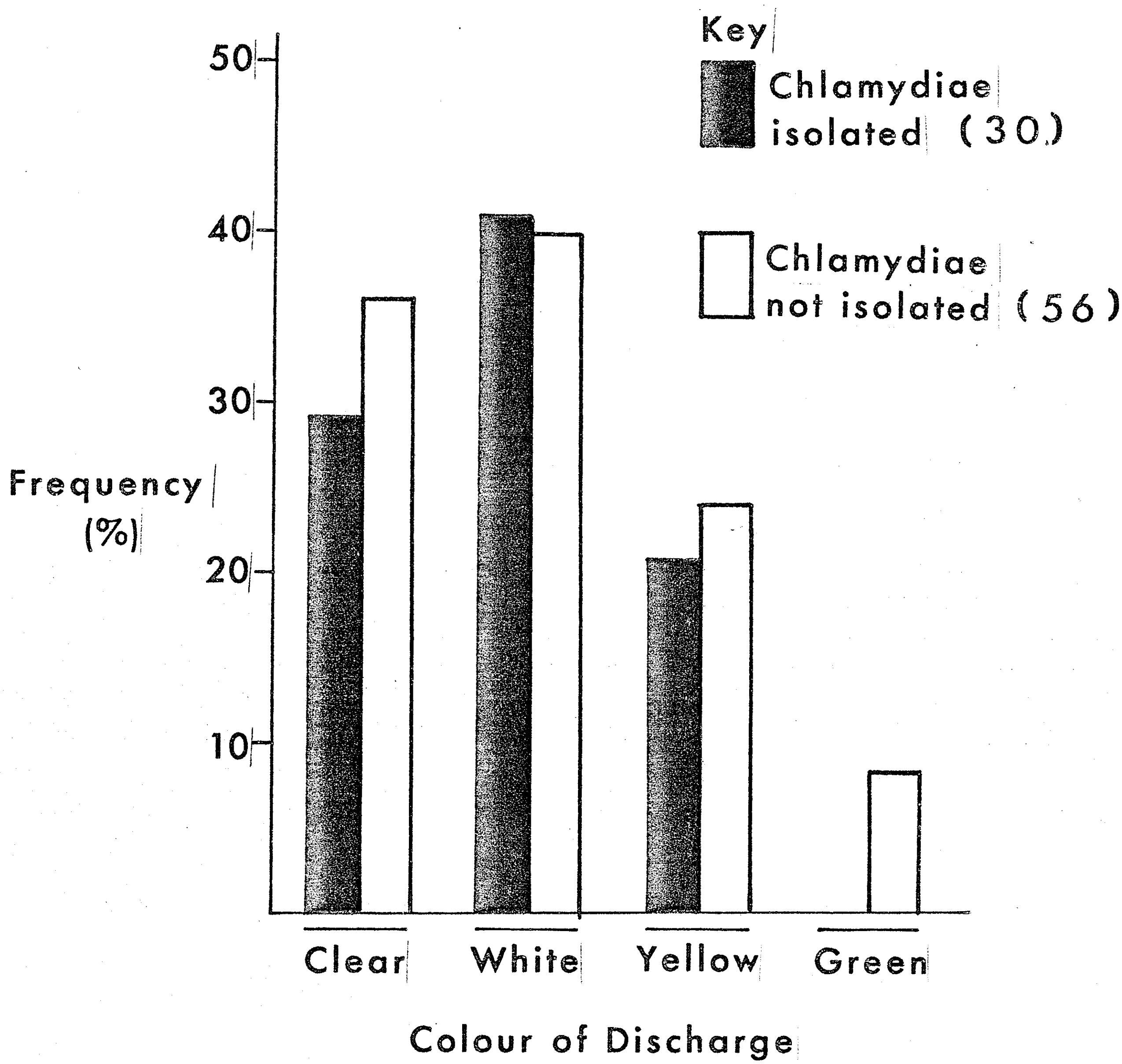

Key
Chlamydiae isolated (30)
Chlamydiae not isolated (56)
50
40
30
Frequency (%)
20
10
Clear
White
Yellow
Green
Colour of Discharge

Figure 10

The amount of discharge in men with nongonococcal urethritis with positive and negative chlamydial isolations.

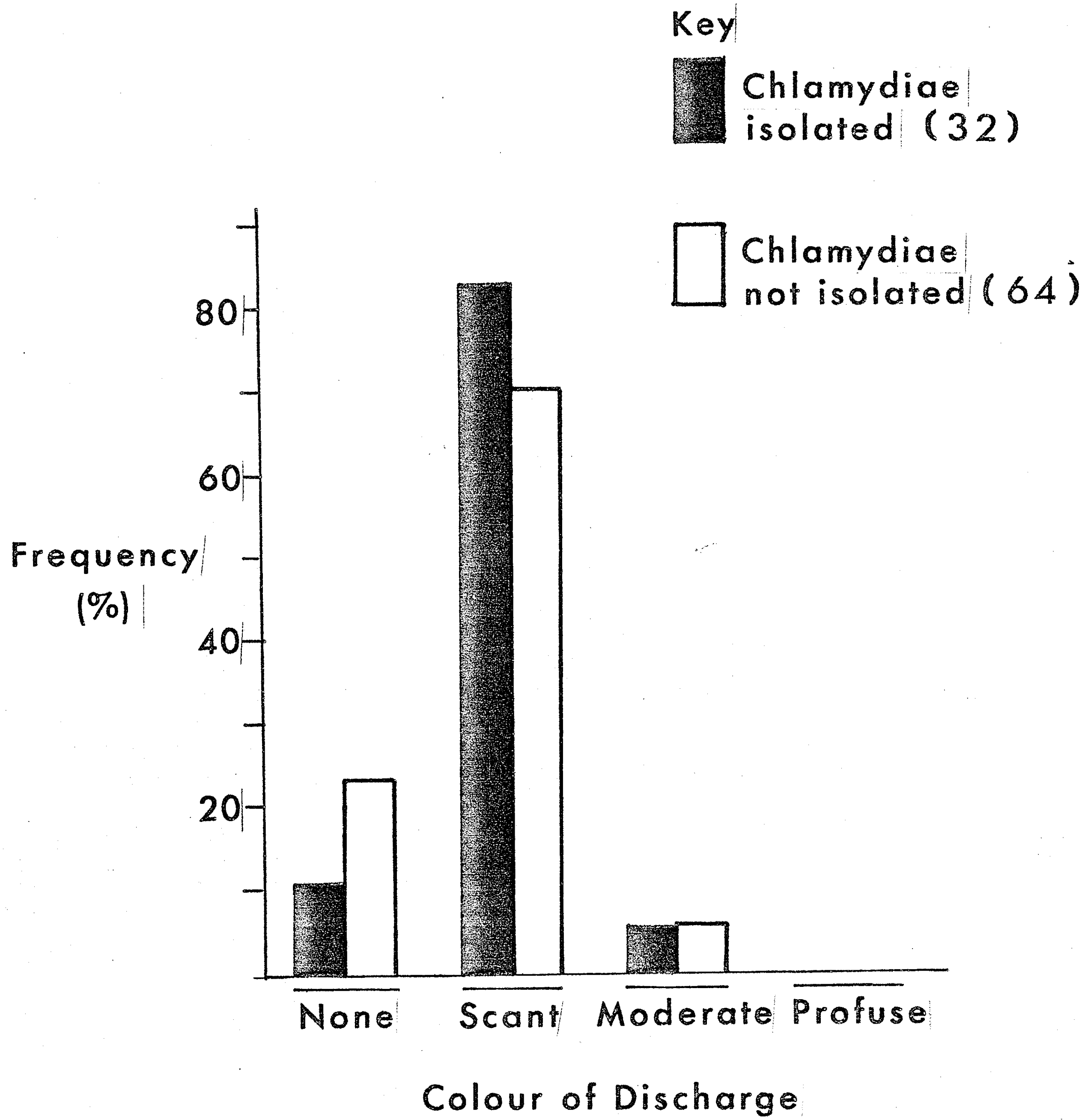

Key
Chlamydiae isolated (32)
Chlamydiae not isolated (64)
80
60
40
20
Frequency (%)
None
Scant
Moderate
Profuse
Colour of Discharge

TABLE X

A COMPARISON OF THE MEN IN

THE THREE DIAGNOSTIC GROUPS WITH RESPECT TO

A PREVIOUS HISTORY OF URETHRITIS

Diagnostic Categories	Previous Episodes of Treated Urethritis		
	Yes	No	Undetermined
Nongonococcal urethritis			
Chlamydiae isolated	12/32 (37.5%)*	13/32 (40.6%)	7/32 (21.9%)
Chlamydiae not isolated	40/64 (62.5%)	11/64 (17.2%)	13/64 (20.3%)
TOTAL	52/96 (54.2%)	24/96 (25%)	20/96 (20.8%)
Gonococcal urethritis			
Chlamydiae isolated	1/6 (16.7%)	4/6 (66.7%)	1/6 (16.7%)
Chlamydiae not isolated	37/80 (46.3%)	20/80 (25%)	23/80 (28.8%)
TOTAL	38/86 (44.2%)	24/86 (27.9%)	24/86 (27.9%)
No urethritis			
Chlamydiae isolated	1/1 (100%)	0	0
Chlamydiae not isolated	8/23 (34.8%)	8/23 (34.8%)	6/23 (26.1%)
TOTAL	9/24 (37.5%)	8/24 (34.8%)	6/24 (25%)

If the patient had not been treated at this clinic for the prior episode of urethritis, then the information as to the classification of the previous urethritis was not considered reliable. No significant difference was found among men in the three diagnostic categories using a chi square test ($X^2 = 1.74$, df = 2, $\alpha = 0.05$). To determine whether a history of prior urethritis affected the isolation of chlamydiae a chi square test was used to test for a significant difference between the men with chlamydial isolates and those who had no chlamydiae isolated within the group of men with nongonococcal urethritis ($X^2 = 4.16$, df = 1). A significant difference between these two groups was found at $\alpha = 0.05$, but not at $\alpha = 0.025$). Therefore, a previous episode(s) does not seem to strongly affect the isolation of chlamydiae.

C Female Data and Analysis.

There were 419 women included in the study. Table XI summarizes the major complaints of the women who attended the clinics. Forty-six per cent of the women were attending for a medical check-up. These women had no symptoms and no complaints. Over one-half of these women were attending for an annual examination, a few were attending for contraceptive advice, and a small number attended for a check-up following gynecological surgery. Seventy-four women attended for a venereal disease (V.D.) check; of these, 22 were asymptomatic. Of the 77 women attending with complaints of vaginal discharge, 52 specifically requested a V.D. check. Fourteen women attended for a follow-up to treatment of a urinary tract infection; 10 of these women had no urinary tract infection symptoms. Fifteen women had complaints of urinary tract infections such as nocturia, dysuria, and increased frequency with no other complaints. Fifteen women who had complaints of urinary tract symptoms also had complaints of vaginal discharge and/or abdominal pain. Thirty-eight women had complaints pertaining to menstrual irregularities. These included complaints of menorrhagia, polymenorrhea and amenorrheae. Included in the group of 37 women who had abdominal pains were women who complained of dyspareunia, or had pelvic findings such as fibroids or

TABLE XI

THE MAJOR REASONS FOR 419 WOMEN

ATTENDING THE CLINICS

	Reason for Attending[1]	Number of women	% of total women
1.	Check-up - no complaints	192	46.0
2.	V. D. checks (symptomatic and asymptomatic)	74	17.8
3.	Vaginal discharge	77	18.5
4.	Urinary tract symptoms and follow-up to treatment	44	10.6
5.	Menstrual problems	38	9.1
6.	Abdominal pains	37	8.9
7.	Follow-up to treated genital infections	17	4.1
8.	Follow-up to known abnormal cytology	12	2.9
	TOTAL	491	

1. Some women who attended for more than one of the above reasons.

ovarian cysts, also some women in whom no abnormality was detected. Seventeen women attended for a follow-up to treatment for genital infections. One woman had been treated for a trichomonas infection, and one woman had been treated for a Herpes Simplex type 2 infection. Fifteen women had been treated for N gonorrhoeae genital infections. There were 12 women whose primary reason for attending the clinic was for a follow-up to atypical findings in a previous Pap smear.

Figure 11 shows the age distribution of the women included in the study. The ages range from 15 years to 75 years with a mean age of 31.1 years (SD 12.4).

Figure 12 shows the different organisms isolated from women attending the clinics. C trachomatis was isolated from 27 women, N gonorrhoeae was isolated from 37 women, and trichomonas from 38 women. Primary syphilis by positive serology was demonstrated in 2 women.

The frequency distribution with respect to the age of the women with isolates of C trachomatis, N gonorrhoeae, and trichomonas is shown in Figure 13. The age distribution of women with C trachomatis and N gonorrhoeae was very similar but trichomonas infections were more evenly distributed among the different age groups.

The mean ages for the different groups of women were 24.1 years (S.D. 7.0) for the women with chlamydiae, 23.4

Figure 11

The age distribution of female patients studied.

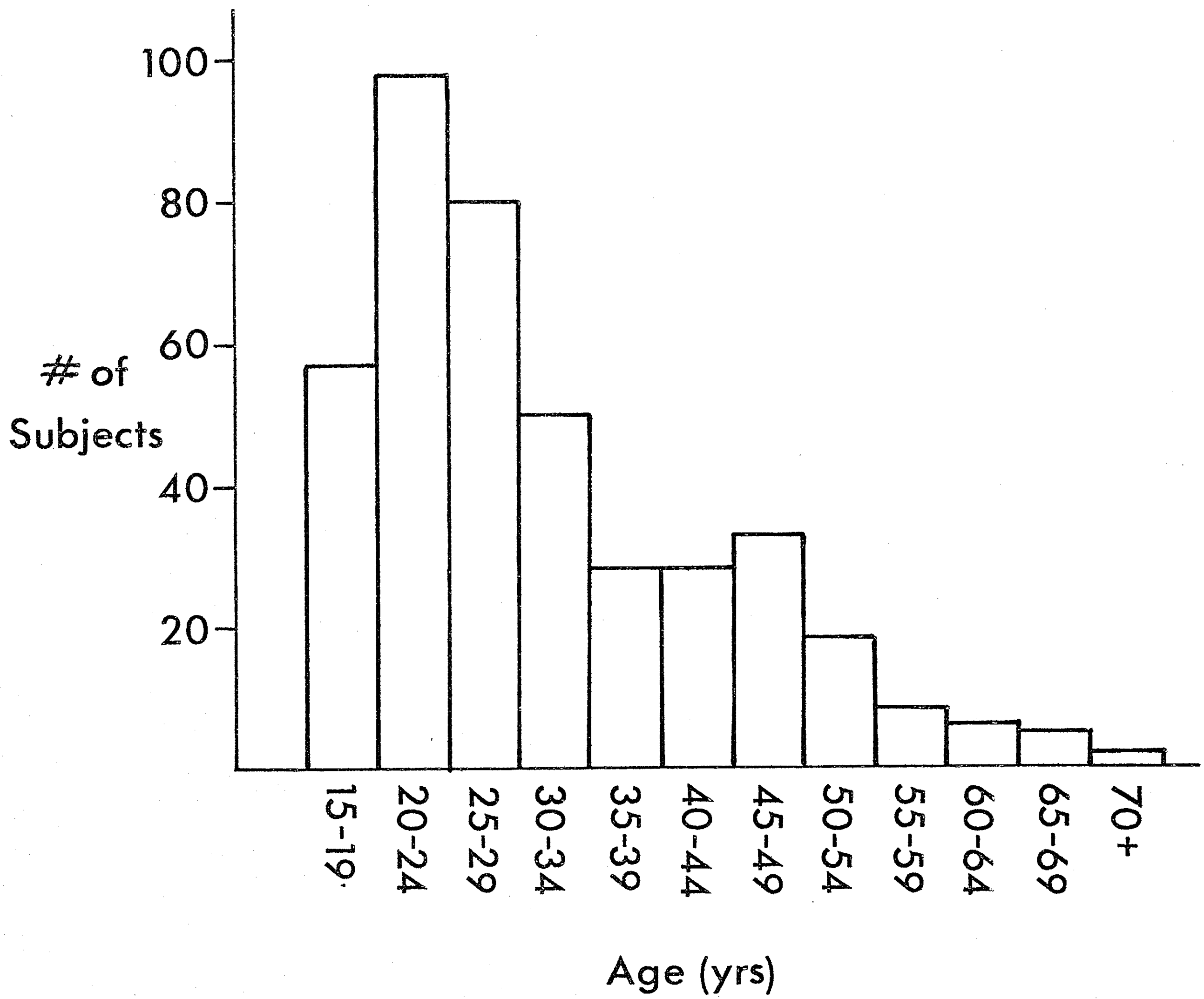
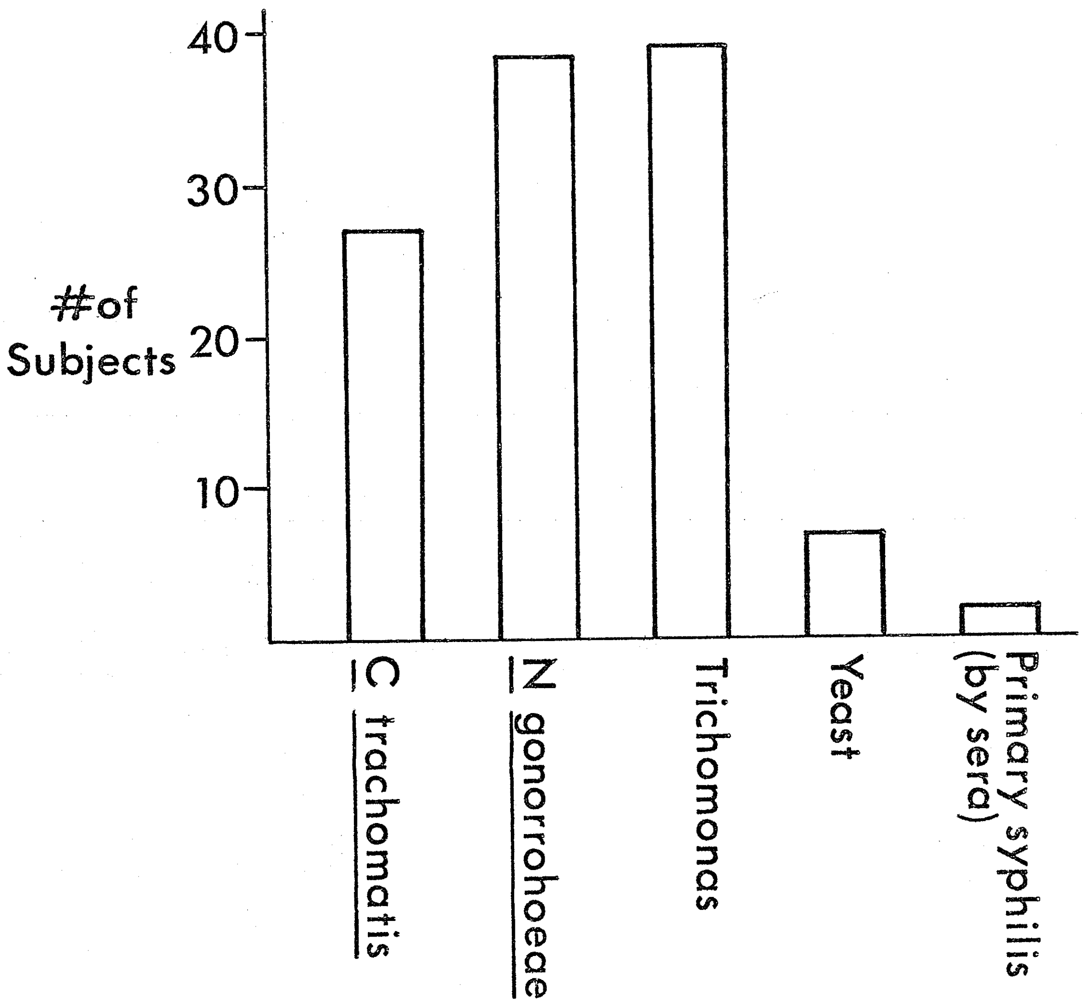

100
80
60
40
20
# of Subjects
15-19
20-24
25-29
30-34
35-39
40-44
45-49
50-54
55-59
60-64
65-69
70+
Age (yrs)

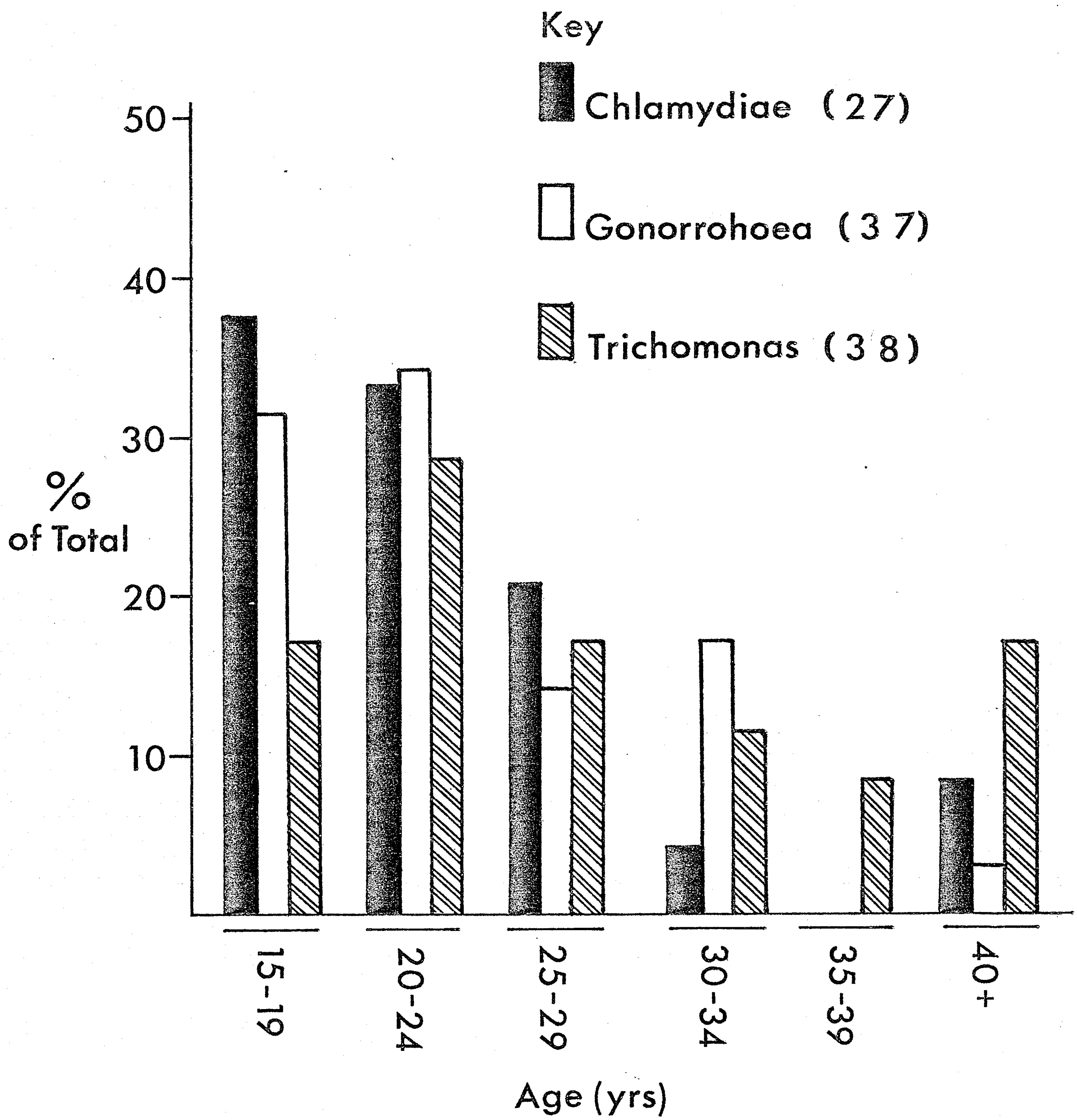
Figure 12

Laboratory findings in female patients.


40
30
20
10
#of Subjects
C trachomatis
N gonorrohoeae
Trichomonas
Yeast
Primary syphilis (by sera)

<u>Figure 13</u>

The frequency of chlamydial, gonococcal and trichomonas infections with respect to age in all female patients.


Key
Chlamydiae (27)
Gonorrohoea (3 7)
Trichomonas (3 8)
50
40
30
20
10
%
of Total
15-19
20-24
25-29
30-34
35-39
40+
Age (yrs)

years (SD 6.6) for the women with gonorrhoea, and 29.0 years (SD 11.2) for the women with trichomonas. Multiple t-tests were used to test for differences between the mean ages of the women with these three organisms. No significant difference was found between the mean ages of women with gonorrhoea and chlamydiae at $\alpha = 0.05$ ($t = 0.46$, $df = 62$). Also no significant difference at $\alpha = 0.05$ was found when comparing the mean age of women with chlamydiae with the mean age of women with trichomonas ($t = 1.95$, $df = 63$). When comparing the mean ages of the group of women with gonorrhoea and with trichomonas ($t = 2.55$, $df = 73$) it is significant at $\alpha = 0.05$ but not significant at $\alpha = 0.01$.

The women with chlamydial isolates came to the clinics for various reasons. Table XII summarizes the major reasons that these women attended the clinics. The majority of women, 16, attended with complaints of vaginal discharge. C trachomatis was isolated from 20.8% of all women who attended complaining of vaginal discharge. Ten of these women specifically requested a venereal disease check. Four women with chlamydial isolates attended requesting a venereal disease check even though they were asymptomatic. C trachomatis was isolated from 18.9% of all the women attending the clinics requesting a venereal disease check. Two women were attending the clinic following a treated gonococcal infection. Both women were asymptomatic. One was a two-week post-treatment visit and the other was a four-week post-treatment visit. Two women with chlamydial isolates were attending for a routine medical examination.

TABLE XII

THE REASONS FOR ATTENDING CLINICS

FOR THE WOMEN WITH C TRACHOMATIS ISOLATES

Major Reason for Attending Clinic	Chlamydiae isolated	
	Number	% of total positive
Vaginal discharge	16	59.3%
Asymptomatic V. D. Check	4	14.8%
Asymptomatic follow-up to treated gonococcal infections	2	7.4%
Asymptomatic - routine medical	2	7.4%
Suffering from abdominal pains	2	7.4%
Follow-up to localization of cystitis	1	3.7%
TOTAL	27	100%

One of these women was normal on examination, the other woman had a slightly atypical Pap smear with the presence of excess pus cells. Two women had complaints of abdominal pains. One of these women had an extremely tender and vascular cervix; in the other, no abnormality was detected. One woman with a chlamydial isolate was examined as a follow-up visit for a localization to cystitis. This woman was asymptomatic and no abnormalities were detected on examination.

To further analyze the data, the women were separated into two major study groups. The first group consisted of all the women who were at a potentially high risk of having a genital infection. Included in this group were all women requesting a venereal disease check, all women with vaginal discharge, all women attending for a follow-up to treatment for genital infections, and all women with abdominal pains with no known pathological cause. There were 138 women in this category.

The second group consisted of all the women who were excluded from the first group. All the women attending the clinics for routine medicals, contraceptive control, follow-up to surgery, urinary tract infections, follow-up to atypical Pap smears, complaints of menstrual irregularities, and women with abdominal pain associated with a pathological cause such as fibroids or ovarian cysts were included in this group. These women were considered the group of women at potentially low risk for genital infections. There were 281 women in this category.

Figure 14 shows the age distribution of all the women studied as they fall into the two study groups. The ages of the group of women at a high potential risk for genital infections ranged from 15 to 60 years with a mean age of 26.8 years (SD 8.4). The range of age of women at a low potential risk for genital infections was from 15 years to 75 years with a mean age of 33.2 years (SD 13.6). There is a significant difference between the mean ages of these two groups ($t = 5.035$, $df = 417$) at $\alpha = 0.05$ using a two-tailed t-test.

By omitting all of the women over the age of 44 years from both study groups the distribution of the ages of the two groups becomes similar (Figure 15) and there is no significant difference between the mean ages of these groups at $\alpha = 0.05$ ($t = 1.76$, $df = 349$). Only 9 women (6.5%) in the group at a high potential risk for genital infection and 59 women (21%) in the group at potentially low risk were over 44 years old. In these women over 44 years old, one in the potentially high risk groups had a gonococcal infection and 3 in the potentially low risk group had trichomonas infections. Dropping the women over 44 years from the further statistical analysis makes the two study groups more similar with respect to age.

<u>Figure 14</u>

The age distributions of the two female study groups.

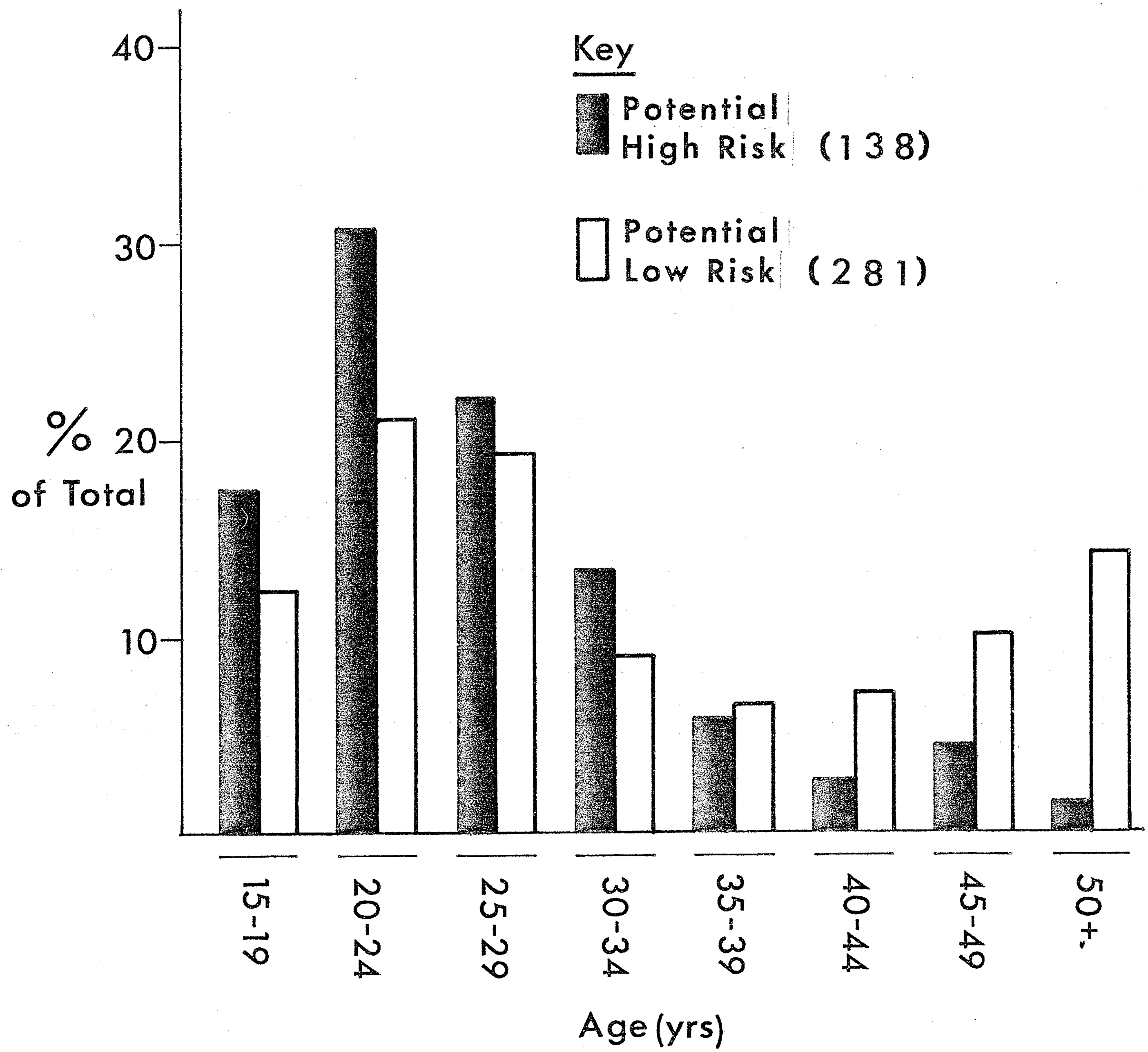

Key
Potential High Risk (138)
Potential Low Risk (281)
40
30
20
10
% of Total
15-19
20-24
25-29
30-34
35-39
40-44
45-49
50+
Age (yrs)

Figure 15

The age distribution of females
in the two study groups (<45 years.)

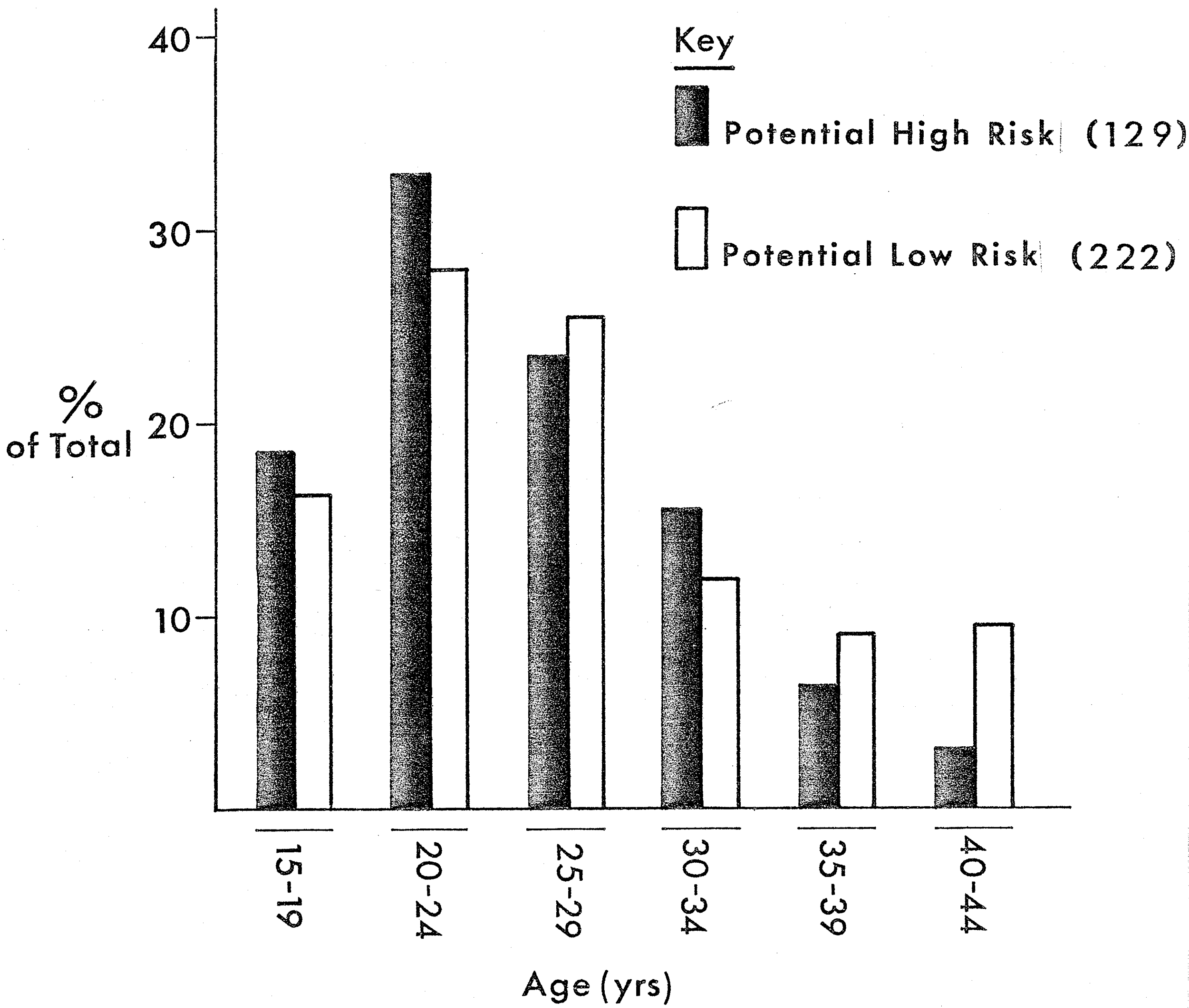
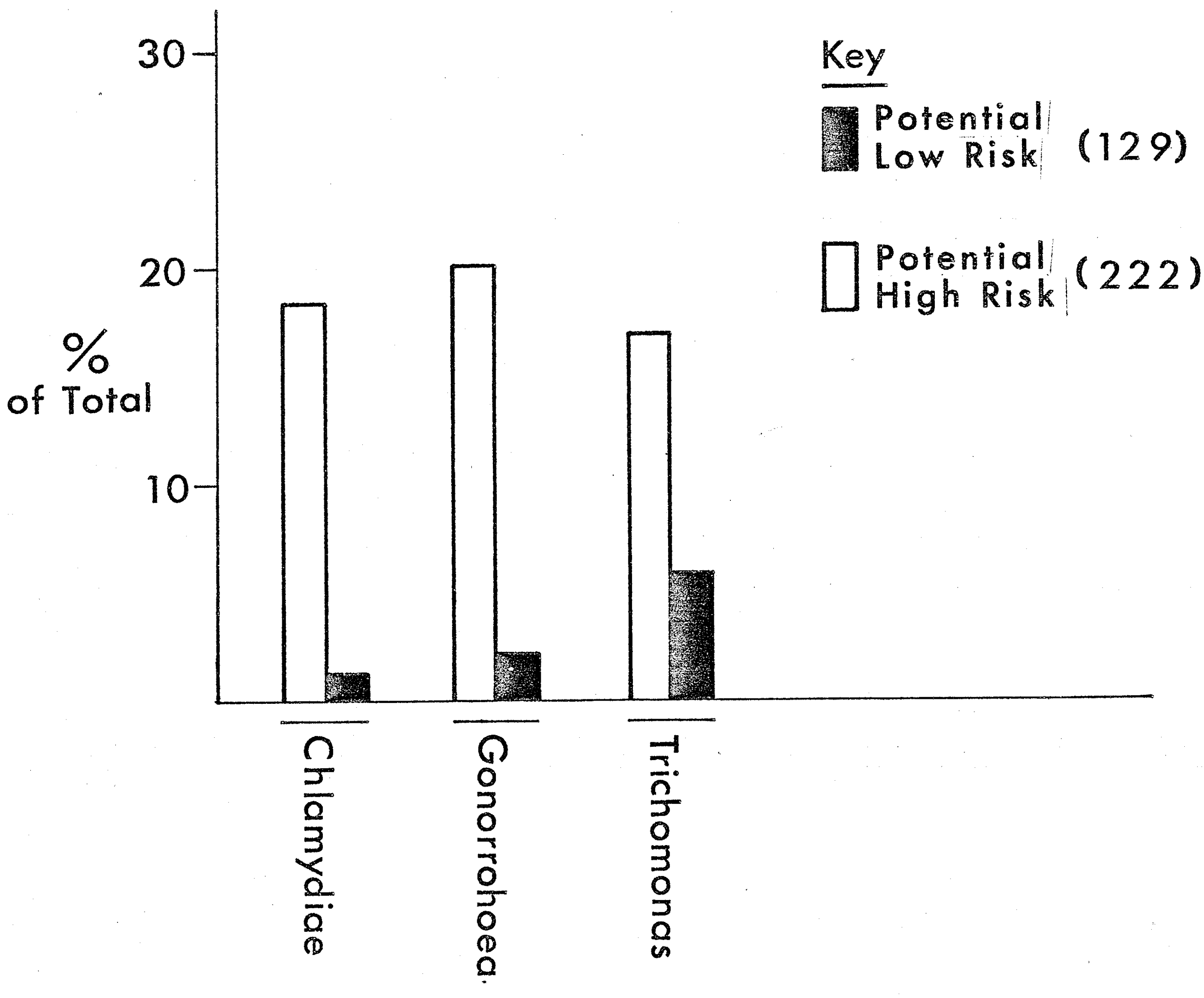

Key
Potential High Risk (129)
Potential Low Risk (222)
40
30
20
10
%
of Total
15-19
20-24
25-29
30-34
35-39
40-44
Age (yrs)

Figure 16 shows the distribution of chlamydial, gonococcal, and trichomonas infections between women (less than 45 years old) in the two major study groups. Chi square test was used to test for significant differences between the isolation rates of each organism in the two study groups. A significant difference at $\alpha=0.05$ was found for the isolation rates of chlamydiae between the group of women at a potentially high risk (18.6%) for a genital infection and the group of women at low risk (1.4%) ($X^2 = 31.82$, df = 1). A significant difference at $\alpha=0.05$ was found for the isolation rates of gonorrhoea between the two study groups ($X^2 = 30.3$, df = 1). When comparing the isolation rates of trichomonas between the two study groups, a significant difference was found at $\alpha=0.05$ ($X^2 = 10.18$, df = 1).

When comparing the number of chlamydial, gonococcal, and trichomonas genital infections within the group of women at a high potential risk for genital infection, no significant difference was found ($X^2 = 0.41$, df = 2), $\alpha=0.05$. Within the group of women at a low potential risk for genital infections no difference at $\alpha=0.05$ was observed in the number of chlamydial and gonorrhoea genital infections ($X^2 = 0.13$, df = 1) or in the number of gonorrhoea and trichomonas infections ($X^2 = 2.84$, df = 1). When comparing the number of chlamydial infections and trichomonas infections within this study group the difference was significant at $\alpha=0.05$ but not at $\alpha=0.01$ ($X^2 = 5.25$, df = 1).

Figure 16

Chlamydial, gonococcal, and
trichomonas infections in the
two female study groups
($\leq$45 years old)



30
20
10
% of Total
Key
Potential Low Risk (129)
Potential High Risk (222)
Chlamydiae
Gonorrohoea.
Trichomonas

Table XIII summarizes the laboratory findings for the two major study groups, indicating the number of mixed infections.

Table XIV shows the association of C trachomatis and other organisms. In the 27 women with chlamydial isolates 66.7% had no other pathogen isolated, 29.6% also had a gonococcal infection, 3 of these 8 women with both gonococcal and chlamydial genital infections also had a trichomonas infection.

Among the 37 women with gonococcal infections, 21.6% also had a chlamydial infection.

TABLE XIII

LABORATORY FINDINGS

IN THE TWO GROUPS OF WOMEN

	High Risk		Low Risk	
	<45	≥45	<45	≥45
No. of Women in Group	129	9	222	59
LAB FINDINGS				
1. C trachomatis	15		3	
2. N gonorrhoeae	16	1	5	
3. T. vaginalis	13		13	3
4. yeast	3		2	
5. primary syphilis (serology)	2			
6. C trachomatis plus N gonorrhoeae	5			
7. C trachomatis, N gonorrhoeae plus T vaginalis	3			
8. N gonorrhoeae plus T vaginalis	6			
9. N gonorrhoeae plus yeast	1			
10. C trachomatis plus yeast	1			

TABLE XIV

ASSOCIATION OF *C. TRACHOMATIS*

WITH OTHER ORGANISMS

Lab Findings	No. of Women	% of Total
No other organism	18	66.7%
N. gonorrhoeae	5	18.5%
N. gonorrhoeae and trichomonas	3	11.1%
Trichomonas	0	0
Yeast	1	3.7%
	27	100%

D. Distribution of Chlamydial Immunotypes in the Study Population

Table XV shows the results of immunotyping 33 of the 66 chlamydial isolates. Figure 17 shows the frequency distribution of all the immunotypes and the distribution of the immunotypes obtained from male and female sources. It can be seen that the immunotypes of the isolates obtained from men and women had a similar distribution, except that there were no type G isolated from men. The numbers involved are too small for a statistical analysis; therefore this difference may be a chance occurrence.

All, except three of the isolates obtained from men were from patients with nongonococcal urethritis. These three men had gonococcal urethritis and the isolates were immunotypes D, F, and J. The isolates from women sources were all from women in the group at a high potential risk for genital infections. Immunotypes D, F, and G were isolated from women who also had a gonococcal genital infection. There is no suggestion of the presence of specific immunotypes associated with gonococcal genital infections in either men or women.

Types D and F were the two most common immunotypes isolated. Immunotypes A, B, Ba, and C, ocular strains, or immunotypes H and K, genital strains, were not found in the isolates typed.

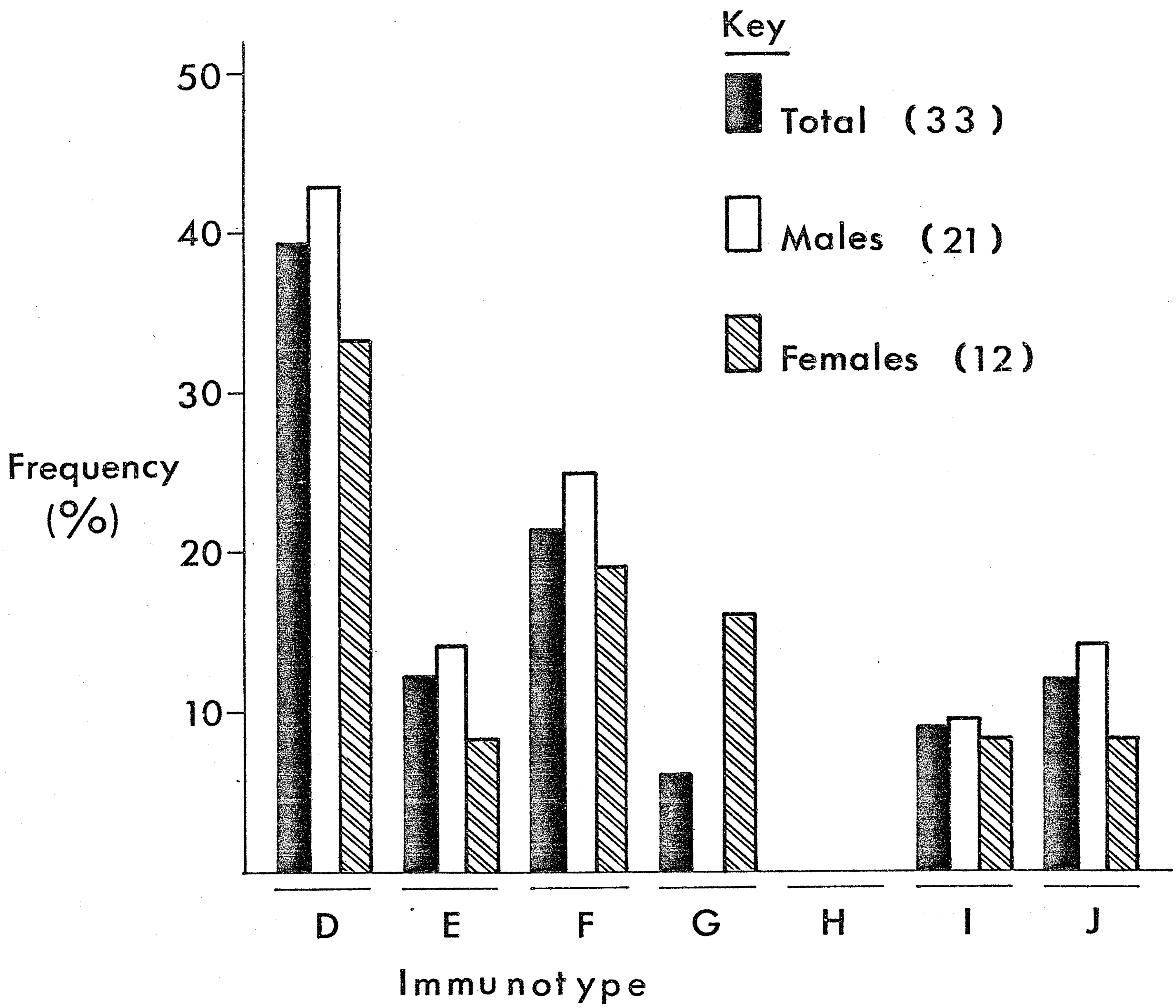
TABLE XV

RESULTS OF IMMUNOTYPING

SEX	IMMUNOTYPES											TOTAL
	A	B	C	D	E	F	G	H	I	J	K	
MALES	-	-	-	9	3	4	-	-	2	3	-	21
FEMALES	-	-	-	4	1	3	2	-	1	1	-	12
TOTAL	-	-	-	13	4	7	2	-	3	4	-	33

Figure 17

The frequency distribution of the chlamydial immunotypes isolated from males and females.


Key
Total (33)
Males (21)
Females (12)
50
40
30
20
10
Frequency (%)
D
E
F
G
H
I
J
Immunotype

DISCUSSION

In this study *C trachomatis* was isolated from urethral swabs obtained from men with nongonococcal urethritis, gonococcal urethritis, and no urethritis. Men in these three diagnostic groups were similar with respect to age and having had a previous treated episode(s) of urethritis. It was demonstrated that the prevalance of chlamydial genital infections among men with nongonococcal urethritis was significantly higher than among men with either gonococcal urethritis or no urethritis. The difference in the percentage of chlamydial isolations between men with gonococcal urethritis and no urethritis was not significant. These results provide new support for the etiological role of *C trachomatis* in nongonococcal urethritis.

The chlamydial isolation rates obtained in men with nongonococcal urethritis, gonococcal urethritis, and no urethritis in our investigation were compared with the chlamydial isolation rates obtained by other investigators as summarized in Table XVI. Chi square tests were used to test for significant differences between the chlamydial isolation rates in our study and those obtained in each study presented in Table XVI. A complete test of all the chi square values is in appendix A.

The percentage of chlamydial isolations described by other researchers (Table XVI) for men with nongonococcal urethritis ranged from 23% to 52%. When using chi square

TABLE XVI

CHLAMYDIAL ISOLATIONS IN MEN

FROM SELECTED STUDIES

STUDY	STUDY GROUPS			REFERENCE
	NGU[1]	GU[2]	NU[3]	
Washington (USA)	35 (23%)[4]	32 (16%)	--	Philip *et al*, (1971)
London (ENG)	135 (36.3%)	--	31 (0)	Oriel *et al*, (1972)
London (ENG)	99 (45%)	--	--	Dunlop *et al*, (1972)
Bristol (ENG)	103 (39%)	99 (32%)	92 (5%)	Richmond *et al*, (1972)
London (ENG)	--	44 (25%)	--	Oriel *et al*, (1975)
Seattle (USA)	113 (42%)	69 (19%)	58 (7%)	Holmes *et al*, (1975)
San Francisco (USA)	76 (35.5%)	18 (11%)	57 (0)	Schachter *et al*, (1975)
London (ENG)	240 (52%)	141 (25%)	74 (4%)	Oriel *et al*, (1976)
Winnipeg (CAN)	96 (33.3%)	86 (7.0%)	24 (4.2%)	

1 Nongonococcal urethritis
2 Gonococcal urethritis
3 No urethritis
4 Number of men studied (% of men with chlamydial isolations)

test to compare the isolation rates obtained from the individual studies with those of our study only the results obtained by Oriel et al, (1976) was significantly different (α=0.05). Oriel et al, (1976) obtained chlamydial isolates from 52% of the men with nongonococcal urethritis.

Other investigators (Table XVI) reported that between 11% and 32% of men with gonococcal urethritis also had chlamydial isolates. In comparing the isolation rates of these studies with our study, chi square tests indicate that, of the six research groups summarized in Table XVI, two had isolation rates very significantly different from that in our study (Richmond et al, 1972: Oriel et al, 1976), two of the research groups had isolation rates only slightly different (Oriel et al, 1975: Holmes et al, 1975), and two of the groups had isolation rates not significantly different (Philip et al, 1971: Schachter et al, 1975).

Chlamydiae has been isolated from 0% to 7% of men with no urethritis (Table XVI). Statistical analysis (chi square test) found no significant difference in the chlamydial isolation rates in men with no urethritis when the isolation rate of each study summarized in Table XVI was compared with that obtained in our study.

Therefore, the chlamydial isolation rates were significantly different at $\alpha = 0.05$ in one (Oriel _et al_, 1976) of seven studies investigating men with nongonococcal urethritis; four (Richmond _et al_, 1972: Oriel _et al_, 1976: Oriel _et al_, 1975: Holmes _et al_, 1975)1975) of six studies investigating men with gonococcal urethritis; and none of five studies investigating men with no urethritis (Table XVI) when compared with the isolation rates obtained in our study. The differences may:

(1) be due to differences in the sensitivity of the isolation technique

(2) be due to differences between the study populations

(3) reflect a true difference in the prevalence of chlamydial genital infections.

Any one, or combination, of the above reasons may contribute to the observed differences in the isolation rates. Of course, some variation in the isolation rate of _C trachomatis_ is expected in different study populations in different geographical locations at different times.

The study by Oriel _et al_, (1976) may have utilized a more sensitive isolation technique. In this study the chlamydial isolation rates in men with nongonococcal urethritis and men with gonococcal urethritis were both significantly higher (α=0.05) than the isolation rates obtained in our study for similarly defined populations. The clinical specimens in our investigation were routinely frozen at -70°C prior to the inoculation of tissue culture whereas Oriel et al, (1976) inoculated the clinical specimens on the McCoy cells without prior freezing. The use of a more sensitive isolation technique cannot explain the differences observed when comparing the chlamydial isolation rate in men with gonococcal urethritis in our study with that obtained in other investigations (Richmond et al, 1972: Oriel et al, 1975: Holmes et al, 1975). Although Richmond et al, (1972) obtained chlamydial isolates from 32% of the men with gonococcal urethritis which is significantly higher than the 7% obtained in our study, the percentage of chlamydial isolations in men with nongonococcal urethritis was not significantly different between the two studies. Therefore a more sensitive technique is probably not responsible for the significant difference in the isolation rates observed in men with gonococcal urethritis. This same argument holds true for the studies by Holmes et al, (1975) and Oriel et al, (1975).

The differences observed may be due to differences in the study population in each investigation. In all the studies, except that by Schachter et al, (1975) reviewed in Table XVI, the clinical specimens were obtained from men attending venereal disease clinics. In the study by

Schachter _et al_, (1975) the specimens were obtained from men attending "drop-in" street clinics. In our study, although the clinic the men were attending was not specifically designed for the treatment of venereal diseases, it was well known as a public clinic in which venereal diseases were treated. Consequently the clinic has a high proportion of the patients attending requesting a venereal disease check. It was from this group of patients that our clinical specimens were obtained.

Not enough information is available to statistically compare the ages of the men attending the clinics in the other investigations (Table XVI) with the age of the men in our study. Four studies (Oriel _et al_, 1976: Schachter _et al_, 1975: Richmond _et al_, 1972: Oriel _et al_, 1972) described the mean age of their study populations. For these studies the mean ages of the men investigated ranged between 24 years to 30 years. The mean age of our study population (27.9 years) was within this age range. In our study, age did not strongly influence the isolation of chlamydiae. This is in agreement with Richmond _et al_, (1972).

In our study there was no difference among the three diagnostic groups with respect to a history of a previous episode(s) of treated urethritis; and this factor did not seem to strongly influence the isolation of chlamydiae.

This was similar to the findings of Richmond et al, (1972) and Oriel et al, (1976). Oriel et al, (1976) concluded that neither a past history of urethritis, the cause of urethritis, nor the interval of time since it had occurred affected the isolation of chlamydiae. It was not possible to compare the different study populations in the studies summarized in Table XVI with respect to a previous history of urethritis. In the study by Oriel et al, (1972) only men attending with their first episode of urethritis were investigated. This difference with our study did not appear to affect the isolation of chlamydiae. Therefore, although it is not possible to define all the study populations of the investigations summarized in Table XVI with respect to a history of previous urethritis it does not seem to be a factor which influences the isolation of chlamydiae.

It may be that there exist undefined differences, such as socio-economic, among the study populations in the different investigations summarized in Table XVI. Jacobs et al, (1975) suggested that men with gonococcal urethritis were of a lower socio-economic status than men with nongonococcal urethritis. Holmes et al, (1975) findings agreed with this. Therefore, since there may be complex socio-economic factors which may affect the distribution of N gonorrhoeae infections throughout the population, it is not inconceivable that these same factors may affect the distribution of C trachomatis infections throughout the population. It is possible and even expected that the studies that have been done in different geographic regions will have socio-economic differences in the populations. This factor may influence the isolation of chlamydiae in the different study populations.

It is difficult to conclude whether the differences in the percentage of chlamydial isolations observed, especially in men with gonococcal urethritis, are due to differences in the study populations or to a true difference in the prevalence of chlamydial genital infections in men with gonococcal urethritis.

C trachomatis was isolated from 27 of the 419 (6.4%) women screened. There was a significant difference found in the percentage of chlamydial isolations in the women less than 45 years of age who were in the group at potentially high risk for a genital infection (18.6%) as compared with those who were not at a potential risk for genital infections (1.4%) (α=0.05).

Of the 74 women who attended the clinics requesting a venereal disease check, 14 (18.9%) had chlamydial isolates. Table XVII compares the isolation results from both women requesting a venereal disease check and women less than 45 years old from our study who were at a potentially higher risk for genital infections with chlamydial isolation results obtained by other investigators. Chi square tests

TABLE XVII

CHLAMYDIAL ISOLATIONS

IN WOMEN FROM SELECTED STUDIES

Study	Study Population	Chlamydiae isolation	Reference
San Fransisco (USA)	symptomatic females	604 (15.6%)*	Schachter et al, (1975)
Bristol (ENG)	women attending a V.D. clinic	279 (31%)	Hilton et al, (1974)
London (ENG)	"	247 (18%)	Oriel et al, (1974)
London (ENG)	"	638 (11.9%)	Burns et al, (1975)
Seattle (USA)	"	385 (21.5%)	Wentworth et al, (1973)
Leeds (ENG)	"	300 (20%)	Nayyar et al, (1976)
Winnipeg (CAN)	V.D. Check	74 (18.9%)	
Winnipeg (CAN)	women at risk of having a genital infection <45 years	129 (18.6%)	

* Number of women studied (percentage chlamydial isolations)

were done to test for significant differences between the chlamydial isolation results in each study with the results obtained from both women requesting a venereal disease check and the women less than 45 years old who were potentially at a higher risk for genital infections. A complete list of the chi square values for the analysis are in Appendix B.

Chlamydiae have been isolated from 11.9% to 31% of the women who were being screened for venereal diseases by the investigations summarized in Table XVII. When comparing the isolation rate of chlamidiae from the women who specifically requested a venereal disease check (18.9%) from our study with the results from each study, no significant differences at $\alpha=0.05$ were found. There was no significant difference at $\alpha=0.05$ when the percentage of chlamydial isolations in the women under 45 years at a potential higher risk of genital infections (18.6%) was compared with the results obtained by Wentworth et al (1973), Oriel et al, (1974), Schachter et al, (1975), and Nayyar et al, (1976). There were significant differences at $\alpha=0.05$ but not at $\alpha=0.01$ when comparing the results by Hilton et al, (1974) and Burns et al, (1975) with that of the women less than 45 years who were at potentially higher risk of a genital infection. This difference is not highly significant and is probably due to differences in the study populations.

Table XVIII summarizes the chlamydial isolation results from selected studies on women who would not be expected to have a genital infection. The percentage of chlamydial isolations ranged from 2% to 4%. These results were statistically compared using chi square tests, with the isolation rate of 1.4% obtained from women less than 45 years old who were at a potentially low risk of having a genital infection. The chi square values are listed in appendix C. No significant differences at $\alpha = 0.05$ were observed when comparing the chlamydial isolation results of the women at low risk for genital infection with the results from each study summarized in Table XVIII.

Even though there were differences in study design between the studies summarized in Tables XVII and XVIII and our study, there was close agreement between the results obtained.

In our investigation the percentage isolation of chlamydiae from women who also had gonorrohea was 21.6%. This is lower than that obtained in other investigations. Oriel _et al_, (1974) obtained chlamydial isolates in 33%, Hilton _et al_, (1974) obtained chlamydial isolates in 63%, and Burns _et al_, (1975) obtained chlamydial isolates in 48.3% of women with gonococcal genital infections. This lower number of mixed gonococcal and chlamydial genital infections in the women in our study is consistent with a lower isolation rate of chlamydiae

TABLE XVIII

C TRACHOMATIS ISOLATION

IN WOMEN FROM SELECTED STUDIES

Study	Study Populations	Chlamydial Isolation	Reference
San Fransisco (USA)	asymptomatic females	665 (3.5%)*	Schachter et al, (1975)
Bristol (ENG)	women attending a family planning clinic	63 (3%)	Hilton et al, (1975)
London (ENG)	women attending a V.D. clinic with no evidence of a genital disease and no known contact with V.D.	49 (2%)	Oriel et al, (1974)
		77 (4%)	Burns et al, (1975)
		48 (2.1%)	Nayyar et al, (1976)
Winnipeg (CAN)	women at low risk for genital infections <45 years	222 (1.4%)	

* Number of women studied (percentage chlamydial isolations)

men with gonococcal genital infections in our study than in some of the other studies. This may reflect a true difference in the prevalence of the simultaneous occurrence of the two organisms or differences in the study populations. It is interesting that the studies which had the highest isolation of chlamydiae in men with gonococcal infections (Oriel et al, 1976: Richmond et al, 1972) and women with gonococcal infections (Oriel et al, 1974: Hilton et al, 1974: Burns et al, 1975) were done in Great Britain. It is conceivable that the prevalence of associated chlamydial and gonococcal infections could be quite different in Great Britain from that in our investigation in Winnipeg, Canada.

Hilton et al, (1974) obtained chlamydial isolates in 63% of women with gonococcal infections, and suggested that this may be due to the gonococcal infection reactivating a latent chlamydial infection. Our investigation does not provide any support for this concept. From our results it appears that multiple infections occur by chance in both women and men.

In our study 21.5% of the women with gonococcal infections had chlamydial isolates and 6.7% of the men with gonococcal infections had chlamydial isolates. This difference is most likely due to a combination of at least two factors. The women had a mugh higher rate of asymptomatic gonococcal genital infections (41.9%) than the men (3.4%) and in Winnipeg the female contacts of men with nongonococcal urethritis are not routinely requested to attend for treatment. These factors may contribute to a higher number of mixed N gonorrhoeae and C trachomatis genital infections in women than men.

In both men and women no distinctive clinical symptoms and/or signs were found to be associated with a chlamydial genital infection. In men with nongonococcal urethritis there were no differences detected between men from whom chlamydiae were isolated and men from whom chlamydiae were not isolated with respect to urinary tract complaints or the characteristics of the urethral discharge. This is in agreement with the findings by Holmes et al, (1975), Richmond et al, (1972) and Oriel et al, (1972). The finding that no distinctive signs or symptoms were associated with chlamydial isolates in women agrees with work by Nayyar et al, (1976): Burns et al, (1975): Oriel et al, (1974): and Hilton et al, (1974). Therefore, laboratory techniques need to be employed to define a chlamydial genital infection.

The 5-iodo-2-deoxyuridine treated McCoy cell isolation technique utilized in our study was first established by Wentworth and Alexander (1974). The sensitivity of this technique, as compared with irradiated McCoy cells, was confirmed by Reeve et al, (1975). The chlamydial isolation results obtained using 5-iodo-2-deoxyuridine treated McCoy cells in our study were similar to studies using the irradiated McCoy cell isolation technique (Schachter et al, 1975: Richmond et al, 1972: Oriel et al, 1972: Oriel et al, 1974). This is further support for the use of this simplified isolation technique.

Of the chlamydial isolates 58% had more than 15 inclusion bodies per coverslip on initial isolation. Approximately 20% of the chlamydial isolates had less than 5 inclusions per coverslip on the initial isolation. Therefore, it is conceivable that there were chlamydial isolates lost due to the lack of sensitivity of the technique utilized.

Reeve et al,(1975) suggested that the maximum number of chlamydial isolates were obtained using a centrifugal force of 3000xg, and that freezing of the specimens at - 70°C decreased the number of isolates obtained. These are two factors which may have influenced the sensitivity of the technique utilized.

Therefore, the number of chlamydial isolates obtained in our study may be a low estimate of the true prevalence of chlamydial genital infections.

The results of the immunotyping of the isolates suggest that serotype D was the most preponderant type with type F the second most prevalent serotype. Together, serotypes D and F constituted 60% of the immunotypes of the isolates.

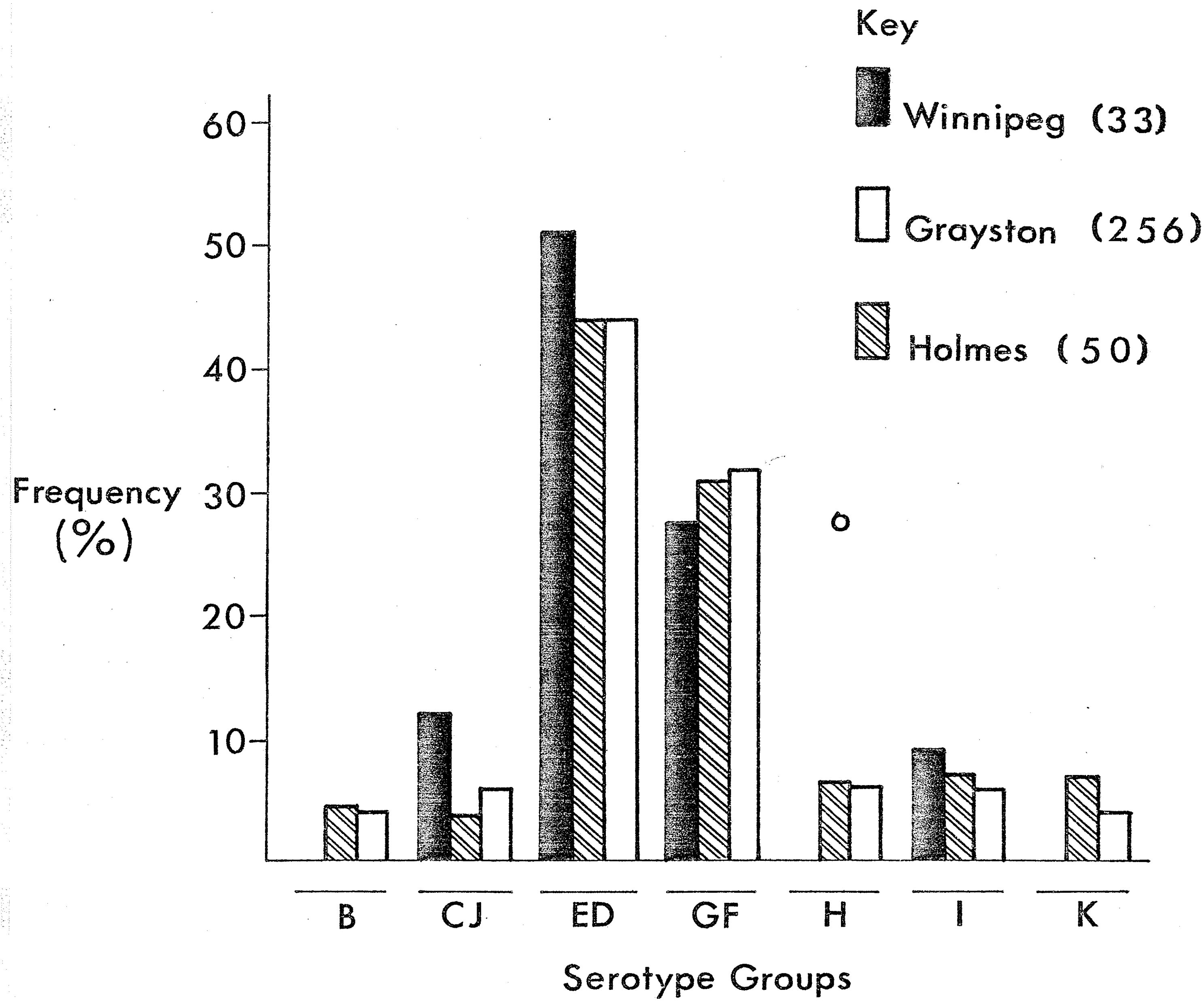
The immunotype of an isolate does not seem to depend upon the diagnostic category. In the males, types D, F, and J were found in three men with gonococcal urethritis. Types D, E, F, I, and J were isolated from men with nongonococcal urethritis. Immunotypes isolated from women were types D, E, F, G, I, and J. The isolates from women with gonorrhoea were two serotypes D, two serotypes F and one serotype G.

The distribution of serotypes of isolates obtained from males and females was quite similar. This is to be expected from an organism that is sexually transmitted. The minor differences observed are probably due to sampling differences (although the numbers involved are too small for a statistical analysis).

Figure 18 shows the distribution of the immunotypes found in this study compared with the distribution of immunotypes Holmes *et al*, (1975) found in 50 men with nongonococcal urethritis and the serotypes described by Grayston and Wang, (1975) for 256 genital isolates from various parts of the world.

Figure 18

Comparison of the distribution of serotypes isolated in Winnipeg with those described by Grayston and Holmes.


Key
Winnipeg (33)
Grayston (256)
Holmes (50)
Frequency (%)
60
50
40
30
20
10
B
CJ
ED
GF
H
I
K
Serotype Groups

It can be seen that in all three investigations, serotype groups ED and FG were the most common found in genital infections. The number of isolates in the other immunotype groups are too small to make it possible to state whether the distribution of isolates observed in this study occurred by chance. It appears that there may be an increased number of serotypes J in the genital infections in Winnipeg as compared to those described by either Grayston and Wang, (1975) or by Holmes *et al*, (1975).

CONCLUSIONS

This investigation has implicated *Chlamydia trachomatis* as an important pathogen of the human genital tract in the study population in Winnipeg. Since chlamydial genital infections do not produce distinctive clinical features specialized culture techniques are necessary to accurately diagnose a chlamydial genital infection. The distribution of the immunotypes of the chlamydial isolates in our population was similar to that described for other study populations.

Having determined that chlamydial genital infections do constitute a significant proportion of human genital infections the next step will be to do extensive follow-ups on the patients with chlamydial isolates and their sexual contacts. This may help to provide some answers to the pathogenicity of chlamydiae in genital infections. Do other bacteria play a role in chlamydial genital infections? Is a second infection a re-infection or the activation of a latent infection? Also there still remains a large proportion of genital infections in which there is no evidence as to the causative agent(s). Much more research is necessary to define the epidemiology of the genital infections in these men and women.

APPENDICES

APPENDIX A

Chi square values for the comparison of chlamydial isolation results of our investigation with that of other studies summarized in Table XVI.

Study	Chi-square values (x^2)*			Reference
	NGU	GU	NU	
Washington (USA)	0.82	1.17	---	Philip et al, (1971)
London (ENG)	0.11	---	0.02	Oriel et al, (1972)
London (ENG)	2.08	---	---	Dunlop et al, (1972)
Bristol (ENG)	0.43	16.60	0.07	Richmond et al, (1972)
London (ENG)	---	6.81	---	Oriel et al, (1975)
Seattle (USA)	1.47	3.97	0.00	Holmes et al, (1975)
San Fransisco (USA)	0.02	0.01	0.20	Schachter et al, (1975)
London (ENG)	8.95	10.25	0.34	Oriel et al, (1976)

* for all x^2, df = 1

APPENDIX B

Chi square values for the comparison of the chlamydial isolation results of our study with that of other studies summarized in Table XVII.

Study	Chi square values * when compared with results of:	
	Women requesting a V.D. Check	Women $<$45 yrs at Potential high risk of genital infection
San Francisco (USA) Schachter *et al*, (1975)	0.33	0.52
Bristol (ENG) Hilton *et al*, (1974)	0.10	6.08
London (ENG) Oriel *et al*, (1975)	0.00	0
London (ENG) Burns et al, (1975)	2.35	3.67
Seattle (USA) Wentworth *et al*, (1973)	0.13	0.35
Leeds (ENG) Nayyar *et al*, (1976)	0.00	0.04

* for all x^2 , df = 1

APPENDIX C

Chi square values for the comparison of the chlamydial isolation results of our study with that of other studies summarized in Table XVIII.

Study	Chi Square Values *
San Fransisco (USA) Schachter _et al_, (1975)	1.91
Bristol (ENG) Hilton _et al_, 1974	0.18
London (ENG) Oriel _et al_, (1975)	0.09
London (ENG) Burns _et al_, (1975)	0.81
Leeds (ENG) Nayyar et al, (1976)	0.08

* for all x^2, df = 1

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