# THE CLINICAL PATTERN OF CHLAMYDIAL INFECTION IN THE CERVIX IN CONTACTS OF MEN WITH NONGONOCOCCAL URETHRITIS AND MOTHERS OF BABIES WITH CHLAMYDIAL CONJUNCTIVITIS

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C O N T E N T S

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This thesis has been composed by myself.

The work throughout represents my own personal activities either alone or in conjunction with my clinical and laboratory colleagues. In the latter case my personal contribution has been made clear in the thesis, and is confirmed in the published work related to this thesis by the inclusion of my name in the list of authors of each relevant paper, to the preparation of which, as well as the work reported, I contributed.

I. Anne Tait.

ABSTRACT

The gradual elucidation of the oculogenital syndrome over the last two hundred years is reviewed in association with recent recognition of the importance of Chlamydia trachomatis as a primary pathogen in both sites. The development of microbiological knowledge of chlamydiae leading to facilities for exact and rapid diagnosis is outlined. Due to increasing doubts of the validity of conventional laboratory investigations, when applied to clinical work, the susceptibility of C. trachomatis to certain antibiotics was measured under less artificial conditions than before. These experiments helped to reveal the stage of the growth cycle where these antibiotics act and explained some previous incongruities.

455 contacts of men with nongonococcal urethritis and 129 babies with neonatal conjunctivitis and their mothers were examined. There was a highly significant correlation between the presence of C. trachomatis and clinical signs of cervical inflammation. The significance of the association of a number of factors with chlamydial infection was studied; these included symptoms, hormonal factors, concurrent infection, clinical signs and the number of partners and their history. The interaction of several variables upon chlamydial infection in the cervix was investigated. The severity of infection in the cervix was related only to the presence of endocervical mucopus. Appropriate chemotherapy was found to eradicate chlamydial infection in the cervix, conjunctivae and pharynx simultaneously with, in almost all cases, the disappearance of signs of inflammation. Epidemiological studies established the prevalence of C. trachomatis in selected populations.

An association of pelvic inflammation, particularly in the puerperium, and chlamydial endocervicitis was found. A significant association of this infection with pre-term deliveries suggests the possibility that chorioamnionitis occurs and may be responsible for antepartum infection in some babies delivered by caesarian section. Discrepancies between mothers' and babies' isolates were not always attributable to previous chemotherapy. In some cases, following prolonged premature rupture of membranes, antibacterial activity in amniotic fluid may have been responsible.

As a large proportion of chlamydia-positive women are asymptomatic and without abnormal clinical signs and since chlamydial infection is sexually transmitted, these patients can only be recognised when they present as contacts of men with nongonococcal urethritis and as mothers of babies with neonatal conjunctivitis.

ILLUSTRATIONS

## INTRODUCTION

### Preliminary Note

Recent statistics show that nongonococcal urethritis (NGU) in the male is becoming the commonest sexually transmitted disease (STD)[1].

Nongonococcal urethritis is merely urethritis where Neisseria gonorrhoeae cannot be found on smears or cultures[2]. Over 70 causes were identified by Harkness (1950)[3] including Trichomonas vaginalis, herpes simplex virus, trauma, chemicals and bacterial urinary infections. Once these have been excluded, the entity nonspecific urethritis (NSU) remains and, with nonspecific cervicitis in women, has been reported to the Department of Health and Social Security (DHSS) since 1971 as nonspecific genital infection (NSGI).

However, in many cases NSU is used synonymously with NGU, little or no attempt having been made to exclude the other causes of urethritis. The term used by the original author has been adhered to in reporting previous work in this thesis.

Since 1951, when notification of cases to the DHSS began, the incidence of NGU in Britain has been seen to rise steadily and has overtaken that of gonorrhoea. Although data is unavailable anywhere else in the world for such a long period, similar trends are to be seen wherever records exist and our unit in Liverpool reflects these national and international patterns. (See graphs p.3a and 3b).

Factors which may be responsible are an increasing awareness of the diagnosis of NGU, quicker eradication of gonococcal infection and probably a true increase in NGU due to a rise in STD generally following the spread of promiscuity and the preferential use of oral over barrier contraception. Certainly, since 1971 when it was generally acknowledged that both sexes were involved and figures for NSGI in both men and women have been published by the DHSS, the incidence has risen markedly.

NGU can mimic gonorrhoea and must be independently diagnosed for evidence[4-6] (which will be discussed later) has accumulated in men that Chlamydia trachomatis is the main pathogen. It is epidemiologically probable that the female is the reservoir for infection in the male (analogous to gonorrhoea) and she should, on these grounds alone, be treated. In fact, many female contacts do have chlamydiae in the cervix[4,7,8,9] and evidence of this will be reviewed in this introduction.

It becomes important to decide whether, as in gonorrhoea, this infection may be a positive risk to women.

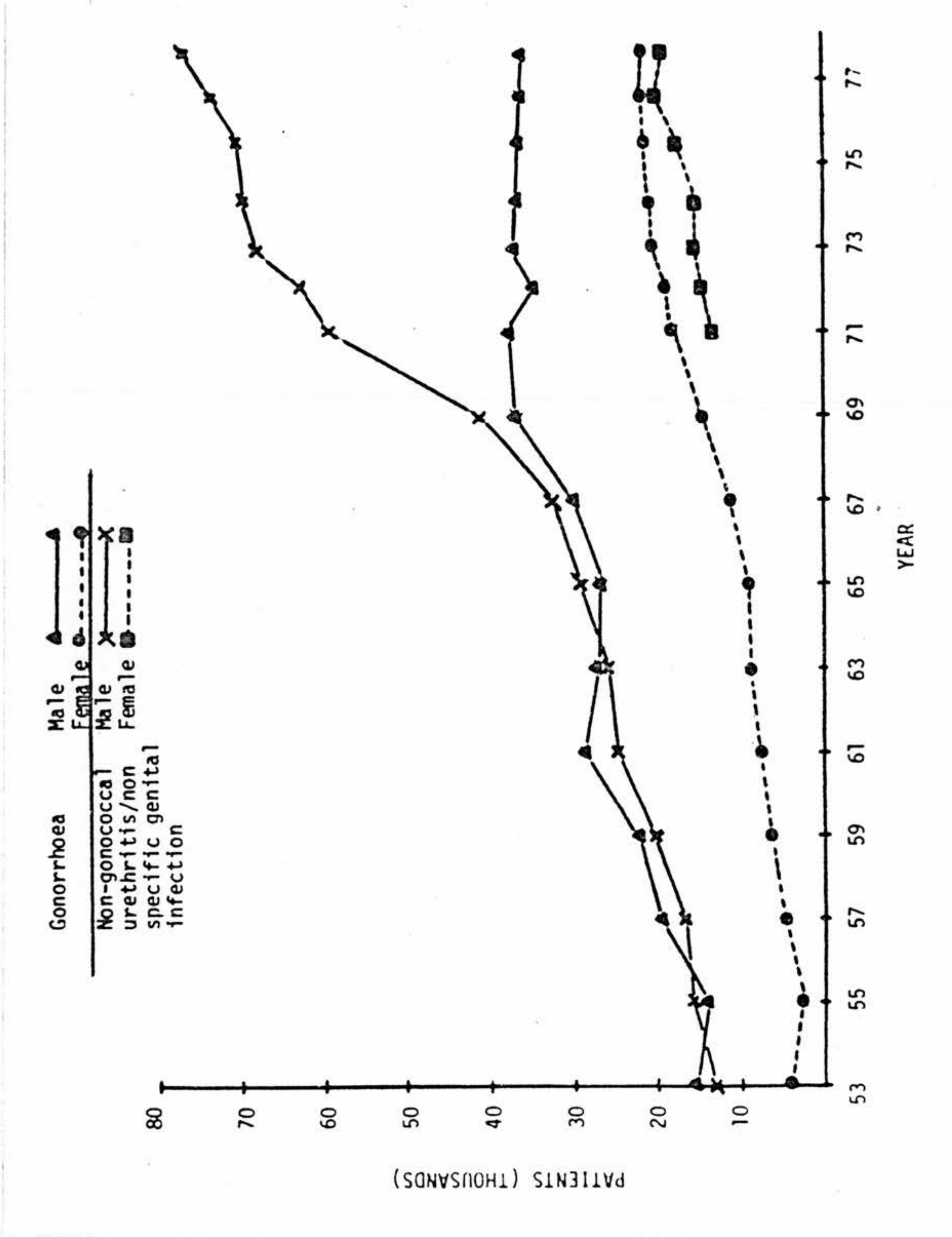
Cervical dysplasia and its sequelae, ascending infection possibly leading to salpingitis or chorioamnionitis and, finally, the spread of infection to the eyes of the patient herself, consort or ~~or~~ infant may all result from cervical chlamydial infection.

Evidence, reviewed later in this introduction or presented in the results section, confirms that C. trachomatis does behave as a primary pathogen in women and infants. It is important however to state at the outset that these syndromes are not new but were clearly and accurately defined in the past. In general medical circles, however, the wider social and health implications of the findings of pioneers in this field have often been disregarded. The first part of this introduction will consist of a historical review of work suggesting the existence and importance of nongonococcal syndromes in genital and neonatal ophthalmic disease in Europe and indirect evidence that chlamydiae played an important role in their aetiology.

It will become obvious from this historical review that early clinical and epidemiological observations were hampered and limited by a lack of background scientific knowledge of the intrinsic properties and biological nature of chlamydiae and other organisms that might be involved (e.g. Ureaplasma urealyticum) and that no reliable diagnostic tests for suspected patients were available. The second part of the introduction will give an outline of the development of microbiological knowledge of chlamydiae and the present state of knowledge and the manner in which it has led to facilities for exact and rapid diagnosis.

In the third part, recent clinical and laboratory observations on chlamydial infections (mainly in women and children) will be evaluated against the large body of evidence of earlier years with the conclusion that chlamydial STD is an entity which has long been with us and merits attention and treatment.

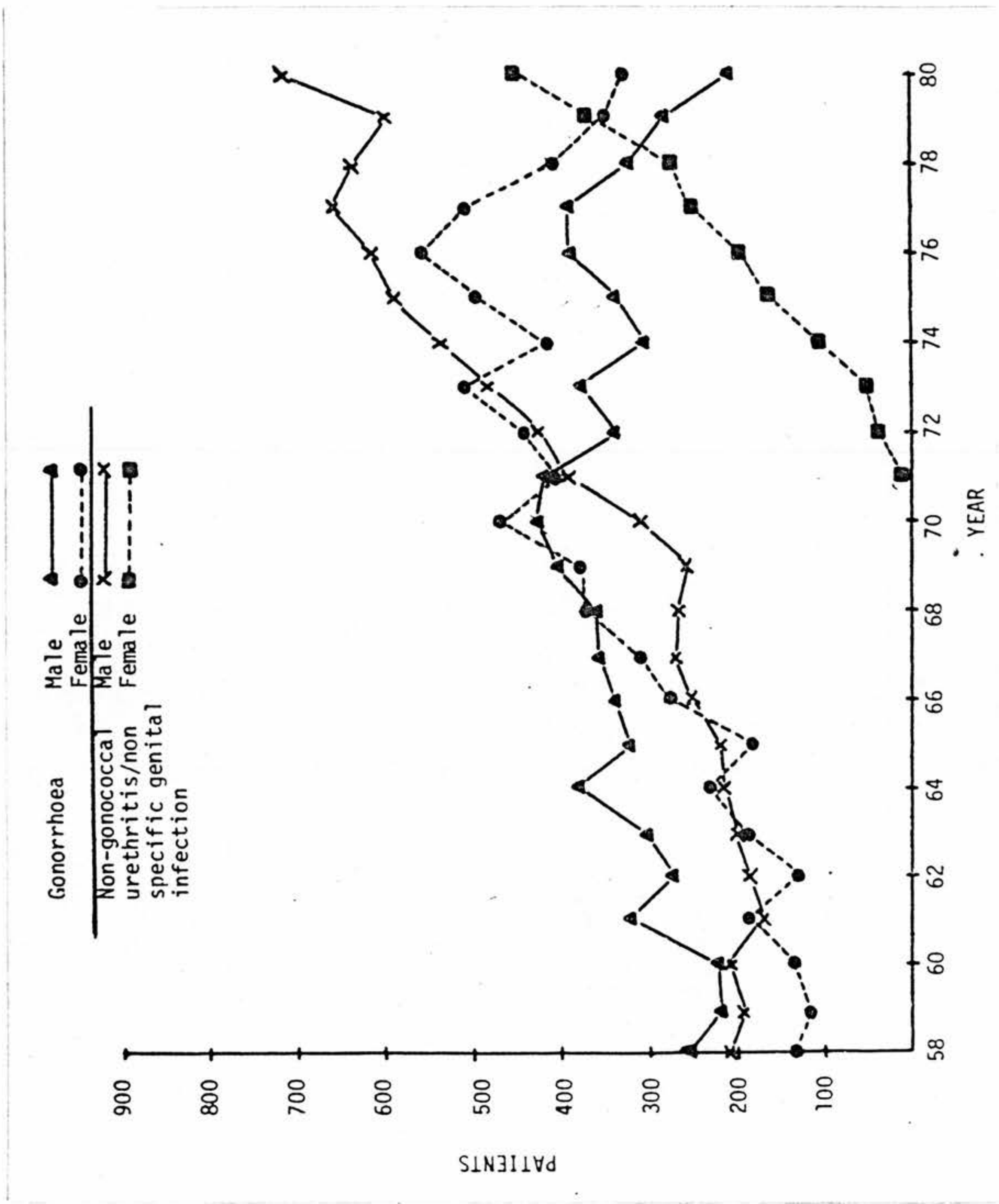
This account includes work by the present author with her colleagues in the Liverpool group, of which she is a member, before and during the time of this thesis.




New cases of nongonococcal urethritis/nonspecific genital infection* and gonorrhoea reported in England and Wales/England** from 1953-1978

*cases of nonspecific genital infection in women were first reported in 1971

**until and including 1968, data in the report of the Chief Medical Officer of the Department of Health and Social Security (from which these figures are taken) referred to clinics in both England and Wales; thereafter it referred only to England.




New cases of nongonococcal/nonspecific genital infection* reported from the Sexually Transmitted Diseases Clinic at Liverpool Royal Infirmary/Royal Liverpool Hospital** from 1958-1980

*cases of nonspecific genital infection in women were first reported in 1971.

**The Sexually Transmitted Diseases Clinic transferred from Liverpool Royal Infirmary to the Royal Liverpool Hospital in December 1978.

## INTRODUCTION: PART I

### HISTORICAL REVIEW OF CHLAMYDIAL INVESTIGATIONS

The fact that maternal genital disease led to ophthalmia neonatorum has been suspected for many years, long before any microbial aetiology of the disease was considered. Quellmaltz[10], in 1750, postulated that it originated from the vaginal secretions of the mother, and Piringer[11] in 1841 demonstrated that genital discharges were infective for the human eye. Much of this disease was probably gonorrhoea and undiagnosable until 1879 when Neisser[12] identified the gonococcus in the mother's vagina and the child's eye.

Historically, attention was drawn to chlamydial infection by evidence of conjunctivitis in babies of mothers later found to be infected. Women did not show clinical signs of chlamydial infection but mothers, later found to be without gonorrhoea, gave birth to babies presenting with nongonococcal neonatal conjunctivitis.

From 1881, the incidence of gonococcal ophthalmia neonatorum was reduced by Credé's manoeuvre[13] - the instillation of 2% silver nitrate solution in the eye of the newborn. However, as this does not affect chlamydiae[14,15], nongonococcal ophthalmia neonatorum continued. In 1884, Kroner[16] investigated nongonococcal ophthalmia neonatorum and thought that this also was due to infection from the mother's genital secretions. In 1890, Schmidt-Rimpler[12], finding gonorrhoea in neither the baby's eye secretions nor mother's genital secretions in some cases of acute conjunctivitis of the newborn, observed "not all blennorrhoea is gonococcal". This was supported by Sattler[18] who, in 1881, had described a case of conjunctivitis in a baby whose mother (in a trachoma-free area) had had slight signs of genital infection but later developed typical clinical trachoma when accidentally infected by secretions from the infant's eye. He concluded that there was a close relationship between the infective agents of infantile conjunctivitis, vaginal discharge and trachoma.

In 1899, Schultz[19] suggested that genital secretions could contaminate public pools resulting in "swimming bath conjunctivitis"[20] which was distinct from trachoma and from infections due to N. gonorrhoea which does not survive in bath water. Later, inclusions were found[21] in the cytoplasm of conjunctival cells of adults with this condition. Chlorination of swimming pools has since eliminated this infection.

In 1897, Guiard[22] claimed that a sexually transmitted urethritis due to "microbes as yet unknown to us" existed and quoted Diday who had noted that this responded much more slowly than gonorrhoea to treatment.

Waelsch (1901)[23] described nongonococcal urethritis as a distinct clinical entity marked by its relatively long incubation period, slight subjective symptoms and almost complete resistance to treatment. In 1903, Morax[24] recognised that maternal genital infections other than gonorrhoea could cause types of ophthalmia neonatorum called "conjunctivitis amicrobienne" from which no bacteria could be cultivated or seen on microscopic examination of stained smears. Thus, only when gonorrhoea could be defined, was it possible to understand the epidemiology of nongonococcal conditions.

In 1907, this epidemiological theory, was confirmed by Halberstaedter and von Prowazek[25]. They had first described their eponymous inclusion bodies in conjunctival epithelial scrapings from experimental trachoma in orang-utans in Java and from patients there with trachoma. Later, they found similar inclusion bodies in Germany in newborn babies with ophthalmia neonatorum and in adults with some forms of conjunctivitis[26] and others confirmed this[27,28,29]. These inclusion bodies, when stained with Giemsa, consisted of small reddish bacteria-like particles embedded in a blue matrix. They were called "chlamydiae" or "mantle bodies" because they crested the cell nucleus like a mantle. Their aetiological importance was not widely believed at the time because this form of intra-cellular parasitism did not seem to conform to the theory of bacteria as then known and they were said to be, among other things, nonspecific reaction products of the epithelial cell, phagocytosed bacteria or intracellular changes due to viral activity[30].

It was obvious, however, that if ophthalmia neonatorum was due to <u>Chlamydia trachomatis</u> then, like gonorrhoea, the infection must originate in the mother, as so many previous workers had postulated. Halberstaedter and von Prowazek's (1909)[26] findings of similar inclusions in the genito-urinary tract (albeit the urethral orifice) of the mother of a baby with inclusion conjunctivitis were soon followed by other confirmatory work[31]. The most important indications that the baby's infection derived from a genital disease of the mother came from (a) Fritsch, Hofstatter and Lindner (1910)[32] when follicular conjunctivitis was experimentally produced in baboons with (i) material from vaginal secretions of mothers whose babies had neonatal conjunctivitis, (ii) material from men with urethritis where gonococci could not be found, (iii) direct transfer of discharge from the eyes of infants with neonatal conjunctivitis and (b) Heymann (1910)[33] who transmitted material from the genito-urinary tracts of parents of babies with inclusion blennorrhoea and produced a similar blennorrhoea in monkeys. In all these examples, inclusions were present and so Koch's postulates were already partially fulfilled.

Lindner[34,35] talked of "genital trachoma" and, considering trachoma and inclusion conjunctivitis to be identical, quoted Arlt who had said conjunctivitis in the adult, originating from genital infection, could progress to trachoma. Wolfrum[36] in 1910, having twice experimentally inoculated an adult's eye with secretion from an infant's inclusion conjunctivitis, said that, on one occasion, trachoma had ensued - however, Gebb[37] (1914) on repeating the experiment said this was not trachoma but a self-limiting conjunctivitis healing without the pannus and scarring typical of trachoma.

By 1911 Lindner[34] suggested that 30% of NGU was due to the same agent as inclusion blennorrhoea and, following the finding of chlamydial inclusions in the female genital tract (urethra and genital secretions), Lindner said the agent of inclusion conjunctivitis was spread in temperate regions mainly via the genital tract and only rarely affected the adult eye. Lindner further said that the sub-clinical nature of cervical infection was probably due to the small area of susceptible cervical epithelium involved and the scanty exudate produced. By 1926, Linder[38] was postulating that genital infecting agents were attenuated forms of the trachoma agents which had lost the ability to produce cicatrisation when they had become adapted to the genital tract.

Hamburger (1934)[39] denied that there could be cervical chlamydial infection because, when gonococcal cervicitis was present in cases of premature rupture of membranes or prolonged labour, children were born with fully developed gonococcal conjunctivitis. This was due to the ease of spread of the gonorrhoea from the mother's infected cervix to the child's conjunctival sac. As this did not happen in similar circumstances with chlamydial infection, he postulated that only vaginal and not cervical infection occurred. (In 1975, the birth of a child with established chlamydial conjunctivitis following rupture of the membranes for over 18 hours was reported[40]).

However, Thygeson and Meingert (1936)[41] emphasised the importance of cervical infection. They examined the cervical discharge of mothers of babies with nongonococcal conjunctivitis and found inclusions in 8 out of 10 but only in the cervix, not in other parts of the genital tract. They proved that adult conjunctivitis (the counterpart of the neonatal infection) was due to auto-inoculation from the genital tract with fingers or to hetero-inoculation by the sexual partner. Thus some adults with inclusion conjunctivitis or their sexual contacts were found to have genital tract infections.

Thygeson and Meingert inoculated the cervix of baboons and of women with material from the eyes of a baby with inclusion conjunctivitis to produce cervical inflammation with, in one woman, demonstrable inclusions. They found no inflammation was produced when the vagina was similarly inoculated.

They concluded (as Lindner had in 1911) that the epidemiology of inclusion conjunctivitis was that of a genito-urinary disease, unlike trachoma with eye to eye transmission. They said that elimination of the genito-urinary disease would cause inclusion conjunctivitis to die out and they pointed out that, although no clinical entity due to chlamydial infection of the female genito-urinary tract was then recognised, it was analogous to NSU in the male.

Braley (1938)[42] studying the cervical biopsies from mothers of babies with inclusion conjunctivitis noticed that the inclusions were confined to the transitional zone (i.e. metaplastic cells) of the cervical epithelium.

It was thus recognised that inclusion conjunctivitis of the newborn and adult inclusion conjunctivitis were pointers to female genital tract infection but, in general, no further investigation and certainly no treatment was forthcoming for the suspected woman. This was because, whereas the diagnosis of ocular infections may be adequately made by finding inclusions by direct microscopy in conjunctival epithelial cells from scrapings stained with Giemsa, iodine or, even better, fluorescent antibody (q.v.), genital chlamydial infections require cultural methods for their routine detection due to the difficulty in differentiating inclusions there from local debris.

When Jones, Collier and Smith, in 1959[43], first isolated the agent (a) from the conjunctiva of a baby with inclusion conjunctivitis and (b) from the cervix of the mother, they followed the method of inoculation of clinical material into the yolk sac of the chick embryo which had been successfully used by T'ang, Chang, Huang and Wang (1957)[44] to isolate chlamydiae from cases of trachoma. They recognised that the agents of inclusion conjunctivitis and those of trachoma were closely similar and for many years they were known collectively as TR-IC (trachoma-inclusion conjunctivitis) agents.

Thus, the epidemiological and microscopical findings of Halberstaedter and von Prowazek in 1907 were now elegantly confirmed by cultural means fifty years later. The early cytological studies whereby the presence of oculo-genital infection was deduced by finding inclusions in smears from babies' eyes and from the genital tract of their parents followed by inoculation of primates' eyes from these sites, with the production of inclusion conjunctivitis[26,27,32], were then repeated using chick embryo isolation methods. Thus direct evidence of the agent in these sites was obtained.

It is now necessary to look at the laboratory evidence and see how it can support clinical observation and routine diagnostic processes in chlamydial infection as in gonorrhoea.

## INTRODUCTION: PART II

### THE MICROBIOLOGICAL NATURE OF CHLAMYDIAE

Halberstaedter and von Prowazek's first observations concerned trachoma in Java. Later, they investigated agents producing genital and ocular infections in temperate countries. All the agents they investigated were known as C. trachomatis.

The microbiology is discussed here because it was mostly discovered from eye infections long before adult genito-urinary chlamydial infections were studied.

It was obvious later that there were other human diseases in which inclusion bodies similar to those of trachoma described by Halberstaedter and von Prowazek could be found in the tissues. For example, in 1929, an apparent "pandemic" of pneumonitis in adult humans occurred synchronously with a lethal epizootic in psittacine birds (e.g. parrots) and apparently originated in South America spreading from Argentina to Europe. Similar inclusions were found in men and birds with this condition and the agent was isolated in 1930, in eggs[45]. Even later, it was realised that similar infections (sometimes called "ornithoses") were common in non-psittacine birds and could also be transmitted to man causing pneumonia. The agents are similar and until recently were called "psittacosis-ornithosis agent" but now are known as Chlamydia psittaci.

C. psittaci are thus primarily animal pathogens, only occasionally transmitted to man but, in contrast, Gamna (1923)[46] discovered similar chlamydial inclusions in patients with lymphogranuloma venereum (LGV) which is purely a human infection, not naturally found in animals. The LGV agent was cultured by Rake[47] in the yolk sac of the developing chicken embryo in 1940 and is now classified with C. trachomatis.

These agents were "fast killers" of chick embryos. Later, when grown in tissue culture, there were dramatic and cytopathic effects due to multiple cycles of growth.

However, trachoma agents had a single growth cycle and were either "slow killers" (up to six weeks) or "non-killers" of the eggs and staining was required to demonstrate their presence.

Culture

T'ang first isolated trachoma in the yolk sac of chick embryos in 1957[44] by previously treating conjunctival scrapings from early endemic trachoma in Peking with streptomycin to suppress bacterial contamination. This had foiled earlier attempts to isolate the agent by killing the embryo before C. trachomatis had had time to grow.

Despite the successful work of Jones et al, yolk sac culture was soon found too insensitive for routine use in European conjunctivitis and genital tract infections. Isolates were made in only 7 of 28 mothers of babies with neonatal inclusion conjunctivitis[48]. Furthermore, egg inoculation is cumbersome; a high titre of infected particles is produced, which is not only potentially dangerous but produces a risk of cross-contamination; chick embryos vary in susceptibility to C. trachomatis infection not only seasonally but even within batches; definite results may take up to six weeks. Thus as tissue culture had been used for many years with C. psittaci and, latterly, LGV[49], attempts were made to grow C. trachomatis on similar tissue cultures. Unfortunately, no gross cytopathic changes occurred, unlike C. psittaci-infected cultures and time had to be spent in close microscopical examination as in gonorrhoea, looking in this case for small intracellular inclusions formed in a single-step growth cycle.

There were however, also problems specific to C. trachomatis. Many routine tissue culture lines are insensitive to C. trachomatis or need special treatment. However, the McCoy cell culture line (MCC) similar to the L cell line of mouse fibroblasts used for C. psittaci for many years is susceptible and it is not unique since tumour or transformed cell lines such as Hela or BHK (baby hamster kidney) are also satisfactory[50].

These tumour or transformed cell lines, which are widely used for C. trachomatis, divide rapidly with no contact inhibition and produce sheets of tiny cells. Central problems in C. trachomatis growth were, firstly, competition between host cell and chlamydiae for available nutrients and, secondly, recognition of inclusions. To cut down rapid cell multiplication, thus producing larger and more slowly metabolizing host cells, various anti-replicative methods were used, attempting to inhibit the eukaryotic host cell and not the prokaryotic chlamydiae. The first was irradiation of the cell sheet before inoculation of the clinical specimen as in Gordon and Quan's modification (1965)[51,52] although this requires sophisticated facilities. Later, chemical anti-replicative agents were employed such as 5-Iodo-2 deoxyuridine (IUdR)[53] or cytochalasin B[54]. More commonly, cycloheximide (an anti-metabolite used in research for many years with C. psittaci) has been found preferable for routine diagnosis[55]. Added with the clinical specimen to the tissue culture, it reduces the biochemical activity of the host and not the parasite. The final effect of all these forms of treatment is to produce monolayers of large non-dividing cells with abundant cytoplasm persisting unchanged for 48-72 hours after incubation following infection with C. trachomatis so that the inclusions are larger and more easily recognisable.

To enhance C. trachomatis growth in tissue culture, close attention is given to pH, carbon dioxide concentration and temperature of the tissue culture monolayer while ensuring a sufficiency of nutrients in excess of that for tissue culture alone[56], particularly of amino acids. Further, with C. trachomatis, unlike C. psittaci and LGV strains, uptake of the parasite must be improved by increasing the effective collisions between chlamydial particles with tissue culture by (1) centrifugation, when the number of inclusions produced increases with the force and duration of centrifugation[57], (2) by modifying the electrostatic effects and increasing phagocytosis using diethylaminoethyl (DEAE)-dextran[58].

In all current tissue culture techniques, C. trachomatis gives a single step growth cycle, i.e. one input-infective C. trachomatis particle produces one inclusion and no secondary growth cycles occur[59]. The number of inclusions in tissue culture thus corresponds directly with the number of viable chlamydial inclusions in the orignal inoculum[60].

## Taxonomy

It is now realised that the growth cycle of all chlamydiae, whether derived from cases of trachoma or inclusion conjunctivitis or LGV or psittacosis, is similar and is complex[61,62,63]. The similarities of behaviour are close enough to suggest that all belong to a single genus.

Chlamydiae are members of the order Chlamydiales[64], the family Chlamydiaceae and the genus Chlamydia. Within the genus, are the two species, Group A (Chlamydia trachomatis) and Group B (Chlamydia psittacci)[65] which share a common antigen, detectable by complement fixation tests.

Group A contains 15 serotypes; in brief, C. trachomatis serotypes A, B and C cause trachoma, C. trachomatis serotypes D to K cause oculo-genital infection and C. trachomatis LGV serotypes I, II and III cause lymphogranuloma venereum (this is discussed more fully elsewhere). In Group B, the number of serotypes is unknown (i.e. it is not known whether diseases of psittacine and non-psittacine birds, sheep and cattle are manifestations of infection by different serotypes or different manifestations of a single serotype infection).

The main laboratory differences between Groups A and B may be summarised as follows:

Group A (1) Form compact micro-colonies (inclusions) within the cyoplasm of the host cell.
(1a) Possess a single-step growth cycle (as discussed above).
(2) Produce an iodine-staining carbohydrate matrix (glycogen).
(3) Lipid is produced in the vesicle.
(4) Sulphonamides inhibit growth.

Group B (1) Form micro-colonies which tend to rupture and the elementary bodies become distributed throughout the host cell cytoplasm.
(1a) Possess a multistep growth cycle.
(2) Do not produce iodine staining carbohydrate or lipid.
(3) Growth is not affected by sulphonamides.

Obviously the most important difference between Group A and B is that Group A (C. trachomatis) are primary human pathogens producing no natural infection in animals and it has been found difficult to induce infection in laboratory animals. In the tropics, where these diseases are endemic, this group causes Trachoma and lymphogranuloma venereum and in temperate zones, in developed countries, oculogenital infections (such as NGU and neonatal conjunctivitis).

Group B (C. psittaci) cause natural infections, generalised and often chronic, in many birds and animals with frequent asymptomatic shedding of the agent in secretions. Man is infected by C. psittaci only following contact with infected animals or birds and so there is an occupational hazard for bird-fanciers and veterinary surgeons. The infections are usually manifest as pneumonia and vague respiratory tract diseases diagnosed as 'flu. Spread from man to man is rare apart from involvement of medical officers or post-mortem attendants.

The general characteristics of chlamydiae will be briefly discussed, their microbiology and structure being well described elsewhere[66-69]. It must be emphasised however, that they are bacteria and not viruses by virtue of possessing a discrete cell wall similar to that of bacteria and are stainable by Giemsa or even by Gram stain. They contain both ribonucleic acid and deoxyribonucleic acid, and primitive enzyme systems and are susceptible to standard antibacterial antibiotics. They have, however, adapted to an intracellular environment and are obligate intracellular parasites.

Growth cycle

For survival and adaptation, a growth cycle has evolved with two distinct morphological forms. The first, the infectious particle or elementary body possesses a rigid cell wall and can survive for long periods extracellularly and enters the host cell by phagocytosis. The intracellular phase is necessary due to the chlamydiae's restricted metabolic activity, in particular, their inability to manufacture adenosine triphosphate. For this reason, they are "energy" parasites of mammalian and other tissue cells. Once intracellular infection has occurred, the original elementary body is transformed to the non-infective initial or reticulate body with a fragile highly permeable cell membrane in place of the rigid cell wall. These are much more active metabolically and multiply by binary fission and, about 24 hours after infection, they resume the elementary body's form, after re-secretion of a rigid cell wall.

Latency

In addition to overt multiplication and rapid destruction of the cell in which they are growing, latent persistent infection of certain cells in the culture or in the body is a universal biological characteristic of chlamydiae, most marked in such subclinical infections as human LGV and avian psittacosis and a well balanced and chronic host-parasite relationship may occur.

In a balanced salt solution, Morgan and Bader maintained TC cells infected with C. psittaci for three days[70]. Neither cell death nor infectious chlamydiae could be detected after 24 hours and yet, when complete medium was added, infectious particles could be seen on the following day. Apparently healthy birds may shed C. psittaci in faeces and have been implicated as sources of human infection. Meyer and Eddie[71] (1951) documented the isolation of C. psittaci in a man with a persistant cough 8-10 years after the original severe acute attack. Hanna[72] (1968) using immuno-fluorescent staining for C. trachomatis showed these agents, too, fitted into the same pattern. Indian children in boarding school, originally with active trachoma, rapidly improved clinically with (or even without) chemotherapy but, in the majority of cases, the agent was not eliminated. Schachter[5a] has postulated that the behaviour of chlamydial genital infection may be similar and, where found in the absence of clinical disease, must not be assumed to be normal flora. As chlamydiae are true intracellular parasites, they are pathogens and cannot merely persist on mucous membrane or cell surfaces. Latent or subclinical infections are low level multiplicates held in check by host defences.

## Antigens

To analyse the antigenic content of C. trachomatis, two problems must be overcome:

(1) contamination by the host cell,

(2) the very small quantity of material produced on tissue culture.

Results from two types of culture method are used to overcome the former (either two different tissue cultures or tissue and yolk sac culture) and microtechniques are used.

### (a) Group antigen

Both members of the genus (C. psittaci and C. trachomatis) share a common heat-stable antigen demonstrable by the lymphogranuloma venereum complement fixation test (LGVCFT)[73]. It is widely used in the diagnosis of LGV and psittacosis where antigen stimulus is considerable and antibody levels are high enough to be detected by such an insensitive test but it is unsuitable for chlamydial infections limited to the epithelial surface where antibody response is poor.

The same group-specific antigen was found responsible for the Frei test[74] demonstrating delayed hypersensitivity to chlamydial infection in LGV. There was an unacceptable incidence of both false negatives and false positives and it was found unsatisfactory in differentiating trachoma from LGV[25].

The radio-isotope precipitation test (RIP)[76] also measures group antibody and is more sensitive than the LGVCFT and more resistant to anti-complementary factor. However, it is less specific than the micro-immunofluorescence (MIF) test (q.v.) and is complicated to perform.

(b) Species antigens

These are heat-labile and more difficult to study. Immunological differences between species have been demonstrated by a form of complement-fixation test once the group antibody was removed and also by double immunodiffusion whereby the species antigens and also group antigens were demonstrated by two-dimensional immunoelectrophoresis[77].

(c) Type antigens

In the mouse toxicity test, intravenous injection of a large dose of live LGV or trachoma organisms into mice causes death within 24 hours. Death can be prevented by prior immunisation with live or formalinised organisms.

This was utilized to classify C. trachomatis into different immunotypes[78]. Ultimately 125 agents were classified into 6 types (A to F). This was a cumbersome method and has been replaced by immuno-fluorescent typing[79,80]. Micro methods using direct immuno-fluorescence (MIF test) have been developed and 15 immunotypes identified and classified[81]. Coversely, the type-specific serological response to chlamydial infection of the eye and genital tract in man has been measured. The micro-immunofluorescence and mouse toxicity tests correlate[82].

The Group A chlamydia strains fall into 12 immuno-types (A to L, plus Ba) and three LGV strains[83]. Using the appropriate antigens, Group A chlamydial infections can now be clearly distinguished from Group B (C. psittaci)[84].

A, B, Ba and C are the predominant types found in the eyes of populations in areas where trachoma is endemic and there is eye to eye transmission. However, only A and C have not also been isolated from the genital tract. Type A is found almost exclusively in the Middle East and North Africa, Ba among the American Indians, and B and C throughout the world. Generally, in Europe and the United Kingdom, types D to K are found in patients with genital infections and ocular strains from non-trachoma-endemic countries share immuno-types similar to the genital isolates[85].

Antibody response - serological and local

There is a regular pattern of serological antibody response in most bacterial infections. Transient immunoglobulin M (IgM) appears soon after the initial infection and is followed by IgG which persists. Further IgG is produced by recurrent homologous serotype infections. IgM serum antibodies have been demonstrated in patients with chlamydial conjunctivitis and in those attending STD clinics and may help in the diagnosis of acute chlamydial infections[83,85].

Locally produced antichlamydial antibody (IgA and IgG) has been found in association with infection of the eye by C. trachomatis serotypes A-C in trachoma-endemic areas and in neonatal conjunctivitis[86] and in cervical infections involving serotypes D-K[87,88].

Antichlamydial antibody levels in sera and tears from schoolchildren in Southern Tunisia with hyperendemic trachoma correlated well with the isolation of chlamydiae from the conjunctivae. Tear antibody reflected serum antibody in titre and type but disappeared sooner.

McComb et al, in a series of 185 women, found cervical antibody in 8 out of 9 women with chlamydial infections of the cervix and in

18 of 176 where chlamydiae had not been isolated. No local antibody was found in the 25 sexually inexperienced women in the study. Serum antibody was found in 38% of 185 women examined but also in 23% of the sexually inexperienced. Cervical antibody, therefore, was more closely correlated with chlamydial isolation rates and sexual experience than was serum antibody.

However, Treharne found significant serum IgG levels in 41% of 272 women with a C. trachomatis isolation rate of 13%. There were significant serum IgM levels in 49% of the chlamydia-positive but only in 23% of the chlamydia-negative women, whereas only 26 out of 35 with isolation positive chlamydial cervicitis had significant cervical antibodies.

80% men and more than 90% women with chlamydial genital tract infection have serum antibodies measured by this technique. Mean levels in men are low due to modest antigenic stimuli as chlamydial urethritis is only a superficial infection whereas titres are higher in women and patients with LGV[89]. In general, results are nonspecific in the sense that they are not correlated with the present illness. 23-40% "normal" adults have antibody as do 10-76% men with chlamydia-negative NGU and 30-50% chlamydia-negative STD clinic women patients and these percentages increase with age. This may be due to failure to isolate C. trachomatis or to antibody from a previous infection[4,6].

For epidemiological screening purposes, the MIF antibody test is invaluable. However, due to those high background levels, for diagnosis in individual patients, it is not sufficiently specific. It may however, be used to confirm the diagnosis in women and, possibly, babies already receiving treatment or to follow the course of the disease. Further investigation of serum IgM activity and local antibody levels might give more specific diagnostic help[90].

A single antigen modification[91] of the MIF test has been developed using either genital serotype D (60-90% of all genital chlamydial infections in the U.K. are type D/E) or an LGV serotype which has been shown to cross-react with antibodies to most genital serotypes.

Immunofluorescence staining

Fluorescein-labelled hyperimmune sera are used, mainly in conjunctival and urethral infections, for this most sensitive and highly specific method[92] of demonstrating inclusions. Again, antibody against a single LGV antigen is used.

## INTRODUCTION: PART III

### RECENT DEVELOPMENTS IN DETERMINING THE AETIOLOGICAL ROLE OF C. TRACHOMATIS

#### (a) Nongonococcal urethritis and cervicitis

Diagnosis by scrapes of epithelial cells from the affected area (as for hyperendemic trachoma) is unsatisfactory in genital infection partly because specimens are too small and also because normal cervical bacteria obscure and contaminate Giemsa and fluorescein stains. (Iodine staining to identify inclusions by their glycogen matrix is not practicable owing to the high glycogen content of many cervical cells).

Previously it was only suspected that C. trachomatis was a genital pathogen, and, until reproduceable isolation methods were used, there was no proof. As previously described in detail, Jones[43], in 1959, isolated C. trachomatis from the conjunctiva of an infant and the cervix of his mother. Dunlop began to apply this method to routine diagnosis and isolates were obtained from conjunctival material from affected babies, cervical material from their mothers and urethral discharge from their fathers, who were suffering from NSU[93,94]. In four out of 28 mothers, salpingitis was diagnosed[95]. By 1967, Dunlop had isolated chlamydiae from 21% of men presenting with NSU and from genital material from their sexual partners[48]. Chlamydiae were also isolated from the genital tract in adults in developed countries who had fresh chlamydial eye infections and from their sexual partners' genital tract[96-99].

When blind volunteers' eyes were inoculated, it was found that an isolate from the eye of a baby with neonatal conjunctivitis and an isolate from the eye of a trachoma patient produced an inflammation indistinguishable by all contemporary means of investigation[100]. The concept of a continuous spectrum of clinical appearances and the usage "TRIC" agent was introduced. (TR-trachoma, IC-inclusion conjunctivitis)[101]. From similar human inoculation experiments in California, with a trachoma and an inclusion conjunctivitis strain, the opposite conclusion was reached, namely that these were two distinct clinical entities[102,103]. However, previous damage to the eyes may have modified that result.

The term "TRIC agents" has been superseded by "chlamydiae". Further studies of the epidemiology of chlamydial infection and its transmission routes in various environments and later demonstration of the different serotypes have helped to relate trachoma to inclusion conjunctivitis. Also, it is now accepted that corneal vascularisation (pannus) and conjunctival scarring (previously considered unique to classical trachoma) do occur in genitally acquired chlamydial eye infections and were reported from Denmark[104] both in children and adults[105] probably initially infected from their mothers' birth canal and re-infected from their own or their mothers' genital tract.

The term "trachoma" is now used only for the hyperendemic eye disease caused by eye to eye transmission. Sexually transmitted chlamydial eye infections are described as "chlamydial inclusion conjunctivitis" and the term "genital trachoma" has fallen into disuse.

Gordon and Quan's replacement of yolk sac culture by tissue culture[52] (with later modifications previously discussed) allowed more sensitive and quicker cultural methods. Using them, by 1972, the urethral C. trachomatis isolation rate had risen to around 40% for men with NSU[7,106,107]. It was slightly higher (45%) in men presenting with conjunctivitis, who were later found to have urethritis[108]. When controls, without urethritis, were tested, chlamydiae were not isolated[7,107].

Richmond[106] found the chlamydial isolation rate in men with gonorrhoea (32%) was very similar to that in NGU (39%) which led her to believe that acute NGU was not primarily due to chlamydiae but that urethritis of any origin (including gonorrhoea) may reactivate a quiescent chlamydial infection.

However, evidence for a direct chlamydial aetiology for NGU in men was increased by King Holmes[4] who found a significantly higher chlamydial isolation rate in men with NGU (42%) than men with gonorrhoea (19%) or men without NGU (7%). Sero-conversion (MIF technique) occurred in 9 of 17 originally sero-negative chlamydia-positive patients. Endocervical C. trachomatis isolation rate was higher in female contacts of chlamydia-positive men (15 of 22) than of chlamydia-negative (2 of 24). The incidence of Trichomonas vaginalis, Ureaplasma urealyticum, herpes simplex virus or anaerobic bacteria was found neither significant nor aetiologically important in NGU.

Bowie (1976)[109] investigating the differential response of chlamydial and non-chlamydial urethritis to different antibiotics supported the aetiological importance both of C. trachomatis and U. urealyticum. He had previously found a higher incidence of ureaplasmas in chlamydia-negative NGU than in chlamydia-positive NGU and was looking at the response of NGU, where ureaplasmas had been isolated, to anti-ureaplasma chemotherapy. He grouped NGU patients according to whether C. trachomatis or U. urealyticum was isolated and then randomly treated members of each group with a sulphonamide (inhibitory to chlamydiae) or an aminocyclitol, spectinomycin or streptomycin (effective against ureaplasmas). Chlamydia-positive NGU responded to the sulphonamides and not the aminocyclitols. Ureaplasma-positive NGU responded only to the aminocyclitols. When neither or both infectious agents were isolated, in a high percentage of cases (73%, 79%) NGU failed to respond to either chemotherapeutic agent singly. This, while confirming that both chlamydiae and ureaplasmas are possible causes of NGU, did not definitely prove that either was aetiologically responsible.

C. trachomatis had been isolated from 12 of 18[7] and 15 of 22[4] sexual partners of chlamydia-positive men with urethritis; the rates were much lower in chlamydia-negative man (1/23[7], 2/24[4]). This suggested that chlamydiae were sexually transmitted and further evidence of this came in 1974 when two important studies on NGU contacts were published[110,111]. Cervical chlamydial isolation rates of 33-34% in NGU contacts, 18-31% in the STD clinic overall and only 3% in Family Planning Association (F.P.A.) clinic attenders who might be considered less promiscuous were found.

Oriel found the incidence was higher in primary (62%) than secondary (25%) contacts and also higher in contacts of men having their first attacks of NGU (33%) than in recurrent episodes (14%)[111].

Hilton and Richmond noted that, in patients taking oral contraception, the C. trachomatis infection incidence was highest in the first and fourth weeks of menstrual cycle (42%) (i.e. when the oral contraception was withdrawn). The isolation rate did not vary throughout the menstrual cycle in patients not taking the pill, but it was generally raised in women taking the pill (45%) compared with women not taking it (24%). The incidence of chlamydial infection was also raised in the presence of gonorrhoea (63%). It was suggested that a normally quiescent chlamydial infection was reactivated not only by gonorrhoea as in men but also by the use of oral contraception[110].

Both Oriel and Hilton et al found that, (as Dunlop had first said in 1964[93], and confirmed in 1966[94,95]) there was an association between the isolation of chlamydiae from the cervix and nonspecific inflammatory changes there. Following these studies, the advisability of treating the sexual partners of men with NGU was generally accepted on an epidemiological basis, without a firm diagnosis.

In 1971, Dunlop had also observed that inflammatory changes in the conjunctivae, the genital tract, and the anorectal area associated with chlamydial infection both in the parents of babies with chlamydial conjunctivitis and in adults with chlamydial conjunctivitis and their sexual partners closely resembled each other in these different sites[112].

Chlamydial inclusions had previously been seen in a cervical biopsy from a mother whose baby had inclusion conjunctivitis[42]. Later, in cervical biopsies from two patients with chlamydial cervical infection (diagnosed by isolation and high serum chlamydial antibody levels) using light and electron microscopy, chlamydiae were found in intra-epithelial vesicles in the cervical mucosa associated with a marked inflammatory response[113]. (Unfortunately, these latter women also had cervical gonorrhoea). The frequency of such multiple infections not only with gonorrhoea but also with other sexually transmitted agents had been recorded previously[114] and, in that study also, the isolation rate of C. trachomatis was found to be twice as high in the presence of cervical abnormality[115].

Burns et al (1975)[116] pointed out that the diagnosis of nonspecific genital infection in women was usually made on epidemiological grounds; contrary to findings in men, polymorphonuclear leucocytes were frequently found in the lower genital tract in varying amounts according to the stage of the menstrual cycle and were not diagnostic of nongonococcal cervicitis. They found the chlamydial isolation rate in 638 women (12%

in the STD clinic population, 22% in NSU contacts, 44% in gonorrhoea) was higher in the presence of cervical erosions and of cytological cervical inflammatory changes, in the primary contacts of men with NSU and in the absence of symptoms.

It was now obvious that there was a genital reservoir of chlamydial infection, mainly asymptomatic, mainly amongst women and possibly associated with cervical inflammatory changes. While the pathogenicity of chlamydiae in NGU in men was under consideration, its role in women had not been properly investigated, although an association with a nonspecific and undefined "cervicitis" had been noted.

In Liverpool, in 1975, the author of this thesis worked in the laboratory, in which all our subsequent investigations were made, for three months during the development here of a method[56] modified and simplified from that of Gordon and Quan (1965)[52]. McCoy cells, untreated by irradiation or by chemical anti-replicative agents, were used. The method will be described later with laboratory work performed by the present author. We investigated the association of clinical signs in the cervix with chlamydial infection and the effect of chemotherapy on them. These results will be discussed in detail in the body of the thesis but the main findings will be considered here in relation to the historical development of our knowledge of chlamydial infection.

For the first time, endocervicitis was defined[8]. Hypertrophic ectopy (or hypertrophic erosion) indicating oedema and congestion of the cervix was, with endocervical mucopus, considered abnormal. However, simple ectopy or erosion (i.e. ectopic columnar epithelium) and the absence of ectopy are normal physiological appearances[117,118]. (This definition was supported by findings at an Edinburgh FPA clinic where no less than 37% of 1400 women had cervical ectopy and ectopy was associated with youth (under 35 years), parity and the use of oral contraceptives[119]).

We selected 127 chlamydia-positive NGU contacts on epidemiological grounds and compared them with an equal number of chlamydia-negative contacts[8]. Patients with gonorrhoea were excluded from this study. There was a significant association between endocervicitis and the presence of chlamydiae. There was, however, no correlation between the clinical findings and the severity of infection as measured by the number of inclusions per coverslip. The highest count was from a cervix without ectopy but containing mucopus.

The presenting symptoms in these chlamydia-positive women were compared with the symptoms in 326 women with gonorrhoea.(Simultaneous C. trachomatis infection had not been excluded and it was thought that the relatively few with a double infection would not invalidate the comparison). There was a similarly high incidence of asymptomatic patients (49% with chlamydial infection and 53% with gonorrhoea). However, more patients with gonorrhoea (9%) than with chlamydial infection (3.9%) had abdominal pain indicating pelvic inflammation. Similarly, in both chlamydial infection and gonorrhoea, there was a large number of women without inflammatory signs but the number was greater in gonorrhoea.

The signs of chlamydial endocervicitis responded to chemotherapy and most women in this selected group were free from any other infection. According to the patients' histories, during the post-treatment surveillance period (up to 20 months) of a larger group of 161 previously chlamydia-positive STD clinic patients, there was a high probability of re-infection in the 16 women with re-isolates. The stress of pregnancy in four and gonorrhoea in another caused no relapse. These results did not support Hilton's[110] theory that chlamydiae were latent and were reactivated by other infections nor, as will subsequently be discussed, did the later investigation of the present author and colleagues on the quantitative aspects of chlamydial infection with and without concomitant gonorrhoea.

By contrast, Nayyar (1976)[120] failed to find any association between cervical changes and chlamydial infection in 300 women attending an STD clinic where the overall chlamydial isolation rate was 20% (30% in NSU contacts, 27% in gonorrhoea patients or contacts).

Oriel (1978)[121] isolated C. trachomatis from 20.4% of 284 unselected women attending an STD clinic. This was the sole pathogen in only 48% but no other pathogen was significantly linked with C. trachomatis. Oriel said that certain factors indicated the possibility of chlamydial infection of the cervix. These were cervical discontinuity, antibodies to C. trachomatis, pus in the cervical exudate, (either observed with the naked eye or microscopically in Gram-stained cervical smears) and signs of inflammation in cervical cytology. In Oriel's terms, "cervical discontinuity" was replacement of "normal" cervical epithelium and was graded to indicate its extent (no variation in the character of this replacement epithelium was described). He found that a history of sexually transmitted disease, the method of contraception, the phase in the menstrual cycle and promiscuity had no effect on the C. trachomatis isolation rate.

In 1978, Terho[122] found chlamydiae in 42% of female contacts of men with NSU but in only two of 22 contacts of chlamydia-negative men, again showing the sexual transmissibility of the agent.

This was in keeping with the results of Paavonen et al[123] (1978) who isolated C. trachomatis from 13 of 144 (9%) women in a gynaecological out-patient clinic. These women might be expected to be less promiscuous than those in STD clinics. Seven (54%) of these chlamydia-positive patients had antibodies on LGVCFT testing and 11 (85%) by the single antigen MIF test compared with 21 (16%) and 70 (53%) of the 131 chlamydia-negative. Eight chlamydia-positive patients showed "cervicitis" but this was a very nonspecific inflammatory picture. They claimed that a significantly higher proportion of ocular and urethral symptoms occurred in the chlamydia-positive patients; C. trachomatis cultures were only taken from the cervix.

Ripa et al (1978)[124] investigated the frequency of sexually transmitted agents in 170 women with symptoms suggesting lower genito-urinary tract infection (discharge, dysuria, irritation and burning, metrorrhagia and pelvic pain). Such organisms were isolated in 56 (32.9%) patients. (C. trachomatis in 31 (19.3%), N. gonorrhoea in 8 (4.7%) and T. vaginalis in 44 (25.9%). In women under 25 years, chlamydiae were found significantly more often in those taking oral contraceptives than in those who did not. In those not using oral contraception, cervical ectopy was found more often in chlamydia-positive than in chlamydia-negative. They found no distinguishing signs between gonorrhoea and chlamydial infections.

(These results will be discussed later in conjunction with the parallel investigations by the author and her colleagues in Liverpool).

McCormack (1979)[125] carried out a prospective study investigating the persistence of untreated chlamydial infection. Chlamydiae were isolated from the cervix in 20 (4.6%) of 439 college students but 62 (14%) had either cervical chlamydial infection or local cervical antibody (micro-immunofluorescence test); only two had gonorrhoea. Fifteen to 25 months later, 25 of these 62 (40.3%) were re-examined. Chlamydiae were then isolated from 7 women. Excluding 11 who had had chemotherapy, re-isolates were made from 4 of 7 originally infected, of whom 3 denied sexual re-exposure. Three of 6, previously possessing cervical antibodies only, were now isolate-positive. Chlamydial infection, present earlier but not detected due to sampling failure or latency could not be differentiated from interim infection.

In view of the possible complications of spread and the risks of infection to sexual partners, such a project where chemotherapy was withheld after already proven chlamydial infection might not be considered advisable. An alternative way of obtaining information about persistent chlamydial infection was provided by studies undertaken by the present author and her colleagues. Nineteen of our chlamydia-positive patients failed to re-attend for varying intervals of up to one year following their first visit or treatment had been withheld during investigation of other conditions. They were re-examined for C. trachomatis and isolates were obtained in all 19[8].

Having established a significant association between our criteria for endocervicitis and chlamydial infection, the present author and her colleagues in Liverpool proceeded to a pilot study of 202 female contacts of men with NGU. The clinical signs of cervical chlamydial infections, their response to chemotherapy and the incidence of cervical infection in the presence of ectopy and oral contraception were examined[9].

Previous studies by the Liverpool group had shown that: (1) When a single culture of C. trachomatis was inoculated on 48 replicate tissue cultures (McCoy cell monolayers), 93% of the inclusion counts fell within ± 20% of the mean value over the whole series[60]. (2) When cervical swabs from 153 chlamydia-positive women were repeated 7 days later before treatment, 141 (92.5%) women were again positive with similar inclusion counts per swab[60]. (3) In a series of 175 chlamydia-positive women, 72% of the swabs yielded inclusion counts of over 100 and only 8% had inclusion counts of 10 or less[56]. For the first time, the effect of certain factors on whole coverslip counts was recorded.

The C.trachomatis isolation rate was 35%. Mucopus was found in 37% chlamydia-positive and 7% chlamydia-negative women, and hypertrophic ectopy in 19% chlamydia-positive and 3% chlamydia-negative. Although 17 patients complained of abdominal pain, only 4 (two chlamydia-positive) had clinical signs of salpingitis. In the presence of oral contraception, ectopy was found to be associated with a significant increase in the chlamydial isolation rate. The latter was also higher in the presence of T. vaginalis but was unrelated to any particular symptom or to any stage of the menstrual cycle.

The signs cleared after chemotherapy in all but one patient. Furthermore, there was in 4 of 5 re-isolates a strong possibility of re-infection. Quantitative isolation studies showed higher counts in the presence of cervical mucopus and in those using oral contraception but not in the presence of hypertrophic ectopy.

Following these results, we made an investigation[126] in Liverpool of the wide variation of counts reflecting high and low grade infections in 580 patients with particular reference to clinical signs in the cervix, the use of oral contraception, the presence of concurrent infections and the patients' age. In 34%, counts were less than 100 per monolayer and in 36% counts were more than 1,000. When the counts were related to above variables, the degree of infection was found to be significantly higher in the presence of cervical ectopy, endocervical mucopus and oral contraception (but only in the presence of ectopy) and in the under 20 age group. Neither the use of oral contraceptives alone nor concurrent gonorrhoea (or any other infection) affected the severity of chlamydial infection as indicated by inclusion counts.

(b) Pelvic infection

Signs of pelvic inflammation associated with the isolation of chlamydiae from the cervix were first reported in mothers of babies with chlamydial conjunctivitis[93,104].

Similarly, in Liverpool, we found pelvic inflammation in 3.9% (5 of 127) NGU contacts with chlamydial cervical infection[8] and, amongst mothers of babies with neonatal chlamydial conjunctivitis in 53% (9 of 17)[8] and 35% (11 of 31)[127]. We had excluded mothers already receiving chemotherapy, possibly for pelvic inflammatory disease (PID) and noted the high incidence of upper genital tract inflammation in the puerperium, particularly in mothers whose diagnosis was only established three to four weeks post-partum.

The later effects of pelvic inflammation had been discussed by Westrom and colleagues[128,129] who used laparoscopy for confirmation of the presumptive diagnosis and later follow-up. After a mean 9 years, of 415 women with a history of acute salpingitis, 21% were involuntarily childless and, in 17% tubal obstruction was responsible. Tubal occlusion was commoner following non-gonococcal (17.3%) then gonococcal (6.1%) salpingitis and commoner too after second (35%) and third (75%) attacks, than after the first. The ratio of ectopic to normal pregnancy was one in 24 (12.8%) following pelvic inflammation and one in 147 in healthy controls. 18% of these women had chronic abdominal pain.

The same workers (1977)[130], in a study of 53 women with acute salpingitis, isolated C. trachomatis from the cervix in 36% (19) and from tubal biopsy using laparoscopy in 6 of 20 (30%). C. trachomatis was isolated from only one of another group of 18 women with lower genital tract infection and not from any of 12 control women without signs of tubal infection. N. gonorrhoeae was isolated in 5 of 19 (26%) also harbouring C. trachomatis in the cervix and in 6 of 34 (18%) chlamydia-negative and from the tube in one of 14 chlamydia-positive cases. They had found an increasing prevalence of acute salpingitis but the proportion associated with cervical gonorrhoea had decreased from 50% to 10% within six years. It was suggested that as C. trachomatis was such a common aetiological agent, salpingitis should be treated primarily with an antibiotic to which C. trachomatis and N. gonorrhoeae were both susceptible.

In 1979, this group inoculated the fallopian tubes of two grivet monkeys with C. trachomatis isolates from the tubes of two women with salpingitis[131]. On examination of the tubes of these monkeys, inflammatory changes similar to those in gonococcal salpingitis were found and sero-conversion occurred and so Koch's postulates were fulfilled. Similar changes were found in tubes removed from two patients with acute salpingitis and chlamydial cervical infection[132]. C. trachomatis was isolated from the tube in one patient and was found on immunofluorescent staining of tubal cells in the other. This contradicts previous general teaching that histological appearances in gonococcal and nongonococcal salpingitis differ.

Paavonen et al (1979)[133] investigated 106 women with salpingitis (not confirmed by laparoscopy). Their findings supported the aetiological importance of chlamydiae. 26% harboured it in the urethra or cervix or both; 43% of 72 from whom paired sera were obtained showed either positive cultures for or sero-conversion in single antigen MIF test to C. trachomatis. The incidence of gonorrhoea was low (26%) and, in 38% of these, concurrent chlamydial infection was present.

In a study of 143 women with salpingitis, verified laparoscopically, high anti-chlamydial antibody titres were found by Treharne (1979)[134] both in serum and in fluid aspirated from the posterior vaginal fornix. 62% had raised serum IgG titres correlating with the clinical severity of the tubal inflammation, whereas only 2 of 19 controls without salpingitis had elevated serum levels. IgM levels were raised in 23% women with salpingitis and one of 19 controls. Raised IgG levels were found in 18 of 27 women with, and only 2 of 9 without, salpingitis who had had fluid aspirated from the posterior fornix.

Sero diagnosis of chlamydial infection by MIF test was used by Simmons et al (1979)[135] to determine the aetiological agent in women with pelvic inflammatory disease without laparoscopy where chlamydial cultures were not taken. Antichlamydial antibody levels (IgM and IgG) were higher where there was salpingitis (particularly in the more severe cases) than in control patients with gonococcal cervicitis and patients with uncomplicated nonspecific genital infection (either NSU contacts or classified on the basis of excess urethral or cervical leucocytes without other pathogens.)

(c) Urethral investigations in women

Dunlop (1972)[136] reported the first isolation of C. trachomatis from the female urethra in a patient who was examined because her husband had a six-month history of chlamydial kerato-conjunctivitis. She had recurrent dysuria and frequency.

A possible association between the urethral syndrome (recurrent increasing frequency and dysuria in the absence of bacteria or significant pyuria) and chlamydial infection was first reported by the present author and her colleagues[137]. Cytoscopic examination in such a woman aged 21 years had revealed intense urethritis and basal cystitis. There was oedema along the length of the urethra palpable through the anterior vaginal wall. Hypertrophic cervical ectopy and endocervical mucopus were present and C. trachomatis was isolated from the urethra and cervix. The urethral oedema and abnormal cervical signs cleared after a three weeks course of tetracycline.

Paavonen (1979)[138] examined both urethral and cervical cultures in 99 chlamydia-positive partners of men with NGU. In 25%, chlamydiae were recovered from the urethra only, in 46% from both urethra and cervix and in 28% from the cervix only. Significantly more women with chlamydial urethral isolates (54%) had urethral symptoms than those where chlamydiae were only obtained from the cervix (21%). In three women with signs of cervical inflammation, chlamydiae were isolated from the urethra but not from the cervix and serum antichlamydial antibody titres were high. It was suggested that cervical chlamydial isolation at that time had been inhibited by high local cervical antibody levels (although these were not tested) and that cultures not only from the cervix but also from the urethra should be taken in chlamydial investigations in women.

However, the Liverpool group found, in a study[139] of 393 women, mainly random STD clinic patients (chlamydial isolation rate $33^1/_3$%) that only in 5% of chlamydia-positive patients was the isolate from the urethra alone. In 37%, only the cervix was infected and, in 58%, C. trachomatis was isolated from both sites. Urethral isolates were not necessarily associated with urinary symptoms. Results suggested that, contrary to Paavonen's conclusions, even in the presence of urinary symptoms, chlamydial tests on the cervix alone were sufficient for the diagnosis in most cases.

The results of tests from 34 chlamydia-positive mothers in the puerperium, previously included in the above series, were examined separately[140]. It was interesting that a similarly small proportion 5.8% (2) had positive isolates from the urethra alone.

The cause of the "acute urethral syndrome" in 59 female patients was investigated by Stamm et al[141] (1980) and compared with findings in 35 women with typical cystitis and in 66 without urinary symptoms. Bacteria at low counts (mainly coliforms) were found in 27 women with the urethral syndrome on catheterisation or supra-pubic catheterisation. In 11 of the other 32 urethral syndrome patients, chlamydial infection was deduced, either by isolation or significant antichlamydial antibody changes. C. trachomatis was present in only one of 35 with cystitis and 3 of 66 without symptoms.

(d) Chlamydial infection in babies with neonatal conjunctivitis and in their parents

As previously discussed in this thesis, the origin of neonatal conjunctivitis by infection from the maternal genitalia had been suspected since the 18th century[10]. First with respect to gonococcal and later to chlamydial infection, this pattern of infection has been confirmed repeatedly[16,18]. The presence of chlamydial or gonococcal neonatal conjunctivitis implies infection in the mother's cervix and probable recent urethritis in the father. Examination of the parents as an epidemiological group who might be asymptomatic is essential to prevent further spread of infection in the community. Furthermore, maternal sequelae such as post-partum infection, in particular, pelvic inflammatory disease, long recognised in gonorrhoea, had already been associated with chlamydial infections[95,104].

Whereas, using yolk sac culture, chlamydiae were isolated from the cervix in only 7 of 28[48] mothers of babies with chlamydial conjunctivitis, with tissue culture methods, isolates were made in 23 of 25 such mothers. Nineteen of 25 had cervical isolates, ten of 20 were positive in the urethra and 5 of 25 in the rectum. Eight had pelvic inflammatory disease in the puerperium. Six of nineteen fathers had a history of urethritis and, in fourteen, urethritis was present (chlamydia-positive in eight)[142].

In Liverpool, on the basis of a preliminary investigation[143] by the present author and her colleagues, we made a more intensive study[127] of the clinical patterns of neonatal conjunctivitis on a selected group of babies referred to us, because there had been no response to routine treatment with saline bathing or neomycin or chloramphenicol eye drops. Cultures were taken from the conjunctivae only. Of 103 babies referred, N. gonorrhoeae was isolated in 10.6%, C. trachomatis in 32% and both were present in 3 cases. There were chlamydial reisolates from 6 babies' conjunctivae after local tetracycline therapy for two to six weeks.

42.4% of chlamydia-positive babies were premature but only 15.5% babies from whom neither gonococci nor chlamydiae were isolated were pre-term deliveries. This difference in prematurity is significant ($\chi^2 = 6.7$, 1 d.f.**). Chlamydial conjunctivitis may have been more readily diagnosed because these premature babies were still in hospital or chlamydial cervicitis may have been an aetiological factor in producing prematurity by spreading to cause chorioamnionitis.

N. gonorrhoeae was isolated from all mothers (10) of babies with gonococcal conjunctivitis and all but one of their fathers who were not already on chemotherapy. Half these women had post-partum infections (pyrexia in one, infected lochia in another and pelvic infection in three).

Where babies had had chlamydial conjunctivitis, chlamydiae were isolated from 20 (87%) of 23 mothers not already taking antibiotics and from 4 of 10 fathers examined, who were all asymptomatic. Post-partum infections were present in 19 of all 31 (61%) mothers examined (pyrexia in 3, infected lochia in 4, pelvic infection in 11 and wound infection in one). Antichlamydial antibodies measured by the LGVCFT test were found to be significantly raised in all nine mothers examined who had evidence of pelvic infection.

Local treatment only, for four weeks, with tetracycline eye ointment, was given to the babies. Of 24 followed up, re-isolates were obtained from six, of whom two had been inadequately treated for three weeks or less.

Neonatal conjunctivitis has often been considered a self-limiting mild condition, but on more careful ophthalmological examination, signs usually associated with classical trachoma, including pannus and conjunctival scarring have been reported as occasional sequelae[144,145].

A distinctive chlamydial pneumonitis in neonates born of infected mothers has subsequently begun to be recognised but there was no evidence of this in our Liverpool series.

In Schachter's single case in 1975[146], where pneumonitis followed local treatment for chlamydial conjunctivitis, C. trachomatis was the only pathogen isolated from the infant's upper airway and antichlamydial serum antibody levels were raised. Incidentally, both parents had chlamydial genital infections.

Beem and Saxon (1977)[147] confirmed the association between chlamydiae and this syndrome, a non-toxic afebrile pneumonia developing the first twelve weeks of life. The clinical picture was of tachypnoea and a distinctive cough with x-ray findings of a diffuse bilateral interstitial pneumonia. C. trachomatis was isolated from tracheo-bronchial aspirates and high serological levels of anti-chlamydial antibody were found. They reported asymptomatic naso-pharyngeal shedders amongst infants with chlamydial conjunctivitis[148].

Harrison et al (1978)[149] estimated C. trachomatis accounted for one third of pneumonias in infants requiring in-patient treatment in Seattle, U.S.A. and found the serotypes were those of typical genital infection. They suggested systemic treatment might eliminate orpharyngeal infection but possibly at the cost of depressing the immune response. Prospective studies[5c], had suggested 5-13% pregnant women in U.S. cities had chlamydial cervicitis and 40-50% of their infants would acquire chlamydial infection during birth so that, by extrapolation, up to 6% of all infants might have neonatal chlamydial conjunctivitis.

Mardh (1980)[150] found that the incidence of chlamydial cervicitis was 6.1% in 231 pregnant women and 8.4% in 273 puerperal women in Sweden. Studying cord blood into which maternal antichlamydial IgG can freely pass, he found 33% of specimens (i.e. five to six times those culture-positive) had antibodies. Only 22% (5 of 23) babies from chlamydia-positive mothers developed chlamydial conjunctivitis. Nasopharyngeal cultures were not taken.

Three prospective studies were carried out in the United States in 1979 in which pregnant women were investigated for chlamydial infection and treatment was withheld until after delivery.

Hammerschlag[151] in Boston examined 322 pregnant women and found a 2% cervical chlamydial isolation rate; 47% had cervical antichlamydial antibodies and 73% were seropositive (i.e. high back-ground positivity). Chlamydiae were later isolated from four of the six babies whose mothers had chlamydial cervicitis. One baby had chlamydial pneumonitis; from another a naso-pharyngeal isolate was obtained; the other two had chlamydial conjunctivitis. Three of 61 babies whose mothers had cervical antibodies had chlamydial conjunctivitis. Of a group of 28 mothers who had neither cervical isolates nor local cervical anti-chlamydial antibodies, 48% were sero-positive but none of their babies had chlamydial infections.

In 81% of 47 babies tested, significant titres of serum anti-bodies were found at two to four weeks old, possibly due to passive transplacental transfer of maternal IgG antibodies with which they correlated well.

Schachter[152], in San Francisco, recovered chlamydiae from the cervix in 4% of 900 women in the fourth month of pregnancy. Twenty babies from chlamydia-positive mothers and eighteen control babies were followed up. Significantly more conjunctivitis and pneumonia occurred in babies exposed to infection. Chlamydiae were isolated from half these babies and none of the controls. 70% of the babies of these chlamydia-positive mothers had serological evidence of infection, i.e. the incidence of chlamydial infection in the new-born was 2.8%.

Frommell[153] screened 340 women in Colorado. Their chlamydial isolation rate was 8.8%. 18 children born to chlamydia-positive women were compared with 16 control babies born to chlamydia-negative women. The overall chlamydial infectivity of mothers for babies (assessed by culture from the conjunctivae and nasopharynx and from anti-chlamydial tear antibody levels) was 61% and there was infant pathology (conjuncti-vitis or pneumonitis) in 44% of the offspring of chlamydia-positive mothers. The cord blood IgM was not raised, suggesting intrauterine infection had not occurred. As the incidence of chlamydial infection in bottle-fed babies was lower than in breast-fed in those exposed, the possibility of post-partum infection during breast-feeding was suggested.

The risk of pelvic infection in pregnancy and the puerperium to mothers in such studies, quite apart from that of sexual transmission to their consorts is unacceptably high and, in Liverpool, retrospective studies[127,143,154] have been undertaken instead, where the baby with chlamydial neonatal conjunctivitis is the index case, enabling the diagnosis and treatment of the mother and consort to be carried out simultaneously.

The present author and others in the Liverpool group investigated the incidence of pharyngeal chlamydial infection in 23 babies with C. trachomatis conjunctivitis and the post-treatment chlamydial isolation rate from eyes and nasopharynx in 34[154]. Babies were treated either (a) with chlortetracycline eye ointment 1%, inserted 5-6 times daily for 28 days or (b) for 14 days (initially only seven) with concurrent erythromycin syrup (30 mg/kg daily in divided doses). Following a reisolate, babies were recalled and given the combined treatment.

Chlamydiae were isolated from the pharynx in 12 of 23 (52%) before treatment and reisolated from the eyes of 4 of 34 (11.8%) and the pharynx of 14 of 34 (41.1%) after treatment. Chlamydiae were reisolated significantly more often (75%) from those who had had only topical treatment than from those given systemic treatment as well (32%). Clinical signs in the eyes were minimal when the reisolates were made.

Routine radiological examination of the chest in babies with cough was carried out latterly and 8 were so examined. Radiological signs of inflammation in the chest were found in two of these babies. Chlamydial antibody levels were raised in neither, although chlamydiae were reisolated from one baby's eye and pharynx, leaving the aetiology of the lower respiratory tract infection unclear in both.

It was obvious that without routine follow-up pharyngeal swabs, the efficiency of treatment would be overestimated. Even the 32% failure rate following combined treatment was not good. Compliance failure may have been responsible as no reisolates were obtained from premature babies whose treatment was carried out in hospital. On the other hand, more reisolates were obtained from the pharynx, whence both anaerobic and aerobic bacterial are difficult to eradicate after standard chemotherapy, than from the eye.

Our Liverpool studies, involving clinical observation, epidemiology, isolation, serotypes and the use of chemotherapy, have confirmed that C. trachomatis is an aetiological agent in oculo-genital infection, similar to N. gonorrhoeae whose pathogenic behaviour it mimics so closely.

Koch's postulates had been reasonably well fulfilled even from the original work of Halberstaedter and von Prowazek[25,26] and later by Jones et al[43] (1959). Further verification of these postulates has come indirectly from clinical experience but even more directly in two sites of infection from animal models.

Firstly, urethritis was produced by experimental inoculation of the urethra of a baboon by an agent isolated from the rectum of the mother of a baby with chlamydial conjunctivitis[155]. The agent was reisolated from the subsequently inflamed urethra of the baboon. These results were later confirmed[156] and extended using genital chlamydiae isolated from men with NGU to infect other baboons' urethras. Urethral lesions, sero-conversion and chlamydial shedding for three months ensued.

Secondly, chlamydial isolates from the fallopian tubes of two patients with acute salpingitis were inoculated directly into the tubes in two grivet monkeys and via the cervical canal in another, resulting in acute salpingitis in all three monkeys[131]. Typical lesions of salpingitis were caused with sero-conversion and reisolation of C. trachomatis.

## INTRODUCTION: PART IV

### THE SENSITIVITY OF C. TRACHOMATIS TO ANTIBACTERIAL CHEMOTHERAPEUTIC AGENTS IN VITRO AND IN VIVO

When its classification was still uncertain, Gordon and Quan[157] suggested that C. trachomatis' sensitivity to chemotherapy was an indication of the agents' biochemical complexity and their relation to bacteria rather than viruses. Thus chemotherapeutic agents which affect the synthesis of bacterial cell walls or activity of bacterial enzymes are often active against chlamydiae. Penicillin is bactericidal and irreversibly destroys sensitive multiplying agents by interfering with cell wall synthesis[158]. Chlamydiae have a cell wall which contains peptidoglycans like bacteria and, in tissue culture, with increasing concentrations of penicillin, normal inclusions are replaced by abnormal ones which do not fluoresce or develop normally. They are arrested at the initial stage and cannot proceed to form elementary bodies with a normal cell wall[159,66,5,6,81]. Other antibiotics, tetracycline, chloramphenicol and erythromycin interfere with protein synthesis in various ways at ribosomal level and are bacteriostatic, temporarily inhibiting bacterial growth. Chlamydiae with their own enzyme systems are sensitive to these antibiotics, erthromycin and tetracycline being very effective against chlamydial genital infections[160,161].

However, clinical practice and conclusions drawn from in vitro experiments do not always agree. Penicillin[162] and chloramphenicol[160] were found inhibitory to certain laboratory-adapted strains of chlamydiae in chick-embryo or tissue culture. However, in Liverpool, in clinical practice, we have found them ineffective.

In a series of 22 women[163], with proven concurrent gonococcal and chlamydial infection, treated with a single injection of procaine penicillin (1.2 mega units intramuscularly) plus amoxycillin (500 mg orally) although gonorrhoea was eradicated in them all, we reisolated C. trachomatis from 17.

Of 106 babies with presumptive chlamydial conjunctivitis[127] (C. trachomatis were isolated either from the baby's eye or its mother's cervix or both) 24 had been treated with local chloramphenicol (for 1-17 days, mean 6.3) before conjunctival chlamydial cultures were taken. C. trachomatis was isolated from 20 of 24 and these all responded either to local tetracycline alone or in combination with systemic erythromycin.

The clinical appearance of chlamydial conjunctivitis was usually modified by chloramphenicol in that the discharge often ceased and mucosal oedema persisted but to a lesser degree, and the signs recurred during or after stopping treatment with chloramphenicol.

It may be that as intra-cellular parasites, chlamydiae are not affected by antibiotics because the host cell wall, inclusion or matrix may be impermeable and related antibiotics' variation in effects may be due to variable cell penetration. Conversely, antibiotics may succeed against chlamydiae because of an indirect effect via the host cell's metabolism. Chlamydiae need amino-acids, nucleic acids and energy from the host cell. Chemotherapeutic agents affecting the host cell nutrition alone may thus arrest chlamydial infection although they are not effective against the organism itself.

As in much of the history of the investigation of chlamydial infection, it was in trachoma that treatment was first described, with copper sulphate and other chemical cauterizing agents by the Egyptians and later with scarification and the expression of follicles in Greek and Roman times[164]. The first effective treatment was with oral sulphonamides in 1938[165] to cure trachoma in American Indians. When wide spectrum antibodies were introduced, the dramatic effects of local and systemic tetracycline were noted[166] and now, provided that it is long-term, (six weeks or, if intermittent, six months) systemic or local therapy with tetracycline or erythromycin is considered satisfactory by the World Health Organisation (W.H.O.) for endemic trachoma, although failure of patient compliance and re-infection may cause persistent or recurrent infection[167]. (To rifampicin, previously recommended, resistant strains have been found to develop).

Although Credé's method of prophylaxis against gonococcal neonatal conjunctivitis (using 2% silver nitrate) has been known since 1881, it is quite ineffective against chlamydial infection and is, in any case, no longer used in British obstetric practice. Where it is employed, the true incidence of gonorrhoea in these babies and their contacts and the sensitivity of the gonococci so killed will not be known and infection in their mothers and possibly their mothers' consorts will not be treated. Furthermore, it has recently been shown to be ineffective in some cases of gonococcal infection[170,14].

Meanwhile, despite a widely held belief that the signs and symptoms of NGU eventually resolve in the majority of cases with no active treatment[171 172], due to a high remission rate (in 50-70% of men, the disease slowly regresses in 1-3 months) the patient's discomfort, anxiety and, occasional complications and spread to others, were strong indications for active treatment. Various trials, with antibiotics empirically chosen and placebos, were carried out. Criteria for diagnosis and cure were often unclear and studies were not controlled. However, placebo trials, unacceptable today, indicated the untreated course of the disease and some useful guidance about possible therapy was obtained[173,174].

Treatment with a combination of streptomycin and sulphonamides was cheap and gave reasonable results and was synergistic, for neither was effective alone. Lyall, in 1953[175], in this department in Liverpool, was looking for a method of treatment for urethritis where preliminary microscopic diagnosis was impracticable (i.e. both gonococcal and nongonococcal). In a large series of 540 patients, he found success rates with this regime of 93% in gonorrhoea and 85% in NGU.

Subsequently, oral medication with tetracycline alone seemed to have a beneficial effect in early trials and Holmes[176] (1967) confirmed this in a classic double-blind trial of tetracycline and a placebo in the closed community of an aircraft-carrier at sea, where heterosexual re-exposure was eliminated. Of 96 men with NGU, there was 86% failure rate in the placebo group and 10-35% failure rate where tetracycline was given from four to seven days, the better results corresponding to the longer course.

Willcox, in a review of NSU therapy in 1977[177], showed an 84% success rate with tetracyclines falling to 68% with a placebo; the streptomycin plus sulphonamides regime was less successful than Lyall had found, at 75% compared with 85% in 1953.

An exhaustive trial of six treatment regimes[178], using three different tetracyclines, was carried out by colleagues in the Liverpool group on 756 men with NGU. This was diagnosed by finding in their urethral discharge or urine threads, more than 15 polymorphonuclear leucocytes in more than five high power fields in the absence of gonorrhoea (cultures for C. trachomatis were not performed). Results of single-dose chemotherapy were unacceptable and a longer (21 days) course produced a better response than shorter courses although the results were not significant. Considering the longer courses, (a) possible effects on the bowel flora with the emergence of resistant organisms, (b) unsuitability for third world countries and (c) the possible failure of patient compliance were all noted. It was concluded that it does not seem possible to exceed around 85% success rate.

A clinical comparison of the efficacy of tetracycline and erythromycin had not been recorded until a trial by Oriel (1977)[179] and, in that, dosage schedules differed. In a series of 148 men with NGU (44% chlamydia-positive), 250 mg oxytetracycline was given every six hours for two weeks whereas 500 mg erythromycin stearate was given 12 hourly for the same period. Despite the variation, there was no significant different in response. After two weeks treatment, only 5 of 35 (14%) treated with oxytetracycline and 4 of 30 (13%) treated with erythromycin still had symptoms. Five weeks later, this was 23-25% and three of four men from whom chlamydiae were then reisolated admitted further exposure. Results did not differ significantly between chlamydia-positive and chlamydia-negative groups.

However, earlier workers[180] had found chlamydia-positive NGU responded better than chlamydia-negative NGU to oxytetracycline. Although chlamydiae had been isolated in about 40% of men with NGU, other aetiological agents were obviously involved. These included the ureaplasmas which had been found significantly more often in men with chlamydia-negative NGU.

Differential chemotherapy studies[109] on men with NGU to show the aetiological importance of ureaplasmas as well as chlamydiae were done using aminocyclitols (ureaplasma-effective) and sulphafurazole (chlamydia-effective). Of 91 men, 40% were chlamydia-positive, 69% were ureaplasma-positive, 23% had both infective agents and in 12% neither was isolated. There was no clinical response by the ureaplasma infection to sulphafurazole nor of the chlamydial infection to the aminocyclitols and very little improvement when either drug was given singly to men from whom both or neither agent was isolated.

A similar double-blind trial[181] used minocycline, a tetracycline to which ureaplasmas and chlamydiae were sensitive, and rifampicin, which inhibited only chlamydiae. Of 217 men with NGU, chlamydiae were isolated from 43%, ureaplasmas from 59%; 68% completely recovered after minocycline, only 37.4% after rifampicin.

These results indicate that, whereas chlamydiae may be a major factor, NGU has multiple aetiological agents and treatment will be more successful if guided by diagnosis dependent on isolation results and may indeed sometimes be an exercise in applied microbiology.

In some men, following treatment for gonorrhoea, their urethral discharge recurs and polymorphonuclear leucocytes (> 10/high power field) are seen on microscopic examination of a Gram-stained smear and gonococci are not found on smear or culture[182]. This is postgonococcal urethritis (PGU) and the chlamydial isolation rate ranges from 15[183] to 81%[106].

In 32 cases, Oriel[182] (1977) isolated C. trachomatis and N. gonorrhoeae before single dose therapy with gentamicin (an aminoglycoside similar to streptomycin) (in 11 men), ampicillin (in 11 men) and spectinomycin (not effective against chlamydiae) (in 10). Gonorrhoea was eradicated in all 32 and in 31 chlamydiae were reisolated and PGU had developed.

Willcox[184] estimated that 44-56% of men with gonorrhoea also had NGU and that 20-30% patients treated with penicillin for gonorrhoea subsequently require treatment for PGU.

These are manifest cases but unfortunately, in women where postgonococcal cervicitis occurs, there is frequently no recurrence of symptoms to alert suspicion.

In a previously mentioned study[163] by the Liverpool group, 22 women with gonorrhoea, later found to have concurrent chlamydial infection, had been given a single treatment with intra-muscular and oral penicillin. Gonorrhoea was eradicated but C. trachomatis was reisolated in 17 (77%). In a later Liverpool survey[185] of a similar group with the double infection who had followed that same treatment regime, chlamydiae were reisolated in 84%.

Oriel[182] also found single dose therapy unsuccessful when gonococcal and chlamydial infection coexisted. In only 5 of 23 such women treated with ampicillin (2 g) and probenecid (1 g) and in only 4 of 18 treated with spectinomycin, were both eradicated.

In a later Liverpool study[185], 262 women with gonorrhoea were given either single dose penicillin treatment (procaine penicillin 1.2 mega unit and either probenecid 1 g or ampicillin 1 g) or a week's course of tetracycline (250 or 500 mg six hourly for seven days). Pelvic infection developed in 11 of 129 treated with penicillin (previous chlamydial isolation rate 52%) and in only one of 133 given tetracycline (previous chlamydial isolate rate 54%). These differences in pelvic infection rate were significant ($\chi^2 = 7.24$, 1 d.f.**). N. gonorrhoeae was not reisolated but C. trachomatis was, in 4 of 8 originally chlamydia-positive treated with penicillin who developed pelvic infection and in the single case treated with tetracycline who developed pelvic infection and who had also been chlamydia-positive originally.

Although tetracycline and erythromycin are effective against chlamydial infection and gonorrhoea, N. gonorrhoeae is less susceptible to these drugs, particularly at the prolonged low dosage suitable for the treatment of C. trachomatis infections. As mentioned previously, in an attempt to treat either gonococcal or nongonococcal urethritis satisfactorily, without differentiating between them, Lyall had, in 1953[175], found streptomycin plus sulphathiazole a most effective regime. However, many strains of N. gonorrhoeae developed resistance to streptomycin and due to that, and its ototoxic effects, it is now an unsuitable form of treatment.

Paavonen (1980)[186] looked at trimethoprim-sulphadiazine, a combination related to cotrimoxazole (trimethoprim-sulphamethoxazole). Sulphamethoxazole had been found satisfactory in gonorrhoea[187] and genital strains of C. trachomatis had been found susceptible[188] although it was said to be less effective in NGU than oxytetracycline and erythromycin[189]. Paavonen compared the effect of the trimethoprim-sulphadiazine combination in 75 men with NGU and their 75 female consorts, with that of a placebo. Thirty-four of each sex had active treatment. Nineteen men and 15 women were chlamydia-positive before active treatment and in one of each sex, C. trachomatis was not eradicated after treatment. In the placebo group, C. trachomatis was reisolated from over 80% of the originally chlamydia-positive after treatment and five chlamydia-positive patients developed complications. Trimethoprim-sulphadiazine is thus effective in the treatment of chlamydial infections provided that the sexual partner is treated simultaneously.

The frequent absence of symptoms and ill-defined signs in women with nongonococcal cervicitis, making diagnostic criteria difficult to assess, was the reason for carrying out most of these studies in men but the need to treat their sexual partners to prevent re-infection has been stressed[111].

As it is likely that chlamydiae producing conjunctivitis in both babies and adults in this country are also present at other sites, local tetracycline alone is rarely satisfactory. Further, systemic tetracycline must be avoided in pregnancy, lactation and in the treatment of young children, due to the effect on bone and teeth formation and so systemic erythromycin is used in the treatment of pregnant and lactating mothers and infants.

The Liverpool group followed up 38 previously chlamydia-positive contacts of men with NGU for 2-14 months after treatment with oxytetracycline hydrochloride 250 mg 6 hourly for 31 days, or, in the case of pregnant women, erythromycin B.P. 250 mg 6 hourly for 21 days[9]. (Treatment was given for 21 days as this had been found most satisfactory in men and because, after only 7 days oral treatment with tetracycline 250 mg 6 hourly, C. trachomatis was reisolated by Dunlop (1980)[190] from the cervix of the mother of a baby with neonatal conjunctivitis), C. trachomatis was reisolated in five and in four there had been a strong possibility of re-infection. The distinction between relapse and re-infection is notoriously difficult after treatment of sexually transmitted disease, especially with long follow-up periods and, in general, treatment failure rates make no distinction between the two.

Work in Liverpool by the present author and her colleagues with NGU contacts and mothers and babies has shown that chlamydial cervicitis and neonatal conjunctivitis respond to treatment with tetracycline and erythromycin as urethritis does. Post-treatment reisolates have generally been due to re-infection or compliance failure. This is supported by our investigation (1980)[9] of serum tetracycline levels in 26 women on a 21 day course of oxytetracycline. They were adequate therapeutically as the range was 0.53-2.01 μg per ml (mean = 1.21) whereas the minimum inhibitory concentration (MIC) of oxytetracycline hydrochloride for C. trachomatis = 0.2 μg/ml. (Minimum inhibitory concentration (MIC) for C. trachomatis is the lowest concentration of a chemotherapeutic agent preventing the appearance of any chlamydial inclusions in the tissue culture cells). Similarly, when Thin (1979)[191] estimated the vaginal oxytetracycline levels from washings in 20 NGU contacts on a 10 day course of oxytetracycline dihydrate, they found them to be 0.6-6.5 μg/ml, well above the MIC.

Earlier work with antibiotics has been extended and now the susceptibility of C. trachomatis can be assayed quantitatively in tissue culture systems[188,193,194,195]. The most active agents are erythromycin and oxytetracycline. Trimethoprim, sulphonamides and chloramphenicol are also inhibitory but C. trachomatis is not killed by aminoglycosides such as spectinomycin, kanamycin or gentamicin, nor by penicillin, ampicillin, lincomycin, clindamycin or metronidazole. Resistance can develop to rifampicin, previously thought most effective[168,169]. However, studies in guinea pig conjunctivitis caused by the related C. psittaci suggest that spiramycin may be even more effective than the tetracyclines[188].

It was noted that some related antibiotics had very different effects on C. trachomatis but the different C. trachomatis serotypes do not differ significantly in their sensitivity to chemotherapeutic agents other than sulphonamides[196]. There is little evidence that resistance develops to the commonly used tetracyclines[195]. This suggested that any patient from whom chlamydiae were isolated would respond to tetracycline therapy and that this would permanently eradicate the infection.

Studies[195,197] had shown that the MIC and the minimum lethal concentration (MLC) (the lowest concentration of the agent required to prevent (a) the tissue culture from being able to transmit infection to further tissue culture on serial passage and (b) infection recrudescing after the antibiotic is removed) may differ widely. This reflects the property of latency previously observed[72] in which there may be arrested growth of the elementary body after adsorption under conditions of nutritional inadequacy. Antibiotics may affect nutrition either by their effect on enzymatic systems or cell wall systems of C. trachomatis.

This work, together with our clinical experience with penicillin and chloramphenicol led us to doubt the validity of MIC results in clinical treatment. The author of this thesis had already worked in the laboratory on the isolation of clinical specimens and routine MIC determinations. We decided to use different laboratory techniques to demonstrate the chemotherapeutic susceptibility of C. trachomatis.

Modifications to previous experiments to determine MIC

Previous tissue culture experiments to determine the MIC of chemotherapeutic agents against C. trachomatis have been rather different from antibiotic investigations in clinical infections. Tissue cultures were infected with highly laboratory-adapted and uncloned (variant containing) strains of C. trachomatis. C. psittaci and LGV and other fast-growing strains were also used. These grow rapidly, producing multiple growth cycles, and are very different in many respects, possibly including antibiotic susceptibility, from genital strains or low tissue culture pass strains of C. trachomatis which very few previous laboratory workers have used.

On the other hand, with clinical isolates of genital strains there is only a single cycle of growth[198] and there are no secondary growth cycles so all inclusions are at the same stage of development and growth is synchronous. It is likely that chemotherapeutic agents may have widely varying effects according to the stage of the tissue culture infection at which they are added.

However, in previous laboratory work, simultaneously with infection of the tissue culture with test chlamydiae, the trial antibiotic was inoculated and remained present at a constant level for the whole single step growth cycle.

Yet, in a clinical case of, for example, conjunctivitis, before any antibiotic is given, the chlamydial infection is likely to be well established with chlamydial inclusions in different developmental phases.

Bowie (1978)[197] described how incubation of infected tissue cultures for 48 hours, prior to the addition of antibiotics, decreased the susceptibility of C. trachomatis so that much higher concentrations than usual of tetracycline, erythromycin and rifampicin were required. It was suggested that these methods would give a much better prediction of the antibiotics' efficacy, particularly in chronic trachoma. A similar plea for laboratory methods more relevant to clinical needs had been made by Jawetz and Hanna in 1961[199].

In clinical practice, the antibiotic level falls between doses and, if not taken regularly or in the case of local treatment not reaching the site of infection, this trough may be even lower. Temporary addition of antibiotic at varying intervals after tissue culture infection, followed by washing of the tissue culture and incubation with fresh antibiotic-free medium, may be used to give a "pulse" effect more similar to clinical dosage and so affect different ages of inclusion[163,198].

Preliminary incubation of tissue culture with the antibiotic before infection with C. trachomatis enables (a) any slowness of the antibiotic in penetrating the tissue culture cell to be compensated for before the infection occurs or (b) any direct effect on cell metabolism to be magnified. Passage after exposure to the antibiotic allows multiplication in tissue culture which is fresh and totally antibiotic free and allows the MLC to be compared with the MIC.

The possibility of mere suppression of infection or "escape" in the form of latent organisms where MLC differs from the MIC or even of resistant forms must be considered when there is a lack of response to conventional treatment or apparent recurrence of infection without re-exposure.

These modifications of the MIC investigation can be used to explain clinical failures with apparently efficacious drugs. (i) Further incubation of duplicate infected cultures at the MIC may cause the inclusions to reappear when chlamydistatic effects wear off.

(ii) Failing that, duplicate cultures after incubation in identical conditions should be washed to remove the antibiotic and inspected after further incubation. (iii) Several passes after incubation of such washed tissue cultures may be needed before some latent chlamydiae multiply again, whereupon their antibiotic sensitivities should be re-tested.

Quantitative assay of the susceptibility of C. trachomatis to commonly used chemotherapeutic drugs was undertaken. The site of activity on the ribosomal system of erythromycin, oxytetracycline and chloramphenicol is similar although their clinical effects are very different. With penicillin, there is a gross discrepancy in that, while the MIC seems achieved well within conventional therapy levels, there may be little or no clinical response. Therefore, these four drugs were studied.

## INTRODUCTION: PART V

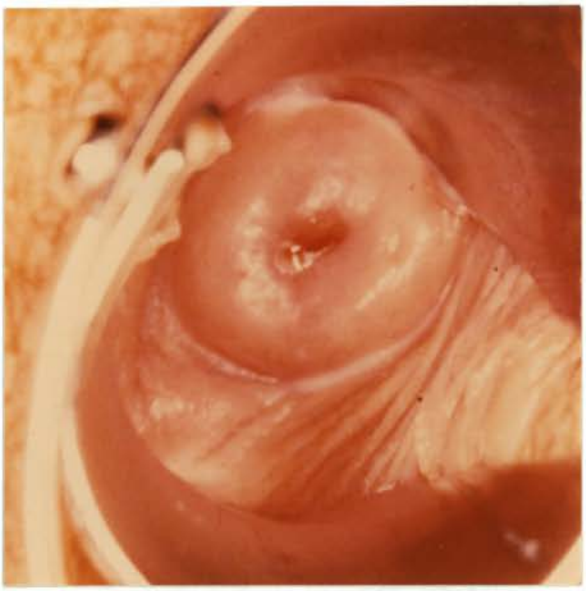
### CERVICAL EPITHELIUM

Cervical epithelium may be either (i) original (native) (ii) squamous metaplastic (physiological) (iii) atypical (dysplastic). Original epithelium may be stratified squamous or columnar. Columnar epithelium lines the endocervical canal and associated glands with a single layer of tall mucous-secreting epithelial cells. Squamous epithelium continues upward from the vaginal lining to meet the columnar epithelium at any point across the ecto- or endocervix[118].

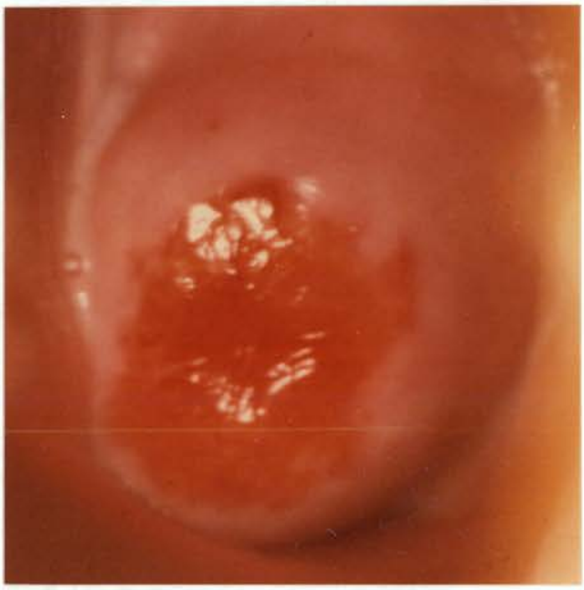
Ectopy or erosion is a cervical condition recognised since the vaginal speculum was introduced. Fischel (1880)[200] described it as congenital, being present in the foetal cervix, absent in childhood, and returning later. He and his contemporaries considered it resulted from loss of squamous epithelium of the ectocervix due to the macerating effects of exudates associated with post-abortal and puerperal discharges and colpo-vaginitis. Gonococcal infections and the trauma of parturition were thought to be common causes. Myer[201,202] (1910, 1930) thought columnar epithelium grew out to cover the damaged area from the os and later was replaced by an ingrowth of squamous epithelium from the ectocervix. Myer based his view that ectopy was due to acute or chronic inflammation on the presence of redness and leucocytic infiltration there. Columnar epithelium is seen to be red, on colposcopic examination, as it is only one cell layer thick overlying the capillary bed. Leucocytic infiltration is universal in the cervix, mainly due to plasma cells, and is associated with sperm destruction, clearing of epithelial debris and antibody production. There need therefore be no inflmmation present although "cervical erosion" and "chronic cervicitis" were both terms used to label ill-defined cervical appearances[119]. (Illustrations p.48a).

Using the colposcope, one finds either ectopic columnar epithelium encroaching from the endocervix on to the ectocervix (previously called "erosion") or columnar epithelium having undergone physiological squamous metaplasia ("chronic cervicitis"). Neither of these is considered an abnormal condition. As erosion and chronic cervicitis imply the presence of inflmmation, the term "ectopy" and "metaplasia" are preferred. "Erosion" is reserved for true erosions found accompanying

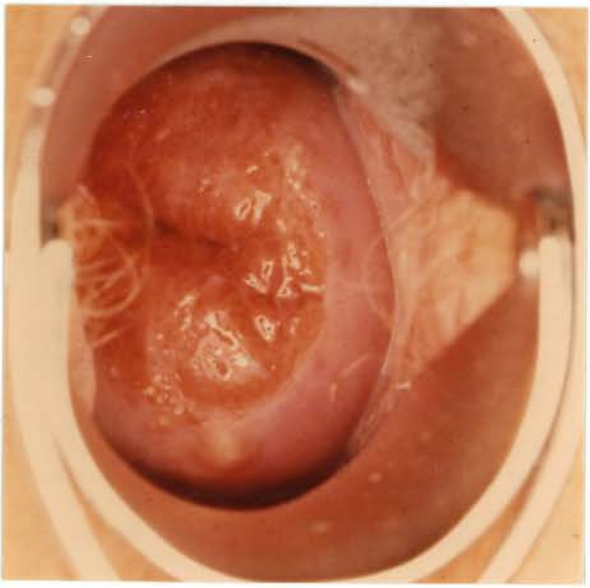
Clinical Signs - Normal cervix with clear endocervical mucus in each case



(a) no ectopy



(b) simple/superficial ectopy



(c) metaplasia

cervical infections with herpes genitalis or syphilis.

Various factors are thought responsible for the presence of ecto-cervical columnar epithelium[203]. The boundary between the stratified squamous epithelium and columnar epithelium internally may coincide with the external os or be either just inside or outside it, the latter producing congenital columnar epithelium on the ectocervix (i.e. ectopy). This has been found in approximately one out of three baby girls. Variations in the circulating oestrogen-progesterone ratio may be responsible for the spontaneous appearance of ectopy in pregnancy and conversely the rarity of post-menopausal ectopy. In pregnancy, prolapse of the endocervix is partly responsible for the production of ectopy. Another factor is the action of oestrogen in causing hyper-plasia of the cervix, in particular increasing the stromal volume.

Columnar epithelium is more receptive to infection by intracellular parasites such as C. trachomatis and N. gonorrhoeae than stratified squamous epithelium. The surface squamous epithelium consists of desquamating dead cells 30 or so layers away from the blood supply and is normally quite resistant to such infecting agents, whereas columnar cells are phagocytic and actively metabolising, a single layer deep. One may consider the frequency of vaginitis due to the gonococcus and other bacteria in the pre-pubertal girl where the vaginal epithelium is only 2-3 cell layers deep compared with that in the menstrual years where superficial cells are prominent and there are many stratified layers of squamous epithelium.

Squamous metaplasia is an irreversible physiological change occuring in areas of columnar epithelium over a short transition period, mainly in pregnancy and adolescence, and, to a lesser degree in late intra-uterine development. The area of metaplasia is known as "the transformation zone" (previously "transition zone"). The commonest form of metaplasia is an essentially physiological process and typical metaplastic squamous epithelium results. Dysplasia and carcinoma-in-situ are forms of abnormal metaplasia where genetic change has occurred[117,118,204].

Studies in adolescence showed that the area of metaplasia in promiscuous girls with accompanying dysplastic features was significantly more than in virgins where only limited change had occurred, suggesting that coital activity stimulates the development of metaplasia[205,206].

In pregnancy, there is a high incidence of metaplastic change in the ectocervix[207]. The pH of vaginal secretions bathing it in early pregnancy drops significantly and this may stimulate metaplasia in ectopic columnar epithelium. Metaplastic islands may so enlarge as to merge with the squamo-columnar junction in later pregnancy. Atypical metaplasia has been observed, with variable neoplastic potential, in pregnancy. However, following the trauma of delivery and the puerperium, the atypical epithelium may be partially or completely removed. After childbearing, there is little alteration in the area of metaplasia until the menopause, when there is shrinkage of the whole cervix.

Coppleson and Reid think that the period of transition from columnar to metaplastic squamous epithelium may be short, hours, days or weeks and it is then that environmental factors may exert their mutagenic action by alteration of the nucleic acids in the dividing cells. Possibly mutagenic nucleic acids may originate from infectious agents or spermatozoa[205].

Much has been written about factors in the genesis of carcinoma of the cervix but Gagnon's report[208] in 1950 of the freedom of virgin women from this condition is surely fundamental. Rotkin (1973)[209] enumerated factors distinguishing cervical carcinoma patients. These included an early age at first coitus, a large number of consorts, sexual promiscuity and a high incidence of sexually transmitted disease. Indeed, carcinoma of the cervix has many characteristics in common with sexually transmitted diseases. In their review of the pathogenesis of cancer of the cervix, Novak and Woodruff[210] state that the evidence is very strongly in favour of the theory that infection, possibly viral, plays a major role in the production of cervical neoplasm.

Herpes simplex virus (HSV) type II fulfills several important criteria as an aetiological agent of cervical carcinoma (a) Naib[211] found an association between viral cervicitis and the presence of pre-malignant lesions of the cervix, (b) Several groups[212,213] in U.S.A. found significantly higher levels of antibody titres to genital herpes in women with carcinoma of the cervix than in matched controls.

(Others[214] in Israel, New Zealand, Taiwan and Colombia did not but the populations they studied differed from those in the original studies in race, age, socio-economic level and other respects), (c) Herpes genitalis viral antigens have been found in cervical cancer tissue[215], (d) Fragments of herpes genitalis DNA have been found in cervical neoplastic and preneoplastic cell nuclei[216]. (DNA from the sperm head, commoner, more abundant and less transient than viral sources, has also been postulated as a cervical carcinogenic agent[217,218]).

The cervix was confirmed as the site of chlamydial infection in the female genital tract when inclusions were reported in smears[41] and biopsy[42]. Accompanying local inflammatory changes were noted in mothers of babies with neonatal chlamydial conjunctivitis[93], women with fresh chlamydial eye infection and female sexual contacts of men with chlamydial conjunctivitis[112]. Hilton and Oriel confirmed this in female contacts of NSU patients[110,111].

Naib (1970)[219] had found a high incidence of atypical cells in cervical smears taken from mothers of babies with chlamydial conjunctivitis.

Alexander (1973)[217] in his review, however, considered that while C. trachomatis was twice as prevalent in the cervix as herpes simplex virus (HSV) and its relationship to sexual activity well known, he had been unable to recognise a clear cut relationship between cervical chlamydial infection and dysplasia.

When Schachter (1975)[220] studied women with cervical dysplasia he found the rate of sero-reactivity to chlamydial antigens (77.6% MIF test, 21.5% LGVCFT), higher than with other clinic populations. The chlamydial isolation rate was not raised in this group and the high antibody levels were unchanged on re-testing, suggesting that this reflects previous exposure to chlamydiae and not recent infection.

Paavonen (1979)[221] found the chlamydial isolation rate in 177 gynaecological patients with cervical dysplasia (Papanicolaou groups III-V, moderate to severe) was 16% and in a control group of gynaecological out-patients 9%. Serum antichlamydial antibody levels in 93 dysplasia patients were significantly more often positive, and at higher levels, by LGVCFT and micro-immunofluorescence testing, than in controls. No such difference was found in testing for anti-HSV type II or cytomegalic antibodies.

Carr (1979)[222] examined cervical secretions for chlamydial antibody by immuno-fluoresence. Where antibody was present, 11 of 15 (73%) had Papanicolaou II or III smears. In the absence of antibody, these abnormal smears were found in only 3 of 18 (16.7%) patients. Following tetracycline treatment, the smears reverted to Class I and antibody disappeared.

These studies do not clarify whether dysplasia and eventually carcinoma of the cervix is related to the long-term effects of latent chlamydial infection or to other consequences of sexual promiscuity.

## MATERIALS AND METHODS

### PART 1: CLINICAL INVESTIGATIONS

### (a) Nongonococcal urethritis contacts

#### Patients

The group of women consisted of 455 contacts of men with NGU. They had attended Liverpool Royal Infirmary consecutively on specified days between January 1974 and December 1977. All the male contacts had been diagnosed on the basis of finding 10 polymorphonuclear leucocytes per high power field (x 100 objective) in a urethral smear with no evidence of N. gonorrhoeae. In those male STD clinics, facilities for the isolation of C. trachomatis were not available. Patients who had received antibiotics recently were excluded and the last occasion on which they had taken antibiotics was recorded for all patients.

404 women presented with contact slips. The others either attended of their own accord or were referred by another doctor and gave a history that their sexual contact was attending a male STD clinic (the diagnosis was then always obtained from the clinic named).

#### Clinical examination

A routine genital examination was carried out. Swabs for detection of N. gonorrhoeae were taken from the urethra and endocervix on at least two separate occasions and used to inoculate on to a selective medium containing antibiotics (London Analytical and Bacteriological Media Ltd. London) and make a smear for Gram staining. Specimens were obtained with cotton tipped swabs. Endocervical swabs were taken after a Cusco's vaginal speculum had been inserted into the vagina and all the vaginal discharge had been removed. The cervix was wiped with sterile cotton and a cotton tipped swab was inserted into the cervical canal and rotated firmly round the mucosa. The swab for C. trachomatis culture was also taken from the endocervix in this manner and preceded that taken for gonorrhoea.

In this group of women and in the mothers of babies with neonatal conjunctivitis, cultures for C. trachomatis were only taken from the cervix. Concurrent and subsequent investigations in Liverpool (mentioned in the introduction[139]) had shown that little more would have been achieved by taking urethral cultures as well, as only 5% were positive only in the urethra.

Swabs taken for C. trachomatis were immersed at once in 3.0 ml transport medium in screw-topped bottles and stored at 4°C until inoculated on to tissue culture (as will be described in "Laboratory methods") usually between two and four hours later but occasionally after storage for 24 hours. All patients were investigated for syphilis, trichomoniasis and candidiasis by conventional means.

## Clinical signs

Clinical signs were recorded on the specially designed request form as well as the case sheet at the time of sampling.* The state of the ectocervix was recorded in three categories: no ectopy, simple ectopy and hypertrophic ectopy. This latter was an area of ectopy which was oedematous, congested and bled. When the area of ectopy was irregular with peninsulae and islands of squamous epithelium and Nabothian follicles, it indicated metaplasia, usually physiological but possibly dysplastic. The contents of the endocervix were recorded as clear mucus, cloudy mucus, mucopus (yellow) and mucoid discharge (white). When examining the cervix, care was taken to remove discharge extruding from the cervix before the contents of the canal were assessed so that vaginal flora might not contaminate clear endocervical mucus. Mucoid or mucopurulent endocervical discharge combined with hypertrophic ectopy indicated cervical inflammation.

## Treatment

Routine treatment of chlamydia-positive women was with oxytetracycline hydrochloride tablets 250 mg 6-hourly for 21 days. Pregnant women were not given oxytetracycline but received erythromycin B.P. 250 mg six-hourly. Treatment was given for 21 days as this is the longest course advised for men with NGU and we did not want possible inadequate treatment to be a cause of persisting clinical signs or chlamydial reisolation or both.

*See p.54a

CHLAMYDIA REQUEST FORM ONLY

Patient's Name and Number | Age | Sex | Previous Lab. Nos.

Type of Specimen | Date and time taken

Consultant | Requested by: | Ward/Clinic

History and Clinical Appearance | LMP | O.C | Preg. | Para.

Recent Antibiotics | Type | Dates | Reason

Concurrent infections | GC | Cand. | Trich. | Other

LAB REPORT

CHLAMYDIA:– NEGATIVE/POSITIVE

whole coverslip inclusion count =

Current Lab. No.

Chlamydial culture request form

As mentioned in the introduction, a reisolate had been reported after only 7 days treatment with oxytetracycline 250 mg six-hourly, from the cervix of a women in the puerperium whose baby had neonatal chlamydial conjunctivitis[190].

Follow up

All chlamydia-positive patients were asked to attend for review to check whether or not candidiasis had developed and to assess the clinical response, on the 14th day of treatment, on day 28 (seven days after completion of treatment), twice more at three weekly intervals and subsequently at six weeks i.e. a total of three months post-treatment observation. Some patients continued to attend at three monthly intervals and others returned within that time with fresh symptoms and requesting re-investigation. On each post-treatment visit, specimens were taken for culture for C. trachomatis, N. gonorrhoeae, Trichomonas vaginalis and Candida species.

(b) Mothers and Babies

Patients (babies)

129 babies were examined between January 1974 and November 1979. They had been referred by paediatricians because of failure to respond to routine treatment with saline bathing and neomycin or chloramphenicol eye drops or because no bacterial growth was reported by the laboratory.

Clinical examination

Specimens were obtained with cotton tipped swabs, as used for the women patients' investigations, from the mucosal surface of the lower lids in all cases and from the upper lids, if possible. Pharyngeal specimens were taken with the same type of swabs by gently stroking the posterior pharyngeal wall.

Other sexually transmitted diseases

These were examined microscopically and by culture for gonococci, other bacteria and for candida. Stuart's medium was used for transport of bacteria but most swabs for gonococcal culture were inoculated directly on to the selective medium described above. Swabs for chlamydial culture were placed in transport medium and processed as before.

## Patients (parents)

Mothers were investigated in the maternity unit where possible but mothers of premature babies and those whose babies had late-onset conjunctivitis were asked to attend as out-patients. Their clinical examination was as described above for NGU contacts. They were investigated for syphilis, gonorrhoea, chlamydial cervicitis, trichomoniasis, candidiasis and, in a few cases genital herpes, by conventional methods.

If possible, fathers were similarly investigated if the mother and/or baby had gonococcal, chlamydial or syphilitic infection.

## Follow-up

Babies were examined during treatment and swabs were taken at each subsequent out-patient attendance. Each mother was asked to bring her baby for examination at ages 4, 8, 12, 18 and 24 weeks. This period of observation was longer if a reisolate was obtained. Babies were treated with either (a) 1% chlortetracycline ointment inserted into both eyes 5-6 times daily for 28 days or (b) for 14 days with concurrent erythromycin syrup 30 mg/kg daily in divided dosage. Treatment was initially given to most babies by nursing staff in the maternity units and was continued at home by their mothers on discharge from hospital.

# PART II: LABORATORY METHODS

In essence, the procedures for the isolation of *C. trachomatis* are similar to those developed by Richmond[223] using irradiated cultures, with modifications by the Liverpool group. The isolation procedures and the methods used for subsequent counts of the total number of chlamydial inclusions recovered have been previously described in detail[56,224,225].

## Preparation of McCoy cells

McCoy cells (initially obtained from FLOW laboratories, Irvine, Scotland) have since been serially propagated in "Medium 199" (Wellcome) plus 10% foetal calf serum (FLOW laboratories) and 0.02 M sodium bicarbonate with vancomycin and streptomycin, each at 100 µg/ml.

Preparation of monolayers

Cell suspensions of 1.5 x $10^5$ cells per ml, obtained from confluent monolayer cultures by conventional trypsinisation techniques, were dispensed in 2 ml amounts in 5 ml (universal) screw-topped glass bottles containing a 16 mm circular glass coverslip covering the bottom of the bottle. Low speed centrifugation was used to disperse the MCC on the coverslip. After incubation at 37°C for 24 hours, the medium was replaced by 3 ml of the same medium plus 0.5% final concentration of glucose and 50 µ per ml nystatin just before inoculation of the clinical specimen.

Initially, and for approximately half the period of this study, MCC which had not been previously treated by x-irradiation or any anti-replicative chemical agents were used. In the latter half, however, cells treated with cycloheximide (BDH) were used. Immediately before inoculation of the clinical specimens, 0.1 ml of an aqueous solution of cycloheximide was added to each culture to give a final concentration of 1.0 µg/ml. Parallel titrations in cycloheximide-treated and untreated cultures with clinical specimens and known laboratory strains of C. trachomatis confirmed that there were no significant differences in the isolation rate between the two types of cell. The purpose of adding cycloheximide was to slow down but not prevent further MCC replication and the advantage was the ease of reading the cultures subsequently.

Inoculation of clinical specimens

Swabs for chlamydial isolation had (as previously mentioned) been taken into 2-3 ml transport medium, and stored at 4°C until inoculated on to tissue culture. The inoculation usually took place between two and four hours later but occasionally after 24 hours. The swab contents were thoroughly dispersed into the transport medium using a mechanical shaker for one minute and 0.4 ml was then inoculated into each of two coverslip cultures of replicating MCC. These were centrifugated at 2500 *g* for one hour on the swing-out head of an MSE major centrifuge at room temperature. Inoculated bottles were then incubated at 37°C in a constant atmosphere of 5% carbon dioxide in air, with the bottle tops loosened, to maintain the medium at a constant pH of 7.0-7.4.

## Inclusions

Under these conditions, C. trachomatis undergoes a single cycle of growth. Each infective particle forms an intra-cytoplasmic inclusion packed with new infective particles (elementary bodies). Inclusions are first detectable after 18 to 24 hours of incubation and reach their maximum number after 38 to 48 hours. Thereafter the size of the inclusions and the number of particles increase and they begin to rupture the host cells between 72 and 96 hours. However, the released elementary bodies cannot establish a fresh growth cycle in other cells in the MCC under these cultural conditions. Thus, the number of inclusions countable in a given MCC is directly proportional to the input number of elementary bodies which were capable of establishing an ongoing intracellular infection.

After 48 hours, therefore, the tissue culture fluid was removed and the coverslip stained by the Giemsa method. Large intracytoplasmic inclusions packed with basophilic particles can easily be seen by light microscopy with the 40 x objective and under dark-ground microscopy, where chlamydia particles give an intense lemon-yellow fluorescence sharply distinct from the dull green-brown appearance of McCoy cells.

## Avoidance of cross-infection

Strict precautions were taken to minimise the risk of cross infection. No standard reference strains of chlamydiae have been used in this laboratory since this work began. Fresh clinical specimens were inoculated at a different time and in a different laboratory from that in which incubated specimens were harvested. During centrifugation, bottletops were tightly sealed. Control McCoy bottles were inoculated with transport medium, centrifuged and incubated with each batch of specimens. All remained negative.

## Counting procedure

The results of inoculating MCC with known laboratory strains of C. trachomatis or with infected clinical material were assessed quantitatively. Having been inoculated for 48 hours and Giemsa-stained, the MCC were examined under dark-ground microscopy at 400 x magnification. The total number of inclusions in the whole coverslip were counted unless preliminary inspection had suggested that the count would be greater than 5,000-6,000 in which case, the inclusions in 60 microscope fields (approx. 1/15th of the coverslip area) were counted, and the number per whole coverslip was derived by multiplication.

The statistical validity of the counting procedure has been discussed by Johnson and Hobson of the Liverpool group[159]. With the strain "STU", it has been calculated that the 95% confidence limits of the counts is ± 23.5%.

Growth of laboratory strains

The "STU" strain of C. trachomatis was isolated here in Liverpool from the cervix of a seventeen-year old girl and the pool used was the first passage in the yolk sac of chick embryos from the positive primary MCC inoculated with the original clinical specimen.

The TC 691 strain was also a Liverpool cervical isolate. It had had two tissue culture passages but had not passed through the yolk sac pool and was of known serotype (D/E).

Preparation of antibiotics

The standard commercially available preparations for intravenous therapy for each antibiotic were used, freshly obtained for the hospital pharmacy. (Benzylpenicillin powder from Glaxo Laboratories, Greenford, Middlesex; tetracycline hydrochloride powder from Pfizer Ltd, Sandwich; erythromycin base powder from Abbott Laboratories, Kent; chloramphenicol sodium succinate powder from Carlo Erba, U.K. Ltd, London). Each antibiotic was freshly dissolved in sterile distilled water from vials for each experiment and working dilutions were made in tissue culture growth medium. Final concentrations of each antibiotic in this medium were assayed in an agar cup-plate microbiological assay system using Staphylococcus pyogenes NTC 6571 or Escherichia coli NTC 10418.

Chloramphenicol

In common with most other workers in this field, the intravenous product chloramphenicol sodium succinate was chosen for convenience. However, following subsequent work in the Liverpool group, it was recognised that, the effectiveness of chloramphenicol sodium succinate in vitro is lower than in the body where the succinate radical must be removed enzymatically for full activity. Subsequently, the Liverpool group have found a convenient source of the pure base chloramphenicol available in sterile ampoules in the form of 0.5% eye drops (Smith and Nephew). The activity of this is approximately ten times that of the succinate form in vitro (personal communication, Dr. Hobson).

Antibiotic sensitivity assays

To avoid any problem of synergy or antagonism with other antibacterial agents, all MCC used in the experiments with antibiotics were maintained throughout in media which did not contain any other antibiotic. Vancomycin and streptomycin (see above) are routinely used at concentrations of 100 µg/ml in normal tissue culture work with C. trachomatis and show no significant antichlamydial activity. However, in the present work, they were used only during the initial preparation of McCoy cells for the experimental MCC. After that, the growth medium was removed and the coverslip washed in antibiotic-free medium three times to remove extracellular vancomycin and streptomycin. The washed coverslip cultures were then incubated with 2 ml antibiotic-free medium for four hours to leach out vancomycin and streptomycin.

In the initial experiments, confluent MCC were washed and changed to fresh growth medium containing various concentrations of the test antibiotic. Control MCC were changed to fresh antibiotic-free medium. All were immediately infected with C. trachomatis test strains (TC 691 or STU strain) at a dose calculated to yield 10,000 to 15,000 inclusions per coverslip and centrifuged without further delay. After varying periods of incubation, these cultures and control antibiotic-free infected cultures were removed, Giemsa-stained and examined under darkground microscopy.

Subsequently, "pulses" of test antibiotic were added to infected MCC by appropriate change of medium or removed at varying intervals of incubation after centrifugation-assisted adsorption of C. trachomatis. The medium of the infected controls, antibiotic-free, was changed at the same time.

## PART III: USE OF THE COMPUTER

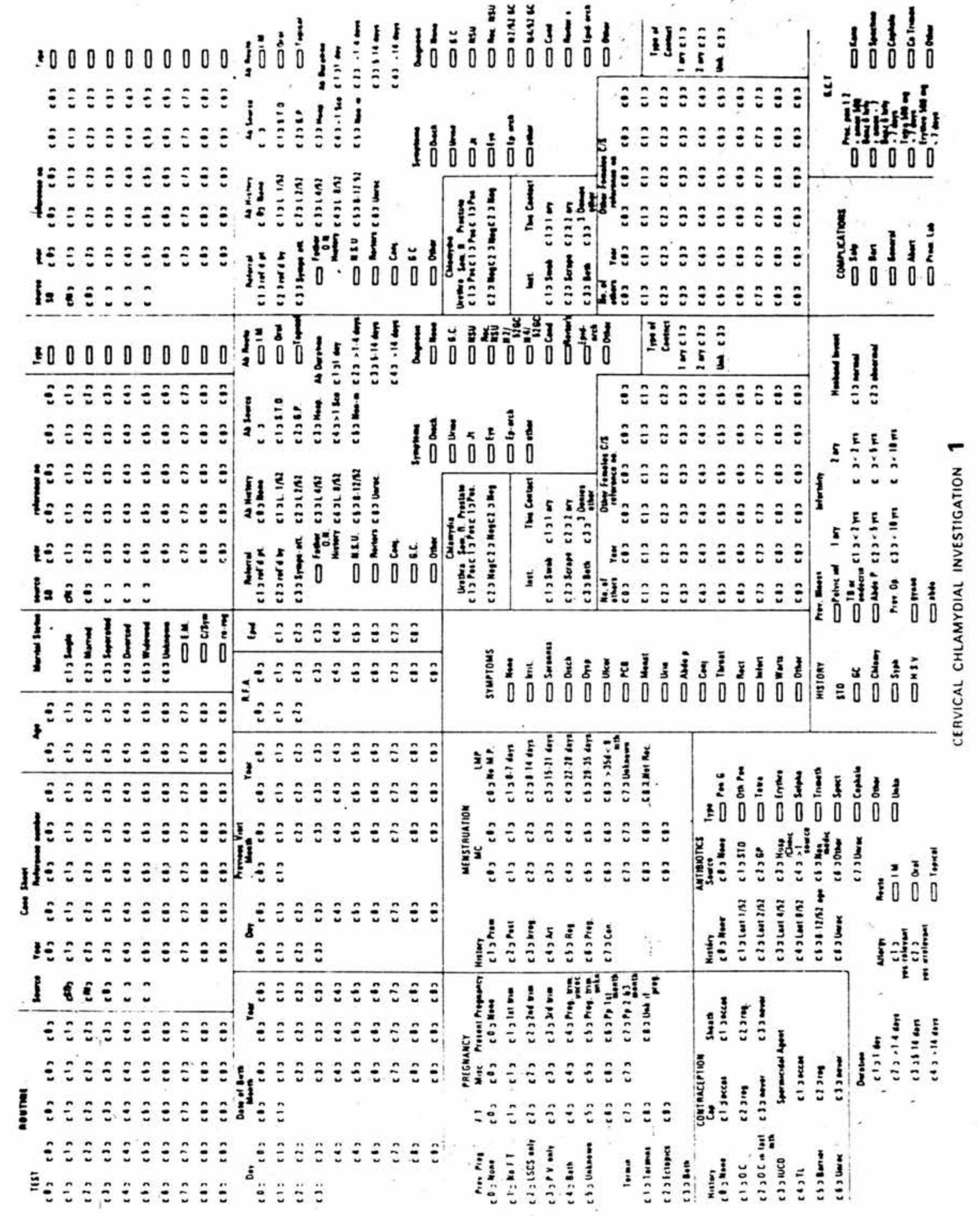
When the information had been abstracted from our case sheets, it was transferred to the $Opscan_{(R)}$ forms* so that the data could be stored in the computer bank. Having written the appropriate programmes (some with the help of the Biostatics Department) various comparisons regarding the incidence of chlamydial infection related to such factors as age, parity, ectopy etc. were made. Missing data including sets of variables (e.g. weight of baby) omitted from the Opscan form was later retrieved from the case sheets, in some cases that of the original maternity hospital.

## PART IV: STATISTICAL METHODS

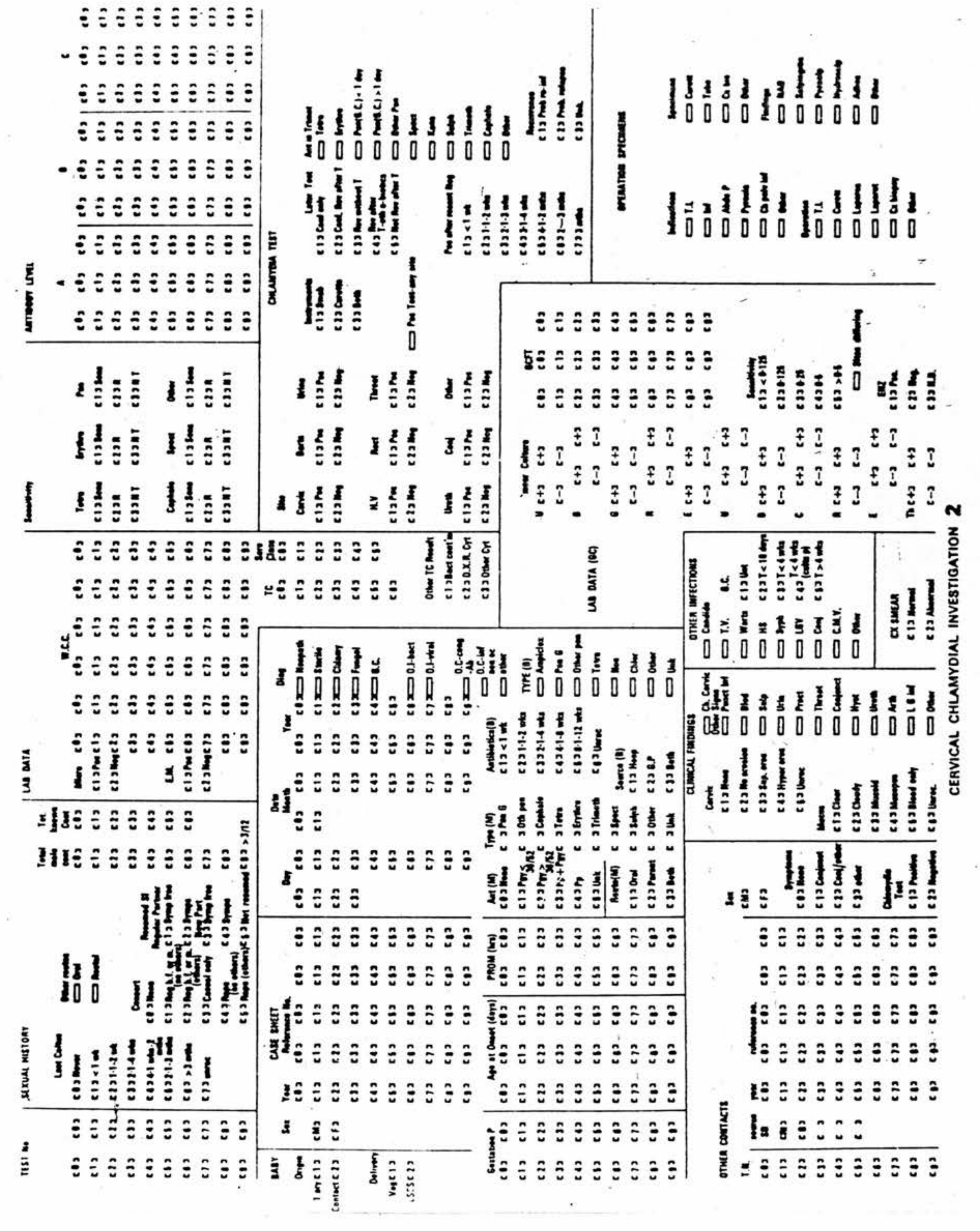
In cases where the data had been reduced to four-fold contingency tables, $\chi^2$ with Yates's correction was used. If data had been reduced to 2 x N contingency tables, ordinary $\chi^2$ (N-1 d.f.) was used to test for any difference in proportions. Where appropriate, $\chi^2$ for linear trend was also applied to 2 x N tables. One-way analysis of variance or unpaired t tests were used on the logarithms of whole coverslip chlamydial counts.

*See p.61a and b

Opscan$_{(R)}$ form - side 1



Opscan$_{(R)}$ form - side 2



RESULTS

PART I: CLINICAL RESULTS

(a) CONTACTS OF MEN WITH NONGONOCOCCAL URETHRITIS

C. trachomatis isolation rate

C. trachomatis was isolated from 160 of 455 women (35.2%) at their first visit.

Reason for attendance - Table 1

394 attended as NGU contacts (C. trachomatis isolation rate 35.2%). 39 attending of their own accord and 22 referred by other doctors were said to be NGU contacts and this diagnosis was confirmed by the relevant male sexually transmitted diseases (S.T.D.) clinic.

Age - Table 2

Their ages ranged from 16 to 62 years (mean 27). In the chlamydia-positive group, the age range was 16 to 37 years (mean 25).

Comparing 5-year groupings from 16-40 years, the chlamydial isolation rate declined significantly (49% to 14%, $\chi^2 = 8.06$, 1 d.f.**) and the total $\chi^2$ was 23.9, 4 d.f.***. $\chi^2$ for linear trend was 23.0, 1 d.f.*** which indicates a significant decline in the chlamydial isolation rate with age.

Marital status - Table 3

50.3% women of the 455 women were single and 39% were married; the remaining 10.7% were divorced or separated. 61.8% chlamydia-positive women were single and 28% were married. The C. trachomatis isolation rate was significantly higher in single (43%) than in married women (26%) ($\chi^2 = 12.73$, 1 d.f.***).

Obstetric history - Table 4

The majority of women (54%) were nulliparous and their isolation rate (41%) was significantly higher than that (26%) of the 38% women who had had full-term vaginal deliveries ($\chi^2 = 9.47$, 1 d.f.**). 62.5% of chlamydia-positive women were nulliparous and 27.5% had had vaginal deliveries.

Of the 181 who had had full-term pregnancies with either vaginal or caesarian section deliveries (39.8% total), 154 (C. trachomatis isolation rate 29.9%) had 1-3 children and 27 had 4-6 children. None of these latter women were chlamydia-positive. Compared with the women who had few or no children, they were older and married. These factors also affected the chlamydial isolation rate.

5.5% of patients had had miscarriages (C. trachomatis isolation rate 32%) and 6.2% had terminations (C. trachomatis isolation rate 43%). Two women had had ectopic pregnancies; one of them was chlamydia-positive.

Hormonal factors

(a) Present pregnancy - Table 5

Eleven were pregnant. One was in the first post-natal month and four were possibly pregnant. Those numbers were too small for any conclusions about the effect of pregnancy on the C. trachomatis isolation rate in NGU contacts to be made.

(b) Menstrual cycle - Table 6

In 414 (91%) women who had a regular menstrual cycle (28 days ± 2) and where the date of the last menstrual period was known, the month was divided into four weeks. Week one started from the first day of menstruation. The week in which the culture for C. trachomatis was taken was recorded. There was no significant difference in the isolation rates in successive weeks of the menstrual cycle. (Total $\chi^2 = 1.06$, 3 d.f. (N.S.)).

This was repeated, only examining data from patients using oral contraception and the rates again did not vary significantly throughout the cycle. (Total $\chi^2 = 0.68$, 3 d.f. (N.S.)).

(c) Contraception - Table 7

The types of contraception used and the chlamydial isolation rates associated are shown in the table. The chlamydial isolation rate (48.1%) in 233 women taking oral contraceptives (then or within the previous month) was significantly higher than in (a) all those not taking oral contraception, excluding those whose method was unknown or unrecorded, (24%, $\chi^2$ = 25.2, 1 d.f.***) (b) those using barrier methods (24%, $\chi^2$ = 4.1, 1 d.f.*) (c) those using contraceptive methods other than barrier methods or oral contraception (21%, $\chi^2$ = 23.5, 1 d.f.***). There was no significant difference in chlamydial isolation rates between those using barrier methods (24%) and those using no contraception (25%, $\chi^2$ = 0).

Amongst chlamydia-positive women, 66.2% used oral contraception, 16.9% used no contraception and 5% used barrier methods. Of the remainder, equal proportions used an intrauterine contraceptive device, had had tubal ligation or had within the previous month stopped using oral contraception.

Symptoms - Table 8

The incidence of symptoms and their distribution appears in Table 8. The C. trachomatis isolation rate amongst NGU contacts was significantly raised when any symptoms were present (41.2%, $\chi^2$ = 5.87, 1 d.f.*) and when there was a vaginal discharge (45%, $\chi^2$ = 7.27, 1 d.f.**).

When, however, 260 women were examined in the absence of concurrent infections which might cause these symptoms (trichomoniasis, candidiasis, gonorrhoea), the presence of any symptoms no longer significantly increased the isolation rate (37%, $\chi^2$ = 2.98, 1 d.f.) although discharge did (46%, $\chi^2$ = 7.11, 1 d.f.**) and post-coital bleeding was suggestive (4/5, $\chi^2$ = 3.78, 1 d.f. N.S.) (Table 9).

Similarly, in the absence of trichomoniasis, the chlamydial isolation rate was higher in women with (44%) than without (29%) a discharge ($\chi^2$ = 7.23, 1 d.f.**) (Table 10).

Coital experience - Table 11

There are difficulties in taking an accurate history in relation to coital experience.

(a) Last intercourse

62% women (isolation rate 39%) admitted having had intercourse within the previous four weeks. Overall, there was no significant difference in chlamydial isolation rates (38.5-39.8%) whether intercourse was one week or three weeks ago.

(b) Number of consorts (in the previous three months)

352 women (77% of total, C. trachomatis isolation rate 32.1%) had only one consort. 52 (11%, 50% chlamydia-positive) admitted to two consorts and two women (one chlamydia-positive) had several casual consorts. The C. trachomatis isolation rate was significantly higher in those with more than one consort than in those with only one ($\chi^2$ = 5.66, 1 d.f.*).

This correlates well with isolation rates in the male partners' primary (55.3%) and secondary (37%) contacts.

(c) Known consorts

443 women (isolation rate 35%) had one or more consorts where case sheets were available for study. Of these 443, 16 (isolation rate 75%) had two such contacts.

The remaining 12 of 455 total women were contacts of men with NGU who had attended STD clinics in other towns whence we had been sent summaries of their histories.

Clinical signs - Table 12 (Illustrations p.65a)

(a) Ectocervix

The appearance of the ectocervix in relation to the chlamydial isolation rate is shown in the table. The isolation rate was significantly higher in those with any ectopy (46.1%, $\chi^2$ = 28.0, 1 d.f.***), in those with simple ectopy (38.5%, $\chi^2$ = 12.7, 1 d.f.***) and in those with hypertrophic ectopy (72.4%, $\chi^2$ = 49.7, 1 d.f.***) than in those with no ectopy (21.5%).

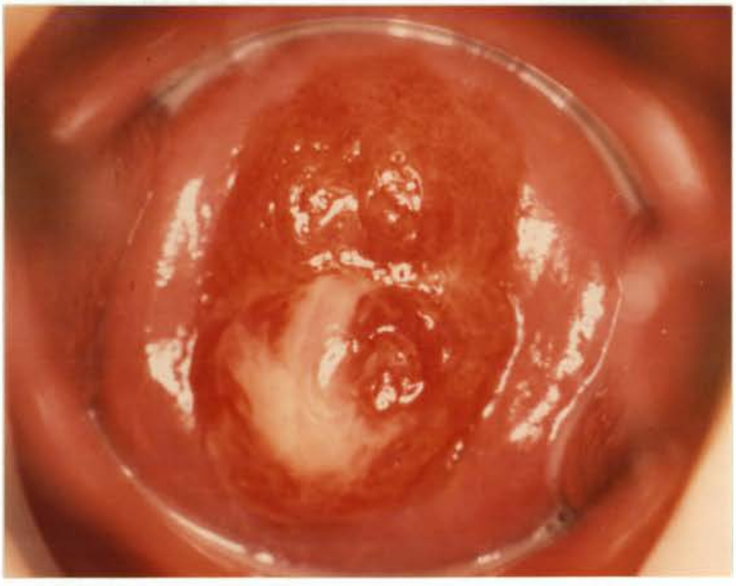
It was significantly higher in those with hypertrophic ectopy than with superficial ectopy ($\chi^2$ = 19.5 with 1 d.f.***) 26% chlamydia-positive women and 51% chlamydia-negative women had no ectopy.

(b) Endocervix

The varying endocervical contents in relation to the chlamydial

(a) endocervical mucopus in the absence of ectopy

(b) endocervical mucopus with superficial ectopy



(c) endocervical mucopus with hypertrophic ectopy isolation rate are shown in the table. The chlamydial isolation rate was significantly higher in those with mucopus (72.4%) than with clear and cloudy mucus (24.6%) ($\chi^2$ = 72.6, 1 d.f.***). 44% of chlamydia-positive women and 9% chlamydia-negative women had endocervical mucopus. 28% chlamydia-positive women and 46% chlamydia-negative women had clear mucus.

In 325 women (71.4% total, chlamydial isolation rate 22.5%) there were no signs of endocervicitis and 32 (7.0% total, 81.3% chlamydial isolation rate) had both hypertrophic ectopy and endocervical mucopus.

(c) Bleeding on examination and post-coital bleeding - Tables 13 and 14 (Illustrations p.66a)

43 women bled when the cervix was examined. The chlamydial isolation rate was significantly raised at 58.1% ($\chi^2$ = 9.91, 1 d.f.**) 15.6% chlamydia-positive women and 6.1% chlamydia-negative women bled on examination.

Eight women had complained of post-coital bleeding. Five were chlamydia-positive and in four of these, there had also been bleeding on examination. There were no associated abnormal smears. Ectopy was reported in all but three of these women.

(d) Abdominal pain and salpingitis - Table 13

27 women had complained of abdominal pain (isolation rate 40.7%) and in 12 there were signs of salpingitis (isolation rate 50%, $\chi^2$ = 0.62, 1 d.f.).

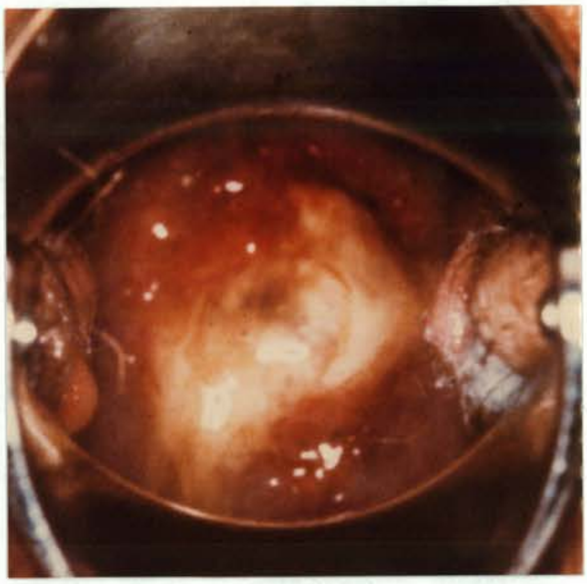
Cervical smears - Table 14a

Ayre smears were taken in 83 women; two were abnormal, both women were chlamydia-negative.

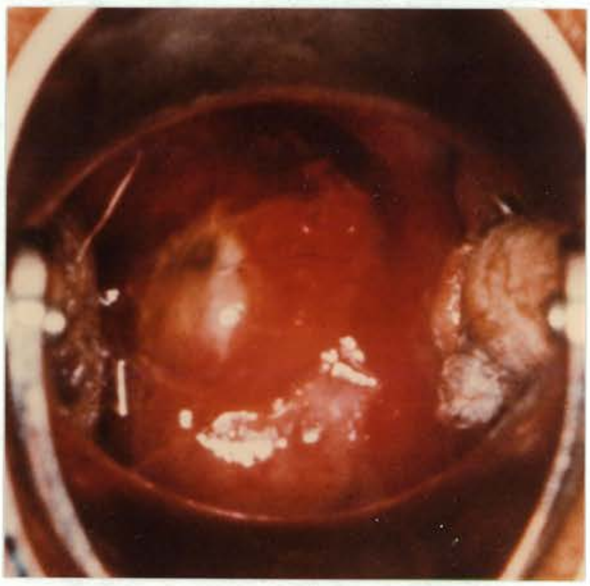
Concurrent infections - Table 15

The presence of other infection in relation to the chlamydial isolation rate is shown in the table. In 211 women, normal flora only was found. (40% were chlamydia-positive). Only with trichomoniasis was the chlamydial isolation rate significantly raised (60.5%, $\chi^2$ = 10.51, 1 d.f.**).

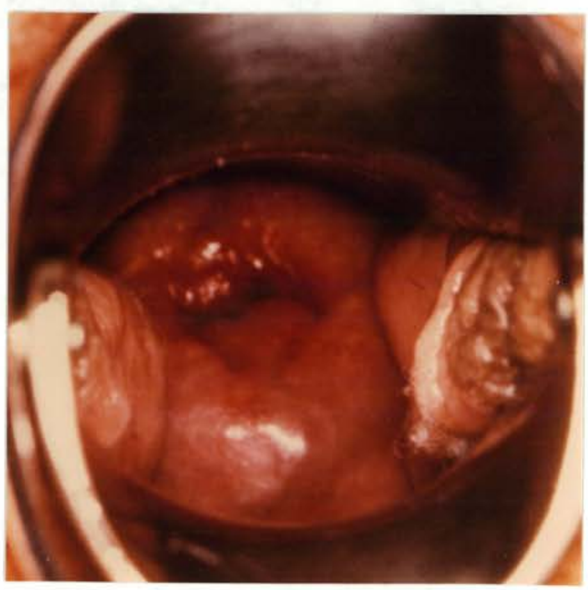
Clinical Signs - Pathological cervix
History of post-coital bleeding
Bleeding on examination



(a) chlamydia-positive
- before mopping



(b) chlamydia-positive
- bleeding after mopping



(c) chlamydia-negative
- post-treatment
- no bleeding after mopping

Although these women were NGU contacts, gonorrhoea was found in seven (1.5% total). Three were chlamydia-positive.

Contraception and ectopy - Table 16

To assess the relationship between oral contraception and ectopy, both of which had highly significant individual effects on the isolation of chlamydiae, the four possible categories were compared and analysed statistically (simple and hypertrophic ectopy were combined).

(1) The isolation rates in patients with (54.2%) and without (35.5%) oral contraception in the presence of cervical ectopy ($\chi^2$ = 6.56, 1 d.f.*).

(2) The isolation rates in patients with (54.2%) and without (34.3%) ectopy in those taking oral contraception ($\chi^2$ = 6.51, 1 d.f.*).

(3) The isolation rates in patients with (34.3%) and without (16.2%) oral contraception in the absence of ectopy ($\chi^2$ = 6.72, 1 d.f.**).

(4) The isolation rates in patients with (35.5%) and without (16.2%) ectopy in the absence of oral contraception ($\chi^2$ = 8.4, 1 d.f.**).

Chlamydial isolation rate relative to age and ectopy - Table 17

As noted above (Table 2) the chlamydial isolation rate lessened significantly with age. Ectopy also decreased significantly (total $\chi^2$ in age range 16-40 years = 21.6, 4 d.f.***, $\chi^2$ for linear trend = 15.27, 1 d.f.***).

Comparing the chlamydial isolation rate in the presence and absence of ectopy, over that age range, there was no significant difference in the curve gradients. (Regression coefficient t = 2.2, 6 d.f.).

Chlamydial isolation rate relative to age and oral contraception - Table 18

The chlamydial isolation rate fell significantly from 37% in age group 16-20 years to 19% in 31-35 years, in the absence of oral contraception (total $\chi^2$ for range = 5.0, 3 d.f. (N.S.) but $\chi^2$ for linear trend = 3.99, 1 d.f.*).

With oral contraception, the isolation rate fell only from 54% to 45% (total $\chi^2$ = 1.51, 3 d.f. (N.S.), $\chi^2$ for linear trend = 1.22 1 d.f. (N.S.)). However, comparing the isolation rates in the presence and absence of oral contraception in that age range, the curve gradients did not differ significantly (regression coefficient t = 0.98, 4 d.f.).

Ectopy relative to age and oral contraception - Table 18

Without oral contraception, the percentage ectopy fell significantly from 51% in age group 16-20 years to 25% in 31-35 years. (Total $\chi^2$ = 7.06, 5 d.f. (N.S.); linear trend $\chi^2$ = 6.33, 1 d.f.*).

With oral contraception, ectopy fell less in this age range, from 71% to 55% (Total $\chi^2$ = 3.27; 2 d.f. (N.S.); linear trend $\chi^2$ = 0.54 1 d.f. (N.S.)). The curve gradients did not differ significantly (regression coefficient t = 0.91, 4 d.f.).

Chlamydial isolation rate relative to oral contraception under and over 25 years - Table 19

The isolation rate was significantly higher in those taking oral contraception (48%) than in the others (28%, $\chi^2$ = 7.24, 1 d.f.**) in those under 25 years, and in those over 25 years (45% and 19%, $\chi^2$ = 11.8 1 d.f.***).

Chlamydial isolation rate relative to parity and ectopy - Table 20

With increasing parity, there was a significant fall in isolation rate from 42% in nullipara to 11% in those with four children. (Total $\chi^2$ = 14.76, 4 d.f.**, $\chi^2$ for linear trend = 11.07, 1 d.f.***).

Ectopy also decreased significantly with increased parity, from 62% in nullipara, to 39% in para 4. (Total $\chi^2$ = 12.87, 4 d.f.; $\chi^2$ for linear trend = 9.26, 1 d.f.**).

Comparing the chlamydial isolation rate in the presence and absence of ectopy over that parity range there was no significant difference in the curve gradients (regression coefficient t = 1.23, 6 d.f.).

Chlamydial isolation rate relative to parity and oral contraception - Table 21

The chlamydial isolation rate fell slightly with increasing parity in the absence of oral contraception 35-25% ($\chi^2$ = 0.31, 1 d.f. (N.S.)) and rose slightly in the presence of oral contraception 47-57% ($\chi^2$ = 0.014, 1 d.f. (N.S.)). A lower percentage of parous women (43%) than nullipara (68%) used oral contraception ($\chi^2$ = 18.4, 1 d.f.***). These curve gradients did not differ significantly (t = 1.46, 4 d.f.).

Chlamydial isolation rate relative to parity and endocervical contents - Table 22

The proportion of endocervical mucopus relative to other endocervical contents was not related to parity. Increasing parity had no definite effect on the incidence of chlamydial infection accompanying mucopus.

Chlamydial isolation rate relative to (a) age, ectopy and parity (Table 23) (b) age, ectopy, parity and oral contraception (Table 24)

Numbers in each category were too small to compare different age groups even when, to judge the effect of parity, nullipara were contrasted with parous women. The groups were even smaller when the effect of oral contraception was also considered. Nothing was added from these studies to knowledge gained from previous comparisons.

Antibiotics - Table 25

56 NGU contacts (12.3% of all) had antibiotics in three months preceding the first visit and chlamydial culture. Numbers were small but the majority had been given them by their family doctors for 5-14 days. Penicillin other than procaine penicillin was the commonest (22/56) (chlamydial isolation rate 36.4%). Of the ten who had taken oxytetracycline or erythromycin, all were chlamydia-negative.

Whole coverslip count - Table 26

The inclusion counts ranged from 1-100,000. There were 158 records. The mean was 5780, median 312. The logarithmic form (LGWCC) was used to compare the counts relative to certain variables.

The count was significantly higher in the presence of mucopus rather than clear or cloudy mucus.

The effects of oral contraceptives and of ectopy on the inclusion counts were examined both separately and in combination. No significant differences in the severity of chlamydial infection were found whatever permutations of these variables were compared.

The counts were not significantly affected by age, parity, marital state, symptoms, cervical ectopy, other concurrent infections, methods of contraception or stage in the menstrual cycle.

These results differ from the other quantitative study[126] in which a significant association was found between high counts and not only the presence of endocervical mucopus but also the under 20 age group, the presence of cervical ectopy and the use of oral contraception (including interaction between these last two variables). In that study, 580 counts were studied (including this 158) and a different hypothesis was being tested namely that the proportions of high and low counts (excluding the middle counts) were related to each factor. Whereas here, the middle counts were included to obtain differences in geometric means of the counts in each category. The two are therefore not directly comparable.

<u>Follow up</u> - Table 27

(i) Repeat Swabs

Five to 7 days after the initial chlamydial cultures had been taken, in a proportion of women, they were repeated before any treatment had been given. Of 160 chlamydia-positive women, swabs were repeated in 128 (80%) and confirmed the previous result (100% positive). Of 285 chlamydia-negative, 38 were retested (13.3%). 36 were again negative but positive swabs with low titre counts (< 10 inclusions per coverslip) were now found in two (0.7% of previous negative women, 5.3% of those retested).

Thus total chlamydia-positive tests in the series was 162 (162 of 455, 35.6%) on twice swabbing. There is therefore little point in taking double swabs as the diagnosis can be firmly made in most instances as a result of a single culture on the first visit.

(ii) Follow up surveillance

Follow up surveillance was carried out on 130 of a possible 162 chlamydia-positive women (80.2%).

Nine were chlamydia-positive at each visit and either treatment had not been completed or reinfection had occurred with no intervening negative culture. The other 121, once positive, were later negative on culture for C. trachomatis (a) following appropriate chemotherapy in 110, (b) after chemotherapy elsewhere in two, (c) without any recorded chemotherapy in 9.

From 18 of these 121 women, chlamydiae were reisolated. In three, this reisolate was made at their final visit. The other fifteen women had again become chlamydia-negative by their final visit.

Thirty-six originally chlamydia-negative women had second or subsequent cultures which confirmed that first negative test.

(iii) Originally chlamydia-positive, later chlamydia-negative women including false negatives (121 women)

Their period of surveillance ranged from 5-632 days (mean 198 days).

(a) Cervical signs (Illustrations p.71a,b)

In this group, when chlamydiae had been present, 27% had had hypertrophic ectopy and 47.9% endocervical mucopus. Examined later, when no reisolates were obtained, only 2.6% still had hypertrophic ectopy and only 5.2% endocervical mucopus.

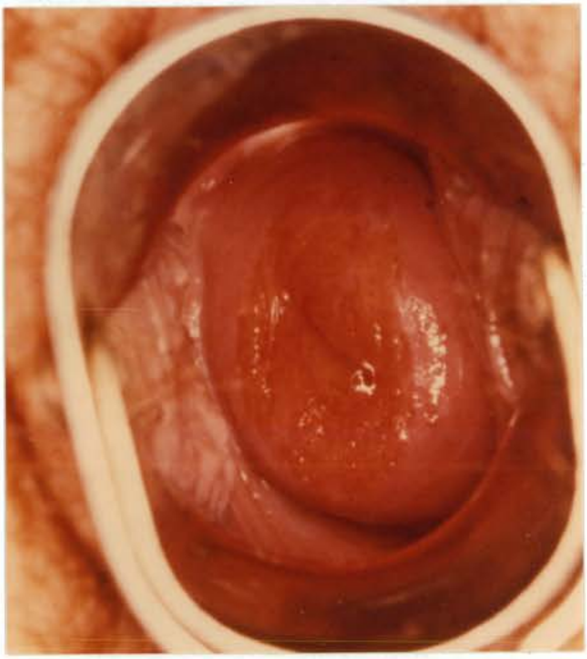
The cervices had reverted from hypertrophic ectopy either to simple ectopy (i.e. mere disappearance of inflammation) or no ectopy. The absence of ectopy, where there had previously been either hypertrophic or simple ectopy, indicated that metaplasia had been completed. Cervical mucopus changed to either clear or cloudy mucus, both physiological appearances.

These changes in signs indicate a response to treatment of chlamydial endocervicitis.

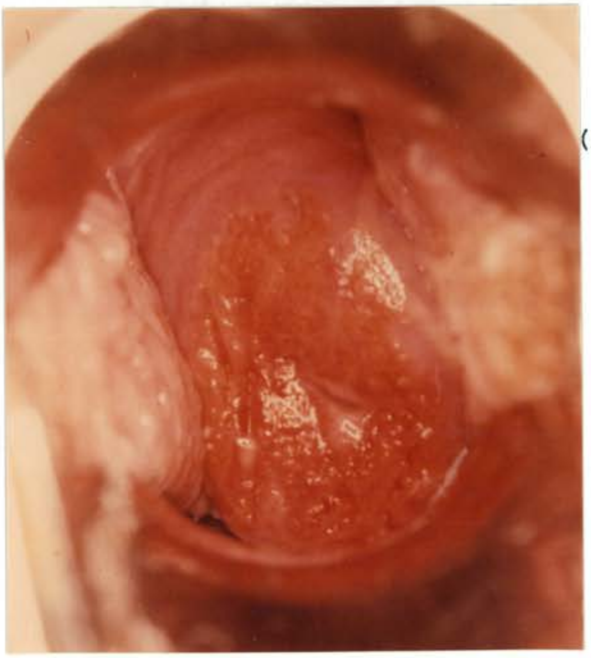
Effect of treatment on signs of inflammation in the cervix (i)

S.A.

(a) pre-treatment; chlamydia-positiv
superficial ectopy with
endocervical mucopus



(b) post-treatment; chlamydia-negati
superficial ectopy with clear
mucus, early metaplasia

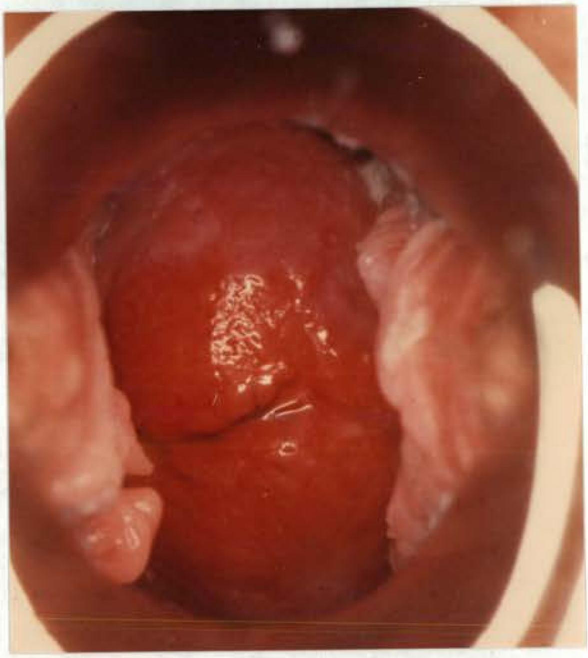


(c) post-treatment; chlamydia-negativ
six months pregnant
superficial ectopy with an
endocervical mucoid plug,
metaplasia

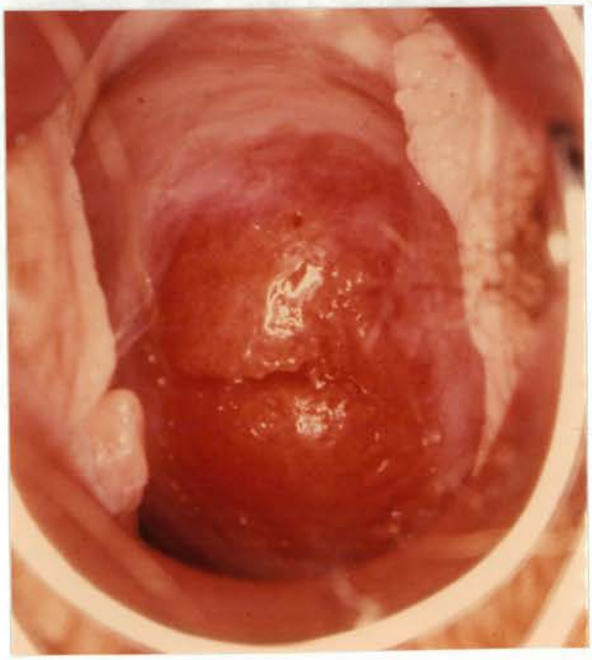
## Effect of treatment on signs of inflammation (ii)

L.B.

(a) pre-treatment; chlamydia-positive
hypertrophic ectopy with endocervical mucopus



(b) post-treatment; chlamydia-negative
superficial ectopy with clear mucus, early metaplasia



(c) post-treatment; chlamydia-negative
superficial ectopy with increased metaplasia and clear mucus

(b) Other infections

*Candida sp.* was isolated in 34% of all 455 women and in 36% chlamydia-positive at their first visit. 38% of this follow-up group had candidiasis at their first visit. By their final visit, this had fallen to 12%. An increase following antichlamydial chemotherapy might have been expected but follow-up surveillance and antifungal treatment avoided this.

Reisolates - Table 28

In 18 women, chlamydiae were reisolated at some time after they had apparently been eradicated. This happened twice with one woman.

(a) Coital activity

Fourteen of these 18 women had resumed intercourse. Nine were with their previous partner and four of these men had recurrent NGU. Four other women had new consorts and these men were said to be symptom free but were not available for examination. The other woman had resumed intercourse within the previous two weeks with a consort regarding whom no information was obtained.

No information was recorded regarding recent intercourse in the other four women with reisolates.

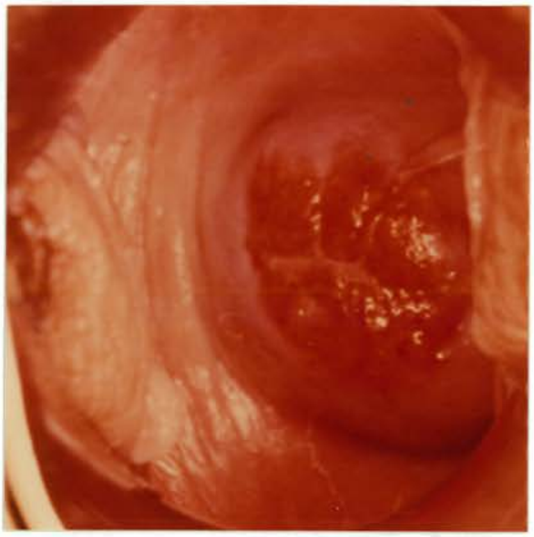
(b) Endocervicitis (Illustrations p.72a,b)

Nine women with reisolates had originally had hypertrophic ectopy and/or mucopus. In two cases, these same signs recurred at the time of reisolation (370 and 87 days later, respectively). In one woman, mucopus with simple ectopy had been present at her first visit and hypertrophic ectopy accompanied the reisolate. Another woman having had the endocervical contents obscured by menstrual bleeding on her first examination, had endocervical mucopus when the reisolate was made. These signs of cervical inflammation were absent when the reisolate was made in the other 14 patients.

Male partners - Table 29

In 443 of these 455 NGU contacts, we had access to their male partner's case sheets. Sixteen of these 443 women had two partners.

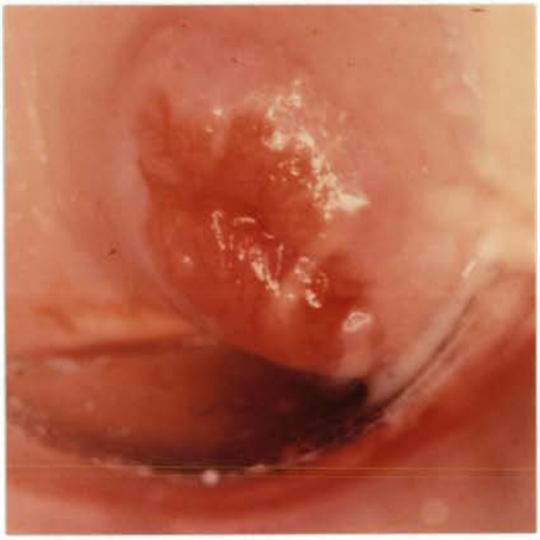
Clinical signs in the cervix accompanying chlamydial reisolation after treatment

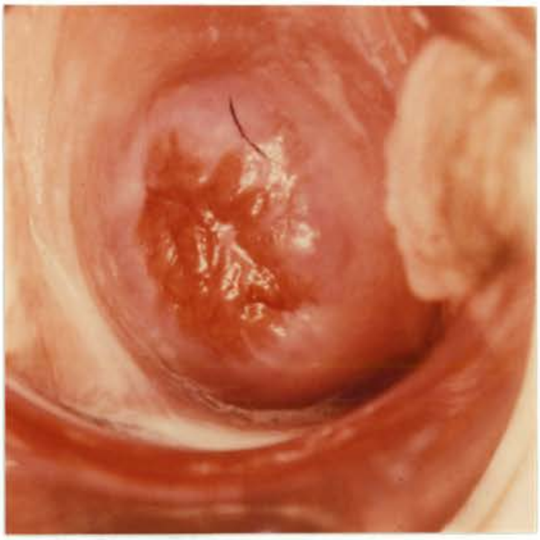


S.H.

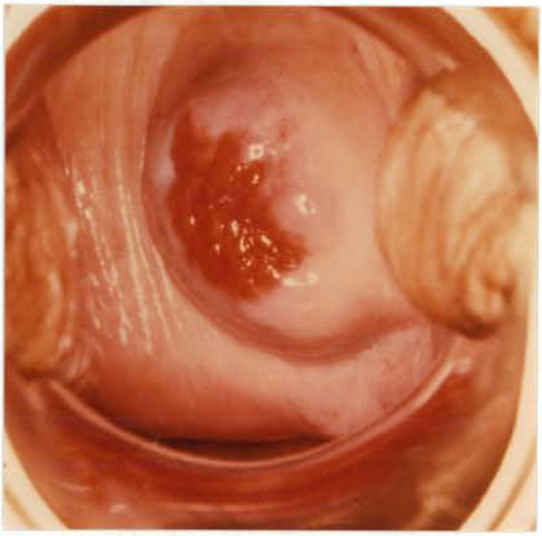
(a) Pre-treatment; chlamydia-positive
hypertrophic ectopy with endocervical mucopus

(b) Post-treatment; chlamydia-negative
superficial ectopy with cloudy mucus





(c) chlamydial reisolate
early recurrence of hypertrophic ectopy and endocervical mucopus



(d) chlamydia-positive; three months later, no chemotherapy meanwhile, hypertrophic ectopy with metaplasia and clear mucus

(e) post-treatment; chlamydia negative metaplasia and superficial ectopy with clear mucus

(consort refused examination)

The first man to attend, whether he referred our patient, attended simultaneously with her or was referred by her to the clinic with some other condition and later was found to have NGU, was named as the first partner (M1). The second to attend, whether he was referred by our patient or had also referred her to the clinic, was named as the second partner (M2). The remaining 427 had only one partner (M1).

(a) Symptoms

67.5% of all women, 69% of the chlamydia-positive group, had consorts who still had symptoms at the time of the woman's first visit.

(b) Diagnosis

427 men were first or only consorts (M1) attending local clinics, 69.8% of these were diagnosed NGU and 27.9% recurrent NGU. However, chlamydial isolation rates in female partners of these two groups (35.6% and 29.4%) did not differ significantly ($\chi^2$ = 1.18, 1 d.f. (N.S.)). In the remaining 2.3% the final diagnosis was ill-defined or unclear.

Of the 16 second male consorts (M2), where two were named, seven had NGU, none had recurrent NGU.

(c) Our patients' relationship with their male partners

154 men (34.8% of the total 443) denied having any other recent partner. The information had not been recorded in 109 (24.6%) case sheets. 38 (8.6% total 443) of our female partners were primary contacts of their partners, 142 (32.1% total 443) were secondary. It was not possible reliably to assess the others. The chlamydial isolation rate amongst primary contacts (55.3%) was higher than amongst secondary contacts (37%) although not significantly so ($\chi^2$ = 3.3, 1 d.f. (N.S.)). However, it was significantly higher than amongst patients whose partner denied any other contact (chlamydial isolation rate 29%, $\chi^2$ = 8.5, 1 d.f.**).

Where there were two male partners, this information was missing from the second partner in over half but five of the other seven men had two partners.

(d) Chlamydial urethral culture

Although not routinely available, it was possible to take chlamydial cultures from the urethra in a small group of 31 male partners.

Of 28 first partners, 18 were chlamydia-postive as were all but two of their female partners. These two negative women had had no chemotherapy. The ten chlamydia-negative men (who also gave no history of chemotherapy) had eight chlamydia-positive female consorts; one of these women had a second male consort who was chlamydia-positive.

Three second male partners were tested. Two, including the above-mentioned, were chlamydia-positive.

(b) BABIES WITH NEONATAL CONJUNCTIVITIS AND THEIR PARENTS

C. trachomatis isolation rates - Table 30

129 selected babies with neonatal conjunctivitis and their mothers were examined. C. trachomatis was isolated from 70 babies. Excluding two chlamydia-negative babies, later found to have been receiving chlamydial chemotherapy already, the isolation rate was 70/127 (55%).

Chlamydiae were isolated from 57 mothers. Excluding those chlamydia-negative mothers who had recently completed or were presently receiving chemotherapy (21), the chlamydial isolation rate was 57/108 (53%) and amongst mothers of chlamydia-positive babies 51/60 (85%) (C. trachomatis pharyngeal cultures were routinely taken only in the last year of the study and are mentioned where relevant).

Caesarian section - Table 31

In the two cases of chlamydial conjunctivitis where the child had been delivered by caesarian section, the mothers were found to be chlamydia-negative.

One mother, examined three weeks after delivery, had already had oral penicillin for seven days (from the fifth to the twelfth post-natal day) for infected lochia. The onset of conjunctivitis was delayed until the eleventh day. There, chorioamnionitis and subsequent ascending infection might have introduced a small intra-uterine inoculum.

The other mother had not received chemotherapy and the onset of conjunctivitis was on the seventh post-natal day. However, the membranes had been ruptured for over 11 hours prior to delivery, the mother's negative cultures, taken 25 days after delivery, were not confirmed prior to chemotherapy and serological antibody testing (MIF test) was indicative of chlamydial infection.

FINDINGS IN MOTHERS OF BABIES WITH NEONATAL CONJUNCTIVITIS

Age

Mean age of the mothers was 22.3 (range 15-34) and, in the chlamydia-positive women, it was 20.6 (range 16-29 years).

Marital status - Table 32

More chlamydia-positive (44%) than negative (28%) were single and less (54%) chlamydia-positive than negative (68%) were married but the differences were not significant ($\chi^2$ = 2.2, 1 d.f. (N.S.)). The other mothers were separated, divorced or widowed.

Parity - Table 33

For most mothers, the affected baby was their first and had been a vaginal delivery.

Contraception - Table 36

None of the chlamydia-positive and only seven of chlamydia-negative were using any contraception at the time of their first examination which for the majority (72%) was in the first month of the puerperium. (Tables 34,35).

Chemotherapy - Table 37

Only five women (3.9%) had had antibiotics in pregnancy, two were chlamydia-positive and a further one had a chlamydia-positive baby.

26 (20.2%) had had chemotherapy in the puerperium. From 10, although their babies were chlamydia-positive, no isolates were obtained. Five remained chlamydia-positive despite the chemotherapy and the remaining 11 and their babies were chlamydia-negative.

Premature rupture of membranes - Table 38

In eight cases, the membranes had ruptured 12 hours or more before delivery (longest period - eight days). Chlamydiae were not isolated from any of these mothers but from seven of their babies. Two of these mothers were known to have had chlamydistatic chemotherapy. Two others had had unknown chemotherapy. There was no history of chemotherapy for the others.

Symptoms - Table 39

Symptoms were absent in 63% of all mothers (chlamydial isolation rate 33%) and significantly more chlamydia-positive (53%) mothers than chlamydia-negative (25%) had symptoms (isolation rate 62.5%, $\chi^2$ = 9.2, 1 d.f.**).

There was an abnormal discharge in 16% of mothers, and in significantly more chlamydia-positive (25%) than chlamydia-negative (isolation rate 67%, $\chi^2 = 4.1$, 1 d.f.*). This discharge might in the puerperium be interpreted as the prolonged appearance of lochia due to mild pelvic inflammation.

Seventeen mothers (13%) complained of abdominal pain. Significantly more (15) had chlamydia-positive than chlamydia-negative babies ($\chi^2 = 7.61$, 1 d.f.**). Nine of these mothers (all had chlamydia-positive babies) were found to have salpingitis on clinical examination.

Cervical signs - Table 40

Simple ectopy was present in 51% of all mothers (36% were chlamydia-positive). There was hypertrophic ectopy in 13% (82% were chlamydia-positive) and mucopus in 40.3% (77% were chlamydia-positive). Fifteen chlamydia-positive mothers (26%) showed no evidence of cervical inflammation (neither hypertrophic ectopy nor mucopus) and so excluding four who had had chemotherapy, which might have modified appearances, 11/53 (20.3%) had no abnormal cervical signs.

The chlamydial isolation rate was significantly higher in hypertrophic ectopy (82%) than in any other appearance of the cervix (38.4%, $\chi^2 = 10.1$, 1 d.f.**), than in superficial ectopy (36%, $\chi^2 = 9.8$, 1 d.f.**) or than in the absence of ectopy (40%, $\chi^2 = 7.6$, 1 d.f.**).

The chlamydial isolation rate was also significantly greater in the presence of mucopus (77%) than in clear or cloudy mucus (26.7%, $\chi^2 = 23.5$, 1 d.f.***).

Other signs - Table 41 (Illustration p.77a)

Salpingitis (q.v.) was found in 9 women, all mothers of chlamydia-positive babies (7% all mothers and 9% of chlamydia-positive). (Chlamydial isolation rate 56%, $\chi^2 = 0.13$, 1 d.f. (N.S.)).

Nineteen mothers (15% total, 21% of chlamydia-positive, isolation rate 63.2%, $\chi^2 = 2.41$, 1 d.f. (N.S.)) bled on examination. In the puerperium as with the symptom of excessive discharge, this is not easy to assess when lochia may have just ceased to flow.

Clinical signs of chlamydial urethritis in the puerperium

(a) Mother B, 6 days after vaginal delivery

C. trachomatis isolated from both urethra and cervix
Mucoid urethral discharge, oedema of the urethral orifice and congestion of the Skene's glands

(b) Mother D, 5 days after delivery; chlamydia-negative in urethra and cervix.
Normal urethra

Other infections - Table 42

Few other infections were found in these women. Five, all chlamydia-positive, had gonorrhoea as did three of their babies. Two of these babies with gonorrhoea also had chlamydial infections. The two babies without gonorrhoea also had chlamydial conjunctivitis although the isolate in one, who had previously had tetracycline eye ointment was from the pharynx only. These babies had had no chemotherapy.

Antibody estimations - Table 43

Serological specimens were examined for antibodies, either by the lymphogranuloma venereum complement fixation test (LGVCFT) or by the micro-immunofluorescence test (MIF) in 16 of the 70 mothers of chlamydia-positive babies. Half of these women were themselves chlamydia-negative but four of these eight had previously had chemotherapy.

Seven of these 16 (three chlamydia-positive, four chlamydia-negative) had had signs and symptoms of pelvic inflammatory disease. In six, there was a single titre of 1:20 or higher, or two or more rising titres.

Among the nine women who had no history of PID, significant titres were found in only three, who were all chlamydia-positive. Rising titres from 1:80 to over 1:1280 were reported in one of this latter group in whom a post-partum haemorrhage had necessitated curettage to remove placental fragments.

Many of these rises in titre in LGVCFT were confirmed by MIF.

Follow-up - Table 44

Of 57 mothers with chlamydial cervicitis, 14 had hypertrophic ectopy, 40 had endocervical mucopus, 42 had one or other sign and 11 had both. Twenty-five of these 42 with signs of inflammation were re-examined following appropriate chemotherapy.

The original signs of inflammation had cleared up after treatment in all but two.

Reisolation - Table 45

Maternal reisolates were made after treatment in three originally positive women where there had been a failure to isolate chlamydiae 42, 46 and 49 days previously. Signs of cervical inflammation had been present in two of three at the original time of isolation and had not recurred with the reisolate. Their babies remained chlamydia-negative. Two of their partners examined at or after the reisolate was made were chlamydia-positive and we failed to secure the third man's attendance.

FINDINGS IN BABIES WITH NEONATAL CONJUNCTIVITIS

Prematurity

There were significantly more premature babies amongst those with chlamydial conjunctivitis. Sixteen of 67 chlamydia-positive babies, whose birth weights were known, were of birth weight less than 2.5 kg. Only three of 58 chlamydia-negative were so small ($\chi^2$ = 6.7, 1 d.f.**).

Sex - Table 46

54% of babies were male and they comprised 60% of chlamydia-positive babies.

Onset and referral dates

The time of onset of conjunctivitis varied between one and 21 days after birth (mean 6.8 days) in chlamydia-positive and between one and 42 days (mean 5.3 days) in chlamydia-negative.

The mean age at onset in seven chlamydia-positive babies where the membranes had ruptured 12 hours or more before delivery was 6.7 days (range 3-12 days).

However, the age at referral was later in chlamydia-positive (mean 13.3 days, range 3-42) than in chlamydia-negative babies (mean 11.0 days, range 1-42).

Signs of infection (Illustrations p.79a,b, 80a, 81a)

All babies with chlamydial conjunctivitis developed oedema of the lids and a discharge. This was generally purulent but often thin and profuse in the absence of concurrent bacterial infection. In the more severe acute infections, it was bloodstained. Localised bubbling of the mucosa was marked in severe cases but mucosal oedema was present

Neonatal chlamydial conjunctivitis - early acute signs (i)

Baby K

(a) born at 32 weeks gestation; had signs of conjunctivitis at 7 days with no response to two days of local neomycin.

C. trachomatis isolated at 9 day

(b)

(c) palpebral oedema and severe sub-mucosal oedema wi bubbling and mucopurulent dis-charge

Mother K

cervix, 9 days after delivery, shows hypertrophic ectopy with endocervical mucopus

C. trachomatis isolated

Neonatal chlamydial conjunctivitis - early acute signs (ii)

(a)

(b)

(c)

Baby H, born at full term had signs of conjunctivitis at 5 days. Left eye was worse than right, with palpebral oedema, severe submucosal oedema with petechial haemorrhages, bubbling and mucopurulent discharge. C. trachomatis was isolated from both eyes.

in all but the most premature infants. In these, signs were minimal with a slight muco-purulent discharge, and little if any oedema, incidentally making examination more difficult.

Topical chloramphenicol had been given in nine cases. There, examination was delayed because typical signs of chlamydial conjunctivitis had been modified but recurred when treatment was stopped.

Previous chemotherapy - Table 47

68% of all babies (88) had had chemotherapy before testing (83% (58) of chlamydia-positive and 51% of chlamydia-negative).

Of the 58 chlamydia-positive, 16% had had systemic chlamydistatic therapy (e.g. penicillin) 22% had local chlamydicidal ointment (tetracycline) or chlamydistatic drops (chloramphenicol). The remaining 62% (36) had had neomycin which does not affect chlamydial isolation.

One-third of the 30 chlamydia-negative babies had had systemic chlamydistatic treatment. Seven of the remaining 20 had chloramphenicol eye drops or tetracycline (two babies) and the other 13 (43%) had neomycin which would not affect chlamydial isolation.

Other infections - Table 48

N. gonorrhoea was isolated from three babies; one was chlamydia-negative. All three mothers had both gonococcal and chlamydial cervicitis. Two other babies were already on chemotherapy for presumptive gonorrhoea (not subsequently confirmed) before being tested for chlamydial infection. Their mothers were found to have chlamydial, but not gonococcal infection.

In 30 babies (23%) with neonatal conjunctivitis, no pathogen was isolated; no cultures were sterile but staphyloccus albus and other commensals were grown and the abnormal eye signs resolved in these babies without any change of treatment.

In 21 (16%) other bacteria were isolated and appropriate chemotherapy was given.

Neonatal chlamydial conjunctivitis - clinical signs modified
(i) by local chloramphenicol

(a) full face

(b) left eye

(c) right eye

Baby R, born at full term; purulent discharge and palpebral oedema had been present at 5 days. A five-day course of local chloramphenicol was completed just before examination. The discharge had ceased and in the left eye, from which C. trachomatis was isolated, peripheral oedema persisted but had lessened.

Follow-up - Table 49 (Illustrations p.81b,c)

Duration of follow-up varied in babies with no reisolate from less than four weeks (one baby) to 36-52 weeks (nine), and from 8-11 weeks (three) to 36-52 weeks (one) in those from whom reisolates had been made.

Reisolates - Table 50

As the Liverpool group had found in a previous study, before routine pharyngeal cultures were taken an erroneous evaluation of the response to treatment was obtained. This is because post-treatment persistence of infection (bacterial as well as chlamydial) tends to occur in the pharynx.

Pharyngeal cultures were not taken until the final year of this study and so these figures probably underestimate the reisolation rate.

After treatment, chlamydiae were reisolated from 20 of 58 babies who attended for follow-up (34.5%).

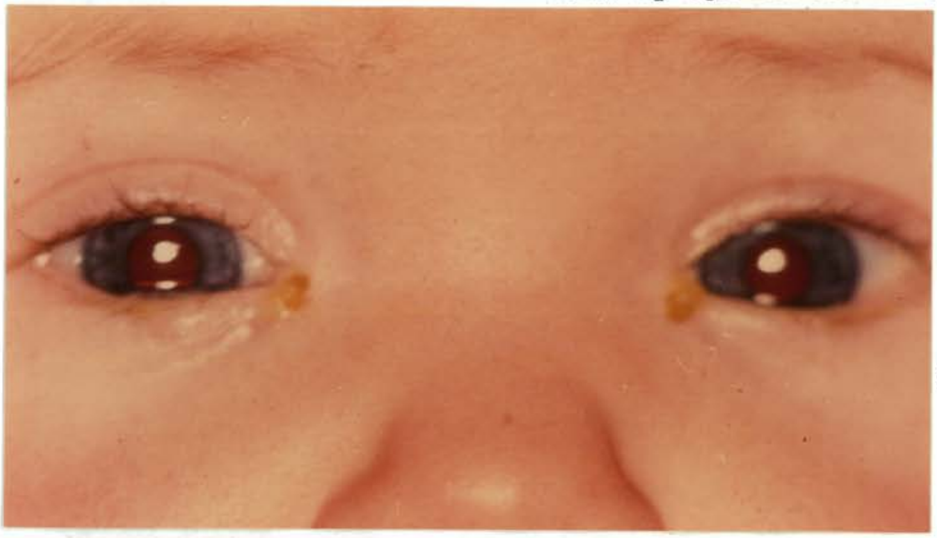
Thirty-one (53.4% of those followed) had had tetracycline eye ointment alone prescribed for four weeks and reisolates were made from 12 of these (38.7%). One of these babies had been treated for only one week. There was a reisolate from the pharynx in seven cases and in four of five reisolates from the eyes, no culture had been taken from the pharynx.

The other 46.6% (27) followed had had tetracycline eye ointment and erythromycin syrup prescribed jointly for two weeks. Reisolates were made in eight of these (29.6%), in six of whom treatment with erythromycin had been inadequate. Equal numbers of reisolates were made from the eyes and pharynx.

However, although combined treatment with chlortetracycline eye ointment and erythromycin syrup appeared more effective than topical tetracycline alone, the two schedules were not comparable because only a minority had had pharyngeal swabs.

The high reisolate rate in babies given only topical treatment led to this practice being stopped and the combined regime of topical tetracycline with systemic erythromycin was given, generally, for two weeks.

Neonatal chlamydial conjunctivitis - clinical signs modified
(ii) by age and chemotherapy



(a) full face

(b) right eye

(c) left eye

Baby J (not in present series) examined at three months, had had signs of conjunctivitis at 4 days and was given various local antibiotics and a 7 day course of ampicillin finishing two weeks previously. There was a history of recent bronchitis and C. trachomatis was isolated from the pharynx but not from the conjunctivae. On the right side was slight unilateral palpebral oedema, with mucopus at both inner canthi and slight submucosal oedema. His mother had thought the right eye looked smaller than the left.

Effect of treatment on neonatal chlamydial conjunctivitis - before treatment

(a)

(b)

Baby B, examined 10 days after delivery, had been born at 36 weeks gestation and had had signs of conjunctivitis at 7 days old, worse in the left eye.

(a) and (b) Pre-treatment
Gross palpebral oedema, upper worse than lower, with a thin purulent discharge and submucosal oedema with bubbling

Effect of treatment on neonatal conjunctivitis - after treatment

(c) after 24 hours treatment with systemic erythromycin and local tetracycline; less oedema, palpebral cartilage visible

(d), (e)

after three days treatment only moderate lid oedema, cannot evert lid, no discharge

Even using the combined treatment, results were not good (30% reisolation rate). Close questioning of their mothers revealed that reisolates from babies given this therapy were usually due to compliance failure. Reisolates have not been obtained from babies who completed their combined treatment in special care baby units.

Discrepancies between mothers' and babies' results - Tables 51 and 52

There was failure to isolate chlamydiae in 19 mothers of babies with chlamydial conjunctivitis. Nine had already had chemotherapy for puerperal pyrexia. Another three had had chemotherapy (penicillin and unknown) in late pregnancy and during delivery. In another the culture was cytotoxic but she had received chemotherapy before it could be repeated. Isolates were later obtained (at 35 and 84 days respectively) in two but they too had been treated for presumptive chlamydial infection meanwhile and as neither husband had till then been examined, these were probably reinfections. Three of the remaining five negative tests were not confirmed; in three, the membranes had been ruptured for from 11 hours to seven days, without recorded chemotherapy and in one the test was not carried out until the third post-natal month.

No isolate was obtained from six babies of chlamydia-positive mothers. Two had had antichlamydial chemotherapy elsewhere and two had had intensive penicillin chemotherapy for assumed or proven gonococcal conjunctivitis. Another when tested had gonococcal conjunctivitis. From the sixth, who had had no relevant antichlamydial chemotherapy, only E. coli was isolated.

FINDINGS IN FATHERS OF BABIES WITH NEONATAL CONJUNCTIVITIS - Table 53

Twenty-seven fathers of chlamydia-positive babies were examined and urethral cultures for chlamydiae were taken. Symptoms were absent or negligible in all but two (both chlamydia-positive).

Nine fathers were chlamydia-positive, 18 chlamydia-negative. Excluding four of the chlamydia-negative who had had relevant chemotherapy, the isolation rate was 9/23 (39.0%).

Although concurrent gonococcal infection in one, a long time interval since original diagnosis in the child in others, and/or other undisclosed chemotherapy may have been contributory factors, this is significantly less than the maternal isolation rate (85%, $\chi^2$ = 15.2, 1 d.f.***).

(c)(i) EPIDEMIOLOGICAL STUDIES IN LIVERPOOL WOMEN - Table 54

In male patients, the prevalence of NGU and the chlamydial isolation rate therein has been seen to vary in different populations studied according to geographical location, race, socio-economic status etc. These studies, therefore, were carried out to investigate the prevalence of chlamydial infection in other groups of women in the population from which our NGU contacts were drawn. They also included an estimated low incidence group of infertility patients used as a control series.

(a) STD clinic population

The prevalence of chlamydial endocervicitis in 314 consecutive female patients in our unit was 24%. The age range was 13-73 years (mean 29.3).

(b) The age range of clinic patients taking oral contraceptives was 16-38 years (mean 22.6). The chlamydial isolation rate in this group was 37.5%.

(c) Pregnancy in STD clinic population

The prevalence of chlamydial infection in 235 pregnant women in our unit was 29%. In the first trimester, it was 33%, in the second, 21% and in the third, 33%.

The chlamydial isolation rate in pregnancy, in the presence of ectopy, was 41%. The age range in pregnancy was 15-38 years (mean 22.6).

(d) Infertility patients

There were no signs of cervical inflammation and no chlamydial isolates from 37 patients examined in the infertility clinic in this hospital who were used as a control group.

(ii) CLINICAL SIGNS AND SYMPTOMS IN THE PRESENCE OF CHLAMYDIAL INFECTION AND GONORRHOEA IN THE STD CLINIC POPULATION - Table 55

Amongst patients in whom gonorrhoea was present but cultures for chlamydia were negative, 48.9% were symptom free.

Clinical signs were studied in four groups of patients.

(1) Those in whom both chlamydial infection and gonorrhoea were present.

(2) Those in whom gonorrhoea was present and cultures for chlamydia were negative.

(3) Those in whom chlamydial infection was present and gonorrhoea was absent.

(4) Those from the general clinic population in whom cultures for gonorrhoea and chlamydial infection had been negative.

Contrary to findings in chlamydial endocervicitis, in gonorrhoea infection was not associated with a higher incidence of ectopy, either superficial or hypertrophic, compared with the normal clinic population. When chlamydial infection was also present the percentage ectopy was significantly increased ($\chi^2$ = 32.3, 1 d.f.***).

There was a significantly higher percentage of endocervical mucopus present in gonorrhoea than in the ordinary clinic population ($\chi^2$ = 28.8, 1 d.f.***), but significantly more again when chlamydiae were also present ($\chi^2$ = 12.7, 1 d.f.***).

## PART I: CLINICAL RESULTS - TABLES

(a) Contacts of men with nongonococcal urethritis

C.t pos = culture-positive for *C. trachomatis*

C.t neg = culture-negative for *C. trachomatis*

Table 1 - Reason for attendance

| | Total n | C.t pos. n | C.t neg. n | C.t isolation rate |
|---|---|---|---|---|
| NSU contact | 394 | 139 | 255 | 35.4% |
| Own accord | 39 | 13 | 26 | 33.3% |
| Referred | 22 | 8 | 14 | 36.4% |

Table 2 - Group age

| Group | Age Range | Total n | Total (%) | C.t pos. n | C.t pos. (%) | C.t neg. n | C.t neg. (%) | C.t isolation rate |
|---|---|---|---|---|---|---|---|---|
| 2 | 16-20 | 114 | (25.1) | 56 | (35) | 58 | (19.7) | 49.1% |
| 3 | 21-25 | 153 | (33.6) | 65 | (41) | 88 | (29.8) | 42.5% |
| 4 | 26-30 | 82 | (18.0) | 22 | (14) | 60 | (20.3) | 26.8% |
| 5 | 31-35 | 53 | (11.6) | 11 | (7) | 42 | (14.2) | 20.8% |
| 6 | 36-40 | 22 | (4.8) | 3 | (2) | 19 | (6.4) | 13.6% |
| 7 | 41+ | 22 | (4.8) | - | - | 22 | (7.5) | - |
| Age unknown | | 9 | (2.0) | 3 | (2) | 6 | (2.0) | |

Significance - C.t isolation rate in group 2/group 6, $\chi^2$ = 8.06, 1 d.f.**. Total $\chi^2$ = 23.9, 4 d.f.***. $\chi^2$ for linear trend = 23.0, 1 d.f.***.

Table 3 - Marital status

| | Total n | Total (%) | C.t pos. n | C.t pos. (%) | C.t neg. n | C.t neg. (%) | C.t isolation rate | $\chi^2$(1 d.f.) |
|---|---|---|---|---|---|---|---|---|
| Single | 229 | (50.3) | 99 | (61.8) | 130 | (44.1) | 43% | 12.73*** (Single vs Married) |
| Married | 179 | (39) | 46 | (28) | 133 | (45.1) | 26% | |
| Div/sep. | 47 | (10) | 15 | (9.3) | 32 | (10.1) | 32% | |

Table 4

Previous pregnancy:

| | Total n | (%) | C.t pos. n | (%) | C.t neg. | C.t isolation rate | $\chi^2$(1 d.f.) |
|---|---|---|---|---|---|---|---|
| Nullipara | 245 | (53.8) | 100 | (62.5) | 145 | 41% | |
| No full term | 29 | (6.4) | 14 | | 15 | 48% | 9.47** |
| Vaginal delivery | 171 | (37.6) | 44 | (27.5) | 127 | 26% | |
| Caesarian section only | 4 | (0.9) | 1 | | 3 | | |
| Both vaginal and C.S. delivery | 6 | (1.3) | 1 | | 5 | | |

Full-term pregnancy:

| | Total n | (%) | C.t pos. n | C.t neg. n | C.t isolation rate |
|---|---|---|---|---|---|
| Parous | 181 | (39.8%) | 46 | 135 | |
| 1 | 59 | | 24 | 35 | |
| 2 | 64 | | 13 | 51 | 29.9% |
| 3 | 31 | | 9 | 22 | |
| 4 | 18 | | - | 18 | |
| 5 | 7 | | - | 7 | |
| 6 | 2 | | - | 2 | |
| Miscarriages | 25 | (5.5%) | 8 | 7 | |
| Terminations | 28 | (6.2%) | 12 | 16 | |
| Ectopics | 2 | | 1 | 1 | |

Hormonal factors

Table 5 - (a) Present pregnancy

| | Total | C.t pos. | C.t neg. |
|---|---|---|---|
| Pregnant | 11 | 3 | 8 |
| 1st trimester | 3 | 2 | 1 |
| 2nd trimester | 6 | - | 6 |
| 3rd trimester | 2 | 1 | 1 |
| Pregnant - uncertain | 4 | 1 | - |
| Post-natal | | | |
| 1st month | 1 | 1 | - |
| 2nd, 3rd month | - | - | - |

Table 6 - (b) Menstrual cycle

| | Total n | (%) | C.t isolation rate | C.t pos. | C.t neg. |
|---|---|---|---|---|---|
| Premenarche | 1 | (0.2) | | - | 0.3 |
| Post-menopausal | 3 | (0.7) | - | - | 1.0 |
| Regular cycle | 414 | (91) | 34.5% | 89.4 | 86.0 |
| Irregular | 26 | (5.7) | 50.0% | 8.1 | 10.0 |
| Pregnant | 11 | (2.4) | 36.0% | 2.5 | 2.6 |

Stage in cycle (known LMP)

(i) All women

| LMP | Total n | (%) | C.t isolation rate |
|---|---|---|---|
| 0-7 days ago | 99 | (23.9) | 33.3% |
| 8-14 days ago | 140 | (33.8) | 35.7% |
| 15-21 days ago | 100 | (24.2) | 40.0% |
| 22-28 days ago | 75 | (18.1) | 34.7% |

(Total $\chi^2$ = 1.06, 3 d.f. (N.S.))

(ii) Oral contraception only

| n | Total (%) | C.t isolation rate |
|---|---|---|
| 49 | (22.2) | 47% |
| 78 | (35.3) | 45% |
| 53 | (23.9) | 51% |
| 41 | (18.6) | 51% |

(Total $\chi^2$ = 0.68, 3 d.f. (N.S.))

Table 7 - Contraception

| Method | Total n | (%) | C.t pos. | C.t isolation rate | C.t pos. % | C.t neg. % |
|---|---|---|---|---|---|---|
| None | 110 | (24.2) | 27 | 24.5% | 16.9 | 28.1 |
| o.c. | 221 | (48.6) | 106 | 48% | 66.2 | 39.1 |
| o.c. within one month | 12 | (2.6) | 6 | 50% | 3.7 | 2.0 |
| IUCD | 26 | (5.7) | 6 | 23% | 3.7 | 6.8 |
| Tubal ligation | 26 | (5.7) | 5 | 19.2% | 3.1 | 7.1 |
| Barrier | 30 | (7.5) | 8 | 23.5% | 5.0 | 8.8 |
| Unknown/ unrecorded | 26 | (5.7) | 2 | 7.6% | 1.2 | 8.1 |

Significance

Oral contraception (including within the past month)/no oral contraception - $\chi^2$ = 25.2 1 d.f.***

Oral contraception (including within the past month)/barrier methods - $\chi^2$ = 4.1, 1 d.f.*

Oral contraception (including within the past month)/contraception other than barrier methods or oral contraception - $\chi^2$ = 23.5, 1 d.f.***

Barrier methods/no contraception - $\chi^2$ = 0, 1 d.f.

Table 8 - Symptoms

| | Total n | (%) | C.t pos. % | C.t neg. % | C.t isolation rate | $\chi^2$ (1 d.f.) |
|---|---|---|---|---|---|---|
| None | 244 | (53.6) | 45.6 | 58 | 29.9% | 5.87* (None vs Any) |
| Any | 211 | (46.4) | 54.4 | 42 | 41.2% | |
| Discharge | 131 | (36.9) | 24.4 | 28.8 | 45.0% | 7.27** |
| Irritation | 40 | (12.5) | 6.8 | 8.8 | 50% | 3.55 N.S. |
| Soreness | 10 | (2.5) | 2.0 | 2.2 | 40% | 0.00012 N.S. |
| Dyspareunia | 7 | (2.5) | 1.0 | 1.5 | 57.1% | 0.69 N.S. |
| Post-coital bleeding | 8 | (3.1) | 1.0 | 1.8 | 5/8 | 1.59 N.S. |
| Menstrual | 19 | (3.1) | 4.7 | 4.2 | 26.3% | 0.34 N.S. |
| Urinary | 46 | (12.5) | 8.8 | 10.1 | 43.5% | 1.17 N.S. |
| Abdominal pain | 27 | (6.9) | 5.4 | 5.9 | 40.7% | 0.17 N.S. |
| Conjunctivitis | 4 | (0.6) | 1.0 | 0.9 | 25% | 0.01 N.S. |
| Rectal | 3 | (0.6) | 0.7 | 0.7 | 1/3 | 0.29 N.S. |
| Infertility | 4 | - | 1.4 | 0.9 | - | - |
| Warts | 10 | (3.1) | 1.7 | 2.2 | 5/10 | 0.43 N.S. |
| Other | 7 | (1.2) | 1.7 | 1.5 | 2/7 | 0.001 N.S. |

Table 9 - Symptoms in the absence of candida, TV, gonorrhoea

| | Total n | (%) | C.t pos. n | C.t isolation rate | $\chi^2$ (1 d.f.) | C.t pos. % | C.t neg. % |
|---|---|---|---|---|---|---|---|
| None | 154 | (59) | 40 | 26% | 2.98 N.S. | 50.6 | 63.0 |
| Any | 106 | (41) | 39 | 36.8% | | 49.4 | 37.0 |
| Irritation | 12 | (4.6) | | 42% | 0.31 N.S. | 6.3 | 3.9 |
| Soreness | 4 | (1.5) | | 50% | 0.09 N.S. | 2.5 | 1.1 |
| Discharge | 57 | (21.9) | 26 | 46% | 7.11** | 32.9 | 17.1 |
| Dyspareunia | 3 | (1.2) | | 33.3% | 0.27 N.S. | 1.3 | 1.1 |
| Post-coital bleeding | 5 | (1.9) | | 4/5 | 3.78 N.S. | 5.1 | 0.6 |
| Menstrual | 12 | (4.6) | | 41.7% | 0.30 N.S. | 3.8 | 5.0 |
| Urinary | 26 | (10) | | 42% | 1.36 N.S. | 13.9 | 8.3 |
| Abdominal pain | 12 | (4.6) | | 41.7% | 0.30 N.S. | 6.3 | 3.9 |

Table 10 - Discharge and ectopy related to C.t and T.V. isolation rates

| TV | Discharge | Total n | C.t pos. n | C.t isolation rate | C.t pos. % | C.t neg. % | Ectopy | C.t in ectopy |
|---|---|---|---|---|---|---|---|---|
| - | - | 307 | 89 | 29% | 56 | 74 | 54.7% | 41% |
| - | + | 110 | 48 | 44% | 30 | 21 | 68.2% | 55% |
| + | - | 17 | 12 | 71% | 7 | 2 | 29.4% | 60% |
| + | + | 21 | 11 | 52% | 7 | 3 | 48.6% | 60% |

1. In the absence of TV, the C.t isolation rate was higher with a discharge than without ($\chi^2 = 7.22$, 1 d.f.**).

2. In the absence of TV, the percentage ectopy and the percentage of chlamydia in ectopy is higher in the presence of a discharge than without ($\chi^2 = 8.07$, 1 d.f.**).

Table 11 - Coital experience

(a) Last intercourse

| | Total n | (%) | C.t isolation rate | C.t pos. % | C.t neg. % |
|---|---|---|---|---|---|
| Less than one week ago | 89 | (19.6) | 39.3% | 21.9 | 18.3 |
| One to two weeks | 117 | (25.7) | 38.5% | 28.1 | 24.4 |
| Two to four weeks | 76 | (16.7) | 39.5% | 18.7 | 15.6 |
| Four weeks to two mths | 19 | (4.2) | 36.8% | 4.4 | 4.1 |
| Two to three mths | 16 | (3.5) | 37.5% | 3.7 | 3.4 |
| Over three mths ago | 3 | (0.7) | - | 0 | 1.0 |
| Unrecorded | 135 | (29.7) | 27.4% | 23.1 | 33.2 |

(b) Number of consorts

| | Total n | (%) | C.t isolation rate | C.t pos. % | C.t neg. % |
|---|---|---|---|---|---|
| One regular | 352 | (77.4) | 32.1% | 70.6 | 81.0 |
| Regular and other | 52 | (11.4) | 50.0% | 16.2 | 8.8 |
| Casual only | 2 | (0.4) | 1/2 | 0.6 | 0.3 |
| Unrecorded | 49 | (10.5) | 40.0% | 12.5 | 9.8 |

(C.t isolation rate in women with one consort/more than one - $\chi^2 = 5.66$, 1 d.f.*).

Table 12 - Clinical Signs

Cervix

(a) Ectocervix

| | Total n | (%) | C.t isolation rate | C.t pos. % | C.t neg. % |
|---|---|---|---|---|---|
| None (no cervix) | 2 | (0.4) | - | - | 0.7 |
| No ectopy | 191 | (42.0) | 21.5% | 25.6 | 50.8 |
| Superficial ectopy | 200 | (44.0) | 38.5% | 48.1 | 41.7 |
| Hypertrophic ectopy | 58 | (12.7) | 72.4% | 26.2 | 5.4 |
| Unrecorded | 4 | (0.9) | - | - | 1.3 |

Significance

No ectopy/superficial ectopy - $\chi^2 = 12.7$, 1 d.f.***

No ectopy/any ectopy - $\chi^2 = 28.0$, 1 d.f.***

No ectopy/hypertrophic ectopy - $\chi^2 = 49.7$, 1 d.f.***

Superficial ectopy/hypertrophic ectopy - $\chi^2 = 19.5$, 1 d.f.***

(b) The endocervical contents

| | Total n | (%) | C.t isolation rate | C.t pos. % | C.t neg. % |
|---|---|---|---|---|---|
| Clear | 181 | (39.8) | 24.3% | 27.5 | 46.4 |
| Cloudy | 143 | (31.4) | 25.2% | 22.5 | 36.3 |
| Mucoid | 8 | (1.8) | 50% | 2.5 | 1.4 |
| Mucopus | 98 | (21.5) | 72.4% | 44.4 | 9.2 |
| Blood only | 22 | (4.8) | 22.7% | 3.1 | 5.8 |
| Unrecorded | 3 | (0.7) | - | - | 1 |

Significance

Clear and cloudy mucus/mucopus - $\chi^2 = 72.6$, 1 d.f.***

Table 12 continued

Cervical signs and chlamydial infection

| | Total n | (%) | C.t isolation rate | C.t pos. % | C.t neg. % |
|---|---|---|---|---|---|
| Hypertrophic ectopy and/or mucopus absent | 325 | (71.4) | 22.5% | 45.6 | 85.4 |
| Ectopy present (hypertrophic or superficial) | 285 | (56.7) | 46.1% | 74.4 | 47.1 |
| Both hypertrophic ectopy and mucopus present | 32 | (7.0) | 81.3% | 16.3 | 2.0 |

Table 13 - Other signs

| | Total n | (%) | C.t isolation rate | C.t pos. % | C.t neg. % | $\chi^2$ (1 d.f.) |
|---|---|---|---|---|---|---|
| Punctate inflammation | 5 | (1.1) | 1/5 | 0.6 | 1.4 | 0.06 N.S. |
| Bleeding on examination | 43 | (9.5) | 58.1% | 15.6 | 6.1 | 9.91** |
| Salpingitis | 12 | (2.6) | 50% | 3.7 | 2.0 | 0.62 N.S. |
| Urinary infection | 1 | (0.2) | 1/1 | 0.6 | - | 0.10 N.S. |
| Proctitis | 5 | (1.1) | 3/5 | 1.9 | 0.7 | 0.49 N.S. |
| Pharyngitis | - | - | - | - | - | - |
| Conjunctivitis | 1 | (0.2) | - | - | - | - |
| Urethritis | 5 | (1.1) | 1/5 | - | - | 0.06 N.S. |
| Lower genital tract infection | 6 | (1.3) | 4/6 | - | - | 1.43 N.S. |
| Other signs | 4 | (0.9) | - | - | - | 0.91 N.S. |

Table 14 - Post-coital bleeding

| C.t pos. | H.E. | S.E. | N.E. | O.C. | Parity | Candida, TV or GC | Bled O.E. | Cx smear |
|---|---|---|---|---|---|---|---|---|
| + | + | - | - | + | 0+0 | C | + | Normal |
| + | - | + | - | - | 1+0 | - | + | - |
| + | - | - | + | - | 0+1 | - | - | Normal |
| + | - | + | - | + | 0+1 | TV | + | Normal |
| + | - | + | - | + | 0+0 | GC | + | - |
| - | - | + | - | - | 4+0 | - | - | - |
| - | - | - | + | - | 3+0 | - | - | - |
| - | - | - | + | - | 3+0 | - | - | - |

O.E - on examination

H.E. - hypertrophic ectopy

S.E. - superficial ectopy

N.E. - no ectopy

O.C. - oral contraception

Table 14a - Ayre smear

| | Total<br>n | C.t pos.<br>n | C.t neg.<br>n |
|---|---|---|---|
| Not performed | 372 | 129 | 243 |
| Normal | 81 | 31 | 50 |
| Abnormal | 2 | - | 2 |

Table 15 - Concurrent infections

| | Total n | (%) | C.t isolation rate | C.t pos. % | C.t neg. % | $\chi^2$ (1 d.f.) |
|---|---|---|---|---|---|---|
| Normal flora | 211 | (46.3) | 40% | 52.5 | 43 | 3.36 N.S. |
| Candidiasis | 154 | (33.8) | 37.7% | 36.2 | 32.5 | 0.48 N.S. |
| T.V. | 38 | (8.4) | 60.5% | 14.4 | 5.1 | 10.51** |
| Warts | 18 | (4.0) | 38.9% | 4.4 | 3.7 | 0.01 N.S. |
| HSV | 3 | (0.7) | 1/3 | 0.6 | 0.7 | 0.29 N.S. |
| Syphilis | 1 | (0.2) | - | - | 0.3 | 0.97 N.S. |
| LGV | - | | | - | | |
| Conjunctivitis | 2 | (0.4) | - | - | 0.7 | 0.91 N.S. |
| Others | 2 | (0.4) | - | 0.6 | 0.3 | 0.97 N.S. |
| Gonorrhoea - untreated | 7 | (1.5) | 3/7 | | | |

Table 16 - Contraception and ectopy

| | Total n | (%) | C.t isolation rate |
|---|---|---|---|
| Ectopy + O.C. | 166 | (39.2) | 54.2% |
| Ectopy - O.C. | 76 | (17.9) | 35.5% |
| No ectopy + O.C. | 64 | (15.1) | 34.3% |
| No ectopy - O.C. | 117 | (27.6) | 16.2% |

Significance -

Ectopy with/without O.C. - $\chi^2 = 6.56$, 1 d.f.*

O.C. with/without ectopy - $\chi^2 = 6.51$, 1 d.f.*

No ectopy with/without O.C. - $\chi^2 = 6.77$, 1 d.f.**

No o.c. with/without ectopy - $\chi^2 = 8.40$, 1 d.f.**

Table 17 - C.t isolation rate relative to age and ectopy

| Age group | 2(16-20) | 3(21-25) | 4(26-30) | 5(31-35) | 6(35-40) | Total $\chi^2$ | $\chi^2$ for linear trend |
|---|---|---|---|---|---|---|---|
| Total cases | 114 | 153 | 82 | 64 | 22 | - | - |
| C.t pos. in age group n(%) | 56(49%) | 64(42%) | 30(37%) | 13(21%) | 3(14%) | 23.9, 4 d.f.*** | 23.01, 1 d.f.*** |
| Ectopy n(%) | 73(64%) | 103(67%) | 42(51%) | 24(38%) | 10(45%) | 21.6, 4 d.f.*** | 15.27, 1 d.f.*** |
| % C.t pos. in ectopy | 58 | 49 | 38 | 35 | 13 | Regression coefficient t = 2.2, 6 d.f. (N.S.) | |
| % C.t pos. in absence of ectopy | 34 | 30 | 16 | 12 | 18 | | |

Table 18 - C.t isolation rate relative to age, oral contraception and ectopy

| Age group | 2(16-20) | 3(21-25) | 4(26-30) | 5(31-35) | Total $\chi^2$ | $\chi^2$ for linear trend | regression coefficient (t) |
|---|---|---|---|---|---|---|---|
| Total cases | 98 | 122 | 71 | 43 | | | |
| Without O.C. | | | | | | | |
| Total | 34 | 35 | 37 | 32 | | | |
| C.t pos. in age group | 13(37%) | 9(26%) | 6(16%) | 6(19%) | 5.0, 3 d.f. (N.S.) | 3.99, 1 d.f.* | |
| Ectopy | 18(51%) | 18(51%) | 13(35%) | 8(25%) | 7.06, 5 d.f. (N.S.) | 6.33, 1 d.f.* | |
| % C.t pos. in ectopy | 50 | 33⅓ | 15 | 37.5 | | | 0.98, 4 d.f. (N.S.) |
| With O.C. | | | | | | | |
| Total | 63 | 87 | 34 | 11 | | | 0.91, 4 d.f. (N.S.) |
| C.t pos. in age group | 34(54%) | 43(49%) | 14(41%) | 5(45%) | 1.51, 3 d.f. (N.S.) | 1.22, 1 d.f. (N.S.) | |
| Ectopy | 45(71%) | 68(78%) | 24(71%) | 6(55%) | 3.27, 2 d.f. (N.S.) | 0.54, 1 d.f. (N.S.) | |
| % C.t pos. in ectopy | 58 | 53 | 50 | 50 | | | |

Table 19 - C.t isolation rates relative to OC, < 25 years and > 25 years

| | | < 25 ys | > 25 ys |
|---|---|---|---|
| Without O.C. | Total | 71 | 68 |
| | C.t pos. n(%) | 20 (28) | 13 (19) |
| With O.C. | Total | 160 | 35 |
| | C.t pos. n(%) | 77 (48) | 19 (45) |
| | $\chi^2$ (1 d.f.) | 7.24** | 11.8*** |

Table 20 - C.t isolation rate relative to parity and ectopy

| Parity | 0 | 1 | 2 | 3 | 4 | 5 | 6 | Total $\chi^2$ | $\chi^2$ for linear trend |
|---|---|---|---|---|---|---|---|---|---|
| Total cases | 166 | 58 | 63 | 30 | 18 | 7 | 2 | | |
| C.t pos. n(%) | 69(42%) | 24(41%) | 13(21%) | 9(30%) | 2(11%) | | | 14.8, 4 d.f.** | 11.07, 1 d.f.*** |
| Ectopy n(%) | 103(62%) | 32(55%) | 36(57%) | 9(30%) | 7(39%) | | | 12.87, 4 d.f.* | 9.26, 1 d.f.** |
| % C.t pos. in ectopy | 51 | 50 | 31 | 33 | 1/7 | | | Regression coefficient t = 1.23, 6 d.f. (N.S.) | |
| % C.t pos. in absence of ectopy | 28 | 32 | 8 | 30 | 9 | | | | |

Table 21 - C.t isolation rate relative to parity, oral contraception and ectopy

| Parity | 0 | 1 | 2 | 3 | |
|---|---|---|---|---|---|
| Total cases | 161 | 57 | 57 | 27 | |
| Without O.C. | | | | | |
| Total cases | 51 | 22 | 38 | 20 | |
| C.t isolation rate | 35% | 36% | 13% | 25% | Regression coefficient t = 1.46, 4 d.f. (N.S.) |
| Ectopy | 51% | 32% | 34% | 15% | |
| % C.t pos. in ectopy | 50 | 35 | 23 | 1/3 | |
| With O.C. | | | | | |
| Total cases | 110 | 35 | 19 | 7 | |
| C.t isolation rate | 47% | 46% | 42% | 57% | |
| Ectopy | 69% | 89% | 71% | 71% | |
| % C.t pos. in ectopy | 53 | 58 | 47 | 50 | |

% O.C. Nullipara 68%, Parous 43% ($\chi^2 = 18.4$, 1 d.f.***)

Table 22 - C.t isolation rate relative to parity and endocervical contents

| Parity | 0 | | 1 | | 2 | | 3 | | 4 | | Total | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Total cases | 166 | | 58 | | 63 | | 30 | | 18 | | | |
| % Total | % Total | C.t isol. rate | % Total | C.t isol. rate | % Total | C.t isol. rate | % Total | C.t isol. rate | % Total | C.t isol. rate | % Total | C.t isol. rate |
| Clear | 45 | 35% | 35 | 35% | 37 | 13% | 43 | 23% | 67 | 8% | 40 | 24% |
| Cloudy | 28 | 26% | 36 | 24% | 32 | 15% | 30 | - | 17 | 33% | 31 | 25% |
| Mucoid | 0.6 | - | 3 | 100% | - | - | - | - | - | - | 2 | 50% |
| Mucopus | 22 | 83% | 21 | 67% | 22 | 50% | 23 | 86% | 17 | 0 | 22 | 72% |
| Blood only | 5 | 25% | - | - | - | - | 3 | - | - | - | 5 | 23% |
| Unknown | | | | | | | | | | | | |
| C.t positive (%) | 42 | | 41 | | 20 | | 30 | | 11 | | | |

Table 23 - C.t isolation rate relative to age, ectopy and parity

Nullipara n = 209

| Age groups | 2(16-20) | 3(21-25) | 4(26-30) | 5(31-35) | 6 |
|---|---|---|---|---|---|
| Total cases | 96 | 85 | 23 | 5 | |
| C.t isolation rate | 49% | 46% | 9% | | |
| Ectopy | 63% | 78% | 35% | | |
| % C.t in ectopy | 56 | 50 | 29 | | |

Parous n = 151

| Age groups | 2(16-20) | 3(21-25) | 4(26-30) | 5(31-35) | 6 |
|---|---|---|---|---|---|
| Total cases | 3 | 40 | 52 | 39 | 17 |
| C.t isolation rate | | 35% | 35% | 23% | 21% |
| Ectopy | | 57% | 59% | 32% | 23% |
| % C.t in ectopy | | 43 | 40 | 38 | 1/2 |

Table 24 - C.t isolation rate relative to age, ectopy, parity and oral contraception

(a) Nullipara n = 202

| Age groups | 2(16-20) | 3(21-25) | 4(26-30) | |
|---|---|---|---|---|
| **Nullipara without O.C.** | | | | |
| Total cases | 34 | 21 | 9 | 2 |
| C.t isolation rate | 38.2% | 23.8% | - | |
| Ectopy | 52.9% | 66.7% | 11.1% | |
| % C.t in ectopy | 50 | 36 | - | |
| **Nullipara with O.C.** | | | | |
| Total cases | 61 | 61 | 11 | 3 |
| C.t isolation rate | 64% | 74% | - | |
| Ectopy | 72% | 80.3% | 45% | |
| % C.t in ectopy | 59 | 55.1 | - | |
| % O.C. | 64 | 74 | 55 | |

Table 24 - C.t isolation rate relative to age, ectopy, parity and oral contraception (continued)

(b) Parous n = 144

| Age groups | 2 | 3(21-25) | 4(26-30) | 5(31-35) | 6(36-40) |
|---|---|---|---|---|---|
| **Parous without O.C.** | | | | | |
| Total cases | 1 | 14 | 27 | 30 | 12 |
| C.t isolation rate | | 28.5% | 22.2% | 20% | 25% |
| Ectopy | | 28.5% | 44.4% | 30% | 16.7% |
| % C.t in ectopy | | 1/4 | 16.7 | 23.3 | 1/2 |
| **Parous with O.C.** | | | | | |
| Total cases | 2 | 26 | 23 | 9 | |
| C.t isolation rate | | 38.5% | 46% | 23% | |
| Ectopy | | 73.0% | 78.3% | 55.6% | |
| % C.t in ectopy | | 47.4 | 55.6 | 2/5 | |
| % O.C. | | 65 | 46 | 23 | |

Table 25 Antibiotics

History

| | Total n | (%) | C.t isolation rate | C.t pos. % | C.t neg. % |
|---|---|---|---|---|---|
| None | 390 | 85.7 | 35.9% | 87.5 | 84.7 |
| Within last week | 2 | 0.4 | 2% | 1.2 | 0.0 |
| Within last two weeks | 2 | 0.4 | 1/2 | 0.6 | 0.3 |
| Within last four weeks | 19 | 4.2 | 36.8% | 4.4 | 4.1 |
| Within last eight weeks | 17 | 3.7 | 23.5% | 2.5 | 4.4 |
| Within last 8-12 weeks | 16 | 3.5 | 18.7% | 1.9 | 4.4 |
| Unrecorded | 9 | 2 | 33.3% | 1.8 | 2.0 |

Source

| | Cases | % Total (56) | % C.t pos. |
|---|---|---|---|
| STD clinic | 7 | 12.5 | 4/7 |
| G.P. | 40 | 71 | 32.5 |
| Hospital/clinic | 5 | 11 | - |
| Non-medic | 1 | 2 | - |
| Unknown | 3 | 6 | 1/3 |

Type

| | No. | C.t pos. |
|---|---|---|
| Penicillin G. | 1 | 1 |
| Other Pen. | 22 | 36.4% |
| Tetracycline | 8 | - |
| Erythromycin | 2 | - |
| Sulphonamide | 4 | 1 |
| Trimethoprim | 4 | 1 |
| Unknown | 19 | 36.8% |

Table 25 - Antibiotics - continued

Allergy Present

| | C.t pos. | C.t neg. |
|---|---|---|
| Relevant | 1 | - |
| Irrelevant | 1 | 2 |

Duration of course

| | Cases | % C.t pos. |
|---|---|---|
| 1 day | 6 | 3/6 |
| 1-4 days | 5 | 1/5 |
| 5-14 days | 39 | 28.2 |
| > 14 days | 1 | - |

Table 26 - Whole coverslip count

| | LGWCC | mean count |
|---|---|---|
| Entire population | 2.4511 | |
| Age range | Mean LGWCC | n |
| Group age 16-20 | 2.340 | 56 |
| 21-25 | 2.417 | 65 |
| 26-30 | 2.962 | 22 |
| 31-35 | 2.559 | 11 |
| 36-40 | 1.127 | 3 |

F = 1.7419 (4 and 156 d.f.) N.S.

| Marital status | | |
|---|---|---|
| Single | 2.309 | 89 |
| Married | 2.811 | 44 |
| Divorced | 2.584 | 9 |
| Separated | 2.563 | 6 |

F = 1.3606 (3 and 144 d.f.) N.S.

| Contraceptive history | | |
|---|---|---|
| None | 2.105 | 27 |
| O.C. | 2.540 | 106 |
| O.C. within month | 3.090 | 6 |
| IUCD | 1.989 | 6 |
| Tubal ligation | 2.480 | 5 |
| Barrier methods | 2.662 | 8 |

F = 0.9472 (5 and 152 d.f.) N.S.

| Last menstrual period | | |
|---|---|---|
| 0-7 days ago | 2.304 | 33 |
| 8-14 | 2.440 | 50 |
| 15-21 | 2.487 | 40 |
| 22-28 | 2.856 | 26 |

F = 1.7171 (3 and 144 d.f.) N.S.

Table 26 - Whole coverslip count - continued

| | Mean LGWCC | n | |
|---|---|---|---|
| **Symptoms** | | | |
| None | 2.385 | 73 | |
| Any symptoms | 2.497 | 87 | F = 0.2848 (1 and 158 d.f.) N.S. |
| No discharge | 2.431 | 101 | |
| Discharge | 2.473 | 59 | F = 0.0380 (1 and 158 d.f.) N.S. |
| No abdo pain | 2.407 | 149 | |
| Abdo pain | 2.979 | 11 | F = 1.9429 (1 and 158 d.f.) N.S. |
| **Cervical signs** | | | |
| **Ectocervix** | | | |
| No ectopy | 2.499 | 41 | |
| Simple ectopy | 2.488 | 77 | F = 0.0702 (2 and 157 d.f.) N.S. |
| Hypertrophic ectopy | 2.390 | 42 | |
| **Endocervix** | | | |
| Clear mucus | 1.833 | 44 | F = 3.6550** (4 and 155 d.f.) |
| Cloudy | 2.719 | 36 | |
| Mucoid | 2.208 | 4 | t = 3.46, 155 d.f.*** |
| Mucopus | 2.680 | 71 | comparing "clear mucus" and "mucopus" |
| Blood | 2.748 | 5 | |
| **Other signs** | | | |
| **Salpingitis** | | | |
| Absent | 2.435 | 154 | F = 0.3015 (1 and 158 d.f.) N.S. |
| Present | 2.737 | 6 | |

The combined effects of ectopy and the pill

| | | |
|---|---|---|
| (a) | Ectopy present, O.C. absent | 2.273 |
| (b) | Ectopy present, O.C. present | 2.516 |
| (c) | Ectopy absent, O.C. absent | 2.162 |
| (d) | Ectopy absent, O.C. present | 2.791 |

Comparing (b) and (c), t = 1.119, 281 d.f. (N.S.)

Table 26 - Whole coverslip count - continued

Other infections

| | | | | |
|---|---|---|---|---|
| TV | Absent | 2.507 | 137 | F = 2.0092 (1 and 158 d.f.)N.S. |
| | Present | 2.087 | 23 | |
| Candidiasis | Absent | 2.298 | 102 | F = 3.6373 (1 and 158 d.f.)N.S. |
| | Present | 2.708 | 58 | |
| GC | Untreated | 1.427 | 3 | F = 2.5169 (1 and 3 d.f.) N.S. |

Table 27 - Follow up (total women - 166)

(a) C.t status sequence

| | First test | | Intermediate test where applicable (may have been repeated) | | | | Last test | |
|---|---|---|---|---|---|---|---|---|
| | + | - | + | - | + | - | + | - |
| -/- | 0 | 36 | 0 | (36) | —— | | 0 | 36 |
| *-/+/- | 0 | 2 | 2 | 0 | —— | | 0 | 2 |
| +/+ | 9 | 0 | (9) | 0 | —— | | 9 | 0 |
| *+/-/+ | 3 | 0 | 0 | 3 | —— | | 3 | 0 |
| *+/-/+/- | 15 | 0 | 0 | 15 | 15 | 0 | 0 | 15 |
| *+/- | 101 | 0 | 0 | 101 | —— | | 0 | 101 |

\+ Ct positive

\- Ct negative

In 121 women originally chlamydia-positive, chlamydia negative later(*)

(b) Signs of endocervicitis

| Original test C.t positive | | | | Later test C.t negative | | | |
|---|---|---|---|---|---|---|---|
| Hypertrophic ectopy | | Endocervical mucopus | | Hypertrophic ectopy | | Endocervical mucopus | |
| n | % | n | % | n | % | n | % |
| 33 | 27.3 | 58 | 47.9 | 3 | 2.5 | 6 | 5.0 |

(c) Other infections

| | Original test C.t positive | | Later test C.t negative | |
|---|---|---|---|---|
| | n | % | n | % |
| Candidiasis | 46 | (38) | 15 | (12) |
| Trichomoniasis | 18 | (15) | 6 | (5) |
| Warts | 3 | (2) | 1 | (1) |
| HSV | 1 | (1) | 0 | - |

Table 27 - Follow up continued

(d) Surveillance

| | | Range (days) | Mean (days) |
|---|---|---|---|
| Total - 121 women | | 5-632 | 198 |
| Interval between first post-treatment culture and reisolate | one reisolate (17) | 11-603 | 166 |
| | two reisolates (1) | - | 77 |
| Interval between first and final cultures after retreatment | one reisolate (17) | 18-432 | 193 |
| Interval between first culture after retreatment and 2nd reisolate | two reisolates (1) | - | 225 |
| Interval between 2nd reisolate and final culture | | - | 0 |

Table 28 - Reisolates

(a) Coital activity

| Total | Resumed S.I. | Same partner Symptoms + | Same partner Symptoms - | New partner Symptoms + | New partner Symptoms - | Unknown partner | Information missing |
|---|---|---|---|---|---|---|---|
| 18 | 14 | 4 | 5 | 0 | 4 | 1 | 4 |

(b) Presence of endocervicitis

| With first isolate | With reisolate | Same signs/other signs | |
|---|---|---|---|
| 9 | 4 | 2 | 2 |

Table 29 - Male partners

1st male consort (M1) 427. Female C.t isolation rate 33.7%

2nd male consort (M2) 16. Female C.t isolation rate 75%

1. Referral route

| | M1: Total n | (%) | Female C.t isolation rate | M2: Total n | Female C.t isolation rate |
|---|---|---|---|---|---|
| Referred patient | 383 | (89.7) | 33.7% | 2/10 | 1/2 |
| Referred by patient | 13 | (3) | 30.8% | 6/10 | 4/6 |
| Attended simultaneously | 20 | (4.7) | 40.0% | 2/10 | 2/2 |
| Missing data | 11 | (2.6) | 27.3% | 6 | - |

2. History

| | M1: Total n | (%) | Female C.t isolation rate | M2: Total n | Female C.t isolation rate |
|---|---|---|---|---|---|
| NGU | 152 | (35.6) | 32.9% | 1/16 | - |
| Reiters | 5 | (1.2) | 2/2 | - | - |
| Conjunctivitis | - | | | - | - |
| GC | 58 | (13.6) | 31.0% | - | - |
| Other | 6 | (1.4) | - | 1/16 | - |

Table 29 - Male partners continued

3. Symptoms (in men) at time of partner's first visit

| | Total n | Total (%) | C.t positive women | Female C.t isolation rate | % C.t positive women |
|---|---|---|---|---|---|
| Absent | 148 | (32.5) | 49 | 33% | 31% |
| Present | 307 | (67.5) | 111 | 36% | 69% |

4. Symptoms

| | M1: Total n | Total (%) | Female Isolation rate | M2: Nos. | Female C.t pos. |
|---|---|---|---|---|---|
| Discharge | 227 | (53.2) | 30.8% | - | - |
| Urinary | 119 | (27.9) | 34.5% | 2 | - |
| Arthritic | 6 | (1.4) | 4/6 | - | - |
| Eye | 4 | (0.9) | 1/4 | - | - |
| Epid. orch | 7 | (1.6) | 0 | - | - |
| Other | 40 | (9.4) | 22.5% | 1 | - |

Table 29 - Male partners continued

5. Diagnosis

| | M1: Total n | (%) | Female Isolation rate | M2: Nos. | Female C.t pos. |
|---|---|---|---|---|---|
| None | - | | | 2 | 2/2 |
| NGU | 298 | (69.8) | 35.6% | 7 | 4/7 |
| Recurrent NGU | 119 | (27.9) | 29.4% | - | - |
| NGU within 2/52 of GC | 1 | (0.2) | 1/1 | - | - |
| NGU within 4/52 of GC | 1 | (0.2) | - | - | - |
| GC | 3 | (0.7) | 1/3 | - | - |
| Candidiasis | 4 | (0.9) | 1/4 | - | - |
| Reiter's syndrome | 15 | (3.5) | 53.3% | - | - |
| Epididymitis | 7 | (1.6) | 1/7 | - | - |
| Other | 28 | (6.6) | 35.7% | 3 | 1/3 |

C.t isolation rate in female partners - NGU/recurrent NGU - $\chi^2 = 1.18$, 1 d.f. (N.S.).

Table 29 - Male partners continued

6. Our patient's relationship with male partner

Information available from 234.

| | Total n | (%) | C.t isolation rate (in our patients) |
|---|---|---|---|
| Primary contact | 38 | (8.6) | 55.3% |
| Secondary contact | 142 | (32.1) | 37.3% |
| Denies other | 154 | (34.8) | 28.6% |
| Information unavailable | 109 | (24.6) | 34.9% |

Isolation rates of primary and secondary contacts do not differ significantly but are suggestive. ($\chi^2 = 3.3$, 1 d.f.).

Isolation rates of primary and "where other denied" do differ significantly ($\chi^2 = 8.5$, 1 d.f.**).

7. C.t isolation rate in women relative to the male consort's diagnosis and her relationship with him

NSU (n = 298)

| Female patient's relationship | Total n | (%) | women's C.t isolation rate |
|---|---|---|---|
| Unknown | 67 | (22.6) | 38.8% |
| Primary | 25 | (8.4) | 52% |
| Secondary | 104 | (34.7) | 38.8% |
| Denies other | 102 | (34.3) | 26.5% |

Table 29 - Male partners (7) continued

Recurrent NSU (n = 119)

| Female patient's relationship | Total n | (%) | women's C.t isolation rate |
|---|---|---|---|
| Unknown | 30 | (25.4) | 30% |
| Primary | 7 | (5.9) | 3/7 |
| Secondary | 32 | (27.1) | 25% |
| Denies other | 50 | (41.5) | 30.6% |

8. Urethral C.t cultures in male partners

| Total tested | | Result | C.t status: Female contact Pos | Female contact Neg | | Other male contact Pos | Other male contact Neg |
|---|---|---|---|---|---|---|---|
| M1 | 28 | C.t pos 18 | 16 | 2 | M2 | - | - |
| | | C.t neg 10 | 8 | 2 | | 1 | - |
| M2 | 3 | C.t pos 2 | 2 | - | M1 | - | 1 |
| | | C.t neg 1 | 1 | - | | - | - |

(b) Babies with neonatal conjunctivitis and their parents

Table 30 - Isolation rates

C.t pos = chlamydia positive

C.t neg = chlamydia negative

| | | | |
|---|---|---|---|
| 1. | Total babies | | 129 |
| | chlamydia positive | | 70 |
| | chlamydia negative, given chemotherapy | | 2 |
| | C.t isolation rate 54% (corrected rate 55% - 70/127) | | |
| 2. | Total mothers | | 129 |
| | chlamydia positive | | 57 |
| | chlamydia negative, given chemotherapy | within 4/52 | 16 |
| | | within 8/52 | 17 |
| | | within 12/52 | 21 |
| | C.t isolation rate 44% (corrected rate 53% - 57/108) | | |
| 3. | Mothers of chlamydia positive babies | | 70 |
| | chlamydia positive | | 51 |
| | chlamydia negative, given chemotherapy | within 4/52 | 8 |
| | | within 8/52 | 9 |
| | | within 12/52 | 10 |
| | C.t isolation rate 73% (corrected rate 85% - 51/60) | | |

Table 31 - Caesarian section

| | C.t status | | | | Chemotherapy | | |
|---|---|---|---|---|---|---|---|
| | Mother | | Baby | | Pregnancy | Puerperium (M) | Baby |
| | - | + | - | + | | | |
| Total | 6 | - | 4 | 2 | | | |
| | 1 | - | 1 | - | 1 | - | 1 |
| | 1 | - | - | 1 | - | 1 | - |
| | 1 | - | - | 1 | - | - | - |
| | 3 | - | 3 | - | - | - | - |

MOTHERS

Table 32 - Marital status

| | Total | | C.t pos | | C.t neg | | $\chi^2$ (1 d.f.) |
|---|---|---|---|---|---|---|---|
| | n | (%) | n | (%) | n | (%) | |
| Single | 45 | (34.9) | 25 | (43.8) | 20 | (27.7) | 2.2 (N.S.) |
| Married | 80 | (62) | 31 | (54.4) | 49 | (68) | |
| Separated | 2 | (1.6) | 1 | (1.8) | 1 | (1.4) | |
| Divorced | 1 | (0.8) | | | 1 | (1.4) | |
| Widowed | 1 | (0.8) | | | 1 | (1.4) | |

Table 33 - Number of pregnancies

| | All mothers | | C.t pos mothers | |
|---|---|---|---|---|
| P1 | 70% | 96% | 80% | 98% |
| 2 | 19% | | 13% | |
| 3 | 7% | | 5% | |
| 4 | 2% | 4% | 2% | 2% |
| 5 | 1% | | 0% | |
| 6 | 1% | | 0% | |

Table 34 - Present pregnancy

| | Total | | C.t pos | | C.t neg | |
|---|---|---|---|---|---|---|
| | n | (%) | n | (%) | n | (%) |
| Pregnant | 1/129 | | 1/57 | | - | |
| 1st postnatal month | 93 | (72) | 49 | (86) | 44 | (61.1) |
| 2nd, 3rd postnatal month | 23 | (17.8) | 4 | (7) | 19 | (26.4) |
| Pregnant again/irreg. MC | 12 | (9.3) | 3 | (5.2) | 9 | (12.5) |

Table 35 - Last menstrual period

| | Total<br>n | C.t pos<br>n | C.t neg<br>n |
|---|---|---|---|
| 0-7 days ago | 1 | - | 1 |
| 8-14 | 3 | - | 3 |
| 15-21 | 2 | - | 2 |
| 22-28 | 3 | - | 3 |
| 29-35 | 1 | - | 1 |
| >35 days, < 9 months | 113 (87.6%) | 55 (97%) | 58 (81%) |
| Unknown/unrecorded | 6 | 2 | 4 |

Table 36 - Contraception

| | Total | | C.t pos | | C.t neg | |
|---|---|---|---|---|---|---|
| | n | (%) | n | (%) | n | (%) |
| None | 117 | (91) | 55 | (96.5) | 62 | (86.1) |
| O.C. | 4 | (3.1) | - | | 4 | (5.6) |
| O.C. past month | 1 | (1) | - | | 1 | (1.4) |
| IUCD | 2 | (2) | - | | 2 | (2.8) |
| Unrecorded/unknown | 5 | (4.3) | 2 | (3.5) | 3 | (4.2) |

Table 37 - Chemotherapy

| | Total n | (%) | C.t pos (%) | C.t neg (%) | Mothers of C.t pos babies |
|---|---|---|---|---|---|
| No chemotherapy | 103 | (79.8) | 87 | 74 | 75 |
| Chemotherapy | | | | | C.t neg mothers of C.t pos babies |
| (a) in pregnancy | 5/129 | (3.9) | n2 | n3 | n1 |
| (b) in puerperium | 26/129 | (20.2) | n5 | n21 | n10 |

Specific chemotherapy in mothers

(a) in pregnancy

B - baby

M - mother

| Time | Type | C.t (M) | Antibiotic (B) | C.t (B) | LSCS |
|---|---|---|---|---|---|
| <36/52 | Penicillin | + | - | + | - |
| >36/52 | Unknown | + | - | + | - |
| <36/52 | Penicillin | - | Penicillin | + | - |
| >36/52 | Penicillin | - | - | - | + |
| <36/52 | Erythromycin | - | - | - | - |

(b) in puerperium

| Time | Type | C.t (M) | Antibiotic (B) | C.t (B) | LSCS |
|---|---|---|---|---|---|
| | Penicillin (3) | - | - | + | - |
| | Unknown (3) | - | - | + | - |
| | Penicillin | - | Chloramphenicol | + | - |
| | Cephaloridine | - | Chloramphenicol/ Penicillin | + | - |
| | Tetracycline | - | Chloramphenicol | + | - |
| | Erythromycin | - | Chloramphenicol | + | - |
| | Penicillin (3) | + | - | + | - |
| | Penicillin/ Tetracycline <1/52 | + | - | + | - |
| | Penicillin | + | Chloramphenicol | + | - |
| | Penicillin (6) | - | - | - | - |
| | Penicillin | - | Chloramphenicol/ Penicillin | - | - |

Table 37 - Chemotherapy continued

| Type | C.t (M) | Antibiotic (B) | C.t (B) | LSCS |
|---|---|---|---|---|
| Cotrimoxazole | - | Chloramphenicol | - | - |
| Cephalosprin | - | - | - | - |
| Unknown (2) | - | - | - | - |

Table 38 - Premature rupture of membranes

| Foetal maturity | Time ruptured | Antibiotics (M) | C.t (M) | C.t (B) | Age at onset (days) of O.N. |
|---|---|---|---|---|---|
| 1. 30/52 | 8 days | Unknown | - | + (father C.t+) | 3 |
| 2. 34/52 | 7 days | - | - | + | 12 |
| 3. 36/52 | 4 days | - | - | + | 4 |
| 4. 34/52 | 72 hours | Unknown | - | + | 7 |
| 5. 36/52 | 55 hours | Penicillin | - | + | 7 |
| 6. 36/52 | 33 hours | - | - | - | 9 |
| 7. 32-34 /52 | 21 hours | Penicillin | - | + | 8 |
| 8. 32/52 | 12 hours+ | - | - | + | 6 |
| 9. Full term (LSCS) | 11 hours | - | - | + | 7 |
| 10. Full term | 8 hours | - | + | + | 9 |
| 11. 36/52 | 6 hours | - | + | + | 7 |

Table 39 - Symptoms

| | Total | | C.t isolation | C.t pos | | C.t neg | | Mothers of C.t pos babies | | $\chi^2$ (1 d.f.) |
|---|---|---|---|---|---|---|---|---|---|---|
| | n | (%) | rate | n | (%) | n | (%) | n | (%) | |
| None | 81 | (63) | 33% | 27 | (47) | 54 | (75) | 35 | (50) | 9.2** |
| Discharge | 21 | (16) | 67% | 14 | (25) | 7 | (10) | 15 | (21) | 4.1* |
| Abdominal pains | 17 | (13) | 59% | 10 | (18) | 7 | (10) | 15 | (21) | 1.1 (N.S.) |
| Dyspareunia | 1 | (0.8) | 0/1 | - | - | 1 | (1.4) | 1 | (1.4) | |
| Menstrual irregularity | 2 | (1.6) | 2/2 | 2 | (3.5) | - | - | 2 | (2.9) | |
| Urinary symptoms | 4 | (3.1) | 2/4 | 2 | (3.5) | 2 | (2.8) | 2 | (2.9) | |
| Warts | 1 | (0.8) | 1/1 | 1 | (1.8) | - | | - | | |
| Other | 15 | (12) | 47% | 7 | (12) | 8 | (11.1) | 11 | (15.7) | |

No irritation, soreness, ulceration, PCB, conjunctivitis, throat or rectal symptoms.

Table 40 - Signs

| | Total n | (%) | C.t isolation rate | C.t pos n | (%) | C.t neg (%) | Mothers of C.t pos babies (%) |
|---|---|---|---|---|---|---|---|
| (a) Ectocervix | | | | | | | |
| No ectopy | 45 | (34.9) | 40% | 18 | (31.6) | (37.5) | (36) |
| Simple ectopy | 66 | (51.2) | 36% | 24 | (42.1) | (58.3) | (41) |
| Hypertrophic ectopy | 17 | (13.2) | 82% | 14 | (24.6) | (4.2) | (23) |
| Unrecorded | 1 | (0.8) | | 1 | (1.8) | - | - |
| (b) Endocervix | | | | | | | |
| Clear | 18 | (13.9) | 16% | 3 | (5.3) | (20.8) | (10) |
| Cloudy | 27 | (20.9) | 33% | 9 | (15.8) | (25.0) | (17) |
| Mucoid | 5 | (3.8) | 1/5 | 1 | (2) | (5.6) | (3) |
| Mucopus | 52 | (40.3) | 77% | 40 | (70.2) | (16.7) | (64) |
| Blood | 27 | (20.9) | 15% | 4 | (7.0) | (31.0) | (6) |

C.t isolation rate in hypertrophic ectopy

(a) relative to other ecto-cervical signs - $\chi^2 = 10.1$, 1 d.f.**

(b) relative to no ectopy - $\chi^2 = 7.6$, 1 d.f.**

(c) relative to superficial ectopy - $\chi^2 = 9.8$, 1 d.f.**

C.t isolation rate in mucopus

(a) relative to clear and cloudy mucus - $\chi^2 = 23.5$, 1 d.f.***

(b) relative to all other endocervical signs - $\chi^2 = 35.6$, 1 d.f.***

Table 41 - Other signs

| | C.t pos n | C.t neg n | Total n | Mothers of C.t pos babies n | (%) | $\chi^2$ (1 d.f.) |
|---|---|---|---|---|---|---|
| Bleeding on examination | 12 | 7 | 19 | 14 | (20) | 2.41 (N.S.) |
| Salpingitis | 5 | 4 | 9 | 9 | (13) | 0.13 (N.S.) |
| Proctitis | 1 | 0 | 1 | 1 | (1.4) | |
| Urethritis | 6 | 1 | 7 | 6 | (8.6) | |
| Lower genital tract infection | 1 | 2 | 3 | 1 | (1.4) | |
| Other | - | 1 | 1 | - | | |

Table 42 - Concurrent infections

(a) Gonorrhoea in mothers and babies

| | Babies | | Mothers | |
|---|---|---|---|---|
| | G.C. | C.t | G.C. | C.t |
| 1. | + | + | + | + |
| 2. | + | + | + | + |
| 3. | + | - | + | + |
| 4. | - | + (pharynx) | + | + |
| 5. | - | + | + | + |

Table 42 - Concurrent infections continued

(b) Other infections/mothers

| | Total | C.t pos | C.t neg |
|---|---|---|---|
| Candida | 1 | 1 | - |
| TV | 3 | 3 | - |
| Warts | 2 | 2 | - |
| Other | 1 | 1 | - |

No HSV, CMV, Syphilis, LGV, Conjunctivitis

Table 43 - Maternal antibodies

| C.t state (M) | PID | LGVCFT | | | | |
|---|---|---|---|---|---|---|
| + | + | 20 | 80 | 160 | 40 | 320 |
| | | Ct+ | PID | Ct- | Ct+ | Ct- |
| + | - | 10 | 10 | 40 | | |
| + | - | 10 | 40 | 20 | | |
| - | - | 10 | | | | |
| + | - | <10 | | | | |
| + | + | <10 | 20 | 40 | | |
| | | Ct+ | Ct- | Ct+ | | |
| + | - | N.S. | | | | |
| + | - | 80 | 1280 | 320 | 160 | |
| | (retained placenta) | Ct+ | Ct+ | Ct- | Ct- | |
| + | + | 40 | | | | |
| - | - | 20 | | | | |
| - | + | 640 | 320 | 80 | | |
| - | + | 40 | 40 | | | |
| - | + | 40 | | | | |
| - | - | 10 | | | | |
| - | (LSCS) - | MIF+ | | | | |
| - | + | 80 | >1280 | | | |
| | | Ct- | Ct+ | | | |

Table 44 - Cervical inflammation in C.t positive mothers (57)

| | Hypertrophic ectopy | Mucopus | Both | Either |
|---|---|---|---|---|
| At first visit | 14 | 40 | 11 | 42 |
| Followed up (25) | 8 | 17 | 6 | 19 |
| At follow up after treatment | 1 | 1 | - | 2 |

Table 45 - Maternal Reisolates

| First treatment | LGVCFT | | | | | Partner examined | Interval since first treatment | Interval since last treatment | Other features |
|---|---|---|---|---|---|---|---|---|---|
| Oxytetracycline, Erythromycin | | 80<br>C.t pos<br>1st | 1280<br>C.t pos<br>2nd | 320<br>C.t neg | | After re-isolate (C.t pos) | 3 months | 42 days | D & C removal of placental fragments |
| Oxytetracycline 3/52 | | <10<br>C.t pos<br>1st | 20<br>C.t neg | 40<br>C.t pos<br>2nd | | With re-isolate (C.t pos) | 3 months | 49 days | - |
| Oxytetracycline 2/52 | 20<br>C.t pos<br>1st | 80<br>C.t neg | 160<br>C.t neg | 40<br>C.t pos<br>2nd | 320<br>C.t neg | (not seen) | 3 months | 46 days | PID during first and second isolation |

BABIES

Table 46 - Sex of babies

| | Total | | C.t isolation rate | | |
|---|---|---|---|---|---|
| | n | (%) | n | (%) | |
| Male | 70 | (54) | 34 | 60% | |
| Female | 59 | (46) | 23 | 40% | ($\chi^2$ = 0.83, 1 d.f. (N.S.)) |

Table 47 - Chemotherapy

| | Total | | C.t pos | | C.t neg | |
|---|---|---|---|---|---|---|
| | n | (%) | n | (%) | n | (%) |
| Any chemotherapy | 88 | (68) | 58 | (83) | 30 | (51) |
| (i) Local | | | 49 | | 20 | |
| (a) ineffective | | | 36 | (62% total) | 13 | (43% total) |
| (b) effective | | | 13 | (tetra x 1) | 7 | (tetra x 2) |
| (ii) Systemic | | | 9 | | 10 | |

("tetra" - chlortetracycline eye ointment)

Table 48 - Other infections and diagnoses

| | Total | | C.t pos |
|---|---|---|---|
| | n | (%) | |
| No pathogen | 30 | (23) | - |
| Sterile | - | - | - |
| Other bacterial infection | 21 | (16) | 4 |
| HSV conjunctivitis | 1 | - | 1 |
| Congenital heart defect | 2 | - | 1 |
| Other infections | 3 | - | - |

Table 49 - Follow up periods after treatment

T = treated with tetracycline

T + E = treated with tetracycline and erythomycin

| Weeks | <4 | 4-7 | 8-11 | 12-23 | 24-25 | 36-52 |
|---|---|---|---|---|---|---|
| T | | | | | | |
| Single isolate | 1 | - | 3 | 5 | 6 | 4 |
| Reisolate | - | - | 1 | 6 | - | - |
| T + E | | | | | | |
| Single isolate | - | 2 | - | 6 | 6 | 5 |
| Reisolate | - | - | 2* | 1 | 1 | 1 |

*(includes one after 3rd reisolate)

Table 50 - Babies' reisolates

T = Chlortetracycline eye ointment

E = Erythromycin syrup

BP = before pharyngeal cultures were taken

AP = after pharyngeal cultures were taken

(a) Chemotherapy

| | Total | | Treatment | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | Local (T) | | | Local and systemic (T + E) | | | |
| | n | (%) | n | (%) | | n | (%) | | |
| Followed up | 58 | | 31 | (53.4) | | 27 | (46.6) | | |
| Reisolate | 20 | (34.5) | 12 | (38.7) | | 8 | (29.6) | | |
| Site | | | Total | (BP) | inadequate | Total | (BP) | inadequate | |
| Pharynx | 8 | | 6 | - | - | 2 | - | 1 | E |
| Eye(s) | 9 | | 5 | (4) | 1 | 4 | (4) | 4 | E, E + T (3) |
| Both | 3 | | 1 | - | - | 2 | - | 1 | E |

(b) Site

| | Eyes | | Pharynx | Both |
|---|---|---|---|---|
| | BP | AP | | |
| Original | 32 | 14 | 2 | 10 |
| Reisolate | 8 | 8 | 1 | 3 |

Table 50 - Babies' reisolates continued

(c) Further reisolates (3 babies)

| | Isolate Eye | Isolate Pharynx | 1st chemotherapy (weeks) | Reisolate Eye | Reisolate Pharynx | 2nd chemotherapy (weeks) | 2nd reisolate Eye | 2nd reisolate Pharynx | 3rd chemotherapy (weeks) | Follow up, etc |
|---|---|---|---|---|---|---|---|---|---|---|
| 1. BP | + | - | $T^{(1)} + E^{(1)}$ | + | - | $T^{(1)} + E^{(1)}$ | + | - | None | None |
| 2. AP | + | + | $T^{(2)} + E^{(1)}$ | - | + | $E^{(1)}$ | + | - | None | None |
| 3. AP | + | + | $T^{(2)} + E^{(1)}$ | - | + | $T^{(2)} + E^{(1)}$ | - | + | $E^{(1)}$ | 3rd reisolate (pharynx)<br>4th chemotherapy<br>($T^{(4)} + E^{(1)}$)<br>2 months follow up |

Table 51 - Discrepancies    Mother (M) C.t neg/Baby C.t pos

PROM = premature rupture of membranes

FT = full term

LSCS = lower segment caesarian section

| | Chemotherapy (M) Type | Chemotherapy (M) Time | PROM | Maturity | Cytotoxic | LSCS | Neg. culture confirmed prior to chemotherapy | Father | Antibodies LGVCFT |
|---|---|---|---|---|---|---|---|---|---|
| 1. | - | - | - | FT | + | - | No | C.t neg | - |
| 2. | Penicillin | Puerperium | 55 hrs. | 36/52 | - | - | - | O | - |
| 3. | Cephalosporin | Puerp (PID) | - | 30/52 | - | - | - | C.t pos | 640,320,80 |
| 4. | Unknown | Puerp (PID) | 8 days | 30/52 | - | - | - | C.t pos | 40 |
| 5. | Penicillin | Puerp (PID) | - | 35/52 | - | Foetal distress | - | O | - |
| 6. | Unknown | Interpartum | 4 days | 36/52 | - | - | - | O | 20 |
| 7. | Penicillin | Puerp (PID) | - | FT | - | - | - | O | 40,40 |
| 8. | Tetracycline | Puerp (PID) | - | FT | - | - | Later isolation | O | 80,>1280 (C.t-) (C.t+) |
| 9. | - | - | 7 days | 34/52 | - | - | No | O | 10 |
| 10. | Unknown | Interpartum | 72 hrs. | 34/52 | - | - | - | O | - |
| 11. | - | - | 12 hrs+ | 32/52 | - | - | Yes | O | 10 |
| 12. | - | - | - | FT | - | - | Yes | O | - |
| 13. | - | - | - | 36/52 | - | - | Yes | O | - |

Table 51 - Discrepancies continued

| | Chemotherapy (M) Type | Chemotherapy (M) Time | PROM. | Maturity | Cytotoxic | LSCS | Neg cultures confirmed prior to chemotherapy | Father | Antibodies |
|---|---|---|---|---|---|---|---|---|---|
| 14. | Penicillin | Pregnancy | - | FT | - | - | Yes | C.t neg | - |
| 15. | - | - | 11¼ hrs | FT | - | Foetal tachy-cardia, failure to progress | No | O | MIF+ |
| 16. | - | - | - | FT | - | - | No (3/12 after delivery) | O | - |
| 17. | Unknown | Puerp. | - | 34/52 | - | - | later isolate | C.t neg (after isolate) | - |
| 18. | Erythromycin | Puerp. | - | FT | - | - | | O | - |
| 19. | Penicillin | Puerp. | - | FT | - | - | | O | - |

Table 52 - Discrepancies Mother (M) C.t pos/Baby (B) C.t neg

| | Maturity | Antibiotics B | Antibiotics M | PROM | LSCS | Onset of ON | Diagnosis (B) | Mat. cultures |
|---|---|---|---|---|---|---|---|---|
| 1. | FT | i.m. Penicillin (?GC) | - | - | - | Day 1 | ?GC ON | C.t pos |
| 2. | 37/52 | - | ? | (ARM for maternal-hypertension) | - | Day 2 | B. Coli conjunct. | C.t pos |
| 3. | 37/52 | i.m. Penicillin (?GC) | - | - | - | Day 1 | ?GC ON | C.t pos |
| 4. | FT | - | - | - | - | Day 1 | GC ON | GCpos, C.t pos |
| 5. | FT | O.C. tetracycline | - | - | - | 1st week | ?C.t pos | C.t pos |
| 6. | FT | O.C. tetracycline | - | - | - | 1st week | ?C.t pos | C.t pos |

FATHERS

Table 53 - Results in fathers (F) of babies with chlamydial conjunctivitis

| | C.t status | | | Chemotherapy (F) | Symptoms etc (F) |
|---|---|---|---|---|---|
| | Father | Mother | Child | | |
| x7 | Pos. | Pos. | Pos. | - | in 1/7 men |
| x2 | Pos. | Neg. | Pos. | - | - |
| x11 | Neg. | Pos. | Pos. | - | - |
| x1 | Neg. | Neg. | Pos. | - | - |
| x1 | Neg. | Pos. | Pos. | - | dysuria 2/12 after baby treated |
| x1 | Neg. | Pos. | Pos. | Penbritin | - |
| x1 | Neg. | Pos. | Pos. | - | Gonorrhoea |
| x1 | Neg. | Pos. | Pos. | Penicillin | - |
| x1 | Neg. | Pos. | Pos. | Oxytetracycline | - |
| x1 | Neg. | Pos. | Pos. | Penicillin | - |

Table 54 - Epidemiology

(a) STD clinic population (n = 204)

(i) Age range 13-73 years (mean 29.3 years)

(ii)

| | |
|---|---|
| Chlamydia positive | 49 |
| Chlamydia negative | 155 |
| Isolation rate | 24% |

(b) Pregnancy in STD clinic

(i) Age range 15-38 years (mean 22.6 years)

(ii) Trimester (n = 235)

| | | 1st | 2nd | 3rd | overall % |
|---|---|---|---|---|---|
| C.t status | Pos | 35 | 17 | 16 | |
| | Neg | 70 | 64 | 33 | |
| C.t isolation rate (%) | | 33 | 21 | 33 | 29 |
| Total (%) | | 45 | 34 | 21 | 100 |

(iii) Ectopy (n = 82)

| Superficial | n | % | C.t isolation rate | C.t isolation rate overall |
|---|---|---|---|---|
| C.t status | Pos | 23 | 37% | |
| | Neg | 30 | | |
| Hypertrophic | | | | 41% |
| C.t status | Pos | 11 | 55% | |
| | Neg | 9 | | |

(c) Oral contraception (n = 80)

(i) Age range 15-38 years (mean 22.6 years)

(ii)

| | |
|---|---|
| Chlamydia positive | 30 |
| Chlamydia negative | 50 |
| Isolation rate | 37.5% |

(d) Infertility patients (n = 37)

| | n |
|---|---|
| Hypertrophic ectopy | 0 |
| Endocervical mucopus | 0 |
| Chlamydia positive | 0 |

Table 55 - Clinical signs in the presence and absence of infection in STD clinic

| | | | | General clinic pop. |
|---|---|---|---|---|
| Infections | C.t+ GC+ | C.t- GC+ | C.t+ GC- | C.t- GC- |
| Cases | 192 | 182 | 160 | 134 |
| No ectopy | 27.6% | 57.1% | 25.6% | 59.7% |
| Superficial ectopy | 56.5% | 36.8% | 48.1% | 36.6% |
| Hypertrophic ectopy | 15.6% | 6.0% | 26.1% | 3.7% |
| Endocervical mucopus | 50% | 31.3% | 44.4% | 6.0% |

Significance

1. Ectopy in gonorrhoea alone/concurrent gonorrhoea and chlamydial infection - $\chi^2 = 32.3$, 1 d.f.***
2. Ectopy in gonorrhoea alone/chlamydial infection alone - $\chi^2 = 33.4$, 1 d.f.***
3. Hypertrophic ectopy in gonorrhoea alone/ chlamydial infection alone - $\chi^2 = 25.0$, 1 d.f.***
4. Hypertrophic ectopy in gonorrhoea alone/ concurrent gonorrhoea and chlamydial infection $\chi^2 = 7.8$, 1 d.f.**
5. Hypertrophic ectopy in gonorrhoea alone/ clinic population - $\chi^2 = 0.44$, 1 d.f. (N.S.)
6. Mucopus in gonorrhoea alone/concurrent and chlamydial infection - $\chi^2 = 12.7$, 1 d.f.***
7. Mucopus in gonorrhoea alone/chlamydial infection alone - $\chi^2 = 5.7$, 1 d.f.*
8. Mucopus in gonorrhoea alone/clinic population $\chi^2 = 28.8$, 1 d.f.***

Symptoms in patients with gonorrhoea whose cultures for chlamydia were negative

| | |
|---|---|
| Total | 88 |
| % symptom free | 48.9 |

PART II: LABORATORY RESULTS

Sensitivity of C. trachomatis to certain antibiotics

Erythromycin

1. To determine the minimum inhibitory concentration

Varying concentrations of erythromycin were added to MCC which were immediately infected with TC 691 at a dose calculated to give 3000 inclusions after 48 hours incubation.

Results show a progressive decrease in inclusion count with increasing concentration of erythromycin. At concentration 0.005 μg/ml, the few persisting inclusions were only 5-6 microns in diameter, (controls 15 microns) and light microscopy was needed to count them. However, the inclusions and elementary bodies within them were not abnormal in any way, unlike the effect of penicillin.

The MIC of erythromycin for Staphylococcus pyogenes is 0.01-1.6 μg/ml. Thus chlamydiae seem very highly sensitive in this system.

2. To determine whether erythromycin has a chlamydistatic or chlamydicidal effect

Duplicates of infected MCC with added concentrations of antibiotic were removed from incubation after 48 hours. The antibiotic was washed out with several changes of antibiotic-free medium and replenished with this medium. They were then re-incubated for a further 48 hours and then Giemsa-stained.

The amount of manipulation, chilling, pH change, mechanical disturbance and extra incubation prevent this experiment from being a true replication of the other. Fewer inclusions were seen in MCC previously containing 0, 0.001, 0.005 μg erythromycin/ml growth medium than in the first experiment. However, inclusions were present (at 96 hours) where, after 48 hours continuous incubation with 0.01 and 0.1 μg erythromycin/ml, the infection had apparently been completely inhibited. The inclusion sizes in the control (antibiotic free) at 96 hours were much larger than at 48 hours

(20-30 microns instead of 15) whereas at concentrations 0.01 and 0.1 they were of a size previously seen at 48 hours (15 microns) as would be expected of late developers resuming growth only when erythromycin was removed.

Therefore during the exposure to erythromycin, some elementary bodies were not killed, merely "latent".

3. To determine the effect of incubating MCC with erythromycin prior to infection and so determine whether prior uptake of erythromycin by the host cell causes C. trachomatis to be more susceptible

The MCC were exposed to varying concentrations of erythromycin for four hours prior to infection at the same dosage as before and left in these concentrations after infection. Inclusions were counted after 48 hours.

The effect was similar to the first experiment and thus there was nothing to suggest that failure to kill all C. trachomatis was due to delayed uptake of erythromycin.

4. To determine whether the effect of erythromycin depends on the stage of C. trachomatis development

MCC were infected and incubated for 24 hours in medium without erythromycin. The medium was then changed and incubation continued in the presence of the range of erythromycin dilutions previously used, for a further 24 hours. Cultures were stained and inclusions counted 48 hours after initial infection.

The results showed that there was no difference in the erythromycin concentration required to inhibit C. trachomatis development from that previously seen when incubation was in the presence of erythromycin throughout. This was a most unexpected result and suggests that the activity of erythromycin unlike chloramphenicol or even tetracycline is not confined to an early biochemical event in chlamydial replication.

In conjunction with other workers in the laboratory, similar experiments were performed with the other antibiotics - which will be described briefly.

Penicillin

1. To determine the minimum inhibitory concentration

Varying concentrations of benzylpenicillin (0.01 - 100 units per ml medium) were added to MCC which were immediately infected with STU strain at a dose calculated to yield 10,000 to 15,000 inclusions per coverslip.

Results show a drop in numbers of typical inclusions at 0.01 units per ml and none were found at 0.1 per ml or above. However, abortive inclusions, small and non-fluorescing (so visible only by light microscopy) and either empty or full of debris or swollen particles were present from 0.01 to 10 penicillin units progressively increasing, with the decreasing count of normal inclusions, from 2,000 to 5,000 per coverslip but reduced at 100 units per ml.

Another experiment using closer spaced penicillin concentrations between 0.01 and 0.1 units/ml determined the MIC more exactly.

2. To determine whether penicillin has a chlamydistatic or chlamydicidal effect

Duplicate MCC from the previous experiment, at penicillin concentrations where large numbers of abortive inclusions had been present, were selected. After incubation for 48 hours, the antibiotic was washed out as in the erythromycin experiment and, after reincubation for 48 hours, the cultures were Giemsa stained.

No normal inclusions were present at 96 hours in cultures where they had been absent in the presence of penicillin at 48 hours and so the abnormal inclusions were assumed, within the limits of these experiments, to have non-viable contents. Later work on this aspect will be described at the end of the chapter.

3. To determine whether the effect of penicillin depends on the stage of C. trachomatis development

Infected MCC were incubated for varying periods before the addition of 0.1 or 1.0 units of penicillin per ml medium. All MCC were stained and examined 48 hours after initial infection. Penicillin was added to certain infected MCC only one hour before the cultures were stained. This was to exclude the possibility that the abortive inclusions might be the result of a direct destructive effect of penicillin against normal inclusions or to chemical interference with their staining properties and fluorescence.

Results suggest that the inclusion-inhibiting activity of penicillin depends on both the concentration of penicillin added and upon the time in the growth cycle at which it is added. The later it was added, the more normal inclusions were present in the final count. The earlier penicillin was added, the more normal inclusions were present. There was no direct effect of penicillin on normal inclusions.

Chloramphenicol

1. To determine the minimum inhibitory concentration

Varying concentrations of chloramphenicol sodium succinate were added to MCC immediately before infection with TC 691.

The results after 48 hours incubation show a progressive decrease in inclusion count with increasing concentrations of chloramphenicol. Inclusions were not abnormal but smaller than usual. The MIC was 1.0 μg/ml whereas that of erythromycin was 0.01. The MIC of chloramphenicol for Staphylococcus pyogenes is 4.0 to 12 μg/ml.

2. To determine whether chloramphenicol has a chlamydistatic or chlamydicidal effect

Duplicate MCC for the MIC estimation experiment were removed from incubation at 48 hours and then the antibiotic was washed out as in the erythromycin experiment. After reincubation for 48 hours, the cultures were Giemsa-stained.

At 96 hours, growth of chlamydiae was re-established in cultures where 48 hours non-stop incubation with 1.0, 2.5, 5.0 and 10.0 μg per ml chloramphenicol, had prevented signs of infection. As with erythromycin, the inclusions sizes were smaller at those concentrations than in the control as would be expected of "latent" inclusions beginning to grow only after removal of chloramphenicol.

Tetracycline

1. To determine the minimum inhibitory concentration of tetracycline

Varying concentrations of tetracycline hydrochloride were added to MCC and infected with STU strain of chlamydiae at a dose calculated to give 4000-6000 inclusions after 48 hours incubation.

The results show an abrupt fall in inclusion count between 0.005 and 0.01 μg tetracycline per ml medium.

2. To determine whether tetracycline has a chlamydistatic or chlamydicidal effect

Two sets of MCC from the previous experiment were removed after incubation for 48 hours. From one set, the antibiotic was washed out as in the erythromycin experiment and the other set was left unwashed. Both were reincubated for 48 hours and then the cultures were Giemsa-stained.

There was no resumption of growth at previously inhibitory levels.

3. To determine whether the effect of tetracycline depends on the stage of C. trachomatis development

MCC were infected with STU strain and incubated. At intervals, certain infected MCC were removed, changed to fresh medium containing tetracycline and then returned to incubation. All MCC were stained after a total of 48 hours incubation from the time of infection.

Tetracycline was added to some cultures only one hour before staining to exclude the possibility that antibiotic effects might be due to chemical interference or degradative effects on the staining properties of the inclusion.

The results suggested that the effects of tetracycline depend on the stage of the inclusion development when it is added. Normal inclusions similar to the controls, were present when tetracycline was added after 24 hours incubation without antibiotics where previously there had been complete inhibition. This was very different from the results with erythromycin. (Tetracycline had no direct effect on the inclusions, the single hour's exposure having made no difference to the count).

Erythromycin

1. To determine the MIC

| Concentration of erythromycin μg/ml | Inclusion count after 48 hours per coverslip |
|---|---|
| 0 | 3,150 |
| 0.001 | 2,940 |
| 0.005 | 240 |
| 0.01 | 0 |
| 0.1 | 0 |
| 0.5 | 0 |
| 1.0 | 0 |

2. To determine whether erythromycin has a chlamydistatic or chlamydicidal effect - removal of erythromycin after 48 hours

| Original erythromycin prior to washing μg/ml | Inclusion count at 96 hours per coverslip - 48 hours after removal of erythromycin |
|---|---|
| 0 | 900 |
| 0.001 | 690 |
| 0.005 | 270 |
| 0.01 | 300 |
| 0.1 | 1 |
| 0.5 | 0 |
| 1.0 | 0 |

3. To determine any effect of erythromycin directly on the tissue culture - pre-incubation with erythromycin 4 hours prior to infection

| Concentration of erythromycin added 4 hours prior to infection μg/ml | Inclusion count after 48 hours |
|---|---|
| 0 | 3,300 |
| 0.001 | 3,180 |
| 0.005 | 900 |
| 0.01 | 0 |

3. <u>To determine any effect of erythromycin directly on the tissue culture - pre-incubation with erythromycin 4 hours prior to infection</u> - continued

| Concentration of erythromycin added 4 hours prior to infection µg/ml | Inclusion count after 48 hours |
|---|---|
| 0.1 | 0 |
| 0.5 | 0 |
| 1.0 | 0 |

4. <u>To determine whether the effect of erythromycin depends on the stage of C. trachomatis development - prior infection for 24 hours before the addition of erythromycin</u>

| Concentration of erythromycin µg/ml | Inclusion count at 48 hours |
|---|---|
| 0 | 2,700 |
| 0.001 | 2,470 |
| 0.005 | 750 |
| 0.01 | 0 |
| 0.1 | 0 |
| 0.5 | 0 |
| 1.0 | 0 |

<u>Penicillin</u>

1. <u>To determine the MIC</u>

| Concentration of penicillin units/ml | Inclusion counts per coverslip - 48 hours after incubation (dark-ground) | Abnormal inclusions (light microscopy) |
|---|---|---|
| 0 | 14,290 | 0 |
| 0.01 | 2,070 | 2,000 |
| 0.1 | 0 | 5,000 |
| 1.0 | 0 | 5,000 |
| 10 | 0 | 5,000 |
| 100 | 0 | 500 |

1(a) To determine the MIC (more accurately)

| Concentration of penicillin units/ml | Inclusion counts per coverslip - 48 hours after incubation (dark-ground) | Abnormal inclusions (light microscopy) |
|---|---|---|
| 0 | 9,300 | 0 |
| 0.01 | 7,100 | 1,500 |
| 0.025 | 4,200 | 2,000 |
| 0.05 | 125 | 4,000 |
| 0.1 | 1 | 5,000 |
| 1.0 | 0 | 5,000 |

2. To determine whether penicillin has a chlamydistatic or chlamydicidal effect - removal of penicillin after 48 hours

| Initial concentration of penicillin 0-48 hours | Count after 48 hours incubation | | Final count - penicillin removed at 48 hours - read at 96 hours | |
|---|---|---|---|---|
| | Normal | Abnormal | Normal | Abnormal |
| 0 | 15,000 | 0 | 5,740 | 0 |
| 1.0 | 0 | 5,000 | 0 | 1,000 |
| 0.1 | 270 | 3,000 | 92 | 100 |

3. <u>To determine whether the effect of penicillin depends on the stage of C. trachomatis development - prior infection before addition of penicillin</u>

| Time of incubation before addition of penicillin (hours) | Hours exposed to penicillin | Concentration of penicillin units/ml medium | Inclusion count (dark ground) | Abnormal count (light) |
|---|---|---|---|---|
| 48 | 0 | 0 | 15,200 | 0 |
| 47 | 1 | 0.1 | 15,000 | 0 |
| 30 | 18 | 0.1 | 8,500 | 2,000 |
| 24 | 24 | 0.1 | 7,500 | 4,000 |
| 6 | 42 | 0.1 | 5,400 | 4,000 |
| 0 | 48 | 0.1 | 1 | 5,000 |
| 48 | 0 | 0 | 15,120 | 0 |
| 47 | 1 | 1 | 14,950 | 0 |
| 30 | 18 | 1 | 870 | 1,000 |
| 24 | 24 | 1 | 270 | 3,000 |
| 6 | 42 | 1 | 15 | 3,000 |
| 0 | 48 | 1 | 0 | 5,000 |

Chloramphenicol

1. To determine the MIC

| Concentration of chloramphenicol μg/ml | Inclusions per coverslip after 48 hours |
|---|---|
| 0 | 7,200 |
| 0.1 | 6,900 |
| 0.5 | 3,810 |
| 1.0 | 0 |
| 2.5 | 0 |
| 5.0 | 0 |
| 10 | 0 |

2. To determine whether chloramphenicol has a chlamydistatic or chlamydicidal effect - removal of chloramphenicol after 48 hours

| Initial concentration of chloramphenicol μg/ml | Count at 96 hours - 48 hours after removal of antibiotics |
|---|---|
| 0 | 7,290 |
| 0.1 | 3,021 |
| 0.5 | 550 |
| 1.0 | 240 |
| 2.5 | 150 |
| 5.0 | 75 |
| 10 | 75 |
| 25 | 0 |
| 50 | 0 |
| 100 | 0 |

Tetracycline

1. To determine the MIC

| Concentration of tetracycline µg/ml | Inclusion count per coverslip |
|---|---|
| 0 | 5,412 |
| 0.001 | 5,148 |
| 0.005 | 4,653 |
| 0.001 | 0 |
| 0.1 | 0 |
| 1 | 0 |

2. To determine whether tetracycline has a chlamydistatic or chlamydicidal effect - removal of tetracycline after 48 hours

| Initital concentration of tetracycline µg/ml medium | Count when washed at 48 hours to remove tetracycline | Count after incubated for 96 hours with tetracycline |
|---|---|---|
| 0 | 793 | 1,551 |
| 0.001 | 726 | 1,584 |
| 0.005 | 660 | 1,518 |
| 0.01 | 1 | 0 |
| 0.1 | 0 | 0 |
| 1 | 0 | 0 |

3. <u>To determine whether the effect of tetracycline depends on the stage of development - prior infection and incubation for varying times before the addition of tetracycline</u>

| Time of incubation before the addition of tetracycline | Hours exposed to tetracycline | Count at each concentration of tetracycline | | | | |
|---|---|---|---|---|---|---|
| | | µg/ml 0 | µg/ml 0.005 | µg/ml 0.01 | µg/ml 0.1 | µg/ml 1.0 |
| Control | - | 5,437 | N.T. | N.T. | N.T. | N.T. |
| 47 | 1 | 5,216 | 5,403 | 5,207 | 5,591 | 4,935 |
| 24 | 24 | 4,967 | 4,847 | 4,247 | 1,024 | * |
| 18 | 30 | 5,206 | 5,697 | 4,897 | * | * |
| 0 | 48 | 5,400 | 5,597 | 4,697 | * | * |

*very small immature inclusions seen - not countable

These laboratory results will be discussed here. The conclusions from the clinical results appear in the main discussion.

## LABORATORY RESULTS: CONCLUSIONS

Penicillin, normally bactericidal against sensitive organisms, showed similar irreversibility against C. trachomatis and abnormal inclusions appeared to be dead chlamydiae. However, in later experiments (D. Hobson, personal communication) the addition of penicillinase to these abnormal inclusions rendered them viable and allowed normal growth to resume once in antibiotic-free medium and Ridgway[195] found they recovered their infectivity after multiple passage in antibiotic-free media.

The inclusion-inhibiting activity of penicillin was greater when added early in the growth cycle indicating that it prevents division of initial bodies where new cell wall growth is involved and so prevents the formation of a new generation of elementary bodies. The discrepancies between tissue culture and clinical findings seem due to the varying susceptibility of inclusions according to their age and to failure to sustain sufficiently high penicillin tissue levels long enough to deal with all the inclusions. A comparison of the results in patients with co-existing gonococcal and chlamydial genital infections treated with a week's course of penicillin with those given only single dose penicillin therapy should support this.

The failure to eradicate chlamydial infection with chloramphenicol is only partially explained. Its MIC was higher than that of erythromycin and tetracycline but easily attainable by chemotherapy and although latency was induced, the MIC and MLC were not widely different. Serial passage, not used in these experiments might show "escape" at even higher concentrations of the antibiotic but that is a cumbersome technique wherein contamination is difficult to avoid.

The excellent response to tetracycline and erythromycin, treatments of choice in chlamydial genital and conjunctival infections, is not entirely explained by their low MIC. Erythromycin's effect seems unrelated to the inclusions' age but that is probably because it acts throughout the cycle not just at the transformation of elementary to initial body but also when the initial bodies divide, whereas tetracycline acts at the first stage when the initial body is formed. Tetracycline's effect seemed chlamydicidal which conflicts with its generally bacteristatic action. However, tetracycline's action on the host cell's protein metabolism may, indirectly, affect C. trachomatis.

It seems probable that all MIC with C. trachomatis in vitro are artificially low. As noted previously, these experiments have been done with replicating cultures which are quite artificial systems with single monolayers, on a single growth cycle, and where there is significant competition between the host cell and chlamydiae. However, later results in our own laboratory with cycloheximide-treated MCC gave similarly highly sensitive results and thus it does not seem that the amount of cell-replication or the degree of protein synthesis affect the activity of antibiotics on C. trachomatis to any significant degree.

# DISCUSSION

At the time that the work for this thesis began in 1974 it was either not known or not generally accepted:

(a) that *C. trachomatis* was a primary pathogen of the female genital tract in its own right,

(b) whether the main site of chlamydial infection was the cervix rather than the urethra or vagina,

(c) whether concurrent infection with other sexually transmitted disease agents could seriously influence the expression of *C. trachomatis* infection or its diagnosis,

(d) whether any physiological factors (e.g. pregnancy, menstruation) in the woman could influence the establishment or outcome of chlamydial infection,

(e) what clinico-pathological changes in the genital tract could be attributed unequivocally to *C. trachomatis*.

(f) what complications of *C. trachomatis* cervical infection might occur,

(g) what frequency of infection with *C. trachomatis* occurred in women in the U.K.,

(h) to what extent this chlamydial infection of women might spread to their babies (i.e. what percentage of neonatal conjunctivitis is caused by *C. trachomatis*),

(i) how efficacious various regimes of antibacterial antibiotics were and why there were marked differences between drugs and between the same drugs in vivo and in vitro.

The prime purpose of the thesis was to obtain answers to some of these problems.

While this investigation in Liverpool has been in progress, there has obviously been an increasing awareness of the importance of *C. trachomatis* in several other large specialized centres in the United

Kingdom and elsewhere. This has given the present author the opportunity to compare her original findings with other concurrent or subsequent observations elsewhere and these will be critically reviewed in the following discussion.

The cervix, signs of inflammation

The cervix was considered as an organ affected by chlamydial infection.

Highly significant positive correlation was found between the isolation of C. trachomatis from the cervix and certain clearly defined abnormal inflammatory signs, hypertrophic ectopy and endocervical mucopus, where they were present. Previous reports (considered in detail in the introduction to this thesis) had not differentiated between simple ectopy which is physiological and abnormal findings described as "cervicitis" and so a high proportion of their control groups were described as showing signs of inflammation. In our control group of 37 infertility patients where no chlamydial isolation was made, no signs of inflammation were found.

In contrast, in 72.4% of women with hypertrophic ectopy, and in 81.3% with both hypertrophic ectopy and endocervical mucopus, C. trachomatis was isolated from the cervix. There was, nevertheless, a large percentage (46%) of women with chlamydial infection of the cervix in whom such signs of inflammation were absent.

Other topics

The characteristics of patients with chlamydial infection of the cervix and their contacts have been studied, along with factors affecting the susceptibility of the cervix to chlamydial infection, the symptoms and other clinical signs of infection, its interaction with other genital infections, the effects of local spread of infection and the serum antibody response.

C. trachomatis, a sexually transmitted agent

Many authors, whose work has been reviewed already in the introduction to this thesis, have suggested C. trachomatis is a major aetiological agent in nongonococcal urethritis, isolated in 25-29% [226,122]

men with that condition. That chlamydial genital infection is a sexually transmitted disease is supported firstly by the increased incidence found here in primary female contacts (55%) of men with NGU compared with secondary contacts (37%) (confirming previous work[111]) and secondly by the different chlamydial isolation rates occurring in populations of varying promiscuity. Amongst our control infertility clinic group, presumably single partner unions of long duration with no barrier or other contraceptives, there was not one isolate. In Family Planning Association (F.P.A.) clinic patients, the isolation rate had been slightly lower (3%)[110] than in gynaecological outpatients (8-9%)[220,128]. It was higher amongst patients in sexually transmitted diseases (STD) clinics (18-31%)[111,110] (currently in our unit 24%) and highest of all in the presence of another sexually transmitted disease, gonorrhoea (48-63%)[227,110].

Susceptibility of columnar epithelium

Columnar epithelium is more susceptible to infection with chlamydiae than stratified squamous epithelium. This may be due to biochemical or physical factors (e.g. the superficial cells in columnar epithelium are active and not overlaid by layers of dead ones). In the other two main sites of chlamydial infection, the urethra and conjunctiva, only columnar epithelium is involved. Columnar epithelium lines the endocervical canal and appears on the vaginal aspect of the cervix in women with ectopy.

Effect of oral contraception

As discussed previously, ectopy is not a response to infection as once was believed[201,202]. For example, it was found in 37% symptom-free women attending an FPA clinic[119]. It was found there and confirmed in this thesis that ectopy was commoner in young women using oral contraception, which was found to lessen the tendency of ectopy to diminish with age and parity. The progestational effect of oral contraception on the cervix (during the time of this study) resembled that of the first trimester of pregnancy, namely the production of hyperplasia and oedema of the columnar epithelium and eversion of the endocervix[228]. There was also an excessive outflow of alkaline mucus from the endocervical glands which might alter the anti-infective effect of the normally low vaginal pH. Delay in the reduction of ectopy might have been due to the alkaline cervical mucus buffering the low vaginal pH which normally stimulates metaplasia in the cervical columnar cells[118a].

Hormonal effects

Direct hormonal effects enhancing chlamydial infection in vitro had been reported[229]. Steroids were added to tissue cell cultures infected with C. trachomatis and their presence throughout the growth cycle increased the size and number of developing inclusions. In the present clinical study, variations in hormone levels throughout the menstrual cycle were not found to affect the chlamydial isolation rate. A significant difference in incidence of C. trachomatis according to the stage in the cycle had been reported by Hilton[110] but only in women using oral contraceptives, whose hormonal constituents have changed over the years. Furthermore, Hilton had found that the highest isolation rates were in the week before, and the week of, the menstrual period. The possible effect of menstrual blood on enhancement or depression of chlamydial growth on tissue culture has yet to be studied.

Profile

The increased cervical ectopy and cervical mucus due to oral contraception thus result in a particularly favourable environment for infection in a young, nulliparous, sexually active girl whose promiscuity exposes her to chlamydial infection. As she would be at peak child-bearing age, she would be likely to infect her babies, resulting in neonatal chlamydial conjunctivitis.

Chlamydial infection in pregnancy/O.C. users

In this present study, the incidence of chlamydial infection in the presence of oral contraception was found higher (48%) than in pregnancy (29%, $\chi^2$ = 16.7, 1 d.f.***) which was not significantly higher than in our clinic population (24%, $\chi^2$ = 1.1, 1 d.f., N.S.). Where there was ectopy in pregnancy, the isolation rate (41%) approached that in the presence of oral contraception ($\chi^2$ = 0.77, 1 d.f. N.S.). The populations had not been matched and although the mean ages of the pregnant women and women taking oral contraceptives were identical (22.6 years), that of the general clinic population was 29.3 years and other variables, such as promiscuity, were not recorded as numbers of partners were unknown. In later pregnancy also, metaplasia tends to replace ectopy which would almost certainly produce a less favourable environment for growth of C. trachomatis; the duration of pregnancy and so time of susceptibility to infection is slight compared with the period for which most women take oral contraception; in pregnancy, there is, as well as persisting vaginal acidity, an endocervical mucous plug discouraging the spread of infection there.

Inclusion counts (a) ectopy

In another study by the Liverpool group[126], the chlamydial inclusion counts, which are a measure of the degree of infection, were raised in the presence of ectopy, although the present study showed no difference in the rate of infection. Possibly columnar epithelium on the ecto-cervix gave a larger area susceptible to chlamydial infection and colonization and for swabbing on examination than when confined to the endocervix.

(b) hypertrophic ectopy

Hypertrophic ectopy, evidence of cervical inflammation which was very significantly associated with chlamydial infection, was not associated with increased counts relative to superficial ectopy either in this or the previous Liverpool studies[9,126]. Further studies are needed to confirm this but, as has been previously found in acute and chronic trachoma[230], clinical signs in primary infections with NGU and chlamydial conjunctivitis[127] are usually acute, whereas recurrent infections produce relatively minor signs, easily missed and are less transmissible.

Some authors[183,181] had found reduced chlamydial isolation rates in men with a previous history of NSU but others[106,231,122] had not. Prentice[232] had postulated that this might be due to the presence of antibodies when he found that a significant number of isolate-negative men (chlamydiae and other possible pathogens) had had previous attacks of NSU. Higher isolation rates were found in our unit in Liverpool in contacts of men with primary than recurrent NGU, confirming previous work[111].

Hypertrophic ectopy may be a host response to a primary infection seen less commonly in later infections because of the presence of local cervical immunoglobulins in immunocompetent hosts. The decreasing incidence and possible severity[126] of chlamydial infections with age may be another aspect of women's altered immunological reaction following previous chlamydial infections. Humoral and cervical antibodies and the cell-mediated immune response may all result in their more rapid control.

(c) Endocervical mucopus

Endocervical mucopus, another indication of cervical inflammation was also significantly associated with chlamydial cervicitis and the inclusion counts were higher in its presence than in its absence in this and previous[126,9] studies in our unit. There are four possible reasons for the importance of mucopus in chlamydial cervicitis. It might yield a larger sample of cervical secretions and so more chlamydial infective agents but, where it is thick and tenacious, a smaller specimen is often obtained from it than from free-running clear mucus. It might enhance the growth of chlamydiae but, added to infected tissue culture, it inhibited their growth in vitro (Hobson D., Lee N., unpublished data). Mucopus might indicate the presence of gonorrhoea or other concurrent infection which encourages chlamydial replication. However, as will be described later, in chlamydial infections, concurrent gonorrhoea seems to be merely a coincidental sexually transmitted disease, common in such a population and signs of cervical inflammation such as endocervical mucopus are attributable more often to the chlamydial infection than to gonorrhoea. The most likely explanation is that mucopus is a direct result of chlamydial cervical infection and, as the inclusion counts suggest, its appearance relates to the severity of the infection.

Cervical inflammation and reisolates

Signs of cervical inflammation were less frequently present following treatment, remaining in only very few cases. In the group of 166 women followed up in this study, reisolates were subsequently made in 18 (10.8%) and, of these, signs of inflammation present in 9 (50%) with the original isolate were only found with the reisolate in 4 (22%). This lends support to the theory above of modified host response and lessened severity of infection in recurrent infections.

Other STD

The incidence of C. trachomatis cervical infection was found higher in the presence of gonorrhoea[110] and in trichomoniasis[233] in this study confirming previous work.

(a) trichomoniasis

Trichomoniasis produces an acute vaginitis and degeneration of squamous epithelium. Endocervical inflammation is not prominent in this infection but trichomoniasis is commonly associated with cervicitis of various aetiologies and is a useful indication of exposure to sexually transmitted diseases, whilst not in itself facilitating chlamydial infection.

(b) gonorrhoea

There are two theories about the effect of gonorrhoea on chlamydial infection. Richmond[106,110] suggested that it caused, as may occur in trachoma, a bacterial reactivation of a chronic latent chlamydial infection. However, the ease of reisolation in untreated genital chlamydial infection over many months, the disappearance of signs of cervical inflammation with the eradication of chlamydiae where those were the sole infective agents and the almost invariable chance of reinfection (by untreated consorts or new partners) where reisolation followed treatment, made this unlikely.

The incubation period for gonorrhoea[234] is shorter than that for chlamydial infection[7] (as is most obvious in neonatal conjunctivitis). When, therefore, there are concurrent gonorrhoea and chlamydial infections, in urethritis and conjunctivitis particularly, clinical signs of gonorrhoea may precede evidence of chlamydial infection. In such double infections, suitable chemotherapy may eradicate chlamydial infection without the diagnosis ever having been made; conversely, abnormal signs, due to gonorrhoea, may attract attention earlier and more urgently but failure of such signs to clear up, after anti-gonococcal chemotherapy, may lead to further investigation with the ultimate diagnosis of chlamydial infection[185] as in post-gonococcal urethritis. For, as these present results show, although there was significantly more mucopus in the presence of gonorrhoea than in its absence, the incidence of mucopus, as of hypertrophic ectopy, was significantly higher with a mixed gonococcal and chlamydial infection (and even with chlamydial infection alone) than with gonorrhoea alone.

Cervical host cell morphology

(a) in gonorrhoea

Electron microscopy studies[235] had suggested gonococcal cervical infection occurs not only in columnar epithelium but in stratified squamous epithelium previously thought resistant to infection. Biopsies had been taken from the squamo-columnar junction, however, and it seems more probable that metaplastic cells were originally infected (using the electron microscope, it may not always be possible to tell whether the infective agent is intra- or extra-cellular). These, at certain phases of metaplasia are actively phagocytic; they have been closely studied in tracing the development of cervical malignancy and pre-malignancy[218].

(b) in chlamydial infection

Similar electron microscopy investigations regarding the site of infection in the cervix would be most interesting.

As early as 1938, Braley[42], an early advocate of the cervix rather than the vagina as the site of genital chlamydial infection, had examined cervical biopsies, using light microscopy, and found chlamydial inclusions only in cells two to three layers deep in the transition (now "transformation") zone, the site of metaplasia. Artefacts in processing may have made the site appear falsely deep but he was definite about the cell morphology and commented that few symptoms could be anticipated when such a narrow area was involved.

Dunlop in 1964[93] had described naked eye changes in the cervix at the cervical margin (i.e. the area of metaplasia) and, on cytological examination, chlamydial inclusions had been found in columnar cells and squamous para-basal cells which are difficult to distinguish from metaplastic epithelium[219].

It would appear that only columnar cells, prior to or undergoing metaplasia, may be infected by C. trachomatis and possibly metaplasia may result from chlamydial infection. Any chronic intracellular infection occurring during metaplasia may be incriminated in the aetiology of carcinoma of the cervix especially when hypertrophic changes occur. N. gonorrhoeae tends to cause a briefer more acute infection and is intracellular only when phagocytosed by polymorphonuclear leucocytes, etc, and so is a less likely carcinogenic agent.

## Cervical signs in gonorrhoea

We have found ectopy in gonorrhoea to be significantly less common than in chlamydial infection. The incidence of ectopy rises significantly when cervical gonorrhoea alone is compared with combined infections. This may be because, as the electron microscopy studies suggested, columnar epithelium does not facilitate gonococcal infections as it does chlamydial. Possibly due to the relative unimportance of superficial ectopy in gonococcal cervical infection, hypertrophic ectopy, unlike endocervical mucopus is unrelated to the presence of gonorrhoea.

## Persistence

We have previously observed the persistence of chlamydial infection untreated over long periods due to patients' failure to keep their follow-up appointments[8], although in this present study only two were untreated for any marked length of time (15 and 54 weeks). A study of the effect of time on cervical inflammation due to chlamydial infection would be interesting but, due to good contact tracing and the patients' cooperation, the numbers available to us would be too small. (The risks of the spread of infection in the patient and to her contacts and children make prospective studies unethical).

## Symptoms of chlamydial infection of the cervix

The ease with which chlamydiae were reisolated in 19 untreated Liverpool women, previously, indicates the reservoir of infection remaining in women and yet, unfortunately, 46% of women with chlamydial infection were symptom-free which is similar to findings in gonorrhoea (49%) in our unit. Nevertheless, the presence of any symptoms was apparently significantly related to chlamydial infection ($\chi^2 = 5.87$, 1 d.f.*).

However, when symptomatic genito-urinary infections such as trichomoniasis and candidiasis were excluded, any symptoms were directly attributable to chlamydial infection alone. In these circumstances, 51% chlamydia-positive women were asymptomatic and the presence of any symptoms was unrelated to chlamydial infection ($\chi^2$ = 2.98, 1 d.f. N.S.). Vaginal discharge ($\chi^2$ = 7.11, 1 d.f.**) and post-coital bleeding ($\chi^2$ = 3.78, 1 d.f., N.S.) alone were suggestively associated with chlamydial infection of the cervix.

The abnormal discharge may be due to the presence of large areas of superficial ectopy. Where no pathogens have been found, these areas, per se, are considered responsible for excessive vaginal discharge due to increased cervical mucus production[236,237]. (There was found in this study significantly more ectopy ($\chi^2$ = 6.05, 1 d.f.*) and a significantly higher chlamydial isolation rate ($\chi^2$ = 7.23, 1 d.f.**) in the presence of a discharge, when trichomoniasis was absent).

Post-coital bleeding had not been associated with ectopy in the Edinburgh F.P.A. study[119] and may be directly related to chlamydial infection and the inflammation that causes. (The numbers are too small to be significant but, in this present study, no dysplasia was found in Ayre smears from chlamydia-positive patients with post-coital bleeding).

Reasons for attendance

Unless, therefore, attention is attracted by obvious pathology in their partners or babies, women's genital chlamydial infection commonly goes unrecognised. The majority of women with chlamydial infection reach medical attention by one of two routes (a) when the partners of men with NGU are treated epidemiologically, with or without chlamydial investigation, or (b) on examining the cervix of the mothers of new-born babies with chlamydial conjunctivitis.

Long-term consequences of neonatal chlamydial conjunctivitis

Full investigation of neonatal chlamydial conjunctivitis before giving possibly suppressive chemotherapy (to mother or baby or both) is even more urgently required than treatment of NGU contacts.

(a) pelvic inflammation

The aetiological role of C. trachomatis has been established in pelvic infection by many authors[95,129] whose work was reviewed in the introduction to this thesis.

As the Liverpool group had found previously[143,127] and this study has confirmed and extended, in the puerperium, the incidence of pelvic inflammation was high in women with evidence of chlamydial infection of the cervix. Although, apparently due to previous chemotherapy, C. trachomatis was not isolated from the cervix in all these women with pelvic inflammation, high antichlamydial serological antibody levels often were present and their babies all had neonatal chlamydial conjunctivitis. The clinical presentation was often later than bacterial post-partum infection[185] due to a long incubation period caused by the unique intracellular developmental cycle and the baby may draw attention to hitherto unsuspected asymptomatic genital infection in its mother.

(b) micropannus

Neglected or incompletely treated chlamydial conjunctivitis has been reported as leaving residual scarring as in the form of micropannus[238,145], but in these families, the most serious consequences are for the mother and already compromised babies who may be susceptible to pneumonitis[147].

(c) pneumonitis

Without systemic treatment, a child handicapped by prematurity, poor nutrition, congenital defect, the presence of other pathogens or any other cause may develop chlamydial pneumonitis. (The aetiology of congenital cardiac defects in babies with chlamydial conjunctivitis was beyond the scope of this study. However, such a child was included in this group and three others, including two with possible pneumonitis have been encountered since. Screening all babies with cardiac defects and their mothers, in cooperation with the paediatric unit, is to be undertaken shortly). The radiological changes in chlamydial pneumonitis are nonspecific and the appearance of chlamydial antibodies, normally late in premature infants may be modified by chemotherapy. In these babies, as with neonatal conjunctivitis, identification of the pathogen and systemic treatment with erythromycin is necessary.

There are two other long-term effects of incomplete care of neonatal conjunctivitis.

(d) pharyngeal infection

Chlortetracycline eye ointment will not eradicate pharyngeal chlamydial infection which was present in 52% babies in a later study in our unit[154]. A baby persistently shedding chlamydiae, apart from being at risk itself, may infect the rest of the family and one infected 18-month old sibling has been seen.

(e) fathers

The other need is to examine the father. We saw 27 of a possible 70 but, particularly where the mother was unmarried, the father often was unknown or not available for examination. Only two of those 27 (7%) had symptoms (previously ignored) but chlamydiae were isolated from the urethra in 9 of 27 (33%). These men would have been a source of reinfection for the mothers.

Premature rupture of membranes

Chorioamnionitis may result from cervical infection[239] and an association between localised chorioamnionitis and premature rupture of membranes has been reported[240]. In the present work there was a significantly high incidence of prematurity in babies with chlamydial (isolate positive) conjunctivitis compared with those in whom chlamydiae were not isolated ($\chi^2 = 6.7$, 1 d.f.**), confirming our previous results[127]. This was not, apparently, because these babies had prolonged surveillance in the special care nursery, since the age at onset of chlamydial conjunctivitis in babies with premature rupture of membranes was no different from that in all chlamydia-positive babies.

Prematurity is a major factor in the infant mortality rate (60% infant deaths occur in 10% infants prematurely born) and in neurological damage in the newborn. As an extension of this work, mothers with spontaneous premature rupture of membranes are presently being screened for chlamydial infection by urethral culture, serological studies and by examination of the placenta for chlamydiae after delivery. Passage of a speculum to examine and take cultures from the cervix from these women in contra-indicated before delivery and we have found elsewhere[139,140] that in the post-natal period urethral culture results correlate well (95%) with those from the cervix.

Amniotic fluid

In fact, in the puerperium, no chlamydiae were isolated from the cervix of 7 mothers of babies with chlamydial conjunctivitis whose membranes had ruptured 12 hours or more before delivery. Previous obstetric teaching recommended oral penicillin to combat ascending infection in these circumstances but, even where no chemotherapy was recorded, the discrepancy between maternal and babies' isolation rates persisted.

The effect of amniotic fluid on the growth of chlamydiae in tissue culture has not been recorded. Obstetricians studying intrauterine infections found that whilst the foetus gradually acquires defence mechanisms of its own, these are inadequate and the amniotic fluid itself exhibits antibacterial activity measurable from 28 weeks gestation. It contains lysozyme, transferrin, a low concentration of immunoglobulin, and, a low molecular weight family of polypeptides of potent broad-spectrum bactericidal properties[241]. Leakage of liquor containing this material through the os for several hours may affect the growth of chlamydiae on the cervix.

Caesarian section

Intrauterine infection following chlamydial chorioamnionitis established either before or after rupture of the membranes may explain the source of infection of some of the babies delivered by caesarian section who had chlamydial conjunctivitis. (There were two in this study). Others may have been indirectly infected postnatally by their attendants. The ante-partum inoculum will be less than that from the cervix in an intrapartum infection which may explain the later onset of conjunctivitis in these circumstances. Of the two babies born by caesarian section included in this study, one developed conjunctivitis aged seven days while still in the maternity hospital and the other at eleven days after discharge from the hospital at ten days.

When, however, children are born following frank spontaneous premature and prolonged rupture of membranes, the onset of neonatal conjunctivitis may be earlier. In both gonococcal[39] and chlamydial[40] infections children have been born with fully developed conjunctivitis. In Liverpool, after completion of this series, a child was seen aged 24 hours with chlamydial conjunctivitis and a history that the membranes had ruptured over eleven hours prior to delivery.

Nevertheless, it was found in this study that the mean age at onset of chlamydial conjunctivitis in seven babies where the membranes had been ruptured for longer than twelve hours before delivery was 6.7 days, similar to that in the group of chlamydia-positive babies as a whole.

Finding the patients

In summary, to reduce the incidence of chlamydial infection in women, thorough investigation of the possible index cases, babies with neonatal conjunctivitis and men with primary as well as recurrent NGU, and their respective mothers and contacts is essential. Screening for chlamydial infection by culture and serological methods of all women with pelvic inflammatory disease or residual post-gonococcal signs of cervical inflammation will contribute to finding and treating the maximum number of infected women.

Chemotherapy epidemiological and ideal

It may be necessary, due to lack of resources, merely to treat NGU contacts epidemiologically and to treat men and women with gonorrhoea with chemotherapy effective against both gonorrhoea and chlamydial infections. The ideal therapy would be at least as efficacious against both as specific conventional therapy against each separate infection is (i.e. eradicating 95% gonorrhoea[242] in men and 85% NGU[178]) and require minimum patient compliance. No treatment has been found to equal Lyall's results[175] from this unit with a combination of streptomycin and sulphathiazole. However, they were achieved in 1953 before the development of decreased sensitivity of N. gonorrhoeae to penicillin and subsequently to streptomycin was reported. Even then, in nonspecific genital infections, aetiological agents, other than chlamydiae, may not have been eradicated.

## Research arising from this study

Further research, projected or already begun, into many aspects of chlamydial infection has already been outlined in this discussion.

### (a) Colposcopy

Using the colposcope, an accurate assessment of the area of ectopy might relate to the severity of chlamydial cervicitis as measured by the inclusion counts. The effects of age, parity and method of contraception could then be compared and their interaction more clearly established.

### (b) Screening studies

Chlamydial infection may be an aetiological factor both in spontaneous premature rupture of membranes and in the production of congenital cardiac defects. Results of screening mothers and babies, where either condition occurs, (by serological and cultural methods for chlamydial infection) will indicate whether there is any significant association.

### (c) Tissue culture

A clinical impression was obtained that the growth of chlamydiae on the cervix was inhibited by amniotic fluid and others' results[110] suggest it might be encouraged by the presence of menstrual blood. In vitro experiments with infected tissue culture in the presence and absence of amniotic fluid and menstrual blood would clarify these points.

### (d) Antibody levels

Chlamydial antibody levels (local and serological) in women with pelvic infection and in babies with neonatal conjunctivitis and their mothers have already been examined[140]; a comparison of levels in primary chlamydial endocervicitis and in later attacks may reveal a pattern of altered host response.

In three other areas, studies in cooperation with the Department of Obstetrics and Gynaecology are planned.

(e) Dysplasia screening

No evidence of dysplasia was found in the presence of chlamydial infection, although the numbers examined were small. Retrospective chlamydial investigation of patients with dysplasia may define a possible effect of chlamydial infection on cervical carcinogenesis. (In the presence of frank carcinoma, this information would be less valuable as the average interval between dysplasia and carcinoma is about ten years).

(f) Electron microscopy

Using electron microscopy, in conjunction with both gynaecologists and pathologists, the site and host cell morphology of chlamydial inclusions will be examined in material taken by punch biopsy from a series of patients with chlamydial infection of the cervix.

(g) Ante-natal colposcopy and screening

Finally, colposcopy and screening for chlamydial infection studies in an antenatal clinic population will enable the manner in which the progestational changes of early pregnancy are replaced by metaplasia to be studied. The effects of incidental chlamydial infection on the cervical epithelium during its most dynamic phase may then be studied.

ABBREVIATIONS

| | |
|---|---|
| ARM | artifical rupture of membranes |
| ATP | adenosine triphosphate |
| BHK | continuous (transformed) tissue culture cell line derived from baby hamster kidney |
| _Candida sp._ | Candida species |
| Ct | _Chlamydia trachomatis_ |
| CMV | cytomegalovirus |
| D and C | dilation and curettage |
| DEAE-dextran | diethylaminoethyl-dextran |
| d.f. | degree(s) of freedom |
| DNA | deoxyribonucleic acid |
| FPA | Family Planning Association |
| FT | full-term |
| GC | gonorrhoea |
| $g$ | (acceleration due to) gravity |
| GP | general practitioner |
| HeLa | continuous tissue cell line derived from human cervical carcinoma |
| HSV II | herpes simplex virus, type II (herpes genitalis) |
| IF | immunofluorescence |
| IgA, IgG, IgM | immunoglobulins, classes A, G and M |
| im | intramuscular |
| IUCD | intrauterine contraceptive device |
| IUdR | 5-Iodo-2-deoxyuridine |
| Kg | kilogram |
| LGV | lymphogranuloma venereum |
| LGVCFT | lymphogranuloma venereum complement fixation test |
| LGWCC | logarithm of whole coverslip count |
| LMP | last menstrual period |
| (LS)CS | (lower segment) caesarian section |
| MIC | minimum inhibitory concentration |
| MLC | minimum lethal concentration |
| ml | millilitre |
| mm | millimetre |
| mg | milligram |
| µg | microgram |

| | |
|---|---|
| μ | micro units |
| M1, M2 | first male consort, second male consort |
| MCC | McCoy cell culture |
| MIF | micro-immunofluorescence test |
| n | number |
| NS | not significant |
| NGU | nongonococcal urethritis |
| NSU | nonspecific urethritis |
| NSGI | nonspecific genital infection |
| N. gonorrhoea | Neisseria gonorrhoea |
| ON | ophthalmia neonatorum |
| Opscan (R) | optical scanning device (registered trade mark) |
| OC | oral contraception |
| PCB | post-coital bleeding |
| PGU | post-gonococcal urethritis |
| PID | pelvic inflammatory disease |
| PRM | premature rupture of membranes |
| RIP | radio-isotope precipitation test |
| RNA | ribonucleic acid |
| SI | sexual intercourse |
| STD | sexually transmitted disease |
| TRIC | trachoma-inclusion conjunctivitis |
| TC | tissue culture |
| TV | Trichomonas vaginalis |
| WCC | whole coverslip count |
| WHO | World Health Organisation |

Significance

NS - $\chi^2$ (1 d.f.) $< 3.841$, $p > 0.05$

* - $\chi^2$ (1 d.f.) $\geq 3.841$, $p > 0.01, \leq 0.05$

** - $\chi^2$ (1 d.f.) $\geq 6.635$, $p > 0.001, \leq 0.01$

*** - $\chi^2$ (1 d.f.) $\geq 10.827$, $p \leq 0.001$

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# Chlamydial infection of the cervix in contacts of men with nongonococcal urethritis

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SUMMARY An investigation of chlamydial infection in sexual contacts of patients with nongonococcal urethritis (NGU) was carried out to determine the clinical signs of infection in the cervix, and their response to chemotherapy, and the incidence of cervical infection in the presence of ectopy and oral contraception.

In 202 consecutive female contacts of NGU the isolation rate of *Chlamydia trachomatis* was 35%. Hypertrophic ectopy and endocervical mucopus were present in 19% and 37% of chlamydia-positive patients respectively and, in all but one, resolved after treatment. Only 14% of those followed up after treatment developed yeast infections. The chlamydial isolation rate was significantly higher in patients with hypertrophic ectopy and endocervical mucopus. Cervical ectopy and oral contraceptives acted additively, each producing a significant effect on the chlamydial isolation rate in the presence of the other but not when present alone.

## Introduction

The clinical diagnosis of nongonococcal urethritis (NGU) in men is often arbitrary and commonly based solely on the finding of 10 polymorphonuclear leucocytes per high power field (× 100 objective) in a urethral smear with no evidence of *Neisseria gonorrhoeae*. However, strong evidence, recently reviewed by Schachter,[1] has now accumulated for a causative role for *Chlamydia trachomatis* in up to 50% of men with NGU and for the sexual transference of this agent.

In women, the commonest site of chlamydial infection (as in gonorrhoea) is the cervix,[2 3] the site of greatest coital contact, but *C trachomatis* may also be isolated—although less frequently—from the rectum,[4] the urethra,[5] and Bartholin's ducts.[6]

Criteria similar to those used in the diagnosis of NGU in men cannot be applied to cervicitis because the endocervical secretion contains polymorphonuclear leucocytes which vary in number according to hormonal factors and coital history. Until the clinical signs of infection and related symptoms were investigated in conjunction with rapid tissue culture procedures for chlamydial isolation (based on methods of Gordon and Quan[7]) the diagnosis of nongonococcal cervicitis in women was unsatisfactory owing to the absence of a known aetiological agent.

The difficulties associated with interpreting clinical signs in the cervix have previously been discussed in detail.[8] The same method of categorising the state of the ectocervix is used here; it is based upon the concept that ectopy (erosion) is not a pathological condition but simply ectopic columnar epithelium which extends from the endocervix to the ectocervix.[9 10] The redness which is associated with such ectopy—and sometimes thought to represent inflammation—results from the sub-mucosal capillary bed being viewed through a single layer of columnar epithelium instead of many layers of squamous epithelium. Irregularity of outline with peninsulae and islands of squamous epithelium together with Nabothian follicles (ducts of mucus glands blocked by metaplastic squamous epithelium) indicates metaplasia which is, in most cases, physiological but may be dysplastic.[11] When an area of ectopy is oedematous and congested and bleeds, we have called it "hypertrophic". This appearance was first described as "cobblestone" by Dunlop,[12] who also reported the presence of "follicles" on colposcopical examination of the cervix. Together with mucoid or

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mucopurulent endocervical discharge these two signs, singly or combined, indicate endocervicitis.

At present there is no consistent management of sexual contacts of men with NGU. Some physicians refer all contacts for investigation and give treatment to all on epidemiological grounds, irrespective of clinical or microbiological findings. Others are selective in both their referrals and their treatment. Many physicians have been deterred from giving epidemiological treatment—in the absence of symptoms—as candidosis might ensue. However, women with gonorrhoea are commonly asymptomatic and without clinical signs. They are treated when *N gonorrhoeae* is isolated because it has been established that failure to treat may lead to serious consequences for the patient herself and for the community. It is therefore important that the pathogenic role of *C trachomatis* in women should be defined; (a) to establish the need for the treatment of known infections; and (b) to provide information for physicians who may have to manage their patients without isolation facilities.

This paper gives the results of an investigation of chlamydial infection in 202 women who were sexual contacts of men with NGU with particular regard to: (a) the incidence of clinical signs and their response to treatment in patients from whom *C trachomatis* was isolated; (b) the presence of concurrent infection; and (c) the incidence of chlamydial infection in patients with cervical ectopy and in those taking contraceptive steroids and the possible interrelationship of these two factors on the isolation rate of this agent.

## Patients and methods

### CLINICAL INVESTIGATIONS

The group of women examined consisted of 202 contacts of men with NGU who attended Liverpool Royal Infirmary consecutively on specified days. All the male contacts had been diagnosed in sexually transmitted diseases (STD) clinics where facilities for isolation of *C trachomatis* were not available. Women who had received antibiotics in the four preceding weeks were excluded. Known cases or contacts of gonorrhoea were also excluded as that infection can also cause endocervicitis.

In this group of women, 169 presented with contact slips; the remainder attended of their own accord, or were referred by another doctor, and gave a history that their sexual contact was attending a male STD clinic. In these cases, the diagnosis was always obtained from the clinic named.

Swabs for detection of *N gonorrhoeae* were taken from the urethra and endocervix on at least two separate occasions and used to inoculate a selective medium containing antibiotics (London Analytical and Bacteriological Media Ltd, London) and make a smear for Gram staining. All patients were investigated for syphilis by conventional methods and for trichomoniasis and yeast infection by inoculation of Feinberg-Whittington medium. (Yeast infection was only recorded if cultures gave positive results even when clinical signs and symptoms suggesting the condition were present.) Swabs for culture for *C trachomatis* were taken from the endocervical canal and, in addition, rubbed on any ectopic columnar epithelium as previously described.[6] Swabs were not taken from the urethra.

### LABORATORY TESTS

Swabs for culture for *C trachomatis* were immediately placed in 2-3 ml of transport medium[13] and stored directly at 4°C until inoculated into tissue culture. This was usually done two to four hours after the swab had been taken but occasionally after storage for 24 hours.

Swab contents were thoroughly dispersed into transport medium and 0·4 ml were inoculated on to each of two 16-mm coverslip cultures of replicating McCoy cells (that is, cells not pre-treated by *x*-irradiation or any anti-replicative chemical agents). Centrifugation-assisted absorption of the inoculum on to the McCoy cells as well as incubation conditions and method of examination of inoculated cultures after 48-72 hours' incubation have all been described previously.[14]

In all cases, the total number of intracellular inclusions of *C trachomatis* recovered from each clinical specimen on the McCoy coverslip culture was counted. The inclusions detected represent a single cycle of development; their number therefore reflects the number of infective particles in the original cervical swab and is a direct measure of the number of particles being shed from the lesion. The purpose of this quantitative procedure was (a) to check the sensitivity and reproducibility of the laboratory method and (b) to have additional data against which the severity, response to treatment, and possibility of relapse might be assessed.

In all cases, the results represent the primary inoculation of specimens in tissue culture. No second passage from tissue culture to further tissue culture was performed to avoid any risk of false-positive results by cross-contamination.

### CLINICAL SIGNS

The clinical signs were recorded on the request form and case sheet at the time of sampling.

The state of the ectocervix has been recorded in three categories: no ectopy, simple ectopy, and hypertrophic ectopy (Figs 1-3). The contents of the

FIG 1 *Cervix showing no ectopy.*

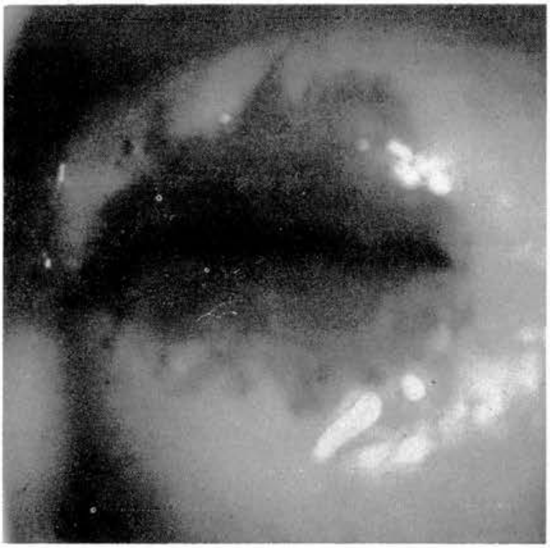

FIG 2 *Cervix showing simple ectopy.*

endocervix have been recorded in three categories; clear mucus, cloudy mucus, and mucoid or mucopurulent discharge (Figs 4 and 5). In examining the cervical contents, care was taken to remove discharge extruding from the cervix before the contents of the canal were assessed and sampled. Cervical secretion spreading from the os into the vagina becomes contaminated by vaginal flora, thus appearing mucopurulent in some cases, whereas behind this clear mucus may be seen in the canal.

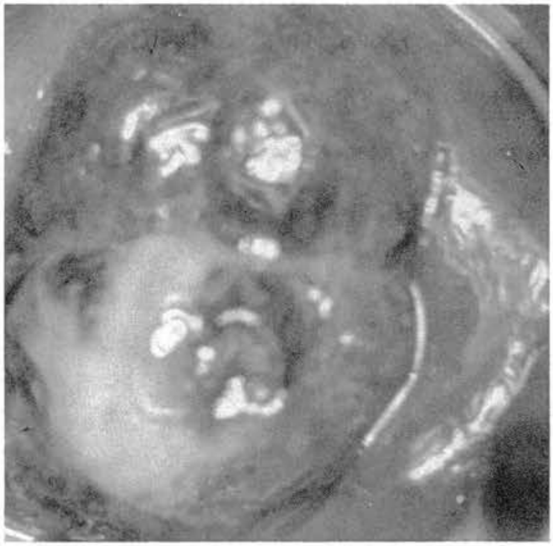

FIG 3 *Cervix showing hypertrophic ectopy with endocervical mucopus. (C trachomatis isolated.)*

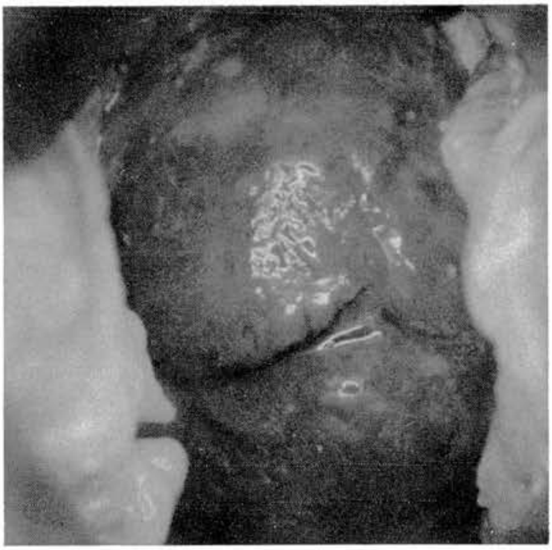

FIG 4 *Cervix showing clear mucus with simple ectopy.*

TREATMENT

Routine treatment of chlamydia-positive women was with oxytetracycline hydrochloride tablets 250 mg six-hourly for 21 days. Pregnant women were not given oxytetracycline but received erythromycin BP 250 mg six-hourly. Treatment was given for 21 days as this is the longest course advised for men with NGU and we did not want possible inadequate treatment to be a cause of persisting clinical signs or chlamydial reisolation or both.

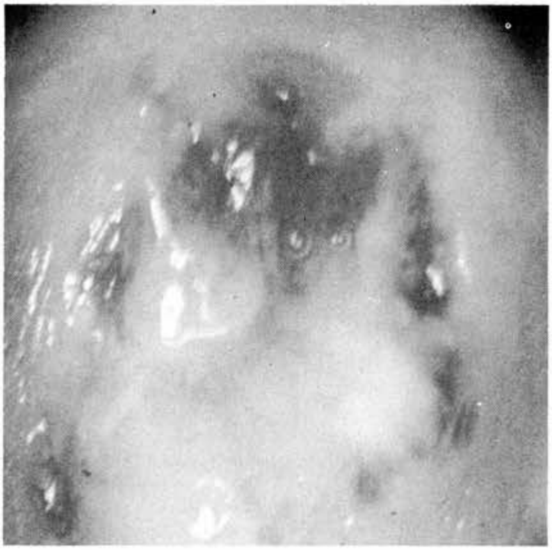

FIG 5 *Cervix showing endocervical mucopus with simple ectopy.*

Patient compliance can be a problem with such prolonged treatment. To assess this, blood samples were taken for serum tetracycline estimation at 14 or 21 days in 26 women. Patients were not told in advance that the test was to be carried out; hence the time of venepuncture varied from two to five hours after the last dose. The range of tetracycline concentrations was $0 \cdot 53$-$2 \cdot 01 \mu g$ per ml (mean $= 1 \cdot 21$). This suggests that patient compliance was good and that the serum concentration was satisfactory (minimum inhibitory concentration of oxytetracycline for *C trachomatis* $= 0 \cdot 2 \mu g/ml$[15]).

FOLLOW-UP

All chlamydia-positive patients were asked to attend for review to check whether or not candidosis had developed and to assess the response to treatment on day 14 (of the course of treatment), on day 28 (that is, seven days after completion of treatment), twice more at three-weekly intervals, and subsequently at six weeks, a total of three months' post-treatment observation. Several patients agreed to co-operate by continuing to attend at three-monthly intervals and others returned with fresh symptoms or requesting reinvestigation within that time.

Specimens were taken for culture for *C trachomatis, N gonorrhoeae, Trichomonas vaginalis,* and *Candida* species on each post-treatment visit.

STATISTICAL METHODS

Statistical comparisons were made using the $\chi^2$ test with Yates's correction.

## Results

*C trachomatis* was isolated from 70 (35%) of the 202 women.

AGE

The age range for all 202 women was 16-46 years (mean 25 years); in the chlamydia-positive group it was 16-37 years (mean 23 years).

MARITAL STATE

One hundred and ten women were single (chlamydia-positive 36%) and 68 were married (chlamydia-positive 28%), 23 were divorced or separated (chlamydia-positive 39%), and the status of one was unrecorded.

HORMONAL FACTORS

*Pregnancy*

Five patients were pregnant; two (one chlamydia-positive) were in the first trimester, two (both chlamydia-negative) in the second, and one (chlamydia-negative) in the third.

*Menstrual cycle*

No significant difference was found in the chlamydial isolation rates in successive weeks of the menstrual cycle.

In those 184 women with a regular cycle of 28 days (± 2), the month was divided into four weeks, week 1 starting from the first day of menstruation. The week in which the swab for *C trachomatis* was taken was recorded in each case. The isolation rates are shown in Table I. In 18 (21% chlamydia-positive) the cycle was irregular.

TABLE I *Isolation of C trachomatis in relation to the week of the menstrual cycle*

| | *Menstrual cycle (week)* | | | | |
|---|---|---|---|---|---|
| *Isolation* | *1* | *2* | *3* | *4* | *Total* |
| No of patients | 45 | 66 | 42 | 31 | 184 |
| Chlamydia-positive (%) | 17 (38) | 23 (35) | 15 (36) | 9 (29) | 64 (35) |

*Oral contraception*

At the time of examination, 99 women ($48 \cdot 7$% chlamydia-positive) were taking oral contraceptives. The methods of contraception for all 202 patients and the distribution of these methods in relation to chlamydial isolation is shown in Table II.

The chlamydial isolation rate was significantly higher in women taking oral contraceptives than in (a) those not using oral contraception ($\chi^2_1 = 10 \cdot 44$, $P = 0 \cdot 0012$); (b) those using barrier methods ($\chi^2_1 = 8 \cdot 51$, $P = 0 \cdot 0035$); and (c) those using other methods, excluding barrier methods ($\chi^2_1 = 5 \cdot 91$, $P = 0 \cdot 015$).

TABLE II *Isolation of C trachomatis in relation to method of contraception*

| Method of contraception | No of patients | Chlamydia-positive | |
|---|---|---|---|
| | | No | % |
| None | 52 | 15 | 29 |
| Oral contraception | 99 | 46 | 49 |
| Stopped oral contraception in past month | 7 | 3 | 43 |
| Barrier | 18 | 1 | 5·6 |
| Tubal ligation and IUCD | 25 | 5 | 20 |
| Not known | 1 | | |
| Total | 202 | 70 | 35 |

IUCD = intrauterine contraceptive device

Despite the difference in percentage isolation between those using barrier methods and those using no contraception, the difference in proportions is not significant at 5% ($\chi^2_1 = 2·90$, P = 0·089) but the lack of significance may be due only to the small numbers in the group using barrier methods.

SYMPTOMS

The incidence of symptoms and their distribution in relation to chlamydial isolation is shown in Table III. No significant difference was found in the isolation rates in relation to any particular symptom.

TABLE III *Distribution of symptoms, appearance of the ectocervix, contents of the endocervix, and incidence of concurrent infections in relation to isolation of C trachomatis*

| | No of patients | No chlamydia-positive | % of positive |
|---|---|---|---|
| Symptoms* | | | |
| None | 103 | 34 | 49 |
| Discharge | 61 | 22 | 31 |
| Irritation and soreness | 31 | 17 | 24 |
| Urinary | 24 | 11 | 16 |
| Abdominal pain | 17 | 5 | 7 |
| Postcoital bleeding | 3 | 1 | 1·4 |
| Dyspareunia | 3 | 2 | 3 |
| Appearance of ectocervix | | | |
| No ectopy | 95 | 21 | 30 |
| Simple ectopy | 87 | 36 | 51 |
| Hypertrophic ectopy | 17 | 13 | 19 |
| Not known | 3 | 0 | 0 |
| Total | 202 | 70 | 100 |
| Endocervical contents | | | |
| Clear | 101 | 27 | 39 |
| Cloudy | 56 | 14 | 20 |
| Mucopus and mucoid | 35 | 26 | 37 |
| Blood | 10 | 3 | 4 |
| Total | 202 | 70 | 100 |
| Concurrent infections | | | |
| None | 110 | 40 | 57 |
| *Candida* spp | 61 | 16 | 23 |
| *Trichomonas vaginalis* | 15 | 10 | 14 |
| Warts (cervical and vulval) | 10 | 4 | 6 |
| Other | 0 | 0 | 0 |
| Total | | 70 | 100 |

*Patients may have had more than one symptom

CLINICAL SIGNS

*Ectocervix*

The appearance of the ectocervix in relation to the chlamydial isolation rate is shown in Table III. The isolation rate was significantly higher in those with (a) ectopy (simple and hypertrophic), 47% ($\chi^2_1 = 12·5$, P = 0·0004); (b) simple ectopy, 41% ($\chi^2_1 = 6·97$, P = 0·0083); and (c) hypertrophic ectopy, 76% ($\chi^2_1 = 17·67$, P = 0·000026) than in those with no ectopy, 22%.

*Endocervical contents*

The distribution of contents in relation to the chlamydial isolation rate is shown in Table III. The isolation rate was significantly higher in those with mucopus (74%) than in those with clear and cloudy mucus (26%) ($\chi^2_1 = 27·14$, P = 0·0000045).

*Salpingitis*

Seventeen patients complained of abdominal pain or discomfort (five were chlamydia-positive). Only four of these had clinical signs of salpingitis (two were chlamydia-positive.)

EFFECTS OF ORAL CONTRACEPTION/CERVICAL ECTOPY ON CHLAMYDIAL ISOLATION

To assess the possible interrelationship between oral contraception and cervical ectopy, both of which individually were found to be highly significant in the isolation of *C trachomatis*, the four possible categories were compared and analysed statistically (for this purpose simple and hypertrophic ectopy were combined) (Table IV):

(1) The isolation rates in patients with (57·1%) and without (30·2%) oral contraception in the presence of cervical ectopy ($\chi^2_1 = 6·06$, P = 0·014);
(2) The isolation rates in patients with (57·1%) and without (27·3%) ectopy in those taking oral contraception ($\chi^2_1 = 6·3$, P = 0·012);
(3) The isolation rates in women with (27·3%) and without (16·4%) oral contraception in the absence of ectopy ($\chi^2_1 = 0·97$, P = 0·32);
(4) The isolation rates in patients with (30·2%) and without (16·4%) ectopy in the absence of oral contraception ($\chi^2_1 = 2·06$, P = 0·15).

Thus neither oral contraception in the absence of ectopy, nor ectopy in the absence of oral contraception, is associated with a significant increase in the isolation rate. On the other hand, a significant increase is associated both with oral contraception when ectopy is present and with ectopy when oral contraception is being used. Oral contraception and ectopy may be said to act additively, neither being associated with a significant increase in the isolation rate when acting alone but jointly being associated with a significant increase when acting simultaneously.

The isolation rates in the above categories (Table IV) differ to a very highly significant extent ($\chi^2_3 = 22 \cdot 95$, $P = 0 \cdot 000041$).

TABLE IV *C trachomatis isolation in relation to ectopy and oral contraception*

| Comparison | No of patients | Chlamydia-positive No | Chlamydia-positive % |
|---|---|---|---|
| Ectopy | 99 | 45 | 45·5 |
| With OC | 56 | 32 | 57·1 |
| Without OC | 43 | 13 | 30·2 |
| No ectopy | 94 | 19 | 20·2 |
| With OC | 33 | 9 | 27·3 |
| Without OC | 61 | 10 | 16·4 |
| Total | | | |
| With OC | 89 | 41 | 46·1 |
| Without OC | 104 | 23 | 22·1 |

OC = oral contraception

CONCURRENT INFECTIONS

The presence of concurrent infection in relation to chlamydial isolation is shown in Table III. Only normal flora was found in only 110 patients (35% chlamydia-positive). A significant increase in the isolation rate was found only in the presence of *T vaginalis* ($\chi^2_1 = 5 \cdot 89$, $P = 0 \cdot 15$). *N gonorrhoeae* was present in seven patients, of whom three were chlamydia-positive at the time of their first visit.

WHOLE-COVERSLIP COUNT (WCC)

The range for the 70 chlamydia-positive patients was 1-100 000 inclusions per whole coverslip. The possibility that the degree of infection (as represented by the WCC) may vary according to conditions such as the presence or absence of oral contraception and clinical signs in the cervix was considered. The mean counts of the WCC in these conditions are given in Table V.

TABLE V *Mean whole-coverslip counts in certain conditions*

| Condition | Mean count |
|---|---|
| Oral contraception | |
| Present | 6185 |
| Absent | 2071 |
| Hypertrophic ectopy | |
| Present | 4895 |
| Absent | 4906 |
| Endocervical mucopus | |
| Present | 10 727 |
| Absent | 1745 |

FOLLOW UP

Thirty-eight women were followed up for 2-14 months. *C trachomatis* was reisolated from five patients (Table VI) and there was a strong possibility of reinfection in four of the five cases.*

TABLE VI *Post-treatment follow up*

| | No of patients | Chlamydial reisolation |
|---|---|---|
| Duration of treatment (months) | | |
| <2 | 8 | 2* |
| 2-3 | 4 | 1 |
| 4-6 | 15 | 1 |
| 7-8 | 7 | |
| 9-10 | 1 | |
| 11-12 | 1 | 1 |
| >12 | 2 | 1 |
| No of visits | | |
| 1 | 4 | 1* |
| 2 | 11 | 3* |
| 3 | 6 | |
| 4 | 8 | 1 |
| 5 | 4 | |
| 6 | 3 | 1 |
| 7 | 1 | |
| 8 | 1 | |

*Two reisolates from one patient

*Clinical signs*

*Ectocervix.* Of 12 patients with hypertrophic ectopy, 11 reverted to simple ectopy and metaplasia was complete in one.

*Contents of the os.* Of those patients followed up after treatment, of 20 endocervices previously containing mucopus, eight became clear, 11 became cloudy, and one contained mucoid material.

*Yeast infection*

Of 70 chlamydia-positive patients, 16 had positive cultures for *Candida* species when the chlamydial infection was diagnosed; twenty-five patients who had negative results for yeasts initially did not attend for follow-up investigations. Of the remaining 29 chlamydia-positive women with negative results for yeasts, who were treated either with tetracycline hydrochloride or erythromycin (two), four (14%) (all taking tetracycline) developed yeast infection.

## Discussion

*C trachomatis* was isolated from 35% of the 202 female sexual contacts of unselected men with NGU. Isolation rates of 22-42%[3 16-18] have previously been reported in different parts of the world. Differences,

*Details of personal case histories may be obtained from Dr I A Tait.

however, occur in the reporting of clinical signs in the cervix associated with chlamydial infection. Signs of cervicitis have been recorded in 62-89%[17 19 20] but were also present in 58% of uninfected women who were contacts of NGU[17] and in 56% of control women attending a family planning clinic.[20] However, cervical ectopy (erosion, cervical discontinuity) was included as an abnormal clinical sign in all these reports and may account for these differences.

The site of the squamocolumnar junction is laid down at the time of organogenesis and ectopy is found in infants, children, and virgins.[9] At certain times, particularly at puberty and first pregnancy, the ectopic columnar epithelium is replaced by squamous epithelium (squamous metaplasia, Fig 6) with the result that ectopy is less common in the older woman.[9] In a study planned to determine the prevalence and significance of cervical ectopy in women attending family planning clinics in Edinburgh, ectopy was found in 37% of 1400 women and was significantly associated with younger age groups, greater parity, and oral contraception.[21] There was no significant association with the presence of aerobic or anaerobic bacteria, *T vaginalis,* or yeasts.

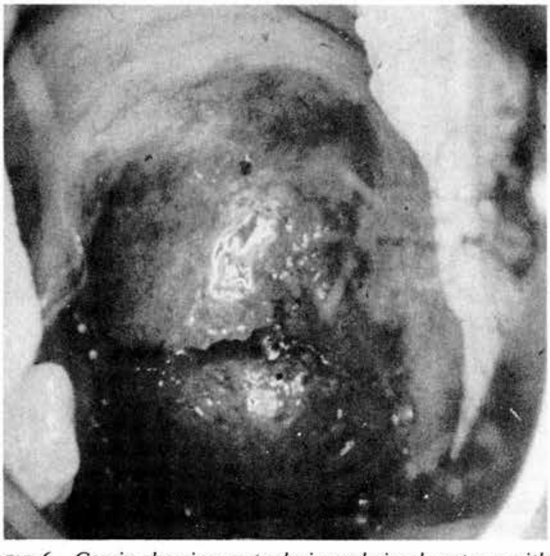

FIG 6 *Cervix showing metaplasia and simple ectopy with clear mucus.*

If signs of cervicitis are narrowly defined on macroscopic examination as endocervical pus within the canal and oedema of the areas covered by columnar epithelium (hypertrophic ectopy or cobblestone appearance as described by Dunlop)[12] there is a highly significant association between cervicitis and the isolation of *C trachomatis.* In this series of consecutive cases, mucopus was found in 37% of chlamydia-positive cases compared with 7% of chlamydia-negative cases ($P = 0 \cdot 0000045$), and oedema (hypertrophic ectopy) in 19% of chlamydia-positive compared with only 3% of chlamydia-negative cases ($P = 0 \cdot 000026$). The incidence of endocervical mucopus is similar to that found in women with chlamydia-negative gonorrhoea examined in this unit. In each infection, macrosopic evidence of the endocervical infection is absent in 50-60%. After treatment of the chlamydial infection, clinical signs of oedema and mucopus disappeared in all but one patient.

Symptoms associated with endocervicitis are difficult to interpret because the area of susceptible columnar epithelium, and therefore the consequent amount of mucopus which may be produced, is relatively small. Excessive mucus production by the cervical columnar epithelium due to the progestational effect of contraceptive steroids, or due to a large area of ectopy, may result in a discharge which is not associated with infection of any kind. In addition, even a mild vaginitis due to common vaginal pathogens such as *T vaginalis* or yeasts may result in a noticeable discharge owing to the large area of squamous epithelium of the vagina affected.

Consequently, symptoms may bear little relationship to cervical pathology in a large number of women. In this series, no significant relationship was found between the presence of chlamydial infection in the cervix and any of the symptoms. Similar findings have been reported by others.[20 22 23] Indeed Burns *et al*[16] reported a significant absence of symptoms in the presence of chlamydial infection. However, Paavonen *et al*[19] found chlamydia-positive gynaecological patients had significantly more ocular and urethral symptoms than chlamydia-negative patients.

Thus, *C trachomatis* infection appears to be associated with certain well-defined signs of cervicitis in some patients and probably these signs are caused by that infection. On the other hand, with ectopy there is also a definite relation to chlamydial infection but, for reasons already discussed, simple ectopy cannot be considered to be part of the sign-complex of cervicitis. It seems possible that ectopy may be one of many possible interrelated factors which allow infection to be more easily acquired. For example, the virulence of particular serotypes of chlamydia, the immune status of the patient, and anatomical and histological variations may all influence the ease with which infection of the tissues is established. Harkness[24] found a higher prevalence of hypospadias (a condition in which columnar epithelium extends to the external urethral surface since the fossa navicularis is absent) in patients with NGU and gonorrhoea than in the normal population. Cervical ectopy may have a similar effect in chlamydial infection and may be more important than in gonorrhoea because of the intracellular developmental cycle of chlamydia.

Our findings indicate that only in patients in whom the two factors, contraceptive steroids and ectopy, are present is there a significant increase in the isolation rate of *C trachomatis* ($P = 0 \cdot 00004$). Neither factor in the absence of the other is significant. Apart from an implied absence of any barrier to infection of the cervix, oral contraceptive steroids may facilitate cervical infection by their progestational effect, which is similar to that produced in the first trimester of pregnancy. They produce an excessive outflow of alkaline mucus from the endocervical glands and increase the bulk of the cervix, producing eversion with further ectopy. It is noteworthy that steroids added to McCoy tissues cultures infected with *C trachomatis* increase the size and number of inclusions developing.[25]

The interdependence of several varying factors may explain some of the disparity about the effect of oral contraceptives on the chlamydial isolation rate previously reported.[16 17 20 23]

Like Oriel *et al*,[23] but unlike Hilton *et al*,[20] we found no variation in the isolation rate according to the week of the menstrual cycle.

The increased chlamydial isolation rate in the presence of *T vaginalis* may reflect the frequency with which multiple sexually transmissible agents are isolated in promiscuous patients.[26] Others have found no relation between the chlamydial isolation rate and any other concurrent nongonococcal infection.[23]

The mean whole-coverslip counts were raised in patients taking oral contraceptive steroids and in the presence of endocervical mucopus but not in the presence of hypertrophic ectopy. It is possible that oral contraceptive steroids facilitate chlamydial cervical infection, and the presence or absence of endocervical mucopus indicates the severity of the infection, whereas hypertrophic ectopy merely indicates a general host-response to infection in some patients. The possible influence of these clinical factors on the degree of chlamydial replication in infected women will be discussed separately.

In addition to causing acute cervicitis *C trachomatis* has been isolated from the fallopian tubes in cases of pelvic inflammatory disease confirmed at laparoscopy and associated with a fourfold rise in chlamydial antibody titre.[27] Clearly, it is potentially a serious pathogen in women.

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# Quantitative aspects of chlamydial infection of the cervix

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SUMMARY In 580 women with *Chlamydia trachomatis* infection of the cervix, the degree of the infection was assessed by counting the number of chlamydial inclusions which developed in McCoy cell monolayers inoculated with cervical swab material under standardised conditions.

In 34% of these women inclusion counts were <100 per monolayer whereas in 36% the counts were ⩾1000. Clinical features in each of these groups were compared to see if certain factors could be identified as the cause, or the result, of high-grade rather than low-grade infection.

A significant association was found between high inclusion counts and the presence of cervical mucopus or cervical ectopy. Oral contraceptives acted additively with ectopy but had no significant effect alone. Concurrent gonorrhoea did not affect the degree of chlamydial infection. High inclusion counts were more common in women under 20 years of age than in older women.

## Introduction

Over the past five years, our combined clinical and laboratory investigations of women with genital tract infections with *Chlamydia trachomatis* have shown large variations between individual infected patients, both in the number and severity of clinical signs[1] and in the number of chlamydial inclusions obtained from cervical swabs inoculated into McCoy cell tissue cultures.[2] In a group of 202 sexual contacts of men with nongonococcal urethritis (NGU), women who had cervical mucopus, or those who had cervical ectopy and were also using oral contraceptives, showed a significantly higher prevalence of chlamydial infection than did women without these clinical features.[3] It seemed of interest to determine if these or other clinical factors might be correlated not only with a high rate of infection but with a more severe infection than in women in whom these factors were absent.

In McCoy cell tissue cultures infected with *C trachomatis* only a single cycle of development of the agent occurs, and each intracellular inclusion found after incubation represents a single infective chlamydial particle in the original inoculum. Thus, when standard amounts of material from cervical swabs are inoculated and incubated under constant conditions in coverslip monolayers of McCoy cells the final total number of inclusions found over the whole cell sheet should give a direct quantitative measure of the number of infective chlamydial particles in the cervical secretions of each patient.

The sensitivity and reproducibility of the laboratory method we have used has been investigated previously;[2 4] for example, in 48 McCoy cultures inoculated with a single sample of *C trachomatis* the counts on individual coverslips varied from the mean by a maximum of only ±23%, and, when a second swab was taken seven days after the first swab from 106 untreated infected women, the inclusion counts were of the same magnitude in both swabs in 92·5% of cases. Thus, the wide variations in inclusion counts we had observed between patients might be the result of differing degrees of infection in the cervical mucosa in individual patients.

The aim of this study was to see if certain clinical factors might be caused by, or be the cause of, high-grade rather than low-grade infection. In particular it seemed essential to consider (a) the physical state of the cervix—that is, whether or not mucopus or ectopy were present; (b) hormonal effects on cervical epithelium—that is, whether or not the patient was using oral contraceptives; (c) if chlamydial infection might be enhanced opportunistically by concurrent infections, especially gonorrhoea; and (d) the age of the patient.

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Accordingly, clinical and laboratory data were compared in the total of 580 women from whom *C trachomatis* had been isolated in this laboratory between January 1975 and December 1978. This series excludes only women with postpartum infection and those who had recently received antibiotic treatment, but it was not restricted to women with a definite history of sexual contact with men with NGU and did not exclude women with concurrent gonorrhoea.

## Patients and methods

### STUDY POPULATION

The 580 women consecutively found to have chlamydial infection on laboratory examination were all patients attending the sexually transmitted disease (STD) clinics at the Liverpool Royal Infirmary or the Royal Liverpool Hospital or who had been referred to the Liverpool Women's Hospital from various sources because of suspected genital tract infections. Only women whose infections were first diagnosed in the puerperium, or who had been given chemotherapeutic agents within the previous four weeks, were excluded.

Clinically, the group consisted of 70 women from a consecutive series of sexual contacts of men with NGU, whose clinical findings have been compared with those of contacts without chlamydial infection,[3] together with 49 infected women examined in a consecutive unselected series of patients attending a single STD clinic of the Royal Infirmary on specified days (Davies *et al,* unpublished data).

The remaining 341 women were not seen consecutively in the clinics; in fact, they had often been deliberately selected for laboratory investigation because they did show certain clinical signs of genital infection, or because they were contacts of men with NGU or with gonorrhoea, or for various other reasons which made it important to confirm or exclude chlamydial infection in their differential diagnoses and to assess its severity if present. This element of clinical selection does not affect the proposed correlation of laboratory findings with the clinical features presented by the patients, since the investigation is based on the laboratory's total number of chlamydia-positive specimens, unselected and completed in advance of the clinical data being known. Obviously, the proportion of women with such features as cervical mucopus, cervical ectopy, or concurrent gonorrhoea cannot be taken as comparable with that in clinically unselected patients seen consecutively in studies designed for clinico-epidemiological purposes.[1 3]

### CLINICAL EXAMINATION

Examination of the patients' clinical signs was made and recorded in the standardised pattern described earlier.[3]

### COLLECTION OF SPECIMENS

Swabs for chlamydial isolation were taken only by medical staff of the clinic experienced in the standardised technique previously described,[5] which ensures that both the contents of the endocervical canal and the whole of any area of ectopic columnar epithelium present are sampled.

Swabs for *Neisseria gonorrhoeae* isolation and investigations for syphilis, trichomoniasis, or yeast infections were performed on all patients as previously described.[3]

### LABORATORY INVESTIGATIONS

The procedures for the isolation of *C trachomatis* and subsequent counts of the total number of chlamydial inclusions recovered have been previously described in detail.[2 4-6] Briefly, particular attention was paid to the quantitative reproducibility of the isolation procedure—for example, immediate immersion of the swab into fluid transport medium, rapid transport to the laboratory and inoculation usually 2-4 hours after the swab was taken but occasionally after overnight storage at 4°C, followed by inoculation of a standardised amount of homogenised swab material with centrifuge-assistance on to McCoy cell coverslip monolayers of constant age and cell density. For the first 260 swabs, untreated McCoy cells were used, but cells treated with cycloheximide were used for the final 200 specimens, and both methods were used simultaneously for the remaining 120 specimens. Cycloheximide treatment has been adopted because chlamydial inclusions can be identified more rapidly and easily than in fresh cells; the total number of inclusions recovered was consistently higher in treated cells, but rarely by more than twofold, so that the log frequency distribution of counts did not vary significantly between the two methods.

In all cases, the inclusion counts recorded represent the primary inoculation of the first cervical swab to be taken from each patient during her attendance in the clinic. All swabs were allocated serial laboratory numbers, and isolation and inclusion counting procedures were completed before the patient's identity and clinical features were reviewed in the laboratory.

### STATISTICAL METHOD

The $\chi^2$ test was used for statistical comparison.

## Results

### INCLUSION COUNTS AND CLINICAL FEATURES

The wide range of inclusion counts found on culture of the primary cervical swabs for the whole group of

TABLE I *Distribution of chlamydial inclusion counts and clinical factors in 580 women with C trachomatis infection of the cervix*

| | | % of women* with positive clinical factor | | | | |
|---|---|---|---|---|---|---|
| iwc | No of women | Mucopus | Ectopy | OC | Gonorrhoea | Aged <20 years |
| <10 | 84 | 36 | 58 | 49 | 38 | 23 |
| 10+ | 112 | 36 | 63 | 50 | 35 | 24 |
| 100+ | 178 | 44 | 67 | 54 | 44 | 26 |
| 1000+ | 133 | 69 | 79 | 62 | 44 | 35 |
| 10 000+ | 73 | 66 | 75 | 66 | 34 | 40 |
| Total No of women | 580 | 290 | 402 | 306 | 232 | 168 |

iwc = No of inclusions per whole coverslip McCoy culture inoculated with cervical swab material
OC = Oral contraceptives
*In each inclusion-count group

580 women is shown in table I. Although the counts ranged from only one inclusion per whole coverslip (iwc) to >75 000 they showed a substantially normal distribution with a median count of 450 inclusions; 476 (85·5%) of the swabs yielded >10 iwc and 207 (36%) gave ≥1000 iwc.

The distribution of the main clinical factors is also outlined in table I. The overall incidence of mucopus, ectopy, and gonorrhoea was high, as was to be expected by the clinical selection of these women. Nevertheless, certain clinical factors were not uniformly distributed among the whole group.

The greater the inclusion count, the greater was the proportion of patients who were under 20 years of age, showed cervical ectopy or mucopus, or were taking oral contraceptives. There did not however seem to be any significant correlation between the height of the inclusion count and concurrent gonorrhoea.

These observations suggest that the degree or severity of infection in the cervical mucosa varied according to the presence or absence of certain clinical factors affecting the cervix. To evaluate their interactions more precisely the clinical features of women at the opposite ends of this frequency distribution of inclusion counts—that is, those with low counts of <100 iwc and those with high counts of ≥1000—were compared (table II).

TABLE II *Distribution of clinical factors in relation to low or high inclusion counts*

| Clinical factor | | No of women with inclusion counts Low (<100 iwc) | High (≥1000 iwc) | Total |
|---|---|---|---|---|
| Mucopus | + | 70 | 140 | 210 |
| | − | 126 | 67 | 193 |
| Gonorrhoea | + | 64 | 79 | 143 |
| | − | 132 | 128 | 260 |
| Ectopy | + | 120 | 160 | 280 |
| | − | 76 | 47 | 123 |
| Oral contraception | + | 94 | 130 | 224 |
| | − | 102 | 77 | 179 |
| Age (years) | <20 | 38 | 76 | 114 |
| | >20 | 158 | 131 | 289 |
| Total (%) | | 196 (34) | 207 (36) | 403 |

+ Present − absent
iwc = inclusions per whole coverslip (10 mm diam)

*Cervical mucopus/concurrent gonorrhoea*

The amount of chlamydial growth in the cervical mucosa was significantly greater in women with mucopus than in those without (table II; $\chi^2_1 = 41·1$, $P<0·001$). A heavy chlamydial infection could be the actual cause of the mucopus but alternatively some other clinical factor could be the cause of both the mucopus and the high chlamydial count. For the latter reason, the fact that 143 (35%) of the 403 women had gonorrhoea concurrently with their chlamydial infection had to be taken into account. Gonorrhoea itself is obviously a common cause of cervical mucopus and chlamydial infection might possibly be enhanced opportunistically in tissues already being damaged by *N gonorrhoeae*.

However, high chlamydial counts were not significantly more frequent in women with associated gonorrhoea than in women with chlamydial infection alone ($\chi^2_1 = 1·33$, $P>0·1$) (table II). Furthermore, the

TABLE III *Prevalence of cervical mucopus in relation to inclusion counts in women with or without concurrent gonorrhoea*

| Clinical factor | No of women with inclusion counts Low (<100 iwc) | High (≥1000 iwc) | Total |
|---|---|---|---|
| Gonorrhoea − | | | |
| Mucopus + | 45 | 82 | 127 |
| Mucopus − | 87 | 46 | 133 |
| Gonorrhoea + | | | |
| Mucopus + | 25 | 58 | 83 |
| Mucopus − | 39 | 21 | 60 |
| Total | 196 | 207 | 403 |

+ Present − absent
iwc = inclusions per whole coverslip (10 mm diam)

association between mucopus and high chlamydial counts was not dependent on a concomitant infection with *N gonorrhoeae*, as was shown by evaluating separately the data in the groups of women with and without gonorrhoea (table III).

Mucopus was present in a high proportion of all the women studied (210/403, 52%); this could be partly due to the clinical selection of the cases before referral for laboratory diagnosis. However, the proportion of cases with mucopus was similar in women with chlamydial infection alone (127/250, 65%) and in chlamydia-positive women with concurrent gonorrhoea (83/143, 70%) ($\chi_1^2 = 3 \cdot 13$, $P > 0 \cdot 05$). Moreover, there was a significant association between the presence of mucopus and high inclusion counts whether or not the woman had a chlamydial infection alone ($\chi_1^2 = 23 \cdot 36$, $P < 0 \cdot 001$) or concomitant gonorrhoea ($\chi_1^2 = 17 \cdot 14$, $P < 0 \cdot 001$).

*Cervical ectopy/oral contraceptives*

When these clinical factors were considered separately (table II) each showed a significant association with the height of the inclusion count. There was little difference between simple ectopy or hypertrophic ectopy in relation to inclusion counts and both have been combined for statistical analysis. Because of the clinical selection cervical ectopy was present in over two-thirds of the 403 women studied (table II). However, 57% of these women had high inclusion counts compared with 38% of women without ectopy ($\chi_1^2 = 12 \cdot 3$, $P < 0 \cdot 001$). Oral contraceptives were being used by 56% of the 403 women and 58% of these patients had high inclusion counts compared with 43% of women not taking oral contraceptives ($\chi_1^2 = 8 \cdot 98$, $P < 0 \cdot 01$).

However, cervical ectopy and oral contraceptives were obviously not independent variables, since only 106 (38%) of the 280 women with ectopy were not taking oral contraceptives and only 50 (22%) women using oral contraceptives did not have cervical ectopy. In fact, the association between the use of oral contraceptives and the presence of ectopy was highly significant ($\chi_1^2 = 47 \cdot 08$, $P < 0 \cdot 001$); thus these two clinical factors probably interact in their effect on the height of the inclusion count, as they have previously been shown to affect the incidence of chlamydial infection.[3] To assess this interaction, the four possible combinations of the two factors were further analysed (table IV):

1. In patients without cervical ectopy, high counts were found in 32% of women not taking, and in 48% of women taking, oral contraceptives. The effect of oral contraceptives alone was not significant ($\chi_1^2 = 3 \cdot 42$, $P > 0 \cdot 05$).
2. In patients with cervical ectopy, high counts were found in 51% of women not taking, and in 61% of women taking, oral contraceptives. The effect of oral contraceptives alone was not significant ($\chi_1^2 = 2 \cdot 67$, $P > 0 \cdot 1$).
3. In patients with oral contraceptives, high counts were found in 48% of women without ectopy and in 61% of women with ectopy. The effect of ectopy alone in these women was not significant ($\chi_1^2 = 2 \cdot 66$, $P > 0 \cdot 1$).
4. In patients without oral contraceptives, high counts were found in 32% of those without ectopy and in 51% of those with ectopy. This effect of ectopy alone was significant ($\chi_1^2 = 6 \cdot 67$, $P = 0 \cdot 01$).

When the patients with neither ectopy nor oral contraceptives were compared with those with both ectopy and oral contraceptives, the difference in the proportion of cases with high inclusion counts, 32% and 61% respectively, was significant ($\chi_1^2 = 17 \cdot 8$, $P < 0 \cdot 001$).

These results suggest that ectopy and oral contraceptives acting jointly are likely to be associated with high chlamydial counts and that ectopy alone can result in high counts even if the ectopy has not been produced by, or the cervical mucosa has not been acted upon by, oral contraceptives. The effect of oral contraceptives on chlamydial counts appears to be indirect by way of its capacity to produce ectopy. When these possible effects are discounted, by dividing the women into groups (table IV), oral contraceptives appear to have no significant direct effect on the degree of chlamydial infection.

TABLE IV *Interaction of cervical ectopy, oral contraceptives, and inclusion counts*

| *Clinical factors* | | *No of women with inclusion count* | | | |
|---|---|---|---|---|---|
| *Ectopy* | *OC* | *Low (<100 iwc)* | *High (⩾1000 iwc)* | *Total* | *% with high count* |
| − | − | 50 | 23 | 73 | 32 |
| − | + | 26 | 24 | 50 | 48 |
| + | − | 52 | 54 | 106 | 51 |
| + | + | 68 | 106 | 174 | 61 |
| Total | | 196 | 207 | 403 | |

+ Present − absent
iwc = inclusions per whole coverslip (10 mm diam)
OC = Oral contraceptives

*Age factor*
Of the total of 403 women with chlamydial infections, 114 (28%) were under 20 years of age (table II); of these, 76 (67%) had high inclusion counts compared with 131 (45%) of the 289 women over 20 years of age ($\chi_1^2 = 14 \cdot 9$, $P<0 \cdot 001$).

Despite this significant difference in the degree of infection between the under-20 age group and older women, no significant differences emerged in any of the other clinical features (table V).

*Other clinical features*
There were 119 women with one or more previous children and 22 pregnant women in the group examined; 36 women had associated trichomonal infections, 89 were infected with yeasts, and 31 had signs or symptoms suggesting salpingitis. There was no evidence that the frequency of high chlamydial counts was greater in these women than in women without these clinical features. However, the small numbers limit conclusive statistical analysis.

No significant difference was found in the proportion of women with high inclusion counts in successive weeks of the menstrual cycle. In the group of 403 women studied, 22 were pregnant, 32 had irregular periods or amenorrhoea, and in 20 the date of the last period was not recorded. In the 329 (82%) women with a regular cycle of 26-30 days, the month was divided into four weeks starting from the first day of menstruation, and the cycle-week in which the primary cervical swab for *C trachomatis* isolation was taken was recorded against the inclusion count obtained on culture (table VI).

## Discussion

Current tissue culture methods for isolating *C trachomatis* from cervical swabs of infected women provide a quantitative means of monitoring the reproducibility and sensitivity of the procedures themselves in continued diagnostic use[2 4] and of detecting differences in the degree of infection between individual patients, since the number of inclusions which develop in McCoy cell culture is directly proportional to the number of viable chlamydial elementary bodies inoculated into the culture.[7]

TABLE V *Distribution of clinical features in relation to age*

| Clinical feature | % of women <20 years | % of women ≥20 years |
|---|---|---|
| Mucopus | 51 | 53 |
| Gonorrhoea | 40 | 39 |
| Ectopy | 75 | 68 |
| Oral contraceptives | 52 | 57 |

In the present series of 580 women with chlamydial genital infections from whom a primary cervical swab was taken for laboratory culture before they had been given chemotherapeutic agents, the inclusion counts were normally distributed with a median value of 450 iwc, but the range of counts obtained was wide, with 73 (13%) of the group having counts of ≥10 000 iwc whereas 84 (14%) had counts of <10 iwc. When the inclusion counts in the individual patients were related to the clinical features found at the time the swab was taken, the degree of infection (indicated by the inclusion count) apparently varied according to the presence or absence of cervical mucopus or ectopy, the use of oral contraceptives, and the age of the patient.

The increase in inclusion counts in women with cervical mucopus may be due to the following: (a) the greater the amount of secretion in the cervical canal the greater is the amount that might be collected on the swab, and hence the greater the number of elementary bodies available to infect the McCoy coverslip culture; (b) the presence of mucopus might non-specifically enhance the sensitivity of McCoy cells to chlamydial infection, resulting in a higher proportion of the potentially infective particles inoculated successfully forming inclusions in McCoy cells; (c) mucopus may be merely an indicator of another concurrent infection, especially gonorrhoea, which might be capable of enhancing the chlamydial infection—that is, *C trachomatis* might thus show the characteristics of a secondary opportunistic invader; and (d) mucopus might in fact be the direct result of chlamydial infection and thus occur most frequently in those with the greater degree of infection, as indicated by the inclusion count.

In our experience the thick tenacious nature of the mucopus often results in rather less than more cervical secretion being obtained per swab and limits the amount of homogenisation of material from the swab before tissue culture inoculation. Suspensions of cervical mucopus from patients with STDs but without current chlamydial infection are slightly but consistently inhibitory to the growth of laboratory strains of *C trachomatis* in McCoy cultures (Hobson and Lee, unpublished data).

TABLE VI *Inclusion counts in relation to menstrual cycle*

| | Week of cycle* 1 | 2 | 3 | 4 | Total |
|---|---|---|---|---|---|
| No of patients | 60 | 112 | 79 | 78 | 329 |
| No with high count (>1000 iwc)† | 31 | 60 | 40 | 42 | 173 |
| % with high count | 52 | 54 | 51 | 54 | 53 |

*At the time of primary cervical swab
†Inclusion count in primary cervical swab

Concurrent infection with trichomonads, yeasts, or *N gonorrhoeae* appeared to have no effect on the degree of chlamydial infection. It is interesting however that women with gonorrhoea have previously been found[8 9] to have a higher rate of chlamydial cervical infection than have contacts of men with NGU or attenders of STD clinics without gonorrhoea. This incidence may be increased because coincidental infections in the more promiscuous STD clinic patients, who must frequently be exposed to both types of infective agent, are highly likely rather than because gonorrhoea predisposes to or stimulates a previously latent or low-grade chlamydial infection. Furthermore, women who had concomitant infections did not develop mucopus more frequently than did women with chlamydial infections alone; in both groups the presence of mucopus was associated with high chlamydial inclusion counts. These results thus suggest that the presence of mucopus in a woman with gonorrhoea may, in certain cases, be the result of an associated chlamydial infection rather than due to the gonococcal infection alone.

There is a highly significant association between the chlamydial isolation rate and the incidence of cervicitis in contacts of men with NGU,[3 10] and treatment of the chlamydial infection is quickly followed by disappearance of mucopus.[3] The present results support and extend these findings—that is, *C trachomatis* can cause endocervicitis and the greater the degree of infection the more likely are the signs of endocervicitis to be present.

In contacts of men with NGU seen in Liverpool[3] a higher rate of chlamydial infection was found when both cervical ectopy and contraceptive steroids were present than when either occurred alone or when neither was present. All the infected women from this smaller study group[3] are included in the present larger and more varied series, which shows that not only the incidence but also the degree of chlamydial infection was greater in women with ectopy and contraceptive steroids than in women with neither. Oral contraceptives alone did not appear to have any influence on the degree of infection but, in contrast, the inclusion counts were significantly higher in those with ectopy alone—even in the absence of oral contraceptives—than in women without ectopy. Since the site of the squamo-epithelial junction is determined during fetal life,[11] infection with *C trachomatis* cannot be a cause of cervical ectopy, although infection may convert a small area of simple ectopy to a larger area of hypertrophic ectopy as a result of oedema and congestion.[3]

The most likely explanation of the significant association between the presence of ectopy and both an increased rate of infection[3] and an increase in the degree of that infection demonstrable in tissue culture is simply that the greater the area of columnar epithelium exposed on the vaginal aspect of the cervix the greater is the chance that infection will be acquired after coitus with an infected man, the greater is the number of susceptible columnar cells available for progressive growth cycles of the infective agent, and the greater is the area that can be sampled by taking swabs.

The role of oral contraceptives is more difficult to determine and is almost certainly multifactorial. The frequency and size of cervical ectopy is itself increased by the use of contraceptive steroids,[12 13] and thus the chlamydial inclusion count is likely to be significantly increased if oral contraceptives are considered overall, but much of this increase may be the result of the ectopy associated with the use of oral contraceptives and not uniquely due to the drugs. However, oral contraceptives may possibly also have direct hormonal effects on columnar epithelium in vivo which increases the chance that a highly productive infection with *C trachomatis* will develop. Contraceptive steroids have a progestational effect, producing a large outflow of alkaline mucus from the endocervical glands.[12] The addition of steroids to McCoy cell cultures increases their susceptibility to infection with laboratory strains of *C trachomatis,* resulting in an increase in the number of infective progeny produced by the inoculation of a given number of elementary bodies.[14] Thus, as suggested by the present findings, the hormonal effects of oral contraceptives would be seen most clearly when acting in conjunction with cervical ectopy, especially since the action of these steroids increases the bulk of the cervix and produces eversion.[12] However, in the present series of women there was no significant association between the height of the inclusion count and other hormonal influences, either in the normal menstrual cycle or in pregnancy, although a much larger series of women would need to be examined to confirm the latter.

A further feature needing more extensive investigation is the highly significant association between the age of the patient and the degree of her infection in the present series of women. This association might possibly reflect anatomical and physiological, including hormonal, differences between women under 20 years of age and older women, but the present findings do not suggest that the increased inclusion counts are the results of a greater use of oral contraceptives or of a greater frequency of cervical ectopy than in women between 21-45 years of age. However, the exact area of ectopy was not measured in these women. The size of cervical ectopy diminishes with age owing to metaplasia, whereby columnar epithelium is gradually replaced by squamous epithelium, and this process is often initiated at puberty or in the first pregnancy.[11] Thus, possibly the area of ectopic columnar cells, rather than the frequency of ectopy, may vary between young and older women to influence their differing degrees of chlamydial infection.

It is also possible that this age difference may have an immunological basis. In girls aged between 14-20 years a greater proportion of the infections are probably the first chlamydial infections to which they have been exposed than is the case in older women with a longer period of sexual activity. Thus, the replication of *C trachomatis* may be less likely to be brought quickly under control by humoral or cervical antibody or by cell-mediated immune responses in young women than in older women with a greater chance of previous immunological experience. Humoral antibody against *C trachomatis* is frequent and persistent in women attending STD clinics and its frequency increases with age.[15-17] Although it is not yet clear that antibody acquired by previous experience will prevent infection, the possibility that it might modify the degree of infection and hence the clinical signs associated with high-inclusion count infection merits further study.

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# Urethral Syndrome Associated with Chlamydial Infection of the Urethra and Cervix

## Case Report

A 21-year-old single girl gave a 3-year history of recurrent increasing frequency and dysuria. She took oral contraceptives and had been treated for gonorrhoea in 1972. There was no significant bacteriuria or pyuria. All other investigations were normal. Cystoscopy revealed only intense urethritis with some basal cystitis.

At the Department of Sexually Transmitted Diseases, oedema of the length of the urethra was palpated through the anterior vaginal wall and discharge was expressed on massage (Fig.). A large hypertrophic erosion and endo-cervical mucopus were found. *Chlamydia trachomatis* was isolated from the urethra and cervix and all other microbiological investigations were negative.

After 21 days oxytetracycline (250 mg 6-hourly) she became symptom-free. The urethral oedema and discharge were minimal, clear mucus was present in the endocervix and the oedema and congestion of the cervical erosion had disappeared, *i.e.*, the hypertrophic erosion had become simple. Follow-up cultures for chlamydia remained negative for a year. Urethral *Chlamydia trachomatis* culture from her consort was negative due, possibly, to recent antibiotic therapy.

Fig.

## Comment

*Chlamydia trachomatis* has been isolated from 50% of men with non-gonococcal urethritis and in one-third of their female partners (Hilton *et al.*, 1974). Its possible role in prostatitis, epididymitis and salpingitis has not yet been evaluated. Dunlop *et al.* (1972) reported the first isolation of chlamydia from the female urethra in a patient who was examined because her husband had a 6-month history of chlamydial kerato-conjunctivitis. She had recurrent dysuria and frequency. The association of chlamydial infection of the cervix with hypertrophic (oedematous and congested) erosion and endocervical mucopus has been reported (Rees *et al.*, 1977).

This case is of interest in that co-existing urethritis and cervicitis was observed by the urologist and that chlamydia was isolated from both sites. It is suggested that the possible association of chlamydial infection with the urethral syndrome requires further investigation.

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# Persistence of chlamydial infection after treatment for neonatal conjunctivitis

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SUMMARY A high incidence of pharyngeal infection was found in babies with isolation-positive chlamydial conjunctivitis. *Chlamydia trachomatis* was isolated from the pharynx of 12 (52%) of 23 babies before treatment, and was reisolated from the eyes of 4 (12%) of 34 and from the pharynx of 14 (41%) of 34 after treatment. *C trachomatis* was reisolated significantly more often from babies treated only with topical tetracycline for 4 weeks (75%) than from those treated with both topical tetracycline and oral erythromycin for 2 weeks (32%). Reisolation from the eyes was associated with only minor clinical signs. Radiological signs of an inflammatory lesion in the chest were found in 2 of 8 babies examined because of persistent cough. These signs were not associated with high or rising titres of serum chlamydial antibody.

Chlamydial infection in children was recently reviewed by Ridgway.[1] Infection in the newborn infant commonly presents as a severe purulent conjunctivitis,[2–3] although mild and subacute cases have been reported. The condition is self-limiting and may resolve spontaneously within a few months. The sight is rarely compromised although micropannus and palpebral scarring can occur particularly in the absence of treatment.[4–5]

An association of chlamydial conjunctivitis with lower respiratory tract infection was first suggested by Schachter *et al.*[6] and was later supported.[7–11] Harrison *et al.*[9] reported that in 9 of 30 babies admitted consecutively to the children's hospital in Seattle with pneumonitis, *Chlamydia trachomatis* was isolated from naso-pharyngeal swabs and aspirates and high levels of chlamydial antibody were associated.

The isolation of chlamydiae from the pharynx when chlamydial conjunctivitis is present could simply reflect their presence in secretions draining from the eye via the lacrimal duct rather than colonisation of the epithelium. However, *C. trachomatis* has a high specificity for columnar epithelium and active infection of the compound columnar epithelium of the pharynx is indicated by isolation from naso-pharyngeal aspirates of infants with pneumonia in the absence of clinical and microbiological evidence of conjunctivitis.[7 9–11] A residual pharyngeal focus may therefore be a source of lower respiratory tract infection and reinfection of the conjunctiva. Theoretically it is more likely to be found in babies in whom conjunctivitis has been treated only with topical tetracycline or with antibiotics only partially active against *C. trachomatis*—such as chloramphenicol.

The purpose of this investigation was to find out (1) the pretreatment isolation rate of *C. trachomatis* from the pharynx of babies with chlamydial conjunctivitis, and (2) the reisolation rate from the eyes and the pharynx of babies who had received topical tetracycline either alone or combined with oral erythromycin.

The symptoms and signs associated with reisolation and the possible role of *C. trachomatis* in the development of lower respiratory tract infections are discussed.

## Patients and methods

Pharyngeal and conjunctival swabs were taken at each follow-up examination from 34 babies treated consecutively for chlamydial conjunctivitis. The last 23 babies also had pharyngeal swabs taken at the time of the primary isolation of *C. trachomatis* from the eyes.

All babies had been referred by paediatricians: 24 had been examined in maternity units, 6 had been referred from outpatient baby clinics of the units, and 4 had been examined in children's hospitals to

Table 1 *Age at onset of conjunctivitis and at referral from paediatric units of 34 babies*

| *Referring unit* | *Age (days)* | | | |
|---|---|---|---|---|
| | *Onset of conjunctivitis* | | *Isolation of C. trachomatis* | |
| | *Range* | *Mean* | *Range* | *Mean* |
| Maternity (n=24) | 1–10 | 6·1 | 4–14 | 8·2 |
| Baby clinic (n=6) | 2–7 | 5·6 | 12–49 | 20·7 |
| Children's hospitals (n=4) | 5–7 | 6·8 | 15–42 | 23·8 |

which they had been admitted after failure of treatment of conjunctivitis prescribed by the family doctor before investigation for *C. trachomatis*. The age at onset of conjunctivitis and at referral from these units is given in Table 1.

For initial investigation of outpatients and for follow-up examinations, babies and their mothers attended the Nonspecific Clinic in the gynaecological outpatient department at the Liverpool Royal Infirmary and the Royal Liverpool Hospital, or the Women's Hospital, Liverpool.

Our routine investigation of babies with conjunctivitis has been described.[3] Pharyngeal specimens were obtained with the same type of cotton-wool tipped swab as that used for taking eye specimens. Care was taken to ensure that the specimen was obtained from the mucosal surface by gently stroking the posterior pharyngeal wall. Swabs were placed in transport medium[12] and immediately delivered to the laboratory; in a few cases swabs were taken in the evening and stored overnight at 4°C.

In the laboratory, specimens were inoculated into cycloheximide-treated coverslip monolayers of McCoy cells and incubated for 48 hours at 35°C, after which they were Giemsa-stained and examined by dark-ground microscopy for chlamydial inclusions. The total number of inclusions which developed in the whole coverslip was counted. Details of all these procedures have been described.[13–14] In most cases the specimens were inoculated into McCoy cells within 2 to 4 hours of being collected from the child, but occasionally they were stored overnight at 4°C.

Babies were examined during treatment and swabs were taken at each subsequent outpatient attendance. Each mother was asked to bring her baby for examination at ages 4, 8, 12, 18, and 24 weeks. This period of observation was longer if a reisolation was obtained. Examinations were carried out by two of us (E R and I A T).

Babies were treated with either 1% chlortetracycline eye ointment inserted into both eyes 5 or 6 times daily for 28 days, or for 14 days with concurrent erythromycin syrup 30 mg/kg daily in divided dosage. A few of the earlier babies received only 7 days' erythromycin with 14 days topical treatment. In most babies treatment was given initially by nursing staff in the maternity units, and was continued at home by the mothers on discharge from hospital 2 or 3 days later.

No baby was left untreated. If a reisolation was obtained the baby was recalled and given combined treatment. There was delay in retreatment if a mother failed to keep an appointment. Swabs were repeated on reattendance to confirm the persistence of infection.

Routine radiological examination of the chest of babies who developed cough in the follow-up period was instituted in the latter part of the study. Eight babies were examined.

Clinical and laboratory investigations were carried out routinely in all mothers and, wherever possible, the fathers were examined for evidence of urethritis. All infected parents were treated.

## Results

The sites of primary isolation and reisolation of *C. trachomatis* are shown in Table 2.

**Primary isolation.** Pharyngeal isolations were more common in older babies, being obtained from 3 of 12 babies aged <11 days, and from 9 of 11 babies aged >10 days. The inclusion count, which is a measure of the degree of infection,[13–14] was low in the pharynx (range 1–21, mean 7·6 inclusions) compared with the eye (range 5–3077, mean 1210 inclusions). There was no apparent correlation between the eye count and the presence or count of *C. trachomatis* in the pharynx.

**Follow-up.** The overall failure rate was 16 (47%) of 34 babies (Table 3). Of the 11 babies whose pharyngeal swabs were negative before treatment, 5 became positive during follow-up after treatment, 4 of whom had negative eye swabs. The reisolation pharyngeal swabs gave much higher inclusion

Table 2 *Sites of primary isolation and of reisolation of C. trachomatis*

| *Isolation* | *Site of isolation* | | |
|---|---|---|---|
| | *Eye* | *Pharynx* | *Eye and pharynx* |
| Primary (n=23) | 11 | 0 | 12 |
| Reisolation (n=34) | 2 | 12 | 2 |

Table 3 *Sites of first reisolation of C. trachomatis after treatment in 34 babies*

| *Treatment* | *Site of reisolation* | | |
|---|---|---|---|
| | *Eye* | *Pharynx* | *Eye and pharynx* |
| Topical (n=12) | 1 | 8 | 0 |
| Topical and systemic (n=22) | 1 | 4 | 2 |

counts than the primary pharyngeal swabs (range 1–1887, mean 166·1 inclusions) suggesting an established infection of the mucosa.

Reisolations from the eyes or pharynx, or both, were obtained from 7 (32%) of 22 babies treated with combined topical tetracycline and systemic erythromycin and from 9 (75%) of 12 treated only with topical tetracycline ($\chi^2 = 4\cdot21$, 1 df; $P = <0\cdot01$). Initially, for the purpose of this investigation, the two treatment schedules were given alternately but the high reisolation rate, particularly from the pharynx, of babies given only topical treatment led to our stopping this practice for ethical reasons.

There was persisting pharyngeal infection in two babies, in one for 6 weeks and in the other for 34 weeks, but there was no reisolation from the eyes of either. One, born by caesarean section to an unmarried mother, was diagnosed aged 18 days in a children's hospital and treated with topical tetracycline and oral erythromycin for 6 days in hospital and subsequently at home by the mother. *C. trachomatis* was reisolated from the pharynx at 1, 14, 18, and 35 weeks after treatment. There was persistent cough but clinical and radiological examinations of the chest were normal. The mother admitted she had had great difficulty in administering both oral and topical treatment.

The second baby received only topical treatment on diagnosis. *C. trachomatis* was isolated from the pharynx 6 and 12 weeks after treatment. No further isolations were obtained after combined treatment. A third baby, treated topically, had reisolations from the pharynx at 5 weeks and from the pharynx and eyes at 12 weeks. In this case there may have been reinfection of the eyes from the pharynx.

Five babies were preterm. Gestation ranged from 32 to 35 weeks and birthweights from 1·63 to 2·23 kg. Four of the 5 received combined treatment and no reisolations were obtained in follow-up periods of 8, 28, 34, and 34 weeks respectively. The fifth baby had topical treatment and *C. trachomatis* was reisolated only from the pharynx when aged 12 weeks.

The age at reisolation and period of follow-up after treatment are given in Tables 4 and 5.

Table 4 *Age at reisolation of C. trachomatis in 16 babies (25 reisolations)*

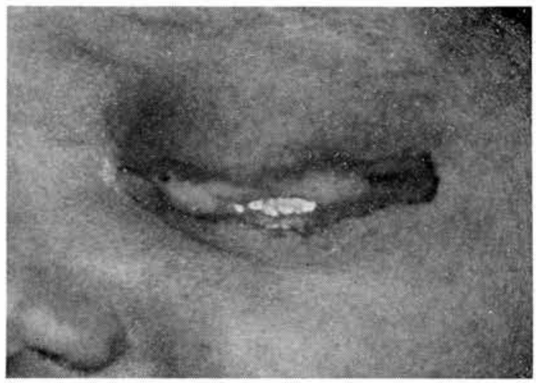
| | *Age (weeks)* | | | | | | |
|---|---|---|---|---|---|---|---|
| | *<4* | *4–7* | *8–11* | *12–15* | *16–19* | *20–23* | *32–35* |
| Number | 2 | 5 | 4 | 6 | 2 | 4 | 2 |

Table 5 *Period of follow-up after treatment*

| *Treatment* | *Time (weeks)* | | | | | |
|---|---|---|---|---|---|---|
| | *<4* | *4–7* | *8–11* | *12–23* | *24–35* | *32–52* |
| Topical | 1 | | | 4 | 6 | 1 |
| Topical and systemic | 3 | 1 | 1 | 6 | 8 | 3 |

## Clinical findings

The initial response to treatment was equally prompt with each treatment schedule. Although all babies had acute purulent conjunctivitis (Fig. 1 a and b),



(*a*)

(*b*)

Fig. 1 (*Case 1.*) (*a*) *Palpebral oedema and purulent discharge.* (*b*) *Severe conjunctivitis with submucosal oedema. C. trachomatis isolated.*

reisolation from the eye after treatment was associated with only minor signs which were generally intermittent. The mothers complained of seeing 'puffiness', slight inflammation, and slight discharge, in some cases only when the baby was waking or crying. Two mothers were very concerned because each believed that one eye of her baby was smaller than the other. This was due to slight unilateral palpebral oedema. The clinical signs noted on eye examination were slight palpebral oedema most easily identified when unilateral, a little mucopus usually at the inner canthi, and slight to moderate conjunctivitis with or without slight mucosal oedema (Fig. 2). One baby reattended 2 weeks after treatment with bleeding from one eye for 8 hours (Fig. 3). Examination of this baby showed only slight palpebral and mucosal oedema, and moderate conjunctivitis. The blood was derived from a single bleeding point on the mucosa of the lower lid. *C. trachomatis* was reisolated from the eye and the pharynx. In our experience this finding differs from the blood-stained discharge occasionally associated with the primary isolation from an acute eye in which blood 'weeps' from the acutely congested and grossly oedematous mucosa. Clinical signs may be absent as reported in an earlier series.[15] Clinical examination of the pharynx was limited to ensuring that satisfactory swabs were taken. Observations on the presence, or absence, of pharyngitis were not made.

Fig. 2 *Slight conjunctivitis with a little submucosal oedema. C. trachomatis reisolated.*

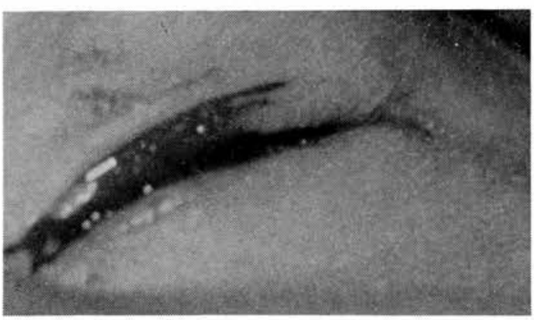


Fig. 3 *Blood presenting between the lids. C. trachomatis reisolated.*

Upper respiratory tract infections were common during the follow-up period. There was no significant difference between their occurrence in babies from whom reisolations were obtained and those from whom they were not. However, many of these babies were treated for cough with one or two courses of ampicillin by the family doctor between follow-up visits. This may have interfered with reisolation although positive swabs were obtained within a few days of the completion of such treatment in 2 cases and during the course of treatment in 1 case.

No baby was admitted to hospital with lower respiratory tract infection during the period of observation. One was admitted with gastroenteritis. None of the babies with cough failed to thrive or had symptoms or clinical signs of serious infection.

**Radiological investigations.** Findings were normal in 6 of the 8 babies examined. Inflammatory shadowing of the posterior segment of the right lower lobe was reported in one baby aged 16 weeks. There was a history of intermittent cough from age 6 weeks and three separate courses of ampicillin had been given by the family doctor. *C. trachomatis* was not reisolated but a course of erythromycin syrup for 2 weeks was prescribed. The chest was *x*-rayed 4 weeks later when it was reported that resolution was well under way but as yet incomplete, and again a further 4 weeks later by which time no active pulmonary disease was found. The second baby who had had a cough, particularly troublesome at night, for several weeks, was *x*-rayed at age 5 months when *C. trachomatis* was reisolated from the eyes and pharynx. A slight increase in the lung markings compatible with a resolving simple inflammatory lesion was reported. Chlamydial antibody levels were low and remained so during follow-up in both babies. IgM was not demonstrated.

## Discussion

The results of this investigation indicate a high incidence of pharyngeal infection in untreated babies with isolation-positive chlamydial conjunctivitis and in those babies treated only with topical tetracycline.

Before treatment pharyngeal isolations were obtained in 52% of babies. After treatment *C. trachomatis* was reisolated from the eyes of 12%, and from the pharynx of 41% of the 34 babies followed up. Failure to include routine pharyngeal swabs would have resulted in a completely erroneous evaluation of the response to treatment, since re-isolation is generally associated with absent or with only minor signs of conjunctivitis. Not surprisingly treatment with topical tetracycline and systemic erythromycin combined for 2 weeks was more effective than the 4-week course of topical tetracycline which has until recently been standard treatment and is still commonly used. *C. trachomatis* was reisolated from 7 (32%) of 22 babies receiving the former schedule and from 9 (75%) of 12 receiving the latter ($\chi^2 = 4 \cdot 21$, 1 df, $P = <0 \cdot 01$).

Reisolations from 32% of babies given combined treatment is not a good result. Possible reasons for this are inadequate treatment or failure in compliance. A lower reisolation rate might have been achieved had a 3-week course of treatment been given, although in adults with genital tract infection due to *C. trachomatis* there was no great difference in cure rate between 2 and 3 weeks of treatment. However, persistence in the pharynx rather than the eye is the problem, and failure to eradicate both aerobic and anaerobic bacteria from the throat after a standard course of the appropriate antibiotic is more common than at other sites. The choice of 2 weeks was based on the general principle that it is desirable to give babies the shortest course of antibiotics consistent with effective treatment. Compliance was certainly a problem with some mothers, even though they were carefully instructed and, in most cases, initially supervised in the maternity units. Such mothers were not irresponsible but many of them found it difficult to insert eye ointment, particularly the unsupported mothers with first babies. Some babies were said to spit out the erythromycin syrup, others to enjoy it. It is perhaps significant that no reisolations were obtained from the 4 preterm babies (birthweights 1·63–2·1 kg) who completed their combined treatment in special care baby units.

Since the radiological signs of lower respiratory tract infection in 2 babies were not associated with high or rising chlamydial antibody titres, we have no clear evidence of extension of infection from the pharynx to the chest. However, our babies received systemic antibiotics at an early age and this may have modified both the clinical and immunological response. In two recent prospective studies in the USA[10–11] maternal chlamydial infection diagnosed during pregnancy was left untreated. Evidence of chlamydial infection was reported in 70 and 61% of the babies. Only babies with conjunctivitis were treated and topical tetracycline was used. Pneumonia developed in 4 of 20 babies in one series and in 2 of 18 in the other.

The apparent rarity of chlamydial pneumonia in babies admitted to hospital in this country compared with the USA may be due to failure to consider *C. trachomatis* as an aetiological agent in Britain. However, this is unlikely in Liverpool, where the referral by paediatricians of babies for investigation of neonatal conjunctivitis has resulted in the isolation of *C. trachomatis* in 90 cases. More probably it results from a different pattern of primary medical care in the UK. Only babies whose condition causes anxiety are likely to be referred to hospital by the general practitioner and chlamydial pneumonia has been characterised as being nontoxic. Nevertheless, persistent cough creates anxiety in the mother and chlamydial respiratory tract infection may be a serious additional factor in babies whose health is compromised by other pathogens or by poor nutrition. The importance of identifying the chlamydial infection lies in the need for treatment of the respiratory infection with erythromycin rather than other more commonly used antibiotics and the need for systemic treatment of conjunctivitis.

Prevention is best achieved by treating infected mothers with erythromycin before parturition. Such treatment is necessary not only to prevent infection of the baby but to reduce the risk of postpartum pelvic infection which is a common complication in untreated mothers.[5 16–17] In addition examination and treatment of the mother's sexual partner is essential if she is not to be reinfected.

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# SIMPLIFIED METHOD FOR DIAGNOSIS OF GENITAL AND OCULAR INFECTIONS WITH CHLAMYDIA

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***Summary*** The diagnosis of infections with chlamydia related to the trachoma agent is best confirmed by isolation of the organism in tissue-culture. A simplified technique using conventional monolayers of McCoy cells can be used as an alternative to current methods involving heavily irradiated cells and appears to be of reasonable sensitivity. Chlamydial infection was confirmed in 38/190 females attending venereal clinics, in 22/35 patients with hypertrophic cervical erosions, and in 7/18 infants with ophthalmia neonatorum.

## INTRODUCTION

NON-GONOCOCCAL genital infection is one of the commonest forms of sexually transmitted disease in the United Kingdom. Evidence that more than a third of these infections may be associated with group-A chlamydia is now being provided by laboratories able to use a somewhat complex tissue-culture procedure. The clinical value of this approach has recently been reviewed.[1] However, these novel techniques[2] involve the pre-inoculation irradiation of McCoy-cell cultures with 4000–6000 rads, a high dosage only likely to be achieved by repeated access to a cobalt-60 source and with the participation of radiophysicists. Smaller general laboratories, distant from specialist radiotherapy centres, have thus not considered it feasible to engage in diagnostic and epidemiological studies on chlamydial infections in the general population.

We suggest here that useful data in this field could be provided by any laboratory with routine tissue-culture capability. There is no specific restriction of chlamydia to growth in irradiated cells or even in McCoy cells. Standard laboratory strains of chlamydia grow well in cell lines such as HeLa or BHK.[3,4] Indeed, the McCoy cell line, once thought to be derived from human synovial tissue, is now recognised to be antigenically similar to the mouse L-cell tumour line, which has long been used for the cultivation of chlamydia.

Our preliminary results with conventional unirradiated cultures of McCoy cells in the clinical investigation of non-gonococcal cervicitis are presented below.

## MATERIALS AND METHODS

Many steps in the general diagnostic procedure are similar to those described by Richmond[5] with irradiated tissue-cultures, but with the following points of difference. McCoy cells (initially obtained from Flow Laboratories, Irvine, Scotland) have since been serially propagated in 'Medium 199' (Wellcome) plus 10% fetal calf serum (Flow) and 0·02*M* sodium bicarbonate, with vancomycin and streptomycin each at 100 $\mu$g. per ml. Cell suspensions of $1{\cdot}5 \times 10^5$ cells per ml., obtained from confluent monolayer cultures by conventional trypsinisation techniques, were dispensed in 2 ml. amounts in 1 oz. (universal) screw-capped glass bottles containing a 16 mm. circular glass coverslip covering the bottom of the bottle. After incubation at 37°C for 24 hours, the medium was replaced by 2 ml. of the same medium plus 0·5% final concentration of glucose and 50 $\mu$ per ml. nystatin just before inoculation of the clinical specimen. Cervical swabs were taken into 2 ml. of transport medium[5]; 0·4 ml. was inoculated into each of two coverslip cultures, which were centrifuged at 2500 *g* for 1 hour on the swing-out head of an M.S.E. Major centrifuge, at room temperature. Inoculated bottles were then incubated at 37°C in a constant atmosphere of 5% carbon dioxide in air, with the bottle tops loosened, to maintain the medium at a constant pH of 7·0–7·4. After 48 hours the tissue-culture fluid was removed and the coverslip stained by the Giemsa method. Large intracytoplasmic inclusions packed with basophilic particles can easily be seen by light microscopy with the 40× objective, and under dark-ground microscopy, where chlamydia particles give an intense lemon-yellow fluorescence sharply distinct from the dull green-brown appearance of McCoy cells.

Strict precautions were taken to minimise the risk of cross-infection. No standard reference strains of chlamydia have been used here since this work began. Fresh clinical specimens were inoculated at a different time and in a different laboratory from that in which incubated specimens were harvested. During centrifugation, bottle-tops were tightly sealed. Control McCoy bottles were inoculated with transport medium, centrifuged, and incubated with each batch of specimens. All remained negative.

## RESULTS AND CONCLUSIONS

Cervical swabs were examined from 190 women with various gynæcological complaints or a history of contact with genital infection. Chlamydia were isolated from 38 (20%), and the number of inclusions ranged from 2 to 18,000 per coverslip (median count= 900). 64 of these women were unselected contacts of males with non-gonococcal urethritis; 15 (23·5%) of them yielded positive cultures. In 35 women with hypertrophic cervical erosions chlamydia were isolated from 22 (63%). Further pre-treatment swabs were obtained within 5–15 days from 21 positive cases, and 20 were again positive, with similar inclusion-counts to those in the first examination. After treatment for 3 weeks with oxytetracycline, repeated swabs from all the previously positive patients gave negative results over the following 2 months.

Chlamydia have also been isolated (60–9000 inclusions per coverslip; median count=1350) from conjunctival swabs from 7/18 (39%) infants found to have a mucopurulent conjunctival discharge within a few days of their birth in hospital. Subsequently, chlamydia were isolated from the cervix of 3 mothers of infected infants, and from the urethra of the husband of one of these women. No other fathers were available; 3 further mothers tested were negative, but 2 had received systemic antibiotics before the swab could be taken. Eye swabs became negative shortly after local chlortetracycline therapy in 5 of the infected babies, but, in the other 2, chlamydia were re-isolated 2 weeks after treatment.

Much more work is needed to determine the frequency of chlamydial infections in ocular and genital disorders in the unselected general population. These preliminary results suggest that such studies are well within the scope of the average laboratory without special facilities for radiobiology, especially overseas, where classical trachoma may also require investigation.

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# Chlamydia in Relation to Cervical Infection and Pelvic Inflammatory Disease

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Assessment of the pathogenic role of *Chlamydia trachomatis* in the cervix is important for three reasons: (i) because of the possible carcinogenic effect of a deoxyribonucleic acid vector which has an intracellular developmental sequence and is associated with coitus; (ii) because of the effect on the fetus—prenatal or intrapartum; and (iii) because of the effect on fertility if ascending infection to the fallopian tubes occurs.

## CERVICAL INFECTION

Novak and Woodruff (1), reviewing the pathogenesis of carcinoma of the cervix, stated that at present the evidence is very strong in favor of the theory that infection, possibly viral, plays a major role in the production of this disease, which accounts for 50% of all primary malignancy in the pelvis. Evidence that an inflammatory process can occur in the cervix in association with the presence of *C. trachomatis* was provided by Dunlop et al. in 1966 (2). They described follicles at the margins of the cervix seen with a colposcope, and later Ashton et al. (3) and Swanson and his colleagues (4) reported inflammatory changes in biopsy specimens from these areas and the identification of chlamydiae on electron microscopic examination.

The association of "cervicitis," variously defined, but including simple erosion, has been reported in several studies. Hilton et al. (5) found the cervix eroded and/or appearing "infected" to the naked eye in 82% of 34 *Chlamydia*-positive women, but 56% of their control group of 54 women attending a family planning clinic showed similar changes in the absence of *Chlamydia* or other organisms, as did 56% of 115 women at a clinic for sexually transmitted diseases. Similar findings were reported by Oriel and his colleagues (6). The high incidence in the control group is probably due to the inclusion of simple erosion in the category "abnormal," although this is not considered to be a pathological condition in its common nonulcerative form, but simply ectopic columnar epithelium (7, 8). The present study was undertaken to determine the possible association of clinical signs in the cervix with the presence of *C. trachomatis*, to determine the effect of treatment on the signs when present and on the subsequent isolation of the organism, and to observe whether or not *C. trachomatis* persists in the cervix in the absence of treatment and of other infection.

### Materials and Methods

**Patients.** We have isolated *C. trachomatis* from 228 of 729 (31.2%) selected patients examined in a clinic for sexually transmitted diseases and from 52 of 145 (35.9%) consecutive contacts of patients with nongonococcal urethritis (NGU) (primary and secondary contacts of first and recurrent attacks). For the purpose of observing clinical signs, we selected the whole group of 127 *Chlamydia*-positive NGU contacts because we wished to exclude known cases or contacts of gonococcal infection, which also causes endocervicitis. They have been paired with 127 consecutive *Chlamydia*-negative NGU contacts, none of whom had received antibiotics for 4 weeks prior to their attendance. All patients were investigated for gonorrhea, trichomoniasis, and candidiasis by conventional methods.

Specimens for culture of *Neisseria gonorrhoeae* were taken on at least two occasions and were inoculated directly onto modified Thayer-Martin medium. Specimens for *C. trachomatis* culture were placed in transport medium and inoculated onto cover-slip cultures of replicating McCoy cells, i.e., not pretreated by irradiation or other antimitotic agents (9). The total number of chlamydial inclusions per cover slip (IFU/WC) was counted on every specimen after 48 h of incubation.

Endocervical smears were not examined for a pus cell count, as pus cells are a normal constitutent of cervical secretion and their presence is not a reliable guide to an inflammatory process. Their numbers exhibit physiological variation with the stage of the menstrual cycle and the presence of degenerating spermatazoa.

The clinical examination of the vaginal cervix (ectocervix) was recorded as no erosion, simple erosion (ectopic columnar epithelium), hypertrophic erosion (edematous and congested erosion), and chronic cervicitis (lacerated with ectropion or nabothian follicles with metaplasia). The endocervical contents were recorded as clear mucus, cloudy mucus, mucoid discharge (white), or mucopus (yellow). The findings were recorded at the time of taking the specimen for *Chlamydia* on both the laboratory request form and the case sheet. Colposcopic examination was not carried out.

As a basis of comparison with a known pathogen of the cervix, the cervical findings in 100 consecutive cases of gonorrhea diagnosed during the period of this study were analyzed. Routine tests for *C. trachomatis* were not carried out in this group, and it will, therefore, contain some cases of concurrent infection.

The presenting symptoms in the 127 *Chlamydia*-positive NGU contacts are compared with those of 326 consecutive gonorrhea patients attending the same clinic. In some cases symptoms may be due to concurrent trichomoniasis or candidiasis rather than gonococcal or chlamydial infection.

**Treatment.** Routine treatment consisted of the oral administration of oxytetracycline hydrochloride, 250 mg every 6 h for 21 days. Pregnant women were given a similar course of erythromycin.

**Follow-up.** All *Chlamydia*-positive patients were requested to attend for observation for 12 months unless it was judged that such a prolonged period of attendance would be bad for psychological reasons. Finnerty et al. (10) have shown the importance of clear communication with the patient in reducing the drop-out rate during observation, and care was taken to explain to patients that we were trying to assess the effect of treatment in a new condition which we were now able to recognize and that their cooperation would be of great assistance. They were asked to refrain from sexual intercourse for the 21 days of treatment, if possible, and then to resume when their husband or consort had permission. Alcohol was not forbidden. Patients were asked to attend for post-treatment tests at 2, 5, 8, 14, 26, 38, and 52 weeks during the latter part of the study, but were seen more frequently during the early part. Some patients volunteered to continue for longer periods and others re-attended with fresh symptoms or following new contact. Swabs for

TABLE 1. *Clinical signs in the cervix in 254 NGU contacts (127 C. trachomatis positive and 127 C. trachomatis negative)*

| Clinical sign | No. of contacts | | Percent positive |
|---|---|---|---|
| | *Chlamydia* positive | *Chlamydia* negative | |
| *Ectocervix* | | | |
| No erosion | 28 | 61 | 31.5 |
| Erosion | | | |
| Simple | 53 | 51 | 50.9 |
| Hypertrophic | 40 | 6 | 86.9 |
| "Chronic cervicitis" | 5 | 9 | |
| Not recorded | 1 | 0 | |
| *Endocervical contents* | | | |
| Clear mucus | 31 | 76 | 28.9 |
| Cloudy mucus | 28 | 36 | 43.7 |
| Mucoid | 5 | 0 | |
| Mucopus | 59 | 11 | 84.0 |
| Blood | 4 | 3 | |
| Not recorded | 0 | 1 | |

culture of *C. trachomatis*, *N. gonorrhoeae*, *Trichomonas vaginalis*, and *Candida* species were taken on each occasion.

## Results

**Clinical findings.** *C. trachomatis* was isolated from cervices exhibiting no clinical signs of infection (Table 1) and in this is closely paralleled by gonococcal infection (Table 2). The high isolation rate associated with hypertrophic erosion (86.9%) and endocervical mucopus (84%) suggested that these signs might be attributable to the presence of the organism and could be observed for a response to treatment.

The number of IFU/WC varied between 1 and 112,000. The number was not directly related to the clinical findings. The highest count was recorded in an uneroded cervix containing sticky mucopus.

**Isolation of other organisms.** In the group of 127 *Chlamydia*-positive NGU contacts, *N. gonorrhoeae* was isolated from 4 (3 on the second time of testing and

TABLE 2. *Clinical signs in the cervix in 100 consecutive patients with gonorrhea*

| Clinical sign | No. of patients |
|---|---|
| *Ectocervix* | |
| No erosion | 57 |
| Erosion | |
| Simple | 34 |
| Hypertrophic | 7 |
| "Chronic cervicitis" | 2 |
| *Endocervical contents* | |
| Clear mucus | 42 |
| Cloudy mucus | 19 |
| Mucoid | 5 |
| Mucopus | 34 |

from the urethra as the only positive site in 2). *T. vaginalis* was present in 17 patients and *Candida* species, in 45.

**Presenting symptoms.** As shown in Table 3, 49% of *Chlamydia*-positive patients and 53% of patients with gonorrhea denied any symptoms (Table 3). When there was more than one symptom, the first or more severe was recorded as the presenting symptom. A lower incidence of lower abdominal pain, which indicates possible pelvic infection, was found in the *Chlamydia*-positive group. Postcoital bleeding, found in 3.2% of the latter group, was associated with hypertrophic erosion or ectopy of endocervical mucosa.

The gonorrhea group was not screened for *C. trachomatis*.

**Effect of treatment on clinical signs.** Thirty-one cases exhibiting hypertrophic erosion have so far been followed for longer than 4 weeks. In 27 cases the hypertrophic erosion became and remained simple (Fig. 1 and 2). In four cases (one of whom was initially pregnant), no erosion was present at the end of the period of follow-up, metaplasia having taken place. Forty-five cases in which mucoid or mucopurulent discharge was found in the endocervix have completed at least 4 weeks of follow-up. The cervical secretion became clear in 25 cases (Fig. 3), became cloudy in 19, and remained mucoid in one patient from whom *Chlamydia* was reisolated. The change in the type of cervical secretion took place during the course of treatment.

**Effect of treatment on the isolation of *C. trachomatis*.** The method of culture for *C. trachomatis* used in this study has been shown to be highly reproducible and sensitive (Johnson et al., p. 309, this volume). A total of 161 *Chlamydia*-positive sexually transmitted disease patients have been followed for periods up to 20 months. Patients who attended for fewer than two post-treatment tests have been excluded. Two to four tests were carried out on those attending for less than 3 months, and 3 to 12 tests were made on those attending for a longer period. *C. trachomatis* was reisolated in only 16 (Table 4). In this group of 16 patients, three re-attended as contacts of men treated for gonorrhea and four as contacts of men with NGU (one *Chlamydia* negative, three not tested). When the asymptomatic consorts of three were examined, pus cells were present in shreds in the urine and *C. trachomatis* was isolated. One consort was said to have attended a clinic with symptoms but was not traced. Three patients had a new consort who did not attend for examination. Both of the remaining two patients were having intercourse with their original consort, neither of whom attended for examination.

TABLE 3. *Presenting symptoms in 127 Chlamydia-positive NGU contacts and 326 consecutive cases of gonorrhea*

| Symptom | NGU contacts (%) | Gonorrhea patients (%) |
|---|---|---|
| None | 48.9 | 54 |
| Discharge | 32.2 | 31 |
| Abdominal pain | 3.9 | 9 |
| Urinary | 3.9 | 5 |
| Irritation/soreness | 6.4 } | |
| Postcoital bleeding | 3.2 } | 2 |
| Warts | 1.5 } | |
| Abscess | 0 } | |

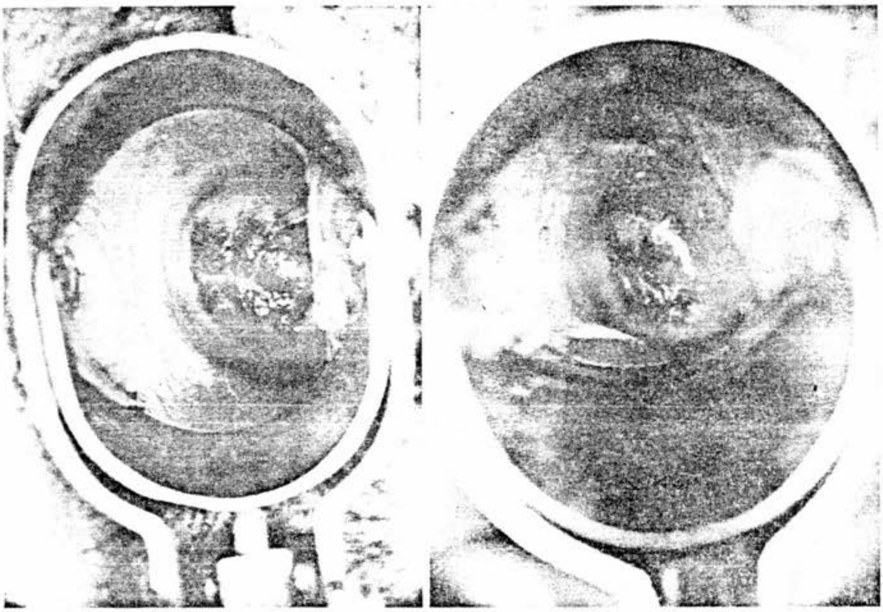

FIG. 1. *(A) Hypertrophic erosion with endocervical mucopus before treatment. C. trachomatis isolated. (B) After treatment. Simple erosion with clear mucus in the endocervix. C. trachomatis not isolated.*

**Effect of physiological stress.** *C. trachomatis* was not reisolated from one patient who became pregnant and three who were pregnant at the time of treatment. All were observed until after parturition, and no isolate was obtained from the mothers or from the babies on conjunctival swabbing. No isolate was obtained from one patient who acquired gonorrhea.

**Persistence of *C. trachomatis* in the absence of treatment.** Having early in the study observed the resolution of clinical signs after treatment, on ethical grounds we did not feel that treatment could be withheld once the diagnosis was made. However, in 19 cases either the patient failed to re-attend in 1 week for the results of her investigation, or treatment was delayed because she was under investigation for another condition, and *Chlamydia* was reisolated on one or two subsequent occasions (Table 5).

## Discussion

The clinical findings suggest that edema, congestion, and mucopurulent discharge are associated with chlamydial infection of the cervix in a proportion of cases and that these signs regress after treatment. In the majority of cases, hypertrophic erosion became and remained simple during the period of observation. In 4 of the 31 cases metaplasia took place. If there should be an association between carcinoma of the cervix and *C. trachomatis* infection, it is possible that these four cases would carry the greatest risk if the hypothesis of Coppleson and Reid (11) is accepted.

The lack of correlation between the number of IFU/WC and the tissue response

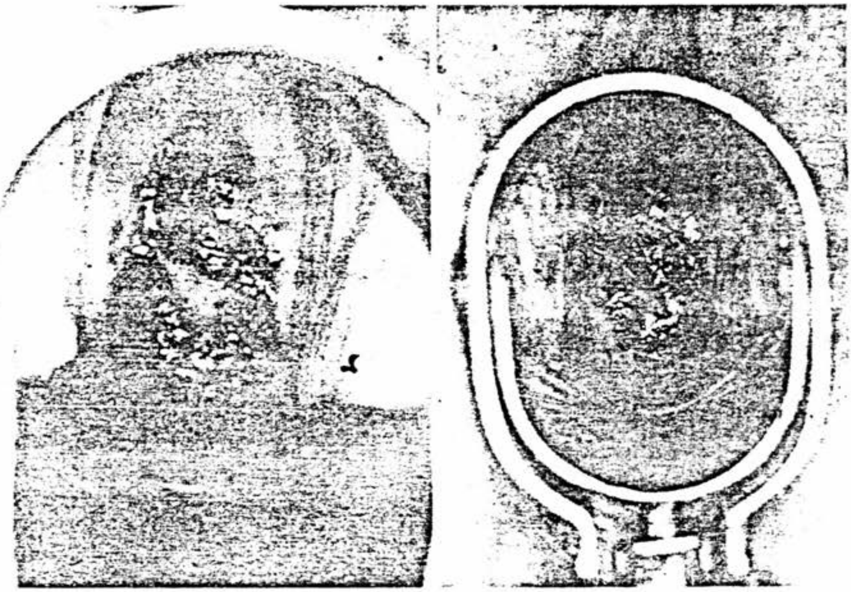

FIG. 2. *(A) Hypertrophic erosion with marginal follicles and endocervical mucopus before treatment. C. trachomatis isolated. (B) After treatment. Simple erosion with areas of metaplasia. Cloudy mucus in the endocervix. C. trachomatis not isolated.*

suggests that the latter may be due to a host factor or to variations in response to different immunotypes of *C. trachomatis*. It is unlikely to be due to sampling, as repeat specimens, usually taken 1 week later, showed little variation in count. The higher isolation rate from NGU contacts with simple erosion compared with no erosion may be due to the fact that columnar epithelium is more receptive to *C. trachomatis* and infection is more easily achieved. This would explain the higher isolation in women on oral contraceptives reported by Hilton et al. (5), in that the development of ectopic columnar epithelium is influenced by hormonal factors (12).

Our findings do not support the view of Hilton et al. (5) that *C. trachomatis* infection is latent but reactivated by other infection. We found signs of severe inflammation in the cervices of a group of women free from other known infection, and these signs responded to therapy. Reisolation of *C. trachomatis* occurred in only 16 of 161 women followed for periods up to 20 months and was associated with a high probability of reinfection rather than relapse. The physiological stress of pregnancy and parturition in four cases and gonococcal infection in one did not result in reisolation. Conversely, the persistence of *C. trachomatis* with isolation on two or three occasions over periods up to 19 months in 18 patients and 12 months in one suggests its persistence in an infective form in this small group of patients available for observation. The isolation of *C. trachomatis* from three asymptomatic male consorts examined because of reisolation of the organism in women on long follow-up suggests that the asymptomatic male may be a source of infection to the female.

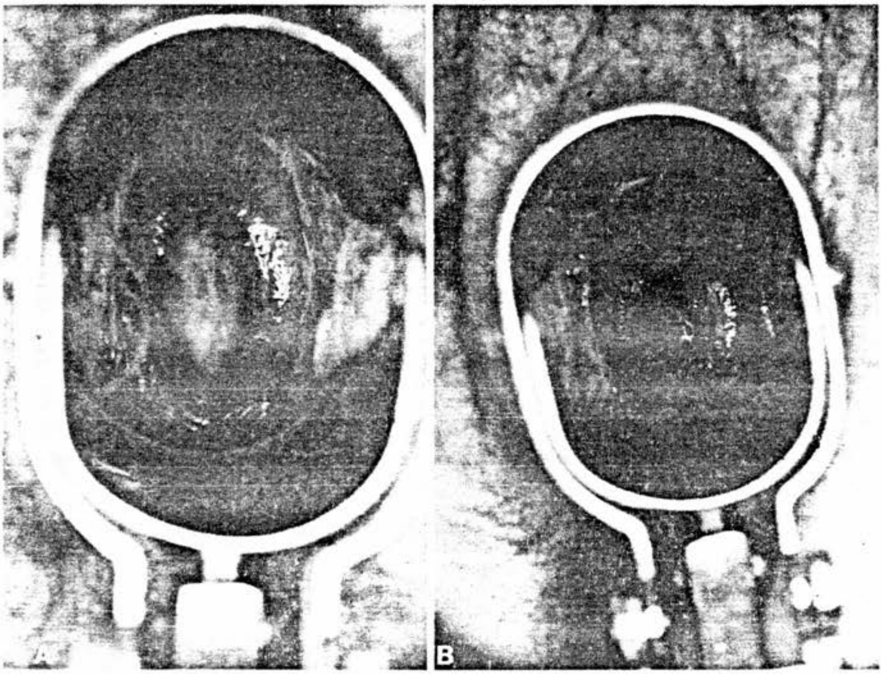


FIG. 3. *(A) Simple erosion with endocervical mucopus before treatment. C. trachomatis isolated. (B) After treatment. Simple erosion, clear mucus in the endocervix. C. trachomatis not isolated.*

## PELVIC INFLAMMATORY DISEASE

There are two major difficulties in considering a possible relationship between the presence of *C. trachomatis* in the cervix and pelvic inflammatory disease: (i) the difficulties associated with the differential diagnosis of pelvic pain of a subacute character, which are appreciated by physicians in all the disciplines involved—gynecologists, urologists, venereologists, surgeons, and general physicians; and (ii) the low isolation rate of organisms from fallopian tubes which show objective signs of infection at laparoscopy or laparotomy, combined with the fact that these procedures are not usually carried out prior to antibiotic therapy when the diagnosis is clear. Curtis (13), in the pre-chemotherapeutic era, investigated the bacteriology and pathology of fallopian tubes removed at operation and concluded that gonococci live only a short time in the tube. Mårdh and Weström (14) confirmed a clinical diagnosis of salpingitis on laparoscopic examination and took swabs for bacteriological investigation from 50 patients in whom there was marked reddening and swelling of the tubes. No growth was obtained in 39 (78%). These findings suggest the presence of potent antibacterial factors in luminal fluid and the difficulties likely to be encountered in isolating *C. trachomatis* from the fallopian tube of a women with salpingitis. There is historical evidence of an association between chlamydial cervicitis and pelvic inflammatory disease, particularly in relation to maternal infection and neonatal chlamydial conjunctivitis (2, 15). We

TABLE 4. *Follow-up of 161 sexually transmitted disease clinic female patients after treatment for C. trachomatis infection of the cervix*

| Time after treatment (months) | No. of cases | No. with *C. trachomatis* reisolated |
|---|---|---|
| <3 | 68 | 9 |
| 3–6 | 37 | 3 |
| 6–9 | 13 | 2 |
| 9–12 | 14 | 2 |
| 12–15 | 17 | 0 |
| 15–18 | 8 | 0 |
| 18–20 | 4 | 0 |
| Total | 161 | 16 |

have investigated the prevalence of pelvic infection in 127 *Chlamydia*-positive NGU contacts and 24 mothers of babies with neonatal conjunctivitis from whom *C. trachomatis* was isolated.

## Results

**NGU contacts.** Pelvic infection was diagnosed in 5 of 127 women with chlamydial cervicitis. Two were wives of men who admitted extramarital contact. Both were found to have a hypertrophic erosion of the cervix and endocervical mucopus. The erythrocyte sedimentation rate (Westergren) (ESR) was 13 and 57, respectively. Tests for *N. gonorrhoeae* were negative. There was no previous history of pelvic infection. The symptoms settled rapidly with tetracycline therapy. Two women were the regular consorts of men with NGU. They admitted no other recent sexual contact. On examination neither cervix was eroded; one contained blood-stained mucus and the other, cloudy mucus. The ESR was 13 and 25, respectively. Tests for *N. gonorrhoeae* were negative. There was no previous history of pelvic infection, but one had a history of gonorrhea. The fifth case gave a history of discharge and of cramplike pain in the right and left iliac fossae for 3 weeks, severe at onset but now low grade. On examination of the lower genital tract, there was a simple erosion of the cervix. The endocervix contained clear mucus but bled easily. Mucopus was present on the rectal mucosa. The ESR was 19. Tests for *N. gonorrhoeae* were negative. *C. trachomatis* was isolated from the cervical and rectal swabs. The

TABLE 5. *Persistence of C. trachomatis in the absence of treatment*

| Time after initial isolation (weeks) | No. of cases | Isolations | |
|---|---|---|---|
| | | ×2 | ×3 |
| 52 | 1 | 1 | |
| 19 | 2 | 1 | 1 |
| 14 | 1 | | 1 |
| 12 | 3 | 2 | 1 |
| 7–8 | 4 | 4 | |
| 5–6 | 3 | 2 | 1 |
| 3–4 | 5 | 2 | 3 |

consort had NGU, and *C. trachomatis* was isolated. The psittacosis/lymphogranuloma venereum complement fixation test was carried out at the start of treatment, and 1 and 4 months later. The titer was 320, 320, and 80, respectively.

**Mothers of babies with neonatal chlamydial conjunctivitis.** Among 24 mothers of babies with neonatal chlamydial conjunctivitis examined, 16 developed postpartum infection. Seven were already receiving antibiotics for infected lochia or pyrexia. Nine of the remaining 17 mothers developed pelvic infection. They had already been discharged from hospital when the diagnosis of chlamydial conjunctivitis was made in the babies who had remained in hospital because of prematurity. The onset of pain varied between 13 and 38 days postpartum and was of such severity in two cases that they were admitted to hospital for curettage to exclude retained products of conception. None was found on histological examination of the curettings. *N. gonorrhoeae* was isolated from two mothers whose babies had concurrent gonococcal and chlamydial conjunctivitis. *C. trachomatis* was isolated from the cervix of seven of the nine mothers. The two negative mothers were already taking antibiotics prescribed by the family doctor for pelvic infection. In four cases blood was sent for psittacosis/lymphogranuloma venereum group antibody estimation before treatment and again after treatment in two cases (Table 6).

## Discussion

The prevalence of pelvic infection in the 127 NGU contacts with chlamydial cervicitis was 3.9%. This is lower than is found with gonococcal cervicitis (16, 17). The frequency of pelvic infection in mothers of infected infants causes concern. The group antibody titers, high in two cases and showing conversion in one, suggest the association of the chlamydial infection with the pelvic inflammatory disease.

These findings lend support to the theory that chlamydiae may ascend to the upper genital tract. Further support is given by the development in a special-care unit of chlamydial conjunctivitis in a baby delivered by caesarean section 11 days previously (Rees et al., p. 140, this volume). If the organism can reach the fallopian tubes and if a similar pathological process can occur there as has been found in the cervix, then its etiological role in nongonococcal salpingitis would not be surprising.

TABLE 6. *Psittacosis/lymphogranuloma venereum group antibody titers in four women with postpartum pelvic infection*[a]

| Patient | Day postpartum | Antibody titer | Contact |
|---|---|---|---|
| M.C. (−, AB) | 39 | 640 | Husband (+) |
| | 90 | 320 | |
| | 135 | 80 | |
| M.W. (+) | 44 | 320 | Husband (+) |
| M.L. (+) | 9 | <10 | Consort (NE) |
| | 26 | 20 | |
| | 46 | 10 | |
| M.F. (−, AB) | 28 | 40 | Consort (+) |

[a] For the patients and contacts: + = *C. trachomatis* isolated; − = *C. trachomatis* not isolated; AB = on antibiotics; NE = not examined.

## CONCLUSION

The findings in this study suggest that *C. trachomatis* is a pathogen in the female genital tract and that it provokes, in some cases, a well-marked tissue response to infection in the cervix.

The treatment of all women from whom *C. trachomatis* is isolated is strongly indicated, and facilities for diagnosis should be generally available.

## ACKNOWLEDGMENTS

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# Perinatal Chlamydial Infection

ELISABETH REES, I. ANNE TAIT, DEREK HOBSON
AND F. WILLIAM A. JOHNSON

The presence of an inflammatory process in the cervix may affect the fetus in the prenatal, intrapartum, or neonatal period. In the prenatal period, the fetal environment may be compromised through bacteremic spread in the mother or ascending infection from the cervix, resulting in chorioamnionitis. The latter commonly develops after prolonged rupture of the membranes, but infection across intact membranes also occurs, and, on examination of placentas, limitation of the chorioamnionitis to the portion of the membranes overlying the os is often found (1). Premature birth is likely, possibly due to rupture of the weakened and inflamed membranes at this site (2).

The most important factor influencing the health of the infant is premature birth. In the neonatal period, 60% of deaths occur in the 10% of infants born prematurely. In addition to increased mortality, neurological sequelae are more frequent (3).

In considering chlamydial cervicitis in pregnancy, therefore, the relative incidence of prematurity not associated with general factors affecting maternal health, such as toxemia, may be of importance. Ascending infection due to *Chlamydia trachomatis*, in either the pregnant or nonpregnant woman, has not yet been established. Chlamydial conjunctivitis in an infant delivered by caesarean section is suggestive, but postnatal transmission by fingers of the attendants cannot with certainty be excluded, although the chances of this are more remote if the infant is transferred to a special-care unit immediately after birth.

Neonatal conjunctivitis and associated infection in the parents has been recognized since 1907, when Halberstaedter and von Prowazek (4) described inclusions in eye and genital exudate. Since the development of sensitive tissue culture techniques, the epidemiological pattern of the disease and the clinical manifestations and sequelae of infection in infants referred to departments of ophthalmology have been defined (5, 6).

An investigation into the presentation of chlamydial conjunctivitis in maternity units was undertaken to examine the perinatal factors influencing the clinical picture, the response to treatment, and the pattern of infection in the parents.

## MATERIALS AND METHODS

Of the 81 babies examined between October 1973 and March 1976, 74 were referred by pediatricians, 3 were referred by ophthalmologists, and 4 were examined because their mothers had been treated for chlamydial cervicitis (3 during the pregnancy and 1 before pregnancy). Four attended as outpatients. The group forms a selected series of cases of conjunctivitis referred mainly because of failure to respond to routine treatment or because no bacterial growth was reported on bacteriological investigation. A few cases were seen because of very early development of signs, the youngest being 6 h. In the United Kingdom, cases of gonococcal conjunctivitis are usually referred to the venereologist.

Specimens were obtained with cotton wool-tipped swabs from the mucosal surface of the lower lids in all cases, and from the upper lids when possible, and were examined microscopically and by culture for *Neisseria gonorrhoeae* and other bacteria. Stuart's medium was used for transport, but most gonococcus swabs were inoculated directly onto modified Thayer-Martin medium. Swabs for *C. trachomatis* were put into transport medium for culture in untreated replicating McCoy cells (7). Specimens for herpes simplex virus identification by electron microscopy and culture were taken in some cases.

Mothers were investigated in the maternity unit when possible, but mothers of premature babies had usually been discharged and were requested to attend as outpatients. All were investigated for syphilis, gonorrhea, chlamydial cervicitis, trichomoniasis, candidiasis, and, in a few cases, genital herpes. When possible, fathers were similarly investigated if the infant and mother had gonococcal, chlamydial, or syphilitic infection. *Chlamydia*-positive mothers and infants were requested to attend once during treatment and then 2, 5, 8, 16, 28, 40, and 52 weeks post-treatment. Swabs for *C. trachomatis* were taken from the infant on each occasion. Swabs from the mother were taken for *C. trachomatis*, gonococcus, *Trichomonas vaginalis*, and *Candida* species at each visit after treatment.

## RESULTS

**Babies.** *C. trachomatis* was isolated from 29 babies, and *N. gonorrhoeae* was isolated from 9. Delivery was by caesarean section in four cases; *N. gonorrhoeae* was isolated from one of these babies, and *Chlamydia* was isolated from one.

In the 29 *Chlamydia*-positive babies, the onset of discharge varied between 3 and 13 days (mean, 6.9 days) (Table 1). Early onset was associated with concurrent bacterial infection, which was not common. *N. gonorrhoeae* was isolated in three cases, *Staphylococcus aureus* in two, *S. albus* in two, *N. pharyngis* in one, diphtheroids in one, and *Streptococcus faecalis* in one. No swab was taken in one case treated by the general practitioner after discharge from hospital. In the remainder, the laboratory reported no bacterial growth or no pathogens grown. Late onset at 9, 11, and 13 days occurred in three babies treated at birth with intramuscular cloxacillin and ampicillin for prematurity associated with prolonged rupture of the membranes.

*C. trachomatis* was isolated from both eyes in 18 cases, the right eye in 7, and the left eye in 4. The inclusion count per whole cover slip (IFU/WC) varied between 1

TABLE 1. *Day of onset of discharge in 29 cases of neonatal chlamydial conjunctivitis*

| Day of onset | No. of babies |
|---|---|
| 3 | 3 |
| 5 | 3 |
| 6 | 8 |
| 7 | 7 |
| 8 | 3 |
| 9 | 2 |
| 11 | 2 |
| 13 | 1 |

and 86,000, and was low in cases which had received prior antibiotic therapy other than topical neomycin.

All babies developed edema of the lids and a purulent discharge, which was often thin and watery in the absence of concurrent bacterial infection. In two cases it was blood stained. Localized "bubbling" of the mucosa was a marked feature in the more severe cases and in those in which the request for investigation was late (Fig. 1).

Twenty-two cases had received prior topical treatment as follows: neomycin, 13; chloramphenicol, 5; neomycin and chloramphenicol, 2; gentamicin and chloramphenicol, 1; penicillin and tetracycline, 1. Neomycin had no effect on the development of the conjunctivitis, but babies receiving chloramphenicol at the time of taking swabs usually had no discharge and were said to be improving. Slight to moderate lid edema and slight to severe conjunctivitis and mucosal edema were found on examination, depending on the duration of therapy (Fig. 2).

Diagnosis was delayed until 30 days in one ill premature baby (30-week gestation) who developed conjunctivitis at 3 days and was treated with intramuscular penicillin and kanamycin for 5 days followed by gentamicin for 5 days for *Escherichia coli* infection. *Streptococcus faecalis* was then isolated, and chloramphenicol eye drops were given for 5 days. The conjunctivitis subsided, but recurred and was retreated twice. *Chlamydia* was isolated on the second and fifth days of the third course of chloramphenicol. Herpesvirus particles were identified on electron microscopy. Only slight edema of the lids and slight conjunctivitis were present. No discharge was seen. The mucosa was edematous, and "bubbling" was present.

One baby (35-week gestation) was delivered by caesarean section because of fetal distress 3 h 15 min after spontaneous rupture of the membranes. The baby was nursed in the special-care unit where, at 11 days, slight edema of the lids of one eye, conjunctivitis, and mucopurulent discharge developed. This failed to respond to neomycin eye drops, and only scanty *Staphylococcus albus* was grown on culture. *C. trachomatis* was isolated from swabs taken on the 13th day (Fig. 3). No other baby was under treatment for chlamydia conjunctivitis in the unit. The mother developed pyrexia on the third day and was treated with ampicillin, 500 mg every 6 h for 7 days.

**Treatment and follow-up.** Tetracycline eye ointment five times daily for 4 weeks was prescribed. Babies attended for follow-up for periods up to 11 months. *Chlamydia* was reisolated from four infants 1 to 6 weeks after cessation of treatment. One had received treatment for 2 weeks, one for 3 weeks, and two for 4 weeks. Isolates were obtained from both eyes of the caesarean baby on relapse, although only one eye had shown clinical or bacteriological evidence of infection initially, and both eyes had been treated. Two mothers reported slight intermittent swelling of the lids and discharge. Slight conjunctivitis was present in only one.

**Prematurity.** Twelve of the 29 babies were premature (41.4%). The gestation period was 30 to 32 weeks in five, 33 to 34 weeks in four, and 35 to 37 weeks in three. None of the mothers of these babies had toxemia of pregnancy. Two babies had a ventricular septal defect. In addition, one *Chlamydia*-positive mother had a baby who died of cardiac failure at 3 days and was found at postmortem examination to have a hypoplastic left ventricle, patent ductus arteriosus, and preductal co-arctation.

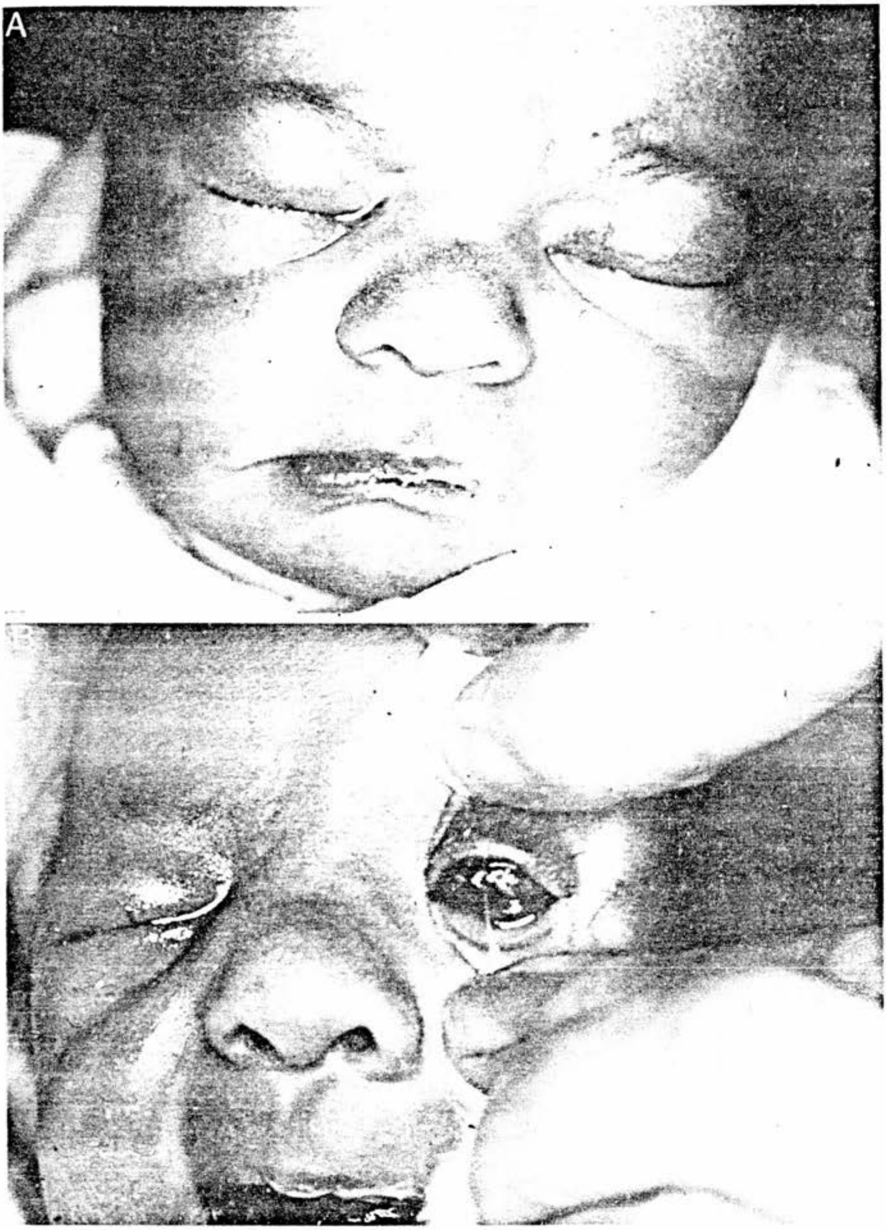


FIG. 1. *Baby K. 32-week gestation. (A) Onset of purulent discharge and palpebral edema at 6 days. No response to topical neomycin for 2 days. S. albus isolated. (B) Severe mucosal edema with bubbling. C. trachomatis isolated at 8 days.*

FIG. 2. *Baby R, 40-week gestation. (A) Onset of purulent discharge and palpebral edema at 5 days. Treated with topical neomycin for 2 days (no response) and topical chloramphenicol for 5 days with improvement. (B) Mucosal edema with bubbling, no discharge. C. trachomatis isolated at 12 days.*

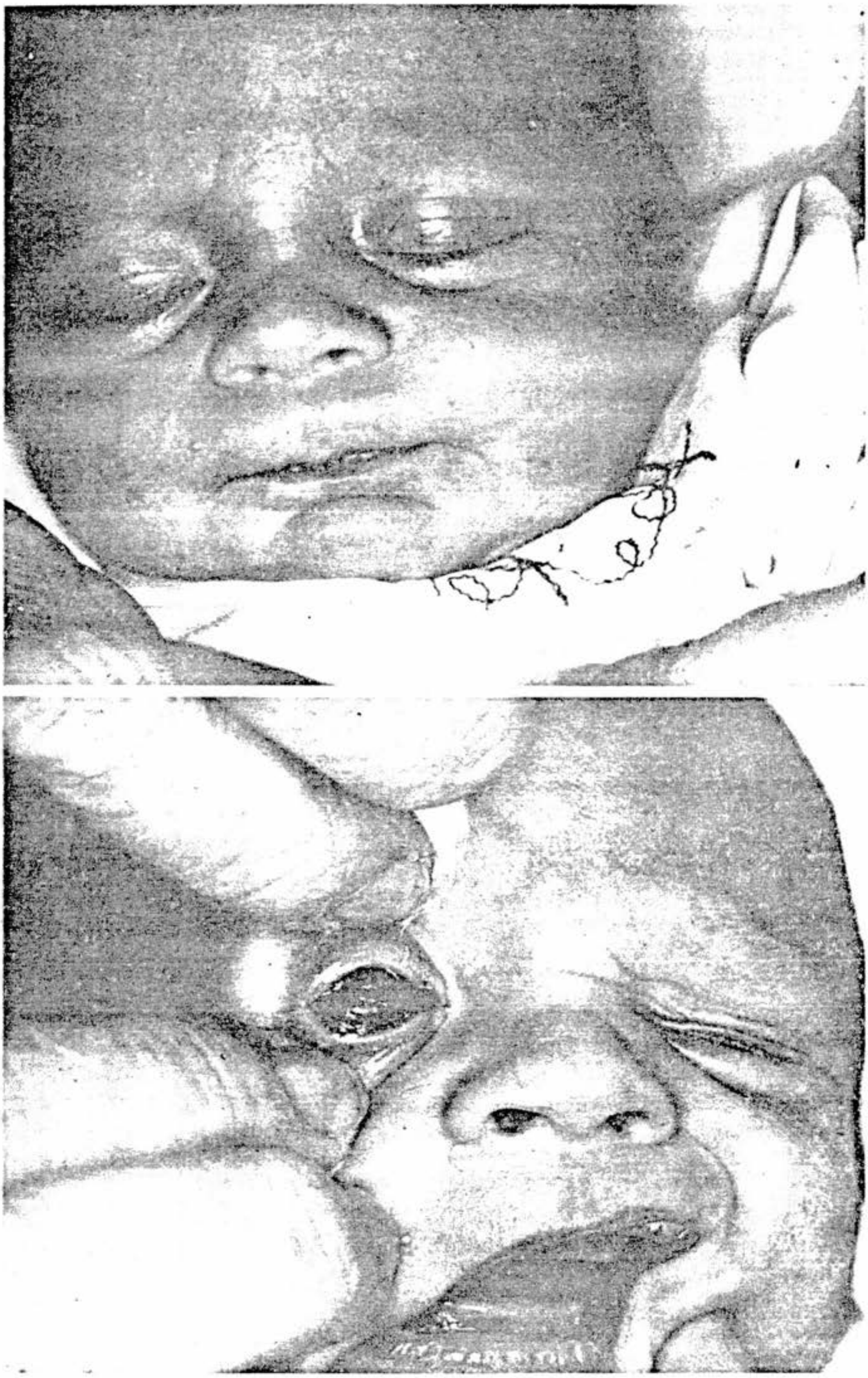

FIG. 3. *Baby C, 35-week gestation, delivered by caesarean section. (A) "Sticky eye" at 11 days. Early palpebral edema of right eye. No response to topical neomycin for 2 days. (B) Typical mucosal edema, scanty mucopurulent discharge in right eye. C. trachomatis isolated at 13 days.*

**Mothers.** Twenty-four mothers, aged 17 to 36 years, were examined, of whom 14 were married and 10 were single. Seven had received antibiotics prior to examination. The cervical specimen from one mother was cytotoxic. Among the 16 remaining patients, *C. trachomatis* was isolated from the cervix of 15 and *N. gonorrhoeae* from 3. Postpartum infection occurred in 16 (66.6%). Seven mothers received antibiotics in the maternity unit for pyrexia (3) or offensive lochia (4). High vaginal swabs were reported as follows: no pathogens, three: *Staphylococcus aureus*, one: gonococci, one: alpha-hemolytic streptococci, one: and anaerobic streptococci, one. Nine mothers developed pelvic infection after discharge from hospital (13 to 38 days postpartum), and details of this group are reported elsewhere in this volume (Rees et al., p. 67). One mother was treated with antibiotics for a wound infection.

**Fathers.** Eleven fathers of *Chlamydia*-positive babies were examined. All were asymptomatic. Two were gonococcus positive, and one of these admitted extramarital contact 3 months earlier. Of 10 examined for *C. trachomatis*, 4 were positive 24, 36, 59, and 84 days after the birth of the baby. The IFU/WC counts were low, being 3, 2, 10, and 420, respectively. No discharge was found, but shreds containing pus cells were present in the urine. One gonococcus-positive father was *Chlamydia* negative.

## DISCUSSION

The actual incidence of neonatal chlamydial conjunctivitis is difficult to assess because of the late onset of clinical signs and the early discharge from hospital of healthy babies.

In the United Kingdom, treatment of conjunctivitis is usually undertaken by the general practitioner unless the condition is refractory, in which case the infant is referred to an ophthalmologist. Investigation of parents is then unlikely to be carried out.

Nine of 24 mothers (37.5%) of infants with neonatal chlamydial infection developed pelvic infection 13 to 38 days postpartum, one having been treated with ampicillin for infected lochia prior to discharge from hospital. Six additional mothers (25.0%) were treated for pyrexia and infected lochia in the maternity unit. This confirms the association of neonatal conjunctivitis with maternal pelvic infection reported in several studies (8, 9). Mordhorst and Dawson (9) found pelvic infection before and after the birth of the infected child in 4 of 16 mothers. They suggest that the physiological stress of childbirth may reactivate a subclinical genital infection. In our group of 81 infants, four of the mothers had been treated for chlamydial cervicitis, three during pregnancy and one 2 months prior to pregnancy. *Chlamydia* was not reisolated during or after the pregnancy, and swabs from the babies were all negative. The four pregnancies continued to term.

The high incidence of prematurity in this series, 12 of 26 babies with chlamydial infection having gestation periods of 30 to 37 weeks, could be attributed to the fact that their longer stay in hospital allows conjunctivitis to develop and an accurate diagnosis to be made. However, the possibility of ascending cervical infection being a contributory factor to premature rupture of the membranes should be considered. The possible infection in utero of one baby delivered by caesarean section lends support to the hypothesis that chlamydial infection may ascend from the cervix and, therefore, may be directly involved not only in chorioamnionitis but also in the development of nonspecific salpingitis.

The asymptomatic nature of both the gonococcal and chlamydial infections in the fathers underlines the importance of investigating them to prevent reinfection of the mother.

It is suggested that the treatment of pregnant women infected with *C. trachomatis* is strongly indicated. Tetracycline should be avoided in pregnancy because of its effect on the bones and teeth of the fetus. Erythromycin is not contraindicated and has given satisfactory results in the small number of cases in which we have used it.

In summary, in a selected series of 81 babies *C. trachomatis* was isolated from 29 in the neonatal period. In the *Chlamydia*-positive group, concurrent gonococcal conjunctivitis was present in three and herpesvirus particles were identified by electron microscopy in one. One baby was delivered by caesarean section. The onset of conjunctival discharge varied between 3 and 13 days postpartum (mean, 6 to 8 days). Concurrent bacterial infection and prior antibiotic therapy influenced the clinical presentation. After treatment with oculentum tetracycline prescribed for 4 weeks, *C. trachomatis* was reisolated in four cases during follow-up extending to 11 months. A high incidence of postpartum maternal infection occurred. Four fathers with chlamydial infection and two with gonococcal infection were asymptomatic. Accurate diagnosis of neonatal conjunctivitis is important in assessing the need for family investigation. The treatment of pregnant women with chlamydial cervicitis is strongly indicated.

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# Effect of Penicillin on *Chlamydia trachomatis* In Vitro and In Vivo

DEREK HOBSON, ELISABETH REES, F. WILLIAM A. JOHNSON, AND I. ANNE TAIT

Concurrent genital infections due to *Chlamydia trachomatis* and *Neisseria gonorrhoeae* are common in women (1, 2). The diagnosis of gonococcal infection can frequently be made immediately by smear and can be confirmed rapidly by bacteriological culture. Hence, many of these patients may be treated immediately by administration of a single large dose of penicillin in the clinic before it is realized that *C. trachomatis* is also present in the genital tract.

Clinical results in men with double infections have suggested that, although single-dose penicillin treatment is effective in curing gonorrhea, it may fail to eradicate concurrent chlamydial urethritis. For example, Richmond et al. found that postgonococcal urethritis occurred more often in men in whom both *N. gonorrhoeae* and *C. trachomatis* had been present before treatment (3), and Holmes et al. isolated *C. trachomatis* from 9 of 20 men who developed postgonococcal urethritis 1 to 6 weeks after treatment of gonorrhea with penicillin (4). Oriel (p. 38, this volume) has shown that even a single dose of 2 g of ampicillin given together with 1 g of probenicid failed to cure *C. trachomatis* infection associated with gonorrhea in 14 of 15 men, although the latter was cured in all cases. It is thus of practical importance to consider the clinical results of using a single dose of penicillin to treat women with concurrent gonococcal and chlamydial infections of the cervix, as well as the effect of this antibiotic on *Chlamydia* in vitro. This report gives preliminary data on both these aspects.

A series of McCoy monolayers was simultaneously infected with *C. trachomatis* SAL strain at a dilution calculated to contain 10,000 inclusion-forming units (IFU) per culture after 48 h of incubation at 37°C. No antibiotics were used other than benzylpenicillin, which was added at the time of infection. Details of our procedure are described elsewhere (5).

The effect of incubation throughout the whole growth period with increasing concentrations of penicillin from 0.1 to 1.0 U/ml was progressive in the sense that normal inclusions, containing Giemsa-stained elementary bodies (EB) which fluoresced brightly under dark-ground microscopy, decreased in numbers and could not be found at penicillin concentrations greater than 1.0 U/ml. However, this decline in the number of normal inclusions was accompanied by a rising number of small abnormal inclusions, which did not fluoresce under dark-ground illumination and which contained only a few large swollen particles staining a reddish color with Giemsa, instead of the normal packed mass of deep blue-stained EB. It was difficult to count these abortive inclusions accurately, but in cultures incubated with 0.5 to 1.0 U of penicillin per ml, where their numbers were highest, they represented over 50% of the number of IFU of *C. trachomatis* originally added to the culture. Even in infected cultures incubated with penicillin concentrations from 5 to 100 U/ml, abnormal inclusions were found, but they became progressively fewer and their contents became increasingly sparse and abnormal, with amorphous red-staining debris replacing the particulate forms. However, even at 100 U of penicillin per ml, the number of abortive inclusions represented approximately 10% of the number of IFU of *C. trachomatis* originally inoculated.

It seems probable that the effect of penicillin on chlamydiae is exerted only at the stage where the progeny growing within the McCoy cell are beginning to synthesize their compact cell wall and become definitive EB. Thus, the abnormal swollen bodies seen within the abortive inclusions in penicillin-treated McCoy cells probably represent initial bodies, the further maturation of which is arrested (Manire, p. 167, this volume). It might thus be expected that normal maturation, with the development of infective EB, would be resumed when the penicillin is removed. So far, we have failed to confirm that reversion can occur in vitro. However, in the very different intracellular environment of chlamydiae growing in columnar epithelium of the genital tract in vivo, it seems probable that similar abnormal inclusions may develop under the effect of penicillin, but that maturation to EB may recommence after the last traces of the antibiotic have disappeared from the system. It is thus of interest to look at the effect of single-dose therapy with penicillin in patients with both gonococcal and chlamydial genital infection.

In our preliminary studies we followed the course of events in 22 women with proven gonococcal infection who were treated at once for this infection with procaine penicillin (1.2 megaunits) plus amoxycillin (500 mg) before it was suspected that a concurrent infection with *C. trachomatis* might be present. In all these patients, penicillin eradicated the gonococcal infection.

In 12 of these women, *C. trachomatis* was in fact isolated from pretreatment swabs, but only after penicillin had already been given for the gonococcal infection. In five of them, *C. trachomatis* was not reisolated after penicillin adminstration during subsequent periods of observation of 22 days to 9 months. The number of post-treatment swabs was five in one case, four in two cases, and three and two in one case each. In the other seven of these women with proven gonococcal plus chlamydial infection, *C. trachomatis* was reisolated in swabs taken 5 to 28 days after penicillin treatment. In one of these patients, although *C. trachomatis* was not isolated 7 days after penicillin administration, it was recovered from later swabs. In the other six patients the first post-treatment swab was positive, and in one case the inclusion count was significantly higher than in the pretreatment swab (18,000 and 900 per cover slip, respectively).

In a further eight women there had been no pretreatment investigation for chlamydiae, but there was persistent pus in the endocervical canal after the gonococcal infection had been eradicated by penicillin. *C. trachomatis* was isolated from all eight women from swabs taken 15 to 34 days after penicillin administration. In five of these cases, *C. trachomatis* remained present in second confirmatory swabs taken from 28 to 130 days after penicillin administration, before the beginning of oxytetracycline therapy for their chlamydial infection.

In the remaining two patients in this series, *C. trachomatis* was isolated 20 and 27 days, respectively, after penicillin administration, although they had had no signs or symptoms of cervical infection. This is commonly the case in women where gonococci or chlamydiae, or both, are found in the cervix (Rees et al., p. 67, this volume).

These preliminary results suggest that many women who present themselves at STD (sexually transmitted disease) clinics, and who are in reality infective reservoirs of both gonococci and chlamydiae, may be recognized and treated only for gonococcal infection. The chlamydial infection may persist undiminished for weeks or months after the gonococcal infection has been cured; in some but not all cases, the clinical signs may persist, and there would seem to be a distinct risk that many of these women, after treatment for gonococcal infection, could return to their community as persistent but unsuspected disseminators of chlamydial STD.

Because of the recent emergence of $\beta$-lactamase-producing strains of *N. gonorrhoeae* (6), the single-dose regimen of penicillin for the routine treatment of gonorrhea may in the near future need to be replaced by some other form of antibacterial therapy. It is to be hoped that any new antibiotic or new regimen devised for this purpose will take account of the frequency with which gonorrhea is accompanied by infection due to *C. trachomatis*, the eradication of which cannot necessarily be guaranteed by treatment with antibiotics active against *Neisseria*.

## ACKNOWLEDGMENT

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# Quantitative Aspects of the Growth of *Chlamydia trachomatis* in Diagnostic Tissue Culture Procedures

F. WILLIAM A. JOHNSON, DEREK HOBSON, ELISABETH REES, AND I. ANNE TAIT

Tissue culture (TC) techniques for isolating *Chlamydia trachomatis* are of proven value in epidemiological surveys in large research centers. In the next few years, they are certain to be widely adopted for the routine investigation of all patients with suspected sexually transmitted disease. In the scaling-up process, some form of quality control is desirable to ensure that the sensitivity and reproducibility of the present procedures is being maintained at a constant high level and that any future technical modifications, to conform with different laboratory facilities and practice, do not affect these parameters.

Comparison of isolation rates of *C. trachomatis* between successive groups of patients or between patients in different clinics can only give information of limited value. Independent proof of infection (e.g., by direct smear or serology) is rarely feasible in all patients (Schachter and Dawson, p. 337, this volume). Hence, the true base rate of chlamydial infection is unknown, and it is difficult to interpret either concordance or divergence of laboratory results with different patients at different times in different cities.

However, the nature of the current TC procedures provides an inbuilt quantitative means of monitoring their efficiency. Since *C. trachomatis* gives only a single cycle of development (1), the number of inclusions finally detected in TC is directly proportional to the number of viable chlamydial elementary bodies originally inoculated. Thus, a sensitive early warning of any change in laboratory precision can be obtained by determining the overall efficiency of plating, by regularly titrating low-passage laboratory strains of *C. trachomatis*, and by testing repeated swabs at short intervals from known positive, untreated patients.

This paper presents such an investigation of a technique with replicating McCoy cells used for the past 3 years in the diagnosis of chlamydial infections of women in Liverpool (2, 3; Rees et al., p. 67, this volume).

## MATERIALS AND METHODS

Details of the preparation of cover slip monolayer cultures of replicating McCoy cells (MCC), media, diluents, and the isolation procedure for clinical specimens have been published (2, 3; Rees et al., p. 67, this volume).

The isolation, propagation, and storage of the laboratory strain SAL of *C. trachomatis* isolated in Liverpool, and methods of investigating its growth under different cultural conditions (pH, incubation temperature, medium, etc.), have previously been described (3). Other technical procedures are described below.

## RESULTS

**Variation between cover slip cultures.** A single batch of MCC was prepared under standard conditions (3). Random samples were stained to confirm that >90% of the cover slip was covered evenly by cells with well-spread cytoplasm. All were then inoculated simultaneously with a single dilution of SAL strain estimated to yield 3,000 inclusions per MCC. After incubation for 48 h at 37°C, all MCC were Giemsa stained, and the total number of inclusions per MCC was counted under ×400 bright-field microscopy. The 48 cover slip counts followed a normal distribution pattern (range, 2,817 to 4,143; mean, 3,200; standard deviation, 325), and 93% of the observed counts were within ± 20% of the mean value.

**Effect of different stains and time of examination.** A single batch of MCC was inoculated simultaneously with a single dilution of SAL strain estimated to yield 40,000 inclusions per cover slip. All were incubated at 37°C, and randomly selected MCC were removed after 24, 48, or 72 h for staining with iodine, Giemsa, or carmine (4). Total inclusion counts per MCC were calculated from counts of 150 randomly chosen microscopic fields in each of two MCC for each stain and time. The mean size of inclusions was determined by measuring the diameter of 100 randomly selected inclusions in each MCC, with the use of an eye-piece micrometer (Table 1).

Giemsa staining at 48 h revealed the highest inclusion count. Even at 24 h, most of the inclusions were already detectable, but their small size made counting slow and fatiguing. By 72 h, inclusions were big and easy to count, but many were bursting, and presumably other infected McCoy cells had already ruptured and dropped off the glass, since the total count had fallen to 25% of the 48-h value.

Carmine staining was less effective than Giemsa for detecting inclusions at 24 h, but gave similar results at 48 and 72 h. The bright-red inclusions were clearly distinguished against the light-brown staining of the McCoy cells.

TABLE 1. *Comparison of number and size of inclusions of C. trachomatis SAL in simultaneously infected McCoy monolayers stained by different methods after different times of incubation*

| Staining procedure | Incubation time (h) | Inclusion data | | |
|---|---|---|---|---|
| | | Count[a] | Diameter (μm)[b] | |
| | | | Range | Mean |
| Iodine | 24 | 10 | 4.25–7.25 | 6 |
| | 48 | 10,250 | 4–18.75 | 8.75 |
| | 72 | 8,700 | 5.5–26.25 | 11.85 |
| Giemsa | 24 | 44,500 | 0.87–12 | 5.25 |
| | 48 | 49,600 | 7–41.75 | 12.75 |
| | 72 | 12,120 | 9.25–38.25 | 24.27 |
| Carmine | 24 | 11,100 | 2.0–11.0 | 5.6 |
| | 48 | 45,100 | 4.5–46.2 | 12.85 |
| | 72 | 12,180 | 8.25–32.75 | 18.15 |

[a] Total count per 16-mm cover slip. Mean of two cover slips.
[b] Mean of two diameters of each of 100 inclusions per cover slip.

Iodine resulted in significant underestimation of the number of inclusions present even at 48 h, and seemed incapable of allowing detection of early inclusions or many of the large late inclusions. This may be because of the variability in timing and amount of the glycogen-rich matrix in *C. trachomatis* inclusions in TC (5, 6).

**Effect of changing laboratory conditions.** Inclusion counts produced by a constant inoculum of SAL strain were determined after various modifications of the standard procedure (3). For each variable factor, two to four MCC were compared with the same number of MCC infected and incubated under standard conditions.

The results summarized in Table 2 indicate the large decrease in the sensitivity of McCoy cells to *C. trachomatis* infection that can result from comparatively small changes in the isolation technique. The only factor which was found to increase the inclusion count significantly was the use of increased force or time of centrifugation. These effects are discussed in greater detail elsewhere (3).

**Clinical specimens.** *C. trachomatis* was isolated from the cervix of 153 contacts of men with nongonococcal urethritis (NGU) within 2 to 3 days of their first visit to the sexually transmitted disease clinic. On their next visit 7 to 10 days later, a

TABLE 2. *Effect of modifications of the standard tissue culture procedure[a] on the efficiency of plating of C. trachomatis SAL*

| Change in procedure | Mean inclusion count per cover slip[b] |
|---|---|
| None[a] | 3,088 |
| Centrifugation | |
| Nil | 62 |
| 2,500 × *g*, 15 min | 2,120 |
| 2,500 × *g*, 3 h | 19,830 |
| 4,500 × *g*, 1 h | 23,400 |
| Incubation temperature | |
| 35°C | 2,014 |
| 39°C | 1,615 |
| pH | |
| 6.6 | 878 |
| 7.8 | 540 |
| $NaHCO_3$ concentration at pH 7.2 | |
| 0.025 M | 2,932 |
| 0.005 M | 405 |
| Medium (+ 10% calf serum, etc.)[c] | |
| Eagle MEM | 1,646 |
| BME | 204 |
| McCoy: initial cell count | |
| $5 \times 10^4$/ml | 633 |
| $5 \times 10^5$/ml | 1,250 |
| McCoy: age of monolayer when infected, 5 days | 1,204 |

[a] In the standard procedure, McCoy cell cultures were prepared from $2 \times 10^5$ cells/ml and incubated for 24 h at 37°C. They were then inoculated, centrifuged at 2,500 × *g* for 1 h, and incubated for 48 h at 37°C. The standard medium was medium 199 plus 10% fetal calf serum and 0.02 M $NaHCO_3$.

[b] Mean of 40 cover slips in replicate experiments by standard procedure. Mean of two to four cover slips for each modification.

[c] MEM = minimal essential medium; BME = Eagle basal medium.

second cervical swab was taken and again examined by the standard procedure. There had been no intervening systemic or local therapy. The repeat swab was again positive in 141 (92.5%) of the women; the 8 patients with negative second swabs had inclusion counts of only 2 to 30 per MCC on the first occasion. In a group of 82 NGU contacts with negative primary swabs, the repeat swab was positive in only 2 (2.5%) women, with counts of two and eight inclusions per MCC.

In 98 women with a constant 7-day interval between two positive swabs, inclusion counts were closely similar in most cases on both occasions (Table 3). The overall distribution of counts in the whole group showed little change; only 8% of all swabs yielded fewer than 30 inclusions, whereas 75.4% of primary swabs and 69.8% of second swabs gave 100 or more inclusions per MCC.

## DISCUSSION

Current TC techniques for isolating *C. trachomatis* are now well within the scope of any laboratory equipped for routine virological diagnosis. However, comparatively small changes in the detailed procedure or in the staining technique can significantly reduce the number of countable inclusions obtained from a given number of viable elementary bodies (3, 9; Rota, p. 314, this volume) and might thus impair the chance of diagnosing a patient with an early, low-grade, or imperfectly treated chlamydial infection.

The present results suggest that quantitative inclusion counts on all clinical specimens, and on low-passage laboratory strains of *C. trachomatis* at regular intervals, could provide a simple check on the confidence limits of the overall working procedure within the laboratory and, indirectly, on the quality of the specimens reaching the laboratory. Such routine counts may be of future clinical value in estimating the extent and severity of the infection and the possibility of relapse after chemotherapy.

The present methods seem capable of isolating a substantial number of infective particles of *C. trachomatis* from the majority of infected women, and it does not seem that small reductions in the sensitivity of the procedures would be reflected by any significant fall in the total number of new sexually transmitted disease patients found positive. However, a sustained high efficiency of plating is necessary (i) to insure against the marked loss in viability of chlamydia in specimens which need to

TABLE 3. *Distribution of inclusion counts in cervical swabs from 98 untreated women with C. trachomatis infection*

| Inclusion counts per cover slip[a] | Percentage of women with stated count in cervical swab taken | |
|---|---|---|
| | On 1st visit | 7 days later |
| 1–10 | 6.3 | 9.9 |
| 10–100 | 17.7 | 18.8 |
| 100–1,000 | 43.1 | 36.3 |
| 1,000–10,000 | 18.3 | 22.6 |
| 10,000–100,000 | 14.0 | 10.9 |

[a] Each cover slip was inoculated with one-twelfth of the secretion recovered from each swab.

be sent to distant laboratories and stored overlong before TC inoculation, and (ii) to confirm that chemotherapy has eradicated the patient's infection and not merely depressed it to levels undetectable by the laboratory but still capable of causing clinical relapse at some later date.

The beneficial effect of increasing centrifugation demonstrated here confirms earlier findings (3, 8, 9) and suggests that current techniques may isolate only a fraction of the total number of infective particles shed by the patient. For most laboratories centrifugation of specimens at more than $2{,}500 \times g$ for 1 h is impracticable, but it seems worthwhile to explore further methods of increasing the efficiency of adsorption of *C. trachomatis* to TC cells, to help minimize the effect of the many other variations in laboratory and clinical conditions which can affect the isolation rate of chlamydiae in genital infections.

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# Neonatal conjunctivitis caused by *Neisseria gonorrhoeae* and *Chlamydia trachomatis*

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SUMMARY In a selected group of 103 babies referred with neonatal conjunctivitis *Neisseria gonorrhoeae* was isolated from 11 and *Chlamydia trachomatis* from 33. Concurrent infection was present in three. One toddler sibling developed chlamydial conjunctivitis. After treatment *C. trachomatis* was re-isolated from six babies during the follow-up period. The discharge started one to three days after delivery in only three babies with gonococcal conjunctivitis and at five to eight days in eight babies. One baby was delivered by caesarean section. *N. gonorrhoeae* was isolated from four asymptomatic fathers, all of whom had urethritis. The mean onset of discharge in the 33 babies from whom *Chlamydia* was isolated was 7·1 days. One baby was delivered by caesarean section. Chlamydial conjunctivitis was associated with a high incidence of prematurity and of postpartum infection in the mother. Ten fathers of *Chlamydia*-positive babies were examined. *C. trachomatis* was isolated from four, all of whom were asymptomatic but had low-grade urethritis. These findings confirm the pathogenic role of *C. trachomatis* in the cervix and indicate the importance to the family of an adequate microbiological investigation of neonatal conjunctivitis.

## Introduction

Infants often develop mild inflammation of the eye a few days after birth. Usually this is due to trauma or transient infection and resolves quickly without treatment. However, more severe forms of purulent conjunctivitis of the neonate are seen and some fail to respond to routine antibiotic treatment.

It has long been recognised that neonatal conjunctivitis could follow intrapartum contamination of the eye with cervical secretion if the mother had a previously unrecognised and untreated genital infection acquired by sexual contact. In the late nineteenth century gonorrhoea was undoubtedly the commonest cause of this condition as well as being the easiest causative organism to identify. As early as 1909, however, Halberstaedter and von Prowazek recognised an abacterial form of infection, and inclusion bodies were identified in the epithelial cells of the infant's conjunctiva and in cellular scrapings of the genital tract of the parents (Lindner, 1910). This was confirmed by Jones *et al.* (1959) when it became possible to grow the agent (trachoma-inclusion conjunctivitis (TRIC) agent, or *Chlamydia trachomatis*) in the laboratory in chick embryos. More recently, *C. trachomatis* has been isolated in 40 to 50% of cases of non-gonococcal urethritis (NGU) in the male by tissue culture techniques (Dunlop *et al.*, 1972; Oriel *et al.*, 1976). Infection is transmitted sexually to female partners of men with NGU, and *C. trachomatis* can be isolated from the cervix in up to 37% of such women (Hilton *et al.*, 1974; Holmes *et al.*, 1975). If left untreated these women can act as chronic reservoirs of infection and can infect more men or their own infants. The increase of NGU in men by 159% during the past 10 years compared with an increase of only 29% in gonococcal urethritis (Department of Health and Social Security, 1965, 1976) is probably a measure of the failure to identify and treat these women. Thus it is likely that paediatricians, obstetricians, and general practitioners will see an increasing number of infants with neonatal conjunctivitis. Those caused by *C. trachomatis* do not respond to conventional treatment with eye

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drops of neomycin, chloramphenicol, gentamicin, or penicillin and persistent infection may result. Some cases progress to palpebral scarring and micropannus similar to, although less in degree than, the sequelae of tropical trachoma (Freedman *et al.*, 1966; Mordhorst and Dawson, 1971). The eyesight is rarely affected.

Infection of the infant with sexually transmissable agents such as *N. gonorrhoeae* or *C. trachomatis* implies that the mother had a cervical infection at the time of birth and that the father may have a persistent but unrecognised urethritis. *N. gonorrhoeae* can cause pelvic infection and an association between pelvic infection and chlamydial cervicitis has been suggested (Weström, 1975; Märdh *et al.*, 1977). A high incidence of postpartum infection in mothers of infants with chlamydial conjunctivitis has been reported (Dunlop *et al.*, 1966; Rees *et al.*, 1977a). Thus failure to establish quickly the aetiology of neonatal conjunctivitis may have serious sequelae in the mother. The purpose of this paper is to present the results of an investigation into the clinical patterns of neonatal conjunctivitis caused by gonococci and *Chlamydia*, and the clinical and microbiological investigation of the parents.

## Patients and methods

One hundred and three babies and three toddlers were examined between October 1973 and July 1976. Ninety-five were referred by paediatricians and four by ophthalmologists. Four babies and one toddler sibling were examined because their mothers had been treated for chlamydial cervicitis, three during and one before pregnancy. Two toddlers with conjunctivitis were investigated as contacts of a neonatal sibling. Seven were seen in children's hospitals, four in an eye hospital, 74 in maternity units, and 21 attended as outpatients.

The group forms a selected series of cases of conjunctivitis referred mainly because of failure to respond to routine treatment or because no bacterial growth was reported by the laboratory. A few cases were seen because of unusual presentation or a history of sexually transmitted disease (STD) in the mother.

Specimens were obtained with cottonwool-tipped swabs from the mucosal surface of the lower lids in all cases and from the upper lids if possible, and were examined microscopically and by culture for gonococci, other bacteria, and for candida species. Stuart's medium was used for transport of bacteria but most swabs for gonococcal culture were inoculated directly on to a selective medium containing antibiotics (Lab M).

Swabs for chlamydial culture were kept at 4°C in transport medium until they were inoculated into tissue culture between 12 and 24 hours later (Hobson *et al.*, 1974). Immediately before inoculation the swab contents were homogenised in the 2·5 ml of transport medium: 0·4 ml aliquots were inoculated on to each of two monolayer coverslip cultures (MCC) of McCoy cells in 1 oz Universal bottles and centrifuged at 2400 *g* for one hour. After 48 hours' incubation at 37° the MCC were Giemsa-stained and examined by light and dark ground microscopy for intracytoplasmic chlamydial inclusions. Details of the methods have already been described (Johnson, 1975; Johnson and Hobson, 1976). In all cases the results described below represent the direct inoculation of the specimen into tissue culture. No second passage from tissue culture to tissue culture was performed to avoid any risk of false positive results by cross-contamination, and known negative material was inoculated, centrifuged, incubated, and stained with each batch of MCC. All specimens were given serial numbers on arrival in the laboratory and examined in random order.

Specimens for identification of herpes simplex virus by electron microscopical examination and culture were taken in some cases.

Mothers were investigated in the maternity unit if possible, but mothers of premature babies had usually been discharged; these, and mothers whose babies had late onset conjunctivitis, were requested to attend as outpatients if either gonococci or *Chlamydia* was isolated from the infant. All were investigated for syphilis, gonorrhoea, chlamydial cervicitis, trichomoniasis, candidiasis and, in a few cases, genital herpes by conventional methods. If possible, fathers were similarly investigated if the infant and/or mother had gonococcal, chlamydial, or syphilitic infection.

## Results

*N. gonorrhoeae* was isolated from 11 babies and *C. trachomatis* from 33 babies and one toddler. Concurrent gonococcal and chlamydial infection was present in three babies. In one case, herpes-like particles were identified by electron microscopy, but not grown in culture.

### GONOCOCCAL CONJUNCTIVITIS

Ten of the 11 babies were delivered at term, and one was premature (birth weight: 2·13 kg): seven were diagnosed in the maternity unit, and four were admitted to hospitals after being discharged from the maternity unit (three to an eye hospital and one to a children's hospital).

The reported onset of exudate varied between one and eight days after birth in those diagnosed in maternity units (1 to 3 days in three cases; 6 to 8 days in four cases) and between five and six days in the four cases admitted to eye or children's hospitals.

The day of onset in the concurrent gonococcal and chlamydial infections was the sixth day after birth in two cases and the seventh day in one. Three of the four babies re-admitted to hospital had developed discharge at six days and one at five days. These four infants had been treated with topical neomycin or chloramphenicol by the general practitioner and were not admitted until seven to twelve days after birth—that is, when it became obvious that the treatment was failing: two of them had concurrent gonococcal and chlamydial infection.

No signs were observed until the eighth day in the baby delivered by caesarean section; the mother had been treated with ampicillin for pelvic infection from the second day postpartum. *N. gonorrhoeae* was isolated from a high vaginal swab taken before the start of antibiotic treatment. One baby had developed an eye infection in the maternity unit at six days. Gram-negative intracellular diplococci were reported in the smear but because of the mildness of the clinical signs treatment was delayed until full identification by culture and fermentation tests was received two days later. No change in the signs developed during this period.

### *Findings in mothers*

All eleven mothers were examined: five were married. *N. gonorrhoeae* was isolated from 10 mothers: no isolate was obtained from one mother already receiving ampicillin for pyrexia in hospital. Five mothers developed postpartum infection: pyrexia (1), infected lochia (1), pelvic infection (3). Three of the five had concurrent gonococcal and chlamydial cervical infection.

*N. gonorrhoeae* was isolated from two other married mothers whose babies were being treated for conjunctivitis with topical chloramphenicol at the time of examination. No isolate was obtained from these babies then or later. The onset of exudate was at three and nine days respectively. One mother had concurrent chlamydial cervicitis and one developed hepatitis during the pregnancy and was hepatitis B antigen positive. One of the two fathers had been treated for gonococcal urethritis, the other was not available for examination.

### *Findings in fathers*

*N. gonorrhoeae* was found in four of five fathers examined. All were asymptomatic. One other father had been treated for gonorrhoea shortly before the birth and another had been treated for urethral discharge.

### CHLAMYDIAL CONJUNCTIVITIS

*C. trachomatis* was isolated from 26 babies in maternity units, one in the eye hospital, four in children's hospitals, and two attending as outpatients. An isolate was obtained from the 18-month-old sister of a baby who developed conjunctivitis at home aged six days. The baby's condition improved after treatment with chloramphenicol, but signs recurred when she was 16 days old and she was admitted to the children's hospital where *C. trachomatis* was isolated. The toddler developed conjunctivitis four days after the baby had been admitted and was treated for several weeks with eye ointment by the general practitioner. *Chlamydia* was isolated eight weeks later. One baby was delivered by caesarean section, and developed conjunctival discharge in the special care unit aged 11 days (Rees *et al.*, 1977b).

The onset of discharge varied between three and 13 days after the birth with a mean of 7·1 days, or 6·7 days if one excludes three cases given a five-day course of cloxacillin and penicillin from birth, in whom the onset was delayed to 9, 11, and 13 days respectively (Table).

Table *Onset of discharge in 33 cases of chlamydial conjunctivitis*

| | *Day of onset* | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | *3rd* | *5th* | *6th* | *7th* | *8th* | *9th* | *11th* | *13th* |
| No. of cases | 1 | 5 | 11 | 7 | 3 | 2 | 2 | 2 |

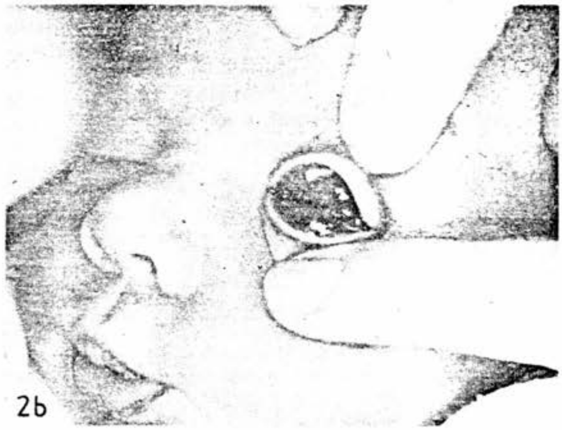
Sixteen infants (48·8 %) were referred for investigation within 10 days of birth: 26 (78·8 %) were referred within 13 days. Isolates were obtained at 17, 19, 20, 24 (2), 30, and 57 days in the remainder. Those in whom referral had been delayed had been treated with topical chloramphenicol with improvement followed by recurrence on cessation of treatment.

All babies developed oedema of the lids and a purulent discharge, which was blood-stained in two cases. Mucosal oedema was present in all cases and was associated with a localised bubbling appearance in the more severe cases and in those in whom the request for investigation was delayed, Fig. 1 a, b. Topical chloramphenicol modified the picture. Discharge often ceased, but mucosal oedema persisted in varying degrees (Fig. 2 a, b, and Fig. 3 a, b). Neither neomycin nor gentamicin had any effect.


1a


1b


2a


2b


3a


3b

*Results of treatment of chlamydial conjunctivitis*

After treatment with tetracycline eye ointment prescribed for four weeks, *C. trachomatis* was re-isolated from six of 24 babies who attended for follow-up. Two of these babies were inadequately treated for only two and three weeks. After four weeks' treatment, *Chlamydia* was re-isolated within a further four weeks in three cases, one of which was the baby born by caesarean section. *Chlamydia* was re-isolated from another baby four weeks after treatment in hospital and again five months after being treated again for six weeks with topical tetracycline. In five cases minor symptoms and signs recurred on relapse.

Fourteen babies were premature (42·4%). The gestation period was between 30 and 34 weeks in 10 and between 35 and 37 in four, birth weight was between 1·5 and 2·7 kg. Excluding the four babies examined as contacts because their mothers had been treated for chlamydial cervicitis before or during the pregnancy, the incidence of prematurity in the group from whom neither gonococci nor *Chlamydia* was isolated was nine of 58 cases (15·5%). The difference in the incidence of prematurity in chlamydial positive and negative babies is significant ($\chi_1^2=6{\cdot}7$ $P<0{\cdot}01$).

*Findings in mothers of babies with chlamydial conjunctivitis*

Nineteen of the 33 mothers were married; 31 were examined. One specimen was cytotoxic: *Chlamydia* was isolated from the cervix in 20 of the remaining 30 mothers. Seven of the 10 negative mothers were receiving, or had received, antibiotics for postpartum infection. Nineteen of the 31 mothers developed postpartum infection: pelvic infection (11), pyrexia (3), infected lochia (4), wound infection (1).

*C. trachomatis* was isolated from eight of the 11 mothers with pelvic infection. All three negative mothers had received or were receiving antibiotics at the time of examination. One *Chlamydia*-positive mother with pelvic infection had been treated with ampicillin for infected lochia before discharge from the maternity unit. Another mother of a premature baby gave a history of severe lower abdominal pain three weeks before she attended as an outpatient, but no tenderness was found on pelvic examination and she is, therefore, excluded from the group. Sera were examined for psittacosis lymphogranuloma venereum group antibody in nine of the 11 cases and all had levels of 1:20 or higher with a rising titre in early cases. Titres of 1:80 rising to >1:1280 were reported in two cases. (Full details of group antibody levels will be reported elsewhere.)

*Findings in fathers*

*C. trachomatis* was isolated from four of 10 fathers examined. All were asymptomatic but low grade urethritis was found. One normal father had recently taken antibiotics.

Fig. 1 *Conjunctivitis aged* 7 *days. Treated with neomycin eye drops for two days without response.* C. trachomatis *isolated aged nine days.*
*(a) Palpebral oedema. (b) Severe mucosal oedema with bubbling and mucopurulent discharge.*

Fig. 2 *Conjunctivitis aged* 5 *days. Treated with neomycin eye drops for two days without response. Chloramphenicol eye drops for five days; discharge ceased, palpebral oedema lessened.* C. trachomatis *isolated aged* 12 *days.*
*(a) Persisting but lessening palpebral oedema indicated by slight wrinkling of skin. (b) Well-marked mucosal oedema with bubbling on upper lid.*

Fig. 3 *Conjunctivitis aged* 3 *days.* Streptococcus faecalis *isolated. Relapsed within* 5 *days of completion of each of two 5-day courses of chloramphenicol eye drops.* C. trachomatis *isolated aged* 30 *days on fourth day of third course in special care unit.*
*(a) Minimal palpebral oedema. (b) Mild conjunctivitis mucosal oedema with bubbling. Two possible early follicles.*

## Discussion

The results of this investigation suggest that neonatal eye infection caused by sexually transmissable organisms is common. This infection is unlikely to lead to impairment of sight unless neglected, but failure to establish the cause before treatment may have serious consequences to the mother in whom risk of postpartum extension of infection from the cervix appears to be high. Furthermore, the woman can be reinfected by the untreated asymptomatic man and both may be a source of infection to other members of the family.

A surprising feature of this group of babies is the late onset of gonococcal conjunctivitis. Only three of the 11 babies developed discharge within three days of birth and for this reason the diagnosis was made after the baby had been discharged from the maternity unit in four cases. It is, of course, possible that infection was not intrapartum but followed contact with infected hands or towels. Nevertheless, in one case of mild conjunctivitis developing in hospital at six days no increase in the slight conjunctival discharge occurred in the 48 hours during which confirmation of the positive Gram-stained smear was awaited. The baby delivered by caesarean section developed conjunctivitis in hospital at eight days. His mother started taking antibiotics 48 hours postpartum and was, therefore, non-infectious from that time. (Gonococci were not isolated again.) These cases suggest that gonococcal conjunctivitis may in some cases have a comparatively long incubation period and may not be acute. Alternatively, frequent postpartum transmission of infection to the eyes of infants must be postulated. Gonococcal conjunctivitis has in the past responded promptly to topical and systemic treatment with penicillin. Difficulties in treatment may be encountered in the future owing to infections with β-lactamase producing strains of gonococci which have been identified in Liverpool since February 1976 (Percival, *et al.*, 1976).

Because of the late onset of chlamydial conjunctivitis (six to seven days after birth) most infected babies will be seen by the general practitioner or at the baby clinic. The high incidence of premature babies in this series may simply mean that they are more likely to be diagnosed than full-term babies because they remain in hospital for a longer time than the incubation period of the infection. However, the possibility that chlamydial cervicitis might involve the membranes overlying the internal os and result in premature rupture has been suggested (Rees *et al.*, 1977b). Some support is given to this suggestion by this investigation in which a significant difference in the prematurity rate for *Chlamydia*-positive and *Chlamydia*-negative babies was found. Thus the association between prematurity and conjunctivitis may be causal—that is, the mother's chlamydial infection may be responsible for both conditions as well as for an increased chance that she may develop postpartum infection.

In an epidemiological study, Watson and Gairdner (1968) reported four definite and four suggestive cases of chlamydial infection in 44 cases of neonatal conjunctivitis screened for inclusions in a maternity unit. Exudate was often scanty and not obviously purulent : inflammation and palpebral oedema were negligible. No attempt was made to culture the agent. No specific treatment was given but in two of the three cases followed for three to 24 weeks postpartum there was scarring of the conjunctiva, and one of the two had a continuing and active pannus. Such cases would not have been referred for investigation in our selected series in which all babies had purulent conjunctivitis with well-marked mucosal oedema and, in untreated cases, palpebral oedema.

Topical neomycin does not inhibit *Chlamydia* and has a broad antibacterial spectrum. It is the first line treatment of choice in non-gonococcal conjunctivitis and babies who fail to respond should be investigated for *Chlamydia*. Inadequate treatment with chloramphenicol or short courses of tetracycline may suppress but not eradicate *Chlamydia*. Palpebral oedema and conjunctival exudate may clear but conjunctivitis often persists. The importance of everting the lids to examine the conjunctiva of all babies treated for neonatal conjunctivitis and as part of the general examination of babies before discharge from the maternity unit is clearly indicated.

The isolation of *Chlamydia* from the 18-month-old sister of one infant admitted to hospital with severe purulent conjunctivitis suggests eye to eye transmission such as occurs in hyperendemic trachoma (Jones, 1975; Grayston *et al.*, 1977).

The asymptomatic nature of both gonococcal and chlamydial infection in eight fathers underlines the necessity for early accurate diagnosis of neonatal conjunctivitis. Only if gonococci or *Chlamydia* are isolated will a full family investigation be carried out and the risk of pelvic infection in the mother be reduced by early diagnosis and treatment. The fact that nearly 60% of the mothers in this series were married possibly reflects the degree of extramarital contact occurring at the present time.

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