The rôle of *Chlamydia trachomatis* in genital-tract and associated diseases

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Introduction

The organisms that cause psittacosis were isolated from affected humans and psittacine birds during the psittacosis pandemic of 1929-30,¹ and the agent of lymphogranuloma venereum was isolated at about the same time. This was followed by a gradual realisation that chlamydial infections were widespread in other birds and mammals. In 1957, the isolation of the aetiological agent of trachoma in chick embryos was reported.² Soon after, the organisms responsible for inclusion blennorrhoea of the newborn and for infection of the genital tract of the parents were recovered,³ and identical organisms were found in the genital tract of men presenting with non-gonococcal urethritis.⁴ Because the organisms from the eye and genital tract could not easily be distinguished, and because the eye disease associated with them formed a continuous spectrum ranging in severity from inclusion conjunctivitis to trachoma, the expression 'TRIC agents' was used for these organisms (TR for trachoma, IC for inclusion conjunctivitis). The TRIC agents belong to the larger group of organisms that has been called the PLT (psittacosis-lymphogranuloma-trachoma) group of agents or an array of other terms including *Bedsonia*. This term was used to recognise the isolation and characterisation of the agent of psittacosis by Sir Samuel Bedson. However, for taxonomic reasons, it has been superseded by the generic name *Chlamydia* (Greek, *chlamys*: a cloak draped from the shoulder, meant to describe the draping of the intracytoplasmic inclusions around the nucleus of the host cell). The genus is in the family chlamydiaceae, of the order *Chlamydiales*⁵ and within the genus two species are recognised: *Chlamydia psittaci* and *Chlamydia trachomatis*.⁶ Recently, Wright⁷ has said that it is wrong to refer to organisms of the genus *Chlamydia* as chlamydiae since chlamydia is already a plural Greek word (singular = chlamydion). However, according to the definitive

rules of taxonomy, generic names of bacteria are regarded as 'Latin' words. *Chlamydia* is thus to be used as a singular, feminine noun, and its Latinised plural becomes chlamydiae. We have therefore used the word 'chlamydiae' in the past and henceforth shall continue to do so.

This review is not concerned with psittacosis or trachoma although they are mentioned where it is pertinent to do so. The discussion is concerned with strains of *C. trachomatis* found in the genital tract, the evidence that is available to show that they are a cause of certain diseases, the proportion of disease they cause, and possible mechanisms underlying disease production. In addition, laboratory diagnosis, the value of serology, and the outlook for a vaccine are considered.

Characteristics of chlamydiae

Some of the properties of chlamydiae, compared with those of mycoplasmas, bacteria, and viruses, are presented in Table 1. In particular, chlamydiae (i) do not stain with the Gram stain, (ii) contain DNA and RNA, (iii) are susceptible to certain antibiotics, (iv) have a rigid cell wall similar in structure and content to that of the Gram-negative bacteria, and (v) multiply by binary fission. In these various respects they are bacteria-like; they may, in fact, be regarded as bacteria that have adapted to an intracellular environment, being (vi) obligate intracellular parasites. In this latter feature, chlamydiae are different from mycoplasmas and bacteria but similar to viruses, needing viable cells for their multiplication and survival, as described below.

CHLAMYDIAL GROWTH CYCLE (Fig. 1)

Despite the occurrence of binary fission as in bacteria, chlamydiae have a complicated growth cycle⁸ involving both an extracellular and intracellular existence. The infectious particle or elementary body is about 300 nm in size, is metabolically inactive, and has a resistant, rigid envelope. It becomes attached to the host cell surface, is taken in by phagocytosis, and undergoes some kind of

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Table 1 Characteristics of chlamydiae in relation to those of mycoplasmas, bacteria, and viruses

Property	Presence (+) or absence (-) of property in:			
	Chlamydiae	Mycoplasmas	Bacteria	Viruses
Ubiquitous nature	+	+	+	+
Multiplication in cell-free medium	-	+	+	-
Multiplication dependent on host cell nucleic acid	-	-	-	+
Smallest reproductive forms less than 350 nm	+	+	-	+
Cell wall or peptidoglycan present	+	-	+	-
Contain DNA and RNA	+	+	+	-
Various metabolic systems	+	+	+	-
Multiplication inhibited by:				
Antibody alone	+	+	-	+
Antibiotics	+	+	+	-

*With a few exceptions.

Fig. 1 Chlamydial growth cycle.

reorganisation to form, by 12 hours, what is known as the initial or reticulate body. This is a strictly intracellular form which is metabolically active and has a fragile envelope. Metabolism of the host cell declines, and binary fission of the reticulate body occurs to form more reticulate bodies. By 20 hours inclusions in the cytoplasm of the host cell contain mainly reticulate bodies. However, these gradually become reorganised into elementary bodies which, by 40 hours, are a major component of the inclusions (Fig. 2). Recognition of the characteristic cytoplasmic inclusions is the means by which chlamydiae are detected. Infectivity increases as the number of elementary bodies increases and by 48 to 72 hours the host cell bursts and liberates these infectious particles. The complete infectious cycle thus takes about two to three days.

Inapparent infections

Chlamydial infections may occur in an inapparent or

subclinical form. Thus, chlamydiae have been isolated from the human genital tract in the absence of symptoms (*vide infra*). Whether chlamydiae can become truly latent is, however, a moot point. Hanna *et al.*⁹ demonstrated inclusions in conjunctival scrapings in the absence of active chlamydial eye disease, but extracellular organisms might have been detected by current sensitive culture techniques. Indeed, Schachter¹⁰ finds no convincing evidence that chlamydiae persist in the intact host in a non-replicating form. It is more likely that 'latent' or subclinical infections represent persistent low levels of multiplication held in check by host defence mechanisms. This is particularly relevant to psittacosis. Apparently healthy birds may shed chlamydiae in their faeces, and such birds have often been implicated as sources of human infection. 'Latent' infection has also been established in cell cultures with various subgroup B organisms (*vide infra*) including those that cause psittacosis.¹¹

Fig. 2 Electron micrograph of a chlamydial inclusion in the mucosal epithelium of a bovine oviduct in organ culture after experimental infection. Bar = 1 μ m.

Table 2 Characteristics of subgroup A and B chlamydiae

Feature	Subgroup A (<i>C. trachomatis</i>)	Subgroup B (<i>C. psittaci</i>)
Association with human disease	Trachoma Inclusion conjunctivitis (adult and neonatal) Genital tract infections (non-gonococcal urethritis, cervicitis, salpingitis, etc.) Reactive arthritis (Reiter's syndrome) Infant pneumonia Lymphogranuloma venereum (LGV)	Psittacosis/ornithosis
Association with animal disease	Mouse pneumonia (Nigg agent)	Psittacosis/ornithosis (birds) Arthritis Conjunctivitis Abortion Intestinal infection Pneumonia Inclusion conjunctivitis (guinea-pigs) Often haematogenous spread with multiple organ involvement Large, diffuse, glycogen-negative Resistant At least three serotypes
Extent of disease	Usually localised	
Cytoplasmic inclusions	Small, compact, glycogen-positive	
Sulphonamide susceptibility	Sensitive	
Serotypes by microimmunofluorescence	A→K (A,B,Ba,C associated predominantly with trachoma and D→K with genital infections) LGV types 1, 2, and 3	

Characteristics of chlamydial subgroups

The characteristics of the two subgroups of organisms which comprise the two species within the genus *Chlamydia* are shown in Table 2. Subgroup A organisms are those within the species *C. trachomatis*, and subgroup B organisms are those within the species *C. psittaci*. Subgroup A organisms are sulphonamide-sensitive, and the compact intracellular inclusions which they produce compress the nucleus of the cell and stain with iodine because they contain glycogen. Within this subgroup are most of the organisms which cause human disease. Subgroup B organisms are sulphonamide-resistant and they produce inclusions which do not stain with iodine. They cause disease mainly in animals, psittacosis in man usually being the result of spread from birds to man. It is possible, therefore, to distinguish between organisms of subgroups A and B by sulphonamide susceptibility, by the kind of intracellular inclusions they produce, by serological means, and, to a lesser extent, by the types of disease they cause.

Laboratory diagnosis of chlamydial infection

Chlamydiae may be detected by cultural and non-cultural methods. The latter include direct microscopy of smears of cells which have been scraped from a lesion and which have been stained, for example, by fluorescent antibody. This is a good technique for seeking inclusions in conjunctival epithelial cells from cases of trachoma or paratrachoma, but it is not regarded as sufficiently sensitive for detecting chlamydial infection in the human genital tract.¹² Electron microscopy without a means of specifically identifying the organisms is

not worth consideration because of its insensitivity. In association with ferritin staining, however, the method has produced results almost comparable with those obtained by means of a cultural technique.¹³ Electron microscopy of this kind has the advantage of being able to detect non-viable organisms but its capacity to detect a few organisms has not been tested.

CULTURAL METHODS

The development of methods for the culture of chlamydiae is presented chronologically in Table 3. To isolate chlamydiae from the genital tract, secretions are taken on cotton-tipped swabs, and it is important to take the swab deeply and vigorously enough to remove epithelial cells. Nasopharyngeal and tracheal aspirates are required from infants with pneumonia. Specimens are placed in sucrose-phosphate (2 SP) medium for transportation.¹⁹ If they are to be tested within a few hours they may be kept at 4°C. Otherwise they are best placed in liquid nitrogen and can then be kept indefinitely.

Inoculation of the yolk sac of embryonated hens' eggs was employed in the past to isolate chlamydiae from genital specimens and still is by some workers in the case of psittacosis. However, this method has been superseded by the use of tissue-cell monolayers¹⁷ in which inclusions are sought. The McCoy cell line has become widely established for this purpose, but the necessity of pretreating the cells with metabolic inhibitors to increase their sensitivity has been a controversial matter. It must be concluded from the published evidence that the success of a single isolation method varies in different laboratories, and that a method regarded as the most sensitive by one group of workers may not prove so in the hands of

Table 3 Development of techniques for the culture of chlamydiae

Technique	Investigators	Year published
Yolk sac of embryonated egg for psittacosis	Bedson <i>et al.</i> ¹	1930
Tissue culture for psittacosis	Bland and Canti ¹⁴	1935
Yolk sac of embryonated egg for LGV	Rake <i>et al.</i> ¹⁵	1940
Yolk sac of embryonated egg for trachoma agent	Macchiavello ¹⁶	1944
Yolk sac of embryonated egg for trachoma agent	T'ang <i>et al.</i> ²	1957
Yolk sac of embryonated egg for inclusion blennorrhoea	Jones <i>et al.</i> ³	1959
McCoy cells for trachoma agent	Gordon <i>et al.</i> ¹⁷	1963
Irradiated McCoy cells for trachoma agent	Gordon and Quan ¹⁸	1965
Sensitivity of irradiated McCoy cells increased by centrifugation of inoculum	Gordon <i>et al.</i> ¹⁹	1969
Simplified irradiated McCoy cell procedure	Darougar <i>et al.</i> ²⁰	1971
DEAE-dextran-treated HeLa 229 cells*	Kuo <i>et al.</i> ²¹	1972
BHK-21 cells*	Blyth and Taverne ²²	1974
IdUR-treated McCoy cells*	Wentworth and Alexander ²³	1974
Cytochalasin B-treated McCoy cells*	Sompolinsky and Richmond ²⁴	1974
Replicating McCoy cells*	Hobson <i>et al.</i> ²⁵	1974
Human thyroid cells*	Hobson <i>et al.</i> ²⁶	1976
Cycloheximide-treated McCoy cells*	Ripa and Mårdh ²⁷	1977
Combination of cycloheximide-treated McCoy cells and immunofluorescence*	Thomas <i>et al.</i> ²⁸	1977
Hydrocortisone-treated McCoy cells	Bushell and Hobson ²⁹	1978

*Reported for *C. trachomatis*.

Table 4 Number of *Chlamydia trachomatis* inclusions in McCoy cells treated in various ways compared to the number in cycloheximide-treated McCoy cells*

Treatment	Number of inclusions (%)	Comments
Untreated	1	Very small dull inclusions
IdUR	12	Bright inclusions
Hydrocortisone	13	Dull inclusions
Cytochalasin B	14	Bright inclusions of variable size and shape
Irradiation	18	Bright inclusions
Emetine	48	Monolayer disrupted: not recommended
Cycloheximide	100	Bright inclusions

*Adapted from Evans and Taylor-Robinson.³¹

another. Regardless of the treatment of the cells, however, there is no doubt that the sensitivity of the cell-culture method has been increased perhaps 100-fold by centrifuging the organisms (2800 *g* for 1 hour) on to the cell monolayers.³⁰

Many workers have found that non-replicating McCoy cells provide the most sensitive culture system. Such cells may be produced by irradiating them with 4500 rads from a cobalt source at least four days before use. Irradiation of the cells inhibits division but allows the cytoplasm to increase in area so that inclusions can be detected more easily. More recently, chemical inhibitors of cell metabolism, such as 5-iodo-2-deoxyuridine, cytochalasin B, and cycloheximide, have been used as alternatives to irradiation for the pretreatment of McCoy cells. Of

these various methods (Table 4), cycloheximide treatment is simple and inexpensive, and, above all, provides the most sensitive system.³¹

Conventionally, the typical inclusions are detected by iodine or Giemsa staining (Fig. 3), and for most purposes this is adequate. However, the use of human LGV antibody followed by fluorescein-labelled anti-human antibody to stain inclusions provides a method that enables *C. trachomatis* genital infections to be diagnosed within 20 hours of a sample being taken.^{28 32}

SEROLOGICAL METHODS

Complement-fixation (CF) test

The CF test detects antibody to the heat-stable lipoprotein-carbohydrate antigen common to all members of the genus *Chlamydia*. The antigen is usually prepared from subgroup B chlamydiae.

Sensitivity Until the development of the more sensitive micro-immunofluorescence (micro-IF) test, the CF test was, and indeed still is, widely used in the diagnosis of two human chlamydial infections, lymphogranuloma venereum (LGV) (subgroup A) and psittacosis (subgroup B). These are systemic diseases in which the antigenic stimulus is considerable and the resulting antibody levels are high enough to be detected satisfactorily by this relatively insensitive test. Thus, high CF antibody titres have been reported in the sera of 98%-100% of patients with LGV.^{33 34} As a corollary, Schachter and colleagues³⁵ reported never having recovered chlamy-

Fig. 3 *Chlamydial* inclusions in McCoy cells after Giemsa staining, viewed by dark ground microscopy (original magnification $\times 900$).

diae from buboes or other sites of patients who had a negative LGV CF test.

The sensitivity of the test is low in those chlamydial infections which are limited to superficial epithelial surfaces where the antigenic stimulus, and hence the antibody response, is poor. For example, in men with chlamydial infections of the genital tract, CF antibody titres rarely exceed 1:8 or 1:16, whereas in patients with LGV, titres in excess of 1:256 are frequently detected.³⁴ Another indication of the lack of sensitivity of the CF test comes from comparing the occurrence of antibody measured by CF and by micro-IF in patients with various chlamydial oculogenital infections.¹² In patients with inclusion conjunctivitis, antibody has been detected in 50% by CF and in 100% by micro-IF; in patients with non-gonococcal urethritis (NGU) it has been found in 15% and 90% by these methods, respectively, and in those with cervicitis in 40% and 99%, respectively. In a further study of patients with urethritis or LGV, antibody measurable by micro-IF was found in 82% of sera, while only 33% had chlamydial group CF antibody, which was generally lower in titre.³⁶

Specificity A complication of the CF test is that it detects group-reactive antibody. This may not be specific to a current oculogenital infection but may be related to a previous group B chlamydial infection or to LGV. This is not, however, a serious problem in the United Kingdom since both psittacosis and LGV are relatively rare conditions. Furthermore, the occurrence of CF reactivity in the sera of patients without disease is low. Thus, only 3% of 'normal' adults and less than 1% of children have CF antibody, compared to 25%-40% and 10%, respectively, who have antibody detected by the micro-IF test.¹² However, despite the high level of specificity of the CF test, its low sensitivity must preclude it from being used as a serological test in chlamydial oculogenital infections. It remains adequate for psittacosis and LGV, and the availability of a commercially produced antigen will ensure its continued use for the diagnosis of these diseases in routine laboratories.

Micro-immunofluorescence (micro-IF) test

The micro-IF test was developed originally by Wang and Grayston³⁷ and used as a means of obtaining basic information about the classification of the 15 serotypes now designated³⁸⁻⁴¹. The test was later developed to measure antibody in experimentally infected monkeys⁴² and in humans with chlamydial infections of the eye⁴³ and genital tract.^{44 45}

Although the original micro-IF method was complicated, involving the application of spots of numerous egg-grown antigens to slides with mapping

pens, it was found to have advantages over the CF test. Thus, it allowed several serum dilutions to be tested on one slide against a number of antigens, the detection of type-specific antibody, and, as it was an indirect test, different immunoglobulin classes to be detected by the use of different antisera. Above all the test was very sensitive.

Sensitivity The results of studies in which antigens comprising either single or multiple serotypes have been used, show that about 80% of men and over 90% of women with chlamydial genital-tract infections have antibody (see later section). Geometric mean (GM) titres of antibody in men are not high, probably because the antigenic stimulus to antibody production by chlamydiae localised superficially in the urethra is minimal; titres are higher in women, and considerably higher in patients with LGV and in some with sexually acquired reactive arthritis.

Specificity In an early study,⁴⁵ antibody was found in the sera of 94% of patients with known chlamydial infections and 79% of these had a type-specific response. However, the results obtained from more widespread use of the micro-IF test suggest that both type-specific and broadly cross-reacting group-specific antibodies are being detected.^{36 46 47} Consequently, for screening studies, several workers have simplified their micro-IF test by using a single serotype.⁴⁷⁻⁴⁹ Other workers still use multiple serotypes but have simplified the test by pooling them.^{50 51} The nature of the broad cross-reactivity remains controversial. The antibody detected by the micro-IF test may be the same as that reacting in the CF test, although an attempt to remove it with a group B chlamydial serotype was unsuccessful.³⁶ Alternatively, repeated exposure to chlamydial antigens, as a result of repeated genital infections, may render the patients' sera reactive to a wide range of chlamydial serotypes.

Although the micro-IF test is sensitive, the 'non-specific' reactivity associated with it remains a problem. Thus, 23%-40% of 'normal' adults have been found to have antibody,^{12 52} while 10%-76% of men with chlamydia-negative NGU and 30%-50% of chlamydia-negative women in a 'venereal disease population' have been found seropositive (see later section). This apparent lack of specificity may be due to failure to isolate chlamydiae efficiently, to antibody remaining from a previous infection, or to non-specific cross-reactivity with antibody against an unrelated microorganism.

Despite the undisputed value of the micro-IF test in seroepidemiology, the problems of specificity raise serious doubts about its use as a diagnostic tool for individual patients with chlamydial infections. This will be discussed further in a later section.

Table 5 Isolation of *Chlamydia trachomatis* from men with non-gonococcal urethritis (NGU) and from control subjects

NGU (No. chlamydia culture-positive/no. examined (%))	Controls (No. chlamydia culture-positive/no. examined (%))	Investigators	Year published	Study in
44/99 (44)		Dunlop <i>et al.</i> ⁵⁴	1972	UK
49/135 (36)	0/31 (0)	Oriel <i>et al.</i> ⁵⁷	1972	UK
40/103 (39)	5/92 (5.4)	Richmond <i>et al.</i> ⁵⁸	1972	UK
27/76 (36)	0/57 (0)	Schachter <i>et al.</i> ⁵⁹	1975	USA
34/131 (26)		Smith <i>et al.</i> ⁶⁰	1975	USA
48/113 (42)	4/58 (7)	Holmes <i>et al.</i> ⁵²	1975	USA
33/133 (25)		Oriel <i>et al.</i> ⁶¹	1975	UK
125/262 (48)	3/74 (4)	Oriel <i>et al.</i> ⁶²	1976	UK
43/136 (32)		Prentice <i>et al.</i> ⁶³	1976	UK
23/69 (33)	1/39 (2.5)	Bowie <i>et al.</i> ⁶⁴	1977	USA
21/67 (31)	3/85 (3.5)	Wong <i>et al.</i> ⁶⁵	1977	USA
71/180 (39)		Segura <i>et al.</i> ⁶⁶	1977	USA
116/385 (30)		Alani <i>et al.</i> ⁶⁷	1977	UK
44/103 (43)		Johannisson <i>et al.</i> ⁶⁸	1977	Sweden
124/238 (52)	1/40 (2.5)	Perroud and Miedzybrodzka ⁶⁹	1978	Switzerland
93/159 (58)	0/64 (0)	Terho ⁷⁰	1978	Finland
73/284 (26)	6/92 (6.5)	Ripa <i>et al.</i> ⁷¹	1978	Sweden
39/75 (52)		Paaavonen <i>et al.</i> ⁷²	1978	Finland
35/107 (33)	6/112 (5.4)	Swartz <i>et al.</i> ⁷³	1978	USA
93/217 (43)		Coufalik <i>et al.</i> ⁷⁴	1979	UK

Radioisotope precipitation (RIP) test

The RIP test was first used to detect group specific chlamydial antibody in sera from patients with various possible subgroup B infections and in some with LGV or trachoma⁵³ and was found to be more sensitive than the CF test. The RIP test has also been used to measure antibody in sera from men with NGU.⁵⁴ It seemed slightly more sensitive than the micro-IF test since it detected antibody in the serum of 94% of patients with chlamydia culture-positive NGU compared with 86% by the micro-IF method. However, the RIP test was less specific than the micro-IF test because antibody was found in 55% of chlamydia-negative NGU patients by RIP but in only 24% by micro-IF, and in 54% and 38% of men without urethritis by the two tests, respectively. Perhaps because of this lack of specificity and the complicated nature of the RIP test, it has not been adopted by other workers.

Enzyme-linked immunosorbent assay (ELISA)

There is a single report⁵⁵ on the use of the ELISA for the detection of chlamydial group-specific antibody in patients with psittacosis or LGV. Single convalescent-phase sera, positive for chlamydial antibody by the CF test, were positive at higher dilutions by the ELISA, and rises in antibody titre were detected in paired sera by both methods. Control sera from persons with no history of chlamydial infection were negative by both tests. The results indicated that the test is more sensitive and rapid than the CF test. The assertion that it is simpler to perform than immunofluorescence

techniques is questionable although it may be easier to read the results.

There are no reports of the ELISA being used to test sera from patients with chlamydial oculo-genital infections. However, if the test were of sufficient sensitivity to detect antibody in this group of patients, it might be a useful adjunct to existing serological tests.

Association of *C. trachomatis* with genital-tract disease of men

The evidence that strains of *C. trachomatis* are a cause of certain genital-tract diseases is presented below (see also Table 10).

NON-GONOCOCCAL URETHRITIS

Isolation studies

Chlamydiae have been isolated from 25%-58% of men with NGU but from only 0%-7% of those without disease (Table 5).

Antibiotic studies

Antibiotics such as sulphafurazole (sulfasoxazole), which inhibit chlamydiae but not ureaplasmas *in vitro*, are effective in treating chlamydia-positive, ureaplasma-negative cases.⁷⁵ In addition, patients from whom only chlamydiae have been isolated respond significantly better to tetracycline therapy than patients who are given a placebo.⁶³

Serological studies

Chlamydial antibody has been found more frequently

Table 6 Relationship between IgG antibody to Chlamydia trachomatis detected by immunofluorescence methods and chlamydial isolation in men with NGU

No. patients studied	Seropositive/chlamydia culture-positive (%)	Seropositive/chlamydia culture-negative (%)	Investigators	Year published
29	15/17 (88)	2/12 (17)	Dwyer <i>et al.</i> ⁴⁴	1972
53	10/12 (83)	31/41 (76)	Philip <i>et al.</i> ³⁸	1974
103*	64/74 (86)	7/29 (24)	Reeve <i>et al.</i> ⁵⁴	1974
113	33/47 (70)	29/66 (44)	Holmes <i>et al.</i> ⁵²	1975
59*	20/20 (100)	7/39 (18)	Bowie <i>et al.</i> ^{64, 76}	1977
65	19/35 (54)	3/30 (10)	Treharne <i>et al.</i> ⁷⁷	1977
69	21/24 (88)	19/45 (42)	Richmond and Caul ⁷⁸	1977
587	156/193 (82)	270/394 (68.5)	Saikku and Paavonen ⁴⁹	1978

*Allegedly first attacks of NGU.

in patients with NGU than in persons without disease.^{12 52 64 76} Antibody is also found more frequently and in higher titres among patients who have disease and from whom chlamydiae are isolated than among those from whom these organisms cannot be isolated (Table 6). Furthermore, IgM antibody and occasionally a four-fold or greater rise in the titre of IgG antibody are found more often in patients suffering from chlamydia-positive NGU, particularly in those who are experiencing a first attack, than in those who have chlamydia-negative NGU (Table 7). Of course, antibody responses cannot necessarily be taken as evidence of a causal relationship since infection might occur and antibody develop without the organisms causing disease.

Animal studies

Intraurethral inoculation of male baboons,⁷⁹⁻⁸¹ rhesus,⁸² and macaque monkeys⁸³ and chimpanzees^{84 85} with genital strains of *C. trachomatis* has often resulted in infection as judged by the ability to recover chlamydiae consistently and, in some instances, detect antibody responses to them. Pathological changes (follicles) have been observed on urethroscopy by some investigators. Polymorphonuclear leucocyte responses have been seen too, but seldom frank urethral exudates. This is not surprising in view of the inability to control urination in these animals. The results, how-

ever, leave no doubt that Koch's postulates have been fulfilled.

POST-GONOCOCCAL URETHRITIS (PGU)

Isolation studies

As in NGU, chlamydiae have been isolated by some investigators from about 50% of men with PGU.^{52 58 67 68 86-88} Furthermore, significantly more men with gonorrhoea who harbour chlamydiae develop PGU than men who are not infected with chlamydiae.⁸⁷⁻⁸⁹ In some studies, the chlamydial isolation rate was greater at the time PGU was diagnosed than at the time of the original gonococcal infection, even though gonococci and chlamydiae were acquired presumably together. This suggests that the urethra was swabbed more efficiently for chlamydiae in the absence of gonococcal discharge and/or that time was required for chlamydiae to multiply sufficiently to allow isolation.

Antibiotic studies

Antibiotics such as ampicillin, which inhibit *Neisseria gonorrhoeae* but are not so effective against chlamydiae *in vitro*, do not prevent the development of PGU, whereas antibiotics which inhibit the multiplication of both these microorganisms do prevent its development.^{58 86 90 91}

Table 7 Occurrence of Chlamydia trachomatis IgG antibody rises and IgM antibody measured by immunofluorescence methods in men with NGU

Chlamydia culture-positive				Chlamydia culture-negative				Investigators	Year published
IgG rise (%)		IgM +ve (%)		IgG rise (%)		IgM +ve (%)			
3/12	(25)	4/12	(33)	NT		4/41	(10)	Philip <i>et al.</i> ³⁸	1974
NT		21/74	(28)	NT		1/29	(3)	Reeve <i>et al.</i> ⁵⁴	1974*
18/37	(49)	NT		3/50	(6)	NT		Holmes <i>et al.</i> ⁵²	1975
3/13	(28)	8/13	(62)	1/13	(8)	1/13	(8)	Richmond and Caul ⁴⁷	1975
11/20	(55)	16/20	(80)	2/39	(5)	3/39	(8)	Bowie <i>et al.</i> ⁷⁶	1977*
NT		29/67	(43)	NT		NT		Thomas (unpublished)	
3/22	(14)	24/37	(64)	1/38	(2.5)	4/35	(11)	Thomas (unpublished)	

*Allegedly first attacks of NGU.

Serological studies

Holmes and colleagues⁸² used the micro-IF technique to look for additional evidence of chlamydial infection in PGU with little success because they found serological evidence of recent infection in only one additional chlamydia-negative patient. In two studies,^{86 87} IgG antibody to *C. trachomatis* was found in 100% and 48%, respectively, of men with chlamydia-positive PGU, and in 45% and 25% of men with chlamydia-negative PGU. In the second study, rises in titre of IgG antibody were sought but not found although IgM was detected in 8 of 11 (73%) chlamydia-positive men who also had IgG, but in only 1 of 6 (17%) chlamydia-negative men. Apart from confirming the presence of chlamydial infection in some men with PGU, none of these results by itself is helpful in indicating a causative rôle for the organisms. The evidence for this is best provided by the isolation and antibiotic-orientated studies.

PROSTATITIS

Surprisingly, there are no reports of studies concerned with the rôle of *C. trachomatis* in acute prostatitis, although the prostate must be involved in some cases of NGU. However, Mårdh and colleagues⁹² investigated the possible rôle of *C. trachomatis* in non-acute (chronic) prostatitis. They studied 53 men but isolated chlamydiae from the urethra of only one, and from none of 28 specimens of prostatic fluid. Only 4 of the 50 men studied serologically by the micro-IF technique had IgM antibody at a titre of 1:8 or greater and, therefore, evidence of a current or recent infection. Examination of prostatic fluid was no more informative. The authors concluded that *C. trachomatis* appears to play a minor aetiological rôle, if any, in non-acute prostatitis. We doubt, however, whether this evidence is conclusive since it seems feasible that chlamydiae could infect initially and trigger the development of chronic disease and yet not be detectable subsequently.

ACUTE EPIDIDYMITIS

Isolation studies

There is obviously a problem in implicating in this disease chlamydiae which have been isolated from the urethra, particularly when a majority of the men have urethritis and other microorganisms are also recovered.⁹³ To avoid contamination from the urethra, Berger and co-workers⁹⁴ obtained aspirates directly from the epididymis and isolated chlamydiae from patients who were all under 35 years of age. This contrasted with the findings in older men from whom only Gram-negative bacilli

(coliforms) were isolated which were thought to be responsible.

Serological studies

Heap⁹⁵ detected chlamydial antibody responses by means of the CF test in two patients with acute epididymitis. Harnisch and colleagues⁹³ and Berger and colleagues⁹⁴ used the micro-IF test. They did not detect IgM antibody but they did find a fourfold or greater rise in the titre of IgG antibody in 3 of 11 patients. These observations are useful in confirming a chlamydial infection but do not, of course, by themselves specifically indicate that the infection is a cause of the epididymitis.

REITER'S SYNDROME

The major problems in attempting to associate chlamydiae with this syndrome and the investigative approaches which are likely to overcome these problems and provide the most useful information have been discussed recently.⁹⁶

Isolation studies

Since chlamydiae have been isolated in most studies from no more than 50% of patients with NGU and since only a few NGU patients develop sexually acquired reactive arthritis (SARA) or Reiter's syndrome (urethritis, arthritis, and conjunctivitis), it would be feasible for all patients with SARA or Reiter's syndrome to be chlamydia-isolate negative. Recovery of chlamydiae from the urethra was infrequent in much of the early work in which isolation was attempted in the yolk sac of embryonated hens' eggs⁹⁷ and even later when cell cultures were used.^{98 99} It seems that this was due largely to patients having been treated with antibiotics before specimens could be taken. However, chlamydiae have been isolated from the urethra of untreated patients with SARA or Reiter's syndrome as frequently as from patients with uncomplicated NGU.^{100 101} Since all patients with extragenital complications could fall into the chlamydia-negative group, these observations do not exclude the possibility that the organisms are associated with the disease.

Serological studies

Early work was based on the CF test, and most investigators, even when they later used the micro-IF technique, reported that the incidence of chlamydial antibody in patients who developed SARA or Reiter's syndrome was about the same as in patients who attended venereal disease clinics. This view was not shared by Schachter,¹⁰² who reported that 28% of patients with Reiter's syndrome had chlamydial CF antibody whereas only 5% of patients

with NGU and 9% of those with gonococcal infections possessed such antibody. This finding is not completely out of line with the results of recent studies in which the micro-IF technique with the relevant chlamydial serotypes has been used.^{101 103} These studies have been the most crucial in unravelling the rôle of chlamydiae. In that by Keat and colleagues,¹⁰¹ *C. trachomatis* IgG serum antibody titres were found to be significantly higher in SARA patients than in patients with uncomplicated NGU, or in patients with rheumatoid arthritis, systemic lupus erythematosus, ankylosing spondylitis, or in healthy persons. Furthermore, rises and falls in IgG antibody titres were detected in SARA patients, and maximal titres were reached in all cases during the period of acute joint disease. Specific IgM antibody was found in the serum of some patients early in their disease. It might be argued that the observations were due to incidental genital chlamydial infections occurring in persons who had a sexually acquired arthritis caused by some other microorganism. However, the responses were atypical of a straightforward chlamydial genital infection; nor did they appear to be anamnestic, occurring in patients who had previously experienced a chlamydial infection, because similar findings are not seen in patients with NGU who have experienced several attacks. The observations are more compatible with dissemination of chlamydial antigen providing the antigenic stimulus for an exaggerated immune response, the infection by *C. trachomatis* occurring at the time of onset of the disease and being the initiating factor in the arthritic process.

The patients in the studies mentioned above were men. Reiter's syndrome is recognised less frequently in women although cases of the incomplete syndrome, that is, cervicitis and arthritis, may be more frequent than currently thought. They need chlamydial investigation, as do patients who attend rheumatology clinics with 'seronegative' arthritis, that is, negative for rheumatoid factor.

Association of *C. trachomatis* with genital-tract disease of women

BARTHOLINITIS

Lee and colleagues¹⁰⁴ examined percutaneous aspirates from 34 intact Bartholin's gland abscesses and 12 cysts for genital mycoplasmas. Although these were recovered from the vagina of most patients, *Mycoplasma hominis* was isolated from only one and ureaplasmas from none of the aspirates. This suggests that alternative causes for the inflammation should be sought and it seems reasonable to focus attention on the possibility that *C. trachomatis*, in addition to *N. gonorrhoeae*, might be involved.

In this regard, Davies and co-workers¹⁰⁵ examined exudate expressed from the Bartholin's ducts of 30 selected patients. Chlamydiae were isolated from nine of the exudates but there was concurrent infection with gonococci in seven. The two patients from whom gonococci were not isolated were sexual contacts of men with NGU; the exudate from the duct of one patient was cloudy, and clear mucus in the case of the other. It is, of course, difficult to exclude the possibility that chlamydial isolation is merely spurious and occurs as a result of contamination of the vulval epithelium by chlamydiae derived from cervical or urethral infection. However, the authors presented three cases in which this did not seem likely since, in each, the number of chlamydiae in the duct exudate was greater than in the cervical secretion. It seems reasonable to conclude, therefore, that a true infection of the duct may occur. However, the proportion of cases of Bartholinitis that may be regarded as chlamydial rather than gonococcal, or due to some other cause, is impossible to assess on currently available data and the possibility that some gland abscesses are chlamydial in origin must remain an assumption.

CERVICITIS

Isolation studies

Jones and colleagues³ were the first to isolate *C. trachomatis* from the cervix of a woman who had given birth to a baby with inclusion blennorrhoea. Subsequently, it has been shown by numerous workers, for example, Paavonen and colleagues,⁷² that chlamydiae are recovered from the cervix of a much greater proportion of women who are partners of men with chlamydia-positive NGU than of those who are partners of men with chlamydia-negative NGU. This association is represented in Figure 4. In addition, there have been numerous studies (Table 8), the results of which show that *C. trachomatis* is recovered from cervical specimens of a greater proportion of women attending venereology clinics than of those attending other types of clinics. There is obviously no problem in isolating chlamydiae from cervical specimens of about one-quarter of women attending venereal disease clinics, but there is in deciding whether the organisms produce cervical disease. A major difficulty is that several potentially pathogenic microorganisms can usually be isolated from cervical specimens taken from women attending clinics.^{106 112} For example, double infections with chlamydiae and gonococci occur frequently: chlamydial infections have been reported in 31%-63% of women with gonorrhoea, and gonorrhoea in 18%-42% of those with chlamydial infections.^{107-109 112-114 118} Despite the problem

Fig. 4 Interrelationship of clinical manifestations of disease caused by genital strains of *C. trachomatis*.

of mixed infections, several studies in which the isolation of chlamydiae from cervical specimens has been correlated with morphological changes in the cervix have been revealing. Rees and colleagues¹¹⁹ found chlamydiae in cervical specimens from a higher proportion of NGU contacts who had 'hypertrophic erosions' (87% positive) and/or endocervical mucopus (84% positive) than of patients without erosions (31% positive) or who had clear mucus in the endocervix (29% positive). However, further insight into the changes produced by chlamydiae in the cervix has come from studies in which colposcopy and/or biopsy examination has been conducted. Dunlop and colleagues¹²⁰ were the first to associate chlamydial infection of the cervix

with lymphoid follicle formation and they likened this to the follicles seen on the conjunctivae in trachoma and inclusion conjunctivitis. They observed follicular cervicitis in 90% of mothers whose babies were suffering from chlamydial ophthalmia neonatorum. Surprisingly, no one quickly resorted to colposcopy in conjunction with microbiological techniques as an investigative procedure, and it was not until much later that Paavonen and co-workers¹¹⁵ reported the result of colposcopy on a series of premenopausal women attending a gynaecological outpatient clinic. These workers isolated *C. trachomatis* from the cervix of 13 (9%) of 144 women but, although colposcopic or biopsy examination revealed non-specific inflammatory changes in the cervix of those

Table 8 Isolation of *Chlamydia trachomatis* from cervical specimens from women attending venereal disease and other clinics and from healthy controls

Women attending venereal disease clinics (No. chlamydia culture-positive/no. examined (%))	Other women (No. chlamydia culture-positive/no. examined (%))	Investigators	Year published	Study in
83/385 (22)		Wentworth <i>et al.</i> ¹⁰⁶	1973	USA
45/247 (18)		Oriel <i>et al.</i> ¹⁰⁷	1974	UK
86/279† (31)	2/63* (3)	Hilton <i>et al.</i> ¹⁰⁸	1974	UK
38/190 (20)		Hobson <i>et al.</i> ²⁴	1974	UK
76/638 (12)		Macd. Burns <i>et al.</i> ¹⁰⁹	1975	UK
31/250 (12)		Schachter <i>et al.</i> ⁹⁹	1975	USA
	7/90** (8)			
	1/127†† (0.8)	Schachter <i>et al.</i> ¹¹⁰	1975	USA
60/300 (20)		Nayyar <i>et al.</i> ¹¹¹	1976	UK
16/45§ (35)		Bowie <i>et al.</i> ⁷⁶	1977	USA
269/1136 (24)		Ridgway and Oriel ¹¹²	1977	UK
117/196 (60)	14/16¶ (87)	Treharne <i>et al.</i> ^{51, 77}	1977	UK
53/200 (27)	2/200† (1)	Woolfitt and Watt ¹¹³	1977	UK
58/284 (20)		Oriel <i>et al.</i> ¹¹⁴	1978	UK
28/75§ (37)	13/144** (9)	Paavonen <i>et al.</i> ¹¹⁵	1978	Finland
		Paavonen <i>et al.</i> ⁷⁸	1978	Finland
	31/161** (19)	Ripa <i>et al.</i> ¹¹⁶	1978	Sweden
	20/439†† (4.6)	McCormack <i>et al.</i> ¹¹⁷	1979	USA
81/382 (21)		Thomas, unpublished		UK
29/106§ (27)		Thomas, unpublished		UK

*Family planning clinic
†Healthy controls
‡NGU contacts included
§NGU contacts
||women with 'cervicitis'

¶mothers of babies with ophthalmia neonatorum
**gynaecology clinic
††cancer screening
‡‡gynaecology clinic on University campus

Table 9 Relationship between IgG antibody to *Chlamydia trachomatis* detected by immunofluorescence methods and chlamydial isolation in women in various population groups

Population group	No. patients studied	Seropositive/chlamydia culture-positive (%)	Seropositive/chlamydia culture-negative (%)	Investigators	Year published
VD clinic	116	51/58 (88)	31/58 (53)	Richmond and Caul ⁴⁷	1975
VD clinic NGU contacts	245	67/76 (88)	83/169 (49)	Richmond and Caul ⁷⁸	1977
'Genital infections'	49	49/49 (100)		Wang <i>et al.</i> ¹²²	1977
'Cervicitis'	196	90/117 (77)	11/79 (14)	Treharne <i>et al.</i> ⁷⁷	1977
Mothers of babies with o n* 16		14/14 (100)	2/2	Treharne <i>et al.</i> ⁷⁷	1977
Gynaecological clinic (mainly NGU contacts)	149	55/58 (98)	61/91 (67)	Saikku and Paavonen ⁴⁹	1978
VD clinic	382	70/81 (87)	86/301 (30)	Thomas, unpublished	
NGU contacts	106	25/29 (86)	19/77 (25)	Thomas, unpublished	

*o n = ophthalmia neonatorum.

who harboured chlamydiae, specific changes that could be ascribed to these organisms were not found. This is difficult to understand in view of the earlier observations of Dunlop and colleagues and the more recent ones of Hare and colleagues.¹²¹ The latter investigators examined the sexual partners of men who had NGU and isolated chlamydiae from the cervix of 5 of 11 women in whom lymphocytic follicular cervicitis was diagnosed by colposcopy and/or biopsy, but from only 1 of 14 women in whom this condition was not found. The correlation between the presence of chlamydiae and the cervical changes was emphasised by a striking lack of association between *M. hominis* or *U. urealyticum* and follicular cervicitis. There seems no doubt, therefore, that chlamydiae produce cervical changes, particularly lymphocytic follicular ones, but how often they infect without causing any pathological change is not known. It is, nevertheless, important to recognise that they may infect without producing symptoms^{109 111 113} in the same way that gonococci do.

Antibiotic studies

The results of antibiotic therapy may throw light on the association of *C. trachomatis* with cervicitis. Rees and colleagues¹¹⁹ treated chlamydia-positive patients with oxytetracycline and found that in 27 of 31 patients 'hypertrophic erosions' regressed to 'simple erosions' and in the remaining four there were no erosions after treatment. In addition, the endocervical content became less purulent. Ripa and co-workers¹¹⁶ used doxycycline or trimethoprim-sulphamethoxazole to treat chlamydia-positive women with 'lower genital tract infection' (excess number of leucocytes in a vaginal smear) and reported the clearance of symptoms and signs. However, interpretation of such findings is difficult because a broad-spectrum antibiotic is likely to inhibit the multiplication of other potentially pathogenic microorganisms. It might be more rewarding to assess the result of treating women who

are chlamydia-positive only, comparing the response of this group with that of other groups of women categorised on the basis of the microorganisms that they possess.

Serological studies

Antibody measured by micro-IF has been detected in a greater proportion of patients with cervicitis who are chlamydia-positive than of those who are chlamydia-negative. Thus, antibody (GM titre of 1:66) was found by Treharne and colleagues⁷⁷ in 77% of chlamydia-positive patients who had the disease but in low titres in only 14% of chlamydia-negative patients. This finding by itself merely indicates that chlamydial infection stimulates antibody production and not that chlamydiae cause cervicitis, because similar serological results have been recorded for other groups of women from whom chlamydiae have been isolated (Table 9).

Animal studies

Some investigators^{123 124} have been able to infect the cervix of baboons by inoculating them with genital strains of *C. trachomatis*, while others⁸¹ have not been successful. Two of 14 pregnant animals inoculated with serotypes E or F by Alexander and Chiang¹²⁴ shed chlamydiae transiently, but cervical lesions were not observed and infection did not develop in the newborn infants. Recently, however, Johnson and colleagues¹²⁵ inoculated the lower genital tract of marmosets with a genital strain of *C. trachomatis* and recovered chlamydiae for several weeks, during which time there was a polymorphonuclear leucocyte response. Apart from fulfilling Koch's postulates, the model is convenient for immunological studies.

CERVICAL DYSPLASIA

Schachter and colleagues¹¹⁰ reported that they had isolated *C. trachomatis* from 4.1% of women with cervical dysplasia but from only 0.8% of all women attending a cervical smear clinic. Antibody measured

by the micro-IF technique was found at a titre of 1:64 or greater in the sera of 43% and 25% of persons in the two groups, respectively. Carr and colleagues¹²⁶ used an immunofluorescence technique to detect chlamydial antibody in cervical secretions. Eleven of 15 patients (73.3%) in whom antibody was found had Papanicolaou class II or III smears but only 3 of 18 patients (16.7%) without antibody. In selected patients, tetracycline treatment resulted in disappearance of antibody and in reversion of the smears from class II or III to class I. These findings suggest a correlation between chlamydial infection and cervical intraepithelial neoplasia, but further work is obviously required to investigate this possibility.

SALPINGITIS

Isolation studies

There have been a number of reports which indirectly have associated acute pelvic inflammatory disease (PID) with chlamydial infection,^{59 108 120 127-130} but the first direct evidence of an association was reported by Eilard and colleagues,¹³¹ who isolated *C. trachomatis* from 2 of 22 tubal specimens. Soon thereafter, Mårdh and co-workers^{132 133} isolated chlamydiae from 6 of 20 specimens taken from inflamed fallopian tubes at laparoscopy but not from tubal specimens obtained from five patients who did not have PID. Although gonococci may be isolated together with chlamydiae from the cervix of women with PID, it is of interest that in the studies by Mårdh and colleagues only one or other of these microorganisms was recovered from the tubal specimens. Specimens taken from the pouch of Douglas of patients with acute salpingitis do not seem to be worth testing for chlamydiae because Mårdh and co-workers^{132 133} did not recover them from 29 such specimens and recovered them from only 1 of 107 specimens when the series was extended.¹³⁴

Serological studies

The most comprehensive assessment of antibody to *C. trachomatis* in patients with acute salpingitis is that by Trehanne and colleagues,¹³⁵ who used the micro-IF technique to test sera from some of the patients examined by Mårdh and colleagues.^{132 133} Although, unfortunately, they did not correlate their serological findings with the recovery of chlamydiae, they did find a correlation between the severity of the salpingitis as judged laparoscopically and the magnitude of the antibody response. Thus, 73% of women with severe salpingitis had an IgG antibody titre of 1:64 or greater (GM titre of 1:122), whereas only 43% of women with mild

salpingitis had such an antibody titre (GM titre of 1:21) and only 10.5% of those without salpingitis (GM titre of 1:2). Furthermore, there was a correlation between the degree of tubal and/or pelvic inflammation and the GM titre of IgM antibody. Fluids from the pouch of Douglas of 27 women with acute salpingitis had high GM titres of chlamydial IgG and IgM antibody, and again the GM titres of both classes correlated with the degree of tubal and/or pelvic inflammation.

Paavonen and colleagues¹¹⁸ detected a fourfold or greater rise in the titre of antibody to *C. trachomatis* measured by immunofluorescence in 10 (46%) of 22 women with symptoms of acute salpingitis from whom chlamydiae were isolated from the cervix and in 7 (14%) of 50 women with symptoms who were chlamydia-culture negative.

Ripa¹³⁴ and Trehanne and colleagues¹³⁵ consider that a *C. trachomatis* serum IgG antibody titre of 1:64 or greater in a patient who has clinical signs of acute salpingitis may be taken as evidence of the disease being caused by chlamydiae, and on this basis they believe that two-thirds of all cases have a chlamydial aetiology. Certainly, such a single antibody titre cannot be regarded as diagnostic in an individual case. Furthermore, since a chlamydial antibody titre of 1:64 or greater is seen in disease of the lower genital tract, it would seem to us that the use of this figure to determine the extent of salpingitis due to *C. trachomatis* epidemiologically might provide an inaccurate assessment.

Animal studies

Ripa and colleagues¹³⁶ inoculated *C. trachomatis* organisms obtained from the fallopian tubes of patients with acute salpingitis directly into the oviducts of two grivet monkeys and through the cervical canal into the uterine cavity of another monkey. A self-limited acute salpingitis occurred in the three animals, chlamydiae being recovered up to three weeks after inoculation. At laparotomy a watery exudate was seen in the abdominal ostia of the tubes and they were swollen and reddened with an infiltrate, mainly lymphocytic, in the mucosal, muscular, and subserosal layers. In addition, there was a serological response with an IgM to IgG antibody conversion. This fulfilment of Koch's postulates adds to the earlier evidence that *C. trachomatis* is capable of causing acute salpingitis in humans.

ABORTION AND INFERTILITY

Chlamydial genital infection is one cause of abortion in various animal species¹³⁷ so it is reasonable to consider whether *C. trachomatis* might be responsible for some cases of spontaneous human abortion. In this regard, Giroud and Dumas¹³⁸ noted a correlation

between high titres of chlamydial CF antibody and the incidence of early spontaneous abortion. In addition, Schachter¹³⁹ reported the isolation of chlamydiae from the aborted products of conception, but these observations do not seem to have been followed up so far by others.

The fertility of a couple depends on the sum of the fertility of each partner. In men, it is possible that acute urethroprostatitis and epididymitis could lead to chronic disease and subsequent infertility; the rôle of chlamydiae in causing these conditions has been discussed. In women, the possibilities for chlamydial involvement are greater but have been little investigated. Chlamydiae were not found in the cervix of 40 patients attending an infertility clinic (GR Hutchinson, unpublished data) so that active chlamydial infection does not seem to have been a likely cause of infertility in this particular group of patients in north-west London. This does not exclude the possibility that active chlamydial infection could be responsible in other groups or that previous infection could lead to an infertility problem. The fact that chlamydiae have been implicated in some cases of acute salpingitis indicates that this is likely to be so. It is interesting to reflect that some of the patients seen by Weström and colleagues,¹⁴⁰⁻¹⁴² from whom gonococci could not be isolated and whose disease, therefore, might have been due to chlamydial infection, had a worse fertility prognosis than those patients from whom gonococci were recovered.

Association of *C. trachomatis* with disease in infants

NEONATAL CONJUNCTIVITIS

It was recognised at the beginning of the century that the agent which caused inclusion conjunctivitis in the newborn was present in the genital tract of the mother; intracytoplasmic inclusions similar to those produced by trachoma were seen in scrapings of the conjunctivae of infants with abacterial conjunctivitis and in those of the cervix of their mothers.¹⁴³⁻¹⁴⁵ However, as mentioned previously, it was not until 1959 that chlamydiae were recovered in the laboratory from neonates with inclusion conjunctivitis and, at the same time, from the maternal cervix.³ This initial isolation was made by inoculating the yolk sac of embryonated hens' eggs, and subsequently there have been numerous reports of isolation from the eyes of newborn infants using this method and the tissue-culture technique,¹³⁰ sufficient to establish beyond doubt that genital strains of *C. trachomatis* are one of the causes of neonatal conjunctivitis. The results of the prospective study by Alexander and colleagues,¹⁴⁶ in which half the babies born to mothers with a cervical

chlamydial infection developed conjunctivitis, mostly chlamydia-positive, while those born to uninfected mothers did not, provide further convincing evidence of this.

PNEUMONIA IN INFANTS

Isolation and serological studies

Schachter and colleagues¹⁴⁷ reported that they had isolated *C. trachomatis* from the sputum of a newborn infant with pneumonia. Contamination of sputum by chlamydiae draining from infected conjunctivae was considered but this seemed an unlikely explanation for their occurrence in sputum because conjunctival cultures were negative. More suggestive evidence to indicate that infection by *C. trachomatis* is a cause of pneumonia in infants was provided by Beem and Saxon.¹⁴⁸ They described a distinct clinical picture in which afebrile illness usually began in the second or third week of life; Harrison and colleagues¹⁴⁹ noted that most of their infants with the same syndrome were 3 to 11 weeks of age. Respiratory findings were usually dominated by tachypnoea and a distinctive cough. In severe cases, this was pertussis-like in tonal quality but differed from it in being a series of staccato coughs, each separated by a brief inspiration, without an inspiratory whoop. The course was protracted, cough and tachypnoea sometimes taking weeks to clear and radiographic changes persisting even longer. Chlamydiae were isolated from the nasopharynx of 18 of 20 infants with pneumonia. Oddly, IgG chlamydial antibody titres in their sera were much higher than those recorded in other chlamydial infections by any other group of workers. Nevertheless, the titres were significantly higher than those in the sera of infants with conjunctivitis alone, and this would be in accord with chlamydial involvement of a larger area of mucous membrane in the lung than in the eye. This finding, together with isolation of chlamydiae from a defined clinical syndrome, suggests that the organisms are one of the causes of infant pneumonia. However, caution needs to be exercised in making the diagnosis. The observation by Beem and Saxon¹⁴² that half of their 20 infants with pneumonia had conjunctivitis, as judged by history or examination, brings into focus the problem of knowing whether chlamydiae isolated from sputum or throat swabs are more than 'contaminants' from infected conjunctival secretions. This may be impossible in the absence of a distinctive clinical picture and suggestive serological data.^{148 149} Observations by other workers are needed and, indeed, are being made,¹⁵⁰ but obviously they must not believe that isolation from the throat necessarily means that the organisms are the cause of the

pneumonia. The isolation of *C. trachomatis* directly from lung tissue of an infant with pneumonia¹⁵¹ is important in this regard, although in the case reported by Frommell and colleagues¹⁵² it was not conclusive, since cytomegalovirus was also isolated. Hobson and Rees¹⁵³ have reported that, even in an area where chlamydial infection of the genital tract and eye is common, infant pneumonia has not been seen. This, of course, might be used as an argument in favour of pneumonia not being caused by *C. trachomatis* and is a good reason for continued investigation.

Animal studies

Harrison and colleagues¹⁵⁴ inoculated three infant male baboons with a strain of *C. trachomatis* that had been isolated from a human infant with pneumonia. Two baboons inoculated intratracheally developed pneumonia and organisms, antigen, and inclusions were found in the lungs of one of them. The third baboon inoculated nasopharyngeally was not sacrificed but organisms were recovered from the nasopharynx for 49 days. All three animals seroconverted. These data support the assertion that *C. trachomatis* is the causative agent in a proportion of cases of pneumonia in human infants.

Association of *C. trachomatis* with other diseases

LYMPHOGRANULOMA VENEREUM

This is a sexually transmitted disease, prevalent in many tropical countries, in which there are generalised constitutional symptoms and a wide spectrum of genital lesions. These may be acute, or chronic with gross destruction of perineal tissue. Direct evidence that the disease is caused by an infectious agent, as opposed to epidemiological evidence, was the transmission of infection to monkeys by intracerebral inoculation¹⁵⁵ and to mice by the same route.¹⁵⁶ Rake and colleagues¹⁵ were the first to isolate a chlamydial agent from the disease in the yolk sac of developing chick embryos. Subsequently, this has been standard practice in diagnosis although tissue-culture techniques are now preferred. Immunologically, three aspects have been valuable in helping to demonstrate the chlamydial cause of LGV: (i) Frei¹⁵⁷ introduced the intradermal test which for many years was the main approach to diagnosis. This test did not necessarily provide evidence for the chlamydial nature of the disease because it was originally performed with human pus so that a positive reaction could have been due to constituents other than chlamydiae. This was less likely when the test was later performed with a heat-inactivated yolk-sac culture of the LGV agent ('lygranum'); (ii) A fourfold or greater rise in the

titre of antibody to an LGV CF antigen in the course of a suspected illness is regarded as diagnostic but is rarely seen because of the chronic nature of the disease. However, in 80% of isolate-positive LGV patients, the serum CF antibody titre is 1:128 or greater¹⁵⁸. A titre of this magnitude is rarely observed in other *C. trachomatis* infections; (iii) The advent of the micro-IF test allowed isolates to be typed and antibody responses to the specific serotypes to be measured. Serotypes specifically associated with the disease have been designated L1, L2, and L3 and a micro-IF antibody titre of 1:512 or greater to one or more of these types is usually recorded. Such an antibody titre is not seen in other sexually transmitted chlamydial diseases except salpingitis, perihepatitis, and Reiter's syndrome.

PERIHEPATITIS AND ENDOCARDITIS

Müller-Schoop and colleagues¹⁵⁹ diagnosed acute peritonitis by laparoscopy in 11 young women, seven of whom also had fibrinous perihepatitis (FitzHugh-Curtis syndrome). A very high titre of IgG antibody to *C. trachomatis* in the serum of one of the patients who had no signs of gonococcal infection prompted a serological investigation of all the patients. Nine of them had evidence of a recent infection by *C. trachomatis* as judged by the presence of IgM antibody or an IgG antibody titre of 1:1024 or greater. The diagnosis in four of these patients was complicated because they had evidence of simultaneous gonococcal infection, but there was no such evidence in the five others so that chlamydial infection seemed a likely explanation for their disease.

C. psittaci is known to be a rare cause of endocarditis, but *C. trachomatis* had not been proposed as a cause until the recent report of van der Bel-Kahn and colleagues.¹⁶⁰ They investigated a 25-year-old pregnant woman who presented with fever and died after a short fulminating illness. Multiple blood cultures were negative but particles which looked like chlamydial elementary bodies on electron micrographs of a vegetation of the aortic valve led to a retrospective serological study by the micro-IF technique. A rise in the titre of IgM and IgG antibody indicated that there had been infection by serotype F of *C. trachomatis*.

GASTROINTESTINAL DISEASE

Schachter and colleagues¹⁶¹ studied 12 infants and isolated *C. trachomatis* not only from the conjunctiva and respiratory tract but also from the vagina and rectum, rectal cultures being positive in four instances. The various anatomical sites may become infected by 'contamination' at birth but in

the case of the rectum the authors regarded this as doubtful since positive rectal cultures were not obtained until the infants were 40-80 days of age. It may be that they were transmitted gastrointestinally from the conjunctivae or respiratory tract to the lower intestinal tract before sufficient gastric acid had been produced to kill them. This and the possibility that chlamydiae may cause gastrointestinal disease in infants need further investigation.

Genital strains of *C. trachomatis* have been isolated from the oropharynx of adults^{162 163} but it is debatable whether they cause pharyngitis.¹⁶⁴ It is unlikely that they are able to negotiate passage through the adult stomach without being killed so that infection of the rectum of adults^{120 165} is probably acquired through anogenital contact. The organisms may cause proctitis but this has not yet been proven unequivocally, although proctocolitis, with the passage of blood, mucus, and pus, and stricture formation possibly developing later, are recognised manifestations of LGV.

Crohn's disease

Schuller and co-workers¹⁶⁶ reported that they had detected *C. trachomatis* antibody by the micro-IF test in the serum of 38 (69%) of 55 patients with Crohn's disease, but in only 2 of 21 patients with other gastrointestinal disorders, and that the antibody was directed specifically against the LGV serotypes. They did not consider that they could necessarily interpret their findings to mean that chlamydiae were important in the pathogenesis of the disease. They did suggest, however, that the presence of antibody in the serum of patients with Crohn's disease might reflect passage of antigen non-specifically across a damaged small bowel and that the antibody test was helpful in distinguishing Crohn's disease from other inflammatory bowel diseases. We also examined by the same technique serum samples from 55 patients with Crohn's disease and from 23 patients with ulcerative colitis for antibodies to *C. trachomatis*.¹⁶⁷ Antibody titres of 1:8 or greater against several serotypes were detected in the serum of 14.5% of patients with Crohn's disease and in 21.7% of those with ulcerative colitis. It will be appreciated that these figures resemble the incidence in a healthy, non-venereal disease population. Furthermore, there was no correlation between the presence of antibody and such factors as duration of symptoms, localisation of disease, or disease activity. Despite the caveat by Munro and co-workers,¹⁶⁸ our findings have been corroborated by those of Swarbrick and colleagues¹⁶⁹ and they do not lead us to believe that chlamydiae are involved in Crohn's disease or that examination for chlamydial antibody is helpful in diagnosis.

How much genital-tract and associated disease is caused by *C. trachomatis*?

A subjective appraisal of the evidence that chlamydiae cause the various diseases previously mentioned is shown in Table 10. This, in effect, is a summary of the previous sections. If chlamydiae are a cause of a particular disease, the question of what proportion of cases is attributable to chlamydiae then arises. On a world-wide basis, the extent to which *C. trachomatis* causes genital-tract and associated disease is difficult to assess because, as shown in Tables 5 and 8, reports of successful attempts to isolate chlamydiae have come entirely from developed countries. Furthermore, in any particular country, the prevalence of chlamydial infection in one geographical location may differ greatly from the prevalence in another. The extent to which chlamydiae might be the cause of each disease which we have considered has been discussed to some extent previously and is presented in Table 10. The problem of making this analysis is seen when an attempt is made to estimate, for example, the prevalence of chlamydial infection in neonatal conjunctivitis and pneumonia. Prentice and colleagues¹⁷⁰ did not isolate chlamydiae from 104 consecutive cases of neonatal conjunctivitis whereas Rees and colleagues¹³⁰ recorded an isolation rate of 32% in a selected series of cases in a different part of the United Kingdom. Alexander and co-workers¹⁴⁶ in the USA found that 12.7% of 142 unselected pregnant women had chlamydial infection of the cervix, and conjunctivitis developed in 9 (50%) of 18 infants born to these women. Schachter¹⁷¹ reported that only 5% of the pregnant women studied by his group had chlamydial infection of the cervix but the conjunctivitis attack rate was similar, 10 of 25 infants at risk acquiring laboratory-proven disease. Thus, based on these figures from the USA, if the cervical carrier rate is 5%-13%, 2%-6% of all newborn infants will develop inclusion conjunctivitis. We suspect that this is an overestimate of the disease throughout the world and it does not, of course, give any indication of the proportion of neonatal conjunctivitis cases that are due to chlamydiae.

Pneumonia in infants, caused by chlamydiae, must occur as a result of transmission of the organisms directly from the maternal genital tract, or indirectly via infectious conjunctival secretions. It seems reasonable to suppose, therefore, that a knowledge of the frequency of neonatal chlamydial conjunctivitis might provide some clue to the frequency of chlamydia-induced pneumonia. Obviously, since the lung is less exposed to infection than the eye, it seems likely that the incidence of

Table 10 Assessment of the extent to which *Chlamydia trachomatis* is involved in various diseases

Disease	Evidence that <i>C. trachomatis</i> is a cause*	Proportion due to <i>C. trachomatis</i>
NGU	++	Up to 50%
PGU	++	Up to 50%
Prostatitis	-	...
Epididymitis	±	?
Reiter's syndrome	+	About 40%
Bartholinitis	±	?
Cervicitis	++	About 50% with follicular cervicitis
Cervical dysplasia	-	...
Salpingitis	++	Up to 66% ??
Abortion	-	...
Infertility	+	?
Neonatal conjunctivitis	++	?
Neonatal pneumonia	+	30% ??
LGV	++	100% by definition
Perihepatitis	+	?
Endocarditis	+	?
Gastroenteritis	-	...
Crohn's disease	-	...

* ++ unequivocal, + good but less convincing, ± dubious, - none

infant chlamydial pneumonia will be less. Indeed, Schachter¹⁷¹ reported the detection of only two cases of chlamydial pneumonia in infants during a prospective study of 500 pregnant women, and based on this and serological data he estimated that there are three to four cases of chlamydial pneumonia per 1000 live births in the USA, a figure later revised to 8 cases per 1000 births.¹⁷² However, as pointed out previously, the incidence is likely to vary widely in different parts of the world and in different population groups within one country, depending upon the incidence of chlamydial infection of the female genital tract. In these circumstances, it is easy to understand why the proportion of infant pneumonia that has a chlamydial aetiology is difficult to assess. *C. trachomatis* accounted for about 30% of all the pneumonias in hospitalised infants less than 6 months of age during the study by Harrison and colleagues in Seattle.¹⁴⁹ This suggests that *C. trachomatis* may be responsible for many pneumonias previously considered to be viral, but how representative this figure is of the prevalence of chlamydial disease in other parts of the world is unknown.

It is clear from the foregoing that the figures shown in Table 10 can be only an estimate. Furthermore, two other factors make an accurate assessment impossible. These may be presented as follows: (i) because chlamydiae have been isolated not only from women but also from men who are not suffering from symptoms, the isolation of chlamydiae

from a patient with disease does not mean that the organisms unquestionably cause the disease, although this is usually the case; (ii) it is possible that the genital-tract disease of patients from whom chlamydiae cannot be isolated is, in fact, chlamydial in origin. Thus, it is possible that in a patient who has experienced one or more episodes of chlamydial infection, chlamydiae could stimulate disease but be eliminated before the opportunity to isolate them arises and so give the illusion of non-chlamydial disease.

Mechanisms underlying the pathogenicity of chlamydiae

ADHERENCE TO CELLS

Cell attachment is necessary for both the pathogenicity and survival of chlamydiae, being the first step in the intracellular part of the growth cycle. Projections have been observed on the surface of *C. psittaci* organisms,¹⁷³ but whether they are concerned in attachment to cells or in phagocytosis is unknown. However, ingestion of chlamydial organisms involves heat-sensitive binding sites on their surfaces, and trypsin-sensitive host cell receptors for *C. psittaci*¹⁷⁴ and sialic acid receptors for *C. trachomatis*.¹⁷⁵ Attachment and infection are also dependent on cell surface properties which can be modulated *in vitro* by growth of cells in different sera¹⁷⁶ and by centrifugation.¹⁷⁷ An understanding of the mechanisms involved in the binding of chlamydiae with cell surfaces *in vitro* may be helpful in designing more effective isolation procedures, but it is difficult to see how centrifugation-induced cell-surface changes are relevant to the pathogenicity of chlamydiae in naturally occurring infections.

TOXIC FACTORS

Large numbers of *C. psittaci* organisms kill mice faster than can be accounted for by multiplication of the organisms.¹⁷⁸ In addition, large numbers of these and *C. trachomatis* organisms, living or ultra-violet light-inactivated, rapidly kill cell cultures.¹⁷⁹ This phenomenon, described as 'immediate toxicity', is apparently not due to either exotoxin or endotoxin activity as seen with various bacteria. It has been suggested that it is due to the formation of a lesion in the host cell membrane each time it ingests a chlamydial organism and that the rapidity of cell death is related to an increasing number of lesions. Whether this mechanism operates under more natural conditions where smaller numbers of organisms are likely to be found is dubious; smaller

numbers of organisms need to multiply for death of the cell to occur.

IMMUNOLOGICAL FACTORS

It is obvious that chlamydiae can cause cell death but dead epithelial cells do not constitute all the pathological changes that are attributable to chlamydial infection. This point is emphasised by the fact that infection of human and bovine oviduct organ cultures by *C. psittaci* or *C. trachomatis* organisms causes minor histopathological changes and negligible functional damage¹⁸⁰ and yet chlamydial infection in women is regarded as a cause of acute salpingitis. Organ cultures do not, of course, incorporate the immunological mechanisms of the host so that by inference it seems likely that such mechanisms play an important part in the disease process.

Whether sensitisation of the immune systems of the host by previous infection is an important factor in chlamydia-induced genital-tract disease is obviously a relevant consideration. Indeed, the question arises whether primary contact with chlamydiae causes disease or whether it occurs only secondarily to a previous clinically inapparent infection. The problem is that it is particularly difficult to determine whether a patient allegedly suffering from a primary genital infection has been infected previously. This difficulty does not arise in the case of infants who develop conjunctivitis soon after birth because infection must result from primary contact with chlamydiae in the maternal genital tract. However, even here sensitisation may play a part because it has been shown that infant monkeys (*Macaca cyclopis*), born to mothers with infected cervixes, develop a more severe conjunctivitis when challenged than those born to uninfected mothers.¹²⁴ Again, infant pneumonia caused by chlamydiae probably occurs as a result of a primary infection, although Beem and Saxon¹⁴⁸ do not discount the idea of a hypersensitivity reaction being involved. Although it is obviously difficult to establish convincingly that human disease is caused by a primary infection in an unsensitised host, the fact that this can be demonstrated in animal models (*vide supra*) suggests that it is likely in man. The question then arises whether successive infections, which are likely in the genital tract, produce progressively more severe disease. There is no definitive answer to this, but it is probable because (i) multiple infections of the eye by strains of *C. trachomatis* which cause trachoma result in more severe disease,¹⁸¹ and (ii) multiple infections of the guinea-pig eye with the guinea-pig inclusion conjunctivitis agent cause conjunctivitis, which becomes more severe with each successive infection.⁸¹

The usefulness and limitations of chlamydial detection procedures and of serology

CHLAMYDIAL DETECTION PROCEDURES

The question often arises whether there is a need to have a laboratory service for isolating chlamydiae from patients with genital infections.¹⁸² In the case of men suffering from NGU, it seems to us that the usefulness of the result would not justify the laboratory effort required, since establishing whether chlamydiae were present or not would not alter the patient's treatment. Most laboratories will provide the results of culture attempts after patients have been treated, although by using a rapid isolation technique and by rearranging clinic routines, it is possible to have culture results before treatment starts.³² Even so, at the moment, knowledge of the culture results will not influence treatment. If chlamydiae develop resistance to tetracyclines, we can foresee that there will be greater need to isolate and perform antibiotic sensitivity tests for the management of patients. A proposal to have a chlamydial culture service for women¹⁸² seems sensible, at least for some. In the case of contacts of men with NGU, since men with chlamydia isolate-negative disease respond to tetracycline therapy, it does not appear logical to treat with tetracyclines only those women who are found to harbour chlamydiae. If this is so, a specific chlamydial diagnosis seems unnecessary. However, many women attend clinics for other reasons, and it is apparent that some who are chlamydia-positive would undoubtedly go untreated if facilities for culture were not available.¹⁸² In addition, because not all neonatal conjunctivitis is chlamydial, it is helpful to know which cases are chlamydia-positive so that those needing treatment receive it and unnecessary broad-spectrum antibiotic therapy is avoided. Of course, chlamydial detection methods are an integral part of any programme concerned with an understanding of the aetiology, pathogenesis, and, indeed, epidemiology of genital infections. We must emphasise, however, that it is necessary to think in terms of 'cost-effectiveness' or 'cost-usefulness', and we do not believe that at the present time the usefulness of a routine chlamydial isolation service for all patients with genital infections justifies the cost of undertaking it.

SEROLOGY

Most chlamydial infections of the genital tract provoke serological responses which are difficult to interpret because (i) most are limited to the epithelial cells of mucous membranes so that the antigenic stimulus is minimal and, in consequence, the humoral antibody response is poor; (ii) infections may be

inapparent, especially in women, and yet provoke chlamydial antibody production. The mere presence of antibody cannot, therefore, be used as confirmatory evidence of chlamydial infection in current disease; (iii) patients with symptoms may not present for treatment until days, or sometimes weeks, have elapsed, when the serological indicators of a current infection, namely, IgM antibody and a rising IgG antibody titre, are no longer detectable; (iv) patients, especially those suffering from NGU, frequently experience a relapse which is often impossible to distinguish from a reinfection. In addition, many patients are repeatedly exposed to chlamydiae and reinfections are common. This makes serological diagnosis of each new attack impossible; (v) there is little information on the time required for antibody to develop or on its duration following cure of disease. Thus, even if a patient is able to give an accurate history of past and present infections, serological data may be difficult to evaluate. Some of these problems may be illustrated by reference to the results of antibody studies of NGU (Tables 6 and 7) and of disease in women (Table 9).

Infections in men

In one study, only 54% of men with chlamydia-positive NGU had IgG antibody⁷⁷ while in another, IgG antibody was found in the sera of all of 20 men who were allegedly experiencing their first attack of chlamydial NGU.⁷⁶ This demonstrates the variation in results of different studies and indicates that not all men with chlamydia-positive NGU are found to be seropositive. It has been our own experience that 10%-20% of men with chlamydia-positive NGU never produce any IgG antibody throughout the course of the disease; serodiagnosis is obviously precluded in this group. Conversely, as shown in Table 6, between 10% and 76% of the NGU patients from whom chlamydiae could not be isolated had IgG antibody but, as we have stressed, it is not possible to attribute this to their current disease. Our data on the persistence of antibody in NGU show that this IgG antibody might be that remaining from a chlamydial infection which had occurred as long as a year previously (PE Munday, personal communication). Overall, between 34% and 77% of all the NGU patients, either chlamydia-positive or chlamydia-negative, in the studies shown in Table 6 had IgG antibody. In the absence of isolation studies, these findings mean only that these men had been exposed sometime in the past to chlamydiae.

In comparison with antibody titres in single serum samples, rising titres of IgG antibody and IgM antibody levels (Table 7) are seen less frequently

among patients in either the chlamydia-positive or chlamydia-negative groups. This is due partly to IgM antibody being produced only in response to a primary chlamydial infection with a particular serotype, and partly to the difficulty of obtaining two appropriately spaced sera to detect a rise in the titre of IgG antibody. However, these serological results, even in the absence of chlamydial isolation studies, are indicative of a current or recent chlamydial infection and they illustrate that the micro-IF test can be useful occasionally for serodiagnosis.

Infections in women

A consistently high proportion of women in most studies have been shown to have IgG antibody (Table 9). This is due in part to asymptomatic chlamydial infections remaining untreated and so providing a continuous antigenic stimulus. In addition, a larger surface area of the genital tract is possibly involved in women than in men, so that the antigenic stimulus and the resulting antibody response are greater. However, IgG antibody is not detected in a proportion of women with chlamydial cervical infection and it may be that they are harbouring the organisms which are not present in sufficient numbers to provoke an antibody response. In the absence of isolation attempts, chlamydial infections in these women would go undetected by serological testing.

As in men, IgG antibody is detected in a variable but generally large number of chlamydia culture-negative women (Table 9). As a corollary, it can be calculated from two studies⁴⁷ (BJ Thomas, unpublished data) involving unselected women attending VD clinics, that 49% of all the sera which contained antibody came from women who were isolate-negative. This antibody does not indicate a current infection; the main reason for its presence is the occurrence of previous chlamydial infections, of which many women will be unaware. Relatively high levels of antibody produced by previous infection probably persist for many months after chlamydiae have been cleared. Serodiagnosis of chlamydial cervical infection is therefore impossible on the basis of a single IgG antibody titre.

Most women are unaware that they have a chlamydial cervical infection unless it becomes severe, or until they have an infant with ophthalmia neonatorum. Thus, it is likely that a diagnosis will be made after IgM antibody has disappeared and a rise in the titre of IgG antibody has already occurred. The study of these antibody responses would, therefore, seem to be of even less value than in men. However, the results of a limited number of studies⁴⁷ ⁷⁸ (BJ Thomas, unpublished data) have shown that IgM antibody is present in about one-third of

women with chlamydial cervical infection and in 5%-35% of isolate-negative women. Although there are currently no data available on the frequency of rising titres of IgG antibody in women, IgM antibody could be assumed to signify recent infection with chlamydiae, even if isolation studies had not been performed.

A serodiagnostic test for chlamydial infection should fulfil the strict criteria laid down in the past for the serodiagnosis of other infectious diseases, namely, detection of IgM antibody in serum and/or demonstration of a fourfold or greater rise in IgG antibody in paired serum samples. These conditions, as we have illustrated, are not frequently encountered in genital chlamydial infections, but this should not be considered a reason for adopting less strict, and thus more dubious, criteria for diagnosis. The presence of IgG antibody to chlamydiae in a single serum sample indicates only that the immune system has, at some time, been stimulated by chlamydial antigens. However, it would not seem unreasonable in certain circumstances, for example Reiter's syndrome of recent onset, to infer that an abnormally high titre of IgG antibody in a single serum sample was suggestive of recent chlamydial infection. Furthermore, determination of antibody titres in single serum samples provides useful information on the extent to which a particular population group, for example patients with Crohn's disease, has been exposed to chlamydial infection, but as we have stressed, it is of little value in the diagnosis of individual infections. However, does an assessment of antibody in local secretions help?

Antibody in local secretions

Tears IgG antibody and lower concentrations of IgA antibody have been found in tears, and titres of both classes are usually lower in tears than in serum. There is little evidence of secretory IgA antibody formation in tears; most evidence suggests that antibody is largely derived by transudation across inflamed mucous membranes. Despite local antibody being only a reflection of serum antibody, many workers have shown that the presence of antibody to *C. trachomatis* in tears is evidence of active trachoma, whereas antibody in serum has little or no correlation with active clinically or microbiologically confirmed disease.^{43 77 181} In patients with active paratrachoma, or 'trachoma of genital transmission',¹⁸³ antibody in tears has been shown to be similarly associated with active disease.

Genital-tract secretions In view of the observations above, it is plausible that antibody in genital-tract secretions might be an indicator of chlamydial genital disease. In this regard, the observations of Trehanne and colleagues¹⁸⁴ are interesting. They

detected IgG and IgA antibodies to *C. trachomatis* in cervical secretions of women with non-specific genital infection and suggested that cervical antibody of either class at a titre of 1:8 or greater is closely associated with isolation of chlamydiae and that its detection may be used diagnostically. Forty-one percent of 272 women were regarded as chlamydia-positive on the basis of isolation, serum IgM, serum IgG antibody at a titre of 1:64 or greater, or cervical secretion IgA or IgG. However, since cervical antibody was found in only 27 of 35 women from whom chlamydiae were isolated, we must conclude that the value of detecting this antibody in diagnosis remains equivocal, at least until better evidence of its correlation with disease is available. Results of such studies in women are awaited with interest.

In men with NGU, Ng and colleagues¹⁸⁵ found local chlamydial antibody in 64% of men from whom chlamydiae were isolated and in 17% of those whose discharge did not yield chlamydiae. To what extent the presence of the antibody can be specifically associated with current disease needs studying. It will also be worth determining whether local antibody can be correlated with recent or current infection in chlamydia culture-negative NGU as a means of assessing whether such cases are in any way associated with chlamydiae.

Other secretions Trehanne and colleagues¹⁸⁵ examined fluids aspirated from the pouch of Douglas of six women with acute salpingitis. High titres of IgG and IgA antibodies were found in half of them, but apart from one case where the antibody titres in the pelvic fluid were greater than in the serum, the titres in the fluids reflected those in the sera. In these circumstances, examination of pelvic fluid seems to hold no advantage over examination of serum. Likewise, from a diagnostic point of view, it is not worth examining synovial fluids from patients with SARA or Reiter's syndrome because titres of IgG antibody to *C. trachomatis* in synovial fluids never exceed those in matched sera from the same patients.¹⁰¹

Vaccination prospects

Patients with chlamydial infections are prone to relapse of disease and, as indicated previously, sequelae may develop in both men and women which would seem to make the prevention of initial disease a worthwhile goal. The question of who should be vaccinated and the public acceptability of a vaccine programme are issues which would inevitably arise if vaccination became a feasible proposition. To what extent it seems feasible may be judged by considering several immunological

aspects. Most of the information concerns chlamydial infection of the eye, but inferences may be drawn in relation to genital tract infections.

WHAT IS THE EVIDENCE THAT CHLAMYDIAL INFECTION OF THE EYE OR GENITAL TRACT LEADS TO RESISTANCE TO REINFECTION?

Observations on animals

Guinea-pigs infected in the eye with the guinea-pig inclusion conjunctivitis agent have often been used as a model for the study of human trachoma, although the disease lacks the chronicity of the human disease. The model has, however, some relevance to the study of human chlamydial oculo-genital infections because intraurethral inoculation of male animals results in an infection which may be transmitted to the genital tract of the female, and thence to the eye of the offspring.¹⁸⁶ Male animals so infected become completely resistant to reinfection of the urethra but are still susceptible to infection of the eye four to six weeks later.¹⁸⁷ Vaginal infection also provides immunity of a similar kind.^{188 189} Infection of the eye, however, results in resistance not only to ocular but also to genital infection six weeks later. These observations suggest that immunity is not dependent entirely upon a local phenomenon, although the presence of serum antibody does not bear a significant relationship to immunity,¹⁸⁸ nor does passively transferred serum antibody confer immunity on a recipient.

Observations on man

Although individuals may experience multiple attacks of NGU, there is a dearth of information on whether successive episodes are chlamydial in origin. Our own experience from observations over several years is that chlamydial urethritis may be followed by further episodes of disease from which chlamydiae cannot be isolated. If we presume that some of the subsequent episodes are due to chlamydiae, it is not known whether they occur as a result of infection with the same chlamydial serotype, immunity having waned, or whether they occur only with other serotypes to which there is no immunity. A second infection with a different serotype from the first has been recognised by detecting specific IgM antibody to the second serotype in the presence of IgG antibody to the first one.⁴⁵ Such observations are, however, sparse, and clearly there is a lack of basic information about immunity produced by chlamydial infection of the human genital tract.

WHAT IS THE EVIDENCE THAT VACCINATION PROTECTS AGAINST DISEASE?

Observations on animals

Early experiments on vaccination of monkeys were encouraging. Protection against ocular disease or a diminution in the severity of disease after challenge with live organisms was attained in monkeys by use of formalised vaccines,¹⁹⁰ and in baboons by subcutaneous and intravenous inoculation of live trachoma elementary bodies.¹⁹¹ However, challenge with a more virulent strain caused disease and, furthermore, Grayston and colleagues¹⁹² noted that monkeys which became infected despite immunisation suffered more severe disease than unimmunised animals as a result of a hypersensitivity reaction. Several factors were found to be vital in producing immunity in monkeys.^{193 194} Vaccines containing at least 10⁹ purified, inactivated elementary bodies per millilitre were necessary to provide protection, lower doses resulting in more severe disease; vaccination protected only against challenge with the same serotype, and multivalent vaccines were less effective because they contained fewer organisms of each serotype; mineral oil-emulsified vaccines were found to be most protective.

Another approach to immunisation was the topical instillation of killed, purified chlamydiae which cause trachoma into the conjunctival sac of owl monkeys. This resulted in a smaller number of conjunctival inclusions after challenge than in control animals and an increase in antibody titres in serum and eye secretions, antibody in both appearing to contribute to protection.¹⁹⁵ However, local antibody appears to be the more important in this regard because, in another study, immunity was found not to be related to serum antibody levels and no protection against challenge with infectious homologous trachoma organisms was conferred on owl monkeys by passively transferring to them sera containing specific antibodies.¹⁹⁶

The guinea-pig model has also been explored for testing the effectiveness of vaccines. Formalin-killed preparations of the guinea-pig inclusion conjunctivitis agent given intraperitoneally and intramuscularly as single doses or with booster inoculations failed to produce any significant immunity to subsequent challenge with the live organisms,¹⁸⁸ although high titres of serum antibody were attained.¹⁹⁷

Observations on man

Blind volunteers with trachoma infection showed a 'greater tendency' to spontaneous cure of their eye infection after being inoculated with formalised, purified trachoma elementary bodies than a similar

group given a placebo.¹⁹² Studies on normal volunteers showed that most vaccinated subjects were immune to challenge. However, as in the monkeys, those who developed disease had symptoms which were much more severe than in a natural infection, especially so if they had high titres of complement-fixing antibody in their sera. It was suggested that the method of immunisation played a vital role; large single doses of vaccine were found to favour a hypersensitivity reaction, while repeated small doses depressed hypersensitivity and provided protection.

Vaccine field trials Results of trachoma vaccine field trials point to the same major and somewhat disappointing conclusions. Firstly, although the incidence of trachoma was reduced in the immunised groups in some studies,¹⁹² in others only the number of conjunctival inclusions found in infected individuals was reduced.¹⁹⁸ Further, while the effects of vaccination were noticeable in the population after six months, protection was very short-lived, varying from one year to 3·5 years after the second dose of vaccine.^{192 199 200}

In summary, resistance to reinfection by chlamydiae is dependent largely on local antibody production. This is stimulated best by natural infection but also by killed vaccines given locally. Parenteral vaccination has not been very successful and may result in more severe disease than experienced by unvaccinated controls. In human subjects, it is very unlikely that administration of a vaccine locally into the genital tract would be either a feasible or acceptable procedure. Recently, it has been suggested that resistance to chlamydial infection of the eye and genital tract may be induced by enteric vaccines.²⁰¹ After oral administration of live guinea-pig inclusion conjunctivitis organisms, guinea-pigs were found to be partially protected against disease produced by live organisms introduced into the conjunctiva and the vagina. Application of antigens (non-chlamydial) to gastrointestinal mucosa has been shown to result in synthesis of secretory IgA at distant sites,²⁰¹ and a similar phenomenon may account for the observations in the guinea-pig. If this approach were to be used in man, prevention of infection rather than just prevention of disease would be desirable. Furthermore, as for all human vaccines, at some stage it would seem necessary to administer enteric vaccines, killed and perhaps live, to human volunteers, followed by infectious challenge. This would appear to be a rather daunting proposition in what, in general, is a bleak outlook. We agree with Grayston and Wang²⁰² that control of chlamydial genital infections through immunisation is not as promising an approach as education, diagnosis, treatment, and contact tracing.

Conclusions

The reasons for believing that certain serotypes of *C. trachomatis* cause genital-tract and associated diseases in men and women, and also disease in infants, have been discussed. A subjective quantitative summary of the evidence is presented in Table 10. It is clear that for some conditions the evidence for a relationship with chlamydiae is poor or non-existent, and further work is required to establish or refute whether the organisms are a cause. An analysis of the proportion of cases of each disease attributable to chlamydiae is also provided in Table 10. In many instances the information is lacking or speculative guesses only can be made. Chlamydial culture techniques have become more sensitive and simple, but thought should be exercised in establishing a routine culture service. This would seem to be of no value in the management of male patients but in some women and infants it would be helpful in pointing to correct management and treatment. Apart from a few circumstances, serological investigations do not help in establishing a diagnosis. Surprisingly, perhaps, almost no information is available on the development or otherwise of immunity to human genital tract chlamydial infections. Studies on trachoma and experiments in animals suggest, however, that the outlook for successful human vaccination, even if acceptable, is bleak. Apart from filling in the gaps in Table 10, further work should be directed towards determining (i) whether isolation techniques are optimal, (ii) whether detection of antibody in local secretions is helpful in diagnosis, (iii) the extent to which chlamydia culture-negative cases of disease might, in fact, be chlamydial, and (iv) the rôle of immunological mechanisms in pathogenesis.

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