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Ureaplasma urealyticum Intrauterine Infection: Role in Prematurity and Disease in Newborns

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INTRODUCTION

Few areas of scientific investigation have been as controversial as that of the role of *Ureaplasma urealyticum* in human disease, particularly as it relates to pregnancy outcome (11). Over the years, ureaplasmas have been implicated in infertility, spontaneous abortion, stillbirth, premature birth, low birth weight, and perinatal morbidity and mortality (11). While it is now clear that ureaplasmal colonization of the lower genital tract is not associated with adverse pregnancy outcome (9, 11), ureaplasmal infection of the chorioamnion is strongly associated with chorioamnionitis (14, 17, 33, 52, 62, 94, 95), premature birth (15, 52, 62), and perinatal morbidity and mortality (14, 33, 62, 94, 95). Case reports indicate that the infection is causal in at least some individuals (14, 16, 38, 96).

Recent evidence indicates that *U. urealyticum* is the single most common microorganism isolated from the central nervous system (CNS) (147, 149) and lower respiratory tract (16) of newborn infants, particularly those born prematurely. Isolation of the organism in pure culture from pleural fluid (5, 16, 146, 152), lung biopsy samples (152), and lung tissue at autopsy (5, 16, 146) from infants with pneumonia and reproduction of similar histologic lesions in lungs of newborn mice (112) and nonhuman primates (151) with these isolates (112) prove that this organism is a cause of respiratory disease in newborns. Prospective studies from six different centers (16, 91, 113, 154, 159, 161) now indicate a significant association between *U. urealyticum* in the lower respiratory tract and development of chronic lung disease (CLD) in low-birthweight infants. While the occurrence of clinically significant hydrocephalus and meningitis is variable in ureaplasmal CNS infections, it is clear that in some cases it is causal (42, 147, 149). Available evidence indicates that *U. urealyticum*-induced CNS and respiratory diseases are uncommon in full-term infants (13, 67, 68, 74, 132, 147, 149).

A major need for future research is the identification of risk factors for chorioamnion infection and risk factor for increased susceptibility of premature infants to ureaplasmal disease in premature infants. Current evidence suggests but does not prove that lack of specific antibody may be a critical determinant. There is limited information concerning guidelines or efficacy of antibiotic therapy of ureaplasmal infections, particularly in neonates. Because of the frequency of these infections and their potential severity in some neonates, there is a critical need for work in the area of therapy.

The present review is not meant to be inclusive; rather, its purposes are to (i) give some insight into why there has been so much controversy concerning the role of *U. urealyticum*

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in adverse pregnancy outcome; (ii) summarize evidence establishing ureaplasmas as a cause of intrauterine infection and premature birth; (iii) summarize evidence of a role for *U. urealyticum* in disease of very low-birth-weight infants; and (iv) discuss considerations for laboratory diagnosis and therapy. Emphasis will be placed on remaining questions and those areas of greatest need for further study.

EXPLANATION OF PREVIOUS CONTROVERSY

Most studies that have addressed the role of *U. urealyticum* in adverse pregnancy outcome have used one of three approaches: comparative rates of isolation from the lower genital tract, comparative rates of isolation from placentas or infants, or outcome of pregnancy following antibiotic therapy. As is often true of clinical studies with any organism, most studies of *U. urealyticum* have suffered from one or more of the following defects: (i) failure to appropriately exclude possible involvement of other infectious agents; (ii) failure to include placebo controls; (iii) inadequate numbers of patients and controls so that statistical reliability is never achieved; (iv) failure to match the groups for factors known to influence ureaplasmal colonization (i.e., race, socioeconomic status with regard to cervical colonization, and duration of membrane rupture and labor with regard to chorioamnion or amniotic fluid infection); (v) inadequate diagnostic evaluations of patients so as to exclude obstetric and gynecologic factors that can confound an association with *U. urealyticum*; and (vi) limitation of the investigation to epidemiological studies, which by themselves can suggest a relationship between ureaplasmas and disease but can never prove a cause-and-effect relationship. Furthermore, adverse outcome of pregnancy has many causes. Failure to incorporate this concept into experimental designs coupled with the high prevalence of the organism almost guarantees negative results. Only in very few studies has the probability that ureaplasmas are likely to be related to adverse outcome only in certain clinicopathologic subsets been considered.

As already mentioned, it is now clear that *U. urealyticum* cervicovaginal colonization is not predictive of adverse pregnancy outcome (9, 11). However, it is also clear that *U. urealyticum* can invade the upper genital tract and does so only in a subpopulation of individuals infected with ureaplasmas in the lower genital tract (14, 20). Herein lies the root of most of the controversy. Most of the earlier studies were limited to culture of the cervix and/or vagina or to surface cultures of infants.

Most available information indicates that if any organism causes adverse pregnancy outcome it most likely does so by infection of the chorioamnion and/or amniotic fluid and fetus. Thus, simply comparing rates of isolation from the lower genital tracts of different patient populations is not likely to establish whether there is an etiologic significance for *U. urealyticum* or, for that matter, any other microorganism in pregnancy-related conditions. In fact, relying on culture of the cervix and vagina would be much like attempting to determine the etiologic agent(s) of pneumonia by culturing the oro- or nasopharynx (9). Harrison and Phil eloquently stated (51) the fallacy of such an attempt: "Assume that 10% of *U. urealyticum* infected women are at risk for invasion and abnormal pregnancy outcome and that this 10% cannot be identified *a priori*. Then the other 90% would carry the same risk of abnormal outcome as uninfected women. Assume also that the prevalence of ureaplasmal endocervical infection in pregnancy is about 75% and that the overall incidence in the population of the outcome to be investigated is about 5%. Using *U. urealyticum* endocervical infection as the risk factor, the minimum relative risk for the adverse outcome that could be detected is a $p = 0.05$ and power of 0.80 is 2.1. For the overall risk in colonized women to be 2.1, the relative risk in the unknown 10% subgroup at risk must be 12.0. This would require 60% ($12 \times .05$) of this subgroup to have the abnormal outcome. It is unlikely, given the multitude of other anatomic, physiologic and microbiologic factors that are probably important in addition to host susceptibility, that the 60% level of abnormal outcome in the subgroup at risk ever occurs."

In contrast to studies limited to isolation of *U. urealyticum* from the lower genital tract or surface cultures of the infant, studies based on infection of the placenta indicate a strong association between isolation of *U. urealyticum* from the placenta and histologic chorioamnionitis (17, 33, 52, 62, 94, 95), premature birth (15, 33, 52, 62), and perinatal morbidity and mortality (14, 16, 38, 96). While most of the earlier studies either did not evaluate the contribution of other microorganisms or did not take into account membrane rupture, duration of labor, or other demographic and obstetric confounding variables, results of more recent studies (15, 52) that have taken these factors into account still show a strong association of *U. urealyticum* infection of the chorioamnion with chorioamnionitis and prematurity. Individual case reports provide compelling evidence that, in at least some individuals, *U. urealyticum* alone plays a causal role in spontaneous abortion (38), chorioamnionitis (14, 17, 38), and premature birth (15). Recent prospective and experimental studies also indicate that *U. urealyticum* is a significant cause of morbidity and mortality in newborns, particularly those of low birth weight (13, 16, 144).

COLONIZATION OF THE LOWER GENITAL TRACT AND EPIDEMIOLOGICAL CONSIDERATIONS

U. urealyticum can be found in the cervix or vagina of 40 to 80% of sexually mature asymptomatic women (12, 18, 77, 78, 80). Colonization is linked to younger age, lower socioeconomic status, sexual activity with multiple partners, black ethnicity, and oral contraceptive use (77, 78, 80). Vertical transmission of *U. urealyticum* occurs at rates of 45 to 66% in full-term infants (29, 114, 132) and 58% in preterm infants (115).

Isolation of *U. urealyticum* in pure culture from placenta, amniotic fluid, and internal fetal organs in the presence of funisitis and pneumonia (14) and a specific immunoglobulin M (IgM) response (96) can be taken as strong evidence that fetal infection can occur in utero. Cassell et al. (16) found that up to 14% of *U. urealyticum* endotracheal isolates collected within the first 12 to 24 h after birth from infants whose birth weight was less than 2,500 g were from infants born by cesarean section with intact membranes, indicating that in utero transmission occurs rather commonly, at least in premature infants. However, the precise rate of in utero transmission and its impact on perinatal morbidity and mortality have not been determined. Nosocomial transmission of mycoplasmas in a newborn or intensive care nursery has not been reported, and whether it occurs is not known.

Colonization of healthy full-term infants is relatively transient, with a sharp drop in isolation rates after 3 months of age (39, 60). Long-term follow-up studies with premature infants have not been conducted. In premature infants with invasive ureaplasmal infection, persistence of the organism in the lower respiratory tract (16) and cerebrospinal fluid (CSF) (149) has been documented for weeks to months.

Fewer than 10% of older children and sexually inexperienced adults are colonized. Colonization after puberty increases with sexual activity.

COLONIZATION OF THE ENDOMETRIA OF NONPREGNANT FEMALES

U. urealyticum can be isolated from the endometria of asymptomatic, nonpregnant females, indicating that the organism can be present at the time of implantation. Three hundred thirteen females undergoing diagnostic laparoscopy for infertility, tubal ligation, or tubal reanastomosis were evaluated microbiologically (20). Endometrial and uterine lavage specimens were collected by methods shown to circumvent cervical contamination. *U. urealyticum* was isolated from the endometria of 3.1% of those individuals who were colonized in the cervix. Bacterial growth was obtained from endometrial samples in 14 of 313 (4.4%) patients but never in association with positive ureaplasmal endometrial cultures; thus, vaginal or cervical contamination was not responsible for ureaplasmal isolations from the endometrium. Interestingly, neither ureaplasmal nor other bacterial colonization of the endometrium was accompanied by microscopic evidence of inflammation or clinical signs of endometritis.

CHORIOAMNIONITIS

U. urealyticum can be isolated from amniotic fluid as early as 16 to 20 weeks of gestation in the absence of labor and the presence of intact membranes (14). Prospective studies indicate that ureaplasmas are only rarely isolated from clear amniotic fluid (14, 139) at 16 to 20 weeks of gestation but that they can be isolated from 4.5% of discolored amniotic fluids (14). Individual case reports (14, 38) indicate that ureaplasmas can persist in the amniotic fluid for as long as 2 months in the presence of intact membranes and an intense inflammatory response and in the absence of labor and other detectable microorganisms. Furthermore, in such cases, ureaplasmas can be demonstrated directly in inflammatory infiltrates in the fetal membranes by immunofluorescence (17). These findings provide a convincing argument that ureaplasmas alone can actually produce chorioamnionitis. The presence of funisitis in such cases provides strong evidence that fetal infection can occur in utero (14, 16).

Studies based on isolation of *U. urealyticum* from the placenta have uniformly shown a significant association with chorioamnionitis (17, 33, 52, 62, 94, 95). While some studies did not rigorously seek other infectious agents and did not take into account duration of labor and/or membrane rupture, recent studies in which all possible agents were sought showed that women whose amniotic membranes were colonized with ureaplasmas were more likely to have chorioamnionitis than were women without *U. urealyticum*, even after rates were adjusted for duration of labor, premature rupture of membranes, duration of membrane rupture, and presence of other bacteria (52). In recent studies conducted in our laboratory, *U. urealyticum* in the chorioamnion was found to be significantly associated with chorioamnionitis, even in the presence of intact membranes in women who delivered by cesarean section (15). In some of these placentas ureaplasmas were the only organism isolated. Thus, there is ample evidence that *U. urealyticum* in the placenta is significantly associated with chorioamnionitis.

Although colonization of the endometrium prior to conception could undoubtedly result in infection of the amniotic sac, either an ascending or a hematogenous route of infection could also occur during pregnancy. *U. urealyticum* has been isolated from maternal blood in cases of septic abortion (38), but it appears that most infections probably occur via the ascending route and generally remain clinically silent. Unlike most other bacterial infections, ureaplasmal infection of the amniotic fluid is not associated with fever and uterine tenderness, and thus it is not a significant cause of clinical amnionitis (17).

CHORIOAMNION INFECTION AND PERINATAL MORBIDITY AND MORTALITY

When considering the potential adverse effect of *U. urealyticum* infection on pregnancy, it is important to consider the following facts. As already discussed, ureaplasmas can cause a clinically silent infection of the endometrium and can invade the amniotic sac as early as 12 to 16 weeks of gestation, even in the presence of a viable, apparently healthy fetus and intact fetal membranes (14, 38).

U. urealyticum has been implicated in spontaneous abortion, in some cases in association with maternal septicemia (12, 38, 80). The organisms have been isolated from fetal lungs, brains, hearts, and viscera (12, 14, 80, 94). *U. urealyticum* has been isolated more frequently from the products of early abortions and midtrimester fetal losses than from products of induced abortions (123, 128). Although rates of isolation of ureaplasmas from the lower genital tract of habitual aborters do not differ from those of normal controls, ureaplasmas are isolated more often from the endometria of habitual aborters (128). However, when only those patients with a positive cervical culture are considered, no higher endometrial colonization rate is found (85). *U. urealyticum* is isolated more frequently from the placentas of aborted fetuses than from those of controls (33, 94). Antibody titers to *U. urealyticum* are higher in mothers with a history of fetal wastage (99). However, the results of all of these epidemiologic studies are difficult to interpret since the comparability of the various groups of women is uncertain and the role of other microorganisms was often not assessed. Some studies have indicated an increase in the number of normal pregnancies following treatment with broad-spectrum antibiotics or erythromycin (98), but either these trials have been uncontrolled or the numbers of patients have been too few to achieve statistical reliability. However, isolation of *U. urealyticum* from amniotic fluid in pure culture from women with preterm labor and subsequent fetal loss in the presence of chorioamnionitis has recently been reported (14, 38). Unlike previous reports, these data clearly show that the fetus was alive prior to ureaplasmal infection and that there were no other apparent causes of abortion. This indicates that, in some cases, the role of *U. urealyticum* is causal.

Chorioamnionitis and isolation of *U. urealyticum* with or without other bacteria from the placenta are associated with stillbirth and perinatal deaths. Quinn et al. (94) studied 33 perinatal deaths (28 fetuses stillborn between 20 and 42 weeks of gestation and 5 neonates who died within 48 h of birth) and 31 random cases of normal term deliveries. They isolated genital mycoplasmas from placentas significantly more often when death of the fetus could not be attributed to a known anatomic or morphologic cause (group 2) than when there were known congenital malformations (group 1) or when the group was a control. The lungs and placentas were more likely to be inflamed in culture-positive cases from group 2 than in those from group 1 or controls, and antibody titers to genital mycoplasmas were more likely to be elevated

in sera from mothers and neonates in group 2. While the control group had membranes ruptured for less than 24 h, it is not clear that duration of membrane rupture was taken into consideration in the study groups or that consideration was given to the time differences of processing cultures from the various groups.

Kundsin et al. (62) cultured 801 placentas of three groups of infants (144 subjects who died in the perinatal period, 452 neonates admitted to the intensive care unit, and 205 case-matched controls). *U. urealyticum* was significantly associated with chorioamnionitis and was isolated from 21% of placentas of premature and term infants who died in the perinatal period, 25% of those admitted to intensive care, and 11% of controls. While no other organism was found to be significantly associated with chorioamnionitis, it is unclear whether appropriate sampling methods were used. There was a 2-day delay in processing bacterial cultures. Other investigators have shown that even shorter delays greatly reduce the recovery of anaerobes (30).

Madan et al. (72, 73) evaluated 432 placentas (201 intrauterine fetal deaths and 231 neonatal deaths occurring within the first week of life) because fetal membranes were grossly infected or there was clinical evidence of prolonged rupture of membranes. *U. urealyticum* was found in 2.2% of placental specimens, 7.4% of lung specimens, and 0.8% of liver specimens. However, interpretation of these results is problematic since the average time between death and obtaining samples for cultures was 21.4 h (range, 1 h to 7 days), with an average of 36 h for cases in which *U. urealyticum* was isolated.

INTRAUTERINE INFECTION AND PREMATURE BIRTH

Preterm birth (gestation of <37 weeks; birth weight of <2,500 g) is the single most common risk factor for infant morbidity and mortality, affecting 8 to 10% of all births and contributing to more than 60% of all perinatal morbidity and mortality (53, 82). Preterm infants are 40 times more likely to die in the neonatal period than are those with normal birth weights (53, 82). Five million hospital days per year are required for the care of those preterm infants who survive (53, 82). Neonatal intensive care costs exceed $5 billion.

A large body of evidence suggests that infection may play a major role in premature birth (43–45, 52), and a significant proportion of that premature birth may therefore be preventable. The first suggestion that infection might be involved in prematurity and low birth weight resulted from studies in which tetracycline was administered to nonbacteriuric pregnant women on a double-blind basis (32). Women who were treated for 6 weeks during pregnancy gave birth to significantly fewer infants weighing ≤2,500 g than women given a placebo. Although no microbiologic investigations were conducted, it was postulated that tetracycline-susceptible microorganisms might be responsible for low birth weight, and mycoplasmas were considered among such microorganisms.

Since 1970, approximately 12 studies involving over 7,133 patients have been conducted to evaluate the role of cervical ureaplasmal infection in prematurity (108). Nine of these studies were cohort (1, 40, 50, 81, 108–110, 130, 141), one was a randomized clinical trial (57, 58, 79), and two were case control studies (63, 64, 108). Almost without exception, these studies do not support an association between colonization of the vagina and/or cervix and premature birth. This was recently confirmed by a multicenter study involving 4,934 females evaluated for vaginal colonization with *U. urealyticum* at between 23 and 26 weeks of gestation (7). After adjustment for medical and sociodemographic factors in a multivariate analysis, there was no difference in the mean birth weights or proportions of low-birth-weight infants delivered by women who carried *U. urealyticum* in the cervix and/or vagina and those who did not.

Studies in high-risk populations indicate a significant association between isolation of *U. urealyticum* from the placenta and low birth weight. Embree et al. (33) isolated *U. urealyticum* from 32% of placentas from infants of <38 weeks of gestation but from only 9% of control infants of >37 weeks of gestation ($P < 0.001$). While the association of *U. urealyticum* and low birth weight was not influenced by maternal smoking, toxemia, or gestational diabetes, other risk factors, including other infectious agents, were not taken into consideration.

In the study by Kundsin et al. (62), gestational age and birth weight were inversely related to isolation of *U. urealyticum* from the placenta. Among infants in intensive care, the percentage of positive placental cultures declined progressively, from a rate of 39% in infants weighing <1,000 g to 16% in infants weighing ≥2,500 g. Among placentas associated with perinatal deaths, the isolation rate of ureaplasmas was also highest in placentas of infants with the lowest birth weights. Among all subjects, recovery of ureaplasmas from the placenta showed a strong inverse relationship to birth weight. Similar results were obtained when data were analyzed by gestational age. There was no association with intrauterine growth retardation. With respect to the strong association between *U. urealyticum* colonization and low birth weight, these investigators assessed possible confounding by maternal characteristics, including spontaneous rupture of membranes, preeclampsia, maternal age of less than 25 years, primagravidity, marital status, race, occupation, diabetes, and smoking. Three of these factors, colonization with *U. urealyticum*, spontaneous rupture of membranes, and preeclampsia, were significantly associated with low birth weight. Neither of the studies by Embree et al. (33) and Kundsin et al. (62) considered the duration of labor. Bacterial and mycoplasmal isolation from the chorioamnion increases after onset of labor (8, 9).

Hillier et al. (52) compared data on women in preterm labor with those on women in labor at term. They used multiple logistic-regression analysis to determine the interrelations among infection of the chorioamnion, histologic chorioamnionitis, and prematurity. One or more organisms were isolated from placentas of 61% of women with preterm labor who delivered before 37 weeks of gestation and from 21% of placentas from women without preterm labor who delivered at term. The most frequent isolates were *U. urealyticum* (47%) and *Gardnerella vaginalis* (26%). The recovery of any organism was strongly associated with chorioamnionitis (odds ratio, 7.2; 95% confidence interval, 2.7 to 19.5). When multiple logistic regression was used to control for demographic and obstetrical variables, premature delivery was still related to the recovery of organisms from the chorioamnion (odds ratio, 3.8; 95% confidence interval, 1.5 to 9.9) and with chorioamnionitis (odds ratio 5.0; 95% confidence interval, 1.6 to 15.3). Thus, infection of the chorioamnion was associated with premature delivery regardless of the time of membrane rupture or the duration of labor. An inverse relation was found between gestational age and both the frequency of microorganisms in the chorioamnion and histologic chorioamnionitis. Bacteria were recovered from 80% and chorioamnionitis was found in 60% of the extraplacental membranes obtained from mothers with very

low-birth-weight infants (<1,500 g), the group with the highest rate of morbidity and mortality. The proportion of placentas with evidence of infection was highest among those infants delivered at the lowest gestational age.

One of the most recent studies to evaluate the role of *U. urealyticum* in prematurity was a randomized placebo-controlled trial based on cervical colonization by ureaplasmas (35). Erythromycin- and placebo-treated women showed no significant differences in infant birth weight or gestational age at delivery, frequency of premature rupture of membranes, or neonatal outcome. On the basis of current evidence, one might have predicted failure of this trial. First, if *U. urealyticum* is involved in premature birth, it probably produces an effect via intrauterine infection. As already discussed, if only subgroups are at risk, then it is unlikely that a prospective study based on cervical colonization will show an association. Another major consideration is that no information concerning the efficacy of erythromycin for treating intrauterine infections is available. Erythromycin, the drug of choice (because of its lack of toxicity), does not effectively penetrate the amniotic sac (46) or eradicate ureaplasmas from the cervix and vagina (probably because of vaginal pH [71]). Nothing is known about its efficacy in reducing numbers of organisms or eradicating them in amniotic fluid or the placenta.

Perhaps a more important reason the treatment trial failed is that the majority of women in this study were treated starting at or beyond week 29 of gestation. It is possible that treatment earlier in pregnancy would have been more effective in preventing invasion of the fetal membranes. Isolation of *U. urealyticum* from the chorioamnion is almost three times higher in infants who weigh <1,500 g at birth and are born before 32 weeks of gestation (15, 52, 62) compared with larger and older infants. Since only 1% of women deliver neonates weighing ≤1,500 g at birth, a very large number of women would have had to be treated to demonstrate a measurable effect.

BV AS A POTENTIAL RISK FACTOR FOR *U. UREALYTICUM* INVASION OF THE CHORIOAMNION

Bacterial vaginosis (BV) occurs in 15 to 20% of pregnant women (75, 76). Symptomatic BV is characterized in part by a watery discharge with a fishy odor. Half of the patients with this infection have no or only very mild symptoms. Patients with BV consistently have an increased prevalence of *G. vaginalis*, selected anaerobic bacteria (most notably *Bacteroides* and *Mobiluncus* spp.), and *Mycoplasma hominis* and a decreased prevalence of facultative lactobacilli (34, 75, 76, 124). A 1,000-fold increase in the concentration of anaerobic microorganisms and a 100-fold increase in the concentration of *G. vaginalis* have been documented (44, 124). Although *U. urealyticum* is not independently associated with BV, the prevalence of vaginal colonization by *U. urealyticum* is increased about 2-fold, and the intravaginal concentration of these organisms is increased 100-fold (44). BV is associated with premature birth. However, the precise relationship among BV, *U. urealyticum*, and premature birth is not known. Some have postulated that the increased intravaginal concentrations of BV organisms may result in increases in the synthesis of phospholipase A_2 and the production of prostaglandins, which may lead to preterm labor or premature rupture of membranes (34, 75). Alternatively (34, 75), *Bacteroides* spp. in the lower genital tract could produce enough proteases to weaken the fetal membrane strength, causing premature rupture of membranes and invasion by other organisms. In addition, it is possible that certain BV-associated microorganisms, such as *U. urealyticum*, may be more likely to invade the intact fetal membranes simply because these organisms are present in larger numbers. However, the latter possibility cannot be the total explanation for *U. urealyticum* association with prematurity, since intravaginal concentrations of *Peptococcus* spp. are also increased in patients with BV but are found infrequently in the chorioamnion and amniotic fluid (15, 34, 52, 75, 76, 124).

The presence of BV is independently and significantly associated with birth at <37 weeks of gestation when cervical organisms and obstetric and demographic factors are taken into consideration (52). However, these studies have not determined whether BV is associated with premature delivery independently of chorioamnion infection (with either organisms associated with BV or those that are not, i.e., *U. urealyticum*). In the study by Hillier et al. (52), multiple logistic regression was carried out to determine the strength of the relation between the recovery of any organism from the chorioamnion and BV. After adjustment for factors related to both BV and the recovery of organisms from the chorioamnion, BV was associated with the isolation of organisms from the chorioamnion (odds ratio, 3.0; 95% confidence interval, 1.1 to 6.6). The most common organisms recovered in their study were *U. urealyticum*, *M. hominis*, *G. vaginalis*, *Peptostreptococcus* spp., and *Bacteroides* spp. Unfortunately, because of the small number of patients (only 38), it was not possible for Hillier and colleagues to determine the effect of individual organisms, to address the question of whether BV is associated with premature delivery independently of chorioamnion infection, or, for that matter, to determine whether *U. urealyticum* chorioamnion infection can occur independently of BV.

While the association between *U. urealyticum* chorioamnion infection and premature birth is strong, this association does not prove a cause-and-effect relationship. If *U. urealyticum* is causal, then one should be able to prove that it precedes onset of labor and rupture of membranes. *U. urealyticum* has been demonstrated in pure culture in the fetal membranes and amniotic fluid in the absence of labor and in the presence of chorioamnionitis (14, 17). A number of published papers indicate that a large variety of microorganisms can be isolated from the chorioamnion of 15 to 20% of women in spontaneous labor who have intact membranes (3, 8, 45, 48, 66, 107, 143, 150). Only a few of the studies have included specific cultures for genital mycoplasmas, but those that have, with rare exceptions, have found *U. urealyticum* to be the most common organism isolated. However, it has been hard to interpret the findings in all of these studies because of the large variety of organisms isolated, the failure to consider the duration of labor, and, more important, the statistically insufficient number of patients included in each report, which makes it impossible to fully determine the etiologic significance of single organisms or to study interactions among organisms. Recent studies in our laboratory indicate that *U. urealyticum* is the single most common microorganism isolated from the chorioamnion of women with spontaneous labor delivering by cesarean section who have intact membranes (15). Furthermore, logistic regression analyses of demographic and obstetric variables indicate that the presence of *U. urealyticum* alone or with other bacteria in the chorioamnion is independently associated with birth at <37 weeks of gestation regardless of the duration of labor (15). As in other studies, isolation of *U.*

urealyticum from the chorioamnion was inversely related to both gestational age and birth weight. In our study, almost 50% of the ureaplasmal chorioamnion isolates were in pure culture. Although *M. hominis* and *G. vaginalis* were the next most common organisms isolated, they, unlike *U. urealyticum*, were not independently associated with birth at <37 weeks of gestation. Thus, the relationship between BV and prematurity with or without *U. urealyticum* infection remains unclear. Current evidence indicates a distinct possibility that both BV and *U. urealyticum* are of etiologic significance independently of each other yet may have an additive effect when present simultaneously. In this regard, it is interesting that in our study the effect of *U. urealyticum* plus other bacteria was additive. *U. urealyticum*, like other bacteria implicated in prematurity, is known to produce phospholipase A_2, a precursor of prostaglandin synthesis that is thought to lead to uterine contractions (26, 27).

CONGENITAL AND NEONATAL PNEUMONIA

Congenital pneumonia or pneumonia acquired during birth is almost always accompanied by chorioamnionitis (31, 86). Since it appears that *U. urealyticum* can cause chorioamnionitis, it is reasonable to suspect that this organism is also a cause of pneumonia in newborns. In fact, retrospective studies indicate an association of ureaplasmas with congenital pneumonia (133). *U. urealyticum* has been isolated from affected lungs in the absence of bacteria, fungi, viruses, and chlamydiae and in the presence of chorioamnionitis and funisitis (14, 38). It has also been demonstrated within fetal membranes by immunofluorescence (17) and in lung lesions by electron and immunofluorescence microscopy (96). A specific IgM response has been demonstrated in individual patients with pneumonia, further documenting in utero infection (96). *U. urealyticum* can induce ciliostasis and mucosal lesions in human fetal tracheal organ cultures (96). Furthermore, ureaplasmas isolated from the lungs of human infants with congenital pneumonia produce pneumonia in newborn animals (24, 112, 151).

We have previously described (146) a series of newborn infants in whom *U. urealyticum* was isolated from the lower respiratory tract and pneumonia and persistent pulmonary hypertension were present. A severely asphyxiated, 2,200-g male infant born after 33 weeks of gestation had multiple *U. urealyticum*-positive cultures from blood, pleural fluid, and tracheal aspirates in the absence of other microorganisms prior to his death on postnatal day 6. At autopsy, severe bilateral pneumonia with a mixed cellular intraalveolar inflammatory exudate and early fibrotic changes of bronchopulmonary dysplasia were confirmed. *U. urealyticum* was isolated in pure culture from nasopharynx, lung, and brain postmortem. The occurrence of histologically proven pneumonia in an infant from whom *U. urealyticum* was the only organism isolated from multiple sites before and after death, including the lungs at autopsy, proves unequivocally that this organism can produce pneumonia in a newborn infant (5, 146).

The association of ureaplasmal infection with persistent pulmonary hypertension of the newborn is of particular interest in view of another study implicating group B streptococci with this condition and experimental studies in which streptococci have been shown to induce pulmonary hypertension when infused into the pulmonary circulation of animals (117). A proposed mechanism relates to thromboxane formation as a result of arachidonic acid activity induced by phospholipases (135). The production of phospholipases by ureaplasmas has been described elsewhere (26, 27).

CLD AND DEATH OF THE NEWBORN

In addition to the acute respiratory distress commonly seen in preterm infants, the survival of a greater number of very low-birth-weight infants than ever before has led to the recognition of the clinical entity bronchopulmonary dysplasia, also known as CLD of prematurity. The pathophysiology and known risk factors for CLD have been reviewed in detail elsewhere (87). Despite multiple intervention strategies, there has been little progress in reducing the mortality of CLD, which affects 15 to 38% of survivors of neonatal pulmonary disease with birth weights of <1,500 g, since the first description of this condition over 20 years ago. This lack of progress has led to consideration of other pathologic mediators of the condition, one of which is *U. urealyticum*.

Four recent independent studies (16, 91, 113, 154) link *U. urealyticum* with CLD. The four studies, performed at different institutions, indicate a significant association between ureaplasmal colonization of the respiratory tract documented within 24 to 72 h of birth and development of CLD in very low-birth-weight infants. The populations studied, entrance criteria, and study designs differed, yet the relative risks of developing CLD in infants colonized with *U. urealyticum* were remarkably similar. Infected infants did not differ from uninfected infants demographically or with respect to other risk factors for development of CLD. In a meta analysis (153) of these four cohort studies (16, 91, 113, 154), relative risk was examined for birth weight groups of infants weighing <750, 750 to 999, 1,000 to 1,249, 1,250 to 1,499, 1,500 to 1,749, and 1,750 to 2,000 g. A significantly increased risk of CLD in ureaplasma-infected newborns was observed in all infants weighing <1,250 g. This risk was no longer observed in infants weighing ≥1,250 g. The magnitude of the relative risk varied from 2.04 to 2.78 times in infected versus uninfected infants.

Sanchez and Regan (113) studied 111 infants in a newborn intensive care unit and found a 30% incidence of bronchopulmonary dysplasia among infants weighing <2,000 g who were colonized with *U. urealyticum* in the throat in comparison to an 8% incidence of the disease in those who were not colonized ($P < 0.05$). They concluded that duration of ventilation and oxygen therapy could not account for the higher incidence of CLD in infected infants. Other potential infectious agents were not sought, and no attempt to culture the actual infected site, i.e., the lung, was made.

Wang et al. (154) reported CLD in 51% of 107 infants weighing <1,250 g who were colonized with *U. urealyticum* in gastric, nasopharyngeal, or tracheal specimens but in only 16% of those not colonized. They concluded that *U. urealyticum* contributed to development of CLD independently of the effects of ventilation, gestational age, and severity of initial respiratory disease.

Payne et al. (91) examined the association between *U. urealyticum* colonization and the development of CLD in 93 premature infants who were treated with a surfactant and who had birth weights of <1,251 g. Nasopharyngeal and tracheal cultures for *U. urealyticum* were obtained at 2 ± 1 and 14 ± 1 days after birth and were positive for 17 of 93 (18%) patients. Infants born vaginally were 4.5 times as likely to be colonized as were those born by cesarean section. Colonization with *U. urealyticum* was associated with 1.66 (95% confidence intervals, 1.24 to 2.20; $P = 0.024$) times the risk of developing CLD and with a greater inci-

dence of ≥2+ polymorphonuclear leukocytes in the tracheal aspirate at 2 ± 1 days of age compared with uncolonized infants ($P = 0.025$). They concluded that *U. urealyticum* colonization is associated with CLD even after surfactant treatment and with inflammatory cells in the tracheal aspirate.

Cassell et al. (16) isolated *U. urealyticum* from 17% of endotracheal aspirates obtained within 12 to 24 h of birth (85% in pure culture) from 200 infants with respiratory disease who weighed <2,500 g. CLD occurred in 82% of infants weighing <1,000 g whose cultures were positive for *U. urealyticum* but in only 41% of those with negative cultures ($P < 0.02$); no association of *U. urealyticum* and CLD was found among infants with birth weights of >1,000 g. Very low-birth-weight, infected infants did not differ from uninfected infants with respect to demographics or other potential risk factors for development of CLD. Fourteen percent of the ureaplasma isolates from endotracheal aspirates were from infants born by cesarean section with intact membranes, indicating in utero infection. We also (16) evaluated death and found that infants weighing <1,000 g who were infected were not only more likely to develop CLD but also twice as likely to die as those who were uninfected or whose birth weights were >1,000 g. These findings reinforce the hypothesis that only a select group of infants, i.e., those with very low birth weights, is very susceptible to disease due to *U. urealyticum*. This fact may account for the seeming disparities in conclusions concerning the role of *U. urealyticum* in neonatal respiratory disease reached in earlier prospective studies (111, 137) which failed to distinguish this high-risk subpopulation from the whole.

The etiologic significance of *U. urealyticum* in development of CLD is strengthened by finding the organisms in pure culture in numbers exceeding 10^3/ml of endotracheal aspirate in 85% of infants, concomitant recovery of the organism from blood cultures in 26% of infants, repeated isolations from endotracheal secretions from the same infants for several weeks (16), and elevated leukocyte counts in infected versus uninfected infants (88, 91). Recently reported studies by Wesenberg et al. (159) and Witman et al. (161) at two additional centers also indicate a role for *U. urealyticum* in pneumonia and CLD in very low-birth-weight infants. Walsh et al. (152) have reported the isolation of *U. urealyticum* directly from lung biopsy samples from infants with CLD and have found that biopsy samples can be positive even when tracheal aspirates are negative. *U. urealyticum* has also been shown to produce pneumonia and in some cases to invade the bloodstream of premature baboons (151).

Current knowledge of the pathophysiology of CLD of prematurity would suggest that *U. urealyticum* is not a primary cause but that *U. urealyticum* produces pneumonia that goes undetected and untreated and results in an increased requirement for oxygen and subsequent development of CLD as a result of oxygen toxicity (13, 24, 91, 151). The occurrence of histologically proven pneumonia in infants from whom *U. urealyticum* was the only organism isolated from endotracheal aspirate, pleural fluid, and lung tissue (5, 14, 16, 146) suggests that this organism can produce pneumonia in newborn infants. Furthermore, *U. urealyticum* isolated from the endotracheal aspirate of an infant in the study of CLD by Cassell et al. (16) and another isolated from the lungs of an infant with proven congenital pneumonia (14) produced pneumonia in two different strains of newborn mice proven to be free of other known pathogens (112). Age was a critical determinant of development and severity of disease. Newborn mice were susceptible, whereas 14-day-old mice were resistant. Furthermore, in newborn mice, infection with *U. urealyticum* and exposure to 80% oxygen resulted in more severe lung lesions, organism persistence, and death than occurred in unexposed infected mice or oxygen-exposed uninfected mice (24). These results suggest that increased oxygen requirements of very low-birth-weight infants might predispose them to lower respiratory tract infection or, alternatively, that *U. urealyticum* infection potentiates oxygen-induced injury. Exposure to oxidants is known to enhance respiratory disease and death due to other mycoplasmal respiratory diseases (90).

That *U. urealyticum* is a cause of pneumonia in newborns can no longer be questioned. Likewise, its true association with CLD is convincing. The available data provide very strong evidence that *U. urealyticum* can actually be a primary cause or a contributing cofactor in development of CLD in humans, but the data are not definitive. Cohort studies allow follow-up of exposed individuals and thus reduce bias, but the designs of these studies cannot rule out the possibility that a third factor associated with *U. urealyticum* is not the true cause of CLD. A randomized trial of exposure to infection in humans is not ethical or practical. While a randomized trial of antibiotic treatment could provide critical information related to patient management, it would still not bring us closer to proving causality. Even if treatment is found to be efficacious, conclusions about causation will be limited by the fact that the third factor might also be susceptible to the antibiotic chosen. Nevertheless, a treatment trial is urgently needed to determine whether appropriate therapy can reduce the incidence of morbidity and mortality associated with CLD.

PNEUMONIA DURING INFANCY

The preterm neonate constitutes a different host from the older, otherwise healthy infant who may be subject to development of pneumonitis. The fact that the majority of infants who present for medical care with respiratory illness never have a precise microbiologic diagnosis has led to a search for other fastidious organisms in addition to the usual bacterial and viral pathogens. Genital mycoplasmas represent only one group of organisms falling into this category.

Stagno et al. (125) performed a microbiologic study of 125 infants aged 2 to 12 weeks who were hospitalized with respiratory syndromes. Infants with CLD or acute pneumonia were excluded. The rate of isolation of *U. urealyticum* from nasopharyngeal aspirates of these infants was compared with that of hospitalized, age-matched controls without respiratory disease. Although the cervicovaginal isolation rate did not differ between mothers of the subjects and those of the controls, *U. urealyticum* was isolated significantly more often from nasopharyngeal aspirates of infants with pneumonitis than from those of controls, while *M. hominis* was isolated from comparable numbers of infants in each group. The majority of ureaplasmal isolates were associated with other organisms, which makes the role of *U. urealyticum*, if any, in the clinical pneumonitis in this population unclear. Moreover, mere isolation from the upper respiratory tract may not accurately reflect the flora of the lower respiratory tract.

Syrogiannopoulos et al. (132) studied 108 full-term infants who were colonized with *U. urealyticum* at birth. They were monitored during the first 3 months of life. These researchers were unable to demonstrate an increased risk of lower

respiratory illness during this period of early infancy in ureaplasma-colonized infants compared with infants who were without pharyngeal ureaplasmal colonization.

Considering that there have been no prospective studies addressing the role of ureaplasmas in lower respiratory tract infections of infants outside the neonatal period that utilized direct cultures from the affected site (i.e., tracheal aspirates, lung biopsy samples, or autopsy material), no compelling evidence suggests that ureaplasmas are significant pathogens in lower respiratory tract infections in this population. Because of the well-documented difference in susceptibility of very low-birth-weight (i.e., extremely premature) infants versus older infants, we do not think that these organisms are likely to be a major cause of respiratory disease in otherwise healthy infants after the first month of life.

INFECTIONS OF THE CNS

The incidence of bacterial meningitis is greater in the neonatal period than in any other period in life (61), yet even in this select group epidemiological surveys place the attack rate at less than 1% (61). The importance of bacterial invasion of the CNS and the need for its prompt investigation when suspected or when found are underscored because of the significant death rate and the incidence of neurologic handicaps in survivors. Only within the past decade has an appreciation of ureaplasmas as pathogens in the newborn gained sufficient acceptance that investigations of their role(s) as pathogens in the CNS are beginning to be undertaken.

We previously (149) studied 100 predominantly preterm infants undergoing lumbar puncture for suspected sepsis and/or meningitis or for treatment of posthemorrhagic hydrocephalus. Infants were derived from a high-risk, university-based obstetric population. *U. urealyticum* was isolated from eight infants and *M. hominis* was isolated from five infants. Only one other CSF infection, in an infant with *Escherichia coli* meningitis, was identified in this group of 100 infants, making ureaplasmas the most common organisms isolated. *U. urealyticum* was isolated from six infants with severe intraventricular hemorrhage and from three infants with hydrocephalus. *U. urealyticum* was isolated from the respiratory tracts of four of eight infants with CSF infections. One infant had clinical pneumonia with pleural effusions, from which the organism was also isolated. Four infants in whom multiple isolations of *U. urealyticum* were made over several weeks had each sustained an intraventricular hemorrhage at or shortly following birth and had large intraventricular blood clots, which may have sequestered organisms over long periods (149). Four ureaplasma-infected infants died. The most striking features of *M. hominis*-induced CNS infection occurred in a full-term infant in whom the clinical features of congenital infection resembled those seen with viral or toxoplasmal infections and in whom major neurological impairment was noted.

A fundamental question that arose as a result of our former study (149) was whether mycoplasmal CSF infections occur with the same frequency in patients of higher socioeconomic levels, i.e., those in private hospitals. We cultured CSF from an additional 318 infants delivered in suburban community hospitals in Birmingham, Ala. (147). *M. hominis* was isolated from nine of the infants and *U. urealyticum* was cultured from five infants. With only three other verified CSF bacterial isolations in this population, mycoplasmas were again the most common microorganisms recovered, although the isolation rates were lower than in the original study.

Particular care was taken to ensure that the microbiologic results were valid. Lumbar skin cultures were taken from 80 newborn infants after the skin had been washed. No mycoplasmas were recovered from any infant. Multiple isolations from the CSF of the same infant over several weeks and the number of organisms (up to 10^5/ml) recovered also make it unlikely that the isolation of ureaplasmas reflects skin or laboratory contamination. The possibility that traumatic lumbar puncture with blood in the CSF specimen may have accounted for some positive cultures cannot be completely discounted. However, a number of isolations occurred in infants with few or no erythrocytes in the CSF and in some with no evidence of intraventricular hemorrhage.

In other prospective studies, Likitnukul et al. (67, 68) and Mardh (74) failed to recover mycoplasmas from CSF of infants. A possible explanation for these negative findings could be that the infants they studied were not really the population in whom mycoplasmal infection is most likely. The study by Likitnukul et al. (67) involved primarily older term infants, all of whom had been previously discharged from the hospital and had returned because of suspected sepsis or meningitis. Mardh did not specify the ages and birth weights of infants in his study (74). Shaw et al. (118) performed a prospective study of 135 preterm infants undergoing lumbar puncture and found only one isolate of *U. urealyticum*. The reason for lumbar puncture was not stated. In some hospitals it has been common practice to evaluate CSF of all infants weighing <2,500 g regardless of clinical evidence of sepsis or meningitis. Despite conclusions by Shaw et al. (118) that in their hospital the single isolation did not justify routine investigation of infants for mycoplasmal infection, a similar or even lower bacterial isolation rate from CSF does not justify withholding diagnostic procedures to identify bacterial infections. As mentioned earlier, the attack rate of bacterial meningitis is greater in the neonatal period than in any other period in life, yet even in this select group the attack rate is less than 1% (61). Not enough is known about the long-term effects of perinatal mycoplasmal infections to ignore their presence. It is obvious from these recent studies that the incidence in various populations differs and, at least in preterm infants, ureaplasmas are the microorganisms most commonly isolated from the CSF.

U. urealyticum may produce CSF pleocytosis, with either polymorphonuclear or mononuclear cells predominating, or the inflammatory reaction in CSF may be minimal or absent (147, 149). Lack of inflammation, when the presence of the organism has been verified on multiple occasions, may logically lead to some skepticism about the significance of mycoplasmal CSF infection. It should be noted that, early in the course of infection with a number of other proven bacterial pathogens that may infect the meninges, inflammatory reactions may be scant or absent (28, 47). The severely ill infant with meningitis may in fact represent only a fraction of the total number of ureaplasma-infected infants, with the majority experiencing only a mild, often subclinical infection that may resolve spontaneously.

That ureaplasmas can invade the CNS of newborn human infants is plausible when one considers that naturally occurring mycoplasmal CNS infections in animals have long been appreciated (144). Furthermore, a wide range of CNS dysfunctions associated with *Mycoplasma pneumoniae* infection has been reported in humans. These include aseptic meningitis, meningoencephalitis, transverse myelitis, cranial nerve palsies, myeloradiculopathy, cerebellar ataxia, cere-

bral infarction, Guillain-Barré syndrome, and acute psychosis (144). The organism has actually been isolated directly from the CSF and brain tissue in some reported cases (144).

The recent production of meningitis and hydrocephalus in newborn mice and beagles by using pure cultures of *U. urealyticum* isolated from CSF of human infants (21a) indicates that these organisms do have the potential to produce CNS disease.

NEONATAL SEPSIS

The factor associated most significantly with sepsis due to any microorganism in the neonate is low birth weight (122). Other factors include prolonged ruptured membranes, traumatic delivery, maternal infection, chorioamnionitis, and fetal hypoxia. In the case of *U. urealyticum*, as with any other bacterium, infection can occur at the time of birth when the infant is inoculated with the organism as it traverses the birth canal. Alternatively, the fetus may acquire the organism in utero through infected amniotic fluid or placenta. Placental infection with involvement of the umbilical vessels could lead to disseminated infection in the fetus, or aspiration of the organisms into the lungs could lead to pneumonitis and septicemia from a respiratory tract focus.

U. urealyticum has been isolated from cord blood (127). However, the possibility of contamination by vaginal organisms or maternal blood cannot be discounted. Waites et al. (149) performed blood cultures for mycoplasmas in 43 newborn infants as part of a study of CSF infections. Two infants were positive for *M. hominis* and two were positive for *U. urealyticum*. Cassell et al. (16) found that 26% of preterm infants with positive endotracheal aspirates had positive ureaplasmal blood cultures. These results suggest that septicemia with ureaplasmas can be rather common in preterm infants. Ureaplasmal septicemia may accompany severe neonatal pneumonia (5, 146), a fact that is not at all surprising when the typical natural histories of better-known bacterial infections such as group B streptococcal infection are considered.

In contrast to findings in neonates, Likitnukul et al. (67, 68) failed to isolate ureaplasmas from the blood in a prospective study of 191 older infants readmitted to the hospital for suspected sepsis. It appears unlikely that ureaplasmas are a significant cause of sepsis outside of the perinatal period in otherwise healthy infants. However, under special circumstances their presence still should be considered.

ASSOCIATION OF *U. UREALYTICUM* SEROVARS WITH DISEASE

Development of disease in only a subpopulation of infected individuals led very early to the hypothesis that only certain strains of *U. urealyticum* may be pathogenic. Thus, subspecies of *U. urealyticum* have been determined by a number of serological methods (105). Eight serovars have been identified by modified metabolic inhibition, growth inhibition, and indirect hemagglutination tests. Fourteen serovars have been identified by immunofluorescence (105). There is a preliminary report that two additional serovars have been found by the mycoplasmacidal assay (69, 70). However, these two serovars have not undergone rigorous comparison with the 14 reference strains and thus have not yet been accepted by the International Research Program of Comparative Mycoplasmology (129). In fact, the investigators who suggested expanding the serotyping scheme to 14 have proposed deleting serovars 11 and 13 (126). In serotyping clinical isolates, they found that repeated tests of serovar 11 showed poor agreement, while serovar 13 not only showed poor agreement but also occurred too frequently to aid in strain differentiation.

The percentages of clinical isolates typeable by using antisera to serovars 1 to 8, 1 to 10, or 1 to 14 are conflicting. It is disconcerting that up to 96% of isolates are typeable with antisera only to serovars 1 to 10 (84) and that up to 94% are typeable with antisera to serovars 1 to 8 (56), while other studies using antisera to all 14 serovars found that serovars 11 through 14 can occur in 20 to 50% of patients (104). These results may be explained by the tendency of clinical isolates to express multiple specificities (54, 84, 126, 157). This is in part because of cross-reactivity alone but is also because of actual common expression of epitopes (54, 126, 157). Admittedly, part of the problem may be lack of standardization of reagents and methods between laboratories.

Unfortunately, little is known concerning the nature of either type-specific or group-specific antigens. Preliminary data suggest that complement-fixing antigens are membrane lipids, whereas metabolism-inhibiting antibodies are directed mainly against membrane protein antigens (106). Type-specific antigens appear to be sensitive to mild protease and Triton X-114 treatment, which suggests that they may be surface-exposed proteins (126).

Two studies involve analysis of *U. urealyticum* proteins by two-dimensional gel electrophoresis. One involved types 1 to 9, and the other involved types 1 to 9, 11, 12, and 14 (83, 131). Although the serovars could be divided into the two genetic clusters (see below), only one serovar-unique protein was found and this was in serovar 9 (131). All other polypeptides were common to all serovars, limited to one of the two genetic clusters, or found in two or more serovars which were not always of the same group. It appeared that about one-third of the polypeptides were common to all serovars and that most of the remainder were group specific. However, it is dangerous to draw conclusions concerning differences in antigenicity without the use of antibody, especially without the use of monospecific or monoclonal reagents. One study using immunoblotting reported detection of serogroup-specific antigens, but only 5 of the 14 serovars were evaluated with a limited number of sera (65).

Previously (19, 54, 157), we have shown that immunoblotting with rabbit antisera reveals an intensely staining, complex "ladder" pattern consisting of a series of proteins with molecular weight heterogeneity when some serovars, serovars 1, 2, 3, 5, and 6, are reacted with homologous antisera. The same ladder pattern also indicates a certain degree of relatedness between some members of the two biotypes of ureaplasmas (between serovars 2 and 5 and between serovars 3 and 14). Using monoclonal antibodies, we have demonstrated the occurrence of a ladder antigen also on serotypes 8 and 10 (Fig. 1). Thus, the ladder antigen has now been identified on 8 of the 14 serovars. Monoclonal antibodies to these ladder antigens have been produced. Detailed analysis of this antigen in serovars 3, 8, and 10 indicates that this antigen (i) is species specific; (ii) contains both serotype-specific and a cross-reactive epitope(s); (iii) is produced not only in vitro but also in vivo; (iv) undergoes a high rate of structural variation in vitro; (v) is present on invasive ureaplasma isolates, i.e., those from placenta, lung, and CSF; and (vi) is one of the most predominant protein antigens recognized during infections in humans (157). Furthermore, we have shown that monoclonal antibodies to this antigen can inhibit the growth of these organisms in vitro, which suggests that antibody to this antigen may be impor-

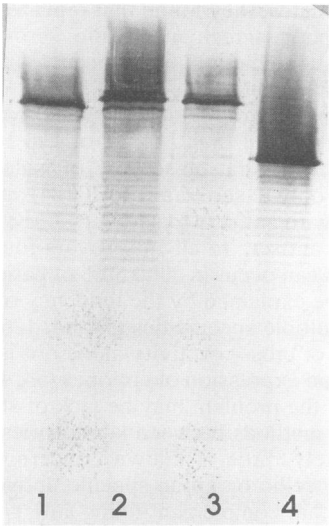

FIG. 1. Immunoblot demonstrating the ladder pattern antigen in *U. urealyticum* serovar 3. Organism proteins were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis, transferred to nitrocellulose, and reacted with a monoclonal antibody which recognizes a serotype-specific epitope on *U. urealyticum* serovar 3. Reactions were visualized by using peroxidase-labeled conjugates. Lane 1 contains the *U. urealyticum* serovar 3 reference strain, lanes 2 to 4 contain three clinical isolates showing size variation with respect to the reference strain, and each lane shows the pronounced ladder pattern characteristic of this antigen.

tant for host defense (157). Data obtained by us for a similar antigen in *Mycoplasma pulmonis*, a genitourinary pathogen of rodents, indicate that the antigen in *M. pulmonis* is associated with interaction with eucaryotic cells (156), lesion character, and severity (134). In vivo variation in this antigen is associated with organism persistence and disease chronicity (134). Continued epitope mapping of this similar antigen in *U. urealyticum* will likely lead to development of serotype-specific reagents against the remaining serovars and will help elucidate the role of this unique antigen in disease pathogenesis.

The original eight serovars fall into two distinct clusters on the basis of DNA-DNA homology (21), restriction endonuclease DNA digestion patterns (100, 101), polyacrylamide gel electrophoresis patterns (83, 131) with either radioautography or silver stain, and sensitivity to manganese salts (103). Cluster A consists of serovars 2, 4, 5, 7, and 8, and cluster B consists of serovars 1, 3, and 6. The more recently described serogroups 9 to 14 can also be fitted into these two groups according to manganese sensitivity as follows: serovar 14 in group 1 and the remaining serovars except 13 in group 2. Type 13 gives an intermediate response to manganese and could not be classified (103). Restriction endonuclease cleavage patterns and Southern blot hybridization both reveal that serovars 9, 10, 11, 12, and 13 belong to cluster A and serovar 14 belongs to cluster B (49). Although the available data provide some justification for designating two separate species within the ureaplasmas of human origin, arguments against doing this were put forward and the decision was postponed pending the finding of differences in disease association (129). Recently developed oligonucleotide primers that differentiate between the two genomic clusters should facilitate addressing this question by using the polymerase chain reaction (2).

Serotyping of clinical isolates by any method is difficult at best. Growth inhibition or mycoplasmacidal assays require passage of primary isolates several times prior to serotyping (126). As these methods are based on the growth of organisms, it is extremely difficult to detect the presence of all serovars in a mixture because certain serovars are more fastidious, grow more slowly, or can be present at lower concentrations (126). Since mixed serovars can be cultured from 53% of patients (93), this problem is a major concern. Immunofluorescence and immunoperoxidase methods allow typing directly on the primary isolation plate, but those who have examined these procedures systematically find that clinical isolates often express multiple specificities (54, 84, 126). Lack of agreement between repeated assays following passage of isolates is also a problem (84, 126). Given these limitations, it is not surprising that results linking specific *U. urealyticum* serovars with certain diseases are conflicting.

Eleven published articles deal with distribution of *U. urealyticum* serovars in different populations. In general, patient and control groups have been poorly characterized and the numbers in the groups have been small. In some instances, detection of serovar-specific antibody has been used in the absence of cultural isolation and documentation of the serovars present (97, 99). Only one group of investigators has used antisera to all 14 serovars, and only one group has performed typing directly on the primary isolation plate (104, 126). Five published reports examined the serovar distribution of *U. urealyticum* in patients with nongonococcal urethritis and in asymptomatic controls (23, 69, 92, 119, 126). No serovar was consistently found to predominate. The remaining six reports deal with the distribution of serovars in various female and neonatal populations. Lin (69) used 11 typing sera and cervical isolates and determined the serovar distribution in normal college women, women with salpingitis, and pregnant women who delivered normal, low-birth-weight, or stillborn infants. There were no differences among the small groups examined. Naessens et al. (84), using 10 typing sera, the first subculture of broth, and immunofluorescence, examined four groups of patients. Group 1 contained 24 couples with a history of recurrent abortion, group 2 contained 25 patients who had had their first spontaneous abortion, group 3 contained 14 pregnant patients with complications (premature delivery or intrauterine death), and group 4 contained 138 patients with uneventful pregnancies. They typed 240 strains (most were from cervices but some were from placentas and trophoblasts). Serovar 4 was significantly more common in the cervices of group 1 patients (20.8%) than in those of control patients (5.1%).

Quinn et al. (99), using the metabolic inhibition assay, measured antibody titers at delivery in 14 women with histories of pregnancy wastage (but who were treated with erythromycin) and in their infants and compared these with titers for 20 normal mothers and their infants. When the mean antibody titers in the normal and pregnancy wastage groups were calculated for serovars 1 to 8 (serovars 9 to 14 were not examined), the infants of mothers with pregnancy losses exhibited significantly elevated mean titers to serovars 6 and 8, while the mothers had elevated mean titers to serovars 4 and 8. A lack of significantly elevated antibody titers to serovar 3 has been used by these investigators to suggest that serovars 4 and 8 may be more pathogenic. In their population serovar 3 is found in 80% of all individuals. They have also suggested that the fact that serovar 8 produces more phospholipase A_2 than serovars 4 and 3 may explain involvement of this serovar in premature birth (26,

27). However, in the study by Naessens et al. (84), there were no isolations of serovar 8 from cervices or placentas of women with intrauterine fetal death or premature delivery.

Even after adjusting for age, mean titers of antibody to *U. urealyticum* are significantly lower in women who are experiencing their first pregnancy than in those who have been pregnant before (70). The study by Quinn et al. (99) is difficult to interpret because no details concerning the parity of the control mothers are given. No information is given concerning the duration of membrane rupture in the two groups (i.e., ureaplasmal amniotic fluid infection resulting from membrane rupture could explain differences in antibody levels). Furthermore, since the *U. urealyticum* isolates from the two groups were not serotyped, the apparent selected increase in antibodies to serovars 4 and 8 cannot be evaluated. Other investigators found that approximately 40% of women demonstrated a significant rise in antibody titer to one or more serovars of *U. urealyticum* during an apparently normal pregnancy (70).

In a somewhat similar study, Quinn et al. (97) evaluated the serologic responses (but not the culture status) of preterm infants with respiratory distress and compared them with the responses and status of normal term infants. Neonates with respiratory disease had significantly elevated mean titers to serovars 4, 7, and 8 compared with mean titers of normal neonates and slight but not significant elevations of titers to serovars 3 and 6. When the respiratory disease cases were assessed according to whether the infant survived or died, the mean titer to serovar 3 was slightly elevated in all groups. With serovars 4 and 8, the mean titers were significantly higher among neonates who died than among the survivors. For serovar 5, a significant elevation occurred only among the survivors. The difficulty in interpreting these results is that sera were collected from infants with respiratory disease from 0 to 20 days after birth and from the control term infants at delivery only. Also, the frequent transfusions received by premature infants were not taken into account. This can affect immunoglobulin levels (25).

Even though there are weaknesses in both studies, it is interesting that Quinn et al. (97, 99) by serology only and Naessens et al. (84) by culture only obtained data that suggest that serovar 4 may be more often isolated from women with recurrent abortion. It is also of interest that Quinn et al. (93, 97, 99) found significantly elevated antibody to serovars 4 and 8 more often in infants with respiratory disease and in infants who died than in those who survived. Some investigators have found serovar 4 more commonly in males with urethritis (23, 119) and in the cervices of females with pelvic inflammatory disease (22). Of eight serovars examined for their abilities to reduce the penetration of zona-free hamster eggs by human spermatozoa, serovar 4 was the only one to produce a significant effect (6). It is not clear whether all serovars were compared within a single experiment, to what degree the assay was reproducible, how many times the experiments were repeated, or how the data were analyzed. Regarding the other studies linking serovar 4 to urethritis and spontaneous abortion, other investigators who used antisera to all 14 serovars, identified isolates directly on the primary isolation plate, and studied larger numbers of patients (104, 126) have not confirmed these results.

From all serotyping studies to date, there appears to be only one consistent finding. Serovar 3 is the most common serovar isolated from females regardless of the patient population and geographic location. The data were obtained by six different investigators from four different countries and were derived by using at least three different typing methods (23, 56, 69, 97, 99, 104, 119). The data implicating serovars 4 and 8 in perinatal morbidity and mortality are by no means conclusive, and further studies are warranted, considering that serovars 4 and 8 are two of the serovars least commonly recovered by culture in all normal female and infant populations examined to date (23, 56, 69, 97, 99, 104, 119). However, these studies should by no means be undertaken until serovar-specific reagents and improved methods of antibody detection are available. Using monoclonal antibodies to the ladder antigen discussed above, we have developed immunoblotting methods for serotyping of clinical isolates (162). We previously isolated *U. urealyticum* from the CSF of 13 of 418 newborn infants; additional bloodstream isolates were obtained from the same population (147, 149). Ten of the 13 CSF isolates and 3 bloodstream isolates were available for serotyping. By the use of serotype-specific reagents, including monoclonal antibodies, 70% of the CSF isolates were identifiable as serotype 1, 3, 6, 8, or 10; i.e., they represented 5 of the 14 established serotypes and both presently defined genomic clusters. One of the bloodstream isolates was identified as serotype 3. These data support the hypothesis that the property of invasiveness for ureaplasmas is likely not limited to one or a few particular serotypes among the 14 established serovars. Additionally, this study showed that, even in isolates of the same serotype from different patients and in isolates from different body sites within the same patient, there can be size variation in the serotype antigens expressed (Fig. 1). Therefore, it appears that many serotypes are invasive and that perhaps antigen variability and host factors may be more important determinants for ureaplasma infections than different serotypes per se.

ROLE OF ANTIBODY IN DEFENSE AGAINST UREAPLASMAS

Available evidence indicates that identification of specific antibody to *U. urealyticum* may be useful in detecting an active infectious process. Specific antibody to *U. urealyticum* can be detected by complement fixation, growth inhibition, metabolic inhibition, and mycoplasmacidal assays (121). However, these methods are limited by technical difficulties and lack the ability to distinguish the responses among immunoglobulin classes. The latter capability may be of utmost importance in ultimately determining the etiologic significance of *U. urealyticum*. We have developed an enzyme-linked immunosorbent assay (ELISA) based on a whole organism lysate as the antigen (4). The assay agrees exceptionally well with results obtained by metabolic inhibition assay (overall agreements of 82 and 95% for acute- and convalescent-phase sera, respectively). Using this assay, we characterized the antibody response in a well-defined population of males with nongonococcal urethritis for whom quantitative cultural results were available over time. Serum antibody levels in nongonococcal urethritis patients were significantly higher than the normal serum standard for the IgG, IgM, and IgA classes. Additionally, the magnitude of change between acute- and convalescent-phase sera was greater for nongonococcal urethritis patients than for normal asymptomatic ureaplasma-positive male controls. A significant change in antibody levels of one or more antibody classes was detected by ELISA in 12 of 18 nongonococcal urethritis patients. Ten of the 12 individuals had a change in the IgM class, which suggests an active infectious process.

We have used the ELISA to analyze sera from various female patient populations. Whereas the mere presence or absence of antibody, especially IgA, agrees well with cervicovaginal isolation of *U. urealyticum* (4), changes in antibody titer do not necessarily agree with cultural isolation of the organism from invasive sites (17). This may be because our ELISA failed to detect serovar-specific antibody increases (54). Two papers purport to demonstrate ureaplasma serovar-specific ELISAs (140, 160). Both assays were tested only with rabbit antisera, and both showed considerable cross-reactions which could be diluted out. However, careful examination of the data suggests that neither assay would prove clinically useful even if human serum reacted in the same fashion as rabbit antiserum. Considerable cross-reactions remained, despite serum dilutions of up to 1:1,000. Furthermore, a serovar-specific antibody response could not be demonstrated with sera from humans presumably exposed to only a single serovar (54).

The unique susceptibility of hypogammaglobulinemic patients to chronic arthritis due to *U. urealyticum* (138) suggests that antibody is important for protection against invasive disease caused by this organism. Increased susceptibility of infants of <30 weeks of gestational age to *U. urealyticum*-induced respiratory disease may be related to their hypogammaglobulinemia (13, 16).

Study of a hypogammaglobulinemic patient with arthritis provides evidence that serovar-specific antibody may be required for protection against invasive disease. A non-serovar-specific ELISA revealed high levels of ureaplasma-specific antibodies in the serum of the patient as well as in two serum donors who served as sources of immunoglobulin replacement during the period of joint infection. However, by the serovar-specific metabolic inhibition assay, no antibodies to serovar 4, which was isolated from the joint and subcutaneous abscesses, were detected (142). In another hypogammaglobulinemic patient with chronic arthritis due to an antibiotic-resistant strain of *U. urealyticum*, treatment with commercial immunoglobulin (Sandoz) was not effective but arthritis and abscesses resolved when the patient was given infusions of goat hyperimmune ureaplasma serum (138, 158). Thus far, the serovars isolated from joints of hypogammaglobulinemic patients include 2, 4, 5, 7, and 8 and an untypeable strain. Generally, these are not the more common serovars isolated from the patient and control populations that have been surveyed. Perhaps commercial immunoglobulin preparations contain antibodies to the more common serovars such as 3 and 6.

That detection of serovar-specific *U. urealyticum* responses is clinically significant has also been suggested by Kass et al. (57). They showed that women who experienced a fourfold or greater rise in antibody titer to any single strain of *U. urealyticum* had a 30% rate of low-birth-weight infants whereas women who did not experience such an increase in antibody titer had a 39% rate. Correspondingly, the birth weights in the two groups differed by 230 g. In most instances of significant increases in antibody titer, the titer increase was specific for a serologic type other than those for which the women had preexistent antibody and the type-specific titer increased from undetectable levels. The data reported by Quinn et al. (96, 97, 99), discussed above, also suggest that selective antibody to certain serovars increases in women with pregnancy wastage and in infants with respiratory disease compared with control patients. Studies by Gallo et al. (41) suggest that the presence of *U. urealyticum*-specific IgM antibody in infants is predictive of disease.

Taken together, the available evidence suggests but does not prove that immunity to *U. urealyticum* invasive infection is type specific. The mechanism of protection afforded by antibody seems to be mediated by metabolic inhibition of the organism and not by opsonization (138, 158). *U. urealyticum* binds spontaneously to neutrophils and also directly activates the first component of complement.

DIAGNOSTIC AND THERAPEUTIC CONSIDERATIONS

There are insufficient data to make specific recommendations related to indications for diagnosis and treatment of *U. urealyticum* infection of the chorioamnion. However, it is obvious from available data that routine culture of the cervix and/or vagina is not indicated. Culture of amniotic fluid alone (15) is unreliable for detection of chorioamnion infection. Only half of those with ureaplasma-positive cultures of the chorioamnion will also have positive culture of the amniotic fluid (15). The fact that analysis by the more sensitive technique of polymerase chain reaction also indicates a much lower frequency of infection in amniotic fluid than in the chorioamnion confirms that reliance on analysis of amniotic fluid will lead to underdiagnosis of chorioamnion infection and hence chorioamnionitis (15). There is no information upon which to base a rational therapy of chorioamnion infection.

Recognition that *U. urealyticum* indeed causes severe disease in newborn infants, including septicemia, meningitis, and pneumonia, is sufficient justification for treatment in most instances when the presence of ureaplasmas is documented in normally sterile sites such as blood, CSF, pleural fluid, or endotracheal aspirates in infants who weigh ≤1,250 g. Techniques for specimen collection, cultivation, identification, and drug susceptibility testing and therapeutic considerations have recently been reviewed elsewhere (10, 18, 120, 121, 145, 148) so only the more important points will be discussed here.

Cultivation

Specimen collection. Liquid specimens, including blood, amniotic fluid, CSF, urine, pleural fluid, and tracheobronchial secretions, are acceptable for ureaplasmal culture. In addition, chorioamnion or any tissue collected from a biopsy or an autopsy can be submitted for culture if there is reason to suspect the presence of ureaplasmas in newborn infants.

Ureaplasmas are extremely susceptible to adverse environmental conditions, especially drying, osmotic changes, and toxic metabolites. Particular care must be taken to ensure that specimens are not subjected to extreme environmental fluctuations. Whenever possible, specific ureaplasmal transport medium such as Shepard's 10B broth (119, 120) should be provided for direct inoculation of clinical specimens at the time they are collected. The medium should then be sent directly to the laboratory and processed immediately. If specimens are allowed to sit at room temperature and are not inoculated into appropriate media, significant loss of mycoplasmal viability or overgrowth of endogenous bacteria is to be expected.

Body fluids should be inoculated in an approximately 1:10 ratio into the transport medium (usually 0.1 ml of fluid per 0.9 ml of broth), but it is also desirable that some uninoculated material be sent to the laboratory. Swabs should also be inoculated into suitable transport medium. Only calcium alginate-, dacron-, or polyester-tipped swabs with plastic or wire shafts should be utilized for sampling of mucosal surfaces, and the swab must always be extracted from the

specimen. Blood should be collected free of anticoagulants and immediately inoculated into transport medium in a 1:5 to 1:10 ratio. Inoculate as much blood as possible, preferably 5 to 10 ml from adults, although smaller volumes from infants are acceptable. Specimens should be kept refrigerated at 4°C and protected from drying in a sealed container until they can be transported to the laboratory. If specimens are collected in a facility that does not have immediate access to ureaplasmal broth for transport, a satisfactory alternative is 2SP medium (10% heat-inactivated fetal calf serum; 0.2 M sucrose in 0.02 M phosphate buffer, pH 7.2). If transport is not possible within 6 to 12 h after collection, the specimen in transport medium should be stored at −70°C and shipped frozen. Ureaplasmas are stable for long periods when kept frozen at −70°C in a protein-containing support medium such as 10B. Storage at −20°C is less reliable and subject to significant loss in titer in a relatively short time.

The original specimen from all swab cultures should be thoroughly mixed on a Vortex mixer. Following mixing, specimens for ureaplasmal culture should always be serially diluted in broth to at least 10^{-3}, preferably 10^{-5}, and an aliquot of the original sample and of each dilution should be inoculated onto A8 agar (120). All dilutions (10^{-1} to 10^{-5}) of the broth should be incubated. The remainder of the original specimen should be frozen (−70°C) for future confirmation. Dilution is necessary to overcome potential inhibitory substances or metabolites, including antibiotics, which may be present in the body fluid or tissue (119, 136) and to facilitate quantitative estimation of the numbers of organisms present. Greater isolation sensitivity may be obtained by centrifuging body fluids such as urine (600 × *g*) to deposit epithelial and other cells and by performing serial dilutions on an aliquot from the sediment. Tissues are preferably minced rather than ground for cultivation to circumvent potential growth inhibitors that are more likely to be released with grinding (136).

Growth and identification. Ureaplasmas are fastidious and demanding in their requirements for special media. For this reason alone, many laboratories choose not to culture mycoplasmas. Although commercially prepared media are now on the market, they have not been thoroughly evaluated in a clinical setting. Growth media are usually enriched with animal serum (fetal calf or horse), peptones, yeast extract, and metabolic substrates, including urea. Shepard's 10B broth and A8 agar are the most widely used media (119, 120), although others are also satisfactory (119, 120).

The relatively rapid growth rate of *U. urealyticum* makes identification of most positive cultures possible within 1 to 5 days. Broth cultures should be incubated at 37°C under atmospheric conditions, and agar plates should be incubated under 5% CO_2. Ureaplasmas are susceptible to a rapid, steep death phase in culture which is likely because of a combination of urea depletion and elevated pH due to urease activity. Ureaplasma broth cultures should therefore be monitored very closely (two to three times daily). The presence of growth in 10B medium is suggested by an alkaline shift due to urease activity of ureaplasmas or arginine hydrolysis by *M. hominis*, causing the phenol red indicator to turn from yellow to pink. Ureaplasmas produce no or minimal turbidity in broth. Broth cultures demonstrating color change should be subcultured to broth and agar just as the pH indicator begins to turn. This combination of broth to agar inoculation technique is the most sensitive for recovery of all mycoplasmas. Positive broth cultures should be frozen (−70°C) immediately after being subcultured for future reference, e.g., for identification and antibiotic susceptibility testing. Plates should be evaluated microscopically for growth every 1 to 3 days and incubated for 7 to 10 days prior to being called negative. Positive agar cultures can be expanded by transfer of an agar plug to broth.

Quality control of culture media. Ureaplasmas are very sensitive to inhibitors present in some lots of horse serum, yeast extract, and even mycoplasma medium base. It is not uncommon for the standard media in a laboratory to be temporarily insufficient for cultivation of ureaplasmas. Rigorous quality control should be performed on media obtained commercially or prepared in the laboratory. Organisms to be used for quality control should be type strain (all 14 serotypes) and low-passage clinical isolates, grown in large volumes, and stored frozen in small aliquots at −70°C. Serial dilutions of the thawed stock strains should be made, and the numbers of organisms growing on the test batch of medium should be quantitated. If the number of CFU on solid medium is less than 90% of that on the medium from the previous batch, the test should be repeated. If it is still less than 90%, the new medium should be considered substandard and discarded. For broth medium, a difference greater than 10 to 100 color-changing units per ml (that is, 1 to 2 10-fold dilutions) between the control and test liquid medium should be considered unacceptable. Each batch of complete medium, both agar and broth, should be tested for sterility by incubation of an aliquot of broth and an agar plate at 37°C for at least 24 h. Finally, uninoculated tubes of the complete medium should be incubated with each series of patient specimens. Observation of mycoplasma colonies and determination of colonial morphology require the aid of a stereomicroscope. *U. urealyticum* colonies appear brown or black on A8 agar because of urease production in the presence of the $CaCl_2$ indicator.

Antibiotic susceptibility testing. Either the microdilution or the agar dilution technique can be employed for testing antibiotic susceptibilities (59, 116, 148). The broth method involves serially diluting antibiotics in liquid media in a 96-well microtiter plate, adding known concentrations of organisms, incubating, and observing for growth. The lowest dilution of antibiotic in which there is no evidence of organism growth is designated the MIC. The agar dilution technique involves incorporating multiple concentrations of antibiotics directly into agar media on which organisms are inoculated. Plates are examined following incubation for the presence of colonies, and a MIC is determined in the same manner as for broth dilution. Each method has relative advantages and disadvantages that have been reviewed by Waites et al. (148) in a comparative study utilizing both methods for testing susceptibility of *U. urealyticum* to erythromycin and tetracycline. Agar dilution typically yields 1- to 2-dilution-higher MICs than broth dilution methods. Unfortunately, there are no universally applied standards for susceptibility testing; therefore, results from different laboratories may not be truly comparable. This lack of standardization has led to confusion about the susceptibility patterns, prevalence, and degree of drug resistance among clinical isolates, particularly among ureaplasmas. Regardless of the technique employed, it is extremely important to have a consistent time point for reading results, since the endpoint may shift over time, and to inoculate the proper number of organisms into the test system. The importance of utilizing standardized inocula has been well documented, particularly with ureaplasmas (55).

The broth dilution method is the most widely used. Pure cultures to be tested should be passaged in SP-4 or 10B broth, aliquoted, and frozen (−70°C), and the color-changing

units or CFU for the frozen stock should be determined subsequently. On the day the assay is to be performed, the specimen should be thawed, diluted in fresh medium to yield 1,000 to 10,000 color-changing units or CFU/0.2 ml, and incubated (37°C) for 2 h prior to assay to allow the organism to begin active growth. A final color-changing unit count should be determined at this time. If results indicate that the number of organisms used in the assay falls outside the acceptable range of 1,000 to 10,000 per microtiter well, the assay must be considered invalid.

Mycoplasma reference strains for which the MICs of the antibiotics being tested are known should be routinely included for evaluation of the suitability of the antibiotic preparations being tested and to help control for any extraneous effects of pH or medium components. When a new drug is to be tested for antimycoplasmal activity, *Staphylococcus aureus* (ATCC 25923) and *Enterococcus faecalis* (ATCC 29212) or other appropriate bacterial reference strains should be tested according to guidelines from the National Committee for Clinical Laboratory Standards, using SP-4 and 10B broth, and results should be compared with results obtained using Mueller-Hinton broth to determine potential drug-mycoplasma medium interactions.

Each drug prepared according to guidelines from the National Committee for Clinical Laboratory Standards should be tested over a range of dilutions from 0.008 to 256 μg/ml. A drug control, positive organism control, and negative broth control should be included with each organism tested.

After inoculation, the microtiter plates should be sealed with acetate tape to prevent the release of ammonia contamination from a positive well to an adjacent negative well and also to prevent evaporation during long-term incubation. Plates should be incubated at 37°C, and results should be determined as soon as the well containing the control organism is positive; for *U. urealyticum*, this could be as early as 16 h. The initial MIC is defined as the lowest dilution of antibiotic in which metabolism of the organism is inhibited as evidenced by lack of color change in the medium at the time the control organism well first shows color change. The final MIC is defined as the lowest dilution of antibiotic in which no further color change occurs at readings on two consecutive days after prolonged incubation.

To ensure reliable results, susceptibility testing should be performed in duplicate. Ideally, tests on those isolates found to be resistant should be repeated.

Therapy

Routine drug susceptibility testing of clinical isolates remains largely the province of a relatively small number of reference laboratories because of cost, time, and labor factors. This lack of diagnostic facilities in many areas coupled with the potentially serious nature of some of the systemic ureaplasmal infections in neonates makes empiric treatment necessary in many instances until proper microbiologic diagnosis and antimicrobial susceptibility information can be obtained.

Since ureaplasmas lack peptidoglycan, they are not affected by beta-lactam antibiotics. They are not susceptible to sulfonamides or trimethoprim because they do not synthesize folic acid. However, they are generally susceptible to certain antibiotics that interfere with protein synthesis, such as tetracyclines and macrolides, but usually not to lincosamides except in high concentrations. Some isolates may be susceptible to streptomycin or other aminoglycosides but not predictably so. Some of the newer investigational quinolones are active in vitro and in vivo against *U. urealyticum* (145), but their roles as therapeutic agents have not been established in pediatric infections because of potential effects on cartilage development.

Aminoglycosides, which are commonly used against gram-negative infections in neonates, do not appear to be effective against *U. urealyticum*. Infants at the University of Alabama Hospital who have previously received >3 days of treatment with intravenous gentamicin for suspected sepsis have frequently had ureaplasmas subsequently isolated from the CSF, the bloodstream, or the lower respiratory tract (145a).

Chloramphenicol has potential bone marrow toxicity. Its clinical use has been relegated primarily to that of a second-line treatment for enteric bacillus infections unresponsive to aminoglycosides. Neither the clinical nor the microbiologic efficacy of chloramphenicol against ureaplasmal infections has been assessed in any population, although its in vitro activity against *U. urealyticum* was favorable in a recent study (145). Data from the studies of Waites et al. (145) suggest that clindamycin should not be used empirically against *U. urealyticum*, but neither should it be used routinely as a selective agent to isolate *U. urealyticum* from mixed cultures, since some strains are apparently susceptible at relatively low concentrations.

In 1974, Ford and Smith (37) published the first report of tetracycline resistance to *U. urealyticum*. In 1978, Evans and Taylor-Robinson (36) reported the incidence of tetracycline resistance among genitourinary ureaplasmal isolates to be approximately 10%. Since that time, a number of additional studies have reported variable resistance rates as high as 42%. Roberts and Kenny (102) increased the understanding of tetracycline resistance in *U. urealyticum* by demonstrating that resistant strains contain DNA sequences homologous to the streptococcal determinant TetM which mediates resistance at the level of ribosomal protein synthesis.

Macrolide resistance in *U. urealyticum* has not been studied nearly as much as tetracycline resistance. High-level erythromycin resistance (>100 μg/ml) has been known to occur but is apparently relatively uncommon when appropriate quantitative techniques to determine MICs are employed (89). Palu et al. (89) speculated that reduced binding to ribosomes may be the most common means by which macrolide resistance arises in *U. urealyticum* since macrolide uptake into the cell depends on passive diffusion.

While it is now clear that antimicrobial resistance in *U. urealyticum* is a definite laboratory as well as clinical phenomenon, the true incidence of resistance to drugs such as tetracyclines and erythromycin is really unknown. This lack of knowledge is due to several factors, such as small sample sizes in many studies, samples often collected in a nonrandom manner without regard for prior exposure to antibiotics, differences in nonstandardized methods, interpretation of endpoints, samples collected from different populations from widely varying geographic and social strata, and the fact that the actual resistance patterns may change over time.

In a recent survey by Waites et al. (145), 43 isolates of *U. urealyticum* obtained from the lower respiratory tracts of neonates were tested against chloramphenicol, ciprofloxacin, clindamycin, erythromycin, doxycycline, and gentamicin by a broth microdilution technique in 10B broth. In vitro resistance was observed in one or more strains for each of the drugs tested except erythromycin (MIC range = 0.125 to 4 μg/ml; MIC for 90% of strains = 2 μg/ml). MICs of the

remaining five antibiotics were 2 μg/ml for doxycycline, 8 μg/ml for chloramphenicol, 8 μg/ml for ciprofloxacin, 16 μg/ml for clindamycin, and 32 μg/ml for gentamicin.

Among the limited options, for 90% of strains erythromycin appears to be the most promising currently available antimicrobial agent for use in neonatal ureaplasmal infections. Because of its toxicity, doxycycline, though active against most strains of *U. urealyticum*, is not a realistic therapeutic alternative except for ill infants from whom *U. urealyticum* is isolated from the CSF. Finding only 7% doxycycline resistance in neonatal ureaplasmal isolates, despite the small sample size, is encouraging (145).

Making specific recommendations for treating ureaplasmal infections is particularly difficult in view of the fact that the spectrum of ureaplasmal disease in neonates has not been fully described. Therefore, not only is choice among the limited drug options for treating ureaplasmal infections controversial, but also the actual indications concerning conditions under which treatment should be offered are debatable. Ureaplasmas can frequently be isolated from the upper respiratory tracts of neonates, but there is no evidence that cultures should be obtained routinely in the absence of symptomatic disease. Neonates clinically ill with pneumonitis or showing signs of CNS disease, particularly progressive hydrocephalus with or without CSF pleocytosis, for whom bacterial cultures are negative or in whom there is no improvement with antibiotic therapy warrant cultures for *U. urealyticum* and treatment if cultures are positive. The higher levels of erythromycin in serum achievable by intravenous administration, with good penetration into tissues including lung tissue, make it likely that the MICs for most strains may actually be exceeded in vivo when the drug is given parenterally (147, 149, 155).

Empiric treatment should be initiated in an unstable infant with a positive culture for *U. urealyticum* from CSF, trachea, pleural fluid, or blood because of the time (several days) required to obtain antimicrobial susceptibility data. Although CSF is normally considered a sterile body fluid in which any bacterial infection can be potentially life-threatening, some ureaplasma-caused CSF infections in term neonates may be asymptomatic, without a CSF inflammatory response, and self-limited (147, 149). However, this may not be the case in low-birth-weight, preterm neonates, who without agressive intervention typically have difficulty in localizing and combatting systemic bacterial infections. Because of poor CSF penetration by erythromycin, tetracyclines are probably the most effective drugs available for ureaplasmal infections of the CSF. It may be prudent to observe stable, asymptomatic infants and to document persistent infection through follow-up CSF cultures before treatment with these potentially toxic antibiotics is initiated.

Several mechanisms may be operative in the development of CLD in neonates with *U. urealyticum* present in the lower respiratory tract. This condition is quite distinct from the more acute entity of pneumonia which may also be caused by *U. urealyticum* in an undetermined number of cases. These organisms may be contributory causes of CLD, initiating an inflammatory response, promoting the production and release of toxic oxygen species, interfering with pulmonary defenses, or promoting injury by other organisms. Therefore, the appropriate treatment may involve eradication of the organisms by antibiotics, modulation of the immune response by steroids, administration of antioxidants, or even surfactant therapy. Until the mechanism of disease production is known, therapy will need to be based on the judgment of each individual clinician.

Since clinically useful antibiotics are only mycoplasmastatic, not mycoplasmacidal, the immune status of the premature infant might well be a crucial component in any successful drug treatment. Experience in treatment of chronic ureaplasmal and mycoplasmal arthritis in hypogammaglobulinemic patients clearly suggests that this is the case (138, 142, 158). Though there are no established guidelines, limited clinical experience with neonates and other persons with systemic infections due to *U. urealyticum* suggests that a minimum of 10 to 14 days of treatment is best. Whether the intravenous or oral route with erythromycin, doxycycline, or alternatives is employed depends on the overall condition of the patient and the nature of the infection being treated. Optimally, isolates from infants treated with antibiotics for ureaplasmal infection should be tested for drug susceptibility and the infants should be monitored for clinical response to the chosen antibiotic. Follow-up cultures of the infected site to document microbiologic efficacy of the drug are suggested when clinical improvement does not occur.

Future studies are needed to assess the utility of antibiotics, especially erythromycin, in treating neonatal ureaplasmal infections and should include pharmacokinetic investigations aimed at determining peak levels, half-life, tolerability, safety, and microbiologic efficacy of intravenous preparations. More information is needed concerning the immune status of the premature infant and the relationship to successful therapy. Such studies are necessary before any definitive recommendations regarding treatment of systemic neonatal ureaplasmal infections are made and before any controlled trials aimed at clarifying the etiologic significance of this organism in pathologic conditions in this population, particularly the important question of its association with CLD of prematurity, are done.

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