ORIGINAL ARTICLES

Role of *Ureaplasma urealyticum* in lung disease of prematurity

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Abstract

***Aim*—To examine the role of *Ureaplasma urealyticum* colonisation or infection in neonatal lung disease.**

***Methods*—Endotracheal aspirates from ventilated infants less than 28 weeks of gestation were cultured for *U urealyticum* and outcomes compared in infants with positive and negative cultures.**

***Results*—*U urealyticum* was isolated from aspirates of 39 of 143 (27%) infants. Respiratory distress syndrome (RDS) occurred significantly less often in colonised, than in non-colonised infants (p=0.002). Multivariate logistic regression analysis showed that in singleton infants, ureaplasma colonisation was the only independent (negative) predictor of RDS (OR 0.36; p=0.02). Both gestational age (OR 0.46; p=0.006) and isolation of *U urealyticum* (OR 3.0; p=0.05) were independent predictors of chronic lung disease (CLD), as defined by requirement for supplemental oxygen at 36 weeks of gestational age. Multiple gestation was also a major independent predictor of RDS and CLD.**

***Conclusions*—Colonisation or infection with ureaplasma apparently protects premature infants against the development of RDS (suggesting intrauterine infection). However, in singleton infants, it predisposes to development of CLD, independently of gestational age. Treatment of affected infants after birth is unlikely to significantly improve the outcome and methods are required to identify and treat the women with intrauterine ureaplasmal infection, before preterm delivery occurs.**

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Keywords: *Ureaplasma urealyticum*; hyaline membrane disease; chronic neonatal lung disease; intrauterine infection

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Although the causes of preterm delivery are still poorly understood, survival of premature infants has improved progressively over the past 30 years because of advances in neonatal intensive care. However, survivors remain at risk from chronic lung disease (CLD).[1 2] The pathogenesis of CLD is poorly understood, but iatrogenic factors, including endotracheal intubation, high peak inspiratory pressures, and high oxygen concentrations during mechanical ventilation, are important.[3 4] The use of antepartum steroids and exogenous surfactant has significantly decreased the incidence and severity of respiratory distress syndrome (RDS). The reduced need for ventilatory support—and consequently its potentially adverse effects—and other improvements in neonatal management, have been reflected in a decrease in the incidence of CLD. However, although administration of exogenous surfactant to infants with RDS has improved the immediate outcome, it has not consistently reduced the incidence of CLD.[5]

Ureaplasma urealyticum is a commensal organism in the lower genital tracts of 40–80% of women. Isolation of ureaplasmas from the placenta or amniotic fluid is consistently associated with preterm delivery, histological evidence of chorioamnionitis, and postpartum endometritis.[6–8] However, prospective studies have shown neither an association between vaginal colonisation and premature delivery, nor any reduction in prematurity resulting from treatment of colonised women with erythromycin.[9 10] Presumably, any adverse effects associated with ureaplasma colonisation are confined to a subset of colonised women with intrauterine infection.

Upper respiratory colonisation with *U urealyticum* in premature neonates is often associated with clinical, radiological, or laboratory evidence of respiratory infection or frank pneumonia.[11–16] An infective and inflammatory basis for CLD has been proposed[17–18]and, in particular, neonatal colonisation or infection with *U urealyticum* has been linked to the development of CLD.[11 19–22] However, some studies have failed to confirm an association and a causal association remains unproved and controversial.[23–26] Differences in results between studies can, in part, be attributed to variability in study design, the characteristics and size of patient cohorts, and the quality of laboratory and statistical methods.

This prospective cohort study aimed to examine the association between *U urealyticum* colonisation and respiratory disease in premature infants most at risk of both; optimal

specimens and culture methods and three different definitions of CLD were used. A newly developed polymerase chain reaction (PCR) was used to determine the biotype and serotypes of *U urealyticum* isolates.

Methods

Westmead and Royal Prince Alfred Hospitals are both teaching hospitals of the University of Sydney. They provide tertiary obstetric and perinatal referral services. The study was approved by the institutional committees of both hospitals.

The study was confined to infants of less than 28 weeks of gestational age. A preliminary study at Westmead Hospital showed that *U urealyticum* was isolated from only one of 53 (2%) infants of 28–32 weeks of gestation, compared with 14 of 60 (23%) of those of less than 28 weeks gestation (unpublished data). The risk of CLD is also significantly higher in infants of less than 28 weeks gestation.[2]

Consecutive infants admitted to neonatal intensive care at Westmead Hospital, who were less than 28 weeks of gestational age and required ventilation, were enrolled during two periods October 1993 to October 1994 and January 1995 to November 1996 (total of 34 months). Consecutive infants admitted to neonatal intensive care at Royal Prince Alfred Hospital who fulfilled the same criteria were enrolled over 16 months from February 1996 to June 1997.

Gestational age was determined by the last normal menstrual period and ultrasound examination before 20 weeks of gestation.

Endotracheal aspirates (ETA) were collected aseptically on the first and fourth days of life and, if the infant was still being ventilated, on day 28 of life (at Westmead Hospital only). The first ETA was collected immediately before administration of surfactant, if indicated, for treatment of hyaline membrane disease (HMD).

After instillation of sterile isotonic saline (0.3 ml) into the endotracheal tube, the infant was ventilated for 10 breaths. Using an appropriately sized catheter, the trachea was suctioned at a point 0.5 cm beyond the tip of the endotracheal tube; after another 10 ventilator breaths suctioning was repeated with a new catheter. ETA were stored at 4°C in a sterile container and processed within 24 hours of collection (average 15 hours), or transported by courier in ureaplasma culture broth to the laboratory and processed immediately.

LABORATORY METHODS

Cultures

Semi-quantitative cultures for genital mycoplasmas were performed using three 10-fold dilutions of the aspirate in ureaplasma broth, containing urea, neutral red indicator, and penicillin. Each dilution was plated on to A8 agar and cultured for 7 days at 35°C in 5% CO_2. If colour change occurred, broths were subcultured on to A8 agar plates, which were examined daily for 7 days for the presence of typical mycoplasma or ureaplasma colonies. Growth of *U urealyticum* was graded as scant, moderate, or heavy if the highest dilution at which it was detected was 10^{-1}, 10^{-2}, or 10^{-3}, respectively.

ETAs from infants at Westmead Hospital were also cultured aerobically for bacteria on horse blood agar for 48 hours in 5% CO_2. *Chlamydia trachomatis* infection is rare in our population (<1%; unpublished data) and cultures were not performed routinely.

Polymerase chain reaction (PCR)

Immediately after colour change had occurred, positive broth cultures (10^{-1} dilution), and a selection of negative cultures after one week's incubation, were stored at −70°C for PCR. After thawing, 0.5 ml of each culture was harvested by centrifugation at 14000 × *g* for 20 minutes. DNA was isolated, as described before.[27]

The methods used for identification, biotyping, and serotyping of *U urealyticum* have been described before.[27] Briefly, primers UMS-125 and UMA226 were used, initially to detect *U urealyticum* and distinguish biovars 1 and 2. Specimens in which *U urealyticum* serovar 1 was detected were reamplified to identify serovars using primers UMS-125 and UMA269 for serovars 3/14, UMS-125 and UMA 269' for serovars 1 and 6 and UMA 54 and UMA 269' for serovar 6.

CLINICAL DATA COLLECTION AND DEFINITIONS

Demographic and clinical data were obtained from the neonatal intensive care clinical databases and individual patients' medical records. Threatened premature labour (TPL) was defined as spontaneous uterine contractions that did not result in the delivery of the fetus during that episode. Pregnancy induced hypertension (PIH) was defined as hypertension first detected during pregnancy with a diastolic blood pressure of 90 mm Hg or more on at least two occasions separated by 6 hours. Antepartum haemorrhage (APH) was defined as clinically significant bleeding from the birth canal after week 20 of pregnancy. Premature rupture of membranes (PROM) was that occurring at any time before delivery. Antenatal steroids (two doses of betamethasone, 24 hours apart) were administered if preterm delivery was anticipated at less than 34 weeks of gestation. Mothers were recorded as having had steroids if they had received at least one dose before delivery. At Westmead Hospital, erythromycin and metronidazole, and at Royal Prince Alfred Hospital, amoxycillin, were normally administered to patients presenting with PROM or clinical manifestations of sepsis. Antibiotics were given at the discretion of the obstetrician.

RDS was diagnosed in ventilated infants with a requirement for supplemental oxygen of more that 40%, to maintain arterial oxygen tension above 60 mm Hg, and radiological changes consistent with HMD (bilateral fine reticular pattern). Surfactant was given for treatment of persistent RDS. Three different definitions of CLD were used: (a) the need for supplemental oxygen at 28 days of age; (b) the

need for supplemental oxygen at 28 days of age with radiological changes consistent with CLD[28]; and (c) the need for supplemental oxygen at 36 weeks postconceptional age. The latter is now regarded as the best predictor of long term outcome in very low birthweight infants.[29] Neonatal sepsis was defined by a positive blood culture.

Differences between groups were compared using Fisher's exact test, the χ^2 test, or the unpaired Student's *t* test, as appropriate. Logistic regression analysis, both univariate and multivariate, was used to test for associations between potential risk factors and the outcome of interest—either HMD or CLD(c). Odds ratios (OR) and their 95% confidence intervals (CI) were used to quantify the degree of association.

The statistical package SPSS for Windows, version 6.01, was used and a 5% level of significance was used throughout the analysis.

Results

One or more ETA cultures were collected from 113 infants at Westmead Hospital and 35 infants at Royal Prince Alfred Hospital, who fulfilled the study criteria. Aerobic bacteria (*Escherichia coli* in four patients and *Klebsiella pneumoniae* in one) were isolated (on HBA and/or in ureaplasma broth) from aspirates of two infants at Westmead and three at Royal Prince Alfred Hospital. These infants were excluded from further analysis. *Mycoplasma hominis* was not isolated from any ETA cultures.

Demographic and clinical details of the remaining infants from each hospital are shown in table 1. The only significant difference between the two units was in the rates of caesarean section. As this was not apparently related to any of the relevant outcomes, the data from both hospitals were combined. Around 30% of infants were twins or triplets.

Predictably, there was a high incidence of RDS and more than 70% of infants were treated with exogenous surfactant; 85% of mothers had been given steroids before delivery and, of these, two thirds had been given a full course. Antibiotics had been prescribed for 73 (59%) mothers in the week before delivery, including 52 (71% of those who received antibiotics or 42% of all mothers) who were given erythromycin alone or with other antibiotics. The duration of antibiotic treatment was not recorded.

U urealyticum was isolated on at least one occasion from 39 (27%) infants; of these, 38 had an ETA cultured on the first and or fourth day of life and 13 on day 28. Positive results were obtained from 25 of 36 (69.4%) infants on day 1, and 27 of 32 (84.4%) on day 4, and nine of 13 (69.2%) on day 28. Four infants were culture positive only on day 28, including three who had had one or more previous negative cultures. There was no difference in the proportion of infants whose mothers had been given antibiotics before delivery, between those with negative cultures on the first day of life who subsequently tested positive (8/10), and those whose cultures were positive on day 1 (22 /25). Cultures collected on day 4 were significantly more likely to have moderate to heavy growth (19/27; 70.3%) compared with those taken on the first (10/25; 40%; p <0.05) or 28th day (2/9; 22.2%; p=0.02).

Oxygen dependence at 28 days was common; it was present in 89% of survivors, of whom 60% also had radiological changes. Oxygen dependency was still present at 36 weeks postconceptional age—CLD (c)—in 34% of surviving infants.

Selected data for all mothers (table 2) and all infants (table 3) and for mothers and infants of singleton births only (table 4) were compared according to whether *U urealyticum* cultures were positive or negative.

Mothers of infants who were colonised with *U urealyticum* were significantly more likely to have been given antibiotics before delivery (p=0.002 for both singleton and multiple births). Mothers of colonised infants, overall,

Table 1 Comparison of infants admitted to neonatal intensive care units at Westmead and King George V Hospitals

	Westmead	*King George V*
Mothers n=	95	28
Antenatal steroids*	78 (82%)	27 (96%)
Antenatal antibiotics†	54 (57%)	19 (68%)
Caesarean delivery	30 (32%)	17 (61%)**
PROM‡ >24 hours	28 (30%)	7 (25%)
Infants (total): n=	111	32
Singleton births	76 (68%)	23 (72%)
F:M ratio	1.11	1.00
Mean birthweight (g)	827 (181)	850 (155)
Range	490–1275	590–1160
Mean gestation: weeks	25.8 (1.1)	25.8–0.9
Range	24–27	24–27
Neonatal sepsis††	38 (40%)	8 (29%)
HMD	84 (76%)	25 (78%)
Surfactant	79 (71%)	23 (72%)
Chronic lung disease (c)¶	28 (25%)	10 (31%)
Deaths§	28 (25%)	4 (13%)
U urealyticum isolated	28 (25%)	11 (34%)

**p<0.05; Caesarean section rates was the only significant difference between the two units.
*Steroids given to mother for fetal lung maturation if delivery anticipated before 34 weeks gestation.
†Antibiotics given to mother before (within 1 week of) delivery.
‡PROM, premature rupture of the membranes.
††Neonatal sepsis defined by a positive blood culture.
¶Requirement for supplemental oxygen at 36 weeks postconceptional age (see text for definitions).
§Deaths that occurred before 36 weeks postconceptional age (2 additional infants died subsequently).

Table 2 Comparison of antenatal data between mothers of all infants with and without U urealyticum colonisation

	*Isolated**	*Not isolated*	*p Value*
Mothers n=	36*	89*	
TPL	22 (61%)	47 (52%)	
PIH	1 (3%)	11 (12%)	
APH	15 (42%)	20 (23%)	0.05
Steroids†	34 (94%)	74 (83%)	
Antibiotics‡	29 (81%)	44/88 (50%)	0.002
Caesarean delivery	11 (31%)	36 (40%)	
Spontaneous labour	30 (83%)	61 (69%)	
PROM <24 hours	22 (61%)	68 (76%)	
PROM 1–7 days	9 (25%)	10 (11%)	
PROM >7 days	5 (14%)	11 (12%)	

**U urealyticum* was isolated from 39 infants. Mothers of two sets of twins, with discrepant culture results, are included in both columns. "Isolated" column also includes a mother of twins and a mother of triplets, all five of whose infants were culture positive.
†Steroids given to mother before delivery for fetal lung maturation.
‡Antibiotics given to mother before (within 1 week of) delivery.

Table 3 Comparison of neonatal data between all infants with and without U urealyticum

	Isolated	*Not isolated*	*p Value*
N=	39 (27.3%)	104	
Hyaline membrane disease	22 (56%)	87 (84%)	0.002
Neonatal sepsis*	15 (39%)	31 (30%)	
Surfactant	20 (51%)	82 (79%)	0.002
Deaths	6 (15%)	26 (25%)	
CLD (a)†	31/34 (91%)	69/78 (89%)	
CLD (b)†	22/34 (65%)	38/78 (49%)	
CLD (c)†	15/34 (44%)	23/78 (30%)	
CLD (c) and deaths	21/39 (54%)	49/104 (47%)	

*As defined by a positive blood culture; clinical significance was not determined.
†CLD (a) are those still requiring supplemental oxygen at 28 days of life; CLD (b) are those still requiring supplemental oxygen at 28 days of life with an abnormal chest *x*-ray; CLD (c) are those still requiring supplemental oxygen at 36 weeks post-conceptional age (PCA); denominators are surviving infants.

were more likely to have had an antepartum haemorrhage ($p<0.05$) but this difference was not significant in the singleton only group.

Infants colonised with *U urealyticum* were significantly less likely to have RDS and to have been given surfactant ($p=0.002$). This difference was confined to singletons ($p=0.001$); proportions of infants of multiple births who had RDS were identical whether or not they were colonised with ureaplasmas (8/9 *vs* 31/35; 89% for both). Among singletons, the mortality was lower in colonised than in non-colonised infants, but the numbers were small and the difference was not significant (7% *vs* 23% among singletons; $p=0.09$). Despite the apparently protective effect of ureaplasma colonisation for HMD, significantly more colonised singleton infants developed CLD ($p=0.03$), as defined by supplemental oxygen requirement at 36 weeks of postconceptional age. However, among infants of multiple births almost identical proportions developed CLD, whether or not they were colonised with ureaplasmas (3/6 *vs* 13/25).

The independent risk factors for the development of RDS and CLD were identified by multivariate logistic regression analysis, using backward elimination. When all infants were included, the independent predictors of RDS were multiple gestation (positive association; OR 3.6; 95% CI 1.2–10.7); administration of antibiotics to the mother (negative association; OR 0.33; 95% CI 0.12–0.88); and a positive culture for *U urealyticum* (negative association; OR 0.36, 95% CI 0.15–0.85). For singleton infants, a positive culture for *U urealyticum* was the only independent (negative) predictor of RDS (OR 0.2, 95% CI, 0.08–0.5). Independent predictors of CLD (c), are shown in table 5. In singleton infants, gestational age and isolation of *U urealyticum* were both independent predictors of CLD (c).

Table 4 Comparison of selected data between singleton infants and their mothers with and without U urealyticum colonisation

	Isolated	*Not isolated*	*p Value*
N = mothers/infants	30 (30.3%)	69	
Antenatal steroids*	28 (80%)	55 (80%)	
Antibiotics†	24 (80%)	31 (45%)	0.002
Spontaneous labour	25 (83%)	44 (64%)	
Cesarean delivery	10 (33%)	30 (44%)	
PROM‡ <24 hours	18 (60%)	49 (71%)	
PROM 1–7 days	8 (27%)	10 (15%)	
PROM >7 days	4 (13%)	10 (15%)	
Mean birthweight, g (range)	850 (161) (620–1245)	825 (181) (490–1275)	
Mean gestation; weeks (range)	25.7 (1.3) (24–27)	25.8 (1.2) (24–27)	
HMD	14 (47%)	56 (81%)	0.001
Surfactant	12 (40%)	53 (77%)	0.001
Neonatal sepsis††	13 (43%)	22 (32%)	
Deaths	2 (7%)	16 (23%)	0.09
CLD (a)	25/28 (89%)	46/53 (87%)	
CLD (b)	18/28 (64%)	25/53 (47%)	
CLD (c)	12/28 (43%)	10/53 (19%)	0.03
CLD (c) and deaths	14/30 (47%)	26/69 (38%)	

*Steroids given to mother before delivery for fetal lung maturation.
†Antibiotics given to mother before (within 1 week of) delivery.
‡PROM; premature rupture of membranes.
††Neonatal sepsis as defined by a positive blood culture.

Table 5 Independent predictors of chronic lung disease (c) by multivariate logistic regression analysis

Factor	*Odds Ratio*	*Confidence Interval*	*Significance*
All infants			
Gestational age	0.47*	0.30–0.75	0.001
Multiple gestation	3.1	1.2–8.1	0.02
Antibiotics	2.7	1.0–7.4	0.05
Singletons only			
Gestational age	0.46*	0.26–0.80	0.006
U urealyticum isolated	3.0	1.0–9.1	0.05

*The odds ratio is the factor by which CLD (c) decreases for each week of gestational age over 24 weeks.

PCR was performed on ureaplasma culture broths, from 50 different infants; *U urealyticum* had been isolated from 22. *U urealyticum* was identified by PCR in 19 (86%) culture positive and no culture negative specimens; biovar 1 was found in 16 (84%) and biovar 2 in four cultures (one culture had both). Serovars 3/14 were detected most commonly (7/16; 43.8%) among cultures containing biovar 1.

Discussion

This study primarily aimed to investigate the independent role of *U urealyticum* infection in the development of respiratory disease in high risk infants (those less than 28 weeks of gestation). The study was limited to infants in the gestational age group in which both ureaplasma colonisation and respiratory disease (RDS and CLD) are most common. Our findings confirmed and extended those of others, in showing significant differences in outcomes in colonised, compared with non-colonised infants.

U urealyticum was isolated from the ETA of 27% of infants, a rate comparable with that reported before. A single culture at birth would have underestimated the colonisation rate. A lower rate of recovery of ureaplasmas from cultures taken on the first day of life, compared with later,[20 25] probably reflects the antibacterial effects of amniotic fluid or intrapartum exposure, rather than nosocomial infection. We have shown that *U urealyticum* is inhibited, in vitro, by surfactant (unpublished observation), therefore we collected the first culture before surfactant was given. Maternal antibiotics did not prevent neonatal colonisation; significantly more mothers of the colonised infants had been given antibiotics—often erythromycin—in the week before delivery.

RDS occurred significantly less often among colonised infants compared with non-colonised

infants, despite the fact that similar proportions of mothers in both groups had been given steroids before delivery. This was masked by the effect of multiple gestation, which was associated with a very high incidence of RDS (89%), irrespective of ureaplasma colonisation. After multivariate logistic regression analysis, colonisation with *U urealyticum* remained the only significant independent predictor of RDS in singleton infants. The lower incidence of RDS in colonised infants was reflected in a lower mortality in the first 28 days of life, although the numbers were too small to be significant. However, despite the lower incidence of RDS, CLD occurred more frequently in colonised infants.

Previous studies have attempted to prove an association between ureaplasma colonisation and CLD, with variable results. CLD correlates best with gestational age and iatrogenic complications of respiratory support, including barotrauma and oxygen toxicity. Lung damage and development of CLD may depend on the balance between pro-inflammatory cytokines—interleukin (IL)-1β, IL-8 and tumour necrosis factor α (TNFα)—which are increased in ventilated infants and anti-inflammatory cytokines—IL-10 and IL-6—which may be reduced in premature infants.[16 31] Respiratory infection can aggravate cytokine mediated lung injury[32] and high IL-1β concentrations and ratios of IL-1β to IL-6 and TNF-α to IL-6 have been found in infants colonised with ureaplasma.[16] This supports other evidence that colonised infants often have congenital pneumonia,[10] but the association between these early effects of ureaplasma infection and CLD is inconsistent and probably indirect.[16 30]

In this study multivariate logistic regression analysis showed that both gestational age and colonisation with *U urealyticum* were significant independent predictors of CLD (oxygen dependence at 36 weeks postconceptional age) in singleton infants. These factors are closely correlated,[8 10] and their individual effects difficult to separate. Previous studies of the role of *U urealyticum* in CLD have shown inconsistent results.[26] Most, however, have shown a higher—but not always significantly higher—incidence of CLD in infants colonised with *U urealyticum*.

A meta-analysis of 17 studies, of variable quality, showed an overall relative risk (RR) of 1.72 (95% confidence interval 1.50–1.96).[33] The mean RR for studies performed since surfactant replacement therapy has been used, was significantly less (1.24; 95% CI, 1.10–1.49) than in earlier studies (1.92; 95% CI, 1.59-2.32). Several well designed studies reported more recently, using a requirement for supplemental oxygen at 36 weeks gestational age as the definition of CLD,[29] have still shown variable results.[22 34]

Circumstantial evidence suggests that *U urealyticum* colonisation in premature infants usually reflects intrauterine infection and a causative role in preterm delivery. *U urealyticum* can cause chronic intrauterine infection,[35] chorioamnionitis,[8] amniotic fluid infection[36] and congenital pneumonia.[14] Its isolation from placentas, following caesarean section with intact membranes,[10] and from infants, correlates strongly with spontaneous preterm birth. However, although colonised infants often have radiological and or laboratory evidence of pneumonia or systemic infection, most do not have obvious clinical signs of sepsis.

The most likely explanation for the protective effect of ureaplasma colonisation against HMD might be a stimulatory effect of subacute intrauterine infection on lung maturation and surfactant production. This is supported by a recent report that only four of nine (44%) infants colonised with *U urealyticum* required surfactant, compared with 84% of 51 non-colonised infants ($p = 0.04$).[30] However, in most previous studies, RDS has not been mentioned specifically or the incidence of RDS has been higher in infants colonised with ureaplasmas.[11 15] No previous study has analysed outcomes in singleton infants separately. In our study, the very high incidence of RDS in multiple births overshadowed the effect of ureaplasma infection, but this did not alter the overall result.

Treatment with both steroids and antibiotics (including erythromycin in many of our patients), at the onset of premature labour or rupture of membranes, is widespread. We suggest that this could suppress the inflammation caused by ureaplasma infection and so delay delivery for long enough to permit lung maturation. This is supported by our observation that colonisation of infants with *U urealyticum* was associated with an interval between membrane rupture and delivery (1–7 days); the usual outcome of intrauterine bacterial infection is prompt delivery.

It has been suggested that a randomised, controlled trial of appropriate antibiotic treatment is needed to determine whether *U urealyticum* has a causative role in CLD.[26] However, inconsistent results, small relative risks in previous studies, and undefined effects of other interventions, mean that large numbers of colonised premature infants would be needed to show a significantly improved outcome. A small, randomised, controlled trial of erythromycin showed no beneficial effect of treatment on the incidence of CLD,[30] suggesting that an antibiotic regimen at birth may be too late to significantly affect outcome.

There have been significant improvements in outcomes following preterm delivery, but less progress in understanding the causes and reducing the incidence. Intrauterine infection with *U urealyticum* has a causative role in a significant proportion of deliveries before 28 weeks. The determinants of adverse outcomes, including CLD, are complex, but gestational age at birth is the most important. Prevention or early treatment of intrauterine infection, to prevent preterm delivery, is only intervention likely to improve outcomes in pregnancies where *U urealyticum* infection is a factor.

Some serovars of *U urealyticum* have been implicated in disease more than others.[37] The use of PCR based serotyping and recombinant antigens for antibody assays, in future studies,

could help to overcome technical difficulties involved in confirming these associations.

Our research has provided important new insights into the complex interactions between *U urealyticum* infection (presumably in utero) and various treatment modalities that can affect the outcome of preterm birth. The challenge is to identify the small subset of women at risk from uterine infection among the much larger proportion who carry *U urealyticum* in the vagina without ill effect, and treat them before premature delivery occurs.

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LETTERS TO THE EDITOR

Outcome in antenatally diagnosed renal pelvis dilatation

EDITOR,—Dr Nicholl raises some pertinent points in his letter[1] regarding our paper.[2] The nub of the matter is whether asymptomatic vesico-ureteric reflux (VUR), detected as a result of antenatal ultrasound findings is clinically important or not. The answer to this question is not yet known and will require a trial that looks at what, if any, difference treatment makes to outcome, as judged by the development of renal scars.

Until this matter is resolved, however, we feel it appropriate to look for VUR when there has been antenatal renal pelvis dilatation, and treat accordingly. As stated in our study,[1] this judgement is partly based on the fact that the prevalence of asymptomatic VUR is around 1%, as described by Bailey, in contrast to an incidence of 20% in our study, implying that our findings were significant.

We accept that in a review of the published findings, from which Bailey acquired his data, the radiological techniques used may have differed from those currently in use, but as can be imagined, it is not easy to acquire information about the incidence of VUR in healthy children, and Bailey's work is, to our knowledge, the currently accepted reference.[2]

With regard to the specific points raised by Nicholl around 50% of the babies with VUR in our study, have now undergone further imaging at the age of 3 years. Their reflux had resolved and, more importantly, no renal scarring had been incurred. In those babies where both postnatal ultrasonography and the micturating cystogram were normal, the infants were discharged from further follow up, as we saw no further indication for continuing their surveillance.

The fact that only one baby required surgical intervention reflects that VUR, which is generally treated medically, was the most common finding, and a more conservative approach is now adopted in cases of pelviureteric junction obstruction.

In table 1 of our study we included, under the diagnosis of "idiopathic dilatation" only those infants in whom persisting renal pelvis dilatation was > 10 mm, because in those (n=22) in whom it was 5–10 mm and the micturating cystogram was normal, we did not feel an MAG III renogram was indicated; therefore, they did not strictly fulfil our criteria for this diagnostic label.

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Unlicensed and off label drug use in neonates

EDITOR,—Most papers in this journal have a commendable clear "take home" message, but this was not really true of the recent paper by Conroy *et al*.[1] They described a 13 week, one unit study in Derby as finding that two thirds of all neonatal prescriptions (294 out of 455) involved the use of a drug in a way that the manufacturers had no license to recommend. The authors do not say what should be done about it.[2]

They note that 84 prescriptions for vitamins and 77 for penicillin or an aminoglycoside used a dose other than the one mentioned in the drug data sheet. But they must be aware, surely, that data sheet information is advisory in nature. Secondly, an immense amount of information has been published on these issues since the data sheets were first prepared. Thirdly, many UK college and American academy guidelines recommend doses that differ from those in the data sheets. The authors note that 36 prescriptions for caffeine, morphine, or parenteral nutrition had to be made up in the local pharmacy aseptic service unit, and the products were therefore classes as unlicensed. They do not suggest, however, how they would prefer to see the prescribing and dispensing of these drugs handled.

What was the intended message when arrangements were made for the news media to latch on to this report before most clinicians had had their chance to read the paper for themselves? Were headlines such as "Doctors raise alarm over drugs given to babies," and "Babies used as drug guinea pigs" really what you hoped to generate? Coming only a week after an article in the *New Scientist*,[3] inspired by a steer from the Derby clinicians, the journal article led the BBC to report that "Doctors are calling for stricter controls to ensure children are not given dangerous doses of adult drugs." Such manipulation of the news media does a serious disservice to a serious subject. Professor Anysley-Green's subsequent letter,[4] contrasting the lack of support for paediatric pharmacology in the UK with the establishment of 13 such centres in North America, rather suggests that it was a simple bid for money.

Readers who turned to Professor Sir David Hull's commentary in the same issue will have found little enlightenment. His main message seemed to be that everyone should buy *Medicines for Children*. However, any suggestion that this would be the first reference text to clearly identify unlicensed and off label paediatric drug use in the UK would be misleading. Even should that be the case, it wouldn't get us very far: the new consensus driven text may tell us what most people currently do, but what most do is not necessarily right.

The neonatal use of gentamicin typifies some of the key issues, as Conroy has herself highlighted.[5] The drug has been in neonatal use for over 30 years, but the best dose is still a matter for debate. High trough concentrations frequently cause concern, but there are actually very few reports of neonatal renal or ototoxicity. Low peak concentrations, on the other hand, often go unremarked.

Six separate papers have been published over the past 10 years, which show that a therapeutic peak concentration will not be acheived for 12 to 24 hours using any standard policy, unless an initial loading dose is given—the volume of distribution being particularly high at birth—but such a strategy is still only recommended in a few reference texts.

This is not an area where more money is needed for research. More than 200 papers have already been published on this topic over the past decade. There is no commercial pressure on the manufacturer to modify the data sheet: they are generic products unprotected by patents. Nor does the Medicines Control Agency believe that it should take the intiative over this, although it would be very willing to review the case for voluntary modification with manufacturers if approached by an appropriate and responsible professional body. Why, then, does the Royal College of Paediatrics and Child Health not do this?

For most of the drugs listed by Conroy, there is no need for further research, or more papers stating that drug data sheets are out of step with current practice. Nor do "they" need to tighten the prescribing rules and restrict what "we" can do. What is needed is sensible, sustained, and constructive dialogue between the profession, the licensing authorities, and the manufacturers, to get drug sheets revised at regular intervals, so that they reflect all the additional information that becomes available in the years after the product first comes on the market. My message is, that it is up to the profession to start the ball rolling.

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Drs Conroy *et al* respond:

We welcome the opportunity to clarify our "take home" message. This is actually very simple: drugs used in children should be tested scientifically to ensure that age dependent changes in pharmacokinetics and pharmacodynamics are known, the likely side effects are anticipated, and that the minimum effective dose can be given.

We expect the Medicines Control Agency to ensure that neonates receive drugs that are as carefully evaluated for efficacy, safety, and quality as the drugs given to adults. We also expect the pharmaceutical industry to provide drugs that are appropriate for use in neonates and children as well as in adults. We accept that health professionals involved in the care of neonates have a responsibility to contribute to this process. It requires a joint effort between healthcare staff caring for children, the industry, and the government. Dr Hey states that data sheet information is "advisory," but this is the only information that the pharmaceutical manufacturer will take responsibility for, anything else is on the head of the prescriber.

There may be few published reports of renal or ototoxicity following the use of gentamicin in neonates, as it is difficult to definitely attribute such problems to the drug. However, this does not mean that gentamicin does not cause such problems. We note that renal insufficiency is not uncommon in acutely ill preterm infants and that long term hearing problems occur in babies who have been through neonatal intensive care. We do not know how many of these problems are associated with gentamicin use because the babies

have many other potentially contributory problems. Research is needed to establish the dose and frequency required to provide therapeutic, non-toxic serum concentrations of this drug for babies of all gestations.[1]

We were surprised by the media interest in our paper and responded to requests for interviews accordingly. Unfortunately, we cannot be held responsible for the headlines or tone of the published newspaper reports.

The extent of drug toxicity from unlicensed and off label drug use in neonates is unknown. We know that severe adverse drug reactions in children are more likely to occur with unlicensed and off label treatment than licensed drugs.[2] The scientific study of drug treatment in neonates has been relatively neglected by both doctors and pharmacists in the UK and Europe. However, there are positive developments: the British Forum for the Use of Medicines in Children and the European Network for Drug Investigation in Children are trying to both encourage and coordinate clinical trials in this area.[3]

It is clear that many health professionals now accept the need for research in paediatric therapeutics. We are not simply bidding for money but trying to raise the profile of a neglected area of research. Historically, research has been centred on disease in specific areas—for example, cystic fibrosis, leukaemia, cardiac defects, etc. When seeking funding for research on the extent and risk of unlicensed and off label drug use in children[2 4] we were told by a major children's charity that they did not consider it an appropriate area for research and that they would not even consider an application for funding. We hope that the studies documenting the extent of unlicensed and off label prescribing[4 5] and the consequences of such prescribing[7] will convince the Department of Health and the major charities that this is an important area of research, and that the use of drugs in the neonate should be evidence based.

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Editors' comments

We issue press releases on articles of public interest with the aim of helping journalists understand the material. The press releases are seen in advance by authors who have an opportunity to make changes, and are issued with an embargo date, to avoid media publicity before the Journal's publication date. However, we have no control over how the media choose to headline this information. The public and the media have access to articles in scientific journals once they are published and if we did not issue press releases we believe there would be even greater scope for misinterpretion.

Glycosaminoglycans in neonatal urine

EDITOR,—Mucopolysaccharidoses (MPS) are a group of lysosomal storage disorders caused by deficiency of the enzymes catalysing the stepwise degradation of glycosaminoglycans (GAG). Bone marrow transplantation can slow down or reverse some of the features of these diseases. Enzyme replacement (ERT) studies in several animal models of MPS disorders have shown promising results[1–3]; human clinical trials of ERT in MPS type I have only recently become possible.[4] The clinical symptoms of MPS usually become evident only between the second and third years of life. This therefore argues for early therapeutic intervention before the development of irreversible changes.

Quantitative measurement of urinary GAG (glycosaminoglycans) can be used to diagnose MPS. We investigated the change in urinary excretion of GAG to use for early diagnosis.

Random urine samples were obtained from 570 neonates on days 2–6 of life. The samples were obtained from 320 boys and 250 girls with birthweights of mean 3137 (SD 374) g and gestational ages of 39.7 (1.1) weeks. Urine specimens were collected from 85 neonates on day 2; 254 on day 3; 92 on day 4; 65 on day 5; and 74 on day 6. The babies had been born after an uneventful pregnancy and delivery and were not known to have any specific clinical abnormalities. Urine samples were also obtained from 1328 infants aged between 1 and 12 months old who had no symptoms of MPS, and from five MPS patients aged 1 month or less (MPS type II, 15 days old, 978 mg GAG/g creatine; MPS type II, 26 days old, 940 mg GAG/g creatine; MPS type II 1 month old, 1177 mg GAG/g creatine; MPS type III, 1 month old, 1180 mg GAG/g creatine; MPS VII, 1 month old), 205 mg GAG/g creatine.

The urine collector (ATOM pediatric urine collector, ATOM medical Co, Japan) was removed as soon as it was full of urine; it was then immediately stored at −20°C until analysis. After thawing at room temperature the urine were analysed as follows. Urinary excretion of GAG was measured using the DMB method[5] and the urinary creatinine concentration was measured using the Jaffe method.[6] Both measurements were performed using an MR 5000 plate reader (Dynatech, USA). The Wilcoxon rank sum test for unpaired data was used to compare groups.

Figure 1 shows the urinary GAG:creatinine ratio for normal neonates and infants and for five MPS patients. Urinary excretion of GAG decreased each day after birth until day 5 of life. The median for the GAG:creatinine ratio was 459.0, 446.4, 400.0, 323.0, and 311.5 mg/g on days 2, 3, 4, 5 and 6, respectively. Between days 2 and day 4 of life, the decrease was significant. Urinary excretion of GAG in the normal neonates was much lower than in the five MPS patients: type II, 15 days of age, 978 mg GAG/g creatinine; type II, 26 days old, 940 mg GAG/g creatinine; type II, 1 month old, 1177 mg GAG/g creatinine; type III, 1 month old, 1180 mg GAG/g creatinine; type VII, 1 month old 1205 mg GAG/g creatinine.

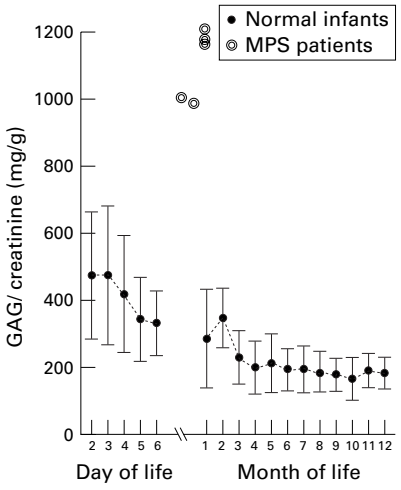

Figure 1 Urinary GAG: creatine excretion ratios for normal infants and MPS patients. Circles indicate means; bars SD.

The GAG:creatinine ratio in MPS patients was much higher than in normal infants. We conclude that these results might be useful for the early diagnosis of MPS.

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CORRECTION

Please note that the authors of Gilbert *et al* (Role of *Ureaplasma urealyticum* in lung disease of prematurity: 1999;81:F162-7) have noted a discrepancy in the reference list for this article. Reference 2 should read:

2 Todd DA, Jane A, John E. Chronic oxygen dependency in infants born at 24-32 weeks' gestation: the role of antenatal and neonatal factors. *J Paediatr Child Health* 1997:**33**:402-7.

From there on all references should be renumbered accordingly.