INFECTION AND IMMUNITY, May 1994, p. 1528–1533
0019-9567/94/$04.00+0

Vol. 62, No. 5

Ureaplasma diversum Infection In Vitro Alters Prostaglandin E_2 and Prostaglandin F_{2a} Production by Bovine Endometrial Cells without Affecting Cell Viability

JULIE J. KIM,[1] PATRICIA A. QUINN,[2,3] AND MICHEL A. FORTIER[1,3]*

Department of Ontogeny and Reproduction, Centre de Recherches du Centre Hospitalier de l'Université Laval, Ste. Foy, Quebec,[1] Department of Obstetrics and Gynaecology, Université Laval, Ste. Foy, Quebec,[3] and Department of Microbiology, The Hospital for Sick Children, Toronto, Ontario,[2] Canada

Received 23 July 1993/Returned for modification 1 December 1993/Accepted 26 January 1994

Bovine epithelial and stromal cells of the endometrium were inoculated with *Ureaplasma diversum*, pathogenic strain 2312, at 10^6 or 10^3 color-changing units (ccu)/ml in the presence of 1% fetal bovine serum (depleted of steroids by dextran-charcoal treatment) to assess the effect of infection on prostaglandin biosynthesis. When the inoculum of *U. diversum* was 10^6 ccu/ml, the concentration of *U. diversum* in the culture medium decreased with time. *U. diversum* was found on the epithelial and stromal cell monolayers, increasing in titer 100-fold, indicating that attachment and eventually growth occurred. When the inoculum was 10^3 ccu/ml, the titer of *U. diversum* remained the same or increased in the supernatant and increased on epithelial and stromal cells. The effect of infection was evaluated by measurement of the primary prostaglandin produced by each cell type, prostaglandin F_{2a} for epithelial cells and prostaglandin E_2 for stromal cells. Infection with *U. diversum* significantly decreased prostaglandin F_{2a} accumulation, by 44.7% ± 6.0% at 10^6 ccu/ml ($P \leq 0.005$) and 15.8% ± 5.3% at 10^3 ccu/ml ($P \leq 0.05$) in epithelial cells. Prostaglandin E_2 accumulation by stromal cells was decreased by 34.0% ± 4.0% at 10^6 ccu/ml ($P \leq 0.001$) and by 13.5% ± 2.7% at 10^3 ccu/ml ($P \leq 0.005$). Infection with 10^6 ccu/ml did not alter endometrial cell viability, as shown by protein measurement, trypan blue dye exclusion, and cell plating efficiency tests. Thus, alterations in prostaglandin production were not due to cell deterioration. These observations suggest that *U. diversum* can alter prostaglandin E_2 and prostaglandin F_{2a} patterns in primary cultures of bovine endometrial cells without affecting cell viability.

Genital ureaplasmas are mycoplasmas belonging to the bacterial class *Mollicutes* (28). Ureaplasmas, like all mycoplasmas, are unique in that they are small, like viruses, have a genome that is 730 to 1,160 kbp in size, and are free living (26). They lack a cell wall; thus, their protein lipid plasma membrane can interact directly with the host cell membrane (27). Clinical studies demonstrate an association between the human ureaplasma *Ureaplasma urealyticum* and a spectrum of reproductive failures, such as infertility, spontaneous abortion, stillbirth, premature delivery, perinatal morbidity, and mortality (3, 24, 30). In parallel, the bovine ureaplasma *Ureaplasma diversum* is associated with a similar spectrum of reproductive failures in the bovine, including vulvitis, endometritis, and salpingitis (6), abortion and premature delivery (21), and infertility (18). The pathogenic mechanisms involved in ureaplasma infection are not known.

It has been suggested that in intrauterine infection, bacterial phospholipases produce arachidonic acid from host tissue phospholipids which artificially stimulate the prostaglandin cascade and induce premature labor (1). Ureaplasmas have phospholipases A1, A2, and C (4) localized in their membranes (5). Since ureaplasmas have no cell wall and attach intimately to host cells (27), such enzymes have direct access to substrates in the hosts. It is possible that through the production of arachidonic acid, diacylglycerol, and lysophospholipids, ureaplasmas have the potential to alter prostaglandin biosynthesis and host cell function.

* Corresponding author. Mailing address: Ontogénie et Reproduction, Centre de Recherches du Centre Hospitalier, de l'Université Laval, 2705 Blvd. Laurier, Ste. Foy, Quebec G1V 4G2, Canada. Phone: 418-654-2244. Fax: 418-654-2714.

Prostaglandins are obligatory for the changes in vascular permeability and decidualization of the endometrium which occur at the time of fertilized-egg implantation. Inhibitors of prostaglandin synthesis, such as indomethacin, delay or inhibit these changes in various species (16, 17). Prostaglandins also play a role in the maintenance of pregnancy. Prostaglandin F_{2a} (PGF_{2a}) released from the uterus is the luteolysin in sheep, pigs, cows, and horses (32). Thus, in early pregnancy, the ability of the uterus to release PGF_{2a} is altered so that reduced amounts reach the ovary.

An in vitro model has been established in which *U. diversum* and primary bovine endometrial cells are used to study the effect of infection on endometrial cell function (25). In this study, this model was modified and the following points were investigated: survival of *U. diversum* in cell culture under the conditions studied, whether *U. diversum* infection decreases endometrial cell viability, and the effect of *U. diversum* infection on the production of prostaglandin E_2 (PGE_2) and PGF_{2a} by bovine endometrial cells.

MATERIALS AND METHODS

Isolation of epithelial and stromal cells. Bovine uteri were obtained from the slaughterhouse and placed on ice until further processing at the laboratory. The day of the estrous cycle of the tissue was estimated by examination of ovarian morphology (15). Early- to mid-luteal-phase (days 1 to 12) tissues were used. The cell cultures were prepared asceptically by the method of Fortier et al. (10). Briefly, the myometrium of the uterine horns was dissected, leaving the endometrium free of vascular and smooth muscle tissue. The endometrium was inverted to expose the luminal epithelium, and sections were

placed in incomplete (Ca^{2+} and Mg^{2+} free) Hanks' balanced salt solution (IHBSS) supplemented with an antimycotic antibiotic (Sigma, St. Louis, Mo.) and containing 0.3% trypsin (Boehringer Mannheim, Laval, Canada). The tissue was digested for 3 h at 22°C with gentle shaking every 30 min. Each section was rinsed with IHBSS and scraped with forceps to remove remaining luminal epithelial cells. The tissue was then placed in a new tube of IHBSS containing 0.02% trypsin, 0.02% collagenase (Sigma; type II), and 0.015% DNase I (Sigma) and incubated at 37°C for 1 h with vigorous shaking in order to recuperate stromal cells.

The cell suspension from the first digestion was inhibited by the addition of 10% calf bovine serum (Flow Laboratories, McLean, Va.), and the epithelial cells were recovered by centrifugation at 1,200 × *g* for 12 min. The cells were washed twice with IHBSS and recovered by centrifugation each time. The final pellet of cells was resuspended in 20 ml of RPMI 1640 (Flow Laboratories). This was then diluted in a final volume of 280 ml of RPMI 1640 containing 5,000 IU of penicillin G, 5,000 μg of streptomycin (Flow Laboratories), and 50 mg of gentamicin (Sigma) per ml plus 10% FBS-DC (fetal bovine serum [Flow Laboratories] depleted of steroids by dextran-charcoal extraction). Stromal cells recovered from the second digestion were processed like epithelial cells. The cells were plated in 24-well culture plates (Becton Dickinson, Lincoln Park, N.J.), and the medium was changed every 4 to 5 days until the cells reached early confluency.

***U. diversum* preparation.** *U. diversum* 2312, passage 6, known to be pathogenic in the bovine (18, 21), was obtained from L. Ruhnke (Veterinary Services Lab, Guelph, Canada). Stock cultures were prepared in complete Hayflick broth (14, 29) consisting of PPLO broth (Becton Dickinson, Cockeysville, Md.) supplemented with 1% phenol red, 20% unheated horse serum, 10% of a yeast extract preparation (all generously donated by L. Ruhnke), 20% urea (Sigma), and 1,000 IU of penicillin G (Glaxo, Ware, England) per ml. The pH was adjusted to 6.0 to 6.5 with 1 N HCl. As a control, uninoculated medium was incubated identically. The prepared ureaplasmas and control broth were aliquoted and stored at −86°C.

Titers of viable *U. diversum* were determined in Hayflick broth with 20% fetal bovine serum (Flow Laboratories). A tube dilution method was used to quantitate ureaplasmas. As defined by Purcell (23), a color-changing unit (ccu) is defined as the highest dilution of a mycoplasma suspension that will produce a color change equivalent to 0.5 pH unit or greater in broth medium with phenol red and urea or glucose or arginine. Thus, 1 ccu is not necessarily equal to one mycoplasma. As ureaplasmas tend to grow in clumps (29), the color-changing unit could actually consist of hundreds of organisms, providing only an estimate of the number of ureaplasmas present. For this reason, all samples were subjected to mild sonication to break up clumps before titration.

Infection of endometrial cells. Infection of epithelial and stromal cells was done at early confluency (25), defined as the time when cultured cells cover the entire growth area of the culture dish as a monolayer. In previous studies (9, 10), we have shown that at this state of early confluency, epithelial cells have reached 85% of the density imposed by contact inhibition. Stromal cells are at only 65% of their maximal density at this time, since they tend to grow in multiple layers after the surface of the dish is covered.

At the time of infection, the medium was replaced with fresh RPMI 1640 containing 40 mM HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid; Sigma), 1% FBS-DC, and no antibiotics. The pH of the medium was adjusted to 7.0. Only 1% FBS-DC was added to the medium because fetal bovine serum itself significantly stimulates prostaglandin production by the endometrial cells. However, the total absence of serum for more than 24 h results in cell deterioration.

Cells were immediately infected with *U. diversum* 2312 at an initial concentration of 10^5 to 10^6 or 10^2 to 10^3 ccu/ml. The concentration of *U. diversum* used that caused reproductive failure in the bovine in the in vivo studies ranged from 10^8 to 10^{12} ccu/ml or CFU/ml (6, 18, 21). The titer of *U. diversum* recovered from these animals can average from 10^6 (18) to 10^7 ccu/ml (13), indicating that these concentrations of ureaplasmas do occur in vivo and cause physiological damage. The maximal titers for *U. diversum* in vitro are 10^6 to 10^7 ccu/ml. Thus, a comparable titer of ureaplasmas was used in the experiments presented in this study. Incubated medium was added to uninfected control cells. Cells were incubated at 35°C with 5% CO_2.

For ureaplasma growth experiments, 100 μl of the culture medium was serially titrated to measure the concentration of *U. diversum* present at various times postinfection. The rest of the culture medium was then discarded, and the cell monolayer was washed twice with IHBSS. This method was sufficient to remove unattached ureaplasmas and to leave the attached ureaplasmas on the cell monolayer, as observed by electron microscopy (25). To determine the concentration of ureaplasmas on the cell monolayer, the cells were scraped off in the presence of 100 μl of IHBSS with a rubber policeman. The total volume was used for assaying the ureaplasma concentration by serial titration. As a further control, *U. diversum* was inoculated in RPMI 1640 with HEPES and 1% FBS-DC with no cells, and the concentration of ureaplasmas was measured by titration.

Cell viability. Cells were grown in 75-cm^2 tissue culture flasks (Becton Dickinson) until early confluency was reached. The medium was changed, and the cells were infected with 10^5 to 10^6 ccu of *U. diversum* per ml. After 48 h of infection, cells were harvested by trypsinization. Cell viability was analyzed by the trypan blue dye exclusion method and the cell plating efficiency method (11). For the latter test, cells were replated onto 24-well plates after trypsinization. After 24 and 48 h and at the time of confluency, the culture medium was discarded and 1 N NH_4OH was placed on the cells. Levels of protein were measured by the protein-dye binding method described by Bradford (2), and DNA content was determined by the 3,5-diaminobenzoic acid-fluorometry method described by Fiszer-Szafarz et al. (8).

PGE_2 and PGF_{2a} accumulation. At various times postinfection, the culture medium was collected in glass tubes and stored at −20°C until further processing. Cells were scraped off and protein content was determined as described above. Radioimmunoassays of PGF_{2a} and PGE_2 were done as described by Fortier et al. (10), omitting the extraction method. Briefly, competition binding occurred between ^{3}H-PGF_{2a} or ^{3}H-PGE_2 (DuPont, Mississauga, Canada) and the prostaglandins in the culture sample in the presence of a fully characterized PGE_2 antiserum (generously donated by T. G. Kennedy) (7) or PGF_{2a} antiserum (BioQuant, Ann Arbor, Mich.).

Statistical analysis. After analysis, the variance between experiments was found not to be homogeneous, and accordingly, the data were transformed to a percentage of the control before analysis by the Student *t* test.

RESULTS

***U. diversum* infection of bovine endometrial cells.** Since the prostaglandin experiments were carried out in 1% FBS-DC, the effect of 1% serum on the survival of *U. diversum* was

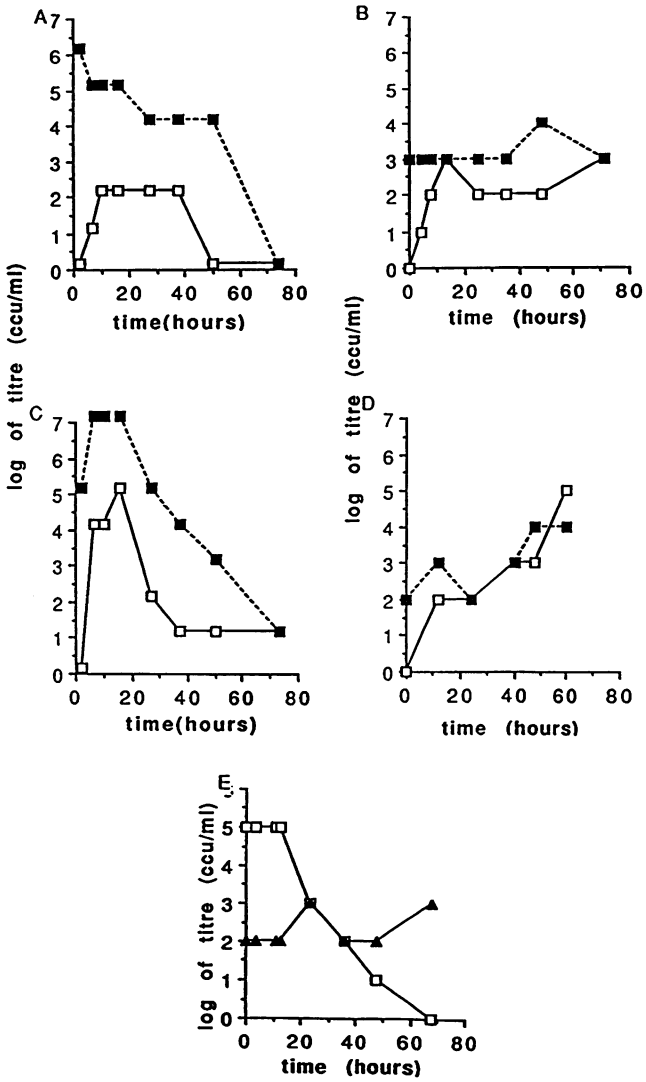

FIG. 1. Evaluation of viable *U. diversum* in the culture medium and on the cell monolayer after infection. Concentrations of viable *U. diversum* are shown after various times of infection for (A) epithelial cells with an inoculum of 10^6 ccu/ml, (B) epithelial cells with an inoculum of 10^3 ccu/ml, (C) stromal cells with an inoculum of 10^6 ccu/ml, (D) stromal cells with an inoculum of 10^3 ccu/ml, and (E) culture medium alone in the absence of cells. Data are representative of three individual experiments. Symbols: (A, B, C, and D) □, cells; ■, culture medium; (E) □, 10^6 ccu/ml; ▲, 10^3 ccu/ml.

determined. Two different concentrations of *U. diversum*, 10^6 and 10^3 ccu/ml, were added to the culture medium of epithelial and stromal cells of the endometrium. Survival of ureaplasmas in the culture medium and on the cells was monitored at various times after infection by titration. When the inoculum of *U. diversum* was 10^6 ccu/ml, the concentration of *U. diversum* in the culture medium of epithelial cells diminished with time until no color-changing units were observed (Fig. 1A). No growth of *U. diversum* in the supernatant was observed. *U. diversum* was also found on the cell monolayer, increasing 100-fold in titer at early times and then declining in titer until no viable ureaplasmas were present. In the culture medium of stromal cells, *U. diversum* increased in concentration from the 10^5 ccu/ml inoculum at early times to 10^7 ccu/ml (Fig. 1C), suggesting that growth occurred followed by a decline in *U. diversum* concentration. On the stromal cell monolayer, *U. diversum* increased to a titer as high as the inoculum and then decreased over time.

When the inoculum was 10^3 ccu/ml, the concentration of *U. diversum* present in the culture medium and on epithelial cells remained at 10^3 ccu/ml for up to 72 h (Fig. 1B). On stromal cells, *U. diversum* appeared to increase in titer with time both in the culture medium and on the cells (Fig. 1D). The survival of *U. diversum* in culture medium alone without cells is shown in Fig. 1E. At a high initial concentration, ureaplasmas die over time but remain viable at the lower concentration.

Cell viability after *U. diversum* infection. The viability of epithelial and stromal cells after *U. diversum* infection was examined in order to ensure that any alteration in cell function was not due to cell death. Protein measurements made 48 h after infection were the same for uninfected and infected cells: 94.8 ± 17.67 and 105.3 ± 19.67 μg per well (mean ± standard error of the mean, $n = 12$), respectively, for epithelial cells and 82.7 ± 8.55 and 89.4 ± 9.55 μg per well, respectively, for stromal cells. Trypan blue dye exclusion tests demonstrated cell viability to be 95% in both control and infected cells. For further confirmation, a cell plating efficiency test was done with control and infected cells. The protein and DNA content of cells subcultured after infection appeared to be the same for infected and control epithelial and stromal cells, suggesting that cell viability was not altered by the infection (Fig. 2).

Effect of *U. diversum* infection on PGE_2 and PGF_{2a} production by endometrial cells. In accordance with previous studies, it was apparent that epithelial cells produced more PGF_{2a} than PGE_2 and that stromal cells produced more PGE_2 than PGF_{2a} (10). Thus, only the accumulation of the major prostaglandin produced by each cell type was measured in control and infected cells. The amount of prostaglandins accumulated over time was measured. Uninfected epithelial cells continually produced PGF_{2a} over time (Fig. 3A). Infection with *U. diversum* at 10^6 ccu/ml dramatically decreased the accumulation of PGF_{2a}, which was observable by approximately 20 h. Infection at 10^3 ccu/ml also lowered the PGF_{2a} levels, but not to the same extent as at 10^6 ccu/ml. Similar results were observed for the amount of PGE_2 accumulated in stromal cells (Fig. 3B).

Since a maximal effect of infection was observed after 48 h of infection, further experiments were performed with this infection time. Since it is commonplace to see variation between different experiments when working with primary cell cultures, the results were expressed as a percentage of the control [(infected − control)/control × 100] for statistical analysis (Table 1). In epithelial cells, PGF_{2a} production was significantly inhibited when infected with *U. diversum* at 10^6 ccu/ml and to a lesser degree when infected at 10^3 ccu/ml. Similar results were observed for stromal cells. PGE_2 production was significantly inhibited at 10^6 ccu/ml and inhibited to a lesser degree at 10^3 ccu/ml.

DISCUSSION

Ureaplasmas are associated with reproductive failures and neonatal respiratory disease (3, 24, 30). Since PGE_2 and PGF_{2a} play a significant role in reproductive processes, it was of interest to determine the effect of *U. diversum* infection on prostaglandin biosynthesis in primary bovine endometrial cells. Studies have suggested that certain mycoplasmas cause cell deterioration when used at high concentrations to infect cell cultures (33, 35). Alternatively, Masover et al. (20) reported that ureaplasmas become cell associated but that the association does not persist or cause extensive cytopathic effects. They

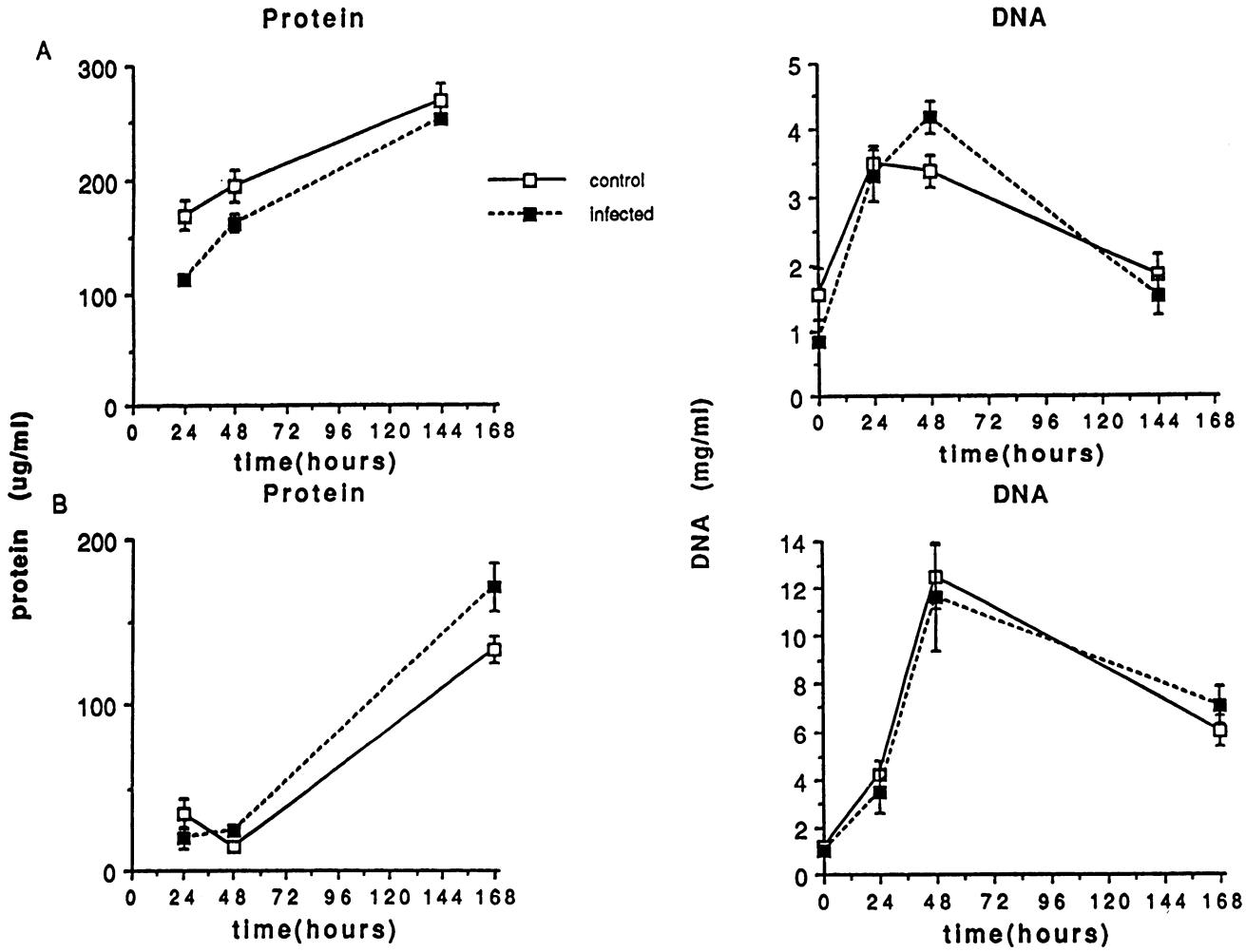

FIG. 2. Cell viability after infection with *U. diversum*, analyzed by the cell plating efficiency test. Epithelial (A) and stromal (B) cells were subcultured after infection with *U. diversum* at 10^6 ccu/ml, and the protein and DNA contents were measured as described in Materials and Methods. Each value represents the average of triplicate determinations.

were also able to subculture their cells after infection without difficulty, showing no apparent effects of prior infection. In this report, *U. diversum* at an infecting concentration of 10^6 ccu/ml did not cause cell death, as demonstrated by the trypan blue dye exclusion method. Measurement of the protein and DNA contents of cells subcultured after infection permitted evaluation of viability by analysis of the growth patterns of these cells. Protein levels estimate cell size as well as number, and DNA levels measure the quantity of cells. DNA and protein levels appeared to be the same for noninfected and infected cells over the entire period of growth. This is indicative of no significant loss in cell viability. However, whether infection retards or modifies the growth process requires further investigation.

It has been reported (25) that in the presence of 10% serum, *U. diversum* increased in concentration, both on the endometrial cell monolayer and in the culture medium, when the starting inoculum was 10^3 ccu/ml. At 10^6 ccu/ml, the ureaplasma titer declined in the supernatant but increased on the cell membranes. In the present study, the above model was modified in that *U. diversum* was inoculated in the presence of a buffer and, more importantly, in the presence of only 1% serum. In the culture medium of the cells, the titer remained the same or increased when the inoculum was low. When the inoculum was high, the titer of ureaplasmas in the culture medium declined, which is characteristic of mycoplasmas in culture (12, 34). Depletion of nutrients from the cell culture could explain their death, since all mycoplasmas require essential nutrients for growth.

On the epithelial and stromal cell monolayers, an increase in titer of *U. diversum* was observed at early times (Fig. 1). It is difficult to determine whether this increase was due to replication of the ureaplasmas already attached onto the monolayer shortly after infection or to a slower rate of adherence that may have occurred due to the lack of nutrients to provide adequate metabolism. Given that ureaplasmas do not grow in such minimal medium (Fig. 1E), it is possible that ureaplasmas obtain nutrients from the cells for growth. Whether growth contributes to the alteration in prostaglandin production requires further investigation. Ureaplasma death could also be explained by an accumulation of toxic products, as shown by Furness (12) in broth cultures as a result of ureaplasmal metabolism. The same may be true for *U. diversum* in cell culture. If the above explanations are true, ureaplasmas would be expected to survive longer in vivo, since nutrients would be supplied continuously and toxic products would be removed.

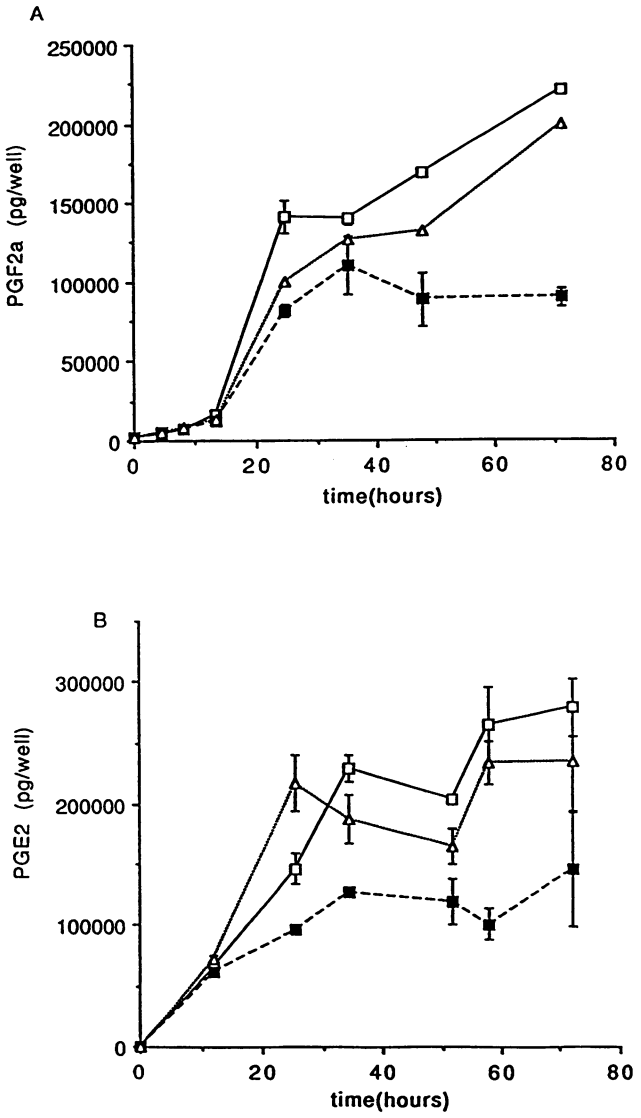

FIG. 3. Accumulation of prostaglandins in endometrial cells at various times after infection. Cells were infected with *U. diversum* for the times shown, and the levels of PGF_{2a} in epithelial cells (A) and PGE_2 in stromal cells (B) were measured by a radioimmunoassay. Each value represents the average of triplicate determinations and also represents one of three individual experiments. □, control (uninfected); ■, 10^6 ccu/ml; △, 10^3 ccu/ml.

Gourlay (13) has shown that ureaplasmas can be maintained in vivo for extended periods at high concentrations. These high concentrations may be necessary to induce a significant pathogenic response, as observed in our in vitro model.

Infection with *U. diversum* significantly inhibited PGF_{2a} production by epithelial cells at infecting concentrations of 10^6 and 10^3 ccu/ml. Infection similarly disturbed PGE_2 accumulation by stromal cells. The reduction in prostaglandin biosynthesis over the 48 h of infection (Table 1) appears to be related to early cellular modifications, since a difference in prostaglandin accumulation was observed as early as 14 h postinfection (Fig. 3). Interestingly, this coincides with an increase in the titer of ureaplasmas on the cell membranes in both epithelial and stromal cells. Given that the maximal effect

TABLE 1. Prostaglandin biosynthesis during infection with *U. diversum*[a]

Cell type (prostaglandin)	Reduction in prostaglandin accumulation (%) with inoculum:			
	10^6 ccu/ml	*P*	10^3 ccu/ml	*P*
Epithelial (PGF_{2a})	44.7 ± 6.0[b]	≤0.005	15.8 ± 5.3[c]	≤0.05
Stromal (PGE_2)	34.0 ± 4.0[c]	≤0.001	13.5 ± 2.7[c]	≤0.005

[a] Endometrial cells were infected with 10^6 or 10^3 ccu of *U. diversum* per ml for 48 h, at which time the levels of PGE_2 and PGF_{2a} were measured. Each value represents the difference between infected and control cultures, expressed as a percentage of the control value.
[b] Mean ± standard error of the mean for four experiments.
[c] Mean ± standard error of the mean for six experiments.

of infection on prostaglandin production was observed at the higher concentration of ureaplasmas, the possibility that the effect is dose dependent suggests that a mass-action relationship may be involved. Stalheim et al. (34) demonstrated that ureaplasmas from both the human genital tract and bovine genital and respiratory tracts stopped ciliary activity. This ciliostasis occurred even after the addition of nonviable ureaplasmas or washed, disrupted organisms, suggesting the production of a cytotoxin by ureaplasmas. It is possible that ureaplasmas secrete a toxic substance during growth in broth or that their cell membrane could act as the toxic factor that, when added to the endometrial cells, could disturb prostaglandin production. Lamont et al. (19) showed that increasing concentrations of ureaplasma-conditioned medium decreased PGE_2 production by amniotic cells. The fact that conditioned medium, presumably containing no ureaplasmas, was able to decrease PGE_2 production also supports the presence of a toxin-like substance.

The second concept is that the ureaplasmas are directly affecting prostaglandin biosynthesis, given that they possess phospholipases in their membranes, including phospholipase A_2, which are active even in nonviable ureaplasmas (5). It has been shown that increasing concentrations of phospholipase A_2 decreased PGE_2 production by amniotic cells (19). Thus, it is possible that increased availability of ureaplasmal phospholipase A_2 could release excessive amounts of arachidonic acid, resulting in substrate inhibition of prostaglandin synthesis.

The increased availability of arachidonic acid could result from several processes. Lipases can act on 1,2-diacylglycerol to liberate intracellular arachidonic acid. Small amounts of 1,2- and 1,3-diacylglycerols are synthesized by all members of the class *Mollicutes* (31). Thus, it is possible that diacylglycerol may provide yet another source of arachidonic acid. Diacylglycerol is also an activator of protein kinase C (22), which then introduces a whole network of signaling systems that may be involved in regulating prostaglandin synthesis. Alternatively, phospholipase A_2 inhibition could explain the inhibition of prostaglandin production, since the liberation of arachidonic acid from phospholipids, carried out by phospholipase A_2, is the rate-limiting step in prostaglandin biosynthesis.

Prostaglandins are potent regulators of cellular activities involved in reproductive processess. They are necessary for implantation and the maintenance of pregnancy (16, 17). A possible mechanism of action of ureaplasmas could involve their ability to disturb prostaglandin production, as shown in this study. Further investigation is under way to understand how this perturbation occurs.

ACKNOWLEDGMENTS

We thank the Microbiology Department at CHUL for allowing the use of their facilities for the infection studies and Cecilia Th'ng, Kit Leung, and Gabriela Mastromonaco for their help in preparing ureaplasma pools and culture medium.

This work was supported by grants NSERC-OGP 46717 (P.A.Q.) and NSERC-OGPIN 001 (M.A.F.).

REFERENCES

1. **Bejar, R., V. Curbelo, C. Dairs, and L. Gluck.** 1981. Premature labour. Obstet. Gynecol. **37:**479–482.
2. **Bradford, M. M.** 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. **72:**248–254.
3. **Cassell, G. H., J. K. Davis, K. B. Waites, P. T. Rudd, D. Talkington, D. Crouse, and S. A. Horowitz.** 1987. Pathogenesis and significance of urogenital mycoplasmal infections. Adv. Exp. Med. Biol. **224:**93–115.
4. **De Silva, N. S., and P. A. Quinn.** 1986. Endogenous activity of phospholipase A and C in *Ureaplasma urealyticum*. J. Clin. Microbiol. **23:**354–359.
5. **De Silva, N. S., and P. A. Quinn.** 1991. Localization of endogenous activity of phospholipases A and C in *Ureaplasma urealyticum*. J. Clin. Microbiol. **29:**1498–1503.
6. **Doig, P. A., H. L. Ruhnke, and N. C. Palmer.** 1980. Experimental bovine genital ureaplasmosis. II. Granular vulvitis, endometritis and salpingitis following uterine inoculation. Can. J. Comp. Med. **44:**259–266.
7. **Evans, C. A., T. G. Kennedy, and J. R. G. Challis.** 1982. Gestational changes in prostanoid concentrations in intrauterine tissues and fetal fluids from pregnant sheep and the relation to prostanoid output *in vitro*. Biol. Reprod. **27:**1–11.
8. **Fiszer-Szafarz, B., D. Szafarz, and A. Guevara de Murillo.** 1981. A general, fast, and sensitive micromethod for DNA determination: application to rat and mouse liver, rat hepatoma, human leukocytes, chicken fibroblasts, and yeast cells. Anal. Biochem. **110:**165–170.
9. **Fortier, M. A., M. Boulanger, A. P. Boulet, and R. D. Lambert.** 1987. Cell-specific localization of prostaglandin E2-sensitive adenylate cyclase in rabbit endometrium. Biol. Reprod. **36:**1025–1033.
10. **Fortier, M. A., L. A. Guilbault, and F. Grasso.** 1988. Specific properties of epithelial and stromal cells from the endometrium of cows. J. Reprod. Fert. **83:**239–248.
11. **Freshney, R. I.** 1983. Quantitation and experimental design, p. 207–215. *In* R. I. Freshney (ed.), Culture of animal cells: a manual of basic technique. Alan R. Liss, Inc., New York.
12. **Furness, G.** 1973. T-mycoplasmas: their growth and production of a toxic substance in broth. J. Infect. Dis. **127:**9–16.
13. **Gourlay, R. N.** 1972. The production of mastitis in cows by the intramammary inoculation of T-mycoplasmas. J. Hyg. **70:**511–521.
14. **Hayflick, L. H.** 1965. Tissue cultures and mycoplasmas. Texas Rep. Biol. Med. **23**(Suppl. 1)**:**285–303.
15. **Ireland, J. J., P. B. Coulson, and R. L. Murphee.** 1979. Follicular development during four stages of the oestrous cycle of beef cattle. J. Anim. Sci. **49:**1261–1269.
16. **Kennedy, T. G.** 1983. Prostaglandin E2, adenosine 3′,5′-cyclic monophosphate and changes in endometrial vascular permeability in rat uteri sensitized for the decidual cell reaction. Biol. Reprod. **29:**1069–1076.
17. **Kennedy, T. G.** 1985. Prostaglandins and blastocyst implantation. Prostaglandin Perspectives **1:**1–3.
18. **Kreplin, C. M. A., H. L. Ruhnke, R. B. Miller, and P. A. Doig.** 1987. The effect of intrauterine inoculation with *Ureaplasma diversum* on bovine fertility. Can. J. Vet. Res. **51:**440–443.
19. **Lamont, R. F., F. Anthony, L. Myatt, L. Booth, P. M. Furr, and D. Taylor-Robinson.** 1990. Production of prostaglandin E2 by human amnion in vitro in response to addition of media conditioned by microorganisms associated with chorioamnionitis and preterm labor. Am. J. Obstet. Gynecol. **162:**819–25.
20. **Masover, G. K., M. Namba, and L. Hayflick.** 1976. Cytotoxic effect of a T-strain mycoplasma (Ureaplasma urealyticum) on cultured normal human cells (WI-38). Exp. Cell Res. **99:**363–374.
21. **Miller, R. B., H. L. Ruhnke, P. A. Doig, B. J. Poitras, and N. C. Palmer.** 1983. The effects of *Ureaplasma diversum* inoculated into the amniotic cavity of cows. Theriogenology **20:**367–373.
22. **Nishizuka, Y.** 1984. The role of protein kinase C in cell surface signal transduction and tumour promotion. Nature (London) **308:**693–698.
23. **Purcell, R. H.** 1966. Color test for the measurement of antibody to T-strain mycoplasmas. J. Bacteriol. **92:**6–12.
24. **Quinn, P. A.** 1988. Mycoplasma infection of the fetus and newborn, p. 107–151. *In* D. G. Scarpelli, and G. Migaki (ed.), Transplacental effects on fetal health, vol. 281. Alan R. Liss, Inc., New York.
25. **Quinn, P. A., and M. A. Fortier.** 1990. Growth and attachment of *Ureaplasma diversum* in co-culture with bovine endometrial cells. IOM Lett. **1:**337–338.
26. **Razin, S.** 1983. Characteristics of the mycoplasmas as a group, p. 3–25. *In* J. C. Tully and S. Razin (ed.), Methods in mycoplasmology, vol. I. Academic Press, New York.
27. **Razin, S.** 1985. Mycoplasma adherence, p. 161–202. *In* M. F. Barile, S. Razin, J. G. Tully, and R. F. Whitcomb (ed.), The mycoplasmas, vol. IV: mycoplasma pathogenicity. Academic Press, New York.
28. **Razin, S.** 1992. Mycoplasma taxonomy and ecology, p. 3–22. *In* J. Maniloff, R. N. McElhaney, L. R. Finch, and J. B. Baseman (ed.), Mycoplasmas: molecular biology and pathogenesis. American Society for Microbiology, Washington, D.C.
29. **Shepard, M. C.** 1969. Fundamental biology of the T strains, p. 49–65. *In* L. Hayflick (ed.), The mycoplasmatales and the L-phase of bacteria. Appleton, New York.
30. **Shepard, M. C.** 1988. *Ureaplasma urealyticum.* Overview with emphasis on fetal and maternal infections. Ann. N.Y. Acad. Sci. **549:**48–55.
31. **Smith, P. F.** 1992. From fats to sugars: an odyssey through the mycoplasma membrane. Emmy Klieneberger-Nobel Award Lecture—1990, p. xxx–xxxvi. *In* IOM Letters, vol. 2: program and abstracts of the 9th International Congress of IOM, 1992.
32. **Smith, S. K., and R. W. Kelly.** 1988. Prostaglandins and the establishment of pregnancy, p. 147–160. *In* M. Chapman, G. Grudzinskas, and T. Chard (ed.), Implantation: biological and clinical aspects. Springer-Verlag, Berlin.
33. **Stadtlander, C. T. K.-H., H. L. Watson, J. W. Simecka, and G. H. Cassell.** 1991. Cytopathic effects of *Mycoplasma pulmonis* in vivo and in vitro. Infect. Immun. **59:**4201–4211.
34. **Stalheim, O. H. V., J. S. Proctor, and J. E. Gallagher.** 1976. Growth and effects of ureaplasmas (T mycoplasmas) in bovine oviductal organ cultures. Infect. Immun. **13:**915–925.
35. **Stanbridge, E. J., and C. J. Doersen.** 1978. Some effects that mycoplasmas have upon their infected host, p. 119–134. *In* G. J. McGarrity, D. G. Murphy, and W. W. Nichols (ed.), Mycoplasma infection of cell cultures. Plenum Press, New York.