Permeation of human chorioamniotic membranes by *Escherichia coli* in vitro

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OBJECTIVE: Our goal was to study the permeation of *Escherichia coli* through human chorioamniotic membranes in vitro.

STUDY DESIGN: Medium was placed in two compartments separated by chorioamniotic membranes obtained from six cesarean sections at term. The compartment faced by the chorion was inoculated with *E. coli.* Both compartments were sampled over 12 hours for observation of bacterial growth. Controls were performed without membranes.

RESULTS: In the compartment that was inoculated, concentration of *E. coli* increased from 10^6 to 10^{10} colony-forming units per milliliter. In the compartment faced by amnion, bacterial growth was observed after 6 hours and reached 10^3 colony-forming units per milliliter. Permeation of *E. coli* was confirmed histopathologically. The change of glucose and lactate was linear. In the controls the concentration of *E. coli* increased to 10^7 ($p < 0.001$).

CONCLUSIONS: *E. coli* organisms permeate viable chorioamniotic membranes. The membranes constitute a weak barrier against ascending infection and do not inhibit bacterial growth. (Am J Obstet Gynecol 1994;170:223-7.)

Key words: Chorioamniotic membranes, in vitro model, *Escherichia coli,* permeation, glucose utilization

There is increasing evidence that genital tract infection is associated with preterm labor, rupture of the membranes, and both chorioamniotic and intraamniotic infection.[1-3] Certain specific microorganisms have been isolated from the genital tract of women with preterm labor and premature rupture of the membranes.[4-6] *Escherichia coli* are frequently recovered in the vaginas of pregnant women.[6] They are found in association with preterm labor,[7, 8] premature rupture of membranes,[9] perinatal[10] and neonatal[11] infectious morbidity. *E. coli* organisms have been shown to produce protease and phospholipase C.[12] This can reduce the strength of the chorioamnion[13] and lead to the production of prostaglandin E_2 in the human amnion,[14] which then can cause cervical softening and uterine contractions.

It is well recognized that intraamniotic infections occur without rupture of the membranes, and there is evidence that the microbes causing these infections are ascending from the vagina into the amniotic cavity.[15] Galask et al.[16] demonstrated that *E. coli* can attach to and invade chorioamniotic membranes in vitro. However, the role of the membranes as a barrier against microbial ascension has not been clearly defined.

The aim of this study was to investigate the permeation of chorioamniotic membranes by *E. coli* and their growth in the maternal and fetal compartments in vitro.

Material and methods

Chorioamniotic membranes from six women undergoing elective caesarean section at term were obtained under sterile conditions at operation. The membranes were immediately placed in sterile containers and washed three times in sterile ice-cold physiologic saline solution to remove blood and debris. They were then cut into square pieces (16 cm^2), and these were mounted onto one end of 10 cylinders with the amnion facing the lumen. Identification of membranes covering the cervix was not possible. Each cylinder was filled with 10 ml of Krebs-Ringer buffer glucose solution (AF) as described elsewhere,[17] which resembled amniotic fluid in its electrolyte composition and osmolality. The cylinders were designated as fetal compartments and placed in a large reservoir designated as a maternal compartment. This reservoir contained 2000 ml of a second buffer (MS) resembling maternal serum[17] (Fig.

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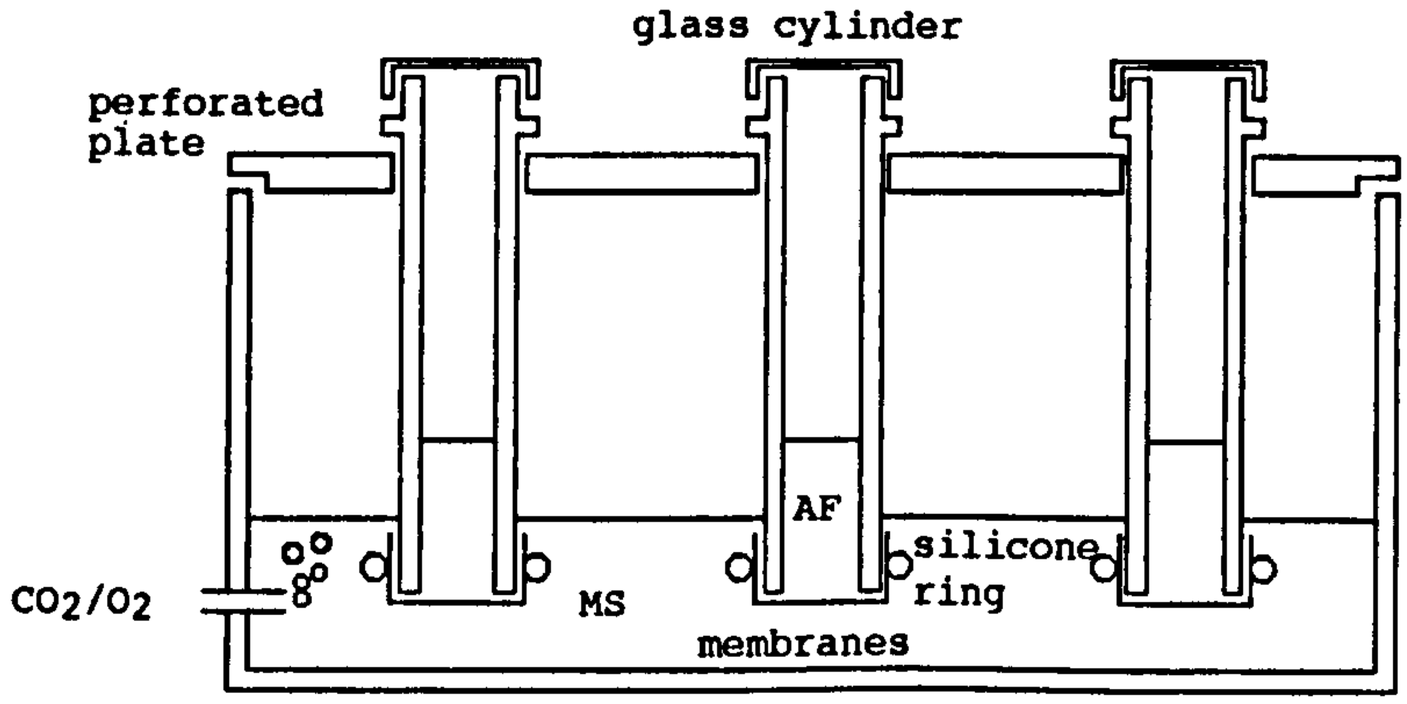

Fig. 1. Diagram of in vitro model. In studies with membranes the amnion was facing the small compartment containing buffer AF (resembling amniotic fluid), and the chorion was facing the large compartment filled with a second buffer (MS) resembling maternal serum. Controls were performed without membranes.

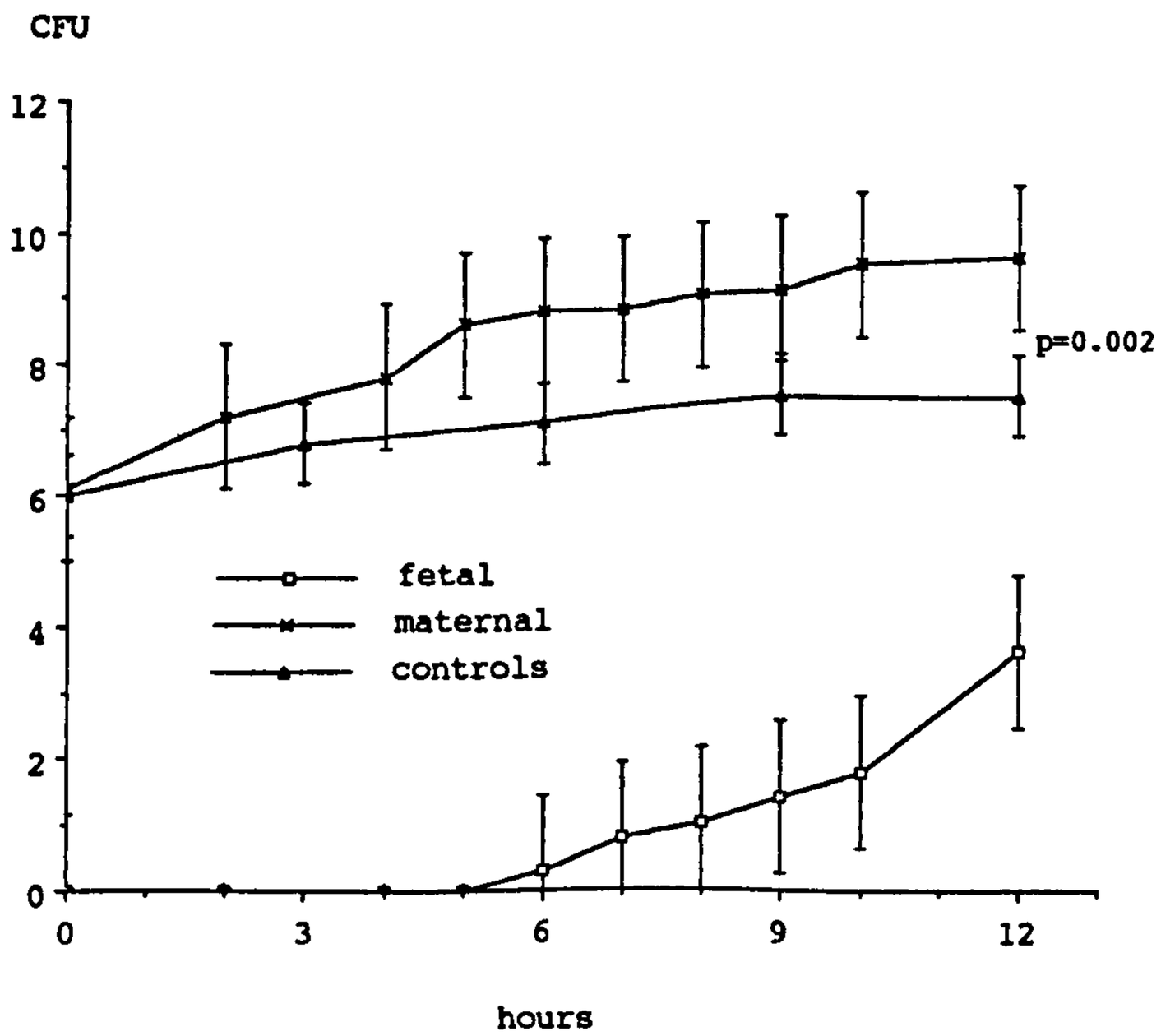

Fig. 2. Increase with time of concentration of *E. coli* in maternal compartment, fetal compartments, and controls.

1). A hydrostatic pressure of 2 cm was exerted on the membrane by maintaining the fluid level inside the cylinders above the fluid level of the maternal reservoir.[17] Oxygenation of the maternal compartment was achieved with a combination of 95% oxygen and 5% carbon dioxide at 0.5 L/min. The system was placed in a shaker at 37° C. *E. coli* were isolated from one urinary sample and kept at −70° C until 10 to 24 hours before inoculation, when they were cultured in thioglycollate broth to achieve concentrations of 10^8 to 10^{10} colony-forming units (CFU) per milliliter. Each of the 10 cylinders was immersed into the *E. coli* suspension with the chorion facing the broth, and after 10 seconds they were placed into the large reservoir. In addition *E. coli* suspension (10 ml) was added to the maternal compartment to reach a concentration of 10^5 to 10^6 CFU/ml. For observation of leaks, 40 μCi (20 μCi/L) tritiated inulin was added to the maternal compartment. Fluid samples (3 ml) were taken from the maternal and fetal compartments at 0, 2, 4, 5, 6, 7, 8, 9, 10, and 12 hours; each cylinder was sampled only once.

For the microbiologic assay, 20 μl of buffers MS and

AF was diluted with 0.9% sodium chloride and spread on Columbia agar with 5% sheep blood and on MacConkey agar (bioMerieux, Marcy, France). The plates were examined after incubation for 24 hours at 37° C.

In the maternal and fetal samples, pH, P_{CO_2}, and P_{O_2} were measured with a gas analyzer (Corning 178, Corning Medical, Medfield, Mass.). Sodium and potassium were determined by flame photometry (Instrumentation Laboratory 243, Milan). Glucose (Uni-Kit III, Roche, Basel) and lactate (Lact, Boehringer, Mannheim, Germany) were measured enzymatically and tritiated inulin was counted in a liquid scintillation system (Beckman LS 1801, Fullerton, Calif.). Biopsy specimens of the membranes were fixed in formaldehyde and stained (Warthin-Starry) for histologic examination.

Six control studies were performed under identical conditions with 2000 ml of buffer MS, 100 ml of buffer AF, and 10 ml of *E. coli* suspension but with the chorioamniotic membranes omitted. The controls were sampled at 0, 3, 6, 9, and 12 hours.

Statistical analysis. Values are given as mean ± SD. Calculation of colony-forming units per milliliter was performed with $\ln(x)$ and e^x transformations. For statistical analysis significance was tested by paired and unpaired Student's *t* test. For changes in concentrations of glucose and lactate, linear regression models were calculated. The regression estimates describing the slopes and intercepts were analyzed. Sign tests were calculated, and Mann-Whitney *U* tests were computed.

Results

Initial concentration of *E. coli* in the maternal compartment was 2.1×10^6 ($+ 11 \times 10^6 - 1.8 \times 10^6$) CFU/ml. Within 12 hours, the number of microbes in the maternal compartment increased to 10^{10} ($+ 25 \times 10^{10} - 0.96 \times 10^{10}$) CFU/ml ($p = 0.001$) in the experiments where membranes were used (Fig. 2). There was no growth of bacteria other than *E. coli* observed on the Agar plates. The samples taken from the fetal compartments before 6 hours were all sterile. Growth of *E. coli* was observed first in one case each after 6, 7, and 8 hours, respectively, in two other cases after 9 hours, and in the last case after 10 hours. Within 12 hours the concentration of *E. coli* in buffer AF increased in all experiments to 2.15×10^3 ($+ 69 \times 10^3 - 0.06 \times 10^3$) CFU/ml (Fig. 2).

Histopathologic examination of the membranes revealed *E. coli* within the chorion after 6 hours, and permeation through the amnion could be demonstrated after 8 to 10 hours (Fig. 3).

At the beginning of the experiments, the mean glucose concentration was 10.9 ± 0.27 mmol/L in buffer MS and 10.02 ± 0.49 mmol/L in buffer AF. The decrease of glucose was linear, indicating that the membranes stayed viable throughout the experiments. After

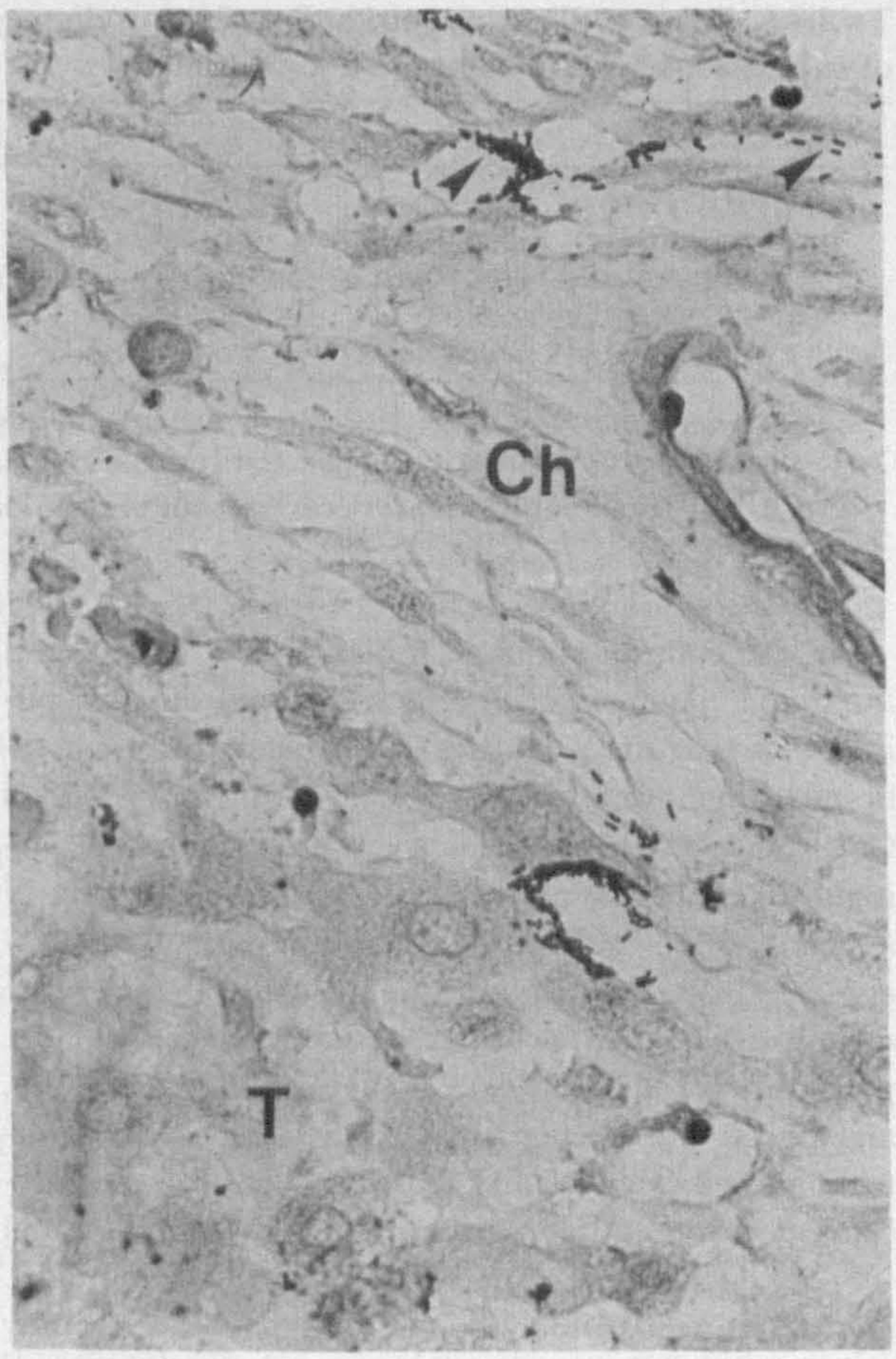

Fig. 3. Isolated organisms and clusters of rod-shaped gram-negative bacteria within chorioamniotic membranes 6 hours after inoculation of the maternal compartment with *E. coli*. Bacteria *(arrow)* penetrate from trophoblastic cell layer *(T)* into chorionic tissue *(Ch)*. (Warthin-Starry. Original magnification ×950.)

12 hours the mean glucose concentration had decreased by 2.67 mmol/L to 8.23 ± 1.03 mmol/L in the maternal compartment and by 0.82 mmol/L to 9.2 ± 0.56 mmol/L in buffer AF ($p < 0.05$). The concentration of lactate in the maternal compartment showed a linear increase by 2.25 mmol/L (from 0.03 ± 0.08 mmol/L at the beginning to 2.28 ± 0.73 mmol/L at the end of the experiments). In buffer AF the increase was 1.35 ± 0.33 mmol/L ($p = 0.02$). The total of tritiated inulin counts in the maternal compartment after 12 hours was 93.9% ± 2.05% of the initial buffer MS value, while fetal compartment inulin increased linearly from 0.8% ± 0.73% to 12.6% ± 4.5% of large compartment values.

There were no significant changes with time in mean pH, P_{O_2}, P_{CO_2}, and concentrations of sodium and potassium (Table I). The concentration gradient for sodium and potassium between the maternal and fetal compartments was kept throughout the experiments.

Table I. Mean ± SD values of Ph, Po_2, Pco_2, sodium, and potassium in fetal and maternal compartments of experiments with membranes and in controls

	Fetal	*Maternal*	*Control*
pH	7.59 ± 0.15	7.27 ± 0.06*	6.96 ± 0.19†
Po_2 (mm Hg)	217.4 ± 49.5	565 ± 30.3*	520.8 ± 48.2
Pco_2 (mm Hg)	14 ± 5.8	29.8 ± 3.8*	42.0 ± 16.0‡
Sodium (mmol/L)	134.8 ± 2	140.1 ± 1.2*	139.2 ± 1.1
Potassium (mmol/L)	5.4 ± 0.10	5.7 ± 0.08*	5.6 ± 0.07

*$p < 0.001$, significant differences between maternal and fetal compartments.
†$p < 0.001$, significant differences between controls and maternal compartment.
‡$p = 0.045$, significant differences between controls and maternal compartment.

For the controls without chorioamniotic membranes the initial count of *E. coli* (Fig. 2) did not differ significantly from that of the experiments with membranes, amounting to 10^6 ($+ 3.3 \times 10^6 - 0.77 \times 10^6$) CFU/ml ($p = 0.44$). The concentration increased during the 12 hours of the experiment to 3.16×10^7 ($+ 7.9 \times 10^7 - 2.3 \times 10^7$) CFU/ml ($p < 0.001$). However, *E. coli* grew less in the controls than in the experiments with the use of membranes ($p = 0.002$). pH was lower and Pco_2 was higher, while Po_2, sodium, and potassium did not differ from maternal compartment values (Table I). The glucose concentration decreased within 12 hours by 0.6 mmol/L (from an initial value of 11.1 ± 0.23 mmol/L to 10.5 ± 0.11 mmol/L). Lactate concentration did not change significantly (from 0.02 ± 0.04 mmol/L to 0.03 ± 0.05 mmol/L).

Comment

This in vitro study has demonstrated that *E. coli* can pass from the maternal to the fetal side of the human chorioamniotic membranes 6 to 10 hours after inoculation. Galask et al.[16] observed that *E. coli* and group B streptococci have the ability to attach to and invade chorioamniotic membranes in vitro. The current investigation further showed that *E. coli* are able to permeate intact and viable membranes in large numbers from the chorionic to the amniotic side and to grow in the fetal compartment. In this study *E. coli* grew less in the controls than in the maternal compartment. There were two major differences between the two experiments: The pH in the controls was lower because of a higher Pco_2, and the control experiments were performed without membranes. Both factors may have influenced growth of *E. coli*.[18] Although the Krebs-Ringer buffer solutions used contained glucose to ensure growth of *E. coli*, substances such as amino acids and proteins derived from the chorioamniotic membranes may have enhanced bacterial growth. This suggests that under the described experimental conditions chorioamniotic membranes do not inhibit bacterial growth, which is in contrast to the observation that bacterial growth is reduced on surfaces covered with chorioamniotic membranes.[19, 20]

Tritiated inulin was used to assess the mechanical integrity of the membranes separating the two compartments. The low rate of diffusion of inulin remained constant during 12 hours, which is in agreement with the low permeability of inulin in the in vitro perfused human placenta.[21] Major leaks within the membranes were ruled out by the continuous slow diffusion of inulin from the maternal to the fetal compartment.

The linear decrease of glucose concentration with time in the maternal compartment is consistent with a continuous consumption of glucose by the membranes, indicating that the membranes remained vital throughout the 12 hours of the experiments. This is further supported by the production of lactate. Glucose concentration decreased by 0.82 mmol/L in the fetal compartment and by 2.67 mmol/L in the maternal compartment. In earlier studies with the same model but without bacteria (unpublished observations) we observed a similar decrease in the fetal compartment but a markedly less pronounced decrease in the maternal compartment (1.05 mmol/L and 1.57 mmol/L, respectively). The decrease seen in the current study corresponds to the sum of the glucose consumption by *E. coli* as seen in the controls plus the membrane glucose utilization observed in the earlier studies. The fetal compartment glucose utilization was not significantly affected by the presence of *E. coli*, which may be due to the low bacterial count in buffer AF and the short observation time.

Low amniotic fluid glucose concentration has been proposed as a diagnostic indicator of intraamniotic infection.[22] The observation that low amniotic fluid glucose can also occur in patients with negative fluid cultures but with histologic signs of chorioamnionitis suggests that not only metabolism of glucose by microorganisms but also an inflammatory host response may be associated with low amniotic fluid glucose concentration.[22] Additional determination of lactate concentrations may be helpful, because there was no change with

time in lactate concentration in the controls with *E. coli* only.

The findings of this study suggest that the chorioamniotic membranes constitute a weak barrier against ascending bacterial infection and do not inhibit growth of *E. coli*. Therefore other defense mechanisms[2, 23] may be important in preventing intraamniotic infection.

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