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Stimulation of Prostaglandin Biosynthesis by Urine of the Human Fetus May Serve as a Trigger for Parturition

Abstract. *Urine of the human fetus stimulated prostaglandin biosynthesis in vitro by increasing the conversion of arachidonic acid into prostaglandins. The stimulatory activity in urine from fetuses delivered at term after labor of spontaneous onset was greater than that in urine from fetuses delivered by cesarean section at term before the onset of labor. Such stimulation of prostaglandin biosynthesis by the fetal membranes, by way of a substance released into the urine and thence into amniotic fluid, could serve as a signal for the initiation of parturition.*

That prostaglandins are involved in the biochemical processes of human parturition is well established (*1*), and there is evidence that the fetal membranes serve a central role in the molecular events that either signal or accompany the onset of labor (*2*). Since the putative signal for the onset of labor may be of fetal origin (*3*), it is also possible that the amniotic fluid serves as a route of communication between the fetus and the fetal membranes. A substance originating in the fetus could be excreted directly into the amniotic fluid through the kidneys, lungs, skin, or umbilical cord and, once there, could trigger parturition.

We recently found that an inhibitor of prostaglandin synthase is present in human amniotic fluid (*4*). The potency of the inhibitory activity in amniotic fluid obtained at term during labor of spontaneous onset was significantly lower than that in amniotic fluid obtained at term before the onset of labor.

These findings suggest that, throughout gestation, substances in amniotic fluid may suppress the production of prostaglandins by the fetal membranes. At term, the fetus may excrete other substances into the amniotic fluid that stimulate prostaglandin biosynthesis and thereby initiate the events that culminate in delivery of the fetus. This hypothetical substance could be released into amniotic fluid from several fetal sources. Since, at term, the amniotic fluid is largely a product of fetal urination (*5*), the kidney seemed to be a likely source of such a stimulatory substance.

By means of established methods (*6*) we evaluated the effect of urine from human fetuses (*7*) on the production of prostaglandin E_2 (PGE_2) (*8*) by a microsome-enriched preparation of prostaglandin synthase from bovine seminal vesicles (*9*). The results with samples of urine from fetuses delivered after spontaneous labor and vaginal delivery (SVD) at term and after cesarean section scheduled at term before onset of labor (not in labor, NIL) are presented in Fig. 1. The results of similar experiments with urine specimens from four adult women and four adult men are shown for comparison.

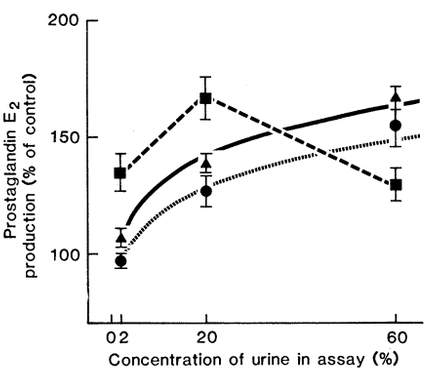

Fig. 1. The effect of human urine on the synthesis of PGE_2 by prostaglandin synthase from bovine seminal vesicle (*6*). The production of PGE_2 is expressed as a percentage of production by controls. Values shown are means (± standard error of the mean) for urine samples from (■) adults (N = 8), (▲) newborns delivered at term (38 to 42 weeks of gestation) after spontaneous labor (*SVD*, N = 10), and (●) newborns delivered after cesarean sections scheduled at term before the onset of labor (NIL, N = 10). The concentrations of urine tested in the assay were 2, 20, and 60 percent (by volume). The curved lines are indicative of best fits (by the method of least squares) to the equation $y = bx^m$. For SVD samples, b = 1.987 ± 0.0169 (± standard error), m = 0.1272 ± 0.0131, and r = .896 ($P < .001$). For NIL samples, b = 1.939 ± 0.0252, m = 0.1323 ± 0.0197, and r = .836 ($P < .001$). By analysis of variance we found that the slopes (m) were not significantly different, but the intercepts (b) were significantly different ($P < .005$).

There were no statistically significant sex-related differences in the stimulatory potency of the adult specimens; the data are therefore combined. Urine from both the fetuses and the adults significantly increased the production of PGE_2 in a concentration-dependent manner. The SVD urine samples, however, stimulated PGE_2 production significantly more than the NIL samples obtained before the onset of labor. The concentration of urine in the incubation mixture that produced a 50 percent increase in PGE_2 production, compared to control incubations (without urine), was 31 percent by volume for SVD samples and 62 percent for NIL samples.

At low concentrations, urine from adults increased PGE_2 production more than either of the SVD or NIL urine samples, but at high concentrations of urine these relations were reversed. This biphasic effect suggests that, compared with urine from fetuses, high concentrations of urine from adults may contain greater amounts of both stimulatory and inhibitory factors, and that at the 60 percent concentration the stimulatory factors were antagonized. The postulated inhibitory factors would have to be present in greater amounts (that is, > 20 percent) to exert a detectable effect. Such inhibitory effects were not apparent in experiments with urine from fetuses.

The stimulatory activity in fetal urine was not significantly altered by boiling the urine for 5 minutes (N = 4), but was not detectable in urine that had been subjected to membrane dialysis (retaining molecules greater than 12,000 to 14,000 daltons) against NaCl (0.15*M*) for 48 hours at 4°C (N = 4). Two treatments with activated charcoal, each for 15 minutes at 4°C, partially reduced the stimulatory activity in urine (40 to 80 percent; N = 4), indicating that the activity may result in part from the action of a steroid or small lipid.

Our interpretation of these results may provide an explanation for our previous finding that the prostaglandin synthase inhibitory activity in amniotic fluid obtained during labor is less than the inhibitory activity of fluid taken before labor. This decreased inhibitory activity could result from the increased production of a stimulatory substance that is released into the urine of the fetus and thence into the amniotic fluid during labor. If this were the case, such a stimulant could cause an increase in prostaglandin production by the fetal membranes and

thereby initiate the sequence of events in the uterus that results in delivery of the infant.

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7. The urine samples were obtained at first voiding from full-term (38 to 42 weeks of gestation) newborn infants immediately after delivery. Such urine had been formed while the infant was in utero and was therefore fetal urine. All infants were healthy and of appropriate size for gestation. Specimens were obtained during spontaneous urination or by gentle suprapubic pressure. The samples were stored at −20°C until tested.
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Bovine × Mouse Hybridomas That Secrete Bovine Immunoglobulin G_1

Abstract. *The interspecific fusion of normal bovine lymphocytes with a nonsecreting mouse hybridoma produced stable cell lines secreting bovine immunoglobulins. One of these lines has continued to secrete immunoglobulin G_1 (5 to 10 micrograms per milliliter) for over 16 months. The bovine × mouse hybrid cells can be expected to provide bovine monoclonal immunoglobulins for sequencing studies and for use as serological standards as well as to provide messenger RNA for cloning bovine immunoglobulin genes.*

The availability of monoclonal immunoglobulins (Ig's) first from myelomas (*1*, *2*) and recently from hybridomas (*3*, *4*) has been of critical importance to the development of our current picture of the structure of human and mouse Ig's and their genes. Antibody-secreting hybridomas have been produced by intraspecific fusion of myeloma cells with normal cells of the B lymphocyte lineage (*5*–*8*) as well as by interspecific fusion of mouse myeloma cells with B lineage cells of nonmurine species (*9*, *10*). However, cell lines secreting monoclonal bovine Ig, derived from normal cells of the B lymphocyte lineage, have not been demonstrated. Hence the field of veterinary immunology has been without a source of bovine monoclonal Ig's for sequence studies and for the preparation and testing of class-specific antisera.

Table 1. Specific immunoabsorption of the hybridoma product. The hybrid cells (1×10^7) were cultured in 2 ml of methionine-free DME medium containing 1 percent fetal bovine serum and 0.5 mCi of [^{35}S]methionine for 6 hours. The culture supernatant was used in a binding assay. Six milligrams of the affinity-purified mouse monoclonal antibody DAS-1, specific for bovine IgG and IgM, was coupled to 1 g of CNBr-activated Sepharose 4B and suspended in 15 ml of PBS containing 0.05 percent polyoxyethylene sorbitan monolaurate (Tween 20). To 1 ml of this suspension was added 100 μl of the labeled culture supernatant, and the mixture was agitated slowly for 30 minutes. The ability of whole bovine serum or whole mouse serum to block the binding of the labeled product in the culture supernatant to DAS-1 was tested by adding 50 μl of each of these sera, respectively, to the DAS-1–conjugated Sepharose prior to the addition of the culture supernatant. The reacted Sepharose was then washed three times by the addition of 10 ml of PBS containing 0.05 percent Tween 20 and centrifuging at 400*g* for 5 minutes. The Sepharose pellet was then counted in a scintillation counter.

Sample treatment	Radioactivity (count/min)*
No incubation	41,610 ± 60
Incubation with bovine serum	3,473 ± 60
Incubation with mouse serum	40,226 ± 370

*Mean ± standard deviation.

We report here the interspecific fusion of a mouse hybridoma with normal bovine spleen cells, resulting in stable hybrid cell lines that secrete monoclonal bovine Ig molecules. Interspecific hybridomas were produced because HAT (hypoxanthine, aminopterin, thymidine)-sensitive bovine myeloma cell lines are not available (*11*). These hybridoma cell lines manufacturing monoclonal bovine Ig will be useful (i) for the production of monoclonal bovine Ig for sequencing studies and as reference reagents for the serology of bovine Ig; (ii) for the production of continuous cultures of hybridomas secreting bovine Ig of predefined antigenic specificity by use of bovine spleen cells from appropriately immunized donors as fusion partners; and (iii) as a source of messenger RNA (mRNA) for the cloning of bovine Ig genes.

Bovine × mouse hybridomas were prepared by polyethylene glycol (PEG)–assisted fusion of normal bovine lymphocytes to the mouse cell line SP-2/0 (*12*), a mouse × mouse hybridoma which does not secrete or internally produce mouse Ig. Bovine lymphocytes were obtained from the spleen of an adult female Holstein cow by first mincing six randomly selected cubes of spleen (~ 2 cm) with scissors and grinding the mince between the frosted faces of microscope slides in serum-free growth medium [50 percent RPMI 1640 and 50 percent Dulbecco's modified Eagle's medium (DME)]. The lymphocytes were washed twice in serum-free growth medium, mixed in a 1:1 ratio with 1×10^8 SP-2/0 cells, and centrifuged at 400*g* for 15 minutes at room temperature. The pellet was then slurried in 2 ml of 52 percent polyethylene glycol 1540 in serum-free growth medium and incubated for 2 minutes at 37°C. The mixture was then diluted over the course of 10 minutes to 50 ml by the addition of serum-free growth medium and centrifuged for 15 minutes at 400*g* at room temperature. The fused cells were resuspended in HAT (*13*) medium (45 percent RPMI 1640, 45 percent DME, 10 percent γ-globulin–free horse serum, 2 m*M* glutamine, penicillin (10 U/ml), streptomycin (100 μg/ml), 1×10^{-4}*M*