Studies on the Role of Interleukin-1 on Prostaglandin Production by Human Fetal Membranes and Decidua

by

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Declaration

I certify that this thesis does not incorporate without acknowledgment any material previously submitted for a degree in any University. To the best of my knowledge and belief, this thesis does not contain any material previously published or written by another author except where due reference is made in text.

Bunan Alnaif

DEDICATION

I dedicate this thesis to my
husband, Durad,
my children, Abed and Ramsey,
and to my parents

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ABSTRACT

Recent studies have indicated the possible importance of cytokines in the onset of term and preterm labour. To understand this further, the effect of interleukin-1 (α and β) on prostaglandin output by dispersed cells from human amnion, chorion laeve, and decidua obtained at term (38-42 weeks gestation) was examined. During the first or second 24 hours of culture, no significant effect of these interleukins on prostaglandin output was observed.

Because it has been previously shown that interleukin-1 (IL-1) stimulates interleukin-6 (IL-6) production by these tissues, we examined the effect of IL-6 on freshly dispersed cell preparations from amnion, chorion laeve, and decidua. IL-6 did not alter prostaglandin (PG) production in the first or second 24 hours of culture.

It is known that the type of preparation influences the tissue response to a given treatment. Therefore we assessed the responsiveness of our *in-vitro* system; dexamethasone significantly inhibited PG output, whereas phorbol ester significantly increased PG release. Thus, our freshly dispersed cell preparations appeared to be functional.

The apparent refractoriness of these cells to interleukin-1 was further investigated by studying the distribution of IL-1 receptors in frozen sections of undisrupted fetal membranes and decidua at

term. Whole-tissue autoradiography indicated that receptors were present in the chorion and decidua but not in the amnion. By using emulsion autoradiography, IL-1 receptors were found in high concentrations in chorion laeve, but absent in amnion, and at low levels in the decidua. Because this study was on dispersed cells, autoradiography was performed on such preparations. Fresh cells and cells cultured for one and two days were labeled for IL-1 receptors. These results confirmed the intact tissue findings. No labeling was found in the amnion; labeling was found in certain populations of the chorion cells.

These studies indicate that under normal circumstances in human pregnancy at term, IL-1 does not stimulate prostaglandin production by dispersed cells. In the case of amnion, this may be due to the absence of receptors, and therefore it would appear that the IL-1 receptor must first be induced in this tissue before it can respond to this cytokine. Furthermore, although chorion laeve expresses the IL-1 receptor, dispersed cells from this tissue did not respond to the cytokine by increasing prostaglandin output.

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ABBREVIATIONS

(In alphabetic order)

ACTH	Adrenocorticotrophic hormone
AF	amniotic fluid
Anova	Analysis of variance
BGG	Bovine gamma globulin
B_o	Reaction without antigen competition
BSA	Bovine serum albumin
c	centi = 10^{-2}
°C	Degree centigrade, measure of temperature
cfm	Cubic feet per minute
ci	Curie
Conc	Concentrated
CS	Cesarean section
DCC	Dextran coated charcoal
Dex	Dexamethasone
DMEM	Dulbecco's modified minimum essential medium
DNase	Restriction endonuclease of deoxyribonucleic acid
EGF	Epidermal growth factor

F12:DMEM	Ham's F12-Dulbecco's modified essential medium
FCS	Fetal calf serum
Fe	Iron
g	gravity
g	gram
h	hour
^{3}H	Triton; a weak β-emitter
H&E	Hematoxylin and eosin
HCL	hydrochloric acid
HEPES	N-2-Hydroxyethylpiperazine-N'-ethanesulfonic acid
HLA-DRα	Human leukocyte antigen HLA-DR alpha
15 HPDH	15-hydroxyprostaglandin dehydrogenase
HPDH	Hydroxyprostaglandin dehydrogenase
hrIL-1	Human recombinant IL-1
IL-1	Interleukin-1
IL-1α	Interleukin-1 alpha
IL-1β	Interleukin-1 beta
IL-1R	Interleukin-1 receptor
IL-1ra	Interleukin-1 receptor antagonist
IL-6	Interleukin-6

IRAP	Interleukin-1 receptor antagonist protein
K	kilo = 10^3
KD	Kilodalton
L	Ligand
l	Liter
L*	Radioligand
µ	Micro = 10^{-6}
m	Milli = 10^{-3}
M	Molar
MW	Millimolar
Mag DCC	Magnatic dextran coated charcoal
min	minutes
µl	microliter
ml	Milliliter
n	Nano = 10^{-9}
ND	Normal delivery
ng	Nanogram
NSAID	Non steroidal anti-inflammatory drug
NSB	Non-specific binding
OCT	Mounting coumpound
p	Pico = 10^{-12}

p mol	Picomolar
PAF	Platelet activating factor
PBS	Phosphate buffered saline
PG	Prostaglandin
PGDH	Prostaglandin dehydrogenase
PGE_2	Prostaglandin E_2
PGEM	11-deoxy-13,14-dihydro-15-keto-11β, 16 cyclo-prostaglandin E_2
$PGF_{1\alpha}$	Prostaglandin F_1 alpha
$PGF_{2\alpha}$	Prostaglandin F_2 alpha
PGFM	13,14 dihydro-15-keto-prostaglandin F
PGI_2	Prostacyclin
pH	- Log_{10} of H^+ ion concentration
PHA	Phytohemagglutinin; a lymphocyte mitogen that is used in vitro to measure lymphocyte mediated cytotoxicity
pM	Picomole
PTL	Preterm labour
R	Receptor
RIA	Radioimmunoassay
RPMI	Revolutions per minute
SA	Specific activity
sec	Second

Smpls	Samples
Stnds	Standards
7TDI cells	mouse-mouse hybridoma cells
TGF-β	Transforming growth factor beta
TNF	Tumor necrosis factor
TPA	Phorbol 12-myrislate 13-acetate
TxA_2	Thromboxane
[^{125}I] IL-1α	Iodine 125 radio-labelled Interleukin-1
u	Units. One unit of IL-1α or β is defined as the amount of IL-1α or β that is required to support half maximal [^{3}H]-thymidine incorporation into mouse thymocytes co-stimulated with low amounts of lectin (e.g. PHA). One unit of IL-6 is defined as the amount of IL-6 that is required to mediate half-maximal cell proliferation with 7TDI cells (mouse-mouse hybridoma cells)
Zn	Zinc

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1.0 INTRODUCTION

1.01 Background

Pre-term birth is a major obstetrical problem; it contributes to around 75% of perinatal mortality and morbidity. Precise methods to prevent or predict its occurrence could result in salvaging a large number of neonatal lives, and an enormous economic saving in neonatal care.

The contemporary era of studies on the fetal membranes' role in the onset of labour started two decades ago, in an effort to explain at the molecular level, the mechanism(s) initiating and regulating human parturition at term (MacDonald *et al.* 1974). It was hoped that understanding normal labour at term might lead to a better understanding of the etiology of preterm labour, and thus its prevention. A sizable fraction of the etiology of preterm labour has been attributed to the products of microorganisms (Helmig *et al.* 1990, Romero *et al.* 1989 *d*) and the host response to them (Mitchell *et al.* 1991*d*), which results in the production of substances that act to increase the concentration of prostaglandins (PGs) in amniotic fluid (Romero *et al.* 1988 *a and b*). PGs are thought to exert an effect on the myometrium and the surrounding tissues in a paracrine and

autocrine fashion, leading to the initiation of human labour (Mitchell *et al.* 1993 *a*).

Although the physiological sequence of events involved in parturition in certain animals, particularly the sheep, is well understood (Liggins *et al.* 1967 and 1977), experimental animals may not be particularly good models for human parturition. Nonetheless, several hypotheses have been formulated to explain the nature of the events leading to the onset of parturition, and PG formation at term is believed to play an obligatory role in each of them.

The objective of this study was to examine certain aspects of the regulation of PG production in term human pregnancy. This project first examined PG production by human fetal membranes and decidua, before and after the onset of labour. It then studied the effects of IL-1 on prostaglandins formation, and examined IL-1 receptor distribution on these tissues and their dispersed cells.

1.02 Development, Structure and Function of the Fetal Membranes and Decidua

This thesis involves the study of tissues derived from the human uterus. Therefore, the development, structure and function of the human intrauterine products of conceptions will be discussed.

1.02.1 Development of fetal membranes and decidua

After the ova is fertilized, it undergoes a series of mitotic divisions, resulting in the formation of the *morula* and then, the *blastocyst*. The outer layer of the blastocyst is called the *trophoblast*; it consists of ectodermal cells, which are directly involved in implantation on about the seventh day after ovulation, and later, placenta formation. Under the effect of progestational substances from the corpus luteum, the endometrium undergoes a series of transformations termed *decidualization* .

Around 12 days after fertilization, the villi which forms the periphery of the conceptus can be easily distinguished. Initially these villi cover the entire surface of the chorion. As pregnancy advances, two separate regions begin to differentiate; the *Chorion frondosum* and the *Chorion laeve*. The *Chorion frondosum* represents the villi of the fetal pole area which contacts *decidua basalis* and continues to grow, forming the fetal component of the placenta. The *Chorion laeve* represents the villi on the abembryonic site of the implantation site which is in contact with the less vascular *decidua capsularis*.

The amnion appears on the dorsal surface of the formed embryo on days 7 and 8 after conception (Fig. 1-1). Therefore, the amnion and the chorion laeve have anatomically different origins, and they are separated until the third month of

gestation by the exocoelomic cavity. Collectively, they form an avascular amniochorion, which is called the *fetal membranes* or the amniotic sac.

The membranes continue to grow by mitotic activity for approximately 28 weeks, after which time the sac is enlarged by passive stretching. The two membranes can generally slide upon each other and are easily separated at birth. The amnion, which lines the entire chorion laeve, placental plate, and the cord, is normally smooth and slippery and its inner surface is in contact with the amniotic fluid. The chorion laeve is in direct contact with the decidua vera.

Decidua is the endometrium of pregnancy, and is so named because much of it is shed after parturition. The decidua reaction encompasses the changes that begin in response to progesterone produced after ovulation, which prepare the endometrium for implantation and nutrition of the blastocyst. The portion of the decidua directly beneath the site of implantation forms the *decidua basalis*. As Fig. 1-1 illustrates, the *decidua capsularis* overlies the developing ovum and separates it from the rest of the uterine cavity. The *decidua capsularis* is in contact with the chorion laeve, and the remainder of the uterus is lined by the *decidua parietalis*.

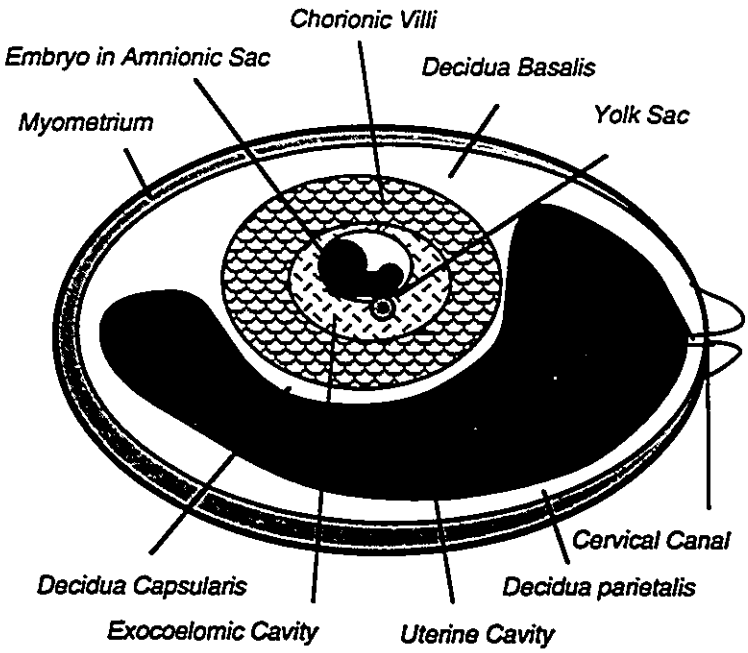


Fig. 1-1
Diagrammatic representation of the human uterus in early pregnancy

By the fourth month of pregnancy, the decidua capsularis fuses with the decidua parietalis to form the *decidua vera*. Because of the uterine distention, the decidua gradually thins from its maximal thickness of 1 cm in the first trimester to only 1 or 2 mm at term and gives rise to the term uterus as shown in Fig. 1-2.

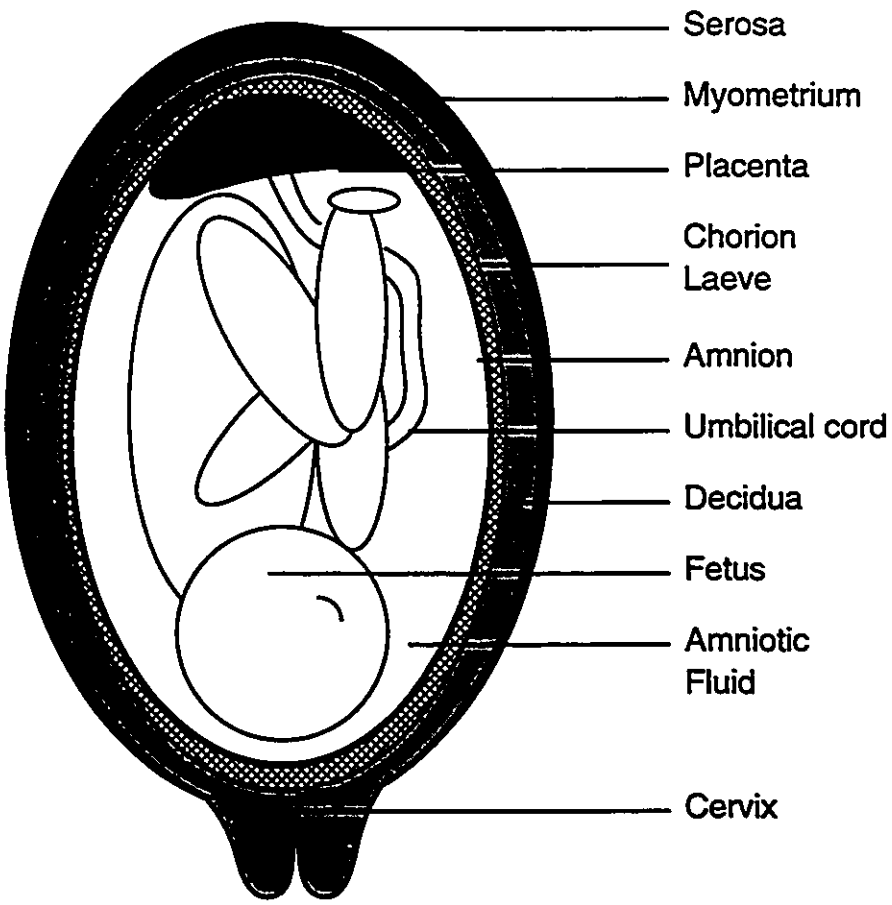


Fig. 1-2
Diagrammatic representation of term gravid human uterus, showing placenta amnion, chorion and decidua in situ

The maternal surface of human placenta and membranes at term is shown in Fig. 1-3. The fetal surface of human placenta and membranes at term is shown in Fig. 1-4.

Fig. 1-3
Maternal surface of human placenta and membranes at term.

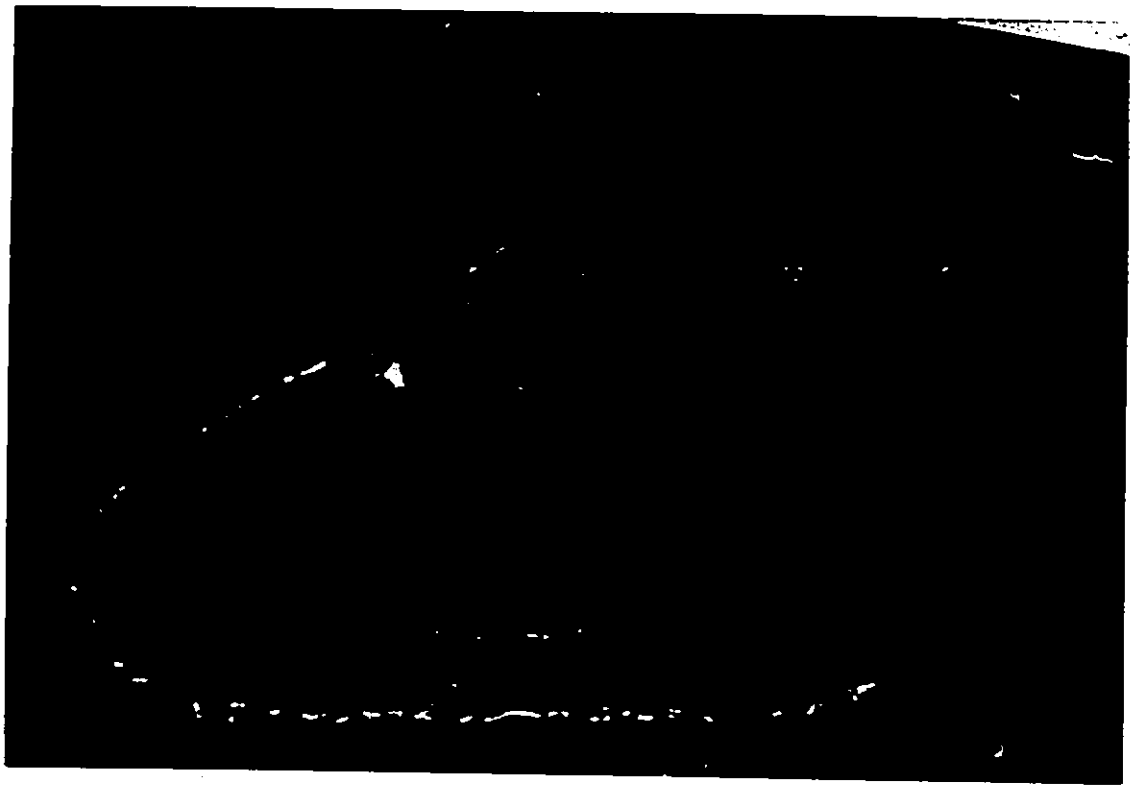


Fig. 1-4
Fetal surface of human placenta and membranes at term.

1.02.2 Morphology

Fig. 1-5 is a schematic illustration of the histological appearance of the human fetal membranes (amnion and chorion laeve). Fig 1-6 is the histological appearance of fetal membranes and decidua at term.

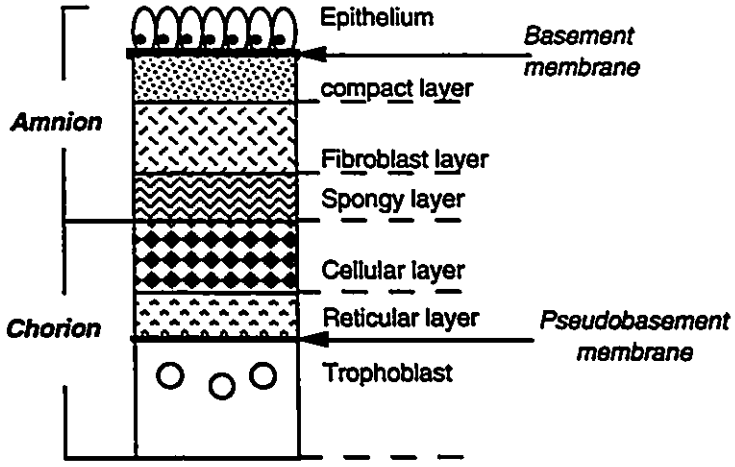


Fig. 1-5
Diagrammatic representation of the histological appearance of the human fetal membranes (amnion and chorion).

(Modified from Danforth and Veland 1986)

1.02.3 Amnion

The amnion is 0.02 to 0.5 mm thick. It consists of a single layer of nonciliated cuboidal epithelial cells with microvilli, a basement membrane (which provides the major strength of the membrane), a compact layer, a fibroblastic layer, and a spongy layer.

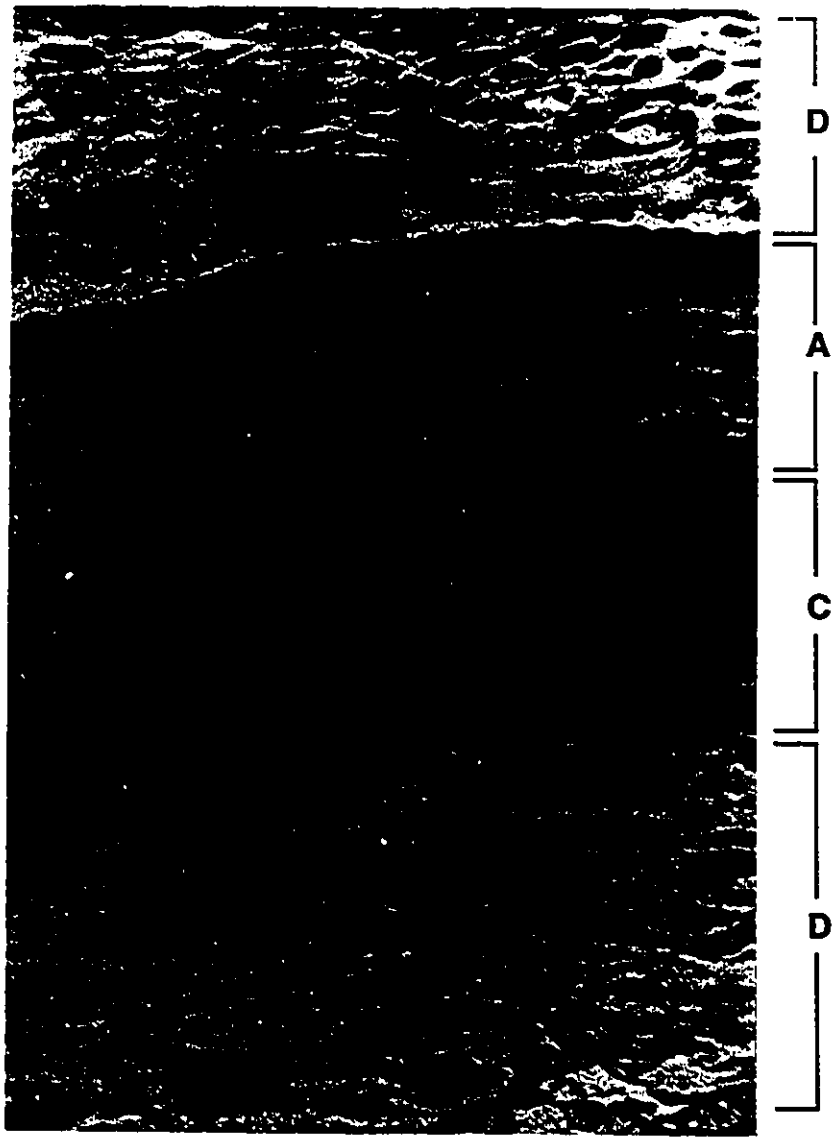


Fig. 1-6

Histological appearance of fetal membranes and decidua at term. The tissue was stained with H&E. Magnified X 20.

A = Amnion

C = Chorion

D = Decidua

1.02.4 Chorion laeve

The chorion laeve is denuded of villi due to direct pressure and interference with its vascular supply early in its development. It is around 1 mm thick, and consists of an inner cellular layer that is formed of loose intracellular matrix containing spindly cells with protoplasmic processes. This layer is followed by the reticular layer, which thickens to form a pseudobasement membrane that provides additional strength to the membranes. The outer layer in contact with the decidua vera contains the trophoblast cells.

1.02.5 Decidua vera

The decidua vera is composed of three layers: a surface, or compact zone (*zona compacta*); a middle portion, or spongy zone (*zona spongiosa*) with glands and numerous small blood vessels; and a basal zone (*zona basalis*). The compacta and the spongiosa together form the functional zone (*zona functionalis*). The basal zone remains after delivery and gives rise to the new endometrium.

1.02.6 Function of the fetal membranes and decidua

Fetal membranes are not simply passive barriers in pregnancy, but also contribute many metabolic factors. The membranes play a critical role in the fetal development and protection throughout pregnancy, and as will be discussed in detail later, in the commencement of labour. The physiological properties of the fetal membranes facilitate this role.

The surface area of the membranes at term is large (approximately 0.6 meters^2). The functional surface area is made even larger by the presence of numerous microvilli on the amnion cells. Such a large surface area is ideally suited for maximum exposure to fetal secretions, and to the secretions emanating from the amnion and chorion cells themselves; and it is also suitable for absorptive processes (as in the intestinal mucosa). At term, almost two thirds of the uterine cavity is in direct contact with fetal membranes, which allows the uniform translocation of substances across the fetal membranes to the decidua and myometrium (Nieder and Augustin 1983, Nakla *et al.* 1986).

Another important property of the membranes is elasticity. This enables them to withstand stresses placed upon them during pregnancy. The elasticity is maintained mainly by two connective tissue components; collagen and glycosaminoglycans (Skinner *et al.* 1981). During the last eight weeks of gestation,

factors associated with collagen synthesis and degradation (cathepsins) are present in amniotic fluid (Vadillo-Ortega *et al.* 1991) and fetal membranes (Halaburt *et al.* 1989, Vadillo-Ortega *et al.* 1990). These factors may become active in preparation for membrane rupture at term. Interleukin-1 has been shown to accelerate hyalurate synthesis, and collagenase production by fibroblast-like cells from confluent cultures of human chorion laeve (Ito *et al.* 1993).

During pregnancy, fetal membranes may play a crucial role in the prevention of parturition though their ability to increase uterotonin destruction, at least *in-vitro* (Germain *et al.* 1994). The presence of fetal membranes, especially chorion, caused a 40% decrease in rat uterine muscle contraction (Collins *et al.* 1993).

The decidua plays an important role in blastocyst implantation, embryo-fetal development, maintenance of pregnancy (Germain *et al.* 1994), acceptance of the semiallogenic fetal graft, and initiation and maintenance of parturition (Romero *et al.* 1989 *a, b, and c*). Human decidua is also capable of producing a number of cytokines, such as interleukin-1, IL-6, Tumor necrosis factor (TNF) (Romero *et al.* 1991 *b*) and platelet activating factor (PAF). The levels of these cytokines are increased during labour, and in response to bacterial toxins. (Hoffman *et al.* 1992, and Romero *et al.* 1989 *a and c*).

1.03 Human Parturition

Human parturition is the process of giving birth, which can occur either at term (term labour), or preterm (preterm labour).

1.03.1 Term Labour

Normally, labour commences at term, which is between 37 and 42 weeks of gestation. Physiologically, it has been divided into three distinctive, but overlapping phases (Williams Obstetrics, 1993): phase one is the time for uterine preparation for labour, phase two is the time of forceful contractions and retraction of active labour and delivery, and phase three is the time of puerperal contraction and involution of the uterus. Fig. 1-7 illustrates the relationship between these phases of parturition.

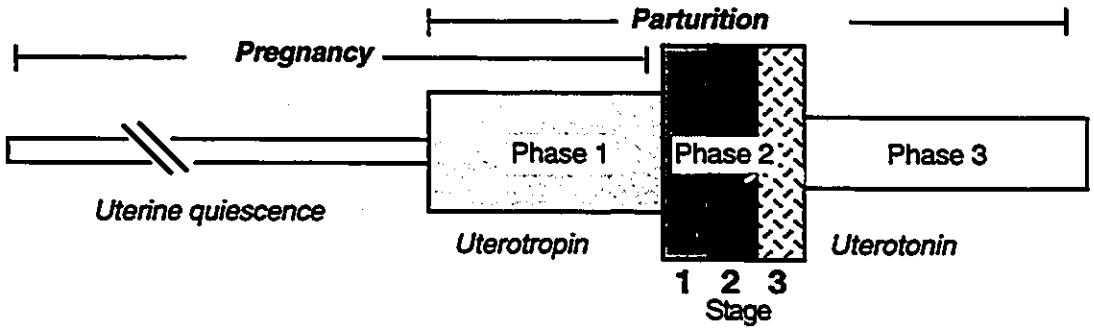


Fig. 1-7
Diagrammatic presentation of the three phases of parturition

(modified from Williams Obstetrics 1993)

Phase one of parturition is the event of transition from uterine quiescence to active labour. Uterine quiescence refers to pregnancy prior to the initiation of labour, when the smooth muscles (myometrium) of the uterus are mostly non-contractile. Active labour refers to the stage of forceful contraction and retraction which results in gradual cervical dilation and expulsion of the conceptus. In phase 1, there is an enhancement of the contractile responsiveness of the myometrium to uterotropins. Uterotropins are agents which cause uterine preparedness and alteration for labour (Casey and MacDonald 1988 *a, b, and c*). Among these alterations are cervical softening and ripening (Kitamura *et al.* 1979, Trofatter 1992), the development of gap junctions between myometrial cells (Garfield *et al.* 1980, Cole and Garfield 1986), an increase in the number of oxytocin receptors in the myometrium (Fuchs *et al.* 1982 and 1984), and increased contractile responsiveness of the myometrium to uterotonins (Garfield and Hayashi 1981, Hayashi *et al.* 1985).

Phase two of parturition is caused by uterotonin agents which act to cause myometrial contractions. These agents include prostaglandins, oxytocin, endothelin-1 and platelet-activating factor. This phase is further divided into three stages of labour, beginning with the onset of regular uterine contractions, and ending with the delivery of placenta and membranes. A particular uterotonin can also be a uterotropin; as for example in the case of PGs.

Phase three of parturition, or puerperium, begins after delivery of the conceptus. It starts with intermittent contraction of the uterus to secure puerperal homeostasis, and then proceeds to a complete involution of the uterus, which returns the uterus to its non-pregnant state.

1.03.2 Preterm labour

Preterm labour occurs between the end of the 20th week and the end of the 37th week of pregnancy. Although it accounts for around 6-10% of all deliveries, it contributes to more than 75% of neonatal mortality and morbidity in North America (Morrison 1990), and is the greatest single cause of neonatal morbidity and mortality. In the majority of instances, the precise cause(s) of preterm labour is not known (*idiopathic*). However, almost one quarter are accompanied by intrauterine infection (Van der Elst *et al.* 1991).

Presently, diagnosis of preterm labour depends mainly on the clinical findings of regular and strong uterine contractions and/or cervical effacement and dilatation. If pregnancy continuation is not contraindicated, an attempt is made to arrest labour. This can range from bed rest and hydration to the use of tocolytic agents such as ritodrine or magnesium sulfate (Smith and Thompson 1993). These agents act by inhibiting the contraction of uterine muscles, and their efficacy is still being

questioned. If the mechanism(s) involved in the initiation of labour were known, more efficacious and physiological ways of arresting labour could be developed, and specific biochemical marker(s) for predicting the onset of labour might be found.

1.04 Human and Animal Parturition

The most complete understanding of the physiological events involved in parturition in mammalian species is that established for the sheep (Liggins *et al.* 1967 and 1973). Maturation of the fetal pituitary-adrenal axis occurs late in gestation and results in an elevation of fetal cortisol (Myers *et al.* 1992 *a* and *b*). The increase of glucocorticoid secretion by the fetal adrenal gland causes a decrease in progesterone and increase in estrogen concentrations in maternal blood (France *et al.* 1987). These changes are thought to provoke an increase in prostaglandin production (Richardson *et al.* 1986), the formation of gap junctions between myometrial cells (Cole and Garfield 1986), and the sensitization of the uterus to uterotonic agents. Estrogens also act to increase the myometrial response to electrical or oxytocic stimuli (Benedetto *et al.* 1990, Kimura *et al.* 1992), as well as to increase muscle action potential and myometrial excitability. They also increase vascularity and tissue permeability which results in cervical ripening. Furthermore, infusion of ACTH and glucocorticoids into the fetal lamb induce premature labour (Langlois *et al.* 1993), while fetal

adrenalectomy and hypophysectomy prolong pregnancy (Liggins *et al.* 1967).

There are a number of differences between human and animal labour. In the human, marked changes in peripheral concentrations of estrogen and progesterone do not occur just prior to labour. Progesterone reaches its highest concentration in peripheral blood late in pregnancy, and estrogen concentration rises gradually throughout gestation (Thorburn and Challis 1979). However, there is a change in the progesterone/ estrogen ratio at term (Romero *et al.* 1988*c*). Also, in contrast to the sheep, the human fetus does not have such an important role in determining the length of human gestation. Anencephaly, which is a congenital anomaly resulting in the absence of the brain, does not result in prolonged gestation (Swaab *et al.* 1977), but does result in more pre-term and post-term deliveries (Malpas 1933), indicating that the human fetus probably fine-tunes the timing of delivery. In the human, glucocorticoids do not induce labour as they do in the sheep, probably because the placental enzyme which glucocorticoids induce in the sheep (17,20 lyase) is not expressed in the human placenta (Challis and Olson 1988).

Although there are major differences in the mechanisms regulating labour in sheep (and other animals) and the human, PG formation appears to be a common factor in the initiation

and maintenance of labour in all species studied (Mitchell 1986).

1.05 Prostaglandins and Parturition

The first suggestion of a contractile effect of PGs (seminal fluid) on human uterus was reported by Kurzrok and Lieb in 1930. Goldblatt in 1933 and Von Euler in 1934 independently discovered that an extract from seminal vesicles stimulated smooth muscle preparations. In 1935, Von Euler named the acidic lipid that caused the effect "Prostaglandin".

The potential importance of PGs in labour and their clinical use was first noted by Karim in 1966, when he reported the presence of PG-rich extracts of amniotic fluid. During labour, PGs of the E and F series were found at concentrations much greater than those obtained prior to the onset of labour (Khan *et al.* 1991). These extracts were capable of increasing the intensity of spontaneous contractions of the uterus. Since the 1970s, PGs have been used to induce labour and abortion (MacLennan 1991).

PGE_2 and $PGF_{2\alpha}$ are PGs of the two double bonds series, and are synthesized from an obligatory precursor, arachidonic acid. Arachidonic acid is released mainly by the action of phospholipase A_2 because of the abundance of arachidonate in position 2 of glycerophospholipids, or via a series of lipases

from phosphatidylinositol (Okazaki *et al.* 1981 *a and b*) see Fig. 1-8.

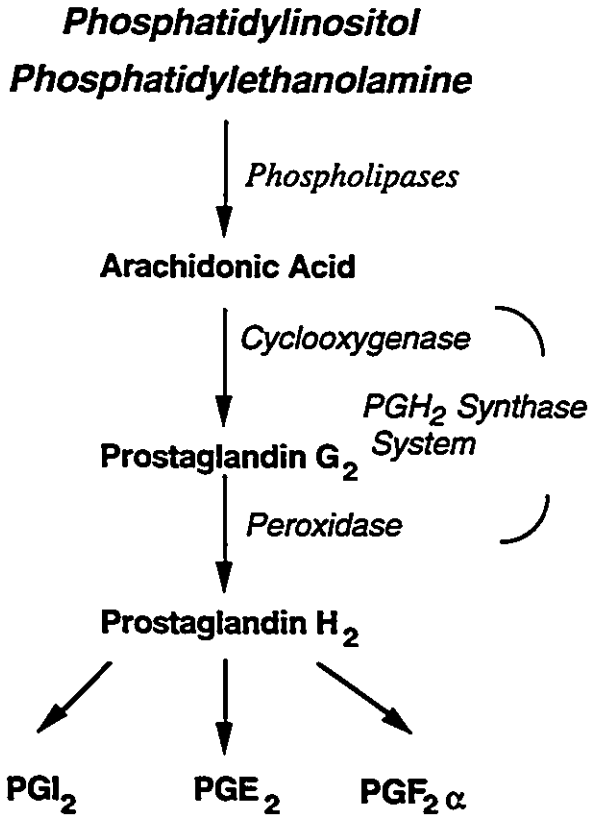


Fig. 1-8
Prostaglandin biosynthesis via PGH synthase (Cyclooxygenase) pathway

Prostaglandins are produced by several uterine tissues including fetal membranes (Keirse and Turnbull 1976), the decidua (Willman and Collins 1976), and myometrium (Weppelmann *et al.* 1980). The normal levels of prostanoids produced by intrauterine tissues are summarized in Table 1-1. PGE_2 and $PGF_{2\alpha}$ stimulate the myometrium to contract at all stages of pregnancy, possibly through increasing intracellular calcium (Rydnert and Joelsson 1985) and prostacyclin (PGI_2)

has been shown to relax human myometrium *in-vitro* (Omini *et al.* 1978). PG action is mediated through specific receptors; $PGF_{2\alpha}$ receptors are present in amnion, decidua and myometrium (Giannopoulos *et al.* 1985) and PGE_2 receptors are present in myometrium and cervix. There is no change in receptor number and affinity for either prostaglandin with the onset of labour (Giannopoulos *et al.* 1985).

Table 1-1

Prostanoid production by human intrauterine tissues at term in ng/min per g dry weight*

Tissue obtained after labour

Prostanoid	Amnion	Chorion	Decidua
PGE	13.2 ± 2.2	2.9 ± 0.5	1.7 ± 0.3
PGF	0.8 ± 0.2	0.5 ± 0.12	0.5 ± 0.1
Thromboxane B_2	1.6 ± 0.4	0.6 ± 0.2	2.1 ± 0.4
6-Keto-PG F1α	6.3 ± 2.4	2.4 ± 0.6	1.5 ± 0.43

Tissue obtained before labour

Prostanoid	Amnion	Chorion	Decidua
PGE	9.6 ± 1.6	3.1 ± 0.6	2.5 ±0.6
PGF	0.7 ± 0.2	0.8 ± 0.2	0.8 ± 0.2
Thromboxane B_2	2.4 ± 0.8	0.9 ± 0.3	2.8 ± 1
6-Keto-PG F1α	2.4 ± 0.6	1.8 ± 0.4	1.4 ± 0.4

*Modified from Mitchell 1984

Inhibitors of PG biosynthesis include corticosteroids and nonsteroidal anti-inflammatory compounds (NSAID) such as aspirin and indomethacin. While aspirin causes irreversible inhibition of PG synthesis, indomethacin inhibition is reversible, both act on PGH synthase (Waltman *et al.* 1972).

The metabolism of PGs occurs mainly in the lung, kidneys, and liver. PGE_2 and $PGF_{2\alpha}$ have short half lives in human plasma, mainly because of the very high rate of metabolism by the lung. Nevertheless, the level of PGFM (a metabolite of $PGF_{2\alpha}$) in maternal blood is increased during labour at term (Romero *et al.* 1989 *a*).

Considerable evidence supports the role of PG in term labour. PG synthesis activity in intrauterine tissue (amnion, chorion laevc and decidua vera) obtained after the onset of labour has been found to be greater than that obtained before the commencement of labour (Neider and Augustin 1983, Cheung and Challis 1989, Keirse 1990, Korte *et al.* 1993). PG levels, particularly PGFM in maternal peripheral blood increase with labour (Green *et al.* 1974, Neider and Augustin 1983, Keirse and Turnbull 1973, Satoh *et al.* 1979, Johnston *et al.* 1993), reaching a maximum at the time of placental separation (Weppelmann *et al.* 1980). The principle source of $PGF_{2\alpha}$ and its PGFM in maternal blood is from the decidua. Mitchell and his coworkers found no evidence of peripheral blood increase in PGE_2 or its metabolites (Mitchell 1986), while Johnston *et al.* 1993 showed

that spontaneous labour is associated with increased concentrations of prostaglandin metabolites (both PGEM and PGFM) in maternal plasma, particularly PGFM. PGFM was significantly higher in spontaneous labour versus dysfunctional labour (Johnston *et al.* 1993). The concentrations of PGE_2, $PGF_{2\alpha}$ and PGFM are greater in cord blood than maternal plasma (Johnston *et al.* 1993). These metabolites are higher in cord blood after labour than prior to labour (Challis *et al.* 1974).

Amniotic fluid PG concentrations increase with labour (Dray and Frydman 1976), and increase with dilatation of the cervix (Bleasdale and Johnston 1985). The concentrations of PGE_2, $PGF_{2\alpha}$ and PGFM in amniotic fluid increase with gestational age. Table 1-2 summarizes the changes in the mean prostanoid concentrations in amniotic fluid with gestational age.

Table 1-2

Changes in prostanoid concentrations in amniotic fluids with gestational age in pg/ml ± SEM (early gestation is 16-20 weeks)*

Prostanoid	Early gestation	Term
PGE	45 ± 9.5	802 ± 77
PGF	44 ± 11	442 ± 102

*From Rehnstrom *et al.* 1983

The ratio of PGE_2 and $PGF_{2\alpha}$ in amniotic fluid changes with labour, such that $PGF_{2\alpha}$ predominates with the progression of labour (Dray and Frydman 1976, Satoh *et al.* 1979). The concentration of unesterified arachidonic acid in amniotic fluid increases 6 to 10-fold during labour (Curbelo *et al.* 1981). Moreover, injection of arachidonate into the amniotic sac initiates parturition (Novy and Liggins 1980).

PG synthesis inhibitors significantly increase the average length of gestation and the incidence of post-maturity in the human (Waltman *et al.* 1972), and sometimes arrest preterm labour (Besinger and Neibyl 1990). This is also true in non-human primates (Novy *et al.* 1974). Stimuli that activate lipase enzymes, such as infusion of hypertonic (Waltman *et al.* 1972) or hypotonic solutions, and mechanical stretching (Grant 1993) result in PG release and augment or induce labour (McColgin *et al.* 1990), and administration of exogenous PGs induce labour (Nimrod *et al.* 1984, Gaucherand *et al.* 1991).

Unlike term labour, the role of PGs in preterm labour does not seem obligatory (Romero *et al.* 1993). Maternal plasma levels of PGFM do not always change. Plasma concentrations of PGFM were significantly lower in preterm labour than early term labour (Mitchell *et al.* 1988, Sellers *et al.* 1981). Furthermore, in women with preterm labour without infection, the amniotic fluid concentration of PGs and their metabolites were only modestly elevated and were not significantly different from

non-labouring women of the same gestational age (Romero *et al.* 1987). However, the amniotic fluid levels of PGs are significantly increased in cases of preterm labour associated with intrauterine infection (Mazor *et al.* 1990). Amniotic fluid median and range of prostanoid accumulations in preterm labour with and without infection are presented in table 1-3. Myometrial exposure to high concentration of PGs, either produced *de-novo* by the tissue or administered, do induce preterm labour.

Table 1-3

Changes in prostanoid concentrations in amniotic fluid in preterm labour with and without infection (pg/ml ± SEM)*

Prostanoid	PTL without infection	PTL with infection
PGE_2		
Median	272	900
Range	175-700	200-2750
$PGF_{2\alpha}$		
Median	72	1475
Range	27-370	80-6525

*Modified from Mazor *et al.* 1990

Although PGs may not change in preterm labour, there could be a reduced 15-hydroxyprostaglandin dehydrogenase (15HPDH) expression in chorionic trophoblasts. PGDH type 1 is the main enzyme responsible for the metabolism of PGE_2 and $PGF_{2\alpha}$.

Immunohistochemical localization of PGDH and its mRNA revealed that it is localized in syncytiotrophoblast and intermediate trophoblasts in placenta and in the trophoblast layer of chorion laeve. Thus a relative deficiency would allow PGs synthesized in the amnion or chorion to escape metabolism in the chorion, thus contributing to preterm labour (Sangha *et al.*, 1994).

1.06 Fetal Membranes, Decidua and Labour

The importance of the fetal membranes in the initiation of human parturition through PG release was first brought forward by MacDonald *et al.* in 1974. Some features of these tissues that render them suitable for their proposed role in the initiation of parturition are:

1. The fetal membranes are rich in arachidonic acid, the obligatory precursor of prostaglandins E_2 and $F_{2\alpha}$ (Van Dorp *et al.* 1964, Bergstrom *et al.* 1964, Okazaki *et al.* 1981 *b*, and Johnston and Howie 1985).

2. The fetal membranes contain lipase enzymes that release free arachidonic acid from phosphatidylinositol and phosphatidylethanolamine (Bleasdale and Johnston 1985).

3. During labour, there is a substantial increase in the concentration of PGE_2, $PGF_{2\alpha}$ and PGFM in amniotic fluid (Neider and Augustin 1983, Keirse 1990). The concentrations of these prostaglandins increase as labour progresses (Dray and Frydman 1976).

4. Manipulation (such as stretching, stripping (McColgin *et al.* 1990) or rupturing (Fraser *et al.* 1991, Grant 1993), or insertion of Foley's catheter, instigate labour (George *et al.* 1993). In fact, mechanical stretching has been shown to increase PGE_2 formation in cultured human amnion cells (Kanayama and Fukamizu 1989).

5. Infection of the fetal membranes leads to increased PGs production and labour (Allot and Palmer 1993).

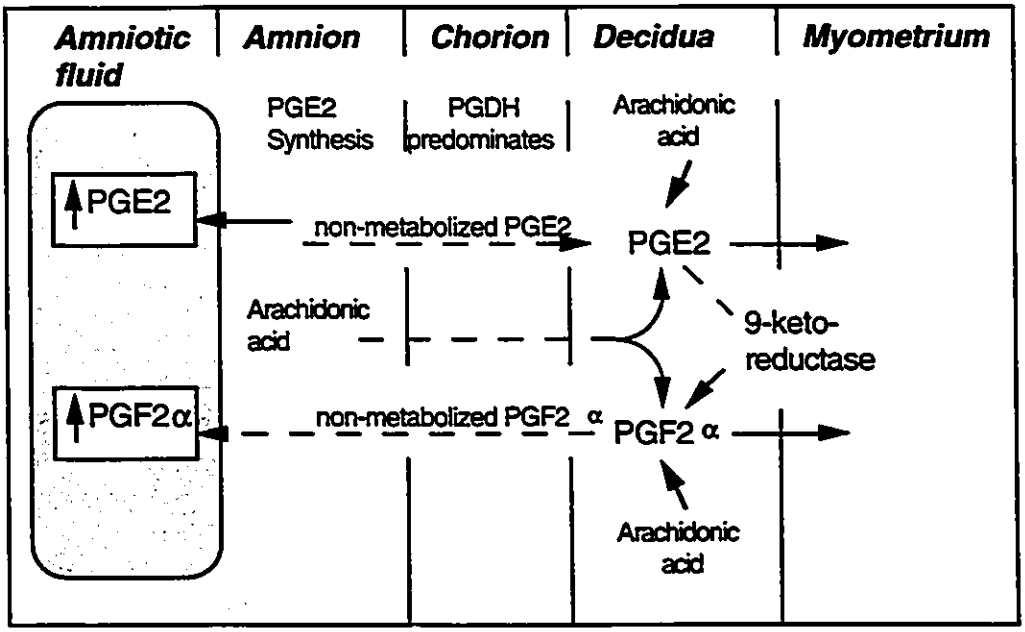


Fig. 1-9

PG production by the fetal membranes and decidua

Fig. 1-9 illustrates the major findings concerning the formation and metabolism of PGE_2 and $PGF_{2\alpha}$ by fetal membranes and decidua. The amnion produces mainly PGE_2 (Challis and Olson 1988). The chorion laeve, lying between the amnion and decidua, produces mainly PGE_2, PGEM and $PGF_{2\alpha}$ (Mitchell 1991 *a*). The chorion possesses high PG dehydrogenase activity (PGDH), such as 15-hydroxyprostaglandin dehydrogenase (15HPDH) (Casey *et al.* 1980) and prostaglandin-9-ketoreductase activity (Okazaki *et al.* 1981 *a*). 15-hydroxyprostaglandin dehydrogenase activity is 2-3 times more potent than prostaglandin-9-ketoreductase activity. The 15-HPDH metabolizes PGE_2 and $PGF_{2\alpha}$ (Okazaki *et al.* 1981 *a*) and is 2-3 fold more active with PGE_2 as substrate than $PGF_{2\alpha}$. This activity is increased significantly around the time of labour (Mitchell *et al.* 1993 *a*).

There is controversy around the importance of PGE_2 production by amnion in human labour. Bennett *et al.* (1990) concluded that PGE_2 synthesized in amnion can cross the membranes by diffusion through the extracellular space to reach decidua and myometrium. However, another *in-vitro* study on PGE_2 transport through fetal membranes using tritiated PGE_2 showed that the PGE_2 increase in amnion after labour does not increase PGE_2 concentration on the myometrial side (McCoshen *et al.* 1990, Roseblade *et al.* 1990). Moreover, while some authors described the amnion as the major source of PGE_2 in labour (Mitchell *et al.* 1991 *b*), another study showed that the amnion

has little contribution to the overall PGE_2 production by fetal membranes (Khan *et al.* 1991).

The decidua produces $PGF_{2\alpha}$ together with PGE_2 (Casey *et al.* 1989, Norwitz *et al.* 1991). $PGF_{2\alpha}$ can be formed in this tissue *de-novo* from arachidonic acid or from PGE_2 via the prostaglandin-9-ketoreductase (Nakla *et al.* 1986). Quantitatively, the decidua contributes more PGE_2 than the amnion (Khan *et al.* 1991). Production of PGE_2 and $PGF_{2\alpha}$ is higher after labour than prior to the onset of labour (Korte *et al.* 1993).

1.07 Regulation of Prostaglandin Formation

During pregnancy, both stimulators and inhibitors of phospholipases and prostaglandin synthetase activities are present in the amniotic fluid and intrauterine tissues (Matsui *et al.* 1989, Mitchell *et al.* 1991 *a*, Newman *et al.* 1991, Myatt *et al.* 1992 and Collins *et al.* 1992). Factors present in amnion have inhibitory effects on endoperoxide synthesis (Matsuda *et al.* 1989). The inhibitory activity decreases with gestation, and this may trigger arachidonic acid release (Mortimer *et al.* 1985, Saeed *et al.* 1982, Wilson *et al.* 1989). Term but not mid-term amniotic fluid stimulates PG production by tissue slices of human myometrium and decidua (Rehnstrom *et al.* 1983). Calcium-binding proteins such as annexins (lipocortins) are produced by placenta and membranes, which may inhibit PG

formation (Wallner *et al.* 1986). However, annexin effects in intrauterine tissue are controversial since an increase in the annexin content of cultured cells is concomitant with stimulation of PG formation (Mitchell *et al.* 1988).

Chorion laeve produces a substance which stimulates PGE_2 output by amnion in term tissues (Lundin-Schiller and Mitchell 1990). The activity of gravidin, an inhibitor of arachidonic acid release, decreases after the onset of labour (Wilson *et al.* 1989). Gravidin is a 68 kilodalton protein which is secreted into the amniotic fluid by the chorion.

Over and above the various inhibitors and stimulators of prostaglandin formation produced *in-situ* by the fetal membranes and decidua, there have been numerous studies on the regulation of prostaglandin formation in these tissues *in-vitro* using cultured cells derived from the amnion, chorion laeve, and decidua. Such approaches are necessary because *in-vivo* studies in human pregnancy are intuitively limited.

Many substances have been found to alter PG formation in these tissues, including; epidermal growth factor, oxytocin, interleukins and glucocorticoids. The reader is referred to a number of excellent reviews which cover this subject (MacDonald *et al.* 1974 & 1978, Lopez-Bernal *et al.* 1993, Schwartz *et al.* 1975, Mitchell 1984 & 1991 *a and b*, Olson *et al.* 1991, Romero *et al.* 1988 *a*, Mitchell and Trautman 1993).

Interleukins are of particular relevance to the present studies, and they will be discussed in the following section. Also, glucocorticoids were found to inhibit PG production in dispersed cells of amnion and chorion laeve (Gibb and Lavoie 1990) but stimulate PG production by confluent cultures of amnion and chorion laeve cells (Potestio *et al.* 1988, Mitchell *et al.* 1988). These studies indicated that the model being studied could markedly affect the response, and thus the conclusions reached. This observation resulted in focusing our studies on interleukin-1 effects on freshly isolated cells from the fetal membranes and decidua, because most previous studies and conclusions were drawn from confluent preparations of these tissues.

1.08 Interleukin-1 (IL-1) and parturition

Interleukin-1 (IL-1) is an immuno-regulatory polypeptide that is produced by the decidua (Romero *et al.* 1989 *c*), the syncytiotrophoblast and the Hofbauer cells (Taniguchi *et al.* 1991). The importance of IL-1 in labour was suggested by observing higher levels of IL-1 in the amniotic fluid in infection associated preterm labour (Romero *et al.* 1988 *a* and 1993, Bry and Hallman 1989, Cox *et al.* 1993). These high levels of IL-1 were associated with increased accumulation of PGE_2 and $PGF_{2\alpha}$.

1.08.1 IL-1 and IL-1 receptor (IL-1R)

The term interleukin 1 (IL-1) describes a family of at least two proteins, termed alpha (α) and beta (β), which both have a similar molecular weight of 17.5 KD, but different isoelectric points (IL-1α = 5.0 & IL-1β = 7.0) (Furutani *et al.* 1985). These two interleukins are produced by two separate genes, but with similar organization of structure (Dinarello 1987 *a and b*). However, although IL-1 has diverse activities *in-vivo* and *in-vitro* (Table 1-4), both IL-1α and IL-1β have almost identical biological action. IL-1 serves as a key mediator of immunological and pathological responses to stress, infection, and antigenic challenge (Oppenheim *et al.* 1986, Mizel 1989). IL-1 is synthesized by a variety of cells, of which the most thoroughly studied are macrophage/ monocyte cells (Dinarello 1984 *a and b*).

IL-1α and β share a wide array of activities (Dinarello 1988 *a* and *b*), and exert their biological activities through interaction with specific receptors on the surface of cells (IL-1R). Two types of receptors exist; they are referred to as type 1 and type 2 (Dinarello *et al.* 1989). The IL-1R are specific in that they do not recognize other cytokines and although IL-1α and IL-1β are poorly homologous in their primary sequences (26% amino acid homology), the two IL-1 isoforms have been shown to bind to the same receptors on a variety of cell types including T and B cells (Dower and Urdal 1987), and fibroblasts (Chin *et al.* 1987).

Table 1-4
The systemic effects of IL-1*

Central nervous system	Fever
	Increase ACTH
	Increased corticosteroid
	Increase sleep
	Decrease appetite
Metabolic	Increase hepatic proteins
	Increase sodium excretion
	Decrease albumin synthesis
	Decreased plasma Zn/ Fe
	Increase or decrease Insulin
	Decrease lipoproteinlipase
	Decrease cytochrome P450
Hematologic	Neutrophilia
	Lymphopenia
	Increase neutrophil TxA_2
	Increase tumor killing
	Increase marrow precursors
Vascular wall	Increase leukocyte adherence
	Increase PGI_2/ PGE_2
	Increase platelets activating factor
	Increase procoagulant
	Decrease plasminogen activator
Hemodynamic	Hypotension
	Decrease systemic resistance
	Decrease central venous pressure
	Increase cardiac output
	Increase heart rate
	Decrease blood pH
Reproductive system	Increase DNA synthesis in spermatogonia
	Inhibit sex steroid production
	? Stimulate labor

*Dinarello 1988 *b*

IL-1R concentration varies with the cell preparations. Cell lines express unusually high numbers of receptors (up to 20,000 per cell) (Lowenthal and MacDonald 1986, Savage *et al.* 1989), whereas non-transformed cells taken from fresh tissue or

circulating blood leukocytes express few receptors (100 to 200 per cell) (Dinarello 1991).

1.08.2 IL-1 role in term labour

A small amount of IL-1 is present in the amniotic fluid of late pregnancy and it is predominantly of IL-1α type (Romero *et al.* 1989 *a*, Tsunoda *et al.* 1991). The physiological activity of IL-1α is cell associated, i.e., the cell-free amniotic liquor possesses non-detectable IL-1 activity. The origin of these cells is mainly from desquamated fetal keratinocytes, which are abundant late in pregnancy but are rare in the second trimester.

During term labour there is a remarkable increase in the concentration of interleukin-1, particularly IL-1β, in amniotic fluid (Cox *et al.* 1988, Romero *et al.* 1990 *a*, Tsunoda *et al.* 1991). The IL-1 content of amniotic fluid has a pattern similar to that of IL-1 formation by macrophages, where IL-1β production greatly exceeds that of IL-1α. Amniotic fluid IL-1β may arise, at least in part, from the macrophage-like decidua (Romero *et al.* 1989 *a*). However, this increase is modest in comparison to that observed in amniotic fluid in cases of intra-amniotic infection (Romero *et al.* 1989 *a*).

Around 10% of tissues obtained at term have histological evidence of inflammation of the chorioamniotic membranes (Guzick and Winn 1985). Thus, an inflammatory process or sub

clinical infection may be one of the mechanisms that triggers spontaneous human parturition at term, at least in a small subset of women. In this subtle intrauterine inflammatory reaction, elevated levels of cytokines such as IL-1 and IL-6 in amniotic fluid correlated well with inflammatory cell infiltrate in fetal membranes, decidua and umbilical cord (Ovigstad *et al.* 1994). IL-1 has been demonstrated to be produced by human decidua in response to bacterial endotoxin (Romero *et al.* 1989 *c*) and could therefore be acting in an autocrine fashion on decidua, and by a paracrine mechanism in the fetal membranes and myometrium (Casey and MacDonald 1988 *b and c*, Cox *et al.* 1988, Word *et al.* 1989). It has also been shown that systemic administration of IL-1 to pregnant animals causes abortion (Romero *et al.* 1991 *a*).

Cytokine secretion from decidua cells has been noted, and interleukin-1 was found to increase PGE_2 and endothelin 1 and 2 secretion from confluent amnion (Romero *et al.* 1989 *b*, Mitchell *et al.* 1991 *a and b*). IL-1 also increased PGE_2 output from cultured chorion cells (Lundin-Shiller and Mitchell 1991 *a*). However, MacDonald *et al.* (1991) suggested that the increase in cytokines and prostaglandins is merely due to influx of mononuclear phagocytes into the forebag of amniotic fluid after the cervix is dilated and the fetal membranes and decidua are exposed to the vaginal secretions, which are mainly bacterial endotoxins and IL-1β. This is how the PGEM and $PGF_{2\alpha}$ come into the amniotic fluid, as the membranes are unable to

manufacture these two PGs. This is also the reason why IL-1β is elevated. Therefore, the accumulation of cytokine and PGs is probably an accompaniment of parturition and not its cause (MacDonald and Casey 1993).

Interleukin-1 receptor antagonist protein (IRAP) opposes the effect of IL-1β in many systems (Baergen *et al.* 1994), and it has been suggested that IRAP could be used to prevent preterm labour. There is more expression of IRAP than IL-1 in normal intrauterine tissue (Baergen *et al.* 1994). The colocalization of IRAP and IL-1 in intrauterine tissues may represent a balance between proinflammatory and anti-inflammatory mediators in normal pregnancy, which possibly changes during labour or inflammation. However, in low doses (<1000 pg/ml), IRAP possesses an action similar to that of IL-1β causing a concentration related increase in PG production by human decidua cells (Mitchell *et al.* 1993 *b*), and it increases the effect of IL-1β (Cole *et al.* 1993). Only very high doses of IRAP (>1000 pg/ml) were found to have limited inhibitory effect on IL-1β action (Cole *et al.* 1993).

1.08.3 IL-1 role in infection-associated preterm labour

One of the pivotal events in infection is the initiation of inflammatory responses which cause the activation of resident tissue macrophages at the site of damage and the infiltration of circulating monocytes. The macrophage activation and the local tissue irritation cause the release of a group of factors and hormones which influence inflammation and regulation of tissue dynamics. These macrophage-derived hormones (or cytokines) such as interleukin-1 (IL-1), interleukin-6 (IL-6), and platelets activating factor (PAF) act on target cells via plasma membrane receptor proteins.

This host response to infection may be the trigger for the initiation of labour. It has been proposed that the secreted cytokine stimulates PG production by intrauterine tissues, which results in uterine contraction (see Fig. 1-10). Large amounts of IL-1 and IL-6, and PAF are found in the amniotic fluid of women in preterm labour with clinical and laboratory evidence of intrauterine infection (Romero *et al.* 1988 *a and b*, Casey *et al.* 1990), and amniotic fluid concentrations of PGE_2 and $PGF_{2\alpha}$ were significantly higher in these cases (Mazor *et al.* 1990). Table 1-5 presents the concentrations of IL-1 in amniotic fluid in infection associated preterm labour. The IL-1 rise is associated with an increase in the concentrations of PGE_2 and $PGF_{2\alpha}$ in amniotic fluid of patients having preterm labour

with intra-amniotic infection (Romero *et al.* 1988 *a* and 1989 *a*, Mitchell *et al.* 1991 *a*). IL-1 has been found to stimulate PG production by amnion, chorion laeve, and decidua cells in monolayer cell culture (Romero *et al.* 1989 *b*, Mitchell *et al.* 1990). This has led to the assumption that cytokines may play a role in the initiation of labour via stimulation of PG production by intrauterine tissues.

Table 1-5
Mean of interleukin-1 concentrations in term and preterm labour (units/ml)*

PTL no infection	PTL with infection	Term labour	Term no infection
5	367	4	<1

Limits of detection = 1 unit.

PTL refers to preterm labour.

*Adopted from Romero *et al.* 1989 *a*.

IL-1β was found to increase endothelin 1 and 2 production in cultured amnion cells (Mitchell *et al.* 1991 *c*). IL-1β was also found to significantly increase 5-hydroxyeicosatetraenoic acid production in primary cell cultures of amnion, chorion laeve and decidua vera (Edwin *et al.* 1993). However the accumulation of leukotrienes B4 and C4, and 12 and 15-hydroxyeicosatetraenoic acid were not changed by treatment with IL-1β (Edwin *et al.* 1993). Using intact tissues, Kent *et al.* in 1993 showed that IL-1β is a potent stimulator of PG synthesis by decidua and by amnion, whereas, IL-1α only stimulates the amnion.

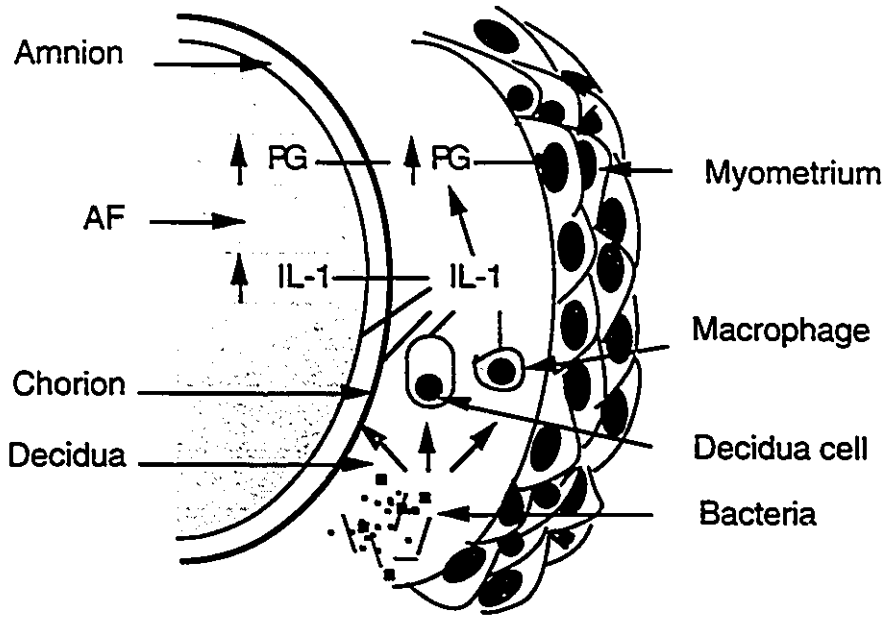


Fig. 1-10
Possible role of IL-1 in the initiation of preterm labour in cases of intra-uterine infection

1.09 Thesis Rationale

Prostaglandin (PG) formation by the fetal membranes (amnion and chorion laeve) and decidua vera is thought to be intimately involved in the onset and progression of human parturition at term and in infection-induced preterm labour. Reports on confluent cell preparation indicated stimulation of PG production by IL-1. However, the type of *in-vitro* preparation influences the response. Dexamethasone inhibits PGs in freshly dispersed cells, whereas it stimulates PGs in confluent

preparations of the same tissue. Therefore this study set out to examine the effect of interleukins on regulation of PG formation in different *in-vitro* preparations of these tissues.

1.09.1 Specific aims

The purpose of this thesis was:

1. To study the effect of IL-1 on prostaglandin production by fetal membranes and decidua.
2. To examine the three intrauterine tissues; amnion, chorion laeve and decidua vera, since most of the previous studies had focused on only one of the tissues.
3. To examine the response of undisrupted tissues and dispersed cell preparations since we had previously found that glucocorticoid action on PG production by freshly dispersed cells and cultured cells was different.
4. To examine the distribution of IL-1 receptors in the human fetal membranes and decidua.

2.0 MATERIALS & METHODS

2.01 Materials

2.01.1 Chemicals

Trypsin inhibitor, DNase 1 (from bovine pancreas, grade II), albumin (from bovine serum), aprotonin Kallikrein inhibitor, and collagenase A lyophilized *(Clostridium histolyticum)* were obtained from Boehringer Mannheim Canada Ltd. (Dorval, Quebec, Canada.). Ham's F12 medium with glutamine and 1.24 L-1 phenol red without sodium bicarbonate, Dulbecco's modified Minimum Essential Medium without sodium bicarbonate (DMEM), RPMI-1640, Trypsin (1:250), (porcine parvovirus tested) penicillin, streptomycin and fungizone were obtained from Gibco Canada (Burlington, Ont., Canada). HEPES, fetal calf serum (FCS), and Jolik's modification of minimum essential medium with L-glutamine, $NaHCO_3$ with penicillin, streptomycin were obtained from Flow Laboratories Inc. (Mississauga, Ont., Canada). L-Isoproterenol hydrochloride, dexamethasone, TPA (12-0-tetra-decanoyl phorbol 13-acetate), penicillin G (Benzylpenicillin) sodium salt, and gentamicin sulfate were obtained from Sigma Chemical Co. (St. Louis, Missouri, U.S.A.). Antisera and standards to PGE and $PGF_{2\alpha}$ were obtained from Advanced Magnetics Inc., (Boston, Massachusettes, U.S.A.), [^{3}H] PGE_2, [^{3}H] $PGF_{2\alpha}$ were obtained from Amersham (Oakville, Ontario, Canada), and ^{125}I-labeled

recombinant human IL-1α (SA,~ 10 μCi/mmol) was obtained from Dupont Canada (Mississauga, Ontario, Canada). Liquid Scintillation cocktail (Ready gel) was obtained from Beckman Canada, (Mississauga, Ont.). A kit to assay 11-deoxy-13, 14-dihydro-15-keto-11β, {16-cyclo-prostaglandin E_2} (PGEM) was obtained from Amersham, (Oakville, Ontario, Canada).

Human recombinant interleukin-6 (IL-6), IL-1α and IL-1β (units per ml), which produced in E. coli and purified by standard chromatographic techniques, were obtained from Boehrienger Mannheim, Canada Ltd (Dorval, Quebec, Canada), and R&D Systems (Montreal, Quebec, Canada). One unit of IL-1α or β is defined as the amount of IL-1α or β that is required to support half maximal [^{3}H]-thymidine incorporation into mouse thymocytes co-stimulated with low amounts of lectin (e.g. PHA). One unit of IL-6 is defined as the amount of IL-6 that is required to mediate half-maximal cell proliferation with 7TDI cells (mouse-mouse hybridoma cells).

Sodium chloride, hydrochloric acid, lithium carbonate, sodium azide, sodium thiosulfate, Eosin Yellowish, buffered neutral formalin 10%, toluene, methanol and isopentane sodium thiosulphate were obtained from BDH Inc. (Toronto, Ontario, Canada). Potassium phosphate (KH_2PO_4), sodium bicarbonate ($NaHCO_3$) and bacitracin were obtained from Aldrich Chemical Companies. Triton X-100 non-toxic surfactant was obtained from Sigma Chem. Co. (St. Louis, Mo.). Harrise modified hematoxylin with acetic acid (mercury free), Permount, ammonium hydroxide, and trypan blue were obtained from Fisher Diagnostics

Canada (Ottawa, Ontario, Canada). Kodak NTB2 autoradiography emulsion, Kodak D19 developer and Kodak OXMAT AR film were obtained from Intersciences (Markham, Ont., Canada). OCT compound-embedding medium for frozen specimens was obtained from Miles, (Ontario, Canada). Poly-L-lysine MW 80,000- 100,000 daltons was obtained from Sigma Chem. Co. (St. Louis, Mo.). Drierite $CaSO_4$ was obtained from Hammond Drierite Co. (Xenia, Ohio, USA). Liquid nitrogen was obtained from Linde Union Carbide, (Ontario, Canada). Polystyrene 24 well plastic culture dishes (Linbro) were obtained from Flow Laboratories (Mississauga, Ontario, Canada) and Corning Can Lab (Montreal, Quebec, Canada). Staining dishes, 50 ml tubes and slide boxes were obtained from Canlab (Montreal, Quebec, Canada). Superfrost Plus microscope slides, premium microscope slides, light safe black autoradiography slide boxes were obtained from Fisher scientific (Ottawa, Ontario, Canada). 100 and 35 µm nylon mesh were obtained from B.S.H. Thompson and Co. (Montreal, Quebec). Number 1 filter paper was obtained from Whatman Glaxo Labs (Toronto, Ontario, Canada). All other reagents used were of American Chemical Society specifications or the best commercial material available.

2.01.2 Equipment

All sterile procedures were carried out inside a laminar flow hood obtained from Forma Scientific Inc, (Marietta, Ohio, U.S.A.). It had a downflow velocity of 75-85 cfm, and face velocity of 106-116 cfm. Radioactivity measurements were carried out with a Packard liquid

scintillation counter model 2200CA Tri-Carb with tritium counting efficiency of approximately 60%, obtained from Canberra Packard, (Canada). Versa-bath S Model 224 Water bath was obtained from Allied Fisher Scientific, (Ottawa, Canada). The IEC Centra-8R Centrifuge, and the Accumet pH meter 910 were obtained from Fisher Scientific (Ottawa, Canada). Spectrophotometric measurements were carried out on a Du-7 spectrophotometer, supplied by Beckman, (Mississauga, Ontario, Canada). The Microm Heidelberg HM 500 M/OM cryostat and the stainless steel C profile cutting knife were obtained from Zeiss Canada, (Ontario, Canada). The Zeiss Photomicroscope Axiophot (Germany) and Olympus Type S (Japan) were obtained from Carsen Medical and Scientific Co, Ltd., (Markham, Ontario, Canada). A Kodak RP X-OMAT Processor, supplied by Kodak (Canada), was used to develop X-ray films.

2.01.3 Buffers and Culture Media

2.01.3a Buffers

- **Buffers For Tissue Preparation and RIA**

Phosphate buffered saline (PBS) was composed of potassium chloride 2.7 mM, potassium phosphate (KH_2PO_4) 1.5 mM, sodium chloride 137.2 mM, disodium phosphate (Na_2HPO_4) 8.2 mM, gentamicin sulfate (16 mg/l) and penicillin (10^{-5} U/l) adjusted to pH 7.4.

- **Buffers for Receptor Labeling and Binding Assay**

Tissue preparation buffer was made of: RPMI-1640; 50 µL/ml gentamicin, 20 mM HEPES, 1 mg/ml sodium azide, aprotonin (100 Kallikrein inhibitor), and 10^{-4} M bacitracin, pH 7.4. Tissue incubation buffer consisted of tissue preparation buffer with 0.15% of BSA.

2.01.3b Culture Media

This consisted of Ham's F12:Dulbecco's modified Minimum Essential Medium (1:1,v/v), containing sodium bicarbonate (2.4g/l), bovine serum albumin (1g/l), HEPES (45 mM) at pH 7.4, gentamicin sulfate (16 mg/l) and penicillin (10^{-5} U/l). Media was supplemented with 10% fetal calf serum (FCS).

Jolik's modification of minimum essential medium with L-glutamine, $NaHCO_3$ with penicillin and streptomycin (1:1,v/v) was used for cell dispersion with trypsin and cell culture.

2.01.4 Radioimmunoassay Solution

- ^3H PGE_2 tracer (at a concentration of 10,000 cpm/50µl).

- Bovine Gamma Globulin phosphate buffer (BGG phosphate buffer) consisted of 0.01M monosodium/ disodium phosphate pH 7.0 containing 0.01%(w/v) bovine gamma globulin and 0.01% (w/v) sodium azide or thimerasol).

• Dextran-coated charcoal (1% (w/v) consisted of activated charcoal added to 1% (w/v) dextran (T70) in H_2O).

2.01.5 Gelatin Solution for Coating Slides

Gelatin 5.0 g was dissolved in warm distilled water (temperature ~ 60°C), then 0.5 g chrome allum (Chromium potassium sulfate) was added, and one or two crystals of Thymol were added to delay bacterial growth.

2.01.6 Hematoxylin and Eosin Solution

• Acid alcohol was made by adding 5 drops of concentrated HCl into around 250 ml of 70% ethanol. This solution was used to differentiate the hematoxylin stain. It causes color change from blue to reddish.

• Ammonia water consisted of 2 ml of concentrated ammonium hydroxide in 1000 ml of distilled water. This solution was used to cause bluing of the sections.

• Eosin-phloxine mix (working solution) was made by mixing the following: 100 ml eosin stock solution (1 g water soluble geosin Y in 100 ml distilled water), plus 10 ml stock phloxine (phloxine B 1 g in 100 ml distilled water), plus 780 ml alcohol (95%), plus 4.0 ml glacial acetic acid.

2.02 Methods

2.02.1 Tissue Preparation

Human placentae were obtained at term (38 to 42 weeks) either after labour from spontaneous deliveries, or before the onset of labour by elective cesarean section. The placentae were obtained within thirty minutes of delivery. All manipulations were carried out under sterile conditions.

2.02.1a Tissue Preparation for PG Study

- **Undisrupted tissue culture for PG**

Tissue separation was carried out as described by Gibb *et al.* (1988). Under completely aseptic conditions, the amnion was peeled away, as shown in Fig 2-1 , and cut 2 inches above the placental disc. The chorion/decidua tissue was cut around two inches above placental plate, and placed on the cutting board. Decidua was carefully scraped from the chorion laeve by the sharp end of scalpel, as shown in Fig 2-2 .

Fig. 2-1
The method of peeling the amnion away from the chorion/decidua

Fig. 2-2

The method of scraping the decidua off the chorion

Top photo.: The knife is pointing at the chorion

Bottom photo.: The knife is pointing at the decidua

Each tissue was processed separately. Large blood clots were removed and the tissues washed several times in phosphate buffered saline (PBS). Individual tissues were then spread over the cutting board. Discs of tissues were obtained using a cork borer number 9 as illustrated in Fig. 2-3 .

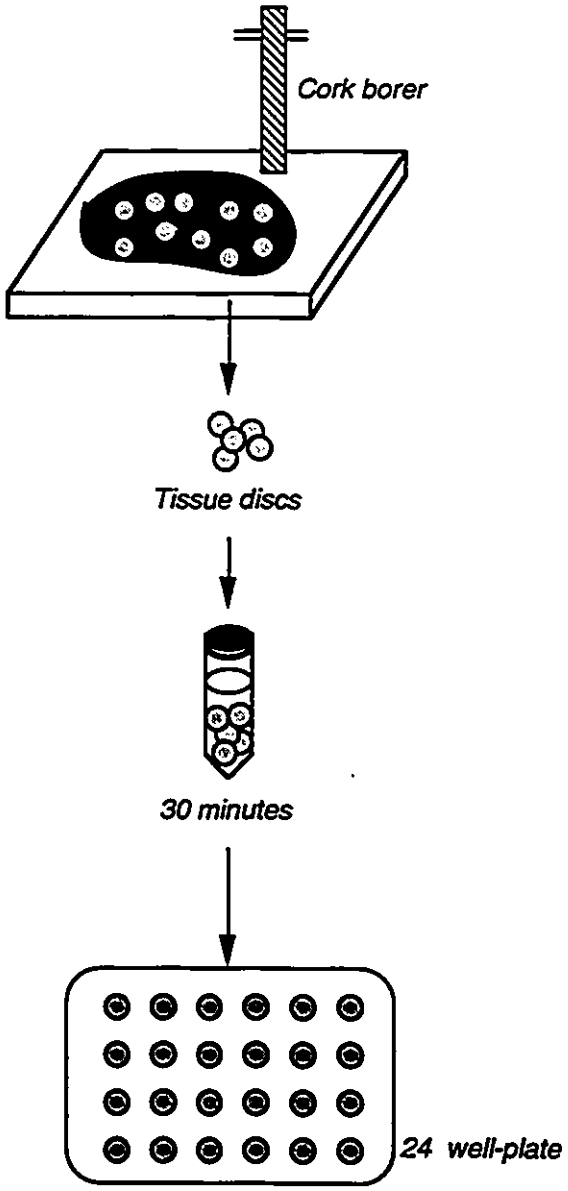


Fig. 2-3
Undisrupted tissue sampling (tissue discs) using the cork borer

The discs were then allowed to rest in F12: DMEM containing 10% Fetal Calf serum at pH 7.4 for 30 minutes. This rest period may help to wash out the potential excess PG produced by the manipulation of the tissue. Each disc of tissue was then placed in one $2cm^2$ well of a 24 -well dish and incubated with various concentrations of IL-1. The discs were maintained in culture at 37°C with H_2O-saturated atmosphere of 5% CO_2 and air.

Recombinant human IL-1α was added either immediately for the first 24 hours of culture or during the second 24 hours of culture after changing the media, in final concentrations of 1, 10, 100 and 1000 units/ml. In all experiments, each concentration was done in quadruplicate, which means four wells were cultured per treatment. Samples of media were taken after the first 24 hours of incubation and stored at -80°C until assayed by radioimmunoassay (RIA). Excess fluid was removed from the tissue discs with filter paper. The tissue discs were weighed and stored at -80°C.

2.02.2 Isolation of cells and culture

Cell dispersion and culture was carried out as follows: The tissues were separated into amnion, chorion and decidua. Each tissue was separately incubated with collagenase. The dispersed cells were washed, centrifuged, counted and plated.

2.02.2a Cell dispersion with Collagenase

Tissue dispersion was carried out as described by Gibb *et al.* (1986). Fig. 2-4a illustrates the steps for this procedure.

Tissue separation was carried out as described in section 2.02.1a . The amnion, chorion and decidua tissues were processed individually. Large blood clots were removed, then the tissue was finely minced with scissors, and washed few times with PBS with antibiotics. The minced tissues were then suspended in 30 ml of F12DME with antibiotics containing collagenase (1.4 mg/ml) and trypsin inhibitor (3 µg/l). The digestion was allowed to proceed in a water bath at 37°C with shaking at 80 - 100 rpm for 2-3 hours. The mixture was filtered through nylon mesh of 100 and 35 µm and the cells were pelted by centrifugation at 800 x g for 20 minutes. The pelted cells were then washed by resuspension in F12:DMEM and centrifuged at 800 x g for 10 minutes. The washed cells were resuspended in a small volume of F12:DMEM and counted by using a hemacytometer (Fig. 2-4b). Cell viability was checked by Trypan Blue exclusion.

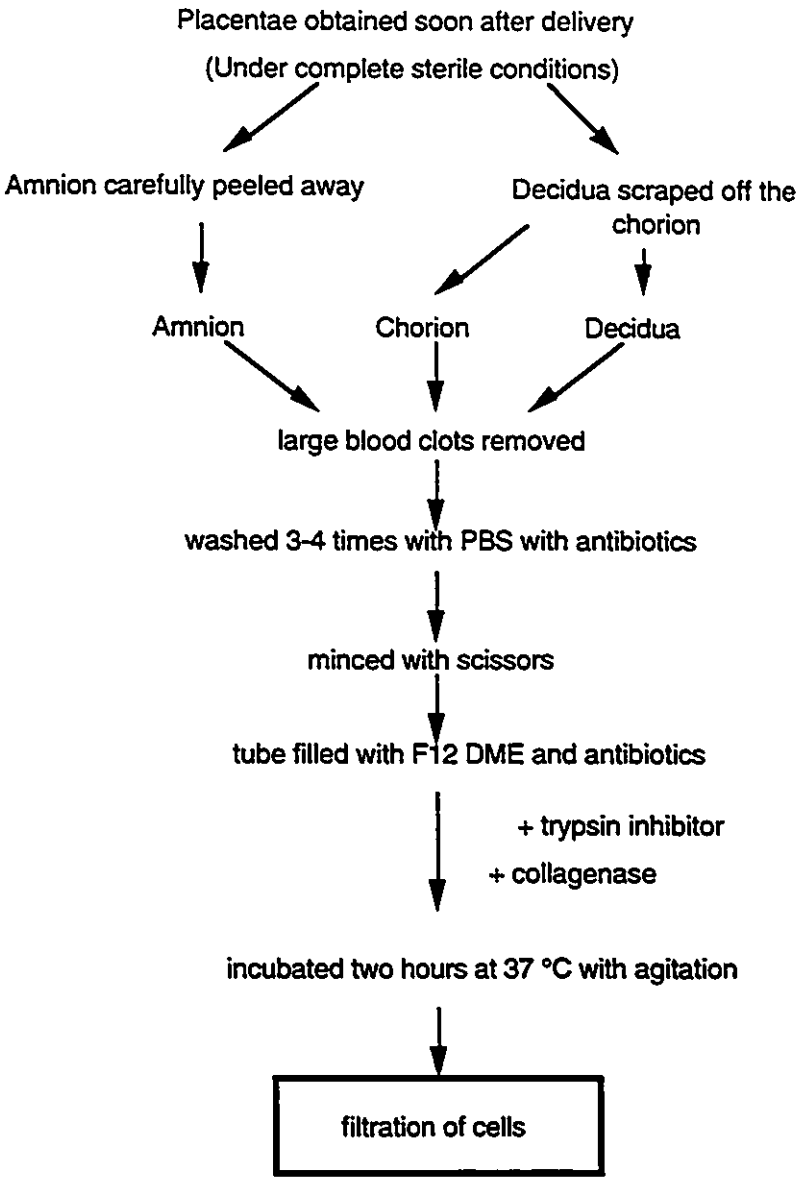


Fig. 2-4a
Dispersed cell preparation
(From tissue separation to cell filtration)

Fig. 2-4b illustrates the steps for cell preparation from cell filtration to cell plating.

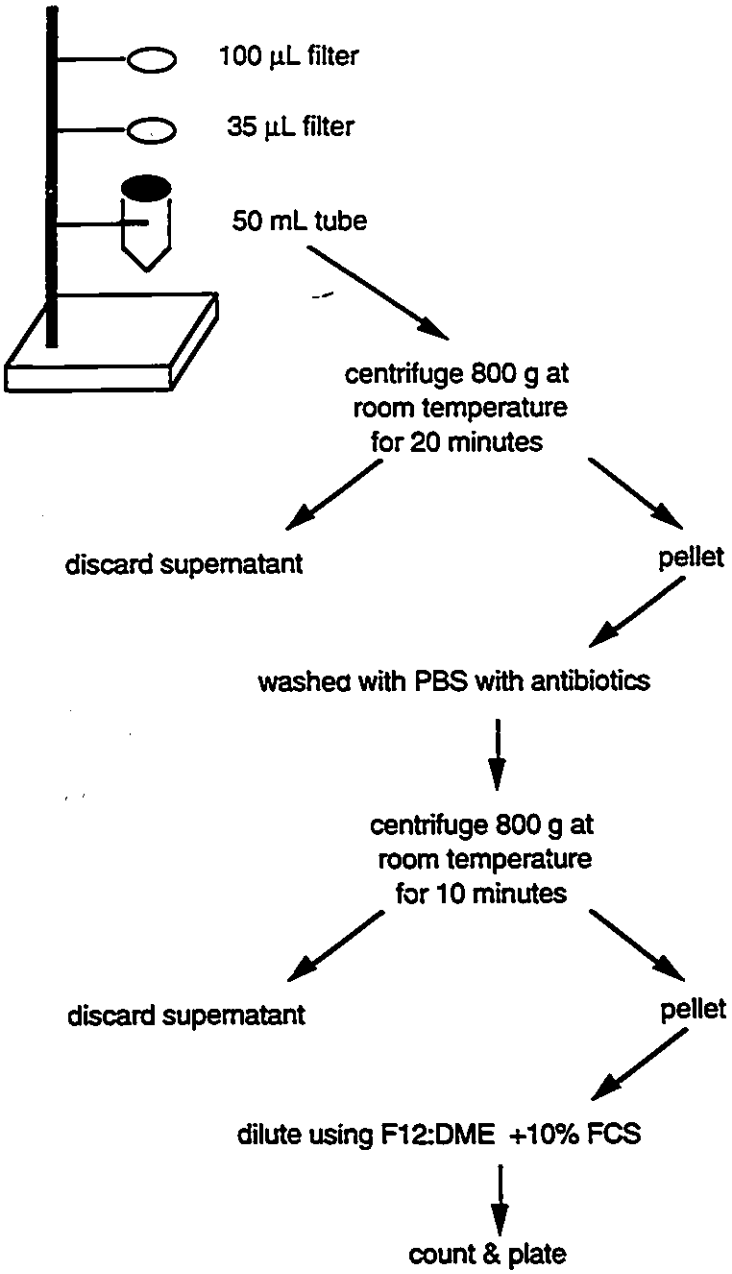


Fig. 2-4b

Dispersed cell preparation

From cell filtration to cell plating. FCS = fetal calf serum

Three hundred thousand cells (3 x 10^5) were plated in $2cm^2$ wells (24 wells/dish). The cells were maintained in culture at 37°C with H_2O-saturated atmosphere of 5% CO_2 and air.

2.02.2b Cell dispersion with Trypsin

Three of the amnion tissues were dispersed with trypsin; Jolik's media was used instead of F12:DMEM. Four grams of minced tissue was added to 80 ml of Jolik's media. This was then mixed with 400 mg of trypsin and 4 mg of DNase. After rapid shaking in a water bath at 37°C for 40 minutes, trypsin inhibitor (25 mg/ml) was added to the mixture. Cells were collected by filtration and centrifugation, and then washed, counted and plated as described in section 2.02.2a .

2.02.3 Cell incubation with interleukins

The culture in each experiment was divided into two time periods; first 24 hours, and second 24 hours. For the first 24 hour period, human recombinant IL-1α, IL-1β, or IL-6 was added to the culture media (F12:DMEM with 10% FCS) immediately in final concentrations of 1, 10, 100 and 1000 units/ml (1 unit is defined as the amount of IL-1 required to double the proliferative response of mouse thymocytes stimulated with 1 μg/ml of phytohemagglutinin alone). The cell free media were obtained after 24 hours and frozen at -80°C.

For the second 24 hours, the cells were cultured in F12:DMEM supplemented with 10% FCS for 24 hours, then the media was

replaced with media containing various concentrations of human recombinant IL-1α or IL-1β. The cell-free supernatants were obtained after 24 hours of incubation, and frozen at -80°C until prostaglandins were assayed by RIA.

With individual tissues, quadruplicate wells were run for every treatment and a minimum of 3 separate tissues were studied for each interleukin.

2.02.4 Measurement of PGE_2 and $PGF_{2\alpha}$

PGE_2 and $PGF_{2\alpha}$ were measured by radioimmunoassays. PGE_2 is the main prostaglandin produced by the amnion and chorion laeve, whereas the decidua produces $PGF_{2\alpha}$ and PGE_2. The methodology employed was that used previously (Gibb and Breton 1993), and was carried out as described by the manufacturer. Radioimmunoassay is used to detect and measure the concentration of a certain biological substance in media. This procedure is highly sensitive and specific, and the results can be obtained within a few hours. The principle of radioimmunoassay is based upon competition of analyte in a test sample with radioactively labeled analyte for a limited number of sites on a specific antibody.

Briefly, ^3H prostaglandin was incubated with culture media and antibody. The incubation was allowed to proceed for 2 hours for PGE_2 and overnight for $PGF_{2\alpha}$ at room temperature. Antibody bound complexes were separated from free PG using dextran-coated

charcoal and the mixture centrifuged. The antibody-bound PG was quantified by liquid scintillation counting. A standard curve was constructed at the same time, using known concentrations of prostaglandin.

The limit of detection of PGE_2 and $PGF_{2\alpha}$ was less than 1 pg/ml, and intra-assay coefficients of variation were 4.5% for PGE_2 and 3.7% for $PGF_{2\alpha}$. PGEM metabolite was measured in media from the chorion laeve following conversion to 11-deoxy-13, 14-dihydro-15-keto-11β, 16-cyclo-prostaglandin E_2 using a kit purchased from Amersham. Prostaglandin output was expressed relative to control cultures, which were taken as 100%. Data in which comparisons were made were assayed at the same time. Cross reactivity of PGE_2 antisera with PGE_2 = 100%; PGE_1 = 26%; other prostaglandins and their metabolites or fatty acids was less than 2.0%, and $PGF_{2\alpha}$ is 15% mainly with $PGF_{1\alpha}$.

Radioactivity measurements were carried out with a β counter and the efficiency for tritium was about 60%. Beckman Ready-Gel MP (10 ml) was used as the scintillation liquid.

A summary of the RIA method is shown in Table 2-1.

Table 2-1
RIA method

Tubes#	*Type*	*Buffer (µL)*	*Standard (µL)*	*sample (µL)*	*Tracer (µL)*	*Antiserum (µL)*	*Mag DCC* (µL)*	*Total (µL)*
1 & 2	Total	350	0	0	50	0	0	400
3 & 4	NSB	50	50	0	50	0	250	400
5 & 6	Bo	0	50	0	50	50	250	400
7-20	Stnds	0	50	0	50	50	250	400
21-n	Smpls	0	0	50	50	50	250	400

* Mag DCC = Magnatic dextran coated charcoal.
Each tube was incubated for 2 hours at room tempeature (25°C) or 16-24 hours at 4°C the centrifuged for 15 minutes at 4°C at 1000 X g.

2.02.5 Tissue preparation for IL-1 receptor labeling

Interleukin receptors were studied using published methods for other systems (Takao *et al.* 1990 *a and b*). Both undisrupted tissue and dispersed cells were examined.

2.02.5a Preparation of the fetal membranes and decidua for frozen sections

The undissected fetal membranes and the dissected tissues (amnion, chorion laeve and decidua vera) were separately rolled, then flash frozen within isopentane slurry (-50°C), and mounted with OCT compound for cryotomy. Fig 2-5 illustrates the procedure for receptor labeling.

• **Frozen sections**

12 µm thick sections were thaw-mounted onto chrome alum/gelatin-coated microscope slides for autoradiography, and on Superfrost Plus microscope slides for histological staining. They were stored desiccated in airtight boxes at -20°C.

Routine hematoxylin and eosin (H&E) staining was performed on a few samples from each placenta to confirm the histological integrity of the preparation. Table 2-2 lists the routine steps for H&E staining.

The H&E procedure for frozen sections is outlined in Table 2-2. Fig. 2-5 illustrates the procedure of receptor labeling for autoradiography.

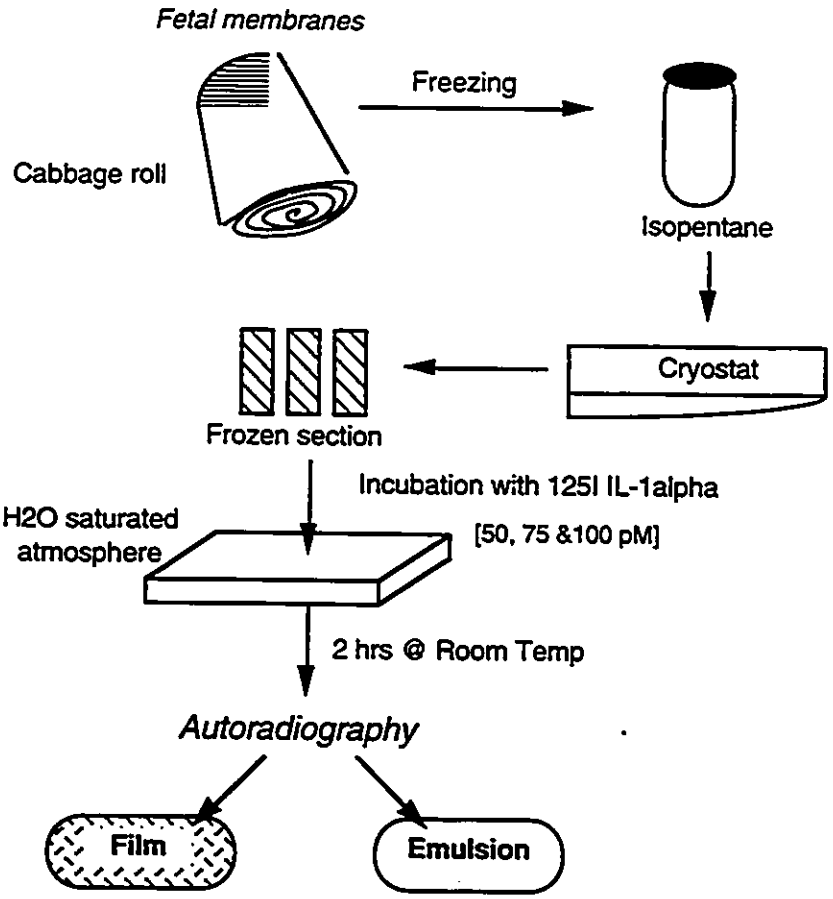


Fig. 2-5

IL-1 receptor labeling for autoradiography

Table 2-2
H & E for frozen sections

Step	*Chemical*	*Time*
1	Fix in 10% Buffered neutral Formalin (pH 7.0)	2 Min
2	Wash out fixative in gently running tap water	5 Min
3	Rinse in distilled water	Quick dip
4	Stain in filtered Harris Haematoxylin with glacial acetic acid (~2mL/ 100mL)	5 Min
5	Wash out excess stain in warm tap water	5-7 dips
6	Differentiate in acid alcohol (5 drops of conc. HCl into ~250 mL of 70% ethanol).	2-3 dips
7	Wash out acid in warm tap water	2-3 dips
8	Blue in ammonia water (2mL of conc. ammonium hydroxide in 1000 mL of dist. water)	2-3 dips
9	Wash in gently running tap water.	10-20 Min
10	Counterstain in Eosin-phloxine Mix (working solutions)	1 Min
11	Dip in 95% ethanol to remove excess eosin	3 dips
12	Dip in absolute ethanol to dehydrate	5 dips
13	Repeat step 12	1 Min
14	Clear in toluene	1 Min
15	Repeat step 14	2 Min
16	Repeat step 14	2-5 Min
17	Coverslip with Permount.	

- **IL-1 receptor labeling**

This procedure was carried out as described by Takao *et al.* (1990 *a* and *b*). The slide mounted tissues were brought to room temperature and incubated for 120 minutes with 50, 75 and 100 pM [^{125}I]IL-1 α (SA ~1500-5000 Ci/mmol) in the incubation buffer (RPMI-1640; gentamicin 50 µl/ml, 20 mM HEPES, sodium azide 1 mg/ml, aprotonin (100 Kallikrein inhibitor), and 10^{-4} M bacitracin, pH 7.4 with 0.15% of BSA) at room temperature in a water-saturated atmosphere. Nonspecific binding was determined by incubating in the presence of a 1000-fold excess of IL-1β (50-100 nM). After incubation, the slides were rinsed and washed for two 5 minute periods at 4°C with PBS without antibiotics at pH 7.4 containing 0.01% Triton X-100, then rinsed in distilled water at 4°C. The slides were then rapidly dried under a stream of cool dry air.

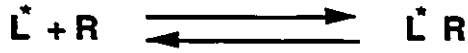
2.02.6 Autoradiography

The autoradiographic localization of receptors is a powerful technique for the study of receptors and their localization, particularly in complex tissue, where correlating the receptors to the underlying anatomical information is desirable. In addition, it can quantifiably localize receptors even in very small pieces of tissue.

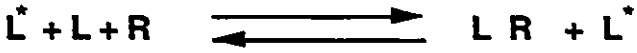
The principles of receptor autoradiography depends on understanding the receptor biochemical behavior. It involves choosing and binding a ligand, and visualization of the receptor. After

the receptors are bound to the radiolabeled ligand, this binding can be seen by either film or emulsion autoradiography. Film autoradiography is used to find whether or not the tissue expresses receptors. Emulsion autoradiography helps to identify the location of these receptors.

Binding of a ligand must be reversible and specific. Fig. 2-6 illustrates the specific and non-specific binding.



Reversible Radioligand-Receptor Complex (specific binding)



Non Specific Binding

Fig. 2-6

Binding of a Ligand

L* = Radioligand

L = Ligand

R = Receptor

L*R = Radioligand-receptor complex

LR = Ligand-receptor complex

Generally, binding can occur at non receptor locations as well as at receptor sites. This binding is termed *total binding*. Binding which takes place at non receptor locations (or not true receptor sites) is termed *non-specific binding*, which contributes to the total

accumulation of labeled hormone in the tissue. To determine the true receptor binding, i.e. *specific binding*, binding with radioligand is allowed to proceed with and without the presence of large quantities (~ 100 to 1000 fold excess) of non-labeled (*cold*) ligand. This will displace any labeled hormone from true receptor sites, but will not alter the non-specific component of binding. Moreover, specific binding usually increases in a linear fashion as the concentration of the radiolabeled ligand increases, until binding reaches a plateau when all the available receptor sites become occupied. However, non-specific binding does not reach a plateau as the concentration of radioligand increases.

In addition, specific binding must assume appropriate distribution. Specific binding can be estimated by subtracting the non-specific binding from the total binding.

The radioligands commonly used for IL-1 receptor labeling are [^{125}I] IL-1β and [^{125}I] IL-1α. These ligands possess highly specific activity with a short half life of 60 days. They both emit gamma radiation. [^{125}I] IL-1α was selected for the present study, because it possesses the above characteristics, and has been used successfully in IL-1 receptor labeling in other studies (Chin *et al.* 1988). It is also commercially available.

Non-labeled IL-1β was chosen to determine the non-specific binding, because IL-1α and IL-1β act on a common class of receptors.

2.02.6a Visualization of receptors

IL-1 receptors were visualized with film and emulsion autoradiography.

- **Visualization of ^{125}I-IL-1 receptors with film autoradiography**

Autoradiography was carried out for receptor visualization and localization as described by Laskey and Mills (1977). The dry labeled slides were placed in an X-ray cassette with a gamma-sensitive film (Kodak X-OMAT AR), and placed at -80°C for 3-7 days. After incubation, the screen was brought to room temperature and the films were developed by a Kodak RP X-OMAT Processor.

- **Visualization of ^{125}I-IL-1 receptors with emulsion autoradiography**

The dry labeled slides were fixed in 10% formalin for 2 minutes and washed for 5 minutes in cold tap water. In a dark room, using a safe light (902 or 904), the labeled slides were coated by dipping for 10 seconds in molten emulsion Kodak NTB2 diluted (1:1 v/v) in water. The slides were dried on a cool tray for one to two hours, then allowed to stand at room temperature for another two hours. The slides were then packed in light safe boxes and stored at 4°C for 7-10 days. They were developed in Kodak D-19 for 4 minutes at 17.5°-18°C, and fixed in 30% sodium thiosulphate for 8-10 minutes as

described by Krantis *et al.* 1991. After rinsing with cold tap water for 30 minutes, the sections were stained with H & E (Table 2-2). The slides were viewed with a Zeiss Photomicroscope Axiophot or an Olympus microscope. Microscopic examination of these sections facilitated the identification of receptor distribution in relation to the underlying cytoarchitecture.

2.02.7 IL-1 receptor labeling on the dispersed cells

The amnion and chorion laeve tissue cells were separated and dispersed as described in section 2.02.2a. The cells were then suspended in F12:DMEM containing 10% FCS. Then one ml of the cell suspension containing around $3x10^5$ cells was placed on a pre-marked circle on a sterile slide. The slides used were either positively charged (Superfrost Plus microscope slides) or coated with poly-L-lysine, or both. Cells were incubated on the slides in an H_2O saturated atmosphere for 24 and 48 hours.

IL-1 receptor labeling followed by film and emulsion autoradiography was carried out as described above. However, some modification of the H & E staining procedure was essential to obtain the desired contrast between the nucleus and cytoplasm. Table 2-3 lists the steps of H&E staining of the cultured cells.

Table 2-3
H & E for Cell Culture

Step	*Chemical*	*Time*
1	Stain in filtered Harris Haematoxylin with glacial acetic acid (~2mL/ 100mL)	5-6 Min
2	Wash out excess stain in warm tap water	2-3 Min
3	Differentiate in acid alcohol (5 drops of conc. HCl into ~250 mL of 70% ethanol).	2-3 dips
4	Wash out acid in warm tap water	2-3 dips
5	Blue in Lithium carbonate (1:1 dilution)	45 sec
6	Wash in gently running tap water. Clean up slides.	20 Min
7	Dip in 50% ethanol	3 dips
8	Counterstain in Eosin-phloxine Mix (working solutions)	1 Min
9	Dip in 95% ethanol to remove excess eosin	3 dips
10	Dip in Absolute ethanol to dehydrate	5 dips
11	Repeat step 10	1 Min
12	Clear in xylene or toluene	1 Min
13	Repeat step 12	2 Min
14	Repeat step 12	>5 Min
15	Coverslip with Permount (or coverbond)	

2.02.8 Statistical analysis

All determinations were done in quadruplicate wells, and a minimum of three experiments, using different patients were studied for each Interleukin. Statistical differences were assessed using one way Anova (one factor with multiple comparison, significance level of 95% and experimental type is factorial), followed by a multiple comparison test, based on Sheffe's Multiple Comparison Test, and Student's t-test (unpaired, one tailed t-test), where applicable. A p value of 0.05 defined statistical significance. In experiments where there were only two variants or two treatments, (e.g. in the dexamethasone experiment, or when the production of PGE_2 was compared before and after labour), unpaired Student t-test was used. Paired Student t-test was used to compare PGE_2 production in the second 24 hours as a percent of the first 24 hours. Systat and Statview programs on a Macintosh computer were employed for statistical analysis. Mac Graphics, Cricket graphics and Microsoft Excel programs were employed for graphics. Drawings were produced with Mac Draw and Superpaint programs.

3.0 RESULTS

3.01 Prostaglandin Production by Human Fetal Membranes and Decidua

3.01.1 Intact Tissues

We attempted to study the action of IL-1 on undisrupted tissues and examined the use of amnion discs in initial experiments. The tissue discs wet weight (after removing the excess media) ranged from 0.0014g to 0.0076g with an average or mean weight of 0.0037g ± 0.0017g.

Discs were incubated in F12:DMEM with human recombinant IL-1α (in final concentrations of 0.1, 10 and 100 Units per ml). The media were collected after 24 hours and PGE_2 accumulation was measured by radioimmunoassay. The results of these experiments are briefly summarized in Table 3-1. The amount of PGE_2 secreted into the media was highly variable. There was no significant effect of IL-1α on PGE_2 output (One way Anova p= 0.7461) and there was also no significant effect of IL-1 on any of the individual tissues examined. The variability and absence of response did not seem to depend on the mode of delivery of the tissue, as two of the tissues were collected before labour by cesarean section and the other two were

obtained after labour by vaginal delivery.

In this system, the variability in PGE_2 output that occurred was not satisfactorily controlled by pre-washing the tissues prior to the treatment. Furthermore, the variability encountered was not improved whether the data was expressed per mg protein or µg DNA. We therefore decided to study a different tissue preparation and examined dispersed cell preparations where the number of cells plated could be controlled and where the PG output might be expected to be less variable.

Table 3-1
PGE_2 production by amnion discs after 24 hour incubation with IL-1α

Treatment	*n*	*PGE_2 Range*	*mean ± SEM*	*% of control*
Control (F12:DMEM)	4	0.2 - 6.8	2.6 ± 1.5	100
IL-1α (0.1 U/ml)	2	5.1 - 8.8	6.9 ± 2.7	230 ± 155
IL-1α (10 U/ml)	4	0.2 - 18	6.3 ± 3.7	452 ± 381
IL-1α (100 U/ml)	4	0.3 - 17	7.2 ± 4.1	453 ± 302

n represents the number of different tissues studied each of which was incubated in quadruplicate with each treatment.
Mean is presented as ng/ml.
Range is presented as ng/ml.
Percent of control refers to the data in each experiment as a percent of the control of that experiment. These data were then presented as mean of the percent data ± SEM.
p=0.7461 by one factor Anova with multi-comparison significance level of 95%.

3.01.2 Dispersed Cells

Viability of Dispersed Cells

Amnion, chorion laeve and decidua vera were dispersed into single cells. Routinely, cell viability was checked by exclusion of trypan blue (as described by Freshney 1987) and found to be greater than 85%. Fig. 3-1 shows a typical result of a trypan blue exclusion test. The viability was also 85% or greater after incubation with the various substances tested in the experiments described.

Fig. 3-1
Trypan Blue Test
This picture shows two dead cells (dark blue), and the rest are considered viable. Magnified X 10.

PGE_2 output by untreated cells

During the first 24 hours of culture, the PGE_2 output was significantly lower from amnion cells dispersed from tissue obtained prior to the onset of labour than from cells obtained after the onset of labour (Table 3-2).

Conversely, as is clearly evident in Table 3-2, the chorion cells which were obtained after the onset of labour produced significantly less PGE_2 in the first 24 hours than the cells harvested prior to the onset of labour. However, with decidua cells there was no significant difference in PGE_2 output prior to or following labour.

The PGE_2 output by freshly dispersed cells obtained before and after the onset of labour in the second 24 hours are shown in Table 3-3. In the second 24 hours, although PGE_2 production by amnion cells was higher post-labour than pre-labour, it did not reach statistical significance ($p= 0.1428$). Chorion and decidua cell production of PGE_2 was also not statistically different ($p= 0.1837$ and 0.3522 respectively).

The PGE_2 output of the cells also varied with the day of culture. Table 3-4 illustrates PGE_2 production by amnion, chorion laeve, and decidua vera cells during the first and second 24 hours of culture. Data from pre and post-labour tissues have been pooled for this evaluation. There was a marked decrease in basal PGE_2 output by amnion cells from the first to the second day of culture, whereas

chorion laeve cells produced more PGE_2 in the second day of culture. In the case of decidua cells there was a trend of increase in PGE_2 production in the second day, but this did not reach statistical significance ($p= 0.058$).

PGE_2 production by amnion cells in the second 24 hours was also examined as percent of the first 24 hours. A significant decrease in PGE_2 production was noted in the second 24 hours ($p< 0.05$). Similarly, the chorion PGE_2 production in the second 24 hours was also studied as a percent of the first 24 hours. A highly significant increase in PGE_2 was noted ($p < 0.001$) using paired t-test. There was no difference in PGE_2 production by decidua cells in the first or second 24 hours. Fig. 3-2 depicts the production of PGE_2 in the second 24 hours as a percent for the first 24 hours for amnion and chorion.

Table 3-2

PGE_2 production by freshly dispersed cells obtained before and after the onset of labour in the first 24 hours

Amnion Cells	*n*	*Mean*	± *SEM*
Before Labour	11	64	± 16
After Labour	9	510	± 132

p= 0.0013 Unpaired student t-test

Chorion Cells	*n*	*Mean*	± *SEM*
Before Labour	7	75	± 26
After Labour	7	19	± 14

p= 0.0456 Unpaired student t-test

Decidua Cells	*n*	*Mean*	± *SEM*
Before Labour	7	73	± 46
After Labour	7	49	± 25

p= 0.3139 Unpaired student t-test

Data expressed as mean in pg/ 3 x 10^5 cells (± SEM).

Table 3-3

PGE_2 production by freshly dispersed cells obtained before and after the onset of labour in the second 24 hours

Amnion Cells	*n*	*Mean*	±	*SEM*
Before Labour	7	74	±	23
After Labour	9	286	±	166

p= 0.1428 Unpaired student t-test

Chorion Cells	*n*	*Mean*	±	*SEM*
Before Labour	4	180	±	73
After Labour	6	69	±	24

p= 0.1837 Unpaired student t-test

Decidua Cells	*n*	*Mean*	±	*SEM*
Before Labour	6	151	±	75
After Labour	4	195	±	76

p= 0.3522 Unpaired student t-test

Data expressed as mean in pg/ 3 x 10^5 cells (± SEM).

Table 3-4

PGE_2 production in the first and second 24 hours of culture

Amnion Cells	*n*	*Mean*	± *SEM*
First 24h	17	278	± 80
second 24h	17	86	± 44

p= 0.0188 unpaired t-test

Chorion Cells	*n*	*Mean*	± *SEM*
First 24h	9	52	± 18
second 24h	9	253	± 104

p= 0.0377 unpaired t-test

Decidua Cells	*n*	*Mean*	± *SEM*
First 24h	16	60	± 24
second 24h	9	131	± 41

p= 0.058 unpaired t-test

The results are expressed as mean pg/ 3 x 10^5 cells (± SEM).

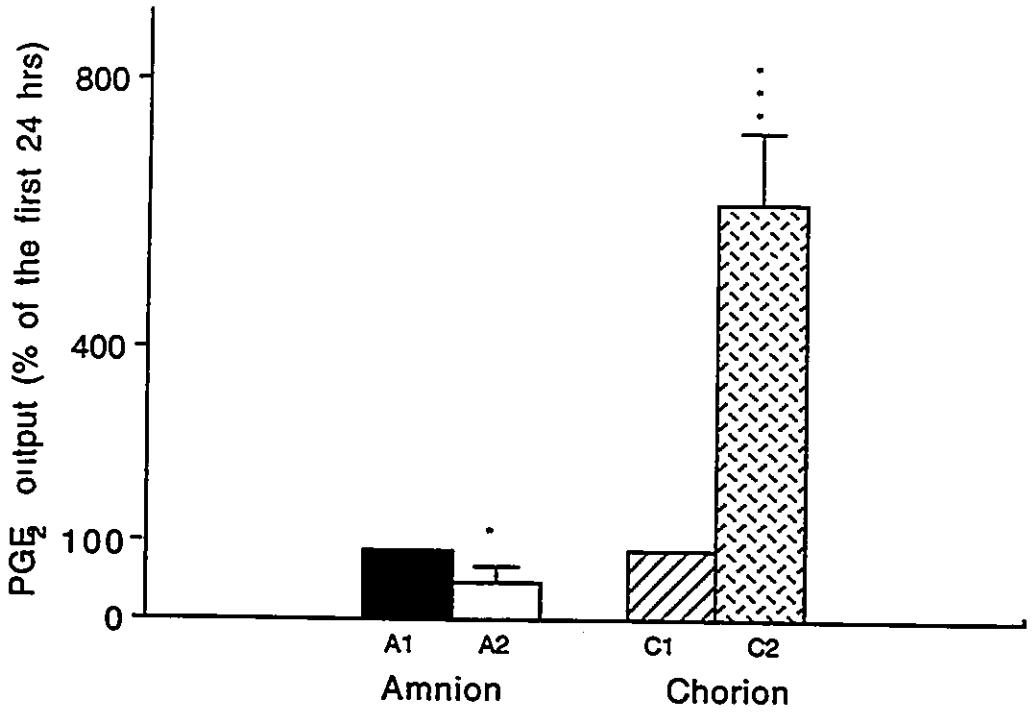


Fig. 3-2
Relative PGE_2 output by dispersed cells from amnion and chorion laeve during the first and second 24 hours of culture

A1: Amnion cells in the first 24 hours expressed as 100%.
A2: Amnion cells in the second 24 hours, presented as a percentage of the amnion in the first 24 hours.
$P < 0.05$ (paired t-test).

C1: Chorion cells in the first 24 hours expressed as 100%.
C2: Chorion cells in the second 24 hours, presented as a percentage of the chorion in the first 24 hours.
$P < 0.001$ (paired t-test).

3.01.2a Effect of IL-1α and IL-1β on PG Production in the First and Second 24 Hours of Culture

Freshly dispersed cells from amnion, chorion laeve, and decidua vera were incubated with IL-1α or IL-1β in final concentrations of 1, 10, 100 and 1000 units per ml. These interleukins were added either in the first 24 hours, or in the second 24 hours of culture, after changing the media. Cell free media were collected after 24 hour incubation with each interleukin, and stored at -80°C until assayed by RIA.

Because PGE_2 production by the amnion and the chorion in the first 24 hours showed significant differences for pre- and post-labour groups, the data were separated into these two groups. However in the second 24 hours, there was no difference, therefore the data were kept together.

All media were assayed for PGE_2. In addition, $PGF_{2\alpha}$ was analyzed for decidua cells both in the first, and in the second 24 hours. Chorion cells were treated with IL-1β for the first 24 hours, and were also assayed for PGEM.

The following sections present the results of the first and second 24 hour cell culture of each tissue studied (amnion, chorion and decidua).

Effect of IL-1α on PGE_2 production by amnion in the first and second 24 hours of culture

Amnion cells in the first 24 hours

Nine tissues were examined; 6 after labour and 3 before the onset of labour. (Fig. 3-3). The data were examined as a percent of the control. PGE_2 output was not influenced by the mode of delivery; $p = 0.5589$ for post-labour and $p= 0.9738$ for pre-labour. The controls of the post-labour data ranged between 52 and 1220 pg/ 3 X 10^5 cells. The controls of the pre-labour data ranged between 19 and 38 pg/ 3 X 10^5 cells.

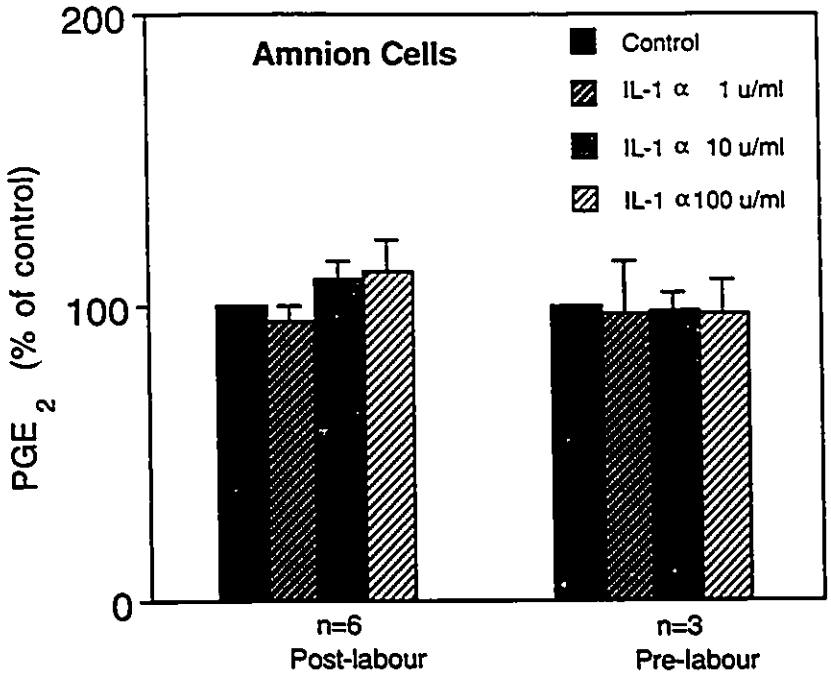


Fig. 3-3

PGE_2 production by amnion cells after 24 hours incubation with IL-1α. Data stratified according to the mode of delivery

Data expressed as percent of the control of that experiment ± SEM.
n; refers to the number of experiments. Six experiments were included in the post-labour bar graph, and three in the pre-labour one.
Each experiment was done in quadruplicate wells per treatment.
p= 0.5589 and 0.9738 by one way Anova.

Amnion cells in the second 24 hours

Table 3-5 summarizes PGE_2 production by amnion cells in response to IL-1α in the second day of culture. There was no significant difference in PGE_2 production between control and IL-1α-treated cells (p= 0.9952 by one way Anova). IL-1α (1 unit/ml) was not included in the analyses because of low number of experiments but it also did not stimulate PGE_2 output. Similarly with the tissue in which basal output of PGE_2 was below the limit of detection no effect of IL-1α was found. Seven tissues were studied; four after the onset of labour and three before the onset of labour by cesarean section.

Table 3-5
PGE_2 production by amnion cells in response to IL-1α in the second 24 hours of incubation.

Treatment	*n*	*PGE_2 Range*	*Mean ± SEM*	*% control*
Control (F12:DME)	7	0 - 926	153 ±112	100
IL-1α (1 U/ ml)*	3	0 - 130	37 ±31	(112 ± 14)
IL-1α (10 U/ ml)	7	3 - 1236	346 ±164	112 ± 19
IL-1α (100 U/ ml)	7	0 -1111	299 ± 158	122 ± 13

* The number of experiments was low and one experiment yielded undetectable levels of PGE_2 and they were not included in the Anova analysis. Their percent of control is put between two brackets.

Data expressed as mean in pg/ 3 x 10^5 cells (± SEM).

Percent of the control refers to the data in each experiment as a percent of the control of that experiment. These data were then presented as mean of the percent data ± SEM.

n represents the number of different tissues studied, each of which was incubated in quadruplicate with each treatment.

p= 0.9952 by one factor Anova with multi-comparison significance level of 95%.

Effect of IL-1α on PGE_2 production by chorion in the first and second 24 hours of culture

Chorion cells in the first 24 hours

Chorion laeve cells were incubated with IL-1α at final concentrations of 1, 10 and 100 U/ml. Two experiments also included IL-1α at a concentration of 1000 U/ml. The media were assayed for PGE_2 by radioimmunoassay. The resulting data were stratified into pre- and post-labour groups, because the production of PGE_2 by untreated cells after labour was significantly less than pre-labour. Five experiments were done; three after labour and two before the onset of labour (Fig. 3-4). No significant change in PGE_2 was observed in either group ($p = 0.7686$ for post-labour and $p= 0.7123$ for pre-labour). The controls of the post-labour data ranged between 4 and 6 pg/ 3 X 10^5 cells. The controls of the pre-labour data ranged between 2.4 and 32 pg/ 3 X 10^5 cells.

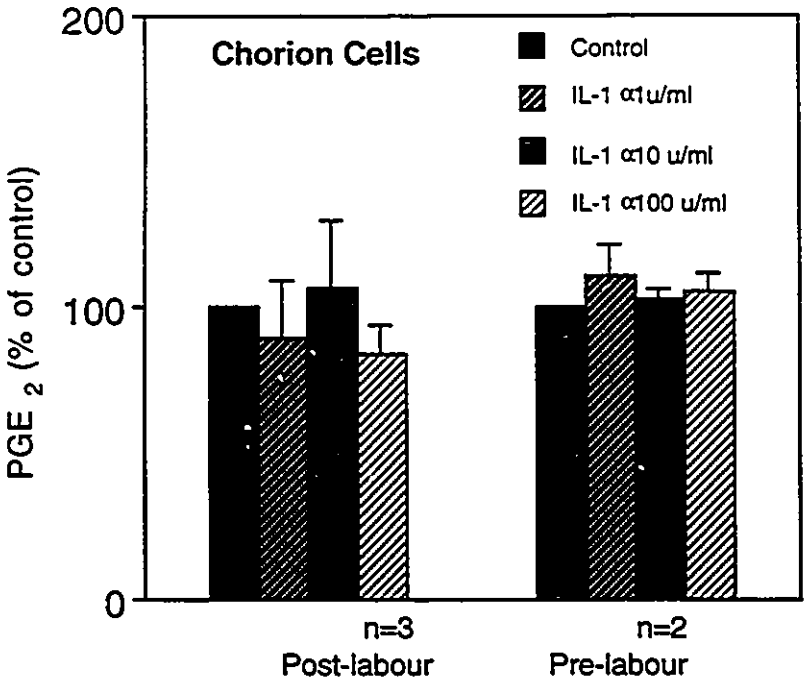


Fig. 3-4

PGE_2 production by chorion laeve cells after 24 hours incubation with IL-1α. Data stratified according to the mode of delivery

Data expressed as percent of the control of that experiment ± SEM.
n; refers to the number of experiments. Three experiments were included in the post-labour bar graph, and two in the pre-labour one.
Each experiment was done in quadruplicate wells per treatment.
p= 0.7686 and 0.7123 by one way Anova.

Chorion cells in the second 24 hours

Media were assayed for PGE_2 after 24 hour incubation with IL-1α in the second day of culture and the results are summarized in Table 3-6. Using one way Anova, the data showed no significant difference in PGE_2 production over control (p= 0.9972). Four experiments were done; two after labour and two before the onset of labour.

Table 3-6
PGE_2 production by chorion laeve cells in the second 24 hours of incubation with IL-1α

Treatment	*n*	*PGE_2 Range*	*Mean ± SEM*	*% of control*
Control (F12:DME)	4	12 - 222	73 ± 50	100
IL-1α (1 U/ ml)	4	10 - 173	63 ± 38	95 ± 10
IL-1α (10 U/ ml)	4	11 - 178	64 ± 39	97 ± 7
IL-1α (100 U/ ml)	4	17 - 196	71 ± 42	117 ± 15
IL-1α (1000 U/ml)	1*	(182)	(182)	(82)

* The number of experiments was low and they were not included in the Anova analysis. Their percent of control is put between two brackets.
Data expressed as mean in pg/ 3 x 10^5 cells (± SEM). Range is also in pg/ml.
Percent of the control refers to the data in each experiment as a percent of the control of that experiment. These data were then presented as mean of the percent data ± SEM.
n represents the number of different tissues studied, each of which was incubated in quadruplicate with each treatment.
p= 0.9972 by one factor Anova with multi-comparison significance level of 95%.

Effect of IL-1α on PGE_2 production by decidua in the first and second 24 hours of culture

Decidua cells in the first 24 hours

The cell free supernatant of decidua vera cells were collected after 24 hour incubation with IL-1α and PGE_2 was measured by RIA. The results obtained are summarized in Table 3-7. Four experiments were carried out; one before labour and three after the onset of labour. There was no significant change in PGE_2 production over the control at any concentration ($p = 0.5703$ one way Anova).

Table 3-7
PGE_2 production by decidua vera cells after 24 hours incubation with IL-1α

Treatment	*n*	*PGE_2 Range*	*mean ± SEM*	*% of control*
Control (F12:DME)	4	0 - 21	14 ± 7	100
IL-1α (1 U/ ml)	4	0 - 20	9 ± 5	100 ± 1
IL-1α (10 U/ ml)	4	0 - 22	10 ± 6	105 ± 4
IL-1α (100 U/ ml)	4	0 - 21	11 ± 6	106 ± 7

One experiment yielded undetectable levels of PGE_2 and was not included in the Anova analysis.
Data expressed as mean in pg/ 3 x 10^5 cells (± SEM). Range is also in pg/ml.
Percent of the control refers to the data in each experiment as a percent of the control of that experiment. These data were then presented as mean of the percent data ± SEM.
n represents the number of different tissues studied, each of which was incubated in quadruplicate with each treatment.
p= 0.5703 by one factor Anova with multi-comparison significance level of 95%.

Decidua cells in the second 24 hours

A preliminary study of two experiments were carried out: one before labour and one after the onset of labour. The media obtained after 24 hours culture of decidua cells with IL-1α in the second 24 hours were assayed for PGE_2 by RIA. The data were analyzed by using one way Anova, and showed no significant difference in PGE_2 production (p value = 0.9987) (Table 3-8).

Table 3-8
PGE_2 production by decidua vera cells in the second 24 hours of incubation with IL-1α

Treatment	*n*	*PGE_2 Range*	*mean ± SD*	*% of control*
Control (F12:DME)	2	7 - 71	39 ± 32	100
IL-1α (1 U/ ml)	2	5 - 76	41 ± 36	90 ± 17
IL-1α (10 U/ ml)	2	5 - 81	43 ± 38	93 ± 21
IL-1α (100 U/ ml)	2	6 - 65	36 ± 29	89 ± 3

Data expressed as mean in pg/ 3 x 10^5 cells (± SD). Range is also in pg/ml.
Percent of the control refers to the data in each experiment as a percent of the control of that experiment. These data were then presented as mean of the percent data ± SD.
n represents the number of different tissues studied, each of which was incubated in quadruplicate with each treatment.
p= 0.9987 by one factor Anova with multi-comparison significance level of 95%.

Effect of IL-1α on prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) production from decidua cells in the first and second 24 hours cf culture

First 24 hours of culture

Because $PGF_{2\alpha}$ is a major prostaglandin produced by decidua cells, it was also assayed in culture media from this tissue after 24 hours incubation with IL-1α at various concentrations. Data of these results represent a preliminary study and are summarized in Table 3-9. Two experiments were carried out; one before labour and one after the onset of labour. Using one way Anova, the results showed no significant difference in $PGF_{2\alpha}$ production ($p = 0.9987$).

Table 3-9
$PGF_{2\alpha}$ production by decidua vera cells in the first 24 hours of incubation with IL-1α

Treatment	*n*	*$PGF_{2\alpha}$ Range*	*Mean ± SD*	*% of control*
Control (F12:DME)	2	10 - 73	42 ± 32	100
IL-1α 1 U/ ml	2	9 - 84	47 ± 38	105 ± 10
IL-1α 10 U/ ml	2	9 - 81	45 ± 36	103 ± 8
IL-1α100 U/ ml	2	9 - 60	35 ± 26	89 ± 6
IL-1α1000 U/ml*	1	8	8	(83)

*The number of experiments was low and were not included in the ANOVA analysis. Their percent of control is put between two brackets.
Data expressed as mean in pg/ 3 x 10^5 cells (± SD). Range is also in pg/ml.
Percent of the control refers to the data in each experiment as a percent of the control of that experiment. These data were then presented as mean of the percent data ± SD.
n represents the number of different tissues studied, each of which was incubated in quadruplicate with each treatment.
p= 0.9987 by one factor Anova with multi-comparison significance level of 95%.

Second 24 hours of culture

Two preliminary experiments were done in the second 24 hours of culture. IL-1α was added at various concentrations and the media was assayed for $PGF_{2\alpha}$ accumulation after 24 hours incubation. As illustrated in Table 3-10 there was no significant difference in $PGF_{2\alpha}$ production when compared to the control (p value = 0.9986, using one way Anova).

Table 3-10
$PGF_{2\alpha}$ production by decidua vera cells in the second 24 hours of incubation with IL-1α

Treatment	*n*	*$PGF_{2\alpha}$ Range*	*Mean ± SD*	*% of control*
Control (F12:DME)	2	0 - 88	44 ± 44	100
IL-1α 1 U/ ml	2	0 - 91	45 ± 45	102 ± 2
IL-1α 10 U/ ml	2	0 - 92	46 ± 46	102 ± 2
IL-1α 100 U/ ml	2	0 - 108	54 ± 54	111 ± 11

One experiment yielded undetectable levels of $PGF_{2\alpha}$.
Data expressed as mean in pg/ 3 x 10^5 cells (± SD). Range also is in pg/ml.
Percent of the control refers to the data in each experiment as a percent of the control of that experiment. These data were then presented as mean of the percent data ± SD.
n represents the number of different tissues studied, each of which was incubated in quadruplicate with each treatment.
p= 0.9986 by one factor Anova with multi-comparison significance level of 95%.

Effect of IL-1β on PGE_2 production by amnion in the first and second 24 hours of culture

Amnion cells in the first 24 hours of culture

As in the procedure for IL-1α, freshly dispersed cells from amnion were treated with IL-1β at various concentrations and PGE_2 measured in the cell free media. Results from amnion cells PGE_2 production in response to IL-1β in the first day of culture were separated to pre and post labour (Fig. 3-5). Six tissues were studied; three after the onset of labour and three before the onset of labour by cesarean section. PGE_2 production by amnion cells in the first 24 hours was significantly higher after labour than before the onset of labour. The mode of delivery did not influence the effect of IL-1α, IL-1β, and IL-6 on PGE_2 production by the amnion cells in the first 24 hours, $p = 0.3049$ for post-labour and $p= 0.7939$ for pre-labour. The control of the post-labour data ranged between 72 and 653 pg/ 3×10^5 cells. The controls of the pre-labour data ranged between 23 and 143 pg/ 3×10^5 cells.

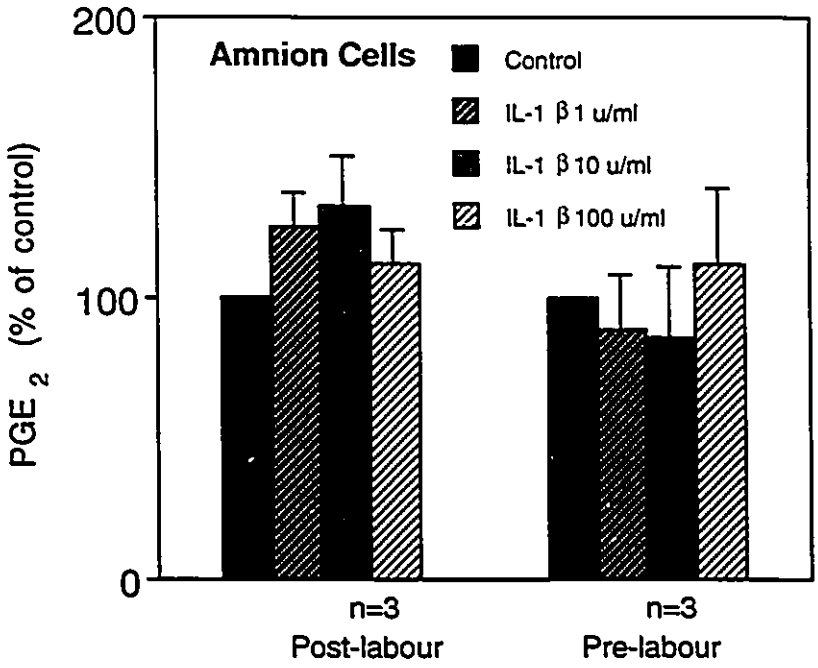


Fig. 3-5

PGE_2 production by amnion cells after 24 hours incubation with IL-1β. Data stratified according to the mode of delivery

Data expressed as percent of the control of that experiment ± SEM.
n; refers to the number of experiments. Three experiments were included in the post-labour bar graph, and three in the pre-labour one.
Each experiment was done in quadruplicate wells per treatment.
p= 0.3049 and 0.7939 by one way Anova.

Amnion cells in the second 24 hours of culture

Results of PGE_2 production by amnion cells in response to IL-1β in the second day of culture are shown in Table 3-11. Six tissues were included in this experiment; three before the onset of labour by cesarean section, and three after the onset of labour. Using one way Anova, no significant change in PGE_2 production under the influence of IL-1β was detected when compared to the control ($p = 0.999$).

Table 3-11
PGE_2 production by amnion cells in the second 24 hours of incubation with IL-1β

Treatment	*n*	*PGE_2 Range*	*mean ± SEM*	*% of control*
Control (F12:DME)	6	0 - 145	23 ± 32	100
IL-1 β (1 U/ ml)	6	0 - 148	23 ± 32	115 ± 22
IL-1 β (10 U/ ml)	6	0 - 148	23 ± 33	102 ± 3
IL-1 β (100 U/ ml)	6	0 - 140	22 ± 31	102 ± 9

One experiment yielded undetectable levels of PGE_2 and was not used in the statistical analysis.
Data expressed as mean in pg/ 3 x 10^5 cells (± SEM).
Percent of the control refers to the data in each experiment as a percent of the control of that experiment. These data were then presented as mean of the percent data ± SEM.
n represents the number of different tissues studied, each of which was incubated in quadruplicate with each treatment.
p= 0.999 by one factor Anova with multi-comparison significance level of 95%.

Effect of IL-1β on PGE_2 production by chorion in the first and second 24 hours of culture

Chorion cells in the first 24 hours of culture

Chorion cells were treated the same way as amnion with IL-1β. Six tissues were studied; three after the onset of labour and three before the onset of labour by cesarean section. Results of PGE_2 production in response to IL-1β in the first 24 hours of culture were stratified according to the mode of delivery. As Fig. 3-6 illustrates, no significant change in PGE_2 was observed in either group ($p = 0.9132$ for post-labour and $p= 0.5708$ for pre-labour). The control of the post-labour data ranged between 0 and 30 pg/ 3 X 10^5 cells. The controls of the pre-labour data ranged between 30 and 134 pg/ 3 X 10^5 cells.

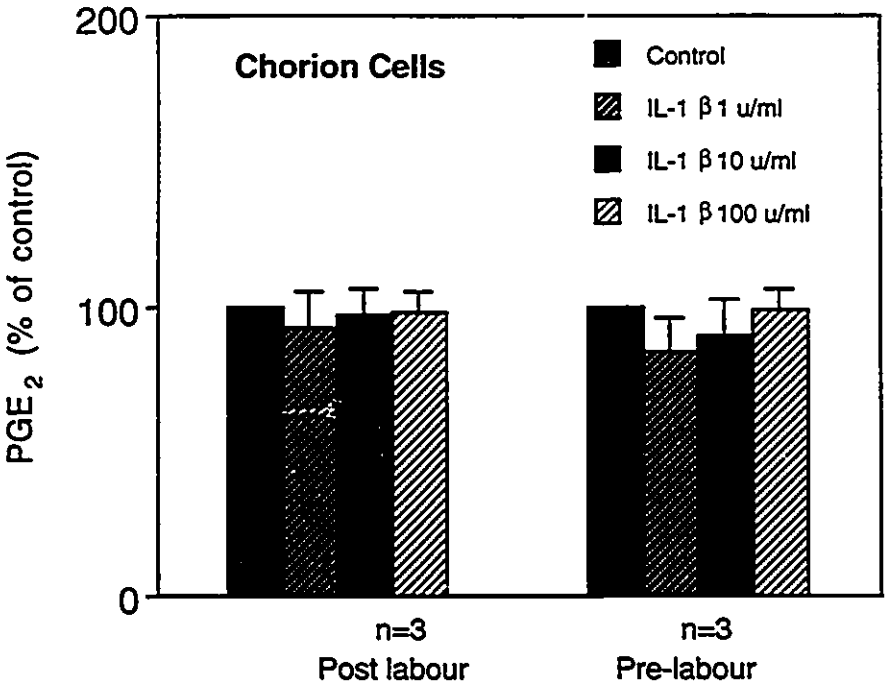


Fig. 3-6

PGE_2 production by chorion laeve cells after 24 hours incubation with IL-1β. Data stratified according to the mode of delivery

Data expressed as percent of the control of that experiment ± SEM.
n; refers to the number of experiments. Three experiments were included in the post-labour bar graph, and three in the pre-labour one.
Each experiment was done in quadruplicate wells per treatment.
p= 0.9132 and 0.5708 by one way Anova.

Chorion cells in the second 24 hours of culture

In the second day of chorion cell culture, the media were changed and IL-1β was added at various concentrations. Results of PGE_2 production in response to IL-1β in the second day of culture are shown in Table 3-12. Six tissues were included in this experiment; three before the onset of labour by cesarean section, and three after the onset of labour. Using one way Anova with multi-comparison significance level of 95%, no significant change in PGE_2 production under the influence of IL-1β was detected when compared to the control ($p = 0.9957$).

Table 3-12
PGE_2 production by chorion laeve cells in the second 24 hours of incubation with IL-1β

Treatment	*n*	*PGE_2 Range*	*mean ± SEM*	*% of control*
Control (F12:DME)	6	0 - 1003	280 ± 162	100
IL-1 β 1 U/ ml	6	0 - 718	319 ± 171	115 ± 7
IL-1 β 10 U/ ml	6	0 - 736	303 ± 166	109 ± 4
IL-1 β 100 U/ ml	6	0 - 899	338 ± 187	105 ± 20

One experiment yielded undetectable levels of PGE_2 and was not included in the statistical analysis.
Data expressed as mean in pg/ 3 x 10^5 cells (± SEM). Range is also in pg/ml.
Percent of the control refers to the data in each experiment as a percent of the control of that experiment. These data were then presented as mean of the percent data ± SEM.
n represents the number of different tissues studied, each of which was incubated in quadruplicate with each treatment.
p= 0.9957 by one factor Anova with multi-comparison significance level of 95%.

Effect of IL-1β on PGE_2 production by decidua in the first and second 24 hours of culture

Decidua cells in the first 24 hours of culture

Decidua cells were treated in the same way as amnion and chorion laeve. IL-1β was added in final concentrations of 0, 1, 10 and 100 units/ml. Media were assayed for PGE_2 by radioimmunoassay after 24 hours incubation. Results of decidua cell PGE_2 production in response to IL-1β in the first 24 hours of culture are shown in Table 3-13. Four experiments were done; two after the onset of labour and two before the onset of labour. Using one way Anova indicated that there was no significant difference in PGE_2 production ($p = 0.902$).

Table 3-13
PGE_2 production by decidua vera cells in the first 24 hours of incubation with IL-1β

Treatment	*n*	*PGE_2 Range*	*mean ± SEM*	*% of control*
Control (F12:DME)	4	2 - 27	13 ± 5	100
IL-1 β (1 U/ ml)	4	2 - 15	10 ± 3	105 ± 9
IL-1 β (10 U/ ml)	4	3 - 18	10 ± 3	91 ± 8
IL-1 β (100 U/ ml)	4	3 - 14	9 ± 2	87 ± 12

Data expressed as mean in pg/ml (± SEM). One ml contains 3.0 $X10^5$ cells.
Range is also in pg/ml.
Percent of the control refers to the data in each experiment as a percent of the control of that experiment. These data were then presented as mean of the percent data ± SEM.
n represents the number of different tissues studied, each of which was incubated in quadruplicate with each treatment.
p= 0.902 by one factor Anova with multi-comparison significance level of 95%.

Decidua cells in the second 24 hours of culture

Results of decidua cells PGE_2 production in response to IL-1β in the second day of culture are shown in Table 3-14. Four tissues were included in this experiment; two before the onset of labour by cesarean section, and two after the onset of labour. Using one way Anova, no significant change in PGE_2 production under the influence of IL-1β was detected when compared to the control (p= 0.9746).

Table 3-14
PGE_2 production by decidua vera cells in the second 24 hours of incubation with IL-1β

Treatment	*n*	*PGE_2 Range*	*mean ± SEM*	*% of control*
Control (F12:DME)	4	0 - 328	102 ± 76	100
IL-1 β (1 U/ ml)	4	0 - 367	113 ± 86	105 ± 3
IL-1 β (10 U/ ml)	4	0 - 313	102 ± 72	101 ± 10
IL-1 β (100 U/ ml)	4	0 - 180	70 ± 41	90 ± 20

One experiment yielded undetectable levels of PGE_2 and was not included in the statistical analysis.
Data expressed as mean in pg/ 3 x 10^5 cells (± SEM). Range is also in pg/ml.
Percent of the control refers to the data in each experiment as a percent of the control of that experiment. These data were then presented as mean of the percent data ± SEM.
n represents the number of different tissues studied, each of which was incubated in quadruplicate with each treatment.
p= 0.9746 by one factor Anova with multi-comparison significance level of 95%.

Effect of IL-1β on $PGF_{2\alpha}$ production by decidua in the first and second 24 hours of culture

First 24 hours of culture

The production of $PGF_{2\alpha}$ by decidua cells after 24 hours incubation with IL-1β was determined by using radioimmunoassay. The results of $PGF_{2\alpha}$ production by decidua vera cells in the first 24 hours of incubation with IL-1 β are summarized in Table 3-15. Four tissues were included in this experiment; two before the onset of labour by cesarean section, and two after the onset of labour. Using one way Anova, no significant change in $PGF_{2\alpha}$ production in response to IL-1β was detected (p=0.9951).

Table 3-15
$PGF_{2\alpha}$ production by decidua vera cells in the first 24 hours of incubation with IL-1β

Treatment	*n*	*$PGF_{2\alpha}$ Range*	*mean ± SEM*	*% of control*
Control (F12:DME)	4	5 - 240	70 ± 57	100
IL-1 β (1 U/ ml)	4	5 - 245	69 ± 59	88 ± 14
IL-1 β (10 U/ ml)	4	4 - 297	82 ± 72	95 ± 17
IL-1 β (100 U/ ml)	4	6 - 328	90 ± 79	95 ± 20

Data expressed as mean in pg/ 3 $X10^5$ cells (± SEM).
Percent of the control refers to the data in each experiment as a percent of the control of that experiment. These data were then presented as mean of the percent data ± SEM.
n represents the number of different tissues studied, each of which was incubated in quadruplicate with each treatment.
p= 0.9951 by one factor Anova with multi-comparison significance level of 95%.

Second 24 hours of culture

The results of $PGF_{2\alpha}$ production by decidua vera cells in the second 24 hours of incubation with IL-1 β are summarized in Table 3-16. Four tissues were included in this experiment; two before the onset of labour by cesarean section, and two after the onset of labour. Using one way Anova, no significant change in PGE_2 production under the influence of IL-1β was detected (p= 0.9572).

Table 3-16
$PGF_{2\alpha}$ production by decidua vera cells in the second 24 hours of incubation with IL-1β

Treatment	*n*	*$PGF_{2\alpha}$ Range*	*mean ± SEM*	*% of control*
Control (F12:DME)	4	0 - 205	56 ± 49	100
IL-1 β (1 U/ ml)	4	0 - 251	68 ± 61	108 ± 5
IL-1 β (10 U/ ml)	4	0 - 267	73 ± 65	114 ± 8
IL-1 β (100 U/ ml)	4	0 - 107	35 ± 25	106 ± 25

One experiment yielded undetectable levels of $PGF_{2\alpha}$. Therefore, it was not used in the statistical analysis.
Data expressed as mean in pg/ 3 X 10^5 cells (± SEM). Range is also in pg/ml.
Percent of the control refers to the data in each experiment as a percent of the control of that experiment. These data were then presented as mean of the percent data ± SEM.
n represents the number of different tissues studied, each of which was incubated in quadruplicate with each treatment.
p= 0.9952 by one factor Anova with multi-comparison significance level of 95%.

Effect of IL-1β on prostaglandin EM (PGEM) production by chorion in the first 24 hours of culture

As there was no significant change in PGE_2 production by chorion laeve cells in response to IL-1β , PGEM levels were measured since this tissue possesses high PG 15-dehydrogenase activity. PGEM levels were determined in cell-free supernatant of chorion cells and the results are shown in Table 3-17. Four tissues were included in this experiments; two before the onset of labour by cesarean section, and two after the onset of labour. Using one way Anova with multi-comparison significance level of 95%, no significant change in PGEM production under the influence of IL-1β was detected when compared to the control ($p = 0.7626$).

Table 3-17
PGEM production by chorion laeve cells after 24 hours incubation with IL-1β , in the first 24 hours

Treatment	*n*	*PGEM Range*	*mean ± SEM*	*% of control*
Control (F12:DMEM)	4	29-118	69 ± 19	100
IL-1 β (10 U/ ml)	4	29-80	59 ± 11	92 ± 8
IL-1 β (100 U/ ml)	4	26-70	55 ± 10	86 ± 9

Data expressed as mean in ng/ 3.0 $X10^5$ cells (± SEM). Range is also in pg/ml.
Percent of the control refers to the data in each experiment as a percent of the control of that experiment. These data were then presented as mean of the percent data ± SEM.
n represents the number of different tissues studied, each of which was incubated in quadruplicate with each treatment.
p= 0.7626 by one factor Anova with multi-comparison significance level of 95%.

Effect of IL-1β on cultured amnion and chorion cells

In order to verify that interleukin-1 was capable of stimulating prostaglandin output in our culture system, we examined the effect of this cytokine on cultured cells. Both amnion and chorion laeve cells were cultured for 5 days. IL-1β was added on the fifth day of culture for 24 hours. As is clearly evident in Fig. 3-7 for amnion, and Fig. 3-8 for chorion, IL-1β did indeed stimulate prostaglandin output by both of these cultures in a dose-dependent fashion.

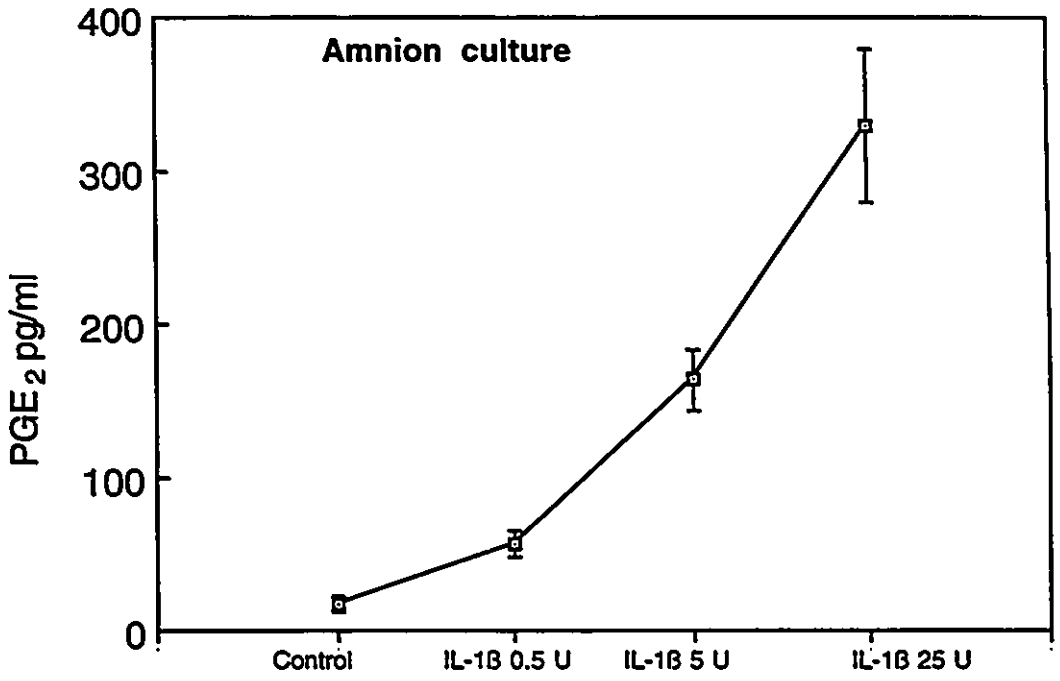


Fig. 3-7

The effect of IL-1β on PGE_2 production by human amnion cells cultured for five days in pre-labour tissue

Data presented as mean ± SD. For each tissue quadruplicate wells were used for each concentration of IL-1. This represents a typical experiment which was repeated with 2 other cultures.
$p<0.05\%$ by Anova, n=3.

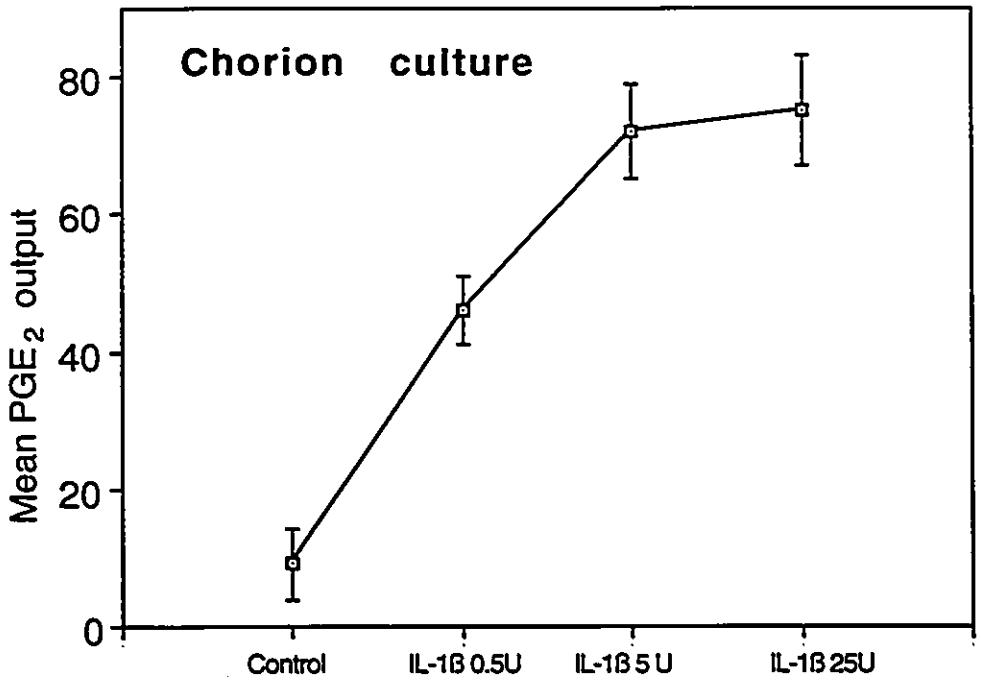


Fig. 3-8

The influence of IL-1β on PGE_2 output by human chorion cells cultured for five days

Data presented as mean ± SD. For each tissue quadruplicate wells were used for each concentration of IL-1. This represents a typical experiment which was repeated with 2 other cultures.
$p<0.05\%$ by Anova.

Conclusion of the effect of IL-1α and IL-1β on PG production by amnion, chorion and decidua cells

Incubation of freshly dispersed cells from amnion, chorion laeve, and decidua vera with IL-1α or IL-1β did not alter their PGE_2 or $PGF_{2\alpha}$ production, either in the first or in the second 24 hours of culture. The basal output of prostaglandin by the control cells was extremely variable, ranging from very high to undetectable levels. However, in no instance was IL-1 found to alter prostaglandin output even when the basal output was below the detection limits of our assays. In addition, no single tissue responded to these interleukins by increasing PG output. However, confluent amnion cells (cells cultured for 5 days) did show an increase in PGE_2 production in response to IL-1β, confirming the previously published results (Lundin-Schiller and Mitchell 1991 *a*).

In addition to PGE_2, $PGF_{2\alpha}$ production by the decidua was also analyzed. Similar to PGE_2 output, IL-1α and IL-1β did not influence $PGF_{2\alpha}$ production by these cells.

There is a clear trend of a decrease in PG production from amnion cells from the first to the second day of culture, whereas there is an increase of the amount of PGE_2 produced by the chorion laeve cells. In the case of decidua cells, there was no significant difference in PGE_2 output between the first and second day of culture.

In the first day of culture, PGE_2 output by amnion was significantly

higher after labour than before the onset of labour, whereas the chorion cell production of PGE_2 was lower after labour than before the onset of labour. Decidua on the other hand showed no significant difference in PG output before and after labour. These differences in PG output by amnion and chorion appeared to level off in the second day of culture. The presence and absence of labour and the mode of delivery had no effect on the response of the tissues to interleukin-1; albeit, it did effect the amount of PG produced by untreated cells.

3.01.2b Effect of IL-6 on prostaglandin production in the first and second 24 hours of culture

IL-6 is a major mediator of the host response to infection and tissue damage. Studies have demonstrated that IL-6 in amniotic fluid originates from both the mother and the fetus (Romero *et al.* 1989) and IL-6 levels in amniotic fluid are higher in preterm labour with intra-amniotic infection than that without infection (Romero *et al.* 1993) Maternal plasma levels of IL-6 are significantly higher in infection associated preterm labour than the matched control (Laham *et al.* 1993). Since IL-1 stimulates IL-6 production by the membranes (Kauma 1994) and IL-6 can stimulate PG formation (Mitchell *et al.* 1991 *b*), it was possible that IL-6 production by the tissues was necessary to stimulate PG production. Therefore, the effect of IL-6 on freshly dispersed cells from these tissues was examined.

IL-6 was added to dispersed cells in final concentrations of 1, 10, 100 and 1000 units per ml in the first or the second 24 hours of culture in a similar fashion to the methods described for IL-1α and IL-1β. The media collected from amnion, chorion and decidua were assayed for PGE_2 by RIA. In addition, $PGF_{2\alpha}$ was assayed for decidua cells only.

PGE_2 production by amnion cells in the first 24 hours of culture

PGE_2 production by untreated amnion cells in the first 24 hours was significantly higher after the onset of labour. Therefore, the data were stratified into pre and post-labour. Eight tissues were studied; five after the onset of labour and three before the onset of labour (Fig. 3-9). The mode of delivery did not influence the response of these cells to IL-6; p=0.8481 for post-labour and p=0.3881 for pre-labour. The data were examined as a percent of the control. The controls of the post-labour data ranged between 0 and 275 pg/ 3 X 10^5 cells. The controls of the pre-labour data ranged between 0 and 194 pg/ 3 X 10^5 cells.

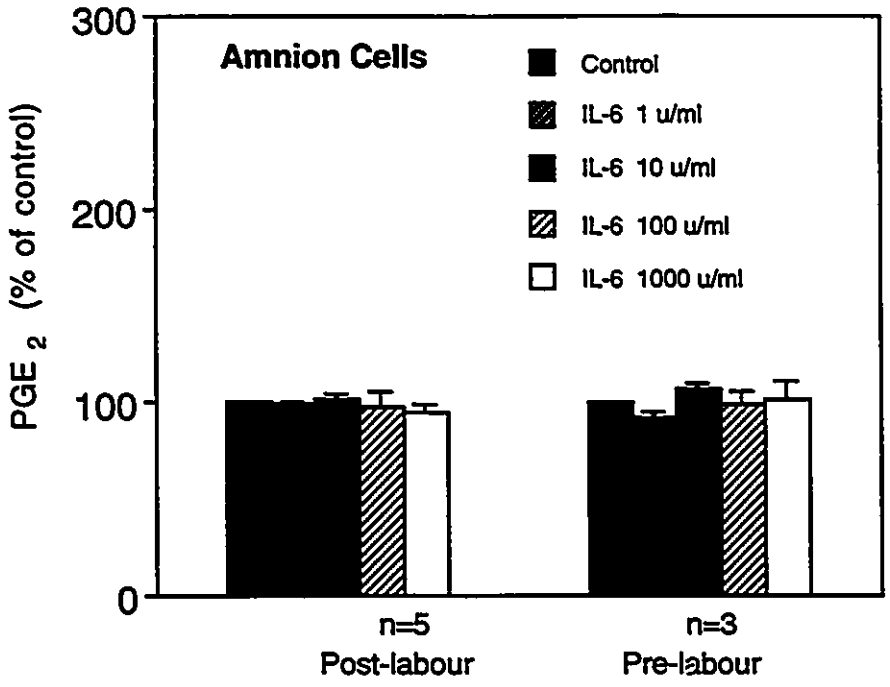


Fig. 3-9

PGE_2 production by amnion cells after 24 hours incubation with IL-6. Data stratified according to the mode of delivery

Data expressed as percent of the control of that experiment ± SEM.
n; refers to the number of experiments, each done in quadruplicate. Five experiments were included in the post-labour bar graph, and three in the pre-labour one. p= 0.8481 and 0.3881 by one way Anova.

PGE_2 production by amnion cells in the second 24 hours of culture

This incubation with IL-6 was done in the second 24 hours after changing the media. Using one way Anova, PGE_2 production showed no significant difference between treated and control cells in the second 24 hours ($p = 0.9073$). These data are summarized in Table 3-18. Eight tissues were studied; five after the onset of labour and three before the onset of labour.

Table 3-18
PGE_2 production by amnion cells in the second 24 hours of incubation with IL-6

Second 24 hours

Treatment	*n*	*PGE_2 Range*	*mean ± SEM*	*% of control*
Control (F12:DME)	8	0 - 135	43 ± 21	100
IL-6 (1 U/ ml)	7	0 - 158	51 ± 32	120 ± 10
IL-6 (10 U/ ml)	8	0 - 122	37 ± 17	113 ± 53
IL-6 (100 U/ ml)	8	0 - 140	45 ± 22	123 ± 43
IL-6 (1000 U/ ml)	8	0 - 139	41 ± 20	95 ± 68

$p = 0.9073$ using one way Anova

One experiment yielded undetectable levels of PGE_2 and was not included in the statistical analysis.
Data expressed as mean in pg/ 3.0 $X10^5$ cells(± SEM).
Percent of the control refers to the data in each experiment as a percent of the control of that experiment. These data were then presented as mean of the percent data ± SEM
n represents the number of different tissues examined each of which was studied in quadruplicate at each interleukin concentration.

PGE_2 production by chorion cells in the first 24 hours of culture

The chorion cells were treated with IL-6 in a similar way to the amnion. The production of PGE_2 by untreated cells after labour was significantly less than pre-labour. Therefore the data was further divided into pre-labour and post-labour Fig. 3-10. No significant change in PGE_2 was observed in either group ($p = 0.7993$ for post-labour). There was only one experiment involving pre-labour tissue. The control of the post-labour data ranged between 0 and 11 pg/ 3 X 10^5 cells. The control of the pre-labour data was 179 pg/ 3 X 10^5 cells.

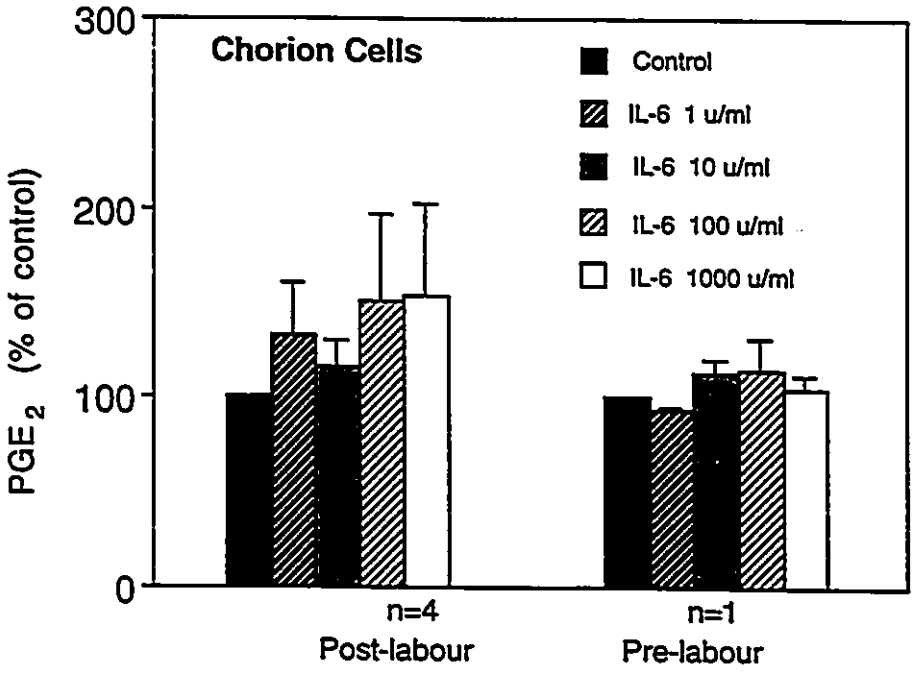


Fig. 3-10

PGE_2 production by chorion laeve cells after 24 hours incubation with IL-6. Data stratified according to the mode of delivery

Data expressed as percent of the control of that experiment ± SEM.
n; refers to the number of experiments, each in quadruplicate wells per treatment. Four experiments were included in the post-labour bar graph, and one in the pre-labour one. p= 0.7993 by Anova for the post-labour group.

PGE_2 production by chorion cells in the second 24 hours of culture

PGE_2 production was determined by RIA in the second day of culture, and after 24 hours incubation with IL-6 in final concentrations of 1, 10, 100, 1000 units per ml. The data is summarized in Table 3-19. Five tissues were studied: four after the onset of labour and one before the onset of labour by cesarean section. Using one way Anova, the results showed no significant difference in PGE_2 production in the second 24 hours ($p = 0.9031$)

Table 3-19
PGE_2 production by chorion cells in the second 24 hours of incubation with IL-6

Second 24 hours

Treatment	*n*	*PGE_2 Range*	*mean ± SEM*	*% of control*
Control (F12:DME)	5	0 - 113	51 ± 25	100
IL-6 (1 U/ ml)*	2	0 - 26	13 ± 13	(79 ± 6)
IL-6 (10 U/ ml)	4	0 - 124	63 ± 30	93 ± 16
IL-6 (100 U/ ml)	5	0 - 128	56 ± 29	102 ± 19
IL-6 (1000 U/ ml)	5	0 - 157	60 ± 32	110 ± 31

$p = 0.9031$ using one way Anova

*The number of experiments were low. Therefore they were not included in the Anova analysis; their percent of control is indicated between two brackets.
One experiment yielded undetectable levels of PGE_2 and was not included in the statistics.
Data expressed as mean in pg/ 3.0 $X10^5$ cells (± SEM). Range is also in pg/ml.
Percent of the control refers to the data in each experiment as a percent of the control of that experiment. These data were then presented as mean of the percent data ± SEM.
n represents the number of different tissues examined each in quadruplicate.

PGE_2 production by decidua cells in the first and second 24 hours of culture

Decidua cells were treated with IL-6 at concentrations of 1, 10, 100, and 1000 units per ml either in the first 24 hours or second 24 hours of culture. The data obtained is summarized in Table 3-20. Three tissues were studied; two after the onset of labour and one before the onset of labour. There was no significant difference in PGE_2 production in pre- and post-labour group. Using one way Anova, no significant difference was detected in PGE_2 production in response to IL-6 treatment in the first 24 hours ($p = 0.9346$), nor in the second 24 hours ($p = 0.923$).

Table 3-20
PGE_2 production by decidua cells in the first and second 24 hours of incubation with IL-6

First 24 hours

Treatment	*n*	*PGE_2 Range*	*mean ± SEM*	*% of control*
Control (F12:DME)	3	10 - 42	30 ± 10	100
IL-6 (1 U/ ml)*	2	16 - 42	29 ± 13	(127 ± 42)
IL-6 (10 U/ ml)	3	14 - 37	23 ± 7	107 ± 78
IL-6 (100 U/ ml)	3	20 - 48	30 ± 9	127 ± 74
IL-6 (1000 U/ ml)	3	9 - 57	36 ± 14	115 ± 34

p = 0.9346 using one way Anova

Second 24 hours

Treatment	*n*	*PGE_2 Range*	*mean ± SEM*	*% of control*
Control (F12:DME)	3	0 - 136	52 ± 42	100
IL-6 (1 U/ ml)*	2	0 - 20	10 ± 10	(97 ± 42)
IL-6 (10 U/ ml)	3	0 - 122	48 ± 38	95 ± 7
IL-6 (100 U/ ml)	3	0 - 83	36 ± 25	93 ± 44
IL-6 (1000 U/ ml)	3	0 - 83	31 ± 26	53 ± 12

p = 0.923 using one way Anova

*The number of experiments was low and one experiment yielded undetectable levels of PGE_2 and was not included in the Anova analysis. Their percent of control is put between two brackets.
Value below the detectable limits of the assay were not included in the statistics.
Data expressed as mean in pg/ 3.0 $X10^5$ cells (± SEM). Range is also in pg/ml.
Percent of the control refers to the data in each experiment as a percent of the control of that experiment. These data were then presented as mean of the percent data ± SEM.
n represents the number of different tissues examined each in quadruplicate.

$PGF_{2\alpha}$ production by decidua cells in the first and second 24 hours of culture

In addition to PGE_2, $PGF_{2\alpha}$ output by decidua cells treated with IL-6 for 24 hours was measured. These cells were treated in a similar fashion to the decidua cells in the previous sections. Table 3-21 summarizes the data of $PGF_{2\alpha}$ output by decidua cells in the first and second 24 hours of incubation with IL-6. In the first 24 hours, five tissues were studied; four after the onset of labour and one before the onset of labour. In the second 24 hours, three tissues were studied; two after the onset of labour and one before the onset of labour. Using one way Anova, the results showed no significant difference between treatment groups in $PGF_{2\alpha}$ production in the first 24 hours ($p = 0.9513$), nor in the second 24 hours ($p = 0.9897$).

Table 3-21
$PGF_{2\alpha}$ production by decidua cells in the first and second 24 hours of incubation with IL-6

First 24 hours

Treatment	*n*	*$PGF_{2\alpha}$ Range*	*mean ± SEM*	*% of control*
Control (F12:DME)	5	0 - 127	37 ± 23	100
IL-6 (1 U/ ml)*	3	0 - 38	16 ± 11	(111 ± 57)
IL-6 (10 U/ ml)	4	0 - 140	49 ± 31	126 ± 22
IL-6 (100 U/ ml)	5	0 - 125	36 ± 23	90 ± 28
IL-6 (1000 U/ ml)	5	0 - 131	37 ± 24	98 ± 8

p = 0.9513 using one way Anova

Second 24 hours

Treatment	*n*	*$PGF_{2\alpha}$ Range*	*mean ± SEM*	*% of control*
Control (F12:DME)	3	0 - 43	14 ± 14	100
IL-6 (10 U/ ml)	3	0 - 53	18 ± 17	123 ± 16
IL-6 (100 U/ ml)	3	0 - 34	12 ± 11	79 ± 7
IL-6 (1000 U/ ml)	3	0 - 38	13 ± 13	89 ± 11

p = 0.9897 using one way Anova

*The number of experiments was low and one experiment yielded undetectable levels of $PGF_{2\alpha}$ and were not included in the statistical analysis. Their percent of control is put between two brackets.
Value below the detectable limits of the assay were not included in the statistics.
Data expressed as mean in pg/ 3.0 $X10^5$ cells.(± SEM). Range is also in pg/ml.
Percent of the control refers to the data in each experiment as a percent of the control of that experiment. These data were then presented as mean of the percent data ± SEM.
n represents the number of different tissues examined each in quadruplicate.

Summary of the effect of IL-6 on PGE_2 production in the first and second 24 hours of culture

Incubation with IL-6 for 24 hours did not influence PGE_2 production by freshly dispersed cells from amnion, chorion, or decidua in the first or second 24 hours of culture, nor did IL-6 alter the production of $PGF_{2\alpha}$ by the decidua cells. At this point we went on to examine the responsiveness of our *in-vitro* system.

3.01.2c Response of fetal membranes and decidua to other substances

In order to verify the responsiveness of the fresh cell preparation from amnion, chorion, and decidua, negative and positive controls were examined. Prostaglandin production by the fresh cells was examined in response to dexamethasone and phorbol ester (TPA).

Dexamethasone has been shown to inhibit PG production by freshly dispersed cells (Gibb and Breton 1993, Potestio *et al.* 1988, Delvin *et al.* 1990). The mechanism of this inhibition is mainly through decreased prostaglandin endoperoxide H synthase activity (Riley *et al.* 1992). Dexamethasone blocks aromatase and phospholipase A_2 enzyme activities (Chatterjee *et al.* 1993), and inhibit the production of PGE_2 from exogenous arachidonate in a concentration dependent fashion (Zakar *et al.* 1994).

TPA; is 12-0-tetradecanoyl phorbol-13-acetate derived from alcohol phorbol obtained from croton oil. TPA is a potent tumor promotor. In addition to its effect on cell growth and development, TPA stimulates prostaglandin production by amnion (Mitchell 1991 *b*) by activation of the Phospholipase C/Protein Kinase C cascade system (Moore *et al.* 1993).

Effect of dexamethasone

Table 3-22 summarizes the data of PGE_2 production by amnion cells in the first and second 24 hours of incubation with dexamethasone at 10^{-7}M. In the first 24 hour of culture, freshly dispersed cells from amnion incubated with dexamethasone at a final concentration of 10^{-7} M showed a highly significant inhibition of PGE_2 production when compared to the control ($p= 0.0014$ using one-tailed unpaired t-test). However, in the second 24 hours of culture, three of the eight tissues did not show inhibition. Therefore, the uncorrected inhibition was not significant ($p= 0.106$), although when corrected for outlier numbers, it was highly significant ($p = 0.0001$). Chorion cells cultured with dexamethasone (10^{-7}M) for 24 hours also demonstrated a highly significant inhibition of PGE_2 production both in the first and second 24 hours of culture ($p <0.0001$ for both), see Table 3-23. Similarly, the decrease in decidua cells production of PGE_2 was highly significant ($p < 0.001$) both in the first and second 24 hours (Table 3-24).

Table 3-22
PGE_2 production by amnion cells in the first and second 24 hours of incubation with dexamethasone (Dex) at 10^{-7}M

First 24 hours

Treatment	*n*	*PGE_2 Range*	*mean ± SEM*	*% of control*
Control (F12:DME)	10	12 - 653	160 ± 74	100
Dex (10^{-7} M)	10	8 - 262	82 ± 24.7	61.3 ± 11

p=0.0014 Student t-test.

Second 24 hours

Treatment	*n*	*PGE_2 Range*	*mean ± SEM*	*% of control*
Control (F12:DME)	8	0 - 145	43 ± 19	100
Dex (10^{-7} M)	8	0 - 46	16 ± 5.	77 ± 17

p=0.106 Student t-test.

One experiment yielded undetectable levels of PGE_2 and was not included in the statistics.
Data expressed as mean in pg/ 3.0 $X10^5$ cells(± SEM). Range is also in pg/ml.
Percent of the control refers to the data in each experiment as a percent of the control of that experiment. These data were then presented as mean of the percent data ± SEM.
n represents the number of different tissues examined, each of which was studied in quadruplicate.

Table 3-23
PGE_2 production by chorion cells in the first and second 24 hours of incubation with dexamethasone (Dex) at 10^{-7}M

First 24 hours

Treatment	*n*	*PGE_2 Range*	*mean ± SEM*	*% of control*
Control (F12:DME)	7	0 - 179	67 ± 24	100
Dex (10^{-7} M)	7	0 - 56	19 ± 8	28 ± 5

$p < 0.0001$ Student t-test.

Second 24 hours

Treatment	*n*	*PGE_2 Range*	*mean ± SEM*	*% of control*
Control (F12:DME)	7	0 - 1000	205 ± 100	100
Dex (10^{-7} M)	7	0 - 81	27 ± 9	21 ± 4

$p < 0.0001$ Student t-test.

One experiment yielded undetectable levels of PGE_2 and was not included in the statistics.
Data expressed as mean in pg/ 3.0 $X10^5$ cells.(± SEM). Range is also in pg/ml.
Percent of the control refers to the data in each experiment as a percent of the control of that experiment. These data were then presented as mean of the percent data ± SEM
n represents the number of different tissues examined, each of which was studied in quadruplicate.

Table 3-24
PGE_2 production by decidua cells in the first and second 24 hours of incubation with dexamethasone (Dex) at 10^{-7}M

First 24 hours

Treatment	*n*	*PGE_2 Range*	*mean ± SEM*	*% of control*
Control (F12:DME)	4	2 - 27	13 ± 5	100
Dex (10^{-7} M)	4	1 - 23	8 ± 5	54 ± 2

$p < 0.0001$ Paired student t-test.

Second 24 hours

Treatment	*n*	*PGE_2 Range*	*mean ± SEM*	*% of control*
Control (F12:DME)	7	0 - 328	87 ± 43	100
Dex (10^{-7} M)	7	0 - 132	44 ± 21	36 ± 5

$p < 0.0001$ Paired student t-test.

One experiment yielded undetectable levels of PGE_2 and was not included in the statistics.
Data expressed as mean in pg/ 3.0 $X10^5$ cells (± SEM). Range is also in pg/ml.
Percent of the control refers to the data in each experiment as a percent of the control of that experiment. These data were then presented as mean of the percent data ± SEM.
n represents the number of different tissues examined, each of which was studied in quadruplicate.

Amnion production of PGE_2 by untreated cells in the first 24 hours was significantly influenced by the mode of delivery, with much higher levels obtained after the onset of labour. Therefore, the amnion data were further stratified to pre and post-labour; these results are depicted in Fig. 3-11. Dexamethasone caused a significant inhibition in PGE_2 output in the pre-labour group and not the post-labour group when compared to the control (post-labour p value = 0.1279, and pre-labour p value = 0.0031 by student t-test).

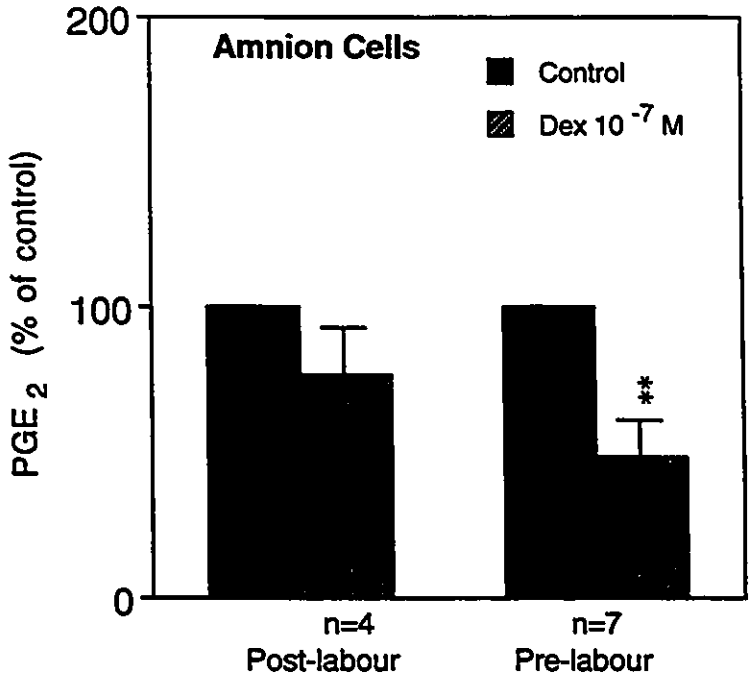


Fig. 3-11

PGE_2 production by amnion cells after 24 hours incubation with dexamethasone 10^{-7} M (Dex). Data stratified according to the mode of delivery

Data expressed as percent of the control of that experiment ± SEM.
n; refers to the number of experiments. Four experiments were included in the post-labour bar graph, and seven in the pre-labour one.
Each experiment was done in quadruplicate wells per treatment.
p= 0.1279 and 0.0031 (**) by t-test for post- and pre-labour groups respectively.

Untreated chorion cells had significantly less PGE_2 production after the onset of labour. Therefore, the chorion data were stratified to pre- and post-labour; these results are depicted in Fig. 3-12. Dexamethasone caused a significant inhibition in PGE_2 output in both groups compared to the control (post-labour p value = 0.0296, pre-labour p value = 0.0009 by Student t-test).

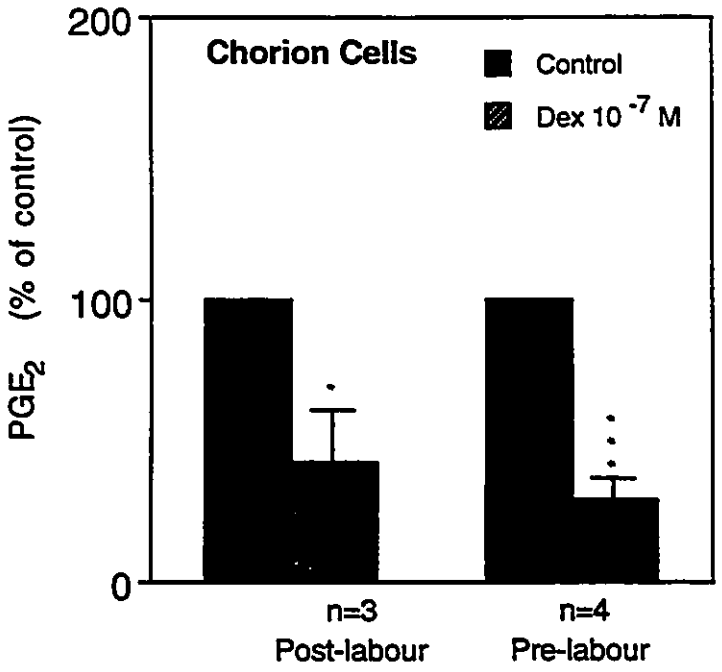


Fig. 3-12

PGE_2 production by chorion laeve cells after 24 hours incubation with dexamethasone 10^{-7} M (Dex). Data stratified according to the mode of delivery

Data expressed as percent of the control of that experiment ± SEM.
n; refers to the number of experiments. Three experiments were included in the post-labour bar graph, and four in the pre-labour one.
Each experiment was done in quadruplicate wells per treatment.
p= 0.0296 (*) and 0.0009 (***) by t-test for post and pre-labour groups respectively.

Effect of Phorbol Ester

TPA (phorbol ester) was added to freshly dispersed cells in three experiments; two after the onset of labour and one prior to the onset of labour. The dispersed amnion, chorion laeve, and decidua vera cells were cultured with TPA in final concentrations of 50 and 100 ng per ml for the first and second 24 hours. Amnion cells treated with TPA in the first 24 hours produced a significant increase in PGE_2 production over the control cells ($p < 0.05$) by Anova. Further, each concentration of TPA was compared to the control using Student t-test. Both 50 and 100 pg/ml concentrations caused a significant increase in PGE_2 output ($p= 0.0425$ and $p= 0.0221$ respectively); these results are presented in Table 3-25. In the second 24 hours, the amnion cells which were incubated with TPA for 24 hours produced significantly more PGE_2 when compared to the control ($p < 0.05$ by Anova). However, using student t-test, only stimulation with TPA 50 pg/ml was significant over the control ($p = 0.0144$), and not the higher concentration of TPA of 100 pg/ml ($p = 0.216$). The chorion cells PGE_2 production was not altered by incubation with TPA (50 or 100 pg/ml) for 24 hours in the first day, nor in the second day of culture ($p = 0.7888$ and $p = 0.6515$ respectively); these results are presented in Table 3-26. Phorbol ester did not influence PGE_2 production by decidua cells, neither in the first nor in the second 24 hours of culture ($p = 0.3775$ and $p = 0.4753$ respectively); these results are presented in Table 3-27.

Table 3-25

PGE_2 production by amnion cells after 24 hours incubation with TPA in the first and second day of culture

First 24 hours

Treatment	n	PGE_2 Range	Mean ± SEM	% of control	t-test
Control F12:DME	3	90 - 752	319 ± 217	100	
TPA 50 ng/ ml	3	173 - 850	407 ± 222	162 ± 31	p = 0.0425
TPA 100 ng/ ml	3	159 - 944	423 ± 261	150 ± 17	p = 0.0221

$p < 0.05$ Anova.

Second 24 hours

Treatment	n	PGE_2 Range	Mean ± SEM	% of control	t-test
Control F12:DME	3	16 - 206	89 ± 59	100	
TPA 50 ng/ ml	3	21 - 386	158 ± 115	155 ± 16	p = 0.0144
TPA 100 ng/ ml	3	22 - 391	148 ± 122	131 ± 36	p = 0.216

$p < 0.05$ Anova.

Data expressed as mean in pg/ 3.0 $X10^5$ cells (± SEM). Range is also in pg/ml.
Percent of the control refers to the data in each experiment as a percent of the control of that experiment. These data were then presented as mean of the percent data ± SEM.
n represents the number of different tissues examined each of which was studied in quadruplicate.

Table 3-26
PGE_2 production by chorion cells after 24 hours incubation with TPA in the first and second day of culture

First 24 hours

Treatment	*n*	*PGE_2 Range*	*Mean ± SEM*	*% of control*
Control F12:DME	3	5 - 201	70 ± 65	100
TPA 50 ng/ ml	3	4 - 716	242 ± 237	184 ± 86
TPA 100 ng/ ml	3	5 - 554	189 ± 182	168 ± 54

p = 0.7888 Anova.

Second 24 hours

Treatment	*n*	*PGE_2 Range*	*Mean ± SEM*	*% of control*
Control F12:DME	3	6 - 1660	559 ± 550	100
TPA 50 ng/ ml	3	4 - 4725	1582 ± 1571	164 ± 64
TPA 100 ng/ ml	3	4 - 4305	2480 ± 1431	154 ± 55

p = 0.6515 Anova.

Data expressed as mean in pg/ 3.0 $X10^5$ cells(± SEM). Range is also in pg/ml.
Percent of the control refers to the data in each experiment as a percent of the control of that experiment. These data were then presented as mean of the percent data ± SEM
n represents the number of different tissues examined each of which was studied in quadruplicate.

Table 3-27
PGE_2 production by decidua cells after 24 hours incubation with TPA in the first and second day of culture

First 24 hours

Treatment	n	PGE_2 Range	Mean ± SEM	% of control	t-test
Control F12:DME	3	7 - 2080	670 ± 690	100	
TPA 50 ng/ ml	3	9 - 448	158 ± 145	98 ± 38	p = 0.9566
TPA 100 ng/ ml	3	6 - 324	115 ± 104	170 ± 54	p = 0.3261

p = 3775 Anova.

Second 24 hours

Treatment	n	PGE_2 Range	Mean ± SEM	% of control	t-test
Control F12:DME	3	4 - 9199	3069 ± 3064	100	
TPA 50 ng/ ml	3	7 - 9318	3116 ± 3101	145 ± 109	p = 0.3155
TPA 100 ng/ ml	3	5 - 6356	2126 ± 2115	178 ± 83	p = 0.4447

p = 4753 Anova.

Data expressed as mean in pg/ 3.0 $X10^5$ cells (± SEM). Range is also in pg/ml.
Percent of the control refers to the data in each experiment as a percent of the control of that experiment. These data were then presented as mean of the percent data ± SEM.
n represents the number of different tissues examined each of which was studied in quadruplicate.

Amnion production of PGE_2 by untreated cells in the first 24 hours was significantly influenced by the mode of delivery, with much higher levels obtained after the onset of labour. Therefore, the amnion data were further stratified to pre-labour (n=1) and post-labour (n=2). As depicted in Fig. 3-13, results clearly show a trend of increase in PGE_2 production in both populations.

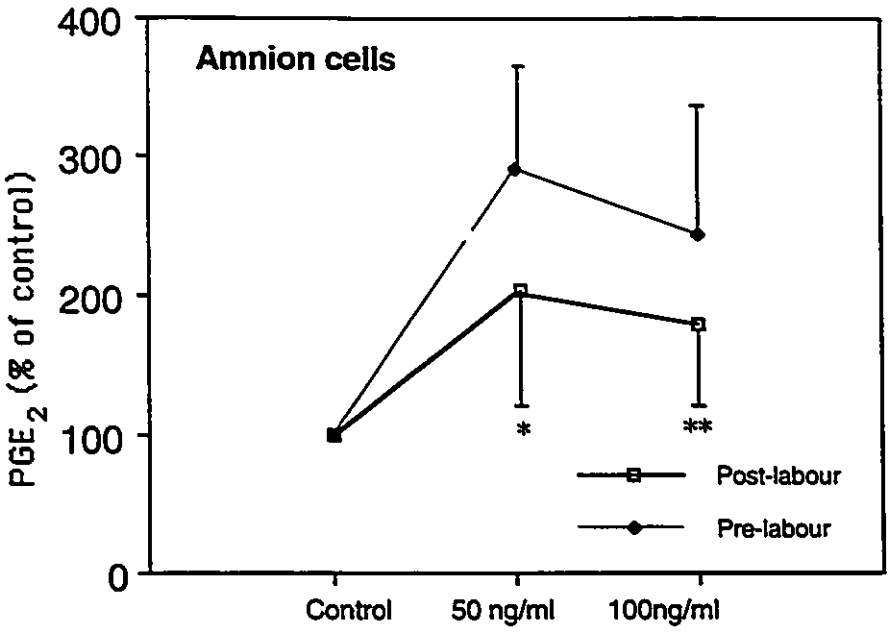


Fig. 3-13

PGE_2 production by amnion cells after 24 hours incubation with phorbol ester (TPA) at a concentration of 50 and 100 ng/ml. Data stratified according to the mode of delivery

Data expressed as percent of the control of that experiment ± SD.
n; refers to the number of experiments. Two experiments were included in the post-labour group, and one in the pre-labour group.
Each experiment was done in quadruplicate wells per treatment.
p= 0.0217 (*) and 0.0268 (**) by t-test for TPA 50 and 100 ng/ml respectively for post-labour tissue.

Untreated chorion cells on the other hand had significantly less PGE_2 production after the onset of labour. Therefore, the chorion data was stratified to pre-labour (n=1) and post-labour (n=2). As depicted in Fig. 3-14, there was no significant increase of PGE_2 output in response to TPA.

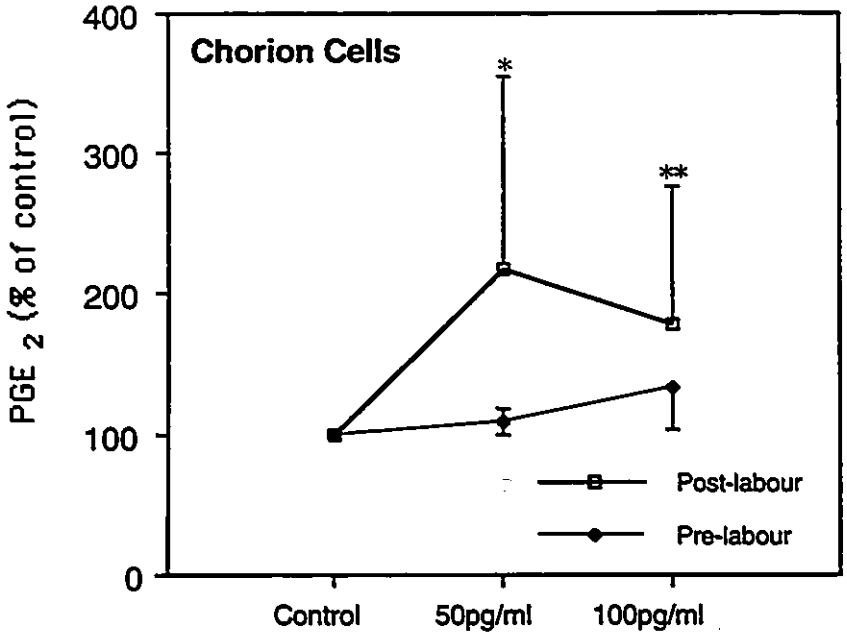


Fig. 3-14

PGE_2 production by chorion laeve cells after 24 hours incubation with phorbol ester (TPA) at a concentration of 50 and 100 ng/ml. Data stratified according to the mode of delivery

Data expressed as percent of the control of that experiment ± SD.
n; refers to the number of experiments. Three experiments were included in the post-labour group, and four in the pre-labour group.
Each experiment was done in quadruplicate wells per treatment.
p= 0.2748 (*) and 0.286 (**) by t-test for 50 and 100 ng/ml respectively for post-labour tissue.

3.02 Interleukin-1 Receptor Distribution on Human Fetal Membrane and Decidua

Intact tissue

IL-1 receptors in undisrupted fetal membranes and decidua were localized by incubating the tissues with increasing amounts of ^{125}I-IL-1α (in final concentrations of 50, 75 and 100 pM). Eight tissues were examined: 5 after labour and 3 before labour. Film autoradiography of a roll of the undisrupted three tissues demonstrated a linear pattern of IL-1 binding which appeared to involve a single layer of the fetal membranes or decidua. The labeling elicited a dose dependent increase in intensity and was eliminated in the presence of 1000-fold excess of unlabeled IL-1β (nonspecific binding), see Fig. 3-15.

To further localize the binding, each of the fetal membranes (amnion and chorion laeve) and decidua were examined separately. The three tissues were separated and IL-1 receptor labeling was carried out as before. The film autoradiography of dissected tissue, showed that the amnion had no detectable IL-1 binding, and was strongly suggestive of IL-1 binding in the chorion. The decidua showed less binding, see Fig. 3-16.

A B

Fig. 3-15

Film autoradiography of undisrupted tissues following incubation with ^{125}I-IL-1α at a concentration of 50 pM. A is the total IL-1 binding, and B is the non-specific binding.

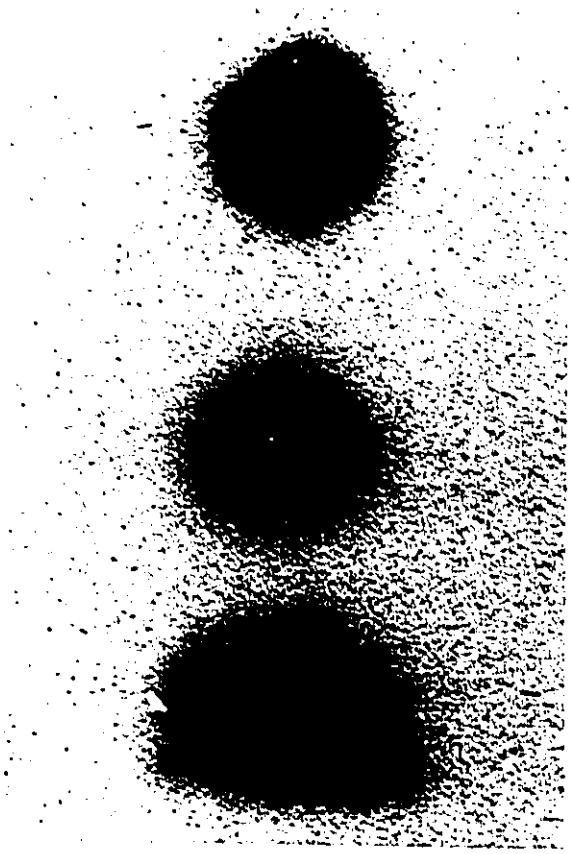


Fig. 3-16

Film autoradiography of separated tissues incubated with ^{125}I-IL-1α at a concentration of 50 pM. W is whole tissue, A is amnion, C is chorion laeve, and D is decidua vera.

In order to visualize the distribution of the putative IL-1 receptor in relation to the underlying cytoarchitecture, we carried out emulsion autoradiography. The histological integrity of the tissue preparation was first confirmed (Fig. 3-17). Using emulsion autoradiography, IL-1 binding was concentrated in the chorion laeve layer rather than the decidua (Figs 3-18, and 3-19) and seemed to target certain populations of cells (Fig. 3-20). The distribution appeared patchy and varied from one area to another (Fig. 3-21). The binding pattern and intensity varied from one tissue to another but amnion tissue did not show any evidence of ^{125}I-IL-1α binding (Fig. 3-22).

The absence of IL-1 receptors in the amnion (and possibly decidua vera) may explain our earlier findings of the inability of IL-1α and IL-1β to stimulate PG release by these tissues. However, receptors appeared to be present in the chorion laeve, yet we were unable to demonstrate any increase in prostaglandin production from freshly dispersed preparations of this tissue. We therefore examined the dispersed cell preparation for evidence of IL-1 binding.

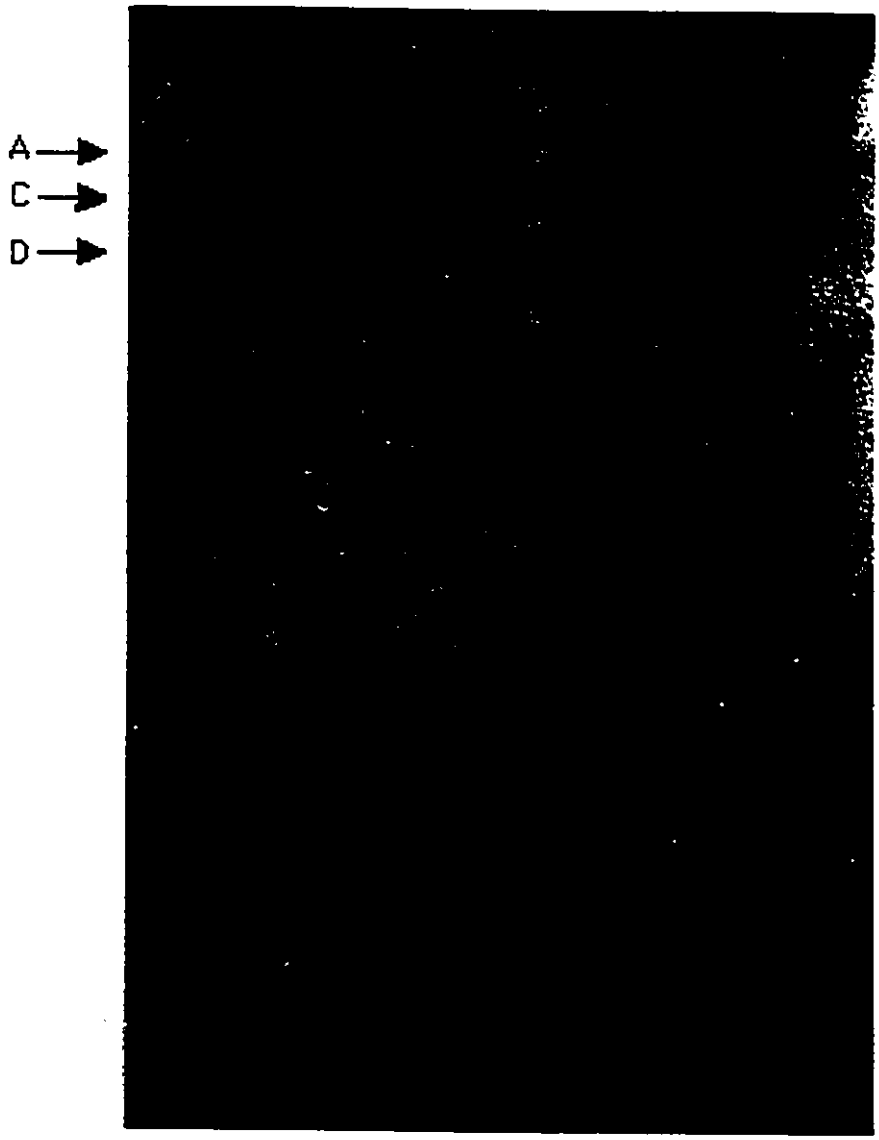


Fig. 3-17

Histological examination of undisrupted tissues with H&E staining. Magnification X 6. A = amnion, C = chorion, D= Decidua.

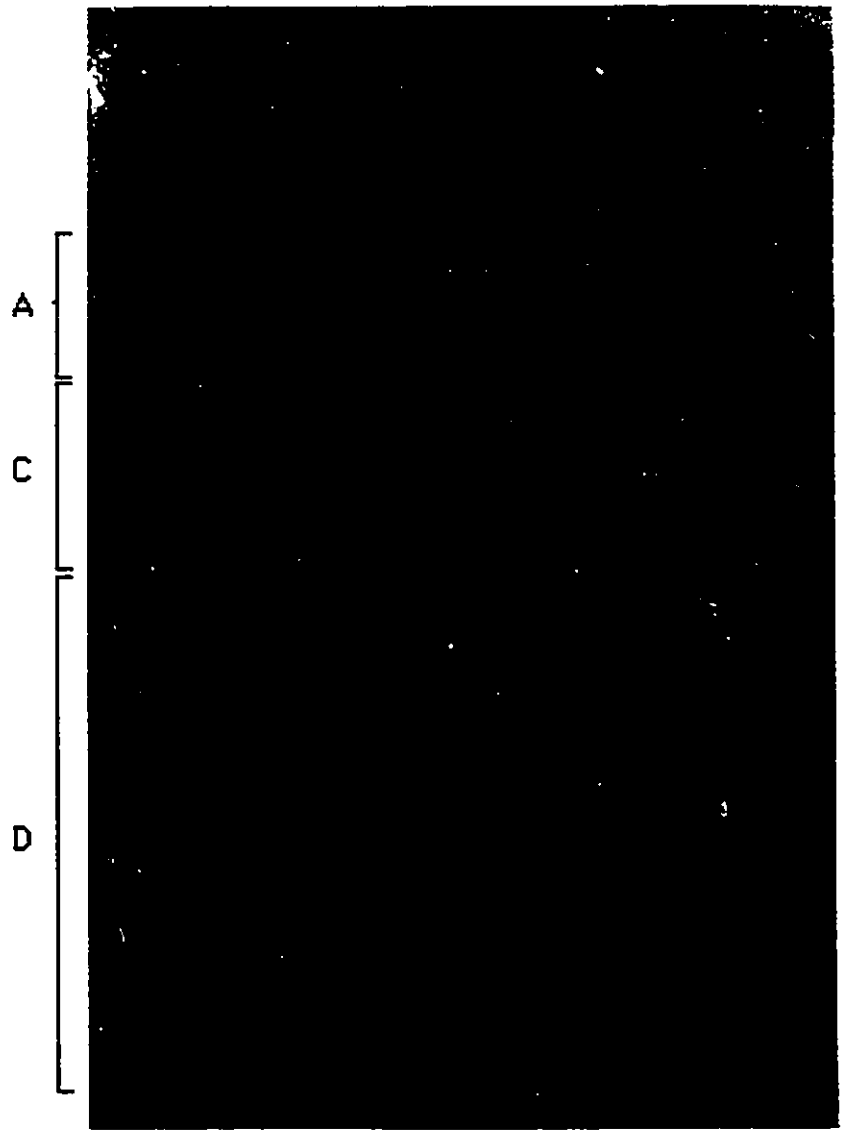


Fig. 3-18

Emulsion autoradiography of undisrupted tissues incubated with ^{125}I-IL-1α at a final concentration of 50 pM. The tissue were stained with H&E. Magnified X 100. A = amnion, C = chorion, D= Decidua.

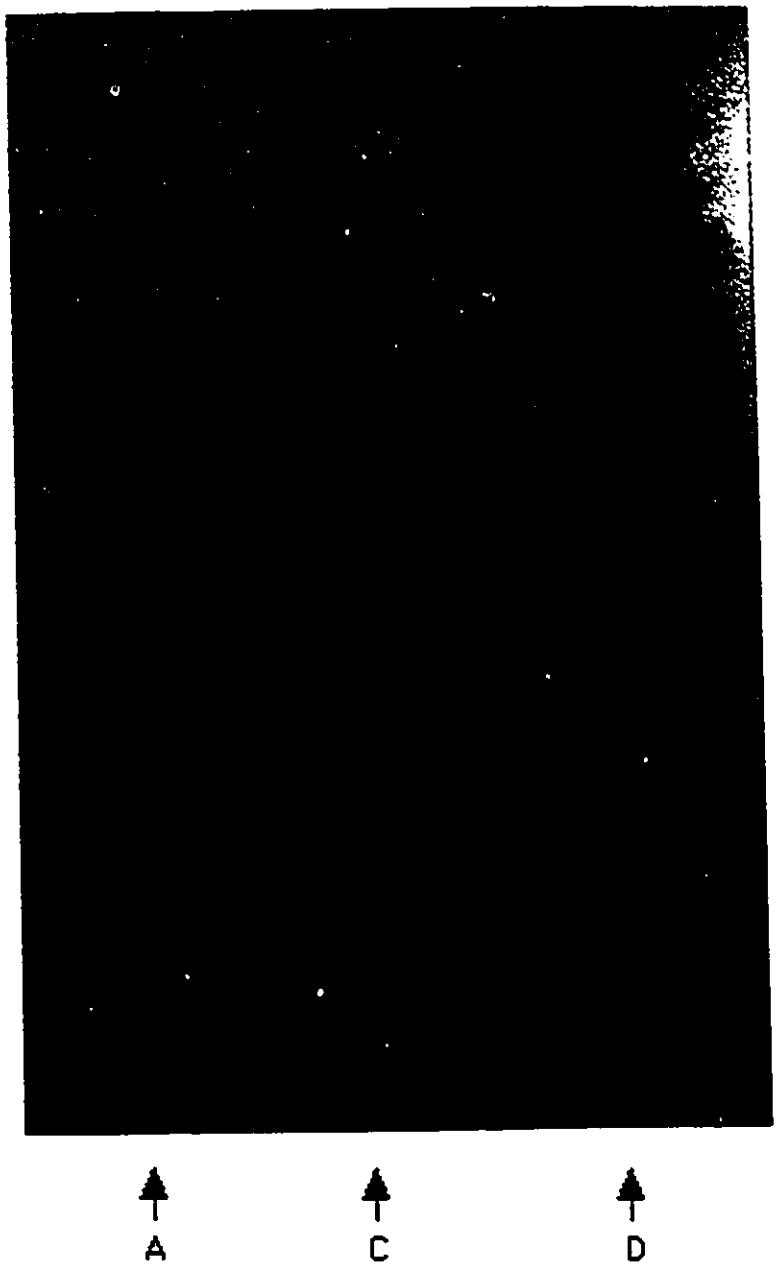


Fig. 3-19

Emulsion autoradiography of undisrupted tissues incubated with ^{125}I-IL-1α at a final concentration of 50 pM. The tissue were stained with H&E. Magnified X 100. A = amnion, C = chorion, D= Decidua.

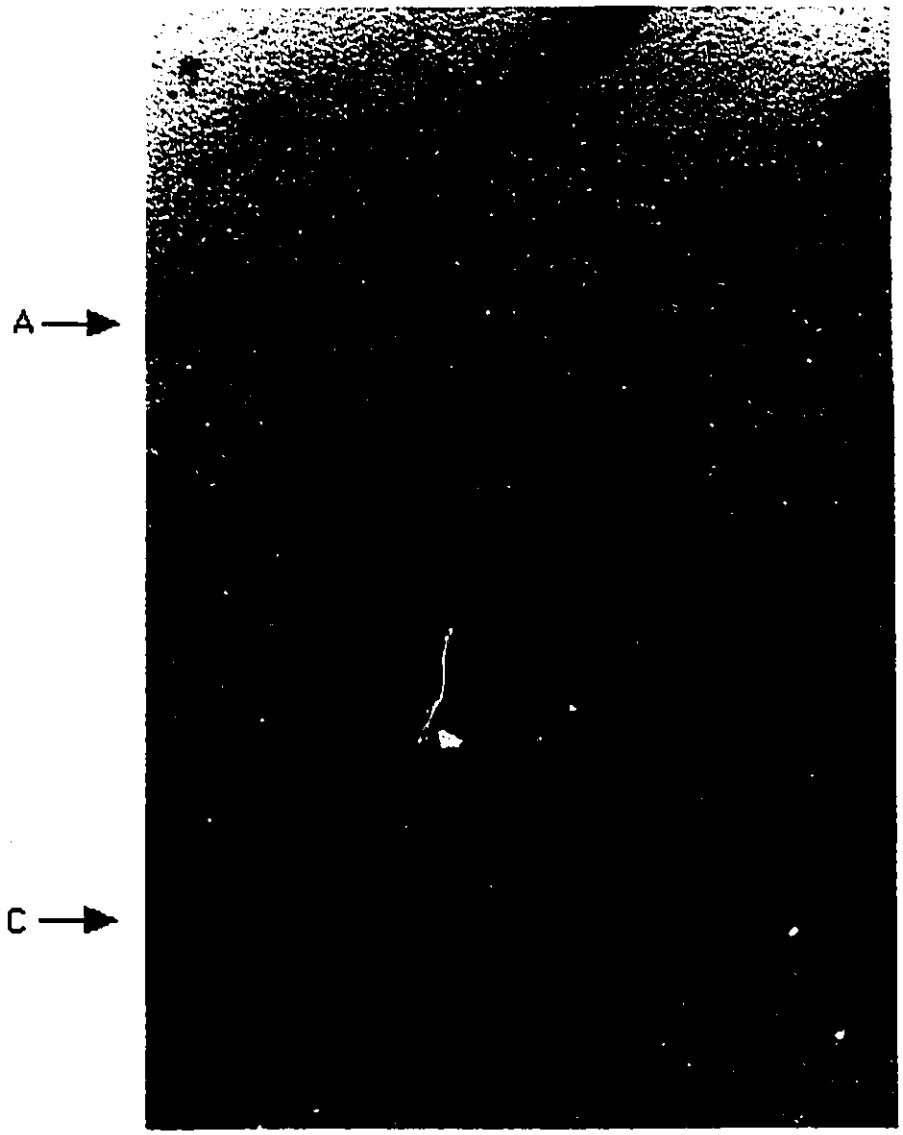


Fig. 3-20

Emulsion autoradiography of undisrupted tissues incubated with ^{125}I-IL-1α at a final concentration of 50 pM. The tissues were stained with H&E. Magnified 400 X. Receptor labeling in chorion appears to be targeting a certain population. A = amnion, C = chorion.

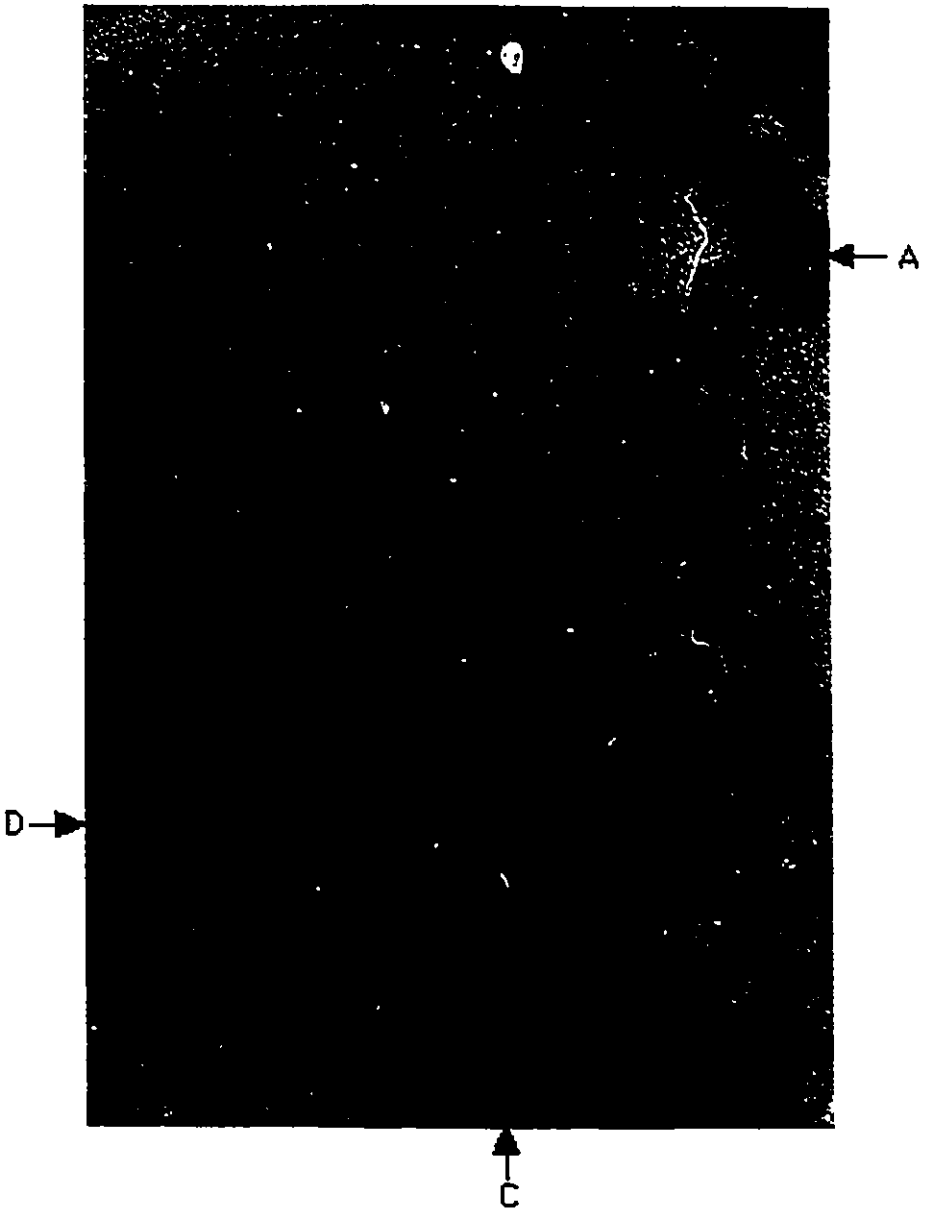


Fig. 3-21

Emulsion autoradiography of undisrupted tissues incubated with ^{125}I-IL-1α at a final concentration of 50 pM. The tissues were stained with H&E. Magnified X 100. The distribution of IL-1 receptors appears patchy. A = amnion, C = chorion, D = Decidua.

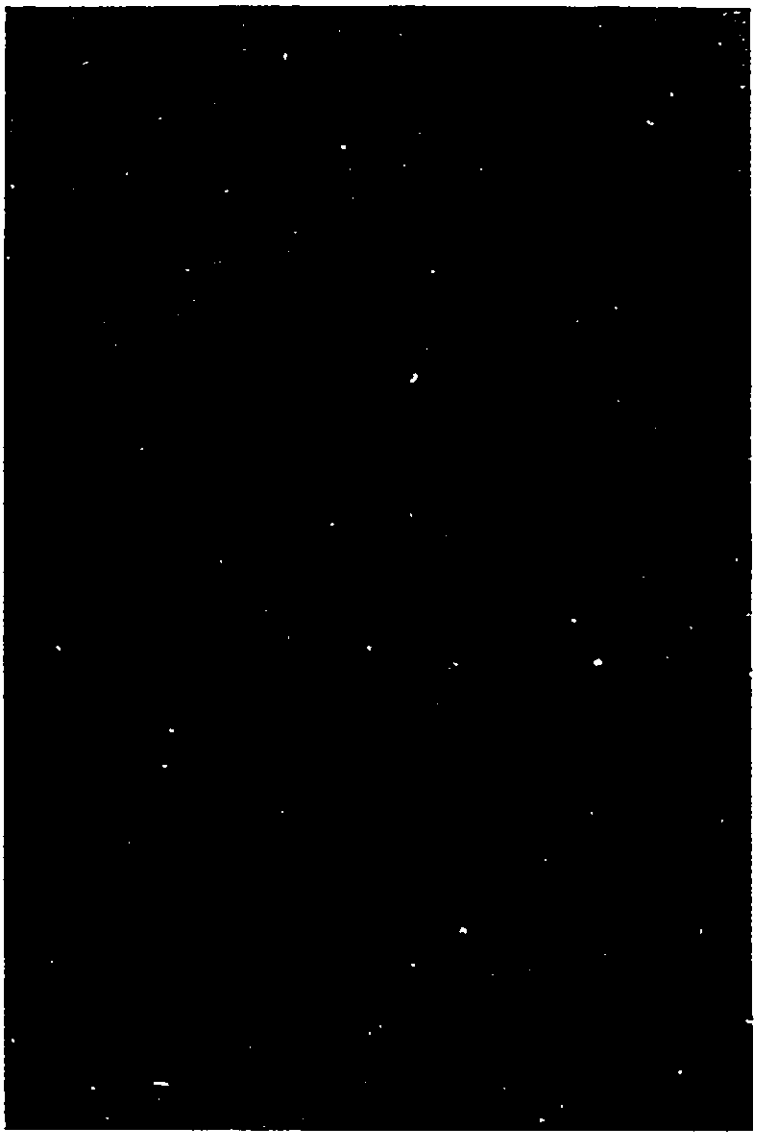


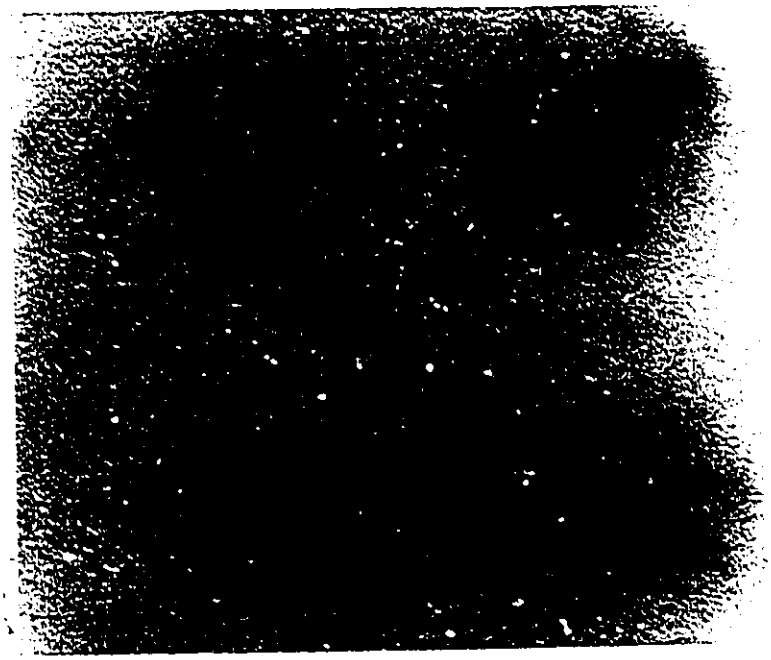
Fig. 3-22

Emulsion autoradiography of intact amnion tissues incubated with ^{125}I-IL-1α at a final concentration of 50 pM. The tissues were stained with H&E. Magnified X 400

Dispersed cells

In order to check the integrity of IL-1 receptors on the dispersed cells from the chorion laeve, IL-1 receptor labeling was carried out on freshly dispersed cells, after 24 and 48 hours of culture of chorion laeve. Three tissues were used; two after the onset of labour and one prior to the onset of labour.

Non-specific binding was determined as described previously in the presence of 1000-fold excess of unlabeled IL-1β . Dispersed amnion cells were studied simultaneously as a negative control, because the undisrupted tissue preparation showed no labeling. The labeling procedure used was the same as that used with the whole tissue, after culturing the cells on slides in water saturated atmosphere. Film autoradiography of dispersed cells indicated that the labeling is present in the chorion laeve cells area rather than in the amnion cells area (Fig. 3-23). Next, emulsion autoradiography was carried out on the chorion and amnion cells; immediately, in the first, and in the second 24 hours of cultures. These experiments indicated the presence of labeling on the chorion cells, and not in the amnion (Fig. 3-24). This labeling could be eliminated in the presence of 1000-fold excess of unlabeled IL-1β . The intensity of the IL-1 receptor labeling varied, some cells exhibited dense labeling while others showed none (Fig. 3-25, Fig. 3-26, and Fig. 3-27).

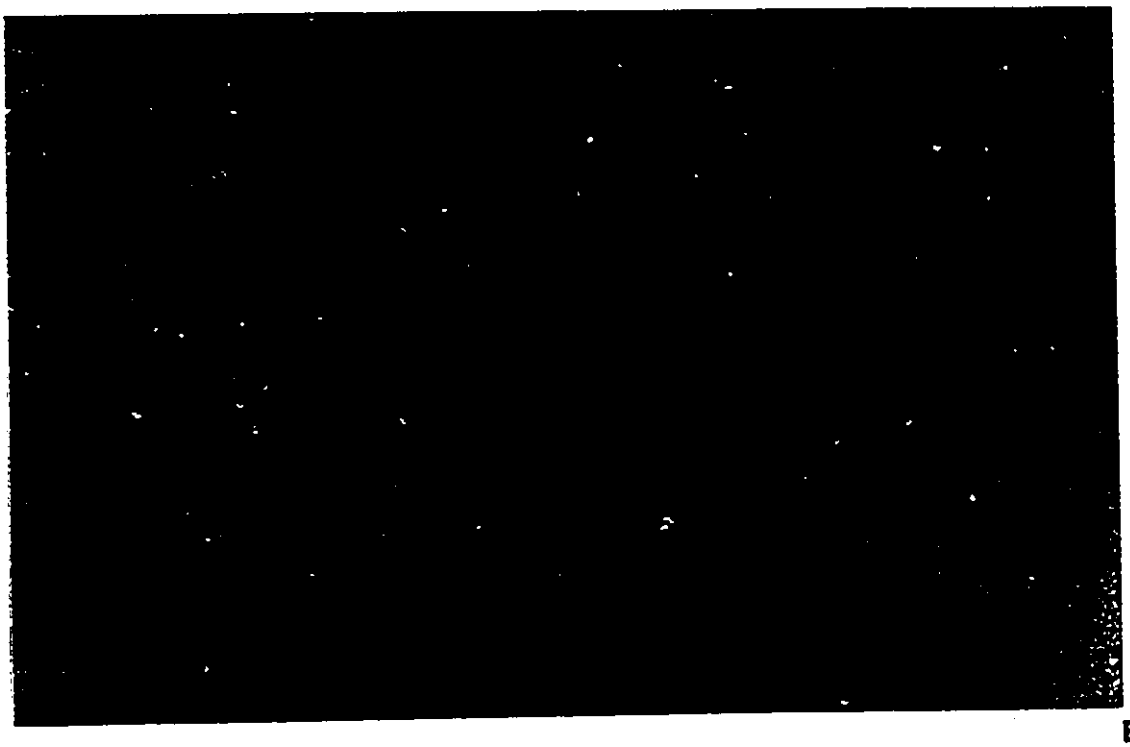


A C

Fig. 3-23

Film autoradiography of dispersed cells after incubation with ^{125}I-IL-1α at a final concentration of 50 pM. A is amnion, C is chorion laeve

A



B

Fig. 3-24

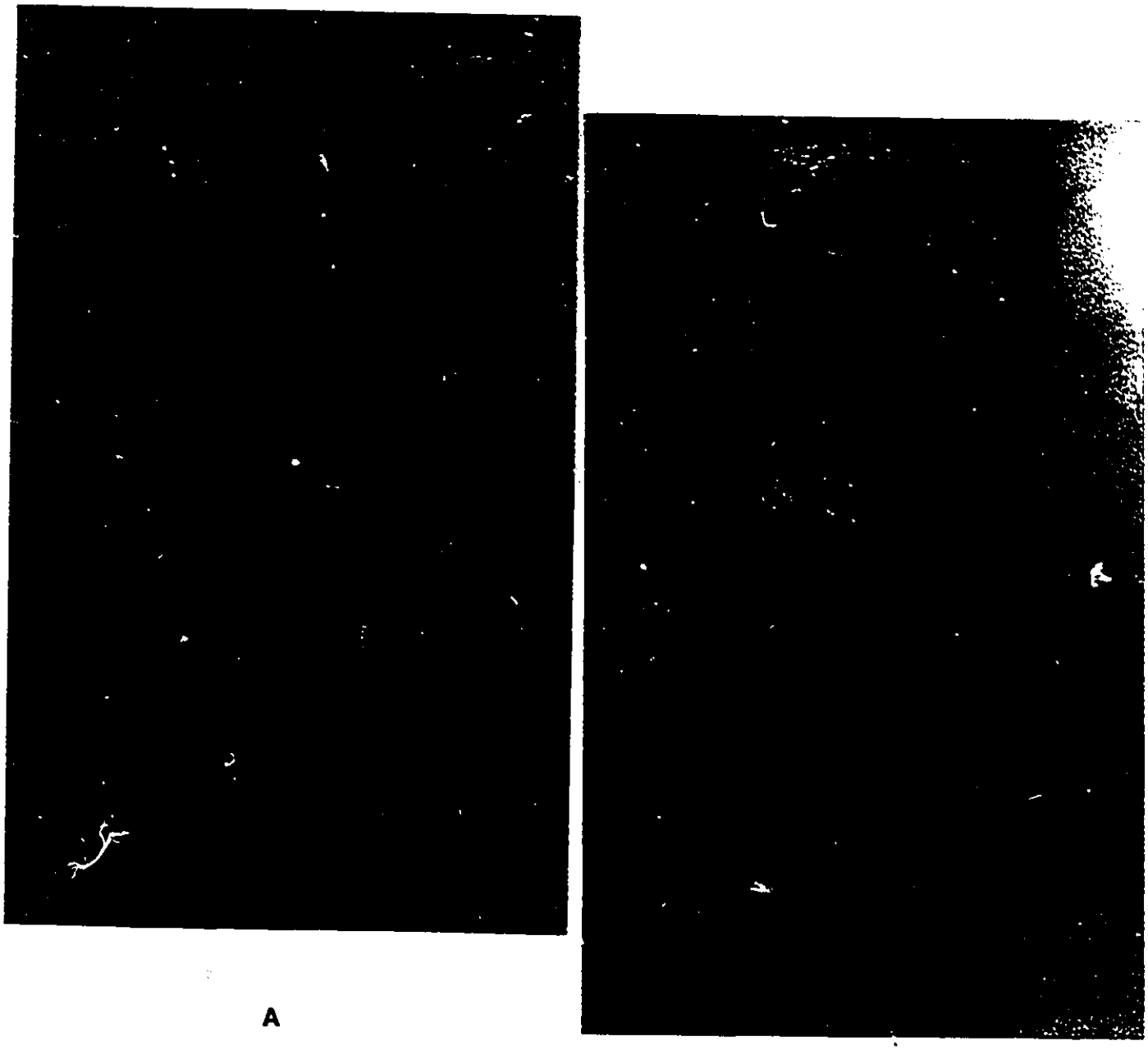
Emulsion autoradiography of freshly dispersed cells from amnion, after incubation with ^{125}I-IL-1α, at a final concentration of 50 pM. H&E staining. A magnified X 200. B magnified X 400

A

B

Fig. 3-25

Emulsion autoradiography of dispersed cells from chorion incubated with ^{125}I-IL-1α at a final concentration of 50 pM. H&E staining magnified X 400. A: Immediately upon plating B: After 24 hours of culture

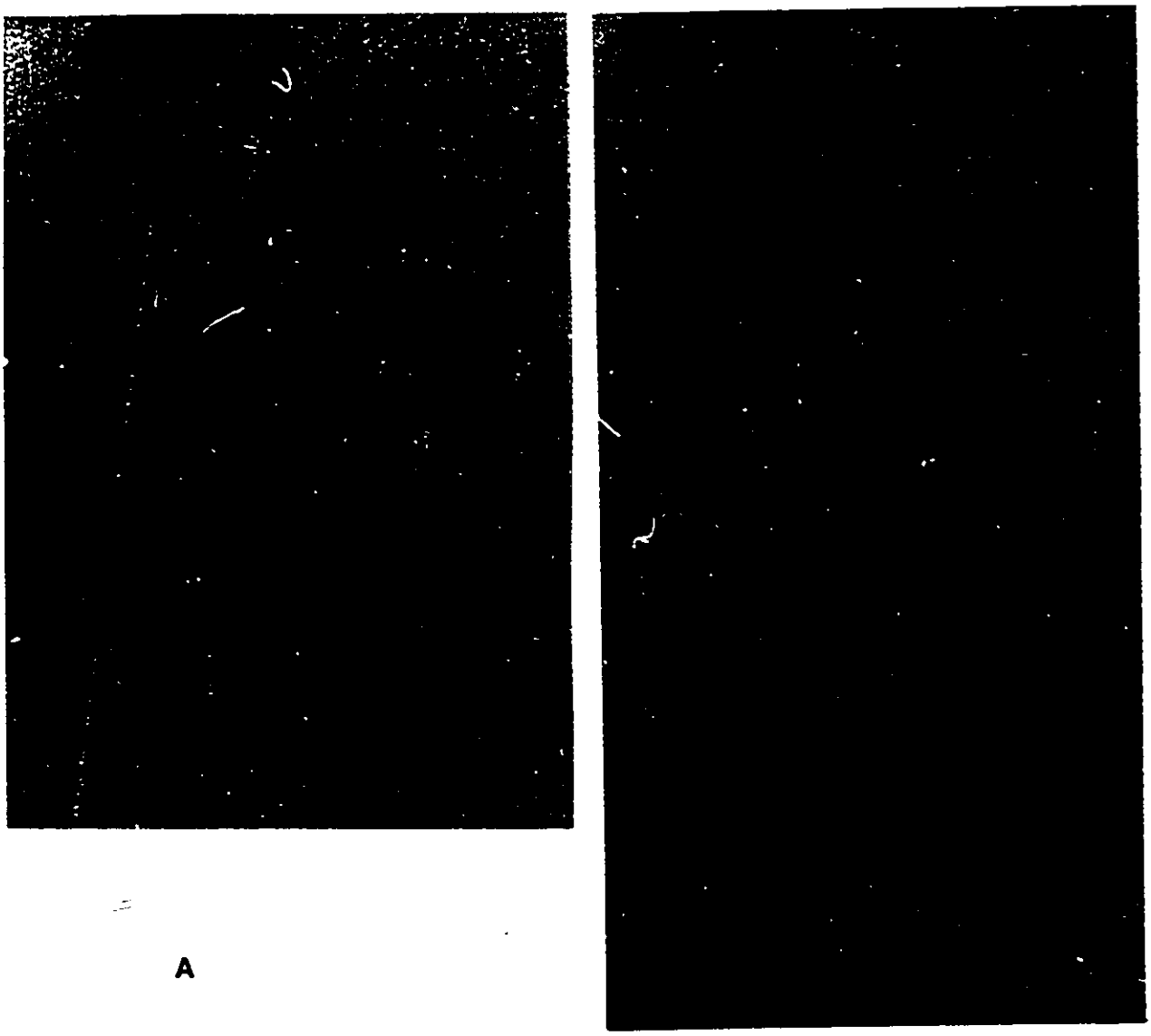


A

B

Fig. 3-26

Emulsion autoradiography of dispersed cells from chorion A: after 24 hours of culture, and B: after 48 hours. Incubation with ^{125}I-IL-1α at a final concentration of 50 pM. H&E staining magnified X 400



A

B

Fig. 3-27

(A and B) Emulsion autoradiography of dispersed cells from chorion after 48 hours of culture. Incubation with ^{125}I-IL-1α at a final concentration of 50 pM. H&E staining magnified X 400

4.0 DISCUSSION

The mechanisms initiating and regulating human parturition remain largely unknown. Although labour in a number of other mammalian species, particularly the sheep, is well understood (Liggins *et al.* 1967 & 1977) there are important differences between labour in the human and in experimental animals. There is however considerable evidence in support of the role of prostaglandins (PGs) as mediators of human parturition, and fetal membranes and decidua are believed to be intimately involved in parturition through their ability to produce PGE_2 and $PGF_{2\alpha}$ (Casey and MacDonald 1988 *a and c*; Valenzuela *et al.* 1993).

As the type of *in-vivo* studies that can be carried out in the human are extremely limited, researchers resorted mainly to the use of *in-vitro* systems to study the regulation of PG output by human intrauterine tissues. In particular, primary cultures of cells from various human intrauterine tissues have been studied, and include amnion (Bry *et al.* 1993) chorion laeve (Ito *et al.* 1993), and decidua (Mitchell *et al.* 1991 *a and b*).

Of the tissues studied, the amnion has been examined the most intensively. Zitcer *et al.* (1955) first reported the use of cultures of this tissue, and since then, primary cultures of human

amnion cells (Gaffney and Grimadi 1981, Dell'Aquila and Gaffney 1982) and cell lines derived from human amnion (Hayflick 1961) have been used extensively for a variety of investigations. In particular, confluent primary cultures of amnion cells are thought to be appropriate *in-vitro* model systems for investigating the regulation of prostaglandin metabolism in this tissue, and a large number of studies with various effectors have been carried out with this preparation.

In the initial studies with amnion cultures, there was some degree of cellular characterization (Okita *et al.* 1983) and some differences were noted between the cultured cells and fresh tissue with respect to arachidonic acid metabolism. In particular, the specific activities of phospholipase A_2 and C, important enzymes involved in arachidonic acid release from phospholoipids, were lower in the cultured cells than in the tissue.

There have been a number of studies in the literature which indicate that the preparation of the tissue studied can influence the responses to various substances. Isoproterenol stimulates PG output in discs of amnion (DiRenzo *et al.* 1984 *a and b*), and amnion and decidua cells which are in secondary culture (Moore *et al.* 1988 *b*, Hassan *et al.* 1992), but not in freshly dispersed cells (Warrick *et al.* 1985). Glucocorticoids inhibit PG output in freshly isolated cells (Gibb and Lavoie 1990) and in amnion-derived WISH cells (Harris *et al.* 1988), but stimulate

PG production by confluent cells (Zakar and Olson 1989, Mitchell *et al.* 1988, Gibb and Lavoie 1990). Epidermal growth factor (EGF) stimulates PG output in amnion-derived WISH cells (Harris *et al.* 1988), and in confluent cells in primary culture (Casey and MacDonald 1988 *c*), but has no effect on freshly isolated cells (Gibb and Lavoie 1990), and platelet-activating factor (PAF) stimulates PG output in discs of amnion, but has no effect on confluent cells (Bleasdale and Johnston 1985). The reason for these altered responses in different tissue preparations is not fully understood, and could be due to a number of different factors, which might include changes in the proportion of different cells types, e.g. fibroblasts versus epithelial cells. Such studies have raised the question of which tissue preparation is the most appropriate model to study PG synthesis in the fetal membranes.

In the present study, we were unable to demonstrate any stimulatory effect of IL-1 on PG output by cells dispersed from amnion, chorion laeve or decidua during the first or second 24h of culture. These findings contrasted with the stimulatory effects of IL-1 which had been reported on cultured amnion cells (Bry *et al.* 1993), chorion cells (Lundin-Schiller & Mitchell 1991 *a*) and decidua cells (Ishihara *et al.* 1992). This stimulatory effect of IL-1 on cultured amnion and chorion cells (more than five days) was also confirmed in the present study.

The refractoriness of dispersed cells to IL-1 has been confirmed by others. In the case of the chorion laeve, Lundin-Schiller & Mitchell (1991 *a*) found that the cells were only responsive to interleukin-1 after a few days in culture, and amnion cultures were refractory until cultured for 4 days.

Both IL-1α and IL-1β were examined since, although poorly homologous, they act upon the same receptors even though they have differing affinities (Dinarello & Savage 1989). However differences in response to IL-1α and IL-1β have been reported. Using intact tissues, Kent *et al.* (1993) showed that IL-1β is a potent stimulator of PG synthesis by decidua and by amnion, whereas IL-1α only stimulates the amnion.

The inability of the dispersed cells to respond to IL-1 did not seem to be due to their non-viability. The dispersed cells were shown to be viable both by their exclusion of trypan blue and by their ability to incorporate radiolabeled amino acid into protein (Gibb *et al.*, unpublished). In the latter instance this was most marked during the second 24h of culture. Also, previous studies have shown that cyclohexamide inhibits basal PG output by these cell preparations indicating that they are indeed capable of protein synthesis at this time. In the case of the chorion laeve the present study found that there was a marked increase in basal PG output from the first to the second 24 hours of culture, indicating that the cells were metabolically active in PG synthesis. The inability of the cells to respond to

the cytokines was also unlikely to be due to them being in a maximally stimulated state, as basal output varied markedly in tissues from one patient to another.

The previously reported increase in PG production by amnion cells following labour (Rice 1990, Lundin-Schiller & Mitchell 1990) was also found in the present study which is consistent with the proposal that in this tissue, prostaglandin production may be important in human labour. In addition, this finding indicated that our dispersed-cell system was behaving in a similar fashion to those described by other investigators.

The responsiveness of the amnion, chorion laeve and decidua was also confirmed using glucocorticoids and phorbol esters. In the case of the glucocorticoids, PG output by all 3 tissues was inhibited, confirming previous studies (Gibb & Breton 1993, Gibb *et al.* 1988). With phorbol ester, the stimulatory effect was found to occur in the amnion as previously reported (Mitchell 1991 *b*) and the chorion, but not the decidua.

Glucocorticoids and phorbol esters act in the interior of the cells. The glucocorticoids presumably through an intracellular receptor (Zakar & Olson 1989) and the phorbol ester by stimulating intracellular protein kinase (Mitchell 1991 *b*). In contrast, IL-1 acts via a plasma membrane receptor and it was possible that the inability of the cells to respond to IL-1 was due to the membrane receptor being destroyed during cell

dispersion. This might be thought to be unlikely to explain the refractoriness of the cells, especially during the second 24 h, since in other systems the turnover of IL-1 receptor is between 2 and 12 hours (Horuk and McCubrey 1989). However, since the tissues under study were quite different from B cells this possibility could not be completely excluded.

It is difficult to design experiments to determine whether the receptors have been altered during dispersion or whether cultured or dispersed cells are the most appropriate models to study the regulation of PG production in human pregnancy. We therefore decided to examine if the undisrupted tissues expressed interleukin-1 receptor. If they did then this would be consistent with this cytokine acting on the tissues in situ. Previous studies in other tissues (Takao *et al.* 1990 *a and b*) had provided evidence for the presence of IL-1 receptors using autoradiography, and we attempted to use this methodology in the present study.

Evidence for the presence of interleukin-1 receptors was found in the chorion-decidua, but not the amnion, which would indicate that the amnion under normal conditions at term is not able to respond to IL-1. Emulsion autoradiography was done to determine the precise localization of receptors in relation to the underlying cytoarchitecture. Microscopic examination of these sections identified the putative IL-1 receptors in the chorion laeve layer of the fetal membranes and decidua. The receptors

had a heterogeneous distribution in chorion laeve cells as the density of the labeling was variable and was mostly localized to the trophoblast cells. The density of the IL-1 receptors appeared to vary within the chorion and was also variable from one placenta to another. Although only a small sample was examined, the variability did not appear to be related to the mode of delivery.

In an attempt to identify the receptors on dispersed cells, we carried out some preliminary experiments with freshly dispersed chorion laeve cells labeled with [^{125}I] IL-1α soon after dispersion and in the first and second days of culture. Non-specific binding was determined by labeling of the chorion cells with [^{125}I]IL-1α in the presence of 1000-fold excess of IL-1ß. Because amnion exhibited no labeling in autoradiography of the undisrupted tissue, amnion cells were also studied as a negative control. There was no evidence for the presence of IL-1 receptors in dispersed amnion cells after one or two days of culture but there was evidence for the receptors being present in the dispersed chorion laeve cells.

The absence of IL-1 receptors in the amnion and the paucity in the decidua is consistent with the inability of IL-1 to alter PGE_2 production by freshly dispersed cells from these tissues. However, IL-1 did not stimulate PGE_2 production by dispersed chorion cells after 24 hours, even though we had evidence of receptors being present in the dispersed cells and undisrupted

tissues. Possibly, IL-1 stimulation of PGE_2 production by chorion cell culture requires more than 24 hours of culture. Studies on the effect of IL-1 on prolactin inhibition revealed that the effect was only noted after 24 hours with maximal effect occurring after 3 to 4 days of culture (Jikihara & Handwerger 1994).

It is also possible that IL-1 action on the chorion requires a cofactor. *In-vivo*, there is certainly interplay between many factors that may augment or attenuate the effect of each other in stimulating PG production. A number of *in-vitro* studies have reflected this interplay. Catecholamines attenuate epidermal growth factor-induced prostaglandin E_2 production in amnion-like (WISH) cells (Su *et al.* 1992). Dexamethasone and EGF appear to have additive effects on the stimulation of PGE_2 output by cultured amnion cells (Su *et al.* 1992). Interleukins have also been found to have modifying effects with other substances in fetal membranes and other systems. IL-1ß synergizes with tumor necrosis factor on PGE_2 production by amnion cells (Bry and Hallman 1991). Epidermal growth factor and transforming growth factor synergize the IL-1 stimulation of PGE_2 production by amnion in monolayer cell culture (Bry 1993), whereas transforming growth factor-beta suppresses the cytokine induced PGE_2 production by amnion (Bry & Hallman 1992).

Alternatively, it is possible that IL-1 may act through the production of other substances. It has previously been shown that IL-1 can stimulate IL-6 production in the amnion and chorion laeve (Kauma *et al.* 1994) and placenta and IL-6 can stimulate PG production (Mitchell *et al.* 1991 *b*). However, we could not find any evidence that IL-1 acted via this cytokine to stimulate PG output, since the tissues did not respond to IL-6 by increasing PG output.

The fact that IL-1 receptors were mainly present in the chorion does not necessarily undermine the role of amnion in the onset of labour in response to IL-1, since there is the potential for many paracrine actions within the membranes and decidua. Several substances that are secreted by the chorion are known to stimulant PG production by the amnion. For example, renin, a protein hormone produced by the chorion, can act on the amnion to increase PGE_2 biosynthesis (Lundin-Schiller and Mitchell 1991 *b*). In a similar fashion, IL-1 may act on the chorion to produce substance(s) which stimulate PG production by the amnion. Substances produced by the chorion in response to IL-1 could also act on decidua PG production in a paracrine fashion.

The present study found that IL-1 was unable to alter prostaglandin production in freshly dispersed cells from human fetal membranes (amnion and chorion), and decidua and that the amnion did not seem to contain receptors for this cytokine

at term. These findings would question the role of the amnion in increased PG production in response to the direct action of IL-1 at term but do not, however, rule out the potential importance of the amnion in IL-1 induced PG production in preterm labour with infection. There is a marked increase in the concentration of IL-1 in amniotic fluid in preterm labour with infection (Romero *et al.* 1988 *a*) and it is possible that IL-1, or other substances produced in the inflammatory reaction might induce the IL-1 receptor in the amnion as occurs in other tissues.

The results of the present study also question the reliability of the confluent preparations of amnion cells as an *in-vitro* model of amnion tissue. The difference in response between freshly dispersed cells and confluent cell preparation could be due to a number of factors. Blocking or destruction of the receptors during cell dispersion may occur. Similarly, these cells may need time to resynthesize and repair the receptors. The confluent preparation also allows the development of cell to cell junctions which may be important for receptor activation or to augment receptor affinity. A cofactor which is removed or attenuated with fresh cell dispersion could reaccumulate in confluent cultures. Indeed this has been proposed by some investigators to explain the fact that the response of amnion cells changes during culture (Gibb & Breton 1993). Our studies also cannot exclude the possibility that high endogenous levels of IL-1 exist in the tissues and are bound to the receptor

and/or are present early in the culture, resulting in receptor saturation and lack of early response to exogenous IL-1.

Another possible explanation might involve the recently described IL-1 receptor antagonist (IL-1ra) which opposes the effect of IL-1 (Baergen *et al.* 1994). IL-1ra expression is an important step in the regulation of IL-1 effect on intrauterine tissues. Normally, there is more expression of IL-1ra than IL-1 in these tissues (Baergen *et al.* 1994). Thus, factors that increase IL-1ra production will inhibit PGE_2 production. IL-4 and tumor growth factor-beta (TGF-ß) enhance IL-1ra production, subsequently IL-4 and TGF-ß inhibit the production of PGE_2 by amnion cells (Bry & Lappalainen 1994). It is possible that other substances are acting in such a fashion in our dispersed cells and that IL-1 receptors in the chorion could be blocked by IL-1ra, resulting in the refractoriness of the chorion cells to IL-1 stimulation.

Although some of the factors described above might explain the inability of the cells to respond to IL-1, it is equally possible, if not more so, that the response produced by the confluent cell preparations may be an artifact due to culture. For example, inadvertently dispersed fibroblasts may out-grow the amnion epithelial cells in culture and thus influence the response.

The present studies question the role of IL-1 in regulating PG formation, but it is possible that this cytokine has other

important actions in the chorion. For example, IL-1 could cause alterations in collagen synthesis and degradation in the fetal membranes. Data suggest that term amniotic fluid but not preterm amniotic fluid is capable of inducing the synthesis of collagenase and other proteases by fibroblasts (Vadillo-Ortega *et al.* 1991), and normal term amniotic fluid but not preterm amniotic fluid have high levels of IL-1 (Romero *et al.* 1989 *a*). Moreover, IL-1 has been shown to increase collagenase and hyaluronate synthesis in cultured fibroblast-like cells from human chorionic tissue (Katsura *et al.* 1989; Ito *et al.* 1993), in the same manner as had been reported in synovial fibroblasts and chondrocytes (Mizel *et al.* 1981; Saklatvala *et al.* 1984, Gowen *et al.* 1984). The stimulatory effect of IL-1 on membrane bound-hyaluronate production is not due to an increase in endogenous prostaglandins (Ito *et al.* 1993). If IL-1 did have such an action *in-vivo* and it promoted collagenolysis in human chorionic membrane, it could have a vital role in connective tissue catabolism required for normal rupturing of fetal membranes at term, and for connective tissue metabolism in fetal membranes associated with chorioamnionitis (Katsura *et al.* 1989). Indeed, the location of the IL-1 receptors in the chorion laeve would facilitate this role.

The IL-1 receptors are localized in the trophoblastic layer of the chorion laeve which is adjacent to the maternal decidua, at the interface between fetal and maternal tissues. These receptors may therefore provide a signal for maternal recognition of the

fetal allograft. Indeed, recent studies indicate that there is an increase in decidua interleukin-1ß and human leukocyte antigen HLA-DR alpha during pregnancy, particularly in the first trimester and at term (Kauma *et al.* 1990). These receptors may play a role in protecting the feto-maternal unit from the adverse effects of maternal immunity to the fetal allograft. Also, the location of the IL-1 receptors would be most suitable for the assumed physiological involvement of IL-1 in implantation (Unanue and Allen 1987), if the receptors have maintained the same distribution on the chorion villi since its development.

Further studies are needed to examine the biological characteristics of different *in-vitro* preparations of human intrauterine tissues. These studies could include examination of cells and tissue biochemical composition, substrates and enzymes activities, response of these preparation to various substances, and receptor distribution in these preparation. In addition, finding an appropriate animal model for human parturition would be most helpful in understanding the mechanism initiating and regulating parturition.

In spite of the high variability in PGE_2 production from undisrupted tissues, I believe that the *in-vitro* tissue model which best represents the physiological situation *in-vivo*, is the undisrupted tissue preparation. It is possible that the highly variable PG production may be due to a number of factors.

Tissue handling might stimulate PG output to varying degrees (McColgin *et al.* 1990). The exposure time during processing of different tissue parts may affect tissue viability, and this could have an important influence on PG production. The location of the tissue in the uterus might result in changing PG output. For example, the distribution of the receptors for a particular substance might vary between the membranes covering the cervix and those close to the placenta. For future studies, it might therefore be appropriate to look at various areas of the membranes with the least invasive procedures such as in situ hybridization and autoradiography as outlined in the present study.

4.01 Future Directions

To better understand the potential importance of inflammatory mediators on the fetal membranes and decidua, further studies on IL-1 action on the tissues is warranted.

Comparison of IL-1 receptor density in normal term and preterm fetal membranes and following the density of the receptors throughout gestation would provide a clue to understanding the role of IL-1 receptors in implantation, pregnancy and parturition.

Mapping of the IL-1 receptors to identify whether the density of these receptors is higher in the lower segment, and more

specifically, in the presenting part, will provide a better understanding of the mechanism leading to cervical ripening that proceed uterine contraction. Correlating the density of the receptors with collagenase synthesis and other proteases may confirm the importance of IL-1 effect on collagen synthesis and degradation. The variation in IL-1 receptor density could be linked to pre-labour rupture of membranes and parturition. Studies on the effect of various potential modulators of receptor expression such as bacteria and their endotoxins may clarify the role of IL-1 in preterm labour with infection. Studying the effect of agents that are believed to have inhibitory effects on the production of interleukin-1 such as glucocorticoids, which are potent anti-inflammatory agents (Krane 1993), may shed a light on potential regulation of this system in pregnancy.

4.02 Conclusions

The role of the inflammatory mediator IL-1 in term labour and preterm labour, associated with intrauterine infection, is not yet fully elucidated. The present study reported the effects of IL-1 on the three intrauterine tissues (amnion, chorion laeve and decidua vera) before and after the onset of labour at term, and compared the response of the various preparations of the same tissue.

Undisrupted tissue preparations from the amnion produced very highly variable amounts of PGE_2 production both in control

incubation and when incubated with IL-1 for 24 hours in the first 24 hours of culture. This variability may have resulted in masking any putative effects of the cytokine on PG production. Therefore, to achieve a better control, we resorted to dispersed cells preparations from the three tissues. Using dispersed cell preparations, IL-1α and ß were unable to alter PG production in amnion, chorion laeve and decidua, although IL-1 did stimulate PG production by confluent cells as has been previously reported (Romero *et al.* 1989 *b*).

In order to further define the potential action of interleukins in these tissues, we examined whether term human fetal membranes and decidua express interleukin-1 receptors. Using previously published procedures the presence and distribution of IL-1 receptors was assessed using [^{125}I] IL-1α binding. The labeling was performed with and without unlabeled IL-1ß to determine the non-specific binding. These studies indicated that receptors were present in the chorio-decidua but not in the amnion. More detailed examination of the tissues using emulsion autoradiography allowed microscopic analysis of receptor distribution. Putative receptors were found to be concentrated in the chorion laeve layer, with less in the decidua and none in the amnion. With freshly dispersed cells from chorion laeve, and cells cultured for one and two days preliminary experiments indicated that IL-1 receptors were present in the cells.

These studies indicate that in normal human pregnancy at term the chorion laeve appears to be the major fetal membrane which can potentially respond to IL-1. However, as we were unable to demonstrate that IL-1 stimulates prostaglandin output by this tissue, it is possible that it regulates the production of other substances. Moreover, at term, under normal circumstances it would appear that amnion cannot respond to IL-1 as we were unable to provide evidence for IL-1 receptors in this tissue or its dispersed cells.

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