

CONTROL OF PROSTAGLANDIN BIOSYNTHESIS

BY THE

INTRAUTERINE TISSUES IN PRIMARY DYSFUNCTIONAL HUMAN LABOUR

by

KOGIE REDDI

ooo0ooo

Submitted in partial fulfilment of the requirements for the degree of
Doctor of Medicine in the Department of Chemical Pathology, University of
Natal

1987

Durban

In this research the statistical analyses and recommendations arising from these analyses have been done in consultation with the Institute for Biostatistics of the Medical Research Council.

PREFACE

The experimental work described in this thesis was carried out in the Department of Chemical Pathology, University of Natal, Durban.

These studies represent original work by the author and have not been submitted to any other University. Where use was made of the work of others, it has been duly acknowledged in the text.

ACKNOWLEDGEMENTS

I wish to thank Professor S M Joubert, Head of the Department of Chemical Pathology and Director of the S A Medical Research Council Preclinical Diagnostic Chemistry Research Unit, who allowed me the opportunity of working in his laboratory. His advice and assistance throughout this work have been greatly appreciated.

To Professor R J Norman, my supervisor during these studies, I owe my deepest appreciation. Besides being a source of endless ideas as supervisor to this project, he has also provided tremendous support and guidance as a friend.

Mr Walter Deppe, Senior Biochemist, freely offered his many practical and academic skills. To him must go the credit of establishing many of the methods described in this text. The skilled and critical technical assistance provided by Mrs Moyra Kemp was greatly appreciated. I also wish to thank Mrs Trudy Machattie for her technical assistance with respect to gas liquid chromatography and the rest of the laboratory staff of the Department of Chemical Pathology for all their assistance offered.

I am very grateful to Mrs Rosemary Pegoraro and Mrs Moyra Kemp who helped in the proof reading, and Miss Arlene Mawhinney and Mrs Louie Vorster who typed this manuscript.

I also wish to thank Professors R H Philpott and J Moodley and the staff of the Department of Obstetrics and Gynaecology for their co-operation

throughout this study and the staff of the Medical Illustration and Electron Microscopy Units for their professional assistance.

To my colleagues who are also deeply involved in their M.D. theses presently, I am deeply grateful for their support throughout this work.

I gratefully acknowledge the financial support received from the South African Medical Research Council and the University of Natal.

Finally, I am grateful to my family for their continued encouragement, and to my daughters Sayuri and Sanusha, who have contributed their own little sacrifices towards making this thesis possible.

LIST OF PUBLICATIONS

ARISING FROM THIS THESIS

1. REDDI, K; KAMBARAN, SR; NORMAN, RJ; JOUBERT, SM; PHILPOTT, RH. (1984). Abnormal concentrations of prostaglandins in amniotic fluid during delayed labour in multigravid patients. Br J Obstet Gynaecol 91 : 781 - 787.
2. REDDI, K; NORMAN, RJ; JOUBERT SM. (1985). Prostaglandin Inhibitory compounds in the initiation of human labour. Lancet 1 : 1508.
3. REDDI, K; KAMBARAN, A; PHILPOTT, RH; JOUBERT, SM; NORMAN, RJ. (1985). Decreased concentrations of PGF₂ in delayed labour. A model for studies on the control of prostaglandin production by the uterus : In Prostaglandins Leukotrienes and Lipoxins. Plenum Publishing Corporation 457 - 464.
4. REDDI, K; NORMAN, RJ; DEPPE, WM; MACHATTIE, CT; KEMP, M; JOUBERT, SM. (1987). Amniotic membrane production of Prostaglandin F₂ is reduced in dysfunctional human labour - results of in vivo and in vitro studies. J Clin Endocrinol Metab Nov (In press).
5. REDDI, K; NORMAN, RJ; JOUBERT, SM. (1987). Differential regulation of prostaglandin production by inhibitors and stimulators in amniotic fluid during normal and dysfunctional labour. Prostaglandins (In press).

6. REDDI, K; NORMAN, RJ; KAMBARAN, SR; PHILPOTT, RH; JOUBERT, SM. (1987)
Intrauterine pressure studies in multigravid patients in spontaneous
labour : effect of oxytocin augmentation in delayed first stage. Br J
Obstet Gynaecol (In press).

LIST OF CONTENTS

ABBREVIATIONS(i)

INTRODUCTION(iii)

REVIEW OF THE LITERATURE

CHAPTER 1 ENDOGENOUS PROSTAGLANDINS IN HUMAN PARTURITION..... 1

1.1 Introduction : An historical note

1.2 Prostaglandins and labour

1.2.1 Measurement of prostaglandins in
 maternal serum

1.2.2 Measurement of prostaglandins in
 umbilical circulation

1.2.3 Amniotic fluid prostaglandins in
 human parturition

1.3 Prostaglandin production by intrauterine tissues

1.3.1 Effect of labour on prostaglandin
 production by intrauterine tissues

1.4 Dysfunctional labour : A model for the study of
 prostaglandins in human parturition

CHAPTER 2 CONTROL OF PROSTAGLANDIN PRODUCTION DURING
 HUMAN LABOUR..... 14

2.1 Introduction

- 2.2 Biosynthesis of prostaglandins
 - 2.2.1 Cyclo-oxygenase activity in the arachidonic acid cascade
 - 2.2.2 Prostaglandin synthetase activity
- 2.3 Catabolism of prostaglandins
- 2.4 Control of prostaglandin synthesis
- 2.5 Availability of free arachidonic acid as substrate for prostaglandin synthesis
 - 2.5.1 Phospholipids as a source of free arachidonic acid
- 2.6 Control of prostaglandin production by the human fetal membranes in relation to parturition
 - 2.6.1 Assessment of cyclo-oxygenase activity in human fetal membranes
 - 2.6.2 Free arachidonate generation from glycerophospholipids in human fetal membranes
 - 2.6.3 Prostaglandin synthetase activity in human fetal membranes during labour

PRESENT STUDIES

INTRODUCTION TO PRESENT STUDIES..... 32

CHAPTER 3 PATIENTS AND METHODS..... 34

3.1 Introduction

3.2 Patients

- 3.2.1 Criteria used for patient selection
- 3.2.3 Patients progressing normally during labour
- 3.2.3 Patients presenting with delay in active labour
- 3.3 Progress and management during labour
- 3.4 Methods
 - 3.4.1 Measurement of uterine activity
 - 3.4.2 Sampling of amniotic fluid during labour
- 3.5 Statistical analyses
- 3.6 Results
 - 3.6.1 Comparative clinical findings in the two groups of patients studied
 - 3.6.2 Oxytocin infusion rates
 - 3.6.3 Uterine pressures in the two groups
 - 3.6.4 Infant morbidity and mortality
- 3.7 Discussion

CHAPTER 4 AMNIOTIC FLUID PROSTAGLANDIN CONCENTRATIONS IN NORMAL AND DELAYED LABOUR..... 47

- 4.1 Purpose of the study
- 4.2 Patients included in the study
- 4.3 Analytical methods
- 4.4 Statistical analyses
- 4.5 Results

4.5.1	Amniotic fluid PGE concentration during normal and dysfunctional labour
4.5.2	Amniotic fluid PGF concentration during normal and dysfunctional labour
4.5.3	Amniotic fluid PGFM concentration during normal and dysfunctional labour
4.5.4	Amniotic fluid PGIM concentration during normal and dysfunctional labour
4.5.5	Amniotic fluid Thromboxane concentration during normal and dysfunctional labour
4.6	Discussion

CHAPTER 5	PROSTAGLANDIN PRODUCTION BY THE FETAL MEMBRANES IN NORMAL AND DYSFUNCTIONAL LABOUR.....	68
-----------	---	----

5.1	Purpose of the study
5.2	Patients included in the study
5.3	Methods
5.3.1	Sample collection and preparation
5.3.2	Superfusion of the fetal membranes
5.3.3	Measurement of prostaglandins in superfusates of the fetal membranes
5.3.4	Histological examination of the fetal membranes
5.4	Statistical analyses
5.5	Results
5.5.1	High performance liquid chromatography of prostaglandins

- 5.5.2 Tissue variation of prostaglandin release by the fetal membranes
- 5.5.3 Histology of the fetal membranes
- 5.5.4 PGE release from the fetal membranes in normal and dysfunctional labour
- 5.5.5 PGF release from the fetal membranes in normal and dysfunctional labour
- 5.5.6 PGFM release from the fetal membranes in normal and dysfunctional labour
- 5.5.7 Pattern of prostaglandin release from the fetal membranes in relation to labour

5.7 Discussion

CHAPTER 6 MOBILIZATION OF ARACHIDONIC ACID FROM SPECIFIC GLYCEROPHOSPHOLIPIDS OF FETAL MEMBRANES DURING NORMAL AND DYSFUNCTIONAL LABOUR..... 89

6.1 Purpose of the study

6.2 Patients included in the study

6.3 Methods

- 6.3.1 Measurement of free arachidonic acid in amniotic fluid and homogenates of the fetal membranes
- 6.3.2 Phospholipase A₂ releasable arachidonic acid content of the homogenates of fetal membranes
- 6.3.3 Measurement of the glycerophospholipid composition of the fetal membranes
- 6.3.4 Phospholipase A₂ releasable arachidonic acid content of the glycerophospholipids of the fetal membrane.

6.4 Statistical analyses

6.5 Results

- 6.5.1 Free arachidonic acid concentration in amniotic fluid
- 6.5.2 Free arachidonic acid content of homogenates of fetal membranes
- 6.5.3 Phospholipase A₂ releasable arachidonic acid content of homogenates of fetal membranes.
- 6.5.4 Glycerophospholipid content of fetal membranes
- 6.5.5 Phospholipase A₂ releasable arachidonic acid content of specific glycerophospholipids of fetal membranes

6.6 Discussion

CHAPTER 7 REGULATION OF PROSTAGLANDIN SYNTHETASE BY AMNIOTIC FLUID FACTORS DURING NORMAL AND DYSFUNCTIONAL LABOUR.. 105

7.1 Purpose of the study

7.2 Patients included in the study

7.3 Methods

7.4 Calculations

7.5 Statistical analyses

7.6 Results

- 7.6.1 Effect of unprocessed amniotic fluid on PGE production by prostaglandin synthetase
- 7.6.2 Effect of unprocessed amniotic fluid on PGF production by prostaglandin synthetase

- 7.6.3 Effect of fractionated amniotic fluid on
PGE production by prostaglandin
synthetase
- 7.6.4 Effect of fractionated amniotic fluid on
PGF production by prostaglandin
synthetase

7.7 Discussion

<u>SUMMARY AND GENERAL CONCLUSIONS</u>	118
--	-----

APPENDICES

Appendix A Patient consent.....	124
Appendix B Statistical analyses.....	125
Appendix C Laboratory methods.....	126

<u>REFERENCES</u>	174
-------------------------	-----

LIST OF ABBREVIATIONS USED

AA	arachidonic acid
B	bound fraction
Bo	maximum binding
BPD	biparietal diameter
Bq	bequerel (1 dps)
BSV	bovine seminal vesicle
cm	centimetre
cpm	counts per minute
DCM	dextran charcoal mixtures
dps	disintegrations per second
EFA	essential fatty acids
g	gram
GLC	gas liquid chromatography
^3H	tritium
h	hour
HCl	hydrochloric acid
HPLC	high performance liquid chromatography
IU	international units
l	litre
mol	moles
MEM	Minimum Essential Medium
min	minute
ml	millilitre
mm	millimetre
mw	molecular weight
N_2	nitrogen
nmol	nanomoles
NSB	non specific binding

p	probability
PC	phosphatidylcholine
PE	phosphatidylethanolamine
PG	phosphatidylglycerol
PGDH	prostaglandin dehydrogenase
PGE	prostaglandin E_2
PGF	prostaglandin $F_{2\alpha}$
PGFM	13, 14 dihydro-15-keto prostaglandin $F_{2\alpha}$
PGG ₂	hydroperoxyendoperoxide
PGH ₂	hydroxyendoperoxide
PGIM	6 ketoprostaglandin $F_{1\alpha}$
PI	phosphatidylinositol
PLA ₂	phospholipase A_2
PS	phosphatidylserine
RIA	radioimmunoassay
SD	standard deviation
SE	standard error
TLC	thin layer chromatography
TXB ₂	thromboxane B_2
vol	volume

INTRODUCTION

INTRODUCTION

Human parturition is a complex process requiring the integration of numerous endocrine and biophysical factors. The successful transition from intrauterine to extrauterine life requires that the fetus be sufficiently mature to adapt to the change of environment and that the mother be able to expel the fetus from the uterus without undue hazard to either her own reproductive organs or the fetus during this process. It is self-evident that these two processes should be closely linked. That the well-being of the fetus is indeed vitally dependent upon the duration of intrauterine development is evidenced by the increased perinatal mortality associated with labour both before and after term. Hence it is reasonable to think that the fetus plays an important, if not a major role in determining the time of its birth. This concept is far from new. As early as 600 BC Hippocrates stated "when the child has grown big and the mother cannot support him with enough nourishment, he becomes agitated, breaks through the membranes and incontinently passes out to the external world".

This signal or mechanism by which maturation of the conceptus is translated to myometrial activity has been the subject of research for many decades.

Studies on domestic animals have confirmed the fetal role in parturition, the classic example being the sheep model. Liggins (1972) demonstrated in a series of elegant experiments that the fetal adrenal gland was central to normal parturition in sheep. Subsequent experimentation has

revealed that the fetal signal, mediated via the fetal adrenal gland, results in the release of contractile prostaglandins which stimulate myometrial activity and the onset of labour.

As a result of these findings much research has been directed at comparing animal models of parturition to that of humans. However, significant differences have emerged in the mechanisms leading to the onset of labour between these models and humans. Serial measurements of hormones or other factors in the human fetus are not possible for ethical reasons, thus making it difficult to define clearly the mechanisms leading to human labour.

In the search for a suitable model for the study of human parturition Norman (1983) examined the human twin pregnancy in a series of studies relating to endocrine factors and labour. He clearly demonstrated some important concepts. The importance of the fetal contribution to the events associated with the initiation of labour was evidenced by the changes in the endocrine milieu of the first twin only in relation to human labour. Increased concentrations of prostaglandin E (PGE) and not prostaglandin F (PGF) in twin I provided strong support that in humans PGE was also part of the final event in the mechanism leading to labour. However no differences in the adrenal function between twin I and twin II were found, thus demonstrating quite clearly that the increased PGE associated with human labour was not mediated via the fetal hypothalamus - pituitary - adrenal axis.

The question then of the control of prostaglandin synthesis in humans remains unanswered. This thesis examines one of the clinically abnormal

labour patterns, primary dysfunctional labour, as a possible model whereby the role and control of prostaglandin synthesis during human parturition may be elucidated.

This model has been used to study the following areas:

- 1) The role of the major prostaglandins (PGE and PGF) in the onset of labour, myometrial activity and progress in labour.
- 2) The production and control of prostaglandin synthesis by the fetal membranes in dysfunctional and normal labour.

LITERATURE REVIEW

- CHAPTER ONE -

ENDOGENOUS PROSTAGLANDINS IN HUMAN PARTURITION

1.1 INTRODUCTION : AN HISTORICAL NOTE

Pharmacological action of prostatic secretions on uterine smooth muscle was first described by Kurzok and Lieb (1930) who observed that human semen caused strips of human myometrium to contract. Goldblatt (1932) made similar observations but it was von Euler (1936) who established that the active compounds were in the lipid soluble fraction. He also established that these substances were fatty acids, and in the belief that they originated in the prostate gland, coined the term prostaglandins.

Bergstrom and Sjövall (1957) isolated and described the first 2 prostaglandins, PGE_1 and PGE_2 . Subsequently a whole host of naturally occurring prostaglandins which play a role in almost every cell in the body, have been identified.

In the sphere of parturition attention has been devoted almost exclusively to the prostaglandins of the 2 series, namely prostaglandin E_2 (PGE) and prostaglandin $\text{F}_{2\alpha}$ (PGF), in part because of their potency in the induction of myometrial contractions. Administration of these prostaglandins precipitate labour at all stages of gestation (Amy and Thiery 1979, Bygdeman 1980) while antiprostaglandin agents reduce myometrial contractility (Lundstrom et al 1976, Wqvist 1979).

1.2 PROSTAGLANDINS AND LABOUR

Unlike studies on the initiation of labour in animals, many obvious restraints apply in man with respect to what may be sampled, the appropriate time for sampling and how the samples may be obtained. These restraints have for a long time limited the scope of studies to an examination of maternal blood, fetal or neonatal cord blood and amniotic fluid which can be obtained without violating ethical norms acceptable in medical practice.

In the following sections these approaches and their limitations will be considered because they apply to the studies of the roles of prostaglandins in the initiation and maintenance of human labour. The prostaglandins most widely studied in parturition are those of the "2" series particularly PGE_2 and $\text{PGF}_{2\alpha}$. Because of difficulties in distinguishing between the 1 and 2 series by some assay methods, PGE_2 and $\text{PGF}_{2\alpha}$ will subsequently be referred to as PGE and PGF in the text.

1.2.1 Measurement of Prostaglandins in Maternal Serum

Measurement of PGE and PGF in peripheral blood of mothers is hampered by their low concentrations due to their rapid clearance by the lung. About 70% of the primary prostaglandins is metabolized in one passage through the pulmonary circulation (Ferreira & Vane 1967). The marked difference in findings between various studies emphasises the problems of interpreting the meaning of changes in maternal blood concentration of the

primary prostaglandins. (Cadwell 1981, Challis 1974, Green 1974, Kinoshita 1977).

Currently the view is held that the measurement of primary prostaglandins in maternal peripheral plasma has no place in the study of the initiation and maintenance of human labour.

1.2.2 Measurement of Prostaglandin in Umbilical Circulation

As in the case of maternal peripheral blood, marked variations of prostaglandin concentrations in the umbilical vessels have prejudiced interpretation of these results (Hertelendy 1973, Keirse 1975, Bibby et al 1979, Norman et al 1981). Thus it would appear that neither maternal peripheral blood nor umbilical cord blood prostaglandin concentration is suitable for studying the role of prostaglandins in the initiation of labour.

1.2.3 Amniotic Fluid Prostaglandins in Human Parturition

To date the most consistent information on prostaglandin levels in biological fluids comes from studies in the changes of prostaglandins in amniotic fluid. Indeed, the first suggestion that prostaglandins may play a role in the initiation of labour came from such studies on amniotic fluid (Karim 1966, Karim and Devlin 1967). Measurement of amniotic fluid prostaglandins is attractive in that minimal synthesis and degradation of prostaglandins occurs in this fluid (Keirse et al 1975, Keirse 1976), and thus their concentrations in amniotic fluid are believed to reflect release from surrounding fetal membranes to the amniotic fluid (Keirse and Turnbull 1976).

Although the actual levels of the primary prostaglandins in amniotic fluid vary from one study to another, all are consistent in showing three trends. Firstly, prostaglandin levels in amniotic fluid are lower in mid pregnancy than at term (Salmon and Amy 1973, Keirse et al 1974, Dray and Frydman 1976); levels are higher following the onset of labour than before labour (Karim and Devlin 1967, Keirse and Turnbull 1973, Salmon and Amy 1973, Hillier et al 1974, Keirse et al 1974, Dray and Frydman 1976) and, lastly, there is a further increase in amniotic fluid prostaglandins during the progress of labour (Keirse et al 1974, Dray and Frydman 1976, Keirse et al 1977). These changes are discussed in greater detail in the following sections.

1.2.3.1 Amniotic Fluid Concentration of Prostaglandins before the Onset of Labour

Although it is generally accepted that prostaglandin levels in amniotic fluid are lower in mid-pregnancy than at term, there is some controversy as to the pattern of prostaglandin concentrations at term immediately before the onset of labour. Dray and Frydman (1976) and Norman et al (1981) found PGE to be the major prostaglandin in amniotic fluid before the onset of labour and noted a significant increase with advancing gestation. No change in PGF concentrations was observed before labour by some authors (Keirse et al 1974, Dray and Frydman 1976, Norman et al 1981) while others found a gradual increase towards term (Salmon and Amy 1973, Hibbard et al 1974).

Mitchell (1977) demonstrated that when amniotic fluid samples obtained by amniotomy and by amniocentesis were considered separately, there was no significant increase in PGF with increasing gestational age and suggested that the apparent contradictions in PGF levels in amniotic fluid may be due to the method of sample collection. This supports the view that increase in PGF occurs only following the onset of labour, (Dray & Frydman 1976, Norman et al 1981) and that it is unrelated to the increasing PGE concentration observed in amniotic fluid before the onset of labour. This implies that PGF production by the intrauterine tissues may be controlled by an entirely independent mechanism from that which regulates PGE production.

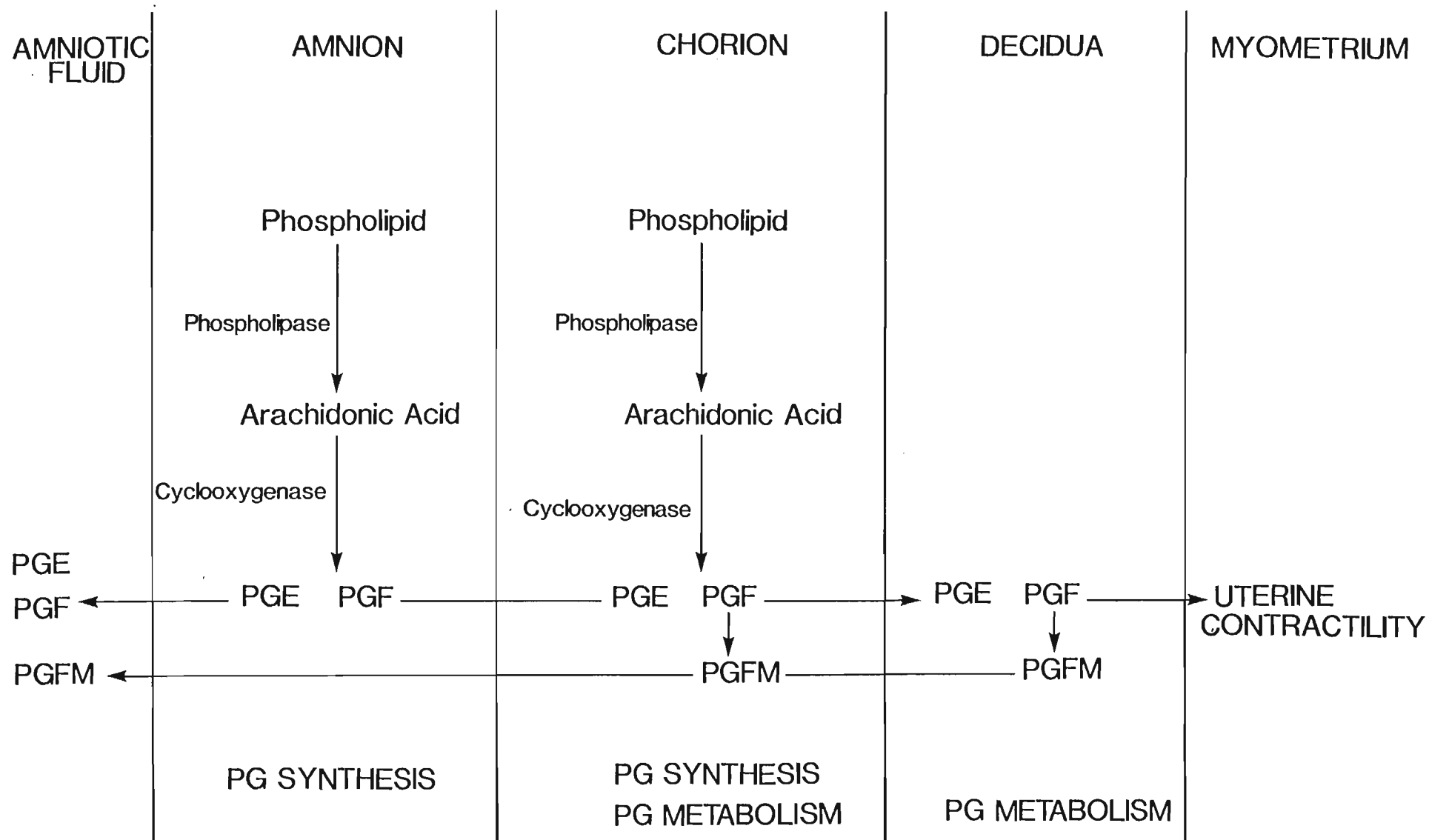
1.2.3.2 Amniotic Fluid Concentration of Prostaglandins after the Onset of Labour

The onset of labour is associated with a significant increase in amniotic fluid concentrations of both PGE and PGF (Karim and Devlin 1967; Keirse and Turnbull 1973, Salmon and Amy 1973, Keirse et al 1974, Hillier et al 1974, Dray and Frydman 1976, Norman et al 1981). The fact that significant increases in both PGF and its metabolite 13, 14 dihydro 15 - keto prostaglandin $F_{2\alpha}$ (PGFM) were found in amniotic fluid following the onset of labour argues for increased prostaglandin synthesis rather than decreased metabolism of the primary prostaglandins (Keirse et al 1977, Norman et al 1981). Synthesis and metabolism of prostaglandins in the amniotic

fluid is negligible (Keirse et al 1975, Keirse 1976). It is thought that the fetal membranes and/or the decidua are the site of metabolism of the primary prostaglandins within the feto-placental unit (Keirse et al 1976, Okazaki et al 1981). Whereas prostaglandin dehydrogenase (PGDH) activity was found to be low in the amnion, significant activity was demonstrated in the chorion and decidua vera tissue suggesting that the metabolites of the primary prostaglandins (specifically PGFM) measured in amniotic fluid originate in the chorio-decidual tissue (Okazaki et al 1981). The metabolite of PGE₂, 13-14 dehydro 15 keto PGE₂ (PGEM), is unstable in aqueous media, thus making it difficult to quantitate in biological fluids. Presumably metabolism of PGE also occurs in the chorio decidual tissue rather than the amnion or amniotic fluid. Figure 1.1 demonstrates the current views on the origin of amniotic fluid prostaglandins.

1.2.3.3 Amniotic Fluid Concentrations of Prostaglandins during Active Labour

During labour concentrations of both PGE and PGF continue to increase and correlate closely with progressing cervical dilation (Fig 1.2 : Keirse and Turnbull 1973, Salmon and Amy 1973, Keirse et al 1974, Hillier et al 1974, Dray and Frydman 1976, Keirse et al 1977). Quite apart from the increased production of PGE and PGF during labour there is also an apparent change in the relative amounts of PGE and PGF produced via the biosynthetic pathway during the progress of labour : before labour, PGE is the major prostaglandin in amniotic fluid



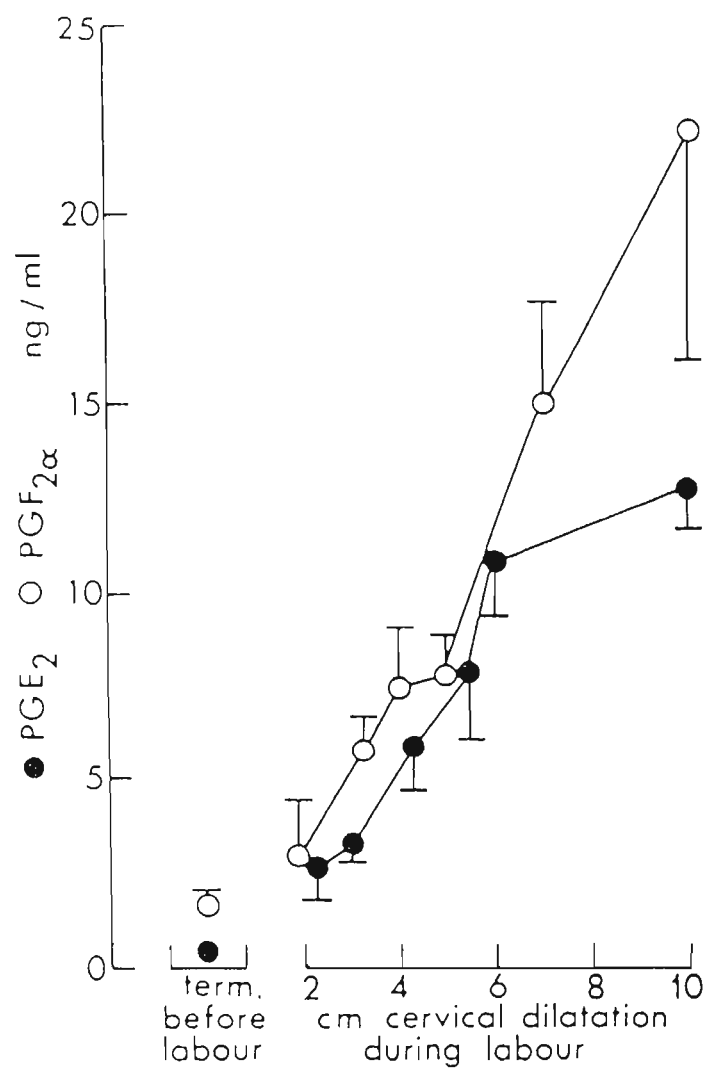


Figure 1.2 Concentration of PGE₂ and PGF_{2α} in amniotic fluid during late pregnancy and labour at term (mean ± SEM) from Keirse & Turnbull (1973) and Keirse et al. (1974) In: Human Parturition, Ed. Keirse et al., Leyden University Press p.125.

(Dray and Frydman 1976, Norman et al 1981); once labour commences PGF concentrations outstrip those of PGE (Dray and Frydman 1976). The changes in PGFM concentrations in amniotic fluid mirror those of PGF, which strongly supports the view that it represents increased prostaglandin production as labour progresses (Keirse 1977).

While these studies on amniotic fluid levels of prostaglandins confirmed increased local production of prostaglandins associated with the onset of labour, attention was focussed on the possible mechanisms involved in the regulation of the increased prostaglandin production. Norman (1983) addressed this problem by using human twin pregnancy as a model for studying endocrine changes related to the onset of labour. He found that increases in PGE levels in twin I but not in twin II were associated with the onset of labour, suggesting some fetal rather than maternal factor/s responsible for the increase in PGE immediately before labour. Concurrent studies on prostaglandins and on adrenal steroid levels in the fetal blood in twins showed that this change in prostaglandin production was not mediated via the fetal adrenal gland (Norman et al 1982). Thus it seems that whereas prostaglandins are integral to the events leading to parturition in both human and animal models, the mechanism which initiates the biosynthesis of prostaglandins in humans is different and, as yet, unidentified.

1.3 PROSTAGLANDIN PRODUCTION BY INTRAUTERINE TISSUES

The origin of amniotic fluid prostaglandins is clearly central to the problem of evaluating their role in labour.

Higher levels of PGF in amniotic fluid obtained following amniotomy rather than amniocentesis (Mitchell et al 1976) and increases in amniotic fluid and serum levels of PGF and PGFM following vaginal examinations (Mitchell et al 1977, Norman et al 1982) certainly suggest local production of prostaglandins by the intrauterine tissues.

To study this aspect more closely a variety of in vitro techniques involving the tissue of the feto-placental unit was developed. The use of such techniques did not however define in unequivocal terms the role of the various tissues in prostanoid production during labour (Williams and Collins 1976, Mitchell et al 1978, Satoh et al 1981, Olson et al 1983, Skinner et al 1985). The equivocal results may well be the result of the difference in methodology employed in these various studies. Membrane or tissue homogenates constitute traumatized tissue with resultant increase in prostaglandin synthesis and release (Piper and Vane). In tissue culture studies of individual cell types (Olson et al 1983, Skinner et al 1985) the cells were removed from the other layers of the fetal membranes, which may profoundly affect control of prostanoid synthesis and hence its relevance to the in vivo situation.

Nevertheless there is consensus that PGE is produced primarily by the amnion (Mitchell et al 1978, Satoh et al 1981, Kinoshita et al 1984). As

far as PGF is concerned the site of production is less clearly defined : decidua, myometrium, chorion and amnion have all been found to produce PGF (Williams and Collins 1976, Keirse et al 1976, Satoh et al 1981, Olson et al 1983, Skinner et al 1985).

1.3.1 Effect of Labour on Prostaglandin Production by the Intra-uterine Tissues

The results of studies on prostaglandin production by various intrauterine tissues after the onset of labour are also conflicting. Mitchell et al (1978) using superfusion of minced tissue found no difference in prostaglandin production between tissue obtained before and after the onset of labour. It may well be that the increased amounts of prostaglandin known to be released as a result of trauma have masked any effect of labour in these experiments. Satoh et al (1981) proposed a switch in myometrial production of prostaglandins associated with labour; before labour myometrial tissue produced primarily 6 keto prostaglandin $F_{1\alpha}$ (PGIM), the stable metabolite of prostacyclin (a non-contractile prostaglandin); following labour PGF was produced in higher concentrations by the myometrium, thus suggesting that a switch in myometrial prostanoid synthesis was linked to the onset of labour in humans. However, this study examined homogenates of tissue and a pre-experimental "wash out" period of only 15 minutes was performed, whereas Mitchell et al (1978) found that 90 minutes was necessary to "wash out" any prostaglandin released by the minced tissue in their

experiments. When traumatized tissue is used in studies on prostaglandin production, interpretation becomes very difficult in the face of known release of prostaglandins secondary to trauma, even though there may be standardisation of experimental conditions. This consideration seems an obvious explanation for the conflicting results in many of these studies.

More recently Olson et al (1983) and Skinner et al (1985), using a tissue culture technique, demonstrated increased PGE and PGF release by the amnion and chorion respectively, following the onset of labour. While this represents whole cells in culture, each tissue type, of necessity, is examined in isolation and as such may not be comparable to the in vivo situation where the fetal membranes are in close proximity to each other.

It may be concluded that for in vitro studies it seems essential that techniques be developed to study untraumatized intact fetal membranes. If this objective is to be achieved it is self-evident that aspects of prostaglandin release by the fetal membranes may be studied under controlled conditions in relation to the onset of labour.

1.4 DYSFUNCTIONAL LABOUR : A MODEL FOR THE STUDY OF PROSTAGLANDINS IN HUMAN PARTURITION

Several studies including that of human twin pregnancies (Norman 1983)

have provided unequivocal evidence of the role of prostaglandins in the initiation of labour. The problems encountered in defining precisely the origin and control of these prostaglandins have been outlined in the preceding sections. There is yet another model which potentially offers scope for the study of the role, origin and control of prostaglandin synthesis during human labour.

Primary dysfunctional labour is a clinical condition in which patients present with delay in active labour. When prolonged and difficult labour occurs in the absence of secondary causes (cephalopelvic disproportion, malpresentation and malposition) it is attributed to faults in the "forces" and is usually called dysfunctional labour (Turnbull 1957). This labour pattern is characterized by hypokinetic uterine contractions, the aetiology of which is unknown. Measurement of uterine activity during labour in these patients confirms lower intrauterine pressures as compared to those in patients with normal progress in labour (Cowan et al 1982, Steer et al 1985). Although clinically dysfunctional labour is corrected by the use of oxytocic agents, no attempt has to date been made to define the biochemical or endocrine changes in patients presenting with primary inefficient uterine activity. In patients who required oxytocin administration for failure to progress in labour there is some evidence that the concentration of PGF and PGFM in amniotic fluid during early labour were significantly lower than in patients with normal progress in labour (Keirse et al 1977). Since prostaglandins are currently thought to play a major role in the events associated with human labour, a study of the production and control of prostaglandin synthesis in patients presenting with dysfunctional labour may elucidate the function of these substances in the progress of human labour.

- CHAPTER TWO -

CONTROL OF PROSTAGLANDIN PRODUCTION DURING HUMAN LABOUR

2.1 INTRODUCTION

It is obvious from the evidence presented in the previous chapter that prostaglandins of the "2" series play a central role in the initiation and maintenance of human labour. The evidence seems to suggest that these prostaglandins are of fetal origin and the all important question which arises is: how is the production of specific prostaglandins initiated and controlled during human parturition?

A major problem in studying the production and catabolism of prostaglandins has been their apparent universal presence in most cells and the fact that the biosynthetic and catabolic pathways may exist within the same cell or in cells in close proximity. This, in physiological terms, accounts for the local rather than distant action of prostaglandins (Vane 1969).

Before considering the control of prostaglandin production during human parturition, some relevant aspects of the biosynthetic pathway of prostaglandins are reviewed.

2.2 BIOSYNTHESIS OF PROSTAGLANDINS

Historically, the discovery of prostaglandins was preceded by the

elucidation of the need for so-called essential fatty acids (EFA) in the diet (Burr & Burr 1930). These subsequently proved to be the precursors of a host of very active, oxidized products of 20-carbon unsaturated fatty acids called prostaglandins. The prostaglandins were first described in terms of the nature of their physiological activity (Section 1).

A review of the eicosanoid biosynthetic pathway from EFA is beyond the scope of this thesis. Rather, the emphasis is placed on biosynthesis of prostaglandins of the 2 series, the precursor of which is arachidonic acid (AA).

2.2.1 Cyclo-oxygenase Activity in the Arachidonic Acid Cascade

The first step in prostaglandin biosynthesis is mediated by cyclo-oxygenase acting on an eicosapolyenoic acid in a reaction which is not rate limited. In Fig. 2.1 hydroperoxyendoperoxide (PGG_2), the product of AA oxidation, and its metabolism to prostaglandins and other active compounds, is schematically presented.

This reaction, catalysed by cyclo-oxygenase, exhibits a number of specificities. Firstly, only naturally occurring polyenoic acids are substrates for cyclo-oxygenase activity (Bakhle 1983). As already indicated, this review is only concerned with the products of the tetraenoic acid, AA.

Another requirement is that only free AA can serve as substrate

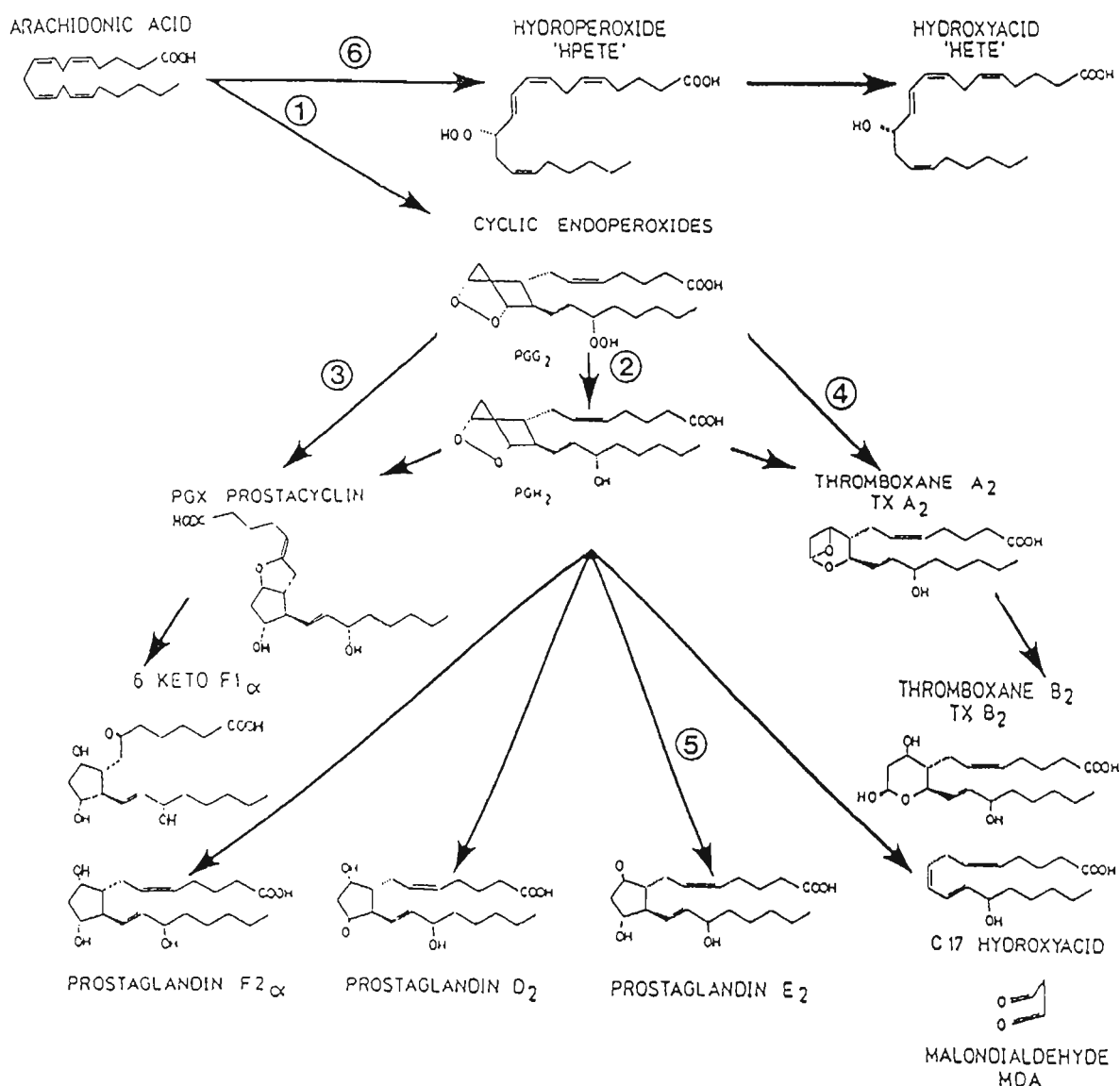


Figure 2.1 Metabolic pathways of arachidonic acid (Vane, J.R. In: Advances in Prostaglandin and Thromboxane Research. Vol. 4, Ed. Coceani F and Olley P.M., Raven Press, New York 1978, p.28.

* PGX (Prostacyclin) - referred to as PGI in text

- 1 Cyclo-oxygenase
- 2 Cyclo-oxygenase (peroxidase activity)
- 3 Prostacyclin synthetase
- 4 Thromboxane synthetase
- 5 Prostaglandin E_2 synthetase
- 6 Lipoxygenase.

for this reaction (van Dorp 1964). Under in vivo conditions hydrolysis of lipid pools which make available the free acid could well be rate limiting. This aspect is considered in greater detail in Section 2.5.

A third consideration is that purified cyclo-oxygenase also has peroxidase-like activity and rapidly generates a hydroxyendoperoxide (PGH_2) from PGG_2 (Lands 1979). The intermediate product PGG_2 may therefore, under in vivo conditions, serve as substrate for other endogenous peroxidases which do not result in prostaglandin synthesis. This could also result in rate limitation of prostaglandin synthesis under in vivo situations.

Another important factor is that the products of cyclo-oxygenase activity show marked specificities for different tissues and different species. Thus, the composition of these products in any tissue type may vary considerably from other tissues. Clear examples of these include the inability of platelets to synthesize prostacyclin (Bakhle et al 1983) and the selective increase in PGE and PGF synthesis by intra-uterine tissues during parturition (Section 1.3). In the light of such specificities it is manifest that animal or tissue models cannot validly be substitute material for the study of prostaglandin biosynthesis in a particular tissue.

2.2.2 Prostaglandin Synthetase Activity

Two well characterized synthetases that catalyse the production of prostacyclin and thromboxane from PGG_2 have been described (Lands 1979). A PGE synthetase has also been isolated (Ogino et al 1977). However, no enzyme system catalysing the production of PGF from PGG_2 has been identified, and it has been suggested that PGF might be a chemical rather than an enzymic product of PGG_2 (Bakhle 1983). A further enzyme capable of reducing PGE to PGF, 9-oxoprostaglandin reductase, has also been described (Lands 1979) but its significance in determining the ratio of PGE and PGF during in vivo production of prostaglandins has not yet been established.

2.3 CATABOLISM OF PROSTAGLANDINS

The products of prostaglandin catabolism are relatively inactive compared to the primary prostaglandins. The rate of catabolism of the primary prostaglandins could therefore influence the biological activity of these substances.

The first enzyme in the catabolic pathway is prostaglandin dehydrogenase (PGDH) which is rate limiting (Bakhle 1983). It is a widely distributed cytoplasmic enzyme and in vitro studies have shown that PGE, PGF, PGA and PGI are all substrates (Hansen 1976). Prostaglandin reductase, however, has specific substrate requirements: only 15 oxo-prostaglandins can serve as substrate (Lands 1979). Although prostaglandin reductase in itself is not rate limiting, it is dependent on the 15 oxo-prostaglandins generated by PGDH.

2.4 CONTROL OF PROSTAGLANDIN SYNTHESIS

The preceding sections clearly demonstrate that prostaglandin biosynthesis is not based on the classical biochemical control mechanisms of rate limiting enzymes sensitive directly to the accumulation of end products or via the rate of enzyme synthesis itself. The obvious conclusion therefore must be that, under in vivo conditions, there exists some sort of control of prostaglandin synthesis which in all probability lies in substrate availability. This may be effected through coupled reactions, intracellular compartmentalization or the presence of enzyme activators or inhibitors at various steps in the biosynthetic pathway.

The study of prostaglandin synthesis in any given tissue therefore involves a systematic examination of every step in the biosynthetic pathway that can feasibly be studied.

2.5 AVAILABILITY OF FREE ARACHIDONIC ACID AS SUBSTRATE FOR PROSTAGLANDIN SYNTHESIS

In Section 2.2.1. it was emphasized that a specific requirement for cyclo-oxygenase activity is the availability of AA in its un-esterified form as substrate. Intracellular levels of free AA are very low (Kunze & Vogt 1971) and therefore could not sustain de novo synthesis of eicosanoids without augmentation.

A number of intracellular lipid pools do exist in the form of cholesterol esters, glycerides and phosphatides which theoretically could supply the substrate needs for prostaglandins synthesis. In most cells, however, it is the phosphatides which supply the free substrate for cyclo-oxygenase activity (Irvine 1982).

2.5.1 Phospholipids as a Source of Free Arachidonic Acid

For the purpose of this study the phospholipids are considered to have the general structure presented in Fig. 2.2. The unsaturated fatty acids, including AA, are usually esterified in position B and are specifically hydrolysed by phospholipase A₂. The four phosphatides, phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylserine (PS) and phosphatidylinositol (PI) vary in their AA content in different tissues. Their importance with respect to human fetal membranes is discussed in greater detail in Section 2.6.2.

An important aspect of prostanoid biosynthesis is that, once the pathway is stimulated, prostaglandins are produced at an extremely rapid rate. Since cyclo-oxygenase activity is limited unless substrate concentration approximates the K_m value of the enzyme (Lands 1979), it has been suggested that availability of the free acid may be a limiting step in prostaglandin synthesis. However, AA is a very apolar compound and even if large quantities of AA were generated, these would rapidly bind to polar regions in protein molecules or become re-esterified by the action of acyl transferases (Lands & Merkel 1963). The concept therefore, is that substrate must necessarily be available in an organised and usable manner. This may include more effective mechanisms like compartmentalization within cells and/or coupling with other enzyme activities. This type of arrangement may well be the basis for the constant compositions of prostaglandins in

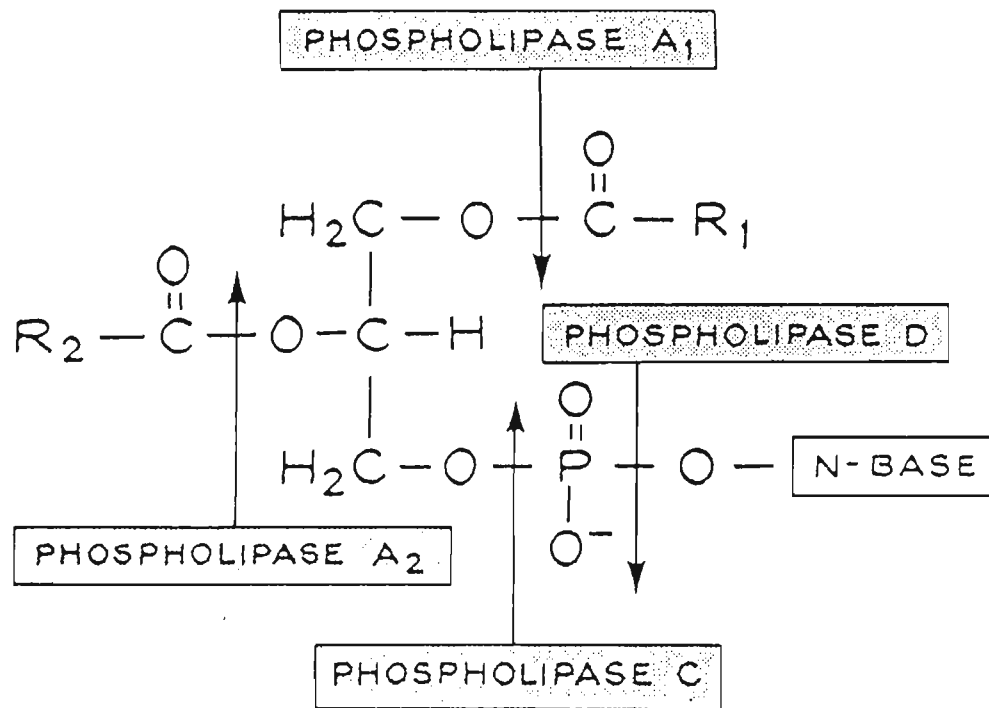


Figure 2.2 The general structure of a phospholipid substrate and the sites of hydrolytic activity of various phospholipases in the metabolism of phospholipids.

specific tissues (Section 2.2.1). Furthermore, while the availability of free AA could be limiting in prostaglandin synthesis, it is important to remember that AA is a substrate from which a variety of other substances is synthesized (Fig. 2.1)

2.6 CONTROL OF PROSTAGLANDIN PRODUCTION BY THE HUMAN FETAL MEMBRANES IN RELATION TO PARTURITION

Evidence has been presented in Section 1 implicating the fetal membranes as the site of production of prostaglandins during human labour. With the above outline of the prostaglandin biosynthetic pathway as it is known today, it seems relevant at this point to examine some of the possible control mechanisms of prostaglandin synthesis in structures within the fetoplacental unit in relation to human parturition.

2.6.1 Assessment of Cyclo-oxygenase Activity in Human Fetal Membranes

The rapid rates of biosynthesis in the AA-prostaglandin pathway and the instability of the intermediate endoperoxides (PGG_2 and PGH_2) limits study of the endoperoxides both in in vivo and in vitro experiments (Nugteren & Hazelhof 1973).

Investigations tend to be limited to studying the availability of the substrate (AA) or factors which affect the synthesis of prostaglandins in tissues and biological fluids. These aspects are reviewed in greater detail in the following sections with respect to the fetal membranes.

2.6.2 Free Arachidonate Generation from Glycerophospholipids in Human Fetal Membranes

Most of the arachidonate in the cell occurs as esters in various lipids, a significant fraction being in phospholipid (Section 2.5). Approximately 66% of the arachidonic acid content of the fetal membrane is present in membrane bound glycerophospholipids (Okita et al 1982). It has also been found that, in the amnion, PE accounted for more than 50% of all esterified arachidonic acid and that PI, although quantitatively less than PE, was also rich in arachidonic acid (Okita et al 1982).

2.6.2.1 Phospholipase A₂ Activity in Fetal Membranes

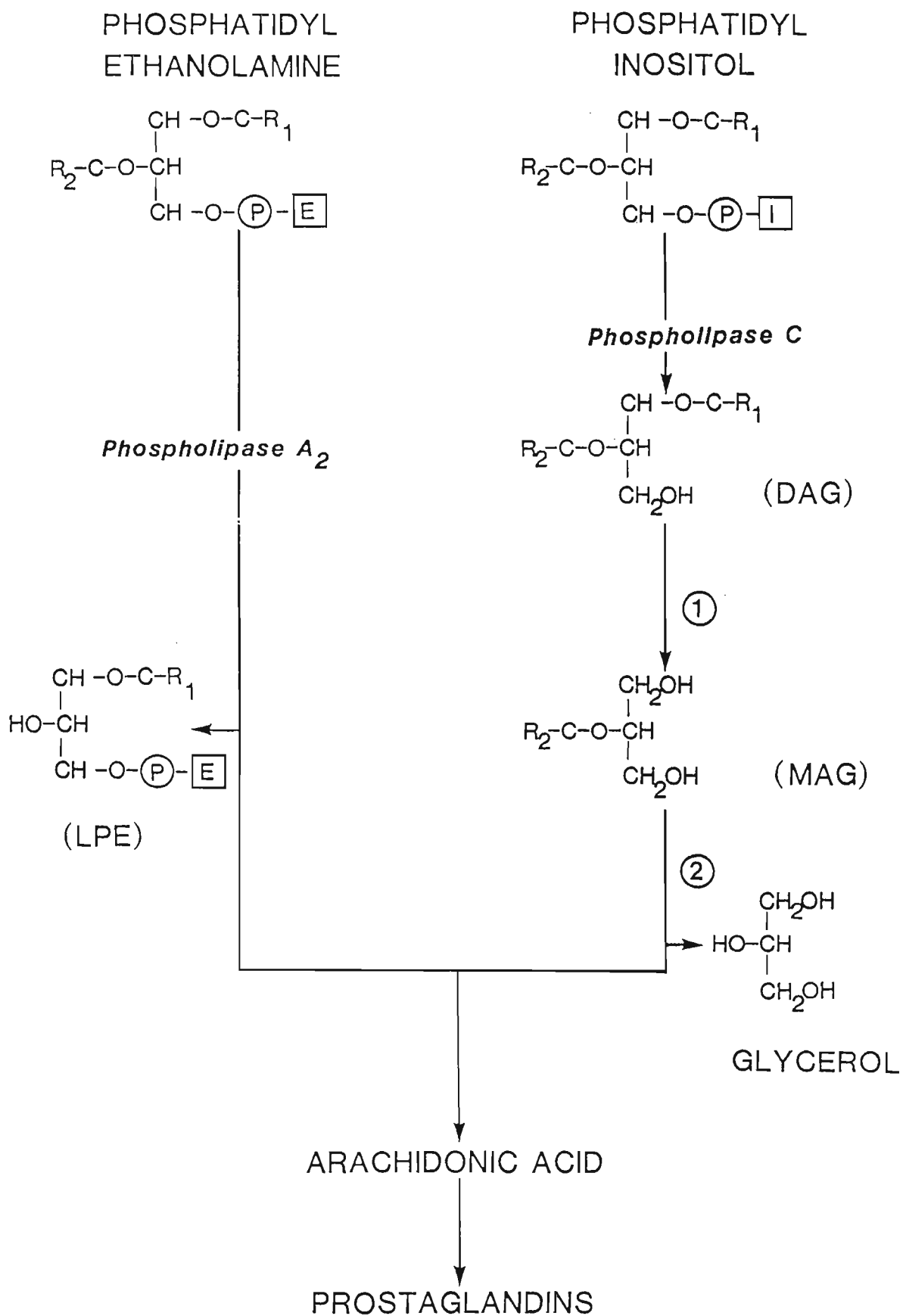
Since the bulk of the arachidonate is esterified in the β position of glycerophospholipids, the finding of arachidonate depletion in these phospholipids in the fetal membranes during the initiation of labour suggests increased PLA₂ activity (Curbelo et al 1981, Okita et al 1982).

Phospholipase A₂ activity has been found by direct measurement in human fetal amnion and chorio-decidua (Schultz et al 1975, Okita et al 1978). Furthermore PLA₂ activity displays variable substrate affinity depending on the acyl substitution in the β position of PE, the highest affinity being found with β arachidonyl substitution. Quite apart from the substrate

affinity of PLA_2 for PE (Okita et al 1978), increasing activity in the amnion has been demonstrated with advancing gestation (Okita et al 1981). However, no further increase in PLA_2 was demonstrated in tissues following the onset of labour (Grieves and Liggins 1976, Di Renzo et al 1981). These in vitro findings, however, do not exclude the possibility that PLA_2 activity could be regulated by some mechanism in vivo such as a fetal factor which would not be present in studies of isolated membranes. Thus although PLA_2 activity in the amnion increases with advancing gestation there seems to be no direct relationship between PLA_2 activity and the onset of labour.

Release of arachidonate from PI appears to depend on a PI specific phospholipase C (PLC) and not PLA_2 (Di Renzo et al 1981). However PLC activity results in the formation of a diacylglycerol and the release of arachidonate would depend on the presence of yet 2 more enzymes, a diacylglycerolipase and a monoacylglycerolipase (Fig 2.3). Both these enzymes have been identified in fetal membranes (Okita et al 1981). Fig. 2.3 demonstrates the current view of the important sources of arachidonic acid in the fetal amnion during labour.

A critical consideration of the role of free AA as a rate limiting factor in prostaglandin synthesis must surely be the quantitative argument. It may be expected that free AA should be low in circumstances where prostaglandin synthesis is unstimulated and higher during increased prostaglandin



production. However measurement of free arachidonate during states of low and stimulated prostaglandin production have shown that the free acid concentrations in amniotic fluid and fetal membranes are in vast excess of prostaglandin concentrations (Schwarz et al 1975, Keirse et al 1977, Filshie et al 1978).

It could thus be argued that the high free AA concentrations relative to prostaglandins in stimulated and unstimulated prostaglandin production states are manifest evidence against substrate limitation as a control mechanism. However, this argument may not be valid.

In Section 2.2.1 it was pointed out that although AA is a substrate for cyclo-oxygenase activity, the endoperoxides that are formed in turn may be substrates for a variety of peroxidation reactions of which prostaglandins constitute one pathway. Furthermore, despite being in excess of prostaglandins in unstimulated states, there is a further increase in the free AA in amniotic fluid and fetal membranes during labour (Schwarz et al 1975, Kerse et al 1977) in keeping with increased prostaglandin production during this time.

Another important consideration to be borne in mind is that the free AA is normally estimated by organic solvent extraction which would strip some protein bound fatty acid. This protein

bound AA would not necessarily be substrate for cyclo-oxygenase activity under in vivo situations. Thus it seems reasonable to conclude that PLA₂ activity is at least coupled to, or linked with, enzymes responsible for prostaglandin biosynthesis for effective utilization of the free acid. PLA₂ activity in fetal membranes would therefore constitute an important area to study in relation to human parturition.

2.6.3 Prostaglandin Synthetase Activity in Fetal Membranes During Human Labour

As discussed in section 2.4 the biosynthetic sequence leading from AA to prostaglandins is not rate limited in the classical biochemical sense. However, a variety of factors may influence the relative amount of products formed, which as pointed out earlier is remarkably constant in any given tissue. For convenience the biosynthetic cascade from AA to prostaglandins will be deemed to be catalysed collectively by an enzyme group called prostaglandin synthetase.

In vivo activity of prostaglandin synthetase can only be inferred from amniotic fluid analyses and these have been discussed in Section 1 but in vitro studies of prostaglandin synthetase activity in fetal membranes have also been described (Kinoshita et al 1981, Okita et al 1981). However, the only product demonstrated in these in vitro experiments was PGE; no PGF was detected (Kinoshita et al 1981). This was due to the

incubation conditions used in the assay system which contained reduced glutathione. It is well known that the presence of reduced glutathione stimulates the production of PGE at the expense of PGF. Thus in vitro experiments utilizing the addition of external co-factors not known to be functional in vivo may result in conclusions about the enzyme activity not necessarily true in the in vivo situation.

Nevertheless, increased prostaglandin synthetase activity has been demonstrated in the amnion following the onset of labour (Kinoshita et al 1981, Okita et al 1981). No change in activity in the chorion or decidua vera was demonstrated. These results imply that the amnion probably has a more important role in prostaglandin biosynthesis than the chorion or the decidua vera.

2.6.3.1 Control of Prostaglandin Synthetase During Human Labour

The control of prostaglandin synthetase activity has been addressed from two points of view. This may be stimulation of the enzyme in states of increased prostaglandin production or removal of an inhibitor of the enzyme, thereby resulting in increased prostanoid synthesis. Saeed and Mitchell (1981) have demonstrated that prostaglandin synthesis by bovine seminal vesicle (BSV) prostaglandin synthetase was stimulated by cytosolic factors prepared from the amnion, chorion and decidua vera. Cohen et al (1985) separated amniotic fluid into 2

fractions; one fraction ($mw > 30\ 000$) was found to have an inhibitory effect on BSV prostaglandin synthetase while the other fraction ($mw < 1\ 000$) was stimulatory. They demonstrated that concomitant increase in stimulatory activity and decrease in inhibitory activity of amniotic fluid on BSV prostaglandin synthetase occurred with advancing gestation and during labour. They concluded that prostaglandin biosynthesis during labour may be dependent on a balance between inhibitory and stimulatory factors present in amniotic fluid.

A more popular approach has been that intra-uterine prostaglandin synthesis may be chronically suppressed by inhibitors of prostaglandin synthetase and that labour is associated with a withdrawal of this inhibitor (Brennecke et al 1981). Human pregnancy plasma has been shown to inhibit prostaglandin synthetase (Brennecke et al 1981), but no significant trends were detected in relation to pregnancy or parturition (Brennecke et al 1984). This excluded the hypothesis of acute removal of circulatory inhibitors of prostaglandin synthesis by the feto placental unit during labour.

The idea of a local mechanism within the feto placental unit in the regulation of prostaglandin synthesis is an attractive one since the production and action of these substances are such that they act as local hormones (Vane 1969). Indeed, acute reduction in the inhibitory effect of amniotic fluid obtained

following the onset of labour on prostaglandin synthetase activity, has been described (Saeed et al 1982, Brennecke et al 1983). None of these studies however, has examined the effect of amniotic fluid obtained at various stages of pregnancy and labour on PGF production by prostaglandin synthetase. This must surely also be an important factor in the light of significant increases in PGF production during normal labour (Section 1.2.3.2).

A relevant question that arises at this point is: What is the origin of these amniotic fluid factors that seem to regulate prostaglandin synthetase activity.

Manzai and Liggins (1984) have demonstrated release of inhibitory factors of prostaglandin synthetase by dispersed amnion cells in culture. This decreased significantly in tissues obtained following the onset of labour. These inhibitors in the amnion may well be related to the 2 proteins isolated in amniotic fluid that display similar inhibitory effect on prostaglandin synthetase activity (Wilson et al 1984).

It seems reasonable to conclude that endogenous inhibitors of prostaglandin synthetase may play a significant role in the regulation of prostaglandin synthesis associated with the onset of labour. However, the evidence presented thus far by no means explains all the changes of prostaglandin production

observed during normal labour (Section 1). The hypothesis, therefore, that the regulation of prostaglandin biosynthesis could be confined to any one factor seems unlikely. Any attempt to study the control of prostaglandin production by the intra-uterine tissues should therefore include an examination of as many factors as may be possible.

INTRODUCTION TO PRESENT STUDIES

INTRODUCTION TO PRESENT STUDIES

The preceding review has critically examined some aspects of the control of prostaglandin biosynthesis and its relevance to human parturition. A major problem facing researchers in the study of human labour has been the availability of suitable material for such studies. In vivo methods applicable in animals are often limited by ethical constraints in humans, and thus many in vitro techniques had to be developed to study aspects of human parturition. However, there is always the problem that such studies may not necessarily reflect the changes in prostaglandins which occur in vivo (Section 1.3).

In an attempt to overcome some of these methodological problems, Norman (1983) studied fetal endocrine changes in human twin pregnancies during the period leading to the initiation of labour and labour itself. His findings that PGE levels increased in the fetal circulation in twin I but not in twin II at the onset of labour, provided clear evidence of a fetal role in the initiation of labour. A study of the pituitary-adrenal gland function in these patients revealed that the increased PGE was not mediated by changes in the function of the fetal adrenal gland, a finding which confirmed that many of the control mechanisms described for ovine parturition (Liggins 1972) are not functional in man.

As a sequel to the studies by Norman (1981, 1982, 1983), the present thesis addresses the problem of control of prostaglandin production during human labour by examining one of the clinically abnormal labour patterns common in obstetric practice.

In primary dysfunctional labour, patients present with delay in active labour or inadequate progress during labour related directly to poor myometrial contractility (Section 1.4). Although the clinical criteria and the pattern of uterine contractions are well described (Turnbull 1957), the biochemical basis of the disorder has not been investigated. In view of the central role that prostaglandins play in the initiation of normal labour, it is reasonable to expect that dysfunctional labour is associated with a concomitant abnormality in the production of prostaglandins by the fetal membranes, as has indeed been documented in part (Section 1.4). The validity of a possible aberration in prostaglandin biosynthesis in dysfunctional labour will be explored in the chapters that follow.

THE PRESENT STUDIES

- CHAPTER THREE -

PATIENTS AND METHODS

3.1 INTRODUCTION

In Section 1, it was suggested that patients presenting with delay in labour due to primary dysfunctional labour might be a model in which to study the control of prostaglandin production by the feto-placental unit.

The practical problem is to distinguish those patients with primary dysfunctional labour from patients presenting with delay in labour secondary to cephalopelvic disproportion or fetal malposition. Objective criteria such as fetal head size, maternal pelvic dimensions and the intrauterine pressures generated during a contraction, assist in the correct categorizing of patients presenting with delay in labour. The present chapter describes the clinical details of multigravid patients presenting with dysfunctional labour and the criteria used in identifying such patients.

3.2 PATIENTS

3.2.1 *Criteria used for Patient Selection*

All patients studied were African multigravidae at term (gestation 38 - 42 weeks) with a history of previous normal vaginal delivery. Patients with a history of antenatal

complications in either past or present pregnancies were excluded from the study. Studies were commenced only in the active phase of spontaneous labour when the cervix was fully effaced, at least 3 cm dilated and accompanied by painful uterine contractions.

On presentation each patient was carefully examined by the same obstetrician for maternal or fetal "risk" factors that might alter management in labour. Patients with evidence of cephalopelvic disproportion on clinical examination were excluded from the study. In the labour ward the patients were personally attended to by the same midwife trained in the techniques used in this study.

A total of 86 patients was selected and they were retrospectively divided into two groups depending on the progress in labour as defined by the rate of cervical dilatation.

3.2.2 Patients Progressing Normally During Labour (Group I)

Patients presenting in spontaneous labour, whose labour progressed at a cervical dilatation rate greater than 1 cm/h, were considered to have a normal labour. All patients in this group had uncomplicated, unassisted vaginal delivery and numbered 54.

3.2.3 Patients Presenting with Delay in Active Labour (Group II)

In those patients who were admitted in spontaneous active labour but failed to progress adequately in the absence of obvious clinical signs of cephalopelvic disproportion, labour was augmented by an intravenous infusion of oxytocin. This was given initially at a rate of 2 mU/min and increased gradually until adequate uterine contractions and progress in labour was achieved. The maximum infusion rate was 14 mU/min. All patients were retrospectively designated dysfunctional labour.

3.3 PROGRESS AND MANAGEMENT DURING LABOUR

During labour patients were instructed to lie in the left or right lateral position. Progress in labour was assessed at hourly intervals using an aseptic vaginal technique. Details of labour were recorded on a specially designed dataform as well as on a routine partogram (Philpott 1972).

Labour was considered normal when cervical dilatation progressed at a rate greater than 1cm/h and terminated in an uncomplicated, unassisted vaginal delivery. When the cervix failed to dilate over a 2h period the patient was classified as delay in active labour.

X-ray pelvimetry was performed in all patients within 24 hours of delivery to establish objective pelvic dimensions. Indices of disproportion were based on a number of factors: infant birthweight;

infant biparietal diameter (BPD); the available brim area of the pelvis; a measure of the fetal head size relative to the pelvic brim area, that is, the cephalopelvic fit (CPF) (Cowan 1982).

Gestational age at delivery was assessed by procedures described by Dubowitz (1970). The BPD was measured by 1 person 12h and 5 days after delivery using engineering calipers and taking the mean of 6 readings (Cowan 1982).

3.4 METHODS

3.4.1 Measurement of Uterine Activity

An intrauterine catheter (Portex reference 700-415-010) was attached to an external pressure transducer (Bell and Howell type 4-327-1) and the system was flushed by gravity feed with a sterile sodium chloride solution (NaCl 150mmol/l). Air bubbles trapped in the tubing were displaced by continuous flushing.

The patient was placed in the lithotomy position and the membranes ruptured if this had not occurred spontaneously. An intrauterine catheter was introduced via the cervix and the tip positioned immediately behind the fetal head. Saline flushing was maintained continuously during insertion. An electrode attached to a cardiotocograph was placed on the fetal scalp to monitor fetal heart rate during labour.

The patient was then placed in the left lateral position and the height of the transducer adjusted to the level of the pubic symphysis. Transducer signals were transmitted to a prototype integrator built at St. Mary's Hospital, London (Steer 1977). Uterine activity was measured at 15 min intervals as a summation of the integrals of uterine pressures above the resting tone of the uterus. Following each 15 min recording the intrauterine catheter was flushed with approximately 5 ml of saline solution. Pressures were recorded until the cervix became fully dilated. In patients presenting with delay in active labour the time of commencement of oxytocin infusion was appropriately marked on the tocograph and measurements were then continued until full dilatation of the cervix.

3.4.2 Sampling of Amniotic Fluid during Labour

In 10 patients in Group I and 12 patients in Group II a second intrauterine catheter was inserted into the amniotic sac at the same time as the one used for intrauterine pressure recordings. This was also positioned immediately behind the fetal head.

Amniotic fluid, approximately 5 ml, was collected at hourly intervals throughout active labour into pre-cooled polypropylene tubes, transported immediately to the laboratory on ice and assayed for prostaglandin and arachidonic acid levels as detailed in Appendix C.3.4 and C.6 respectively.

3.5 STATISTICAL ANALYSES

When the recorded data had a parametric distribution it was analysed for significance using the Students t test; in non parametric distribution the Mann Whitney U test was applied. Differences in mean values were regarded as significant when p values were less than 0,05. Details of statistical analyses are presented in Appendix B.

3.6 RESULTS

3.6.1 Comparative Clinical Findings in the Two Groups of Patients Studied

In Table 3.1 relevant clinical details in the two groups under study are set out. There were no differences in maternal age, parity or pelvic dimensions between the 2 groups. Although the mean BPD of babies in Group II was marginally greater than that in Group I ($p = 0,05$), there was no difference in the CPF measurements between the 2 groups of patients.

Occipito-posterior position of the fetal head, which may result in prolonged active labour was present in only one patient in each of the 2 groups.

3.6.2 Oxytocin Infusion Rates

The dose of oxytocin required to effect progress in labour varied between patients (Fig 3.1). Although the maximum infusion rate was 14 mU/min, 24 patients (75 %) delivered spontaneously on a dose of oxytocin ≤ 8 mU/min.

TABLE 3.1

Clinical findings in mothers and babies in groups I and II. Results are expressed as mean and standard deviation $M \pm SD$.

	<u>Normal Labour</u>	<u>Dysfunctional Labour</u>
	<u>Group I</u>	<u>Group II</u>
<u>Maternal</u> : Number	<u>54</u>	<u>32</u>
Age (yrs)	26,7 \pm 5,2	26,5 \pm 6,5
Parity	2,6 \pm 2,0	2,8 \pm 2,0
Conjugate vera (mm)	106,3 \pm 9,0	103,9 \pm 8,7
Transverse brim (mm)	117,1 \pm 6,9	118,3 \pm 9,0
Brim area	98,6 \pm 11,2	99,4 \pm 13,3
Cervical dilatation on admission (cm)	4,7 \pm 1,1	4,2 \pm 1,3
Cervical dilatation at arrest		5,6 \pm 1,3
<u>Neonatal</u> : Number	<u>54</u>	<u>32</u>
Weight (kg)	3,3 \pm 0,4	3,4 \pm 0,4
Biparietal diameter (mm)	94,4 \pm 3,5	95,9 \pm 3,3*
Suboccipito-bregmatic (mm)	99,6 \pm 4,6	99,2 \pm 3,4
Head area	74,4 \pm 5,0	74,3 \pm 5,0
Cephalopelvic fit	76,7 \pm 9,3	79,1 \pm 9,2
Apgar Score	9,0 \pm 0,9	8,7 \pm 1,1

* p = 0,05 compared to Group I

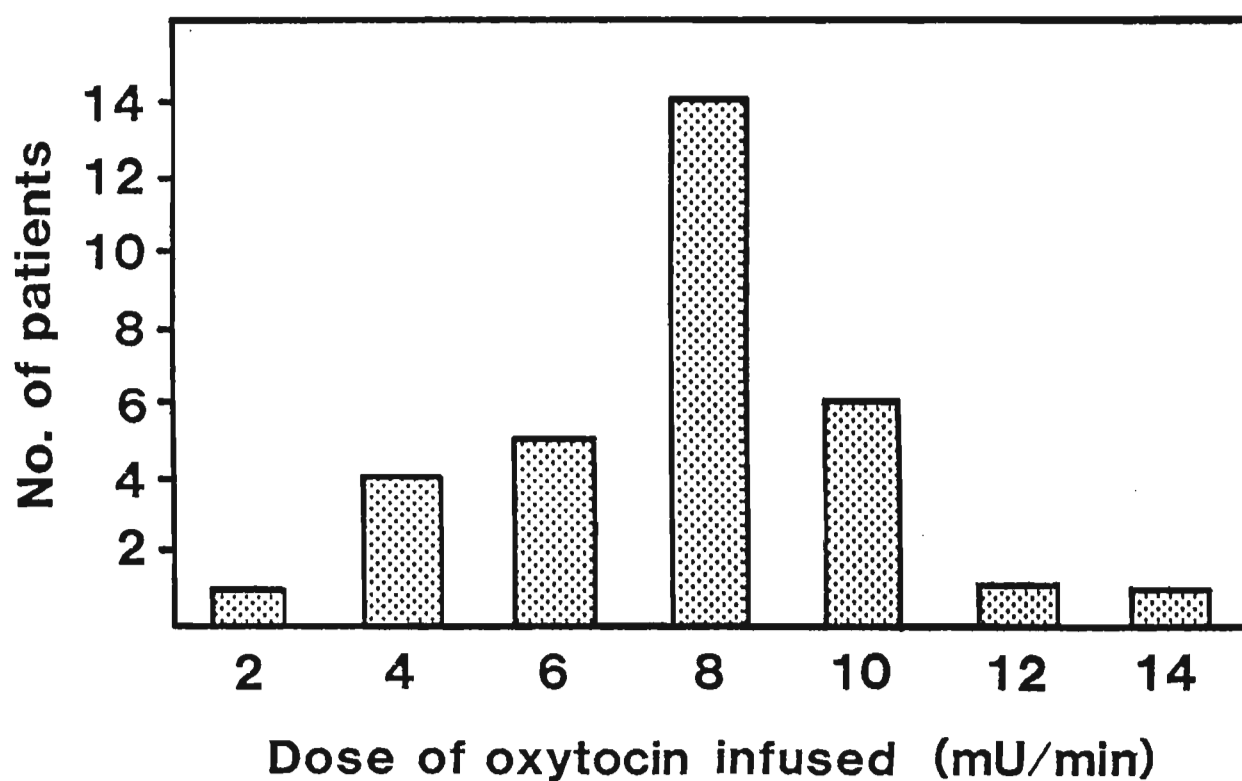


Figure 3.1 The maximum dose of oxytocin required to effect progress in labour in patients presenting with primary dysfunctional labour. Oxytocin infusion was commenced at 2 mU/min in all patients and increased gradually until progress in labour was achieved.

3.6.3 Uterine Pressures in the Two Groups

The mean intrauterine pressures (IUP) recorded in each group are presented in Table 3.2. In patients in Group II the mean IUP before oxytocin infusion was significantly less than that of normal labour. (Group I 1640 ± 40 kPa/15 min; Group II 1040 ± 32 kPa/15 min $p < 0.002$). During oxytocin infusion the mean IUP increased in all patients in Group II to levels similar to the mean value in Group I (Table 3.2).

In Fig 3.2 the distribution of the mean levels of IUP for individual patients in Group I and in Group II (before and after oxytocin administration) are presented. The wide range of IUP associated with normal labour is obvious from the figure. Only 4 patients presenting with dysfunctional labour had mean IUP levels greater than the mean level observed in normal labour (Fig 3.2); in all patients in Group II an increase in mean IUP occurred following oxytocin administration.

In Fig 3.3 the relationship between IUP and cervical dilatation is presented. During normal active labour there is a progressive and significant increase in IUP with maximum values recorded when cervical dilatation reached 9 cm. In patients with dysfunctional labour (Group II), the mean IUP before oxytocin infusion was significantly lower at 4,5,7 and 8 cm cervical dilatation than the corresponding levels in Group I ($p < 0.001$; Fig 3.3). Mean IUP levels following oxytocin

TABLE 3.2

Intrauterine pressure recordings (kPa/15min) in patients in Groups I and II. In the latter group the mean IUP before and after the administration of oxytocin are given.

	<u>No of Patients</u>	<u>Mean IUP</u> <u>(kPa/15min)</u>	<u>Standard</u> <u>Error of</u> <u>Mean</u>	<u>No of recordings</u>
<u>Group I</u>	54	1 640	40	319
<u>Group II</u>				
Before Oxytocin	32	1 040 *	32	172
After Oxytocin	32	1 840 **	40	197

* p < 0,002 compared to Group I

** p < 0,001 compared to mean level before oxytocin therapy.

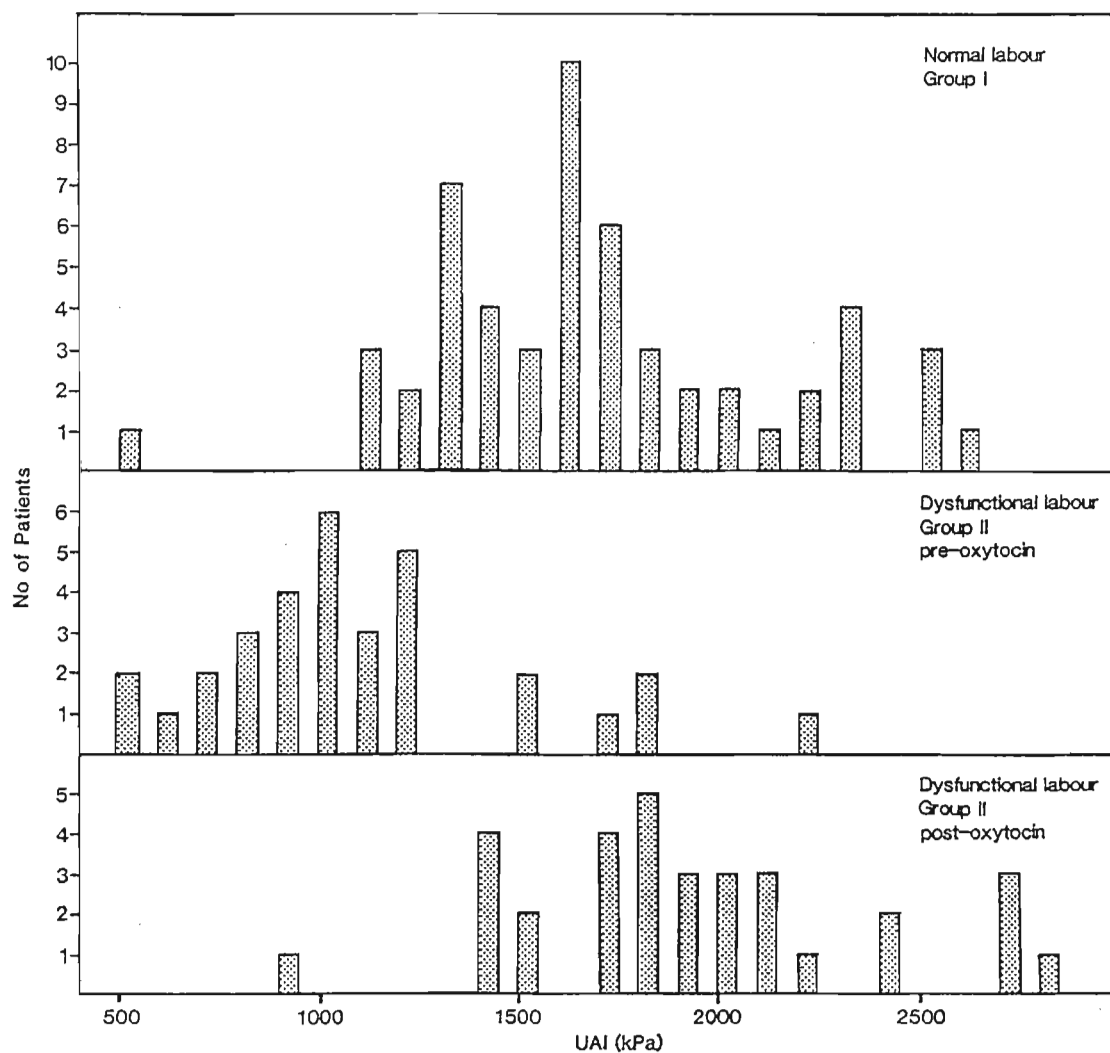


Figure 3.2 The distribution of the mean levels of intrauterine pressure recordings for individual patients in group I (normal labour) and group II (dysfunctional labour). In patients of group II the mean levels before and after oxytocin therapy have been plotted separately.

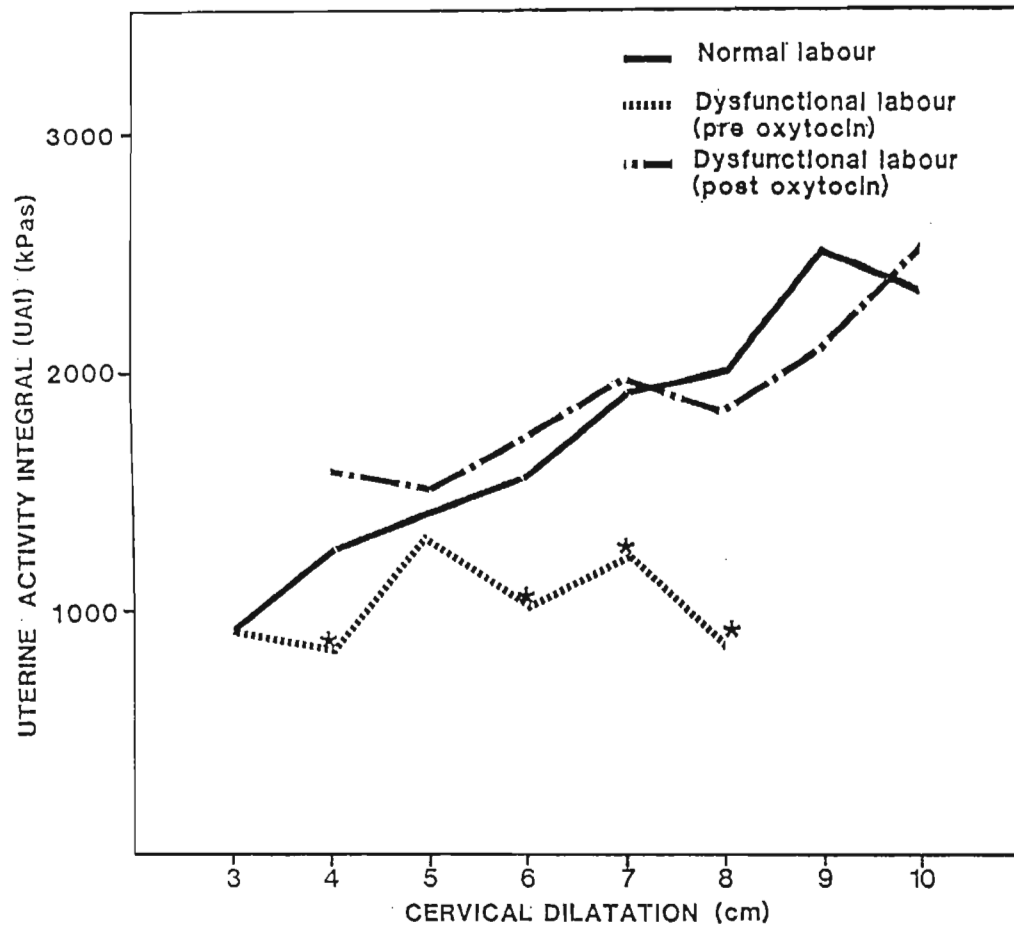


Figure 3.3 Relationship between the intrauterine pressure recordings and cervical dilatation in patients during normal and dysfunctional labour. In patients presenting with dysfunctional labour the mean intrauterine pressures before and after oxytocin therapy are plotted.

infusion were not different between the 2 groups at all stages of cervical dilatation (Fig 3.3).

3.6.4 Infant Morbidity and Mortality

The infants borne to mothers in this study were all in clinically sound condition at birth; Apgar scores were greater than 7/10 at 5 min after delivery and neonatal progress was uneventful.

3.7 DISCUSSION

In the present study there were no clinical differences in the mothers or babies between the two groups of patients. It can therefore reasonably be concluded that delay in labour was a primary uterine dysfunction and not related to any secondary cause.

Analysis of the uterine activity confirmed the hypokinetic nature of the myometrial contractions in patients presenting with dysfunctional labour. Correction of uterine activity following oxytocin infusion resulted in adequate progress in labour. Subsequent unassisted vaginal delivery in those patients provided unequivocal evidence that the cause for the delay in active labour in these patients was primary uterine dysfunction.

Thus, for the purposes of this study, patients with primary dysfunctional labour were readily identified and, as such, would provide a suitable model in which to study further some of the biochemical events known to be associated with human labour.

CHAPTER 4

AMNIOTIC FLUID PROSTAGLANDIN CONCENTRATIONS IN NORMAL AND DYSFUNCTIONAL LABOUR

4.1 PURPOSE OF THE STUDY

In the preceding chapter it was concluded that there is no difficulty in defining patients presenting with primary dysfunctional labour retrospectively, and as proposed in Section 1.4, such a group of patients would be eminently suitable for the study of the role of prostaglandins in the regulation of myometrial function.

It is generally accepted that amniotic fluid concentrations of prostaglandins reflect local production by the fetal membranes and in Section 1.2.3 it was pointed out that the lack of significant synthesis and metabolism of prostaglandins in amniotic fluid, makes it eminently suitable for studying prostaglandin changes during labour.

The purpose of this study is therefore to examine the changes in prostaglandin concentrations in amniotic fluid obtained from patients presenting with primary dysfunctional labour and to compare these results with those obtained from a matched group of patients during normal active labour.

As a first step the concentration changes of PGE and PGF in amniotic fluid were studied in patients presenting with dysfunctional labour. As described in Section 1.2.3, metabolism of the primary prostaglandin occurs in the fetal membranes and these metabolites may also be measured

in amniotic fluid. Since prostacyclin and thromboxane are synthesized from the common precursor, arachidonic acid, the respective metabolites of these two unstable compounds, 6 ketoprostaglandin $F_{1\alpha}$ (PGIM) and thromboxane B_2 (TXB₂) were also measured in amniotic fluid of these patients.

4.2 PATIENTS INCLUDED IN THE STUDY

The patients included in the study are described in detail in Section 3.2. A total of 22 patients were studied, 10 during normal active labour with spontaneous vaginal delivery (Group I) and 12 patients presenting with primary dysfunctional labour as identified retrospectively (Group II).

Amniotic fluid was collected at hourly intervals through an indwelling intra-uterine catheter as described in detail in Section 3.4.1. All samples were collected immediately before the next vaginal examination or the flushing of the intra-uterine catheter used for recording intra-uterine pressures as described in Section 3.4.1.

4.3 ANALYTICAL METHODS

Prostaglandins E, F, FM, PGIM and TXB₂ were measured by radio-immunoassay as described in Appendix C.3.4. Samples were stored at - 20° C and assayed within 2 weeks of collection.

4.4 STATISTICAL ANALYSES

Details of statistical analyses are presented in Appendix B.

The data were found to be non-parametric in distribution. Analysis of the significance of differences between the groups was therefore tested by means of the Mann Whitney test. Correlation coefficients were calculated on logarithmically transformed data. Differences in mean values were regarded as significant when the p value was less than 0,05.

4.5 RESULTS

4.5.1 Amniotic Fluid PGE concentration during normal and dysfunctional labour

In Figure 4.1 the concentration changes of PGE in amniotic fluid from patients in Group I (normal labour) and Group II (dysfunctional labour) are presented.

During the progress in normal labour, (Group I) PGE concentration in amniotic fluid increased significantly and could be correlated with the degree of cervical dilatation ($r = 0,82$; $p < 0,001$). The mean level of PGE in early labour (cervical dilatation 3-5 cm) was $41,4 \pm 23,3$ nmol/l ($M \pm SD$) and $78,8 \pm 25,3$ nmol/l in late labour (cervical dilatation 9 - 10 cm, $p < 0,05$).

In the 12 patients in Group II, a wide range of PGE concentrations in amniotic fluid was found throughout labour (Fig 4.1) but no statistical differences were demonstrated in the mean concentrations between the 2 groups of patients at all stages of cervical

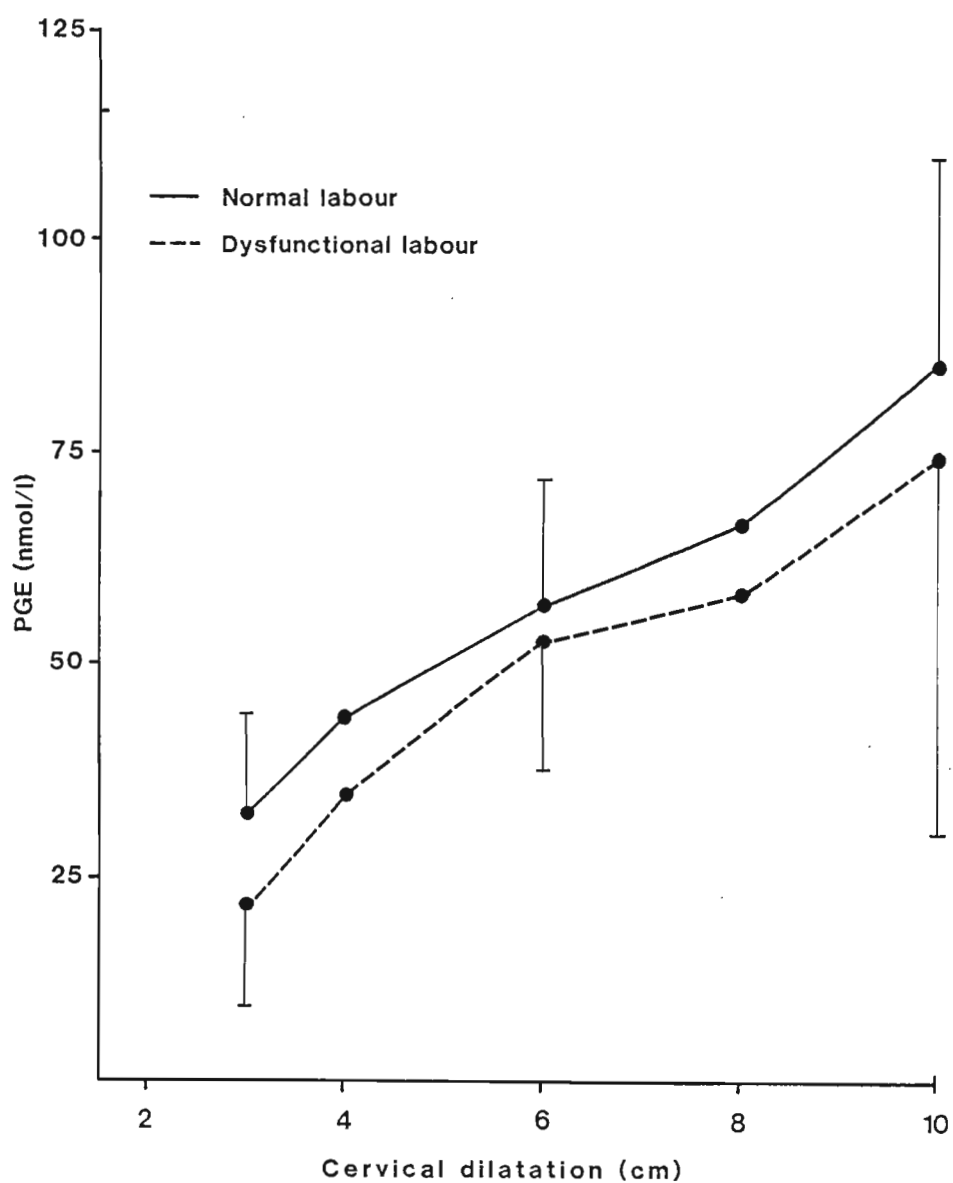


Figure 4.1 Comparison of PGE concentration in amniotic fluid during normal and dysfunctional labour in multigravid patients plotted against cervical dilatation. Values are expressed as nmol/l ($M \pm SD$).

dilatation. The mean concentrations of PGE in amniotic fluid in patients in Group II were $30,0 \pm 23,1$ nmol/l in early labour and $69,1 \pm 29,2$ nmol/l in late labour (Fig 4.1).

Administration of oxytocin did not have any effect on PGE concentration in amniotic fluid. In 3 of the 12 patients in Group II, PGE levels were lower than the rest of the group and despite oxytocin treatment levels did not reach those found in normal labour. The PGE concentrations in amniotic fluid during labour in these 3 patients are demonstrated in Figure 4.2.

4.5.2 Amniotic Fluid PGF concentration during normal and dysfunctional labour

During early labour in Group I, PGF levels were less than that of PGE. The mean levels for each prostanoid were PGE $41,4 \pm 23,3$ nmol/l and PGF $21,7 \pm 15$ nmol/l respectively, $p < 0,05$. As labour progressed PGF levels in amniotic fluid in Group I increased to levels higher than those of PGE with a close correlation to cervical dilatation ($r = 0,93$; $p < 0,001$). In late labour the respective values for PGE and PGF were $78,8 \pm 25,3$ nmol/l and $88,4 \pm 33,3$ nmol/l. The changing ratios of PGF to PGE during the progress in active labour are presented in Table 4.1.

In Figure 4.3 the changes in amniotic fluid PGF concentration throughout labour in patients in Groups I and II are presented.

Patients presenting with dysfunctional labour (Group II) had significantly lower PGF concentrations at all stages of cervical

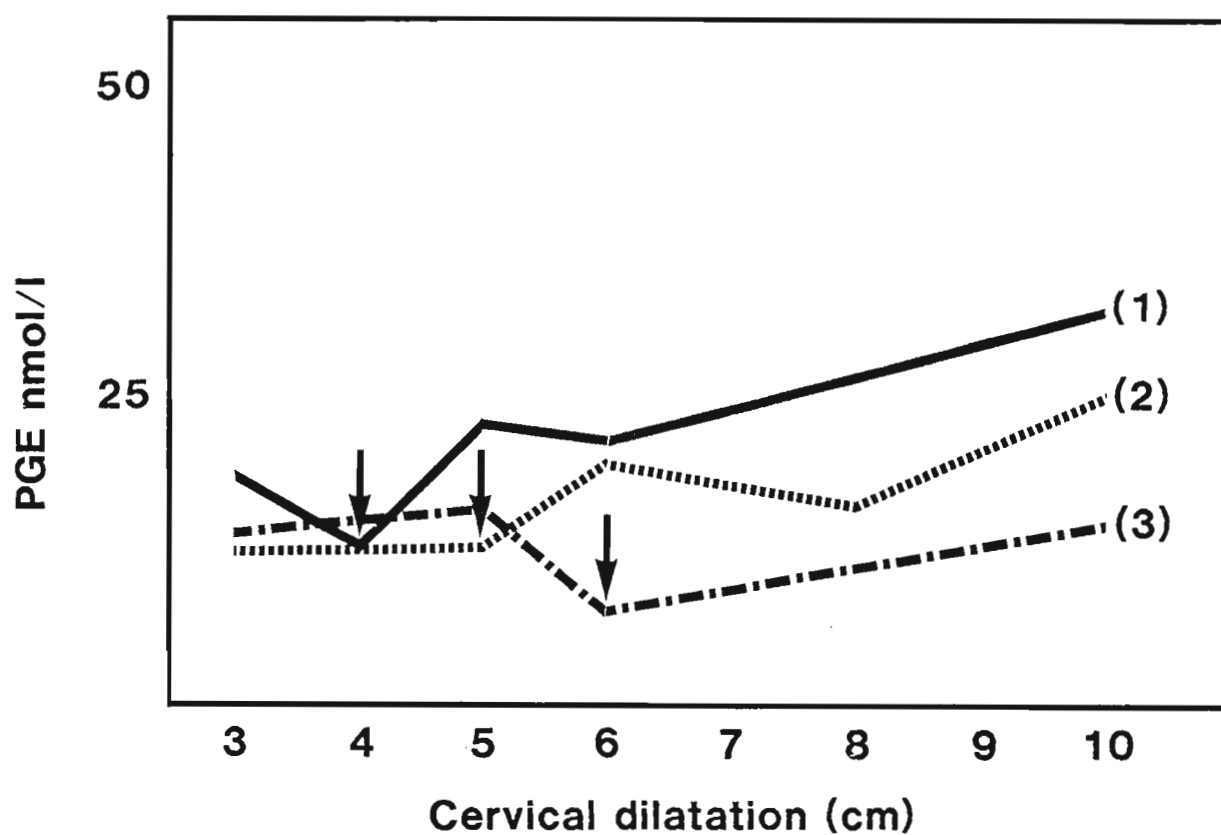


Figure 4.2 Concentration of PGE in amniotic fluid during labour in the 3 patients in group II in whom no increase in PGE was observed. Arrow indicates point at which oxytocin was administered in each patient.

TABLE 4.1

Changes in the PGF : PGE ratio in amniotic fluid during normal and dysfunctional labour.

		<u>Cervical Dilatation</u>		
		<u>3 - 5 cm</u>	<u>6 - 8 cm</u>	<u>9 - 10 cm</u>
Normal Labour				
PGF : PGE		0,44	1,05	1,16
Dysfunctional Labour				
PGF : PGE		0,27*	0,42*	0,32*

* = $p < 0,001$ at all stages of cervical dilatation during labour.

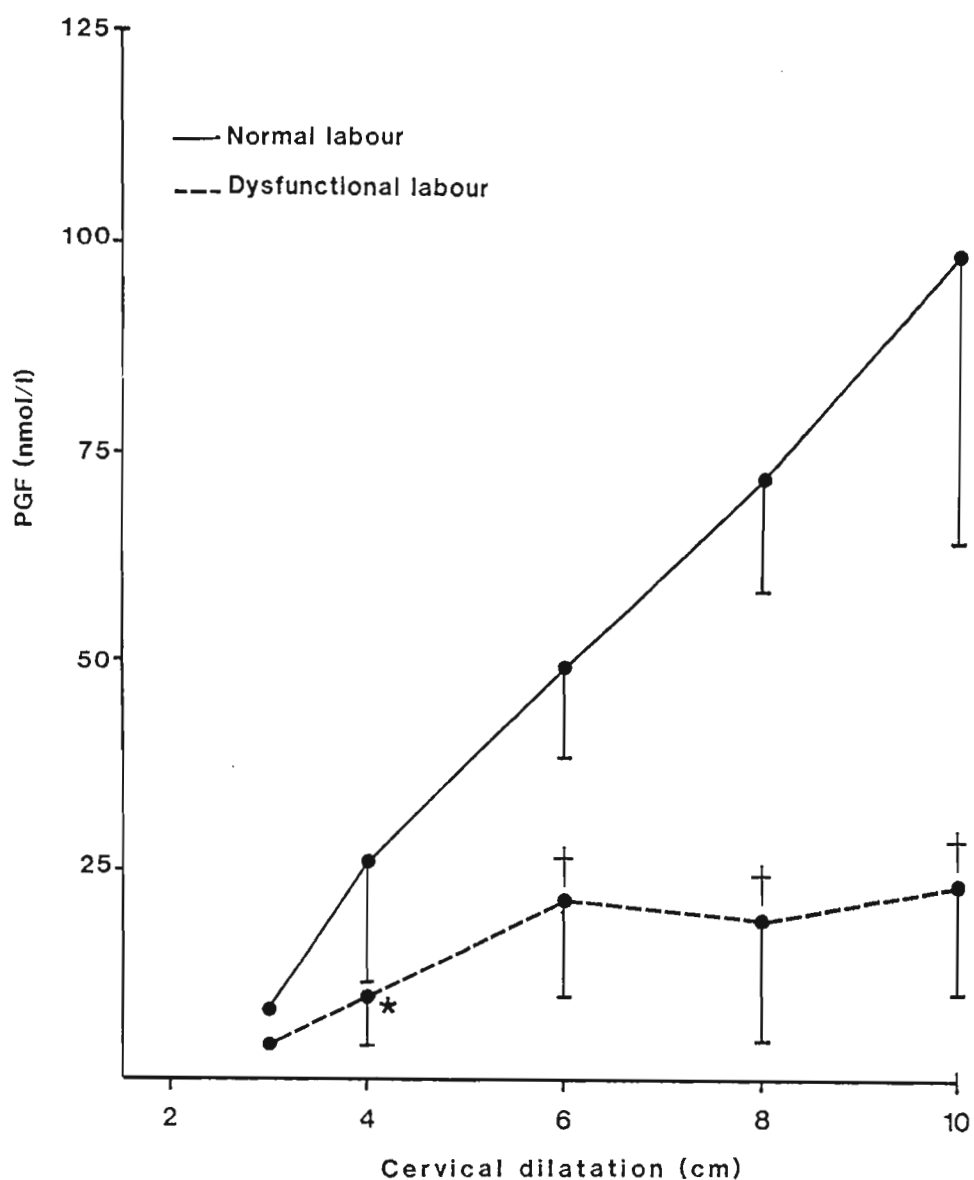


Figure 4.3 Comparison of PGF concentration in amniotic fluid during normal and dysfunctional labour in multigravid patients plotted against cervical dilatation. Values are expressed as nmol/l ($M \pm SD$)

* $p < 0.05$ compared to normal labour

† $p < 0.001$ compared to normal labour.

dilatation during labour compared to patients in Group I ($p < 0,001$; Fig 4.3). The ratio of PGF to PGE remained less than 1 throughout labour in this group and was significantly less than that in Group I ($p < 0,001$; Table 4.1).

Concentration of PGF did not alter significantly following the administration of oxytocin to patients in Group II. The relationship between IUP and PGF concentrations in amniotic fluid in 2 patients each in Group I and II are shown in Figs 4.4 and 4.5, respectively. A rapid increase in IUP occurred following oxytocin administration but no significant change in PGF concentration in amniotic fluid was demonstrated. (Fig 4.5).

4.5.3 Amniotic Fluid PGFM concentration during normal and dysfunctional labour

The change in PGFM concentrations in amniotic fluid paralleled those of PGF both during normal and dysfunctional labour (Fig 4.6). The mean level of PGFM in amniotic fluid in patients in Group I during early labour was $30,0 \pm 23,3$ nmol/l and in late labour was $70,6 \pm 48,6$ nmol/l ($p < 0,05$). In Group II the concentrations during early and late labour were $15,6 \pm 9,7$ nmol/l and $26,1 \pm 11,1$ nmol/l respectively ($p < 0,05$ compared to levels obtained in Group I). The concentration of PGFM in amniotic fluid in patients in group II did not change significantly following the administration of oxytocin.

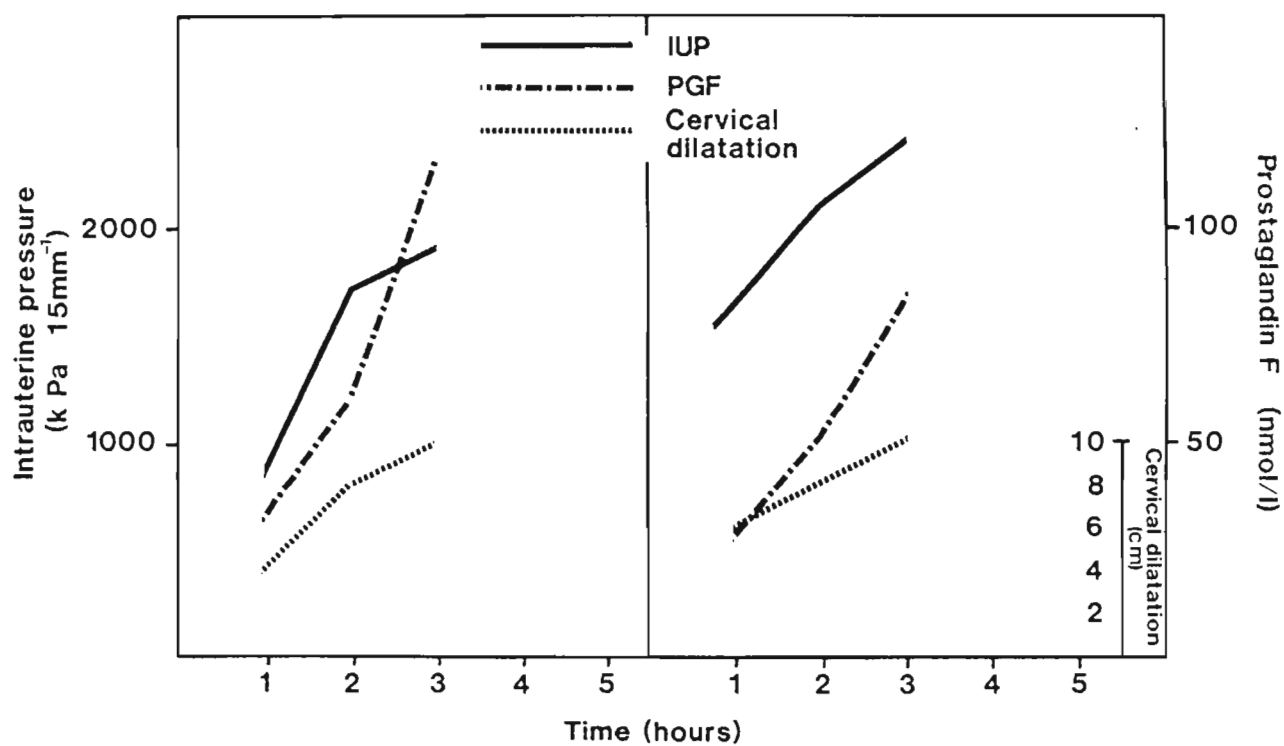


Figure 4.4 Changes in intrauterine pressure recordings and PGF concentrations in amniotic fluid during normal active labour (group I) in 2 patients.

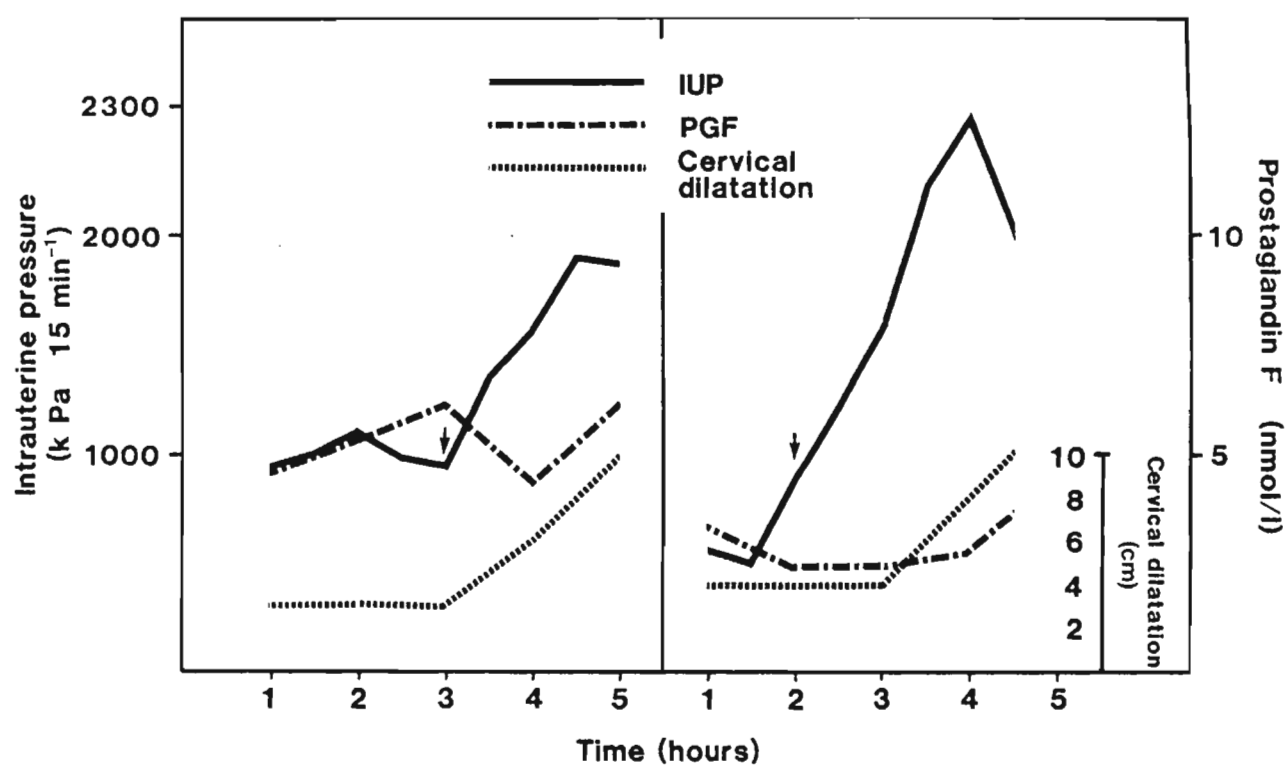


Figure 4.5 Changes in intrauterine pressure recordings and PGF concentrations in amniotic fluid in 2 patients with dysfunctional labour. Arrow indicates point at which oxytocin was administered.

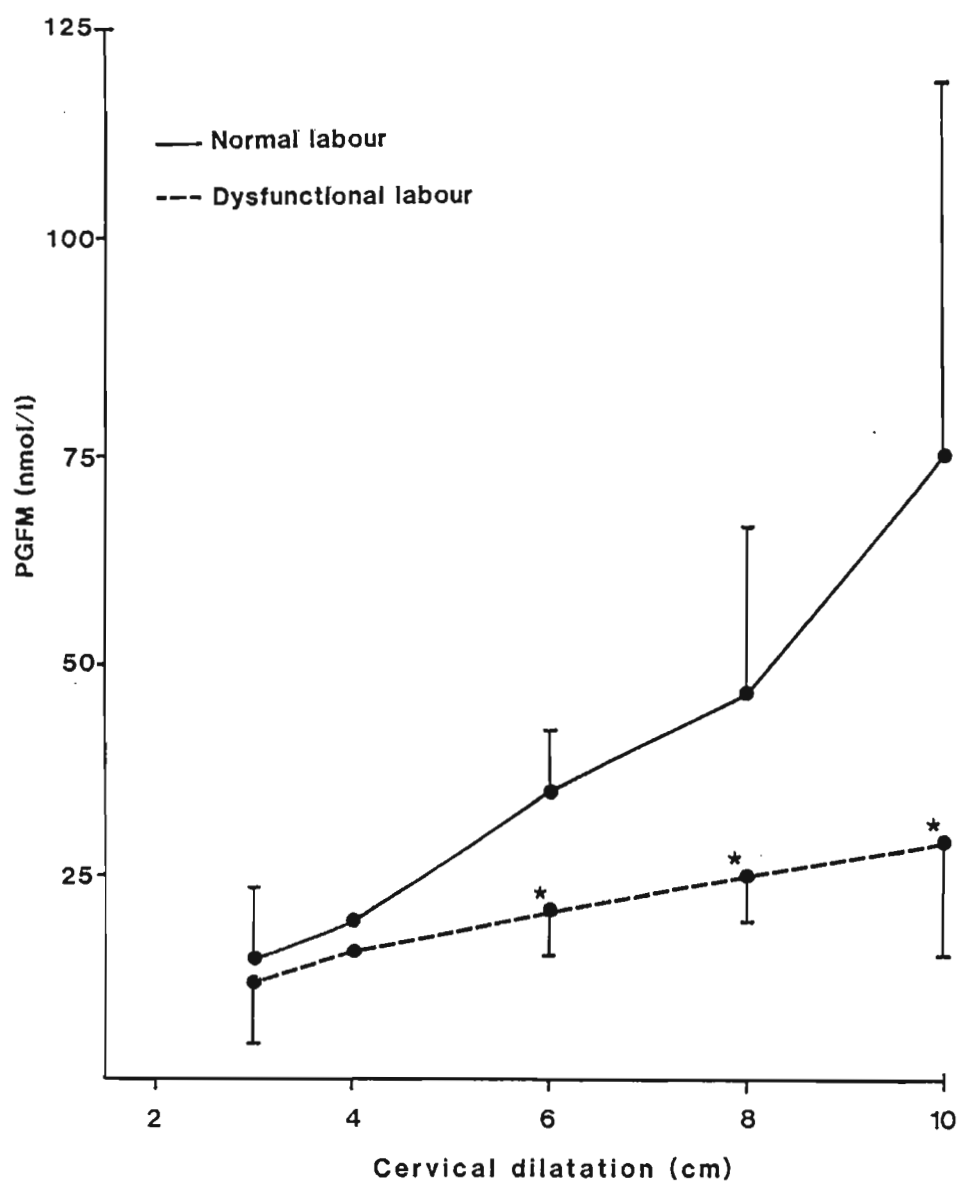


Figure 4.6 Comparison of 13,14 dihydro-15-keto prostaglandin $F_{2\alpha}$ (PGFM) during normal and dysfunctional labour plotted against cervical dilatation.

Values are expressed as nmol/l ($M \pm SD$)

* $p < 0.05$ compared to normal labour.

4.5.4 Amniotic Fluid PGIM concentration during normal and dysfunctional labour

The concentration of PGIM in amniotic fluid was significantly less than both PGE and PGF throughout normal active labour (Group I; $p < 0,001$ at all stages of cervical dilatation). The mean levels of PGIM fluctuated during labour and displayed no particular trend as labour progressed (Fig 4.7). No differences were demonstrated between levels obtained in patients from Groups I and II throughout labour (Fig 4.7). The administration of oxytocin did not have any effect on amniotic fluid PGIM concentrations.

4.5.5 Amniotic Fluid Thromboxane concentration during normal and dysfunctional labour

Absolute concentrations of TXB_2 in amniotic fluid were also significantly lower than PGE and PGF levels in patients in Group I ($p < 0,001$ at all stages of cervical dilatation). Levels remained low throughout labour and showed no correlation to cervical dilatation (Fig 4.8). No differences in TXB_2 levels in amniotic fluid were found between patients in groups I and II (Fig 4.8) and oxytocin infusion in patients in Group II had no effect on TXB_2 levels in amniotic fluid.

4.6 DISCUSSION

The results reported in the current study demonstrate clearly that PGE and PGF levels increase in amniotic fluid during active labour; PGFM, the membrane produced metabolite of PGF showed parallel increases. These

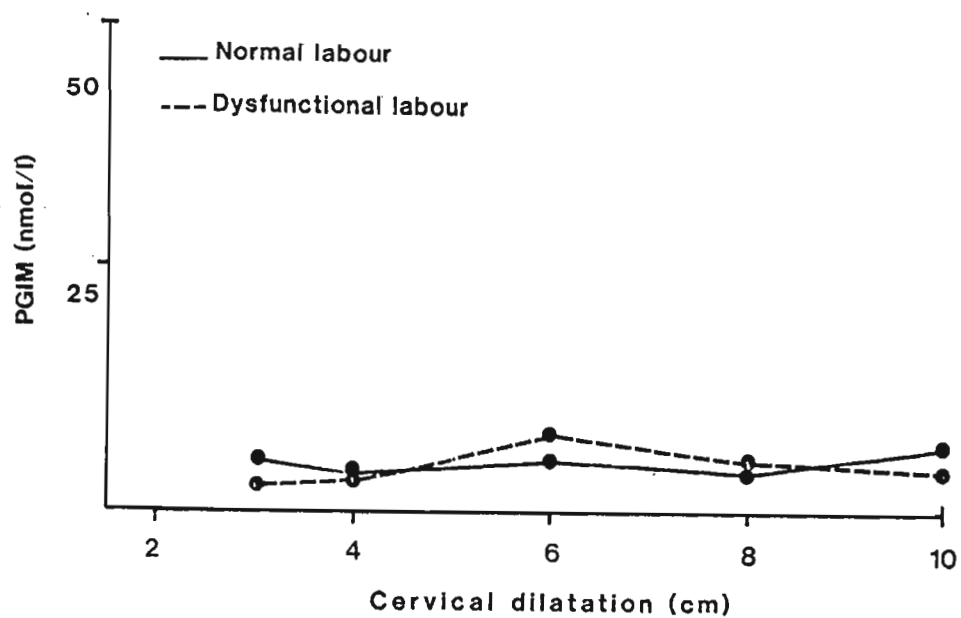


Figure 4.7 Comparison of 6-keto prostaglandin $F_{1\alpha}$ (PGIM) concentration in amniotic fluid during normal and dysfunctional labour plotted against cervical dilatation. Values are expressed as nmol/l.

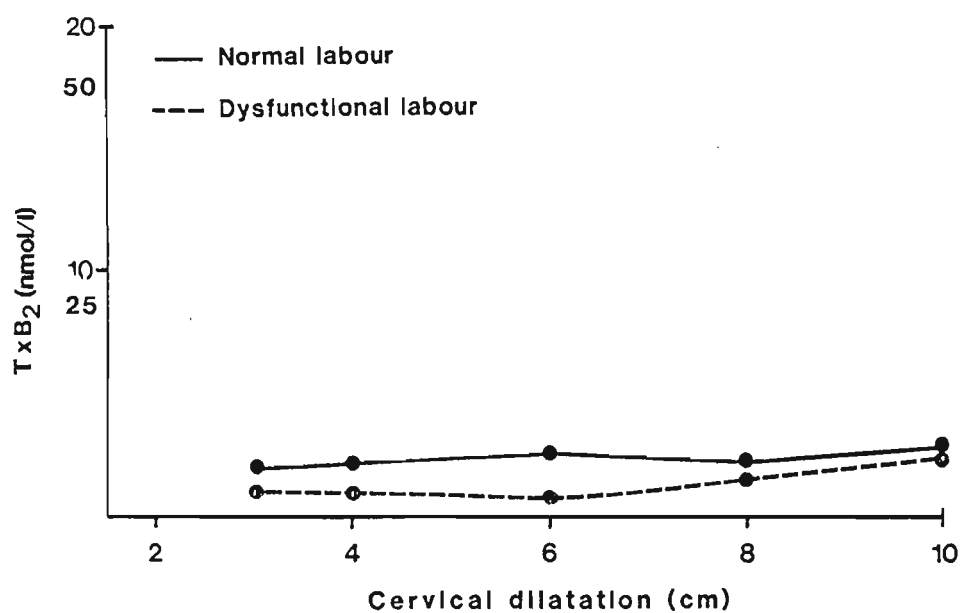


Figure 4.8 Comparison of thromboxane B₂ concentration in amniotic fluid during normal and dysfunctional labour. Values are expressed as nmol/l.

results confirm previous reports on the behaviour of these prostaglandins in amniotic fluid during normal active labour (Section 1.2.3.3).

With respect to dysfunctional labour however, the interesting phenomenon observed is the segregation in the behaviour of PGE and PGF during labour. The increase in PGE levels in amniotic fluid during dysfunctional labour was not significantly different to that found during normal active labour. There are two possible explanations for this finding.

Norman (1983) studied the behaviour of PGE and PGF in amniotic fluid during late gestation before the onset of labour. He demonstrated clearly that during the last 5 weeks of pregnancy PGE levels in amniotic fluid gradually increased whereas that of PGF showed no change at all. These findings are illustrated in Figs 4.9 and 4.10 respectively (Norman 1983). This evidence supports the interpretation that PGE is related to events necessary in preparing both mother and baby for parturition.

This interpretation is further supported by the fact that the patients presenting with dysfunctional labour went into spontaneous labour and the arrest of cervical dilatation that occurred later seemed independent of PGE levels in amniotic fluid. Further evidence for the preparatory role of PGE is based on the findings that PGE can reproduce all the structural and biochemical changes that characterize cervical maturation (Huszar 1981), findings which are consistent with the cervical status of the patients with dysfunctional labour in the present study. It is also known that PGE can influence many aspects of organ maturation in the fetus, including preparation of the fetal blood supply for the haemodynamic changes consequent during parturition (Coccaciuo et al 1977,

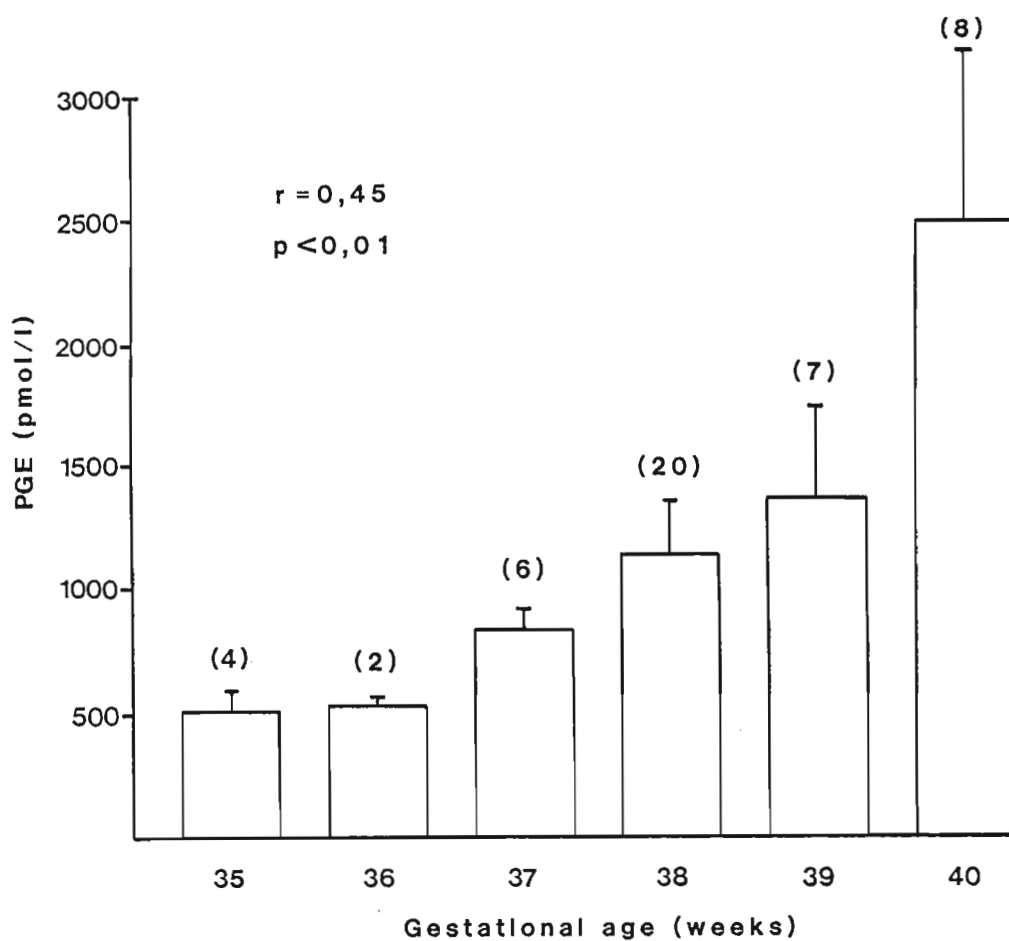


Figure 4.9 Prostaglandin E concentrations in amniotic fluid before the onset of labour (mean \pm SE). Twins I and II combined (from Norman In: The Twin Pregnancy as a Model for Studies in the Endocrinology of Parturition and Development. M.D. Thesis, 1983).

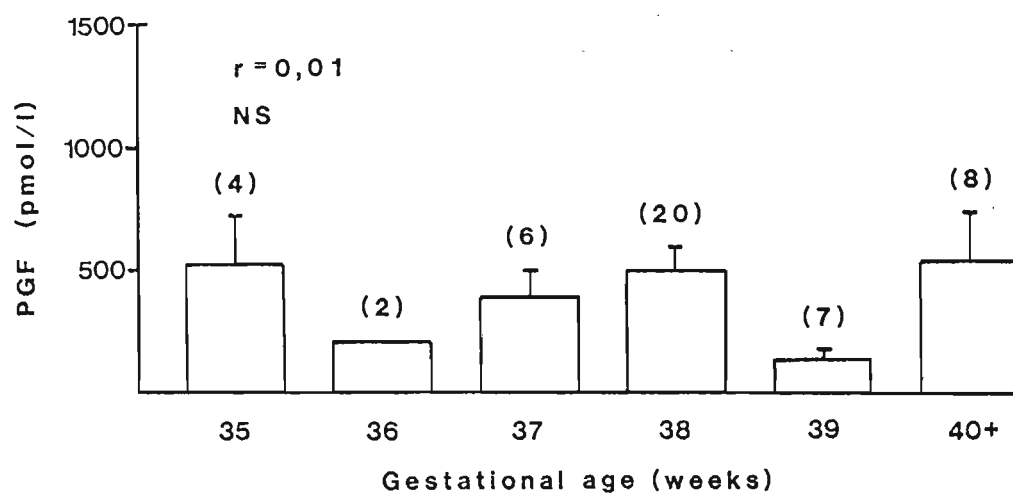


Figure 4.10 Prostaglandin F concentrations in amniotic fluid before the onset of labour (mean \pm SE). Twins I and II combined (from Norman In: The Twin Pregnancy as a Model for Studies in the Endocrinology of Parturition and Development. M.D. Thesis, 1983).

Rankin 1978), circumstances which again are manifest in the present study in that all the patients presenting with dysfunctional labour gave birth to healthy infants. Thus if the role of PGE is primarily that of preparation of mother and baby for labour rather than a role in the maintenance of uterine contractions during labour, then the continued increase in amniotic fluid PGE levels in dysfunctional labour would be entirely consistent with the cessation in the progress of active labour.

An alternative interpretation however is suggested by the 3 patients presenting with dysfunctional labour who showed no significant increases in amniotic fluid PGE levels during labour although levels in early labour were no different to those in normal labour. It may well be that if larger numbers of patients were studied that a subgroup in dysfunctional labour may segregate in which both PGE and PGF levels fail to increase. This may then be representative of a spread, in quantitative terms, of the abnormality which would then suggest a more general limitation in prostaglandin biosynthesis.

With respect to the behaviour of PGF levels during dysfunctional labour, the evidence is unequivocal that they do not follow the rapid increases seen during normal labour. This phenomenon may be the result of decreased production of PGF or an increased rate of metabolism. However, the finding of low levels of the metabolite of PGF, that is PGFM, in amniotic fluid from patients in dysfunctional labour excludes increased metabolic disposal as an explanation for the observed results. In as much as PGF is known to stimulate uterine contractions and since PGF failed to increase in dysfunctional labour, a cause and effect relationship between inhibition of PGF production and poor uterine contractions in dysfunctional labour seems a reasonable assumption.

As to the question of the cause of the diminished PGF levels in dysfunctional labour, no positive clues have emerged from the present study. However it has illustrated some important aspects of prostaglandin production in relation to labour.

Although this study was not primarily designed to examine the converse of the proposal by Satoh et al (1981; Section 1.3), that parturition may be associated with a switch to myometrial production of PGF at the expense of PGIM (the non contractile prostanoid), it is quite obvious that in the present study PGIM levels did not change in amniotic fluid during either normal or dysfunctional labour.

The suggestion made in Section 2.2.1 that switches in the biosynthetic pathway to the various products of cyclo-oxygenase activity could possibly be a controlling mechanism in specific prostaglandin biosynthesis, also finds little support from the current study since no differences in the levels of PGIM and TXB_2 were found between normal and dysfunctional labour.

Although not germane to the purpose of the present study, the effect of infused oxytocin on the prostaglandin levels in amniotic fluid is interesting. Oxytocin was used to effect normal unassisted delivery in patients presenting with dysfunctional labour at therapeutically low doses and this objective was manifestly achieved. Yet it had no effect whatsoever on PGE and PGF concentrations in amniotic fluid and thus presumably on the biosynthesis of these two prostanoids in vivo. Nevertheless, in patients presenting with dysfunctional labour, progress in labour was achieved under the influence of oxytocin and it therefore seems that oxytocin affects uterine contractions by a mechanism(s) independent of the action of prostaglandins.

It may be concluded from the present study that PGF biosynthesis is unequivocally severely limited in dysfunctional labour, and that arrest in progress in labour and deficient PGF synthesis are related as cause and effect. If the reasons for this limitation of PGF synthesis are to be further investigated, a systematic study by in vitro techniques of the biosynthetic pathway of prostaglandins in the fetal membrane is clearly necessary.

CHAPTER 5

PROSTAGLANDIN PRODUCTION BY THE FETAL MEMBRANES IN NORMAL AND DYSFUNCTIONAL LABOUR

5.1 PURPOSE OF THE STUDY

In the preceding section (4.5) patients presenting with dysfunctional labour were found to have significantly lower amniotic fluid concentrations of PGF and PGFM as compared to those in normal active labour. One possible explanation for this finding would be decreased PGF production and/or release by the fetal membranes in this condition. The present study therefore examined prostaglandin release by the fetal membranes obtained from patients presenting with primary uterine dysfunction.

In Section 1.3 various studies employing different techniques in assessing prostaglandin production by the fetal membranes have been critically reviewed and comment has been made on the interpretation of the results of these studies. In an attempt to overcome some of these technical problems, a method was developed for the continuous superfusion of intact untraumatized full thickness fetal membranes (amnion and choriondecidua), thus approximating the membrane status under in vivo conditions.

In the present chapter this methodology is described in detail. The effect of labour on prostaglandin release by the fetal membranes was also examined by comparing tissues obtained following spontaneous normal labour to that obtained at elective caesarean section.

5.2 PATIENTS INCLUDED IN THE STUDY

Patients included in the study have been described in Section 3.2.

Placentae were collected from 8 patients in Group I (normal labour) and 6 patients in Group II (dysfunctional labour). In addition, placentae were obtained from 10 patients undergoing elective caesarean section (Group III prelabour group).

5.3 METHODS

5.3.1 Sample Collection and Preparation

The placentae were collected immediately following delivery, placed on ice and transported to the laboratory where the fetal membranes were carefully cut from the placenta at the site of attachment. A piece of membrane (amnion and choriodecidua intact) approximately 50mm by 30mm was then cut from an area midway between the site of rupture and attachment of the membranes to the placenta using a pair of sharp scissors. This was washed in ice cold saline (9g/l) until free of loose debris and blood. No attempt was made to dissect off the decidua from the chorionic side of the fetal membranes.

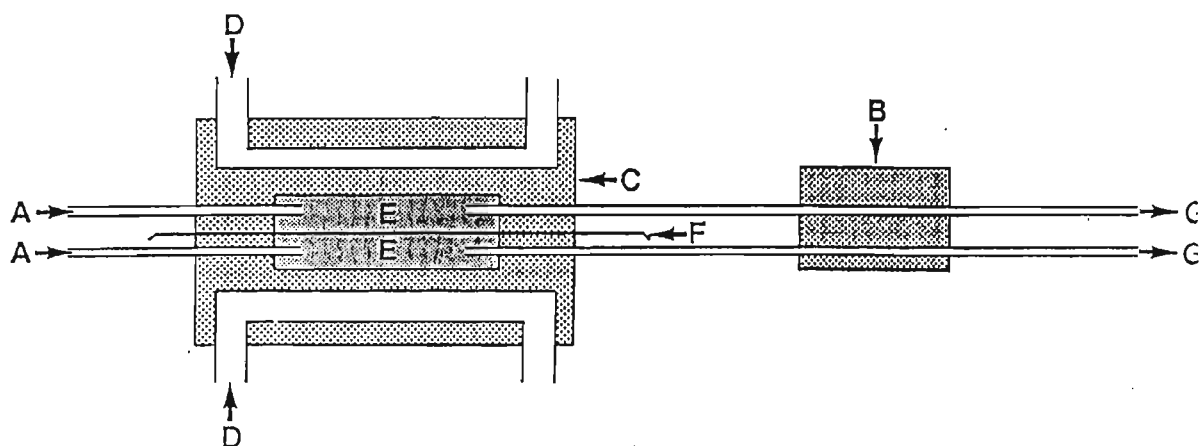
To assess any variation in prostaglandin production which may be dependent on the site of the membranes from which the sample was taken, 3 samples of membrane were taken from each of 4 different placentae. After the membranes were removed from the placentae as described above, the 3 samples were clamped in 3 separate superfusion chambers, superfusion and sampling were then synchronous.

5.3.2 Superfusion of the Fetal Membranes

The superfusion chamber comprised 2 identical halves made of perspex, which, when held together by a clamp, enclosed a chamber of total volume of 1,92 ml (Fig 5.1). Insertion of the fetal membrane sample between the 2 halves of the superfusion apparatus resulted in 2 independent chambers of equal volume on either side of the membrane, fluid in one chamber being in contact with the amnion layer of the membrane, and on the other side with the choriodecidual layer of the membrane (Fig 5.1).

The tissue in the chamber was superfused with Eagles Minimum Essential Medium (MEM with Earles Salts, L-glutamine and non essential amino acids, without sodium bicarbonate, Gibco Ltd, Catalogue No. 072-1500). Only freshly prepared medium was used and sodium bicarbonate (2,2 g/l of MEM) was added immediately before reconstitution of the fluid medium and the pH adjusted to 7,4 with sodium hydroxide (0,1 mol/l).

The MEM perfusion medium was pumped through the two chambers separated by the fetal membranes at a flow rate of about 8 ml/h. The temperature of the medium was kept constant at 37°C; the medium was continuously gassed with a 95% O₂ 5% CO₂ mixture; total superfusion area was 480mm² and the depth of the chamber on either side of the membrane was 2mm (Fig 5.1).



- A** Eagles MEM gassed with 95% O₂ 5% CO₂
- B** Pump at flow rate of approximately 8mls/hr
- C** Superfusion cell comprised of 2 identical halves held together by a clamp
- D** Water at 37°C
- E** Superfusion chamber divided into 2 compartments by membrane
- F** Fetal membrane
- G** Fractions of superfusates collected from either side of membrane

Figure 5.1 Schematic representation of the apparatus used in the superfusion experiments.

Even though the samples were collected with minimum trauma to the membranes, a superfusion period of 1h was allowed for equilibration of the system and to wash out any prostaglandins released as a result of sample handling. Thereafter, superfusates were collected from either side of the fetal membrane at 15min intervals for a period of 3h; samples collected were immediately frozen using dry ice and alcohol, and stored at -20° C until assay.

5.3.3 Measurement of Prostaglandins in Superfusates of the Fetal Membranes

Prostaglandin E, PGF and PGFM were measured in the superfusates by specific RIA as described in Appendix C.3.4. The validity of the RIAs in solutions containing Eagles Medium was established by comparing the assay results with values obtained after HPLC of the samples as detailed in Appendix C.4.1

5.3.4 Histological Examination of the Fetal Membranes

The fetal membranes were examined by light and electron microscopy before and after the superfusion experiments.

5.4 STATISTICAL ANALYSIS

The data obtained from the superfusion experiments proved to be non - parametric in distribution and the Mann Whitney U test was applied to test the significance of observed differences in the means.

5.5 RESULTS

5.5.1 High Performance Liquid Chromatography of Superfusates

The results obtained following RIA on HPLC fractions of the superfusates in the 4 experiments performed are shown in Table 5.1. The amounts of the PGE and PGF measured in the samples following HPLC correlate with that measured following RIA of the superfusates after extraction alone as described in Appendix C.3 (Table 5.1).

5.5.2 Tissue Variation of Prostaglandin Release

In Table 5.2 the amounts of PGE and PGF released from samples of fetal membrane obtained from 3 different sites of the same tissue when superfused under similar conditions, are shown. The release of prostaglandins from both the amnion and chorion sides of the membrane were mostly comparable, this applied to both PGE and PGF values.

5.5.3 Histology of the Fetal Membranes

Light microscopy of the tissues confirmed the presence of all 3 tissue types; amnion, chorion and decidua. There were no gross histological changes observed between tissues before and after the superfusion experiments. Electron microscopy confirmed the viability of cells of the amnion and chorion (Appendix C.4.1.2, Figs C7 - 10).

TABLE 5.1

Comparison of results of radioimmunoassay on superfusates from membranes following extraction plus high performance liquid chromatography and extraction alone.

	Normal Labour	Prelabour
RIA following HPLC of superfusate (n=2)		
* PGE (pg/cm ² /3h)	4525	1119
* PGF (pg/cm ² /3h)	840	203
RIA following extraction alone (n=8)		
PGE (pg/cm ² /3h)	1742 - 10529	550 - 1646
PGF (pg/cm ² /3h)	338 - 972	205 - 627

* Mean of 2 values presented.

TABLE 5.2

Release of prostaglandin E and F ($\text{pg}/\text{cm}^2/3\text{h}$) from the amnion and choriodecidual surfaces of fetal membranes taken from 3 different sites in 4 different placentae. The membrane samples were obtained from placentae a,b,c and d following spontaneous normal labour (1,2 and 3 denote the 3 membrane samples obtained from each placenta)

	<u>PGE</u>			<u>PGF</u>		
	<u>($\text{pg}/\text{cm}^2/3\text{h}$)</u>					
	1	2	3	1	2	3
Amnion side of fetal membrane						
a	1113	958	965	340	550	590
b	3542	3119	3426	502	476	550
c	3840	3470	6840	680	806	1080
d	3383	2859	2330	671	456	439
Choriodecidual side of fetal membrane						
a	525	352	342	235	196	183
b	455	492	507	348	298	317
c	846	927	1217	784	681	602
d	642	647	738	778	682	427

5.5.4 PGE release from the Fetal Membranes in Normal and Dysfunctional Labour

The release of PGE from the fetal membranes in the 3 groups of patients studied is shown in Table 5.3 and it is obvious that the amnion surface of the fetal membrane produced about 2 - 3 times more PGE than the choriodecidual surface.

In tissues obtained following normal labour (Group I) there was a 3 fold increase in PGE released by the amnion surface of the membrane only (Table 5.3). The release of PGE from the amnion surface of the membranes in tissues obtained from patients with dysfunctional labour was also greater than that of prelabour tissue ($p < 0,05$ Table 5.3) but remained less than that released by the amnion surface of membranes from patients in normal labour ($p = 0,05$; Table 5.3). The release of PGE from the amnion in the 3 groups of patients studied is depicted in Fig 5.2.

No statistical differences were observed in the release of PGE by the choriodecidual sides of the fetal membrane between the 3 groups of patients (Fig 5.3).

5.5.5 PGF release from the Fetal Membranes in Normal and Dysfunctional Labour

The release of PGF from the amnion surface of the membranes was less than that of PGE in all 3 groups of patients studied (Table 5.3).

In tissues obtained following normal labour there was a significant increase in PGF release by the amnion surface as compared to that released by the amnion surface in tissues obtained before the onset of labour ($p < 0,02$; Table 5.3).

TABLE 5.3

Release of PGE and PGF by the amnion and choriodecidual surfaces of fetal membranes obtained from the patients in the three groups studied. Results expressed as median and range of prostaglandin production during a 3h superfusion.

	<u>Group I</u>	<u>Group II</u>	<u>Group III</u>
	Normal Labour	Dysfunctional	Prelabour
	n = 8	Labour n = 6	n = 10
Amnion side of fetal membrane			
PGE (pg/cm ² /3h)	2993 [*] (1742-10529)	1806 ^{**} (1127-2867)	918 (550-1646)
PGF (pg/cm ² /3h)	662 [*] (338-972)	205 ^Φ (129 -259)	370 (205-627)
Choriodecidual side of fetal membrane			
PGE (pg/cm ² /3h)	983 (456-1280)	845 (348-1432)	530 (349-1223)
PGF (pg/cm ² /3h)	459 (222-707)	310 (205-824)	537 (210-757)

* p < 0,02 compared to prelabour

** p < 0,05 compared to prelabour

** p = 0,05 compared to normal labour

Φ p < 0,001 compared to normal labour

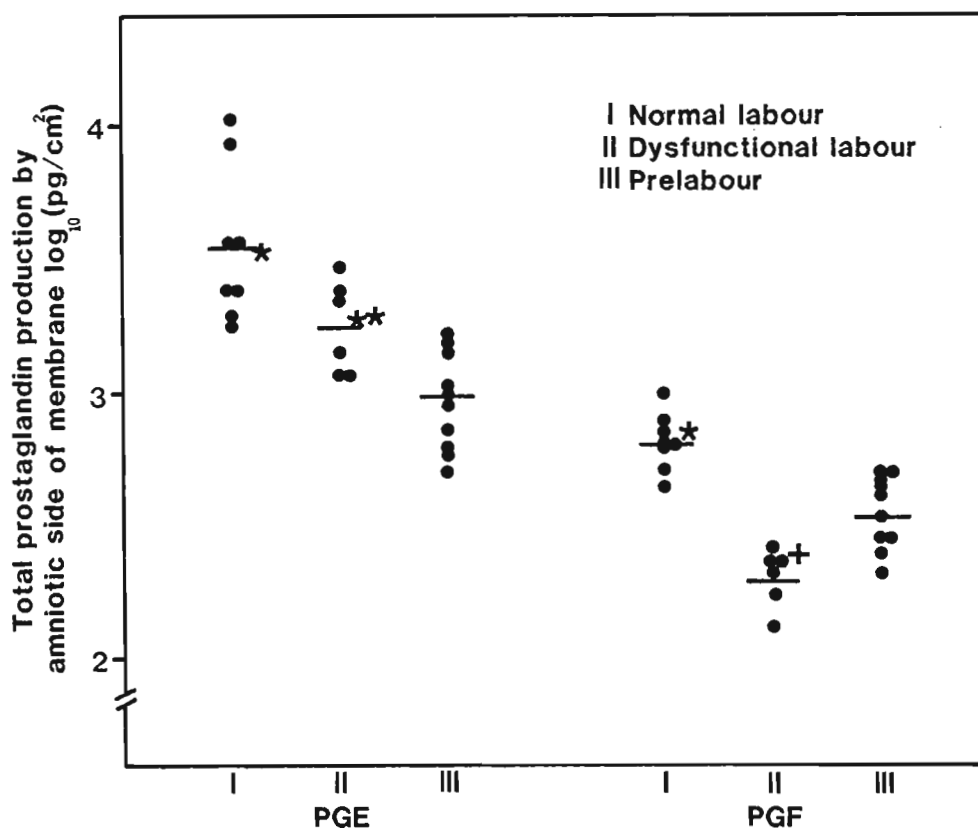


Figure 5.2 Total prostaglandin E and F release by the amnion side of the fetal membrane during 3 hours superfusion in the 3 groups of patients studied (expressed as $\log_{10} \text{pg/cm}^2$ of membrane). Horizontal bars indicate median value.

* $p < 0.02$ compared to prelabour (group III)

** $p < 0.05$ compared to prelabour (group III)

** $p = 0.05$ compared to normal labour (group I)

+ $p < 0.001$ compared to normal labour (group I).

The release of PGF from the amnion surface obtained from patients with dysfunctional labour however was significantly less than that of normal labour ($p < 0,001$ Fig 5.2). No statistical difference in the release of PGF by the choriodecidual sides of the membranes was observed between the 3 groups of patients (Fig 5.3).

5.5.6 PGFM release from the Fetal Membranes in Normal and Dysfunctional Labour

The amount of PGFM released from the fetal membranes in the few experiments performed in the 3 groups of patients is tabulated in Table 5.4. The amount of PGFM released by the amnion surface was less than that of PGE and PGF in all 3 groups of patients studied and was undetectable in 2 patients. No obvious differences in the release of PGFM by the amnion of choriodecidual surfaces were observed between the 3 groups of patients studied.

5.5.7 Pattern of Prostaglandin Release from the Fetal Membranes in Relation to Labour

A difference in the pattern of prostaglandin release by the amnion surface of the fetal membranes was observed in tissues obtained following normal labour, evidenced by the apparent pulsatile or intermittent release observed in the prelabour group. In Figures 5.4 and 5.5 the typical patterns of PGE and PGF release from 2 patients each before and after normal labour are demonstrated.

In tissues obtained following dysfunctional labour there was an apparent lack of the pulsatile release of PGF from the amnion surface observed in normal labour. This was less clearly defined

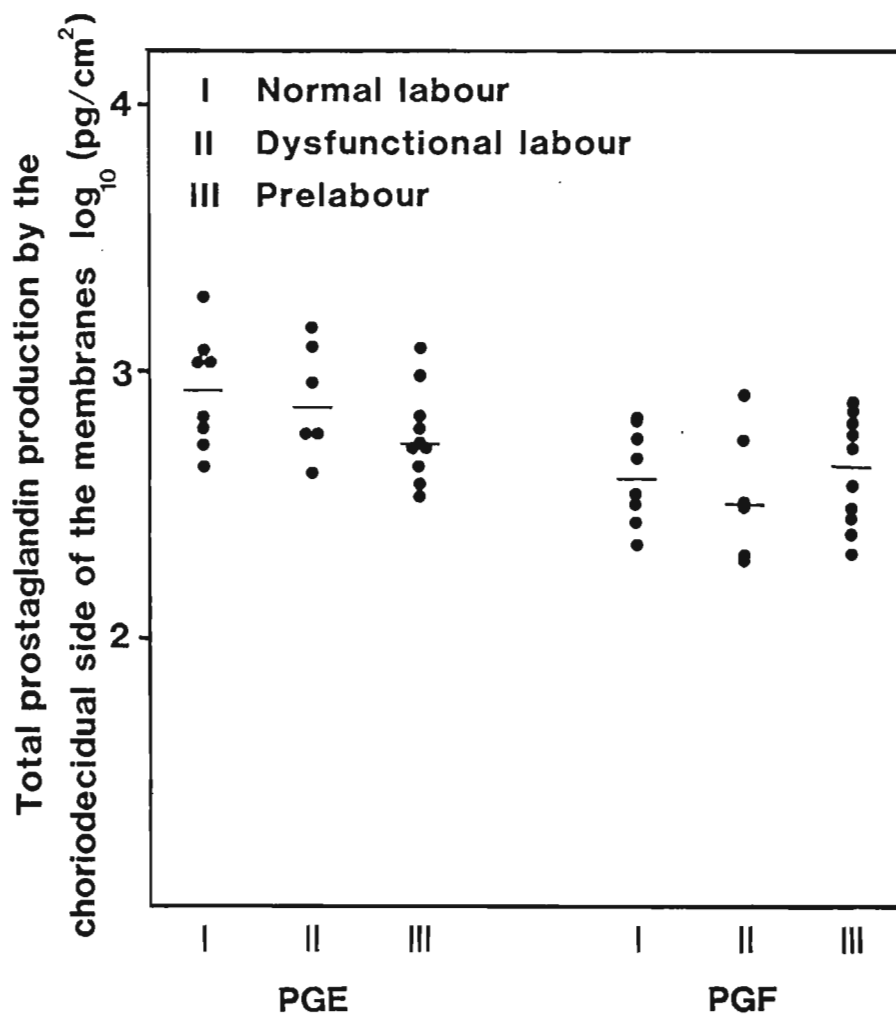


Figure 5.3 Total PGE and PGF release by the choriodecidual side of the fetal membrane during 3 hours of superfusion in the 3 groups of patients studied (expressed as $\log_{10} \text{ pg/cm}^2$ of membrane). Horizontal bars indicate median value.

FIG 5.4

Release of PGFM by the amnion and choriodecidual surfaces of the fetal membranes in prelabour, normal labour and dysfunctional labour

	<u>Group I</u>	<u>Group II</u>	<u>Group III</u>
	Normal Labour	Dysfunctional	Prelabour
	n = 2	Labour n = 2	n = 2
Amnion side of			
fetal membrane			
PGFM (pg/cm ² /3h) 1	158	121	106
2	122	undetectable	undetectable
Choriodecidual			
side of fetal			
membrane			
PGFM (pg/cm ² /3h) 1	228	232	133
2	248	186	164

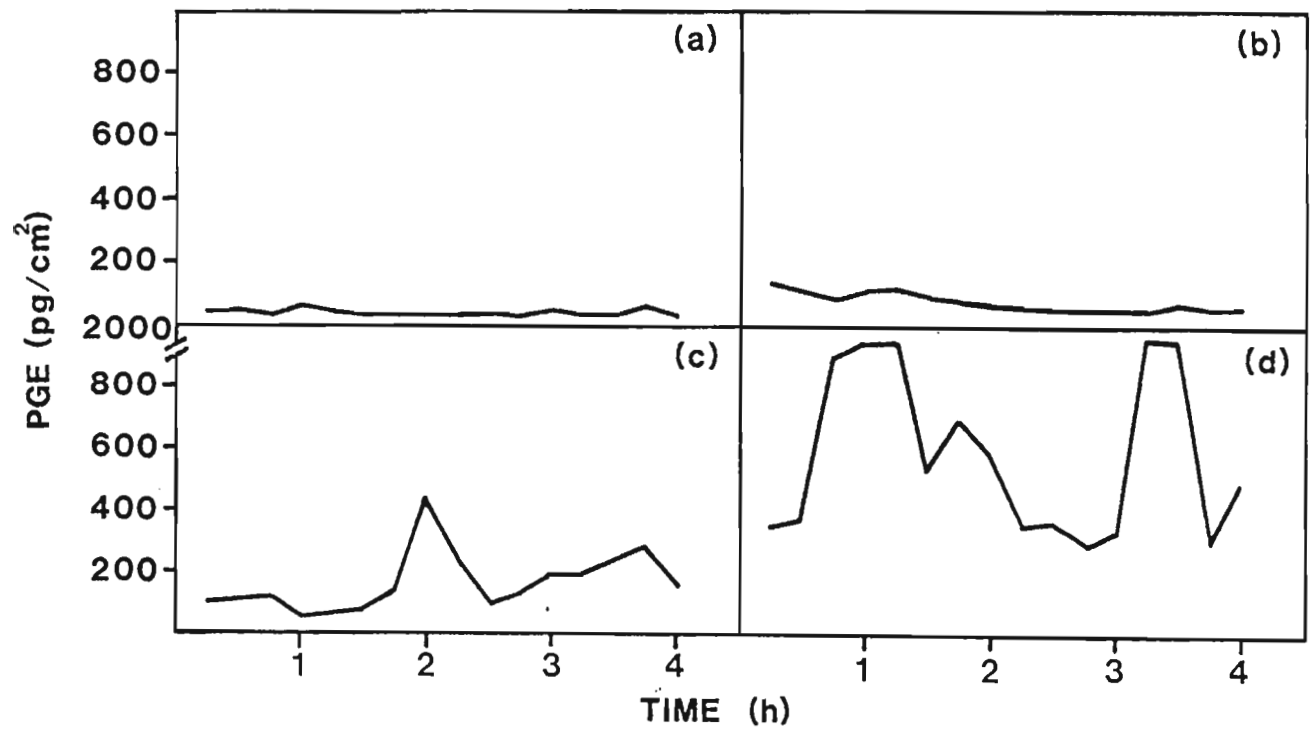


Figure 5.4 Release of PGE by the amnion side of the fetal membrane during 4 hours of superfusion in 4 patients. Superfusates were assayed for PGE every 15 min.

a & b) Results from tissue obtained following elective caesarean section (prelabour)

c & d) Results from tissue obtained after spontaneous normal labour.

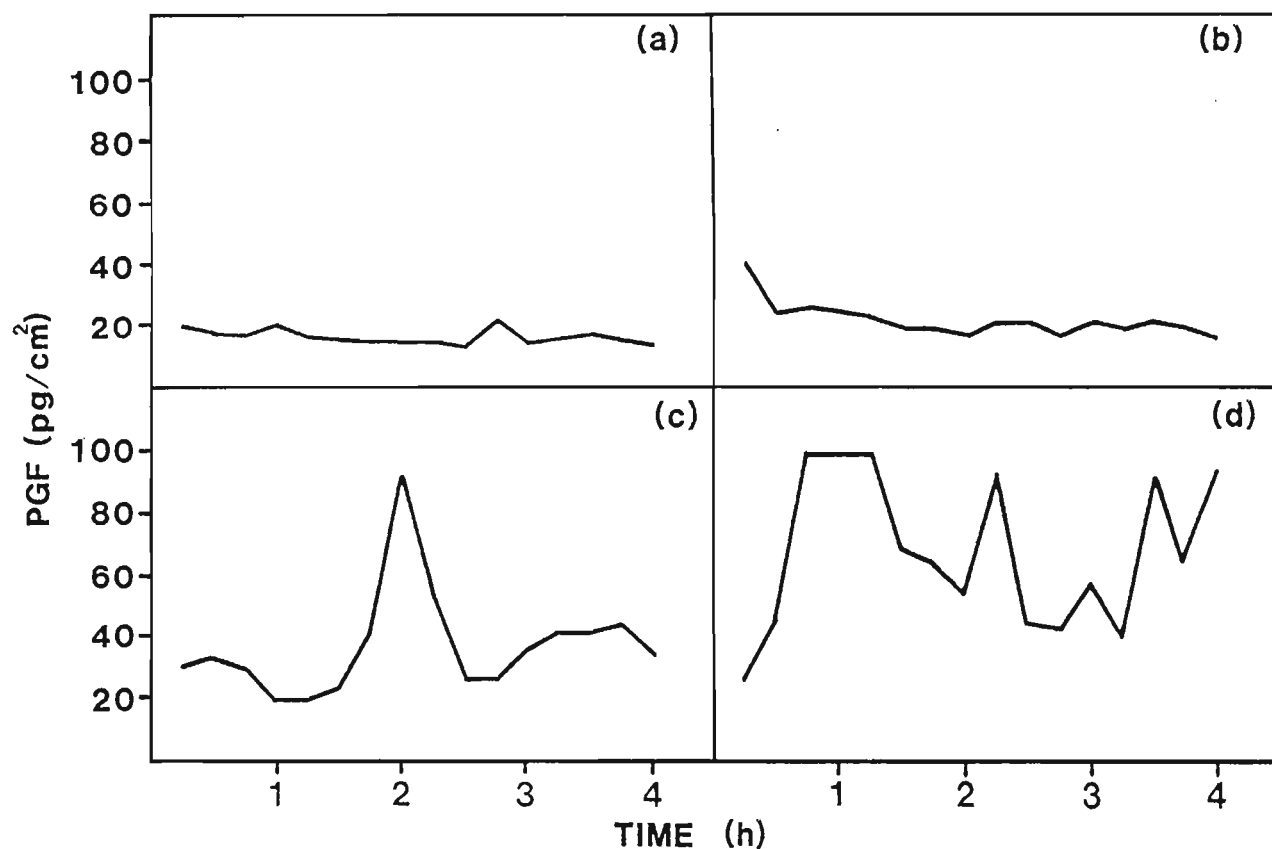


Figure 5.5 Release of PGF by the amnion side of the fetal membrane during 4 hours of superfusion in 4 patients. Superfusates were assayed for PGF every 15 min.

a & b) Results from tissue obtained following elective caesarean section (prelabour)

c & d) Results from patients obtained after spontaneous normal labour.

for PGE release from the amnion surface of tissue obtained following dysfunctional labour. In Figures 5.6 and 5.7 the pattern of PGE and PGF release from the amnion surface of tissue obtained from patients presenting with dysfunctional labour is shown.

5.6 DISCUSSION

The results of the present in vitro study of prostaglandin release by the fetal amnion clearly confirmed the pattern of prostaglandin concentrations observed in amniotic fluid from patients presenting with dysfunctional labour. In membranes obtained following normal labour, increased production of both PGE and PGF by the amnion was observed as compared to prelabour tissue. However, in primary dysfunctional labour PGE production was significantly increased as compared to prelabour tissue but no change in PGF production was observed.

In quantitative terms the amount of PGF released from the amnion surface of the membranes was significantly less than that of PGE, even in tissues obtained following spontaneous normal labour. This quite obviously differs from the previous in vivo study which clearly demonstrated a greater increase of PGF as compared to PGE in amniotic fluid during normal active labour. Admittedly, as pointed out in Section 1.3, a major disadvantage of in vitro studies is that they may not reflect what is happening in vivo.

Firstly, amniotic fluid concentrations of prostaglandins reflect accumulation of these substances over time whereas the superfusate in the present study is continuously removed and is expressed as production per unit surface area/per unit time. Also, very little is known about the

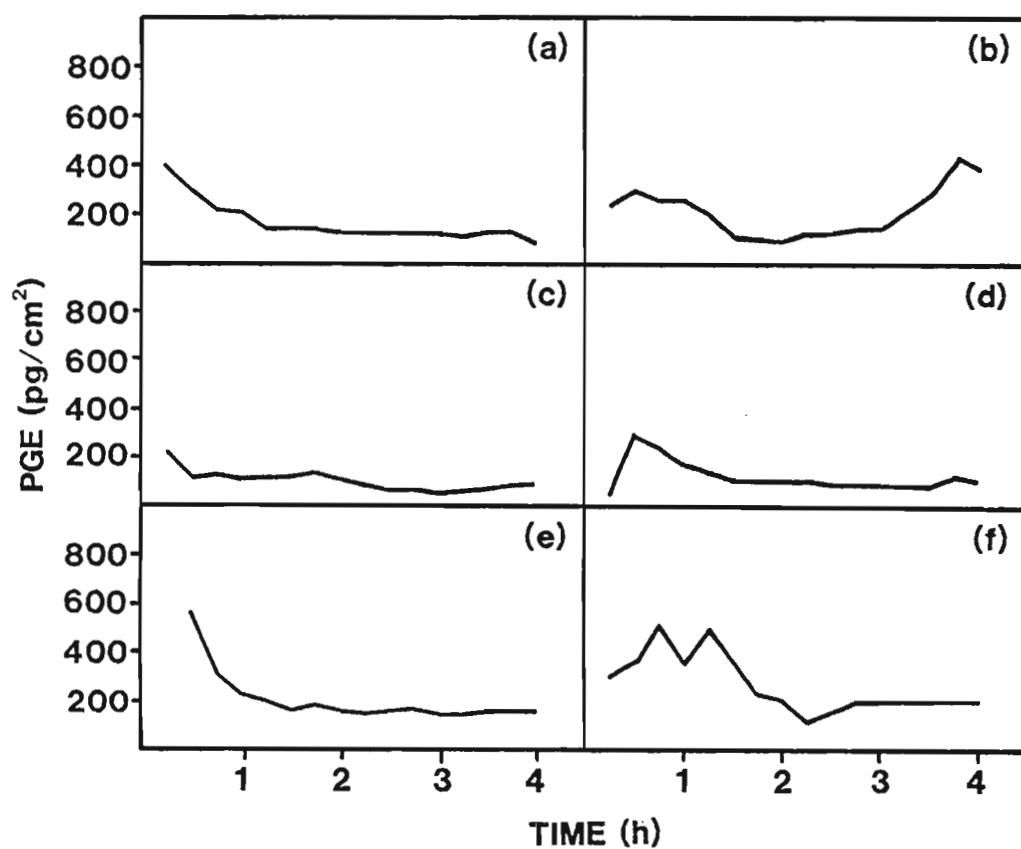


Figure 5.6 Pattern of PGE release by the amnion side of the fetal membrane in patients with dysfunctional labour during 4 hours of superfusion (pg/cm^2 plotted against time). Superfusates were assayed every 15 min.

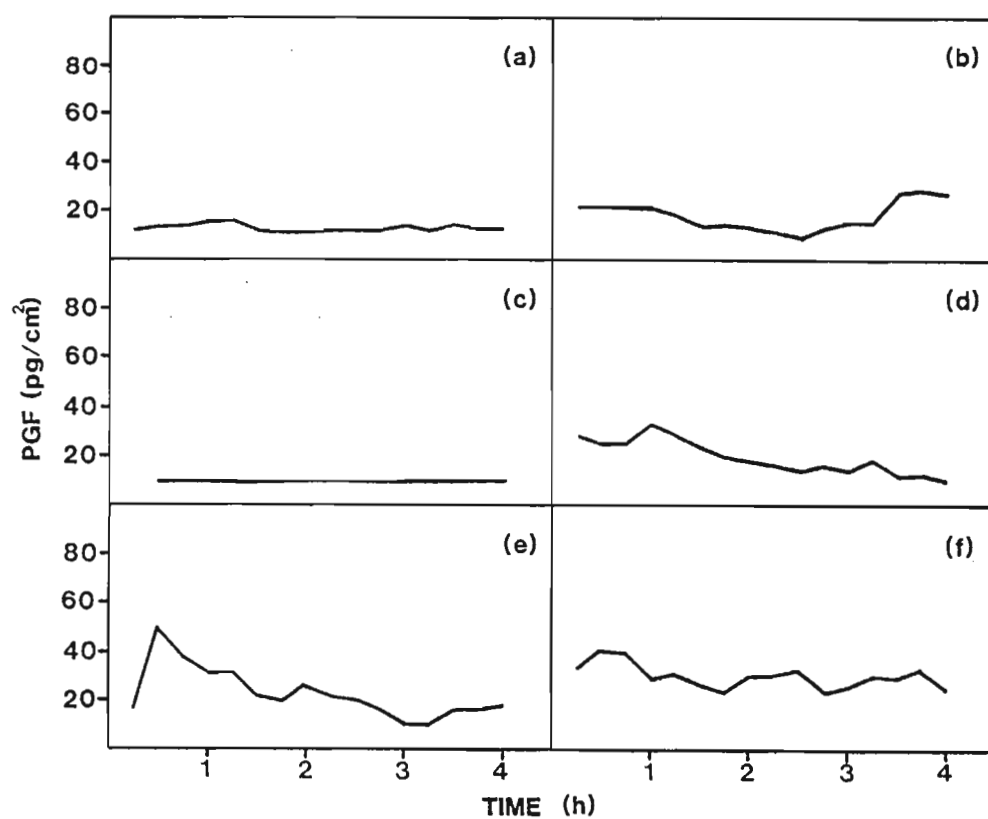


Figure 5.7 Pattern of PGF release by the amnion side of the fetal membrane from patients with dysfunctional labour (pg/cm^2 plotted against time). Superfusates were assayed every 15 min.

rate of diffusion of prostaglandins out of cells or whether the presence of one prostanoid influences the production of the other prostanoids in the arachidonic acid cascade. Another possible explanation for this discrepancy could be that isolation of the fetal membranes for in vitro studies necessarily removes any endogenous stimuli or control mechanisms that may have contributed to PGF synthesis under in vivo conditions during labour (Section 2.6.3).

It is also possible that rapid metabolism of PGF by the amnion resulted in the lower PGF measured in the superfusate. However this argument does not seem valid. Firstly, as reviewed in Section 2.3 the activity of the enzyme PGDH is minimal in the amnion (Keirse et al 1976). Secondly, although the measurement of the metabolite of PGF, namely PGFM, released from the membranes was performed in only a few experiments in the present study, the results obtained do not support this view.

An interesting phenomenon arising from the present study was the apparent intermittent or periodic release of prostaglandins from the amnion surface of the membranes in tissues obtained following the onset of labour. This is unlikely to be related to trauma since none of the prelabour tissue exhibited this phenomenon. An advantage of the method described in the present study is that the relationship between the various layers of the fetal membrane was maintained during the superfusion experiment and the tissue was subjected to minimal handling and trauma. This was borne out in the results of the histological examination. Furthermore, the consistency of the method was confirmed by comparing results of tissue superfused simultaneously. Again, this is probably related to the fact that prostaglandin release as a result of tissue trauma was not a factor in the present method employed.

Quite obviously, this method confirms the amnion to be the major site of prostaglandin synthesis during labour. With respect to the choriodecidua however, the superfusion technique described in the present chapter has failed to define any significant role of this tissue either in normal or dysfunctional labour. Whether the chorion and/or decidua has some permissive role in prostaglandin production by the amnion, is speculative at this stage. Alternatively, the choriodecidual tissue may serve as the local site of metabolism of the vast amount of prostaglandins produced by the amnion during labour. Indeed, higher levels of PGDH activity have been found in the chorion and decidua as compared to the amnion (Keirse et al 1976).

In tissues obtained following dysfunctional labour there was an apparent lack of the pulsatile pattern observed in normal labour, more especially that of PGF by the amnion. This emphasizes the possibility that there is some intrinsic control mechanism within the amnion tissue which, at least in part, is responsible for prostaglandin production by this tissue during labour.

The results of the present study, together with that of the previous in vivo study provide unequivocal evidence of a lack of PGF synthesis by the amnion in dysfunctional labour. It seems reasonable therefore to investigate the availability of free arachidonate as substrate for prostaglandin synthesis in the amnion in patients presenting with dysfunctional labour.

CHAPTER 6

MOBILIZATION OF ARACHIDONIC ACID FROM SPECIFIC GLYCEROPHOSPHOLIPIDS OF FETAL MEMBRANES DURING NORMAL AND DYSFUNCTIONAL LABOUR

6.1 PURPOSE OF THE STUDY

In the previous section it has been shown that the amnion is an important site of prostaglandin production during spontaneous normal labour (Section 5.5.4). In patients presenting with dysfunctional labour, a deficiency of prostaglandin release, especially PGF, from the amnion was demonstrated in vitro (Section 5.6). One of the possible explanations for this deficiency could be limitation in substrate availability for prostaglandin synthesis in the fetal amnion.

Free arachidonic acid (AA) is the obligatory precursor of prostaglandins of the "2" series. As discussed in Section 2.5 most of the AA in fetal membranes is bound to glycerophospholipids. In Section 2.6 the role of phospholipase A₂ (PLA₂) in making available AA for prostaglandin biosynthesis was discussed. In the light of the demonstrated decreased production of PGF in dysfunctional labour, information on the free AA content of the fetal membranes in normal and dysfunctional labour and its release from phospholipids as substrate is essential.

Since amniotic fluid concentrations of AA are thought to reflect release from surrounding fetal membranes (Keirse et al 1977), greater insight into the in vivo events may be obtained by measuring AA in amniotic fluid from the same patients during normal and dysfunctional labour. In the

following sections these aspects will be examined in patients presenting in normal and dysfunctional labour.

6.2 PATIENTS

Patients included in the present study have been described in detail (Section 3.2). Placentae were collected immediately after delivery from patients following spontaneous normal labour (n = 7) and those presenting with dysfunctional labour (n = 6). Placentae were also collected from patients at elective caesarean section (n = 7) as representing prelabour status of the fetal membrane. Amniotic fluid was collected from patients during labour via an indwelling intrauterine catheter as described in Section 3.2 and by amniocentesis from patients before the onset of labour.

6.3 METHODS

6.3.1 Measurement of Free Arachidonic Acid in Amniotic Fluid and Homogenates of the Fetal Membranes

The free AA content of the fetal membranes was measured by initial extraction of the lipid content of homogenates of the amnion and choriondecidua using a chloroform : methanol solvent mixture as described in Appendix C.5.4. A portion of this extract and portions of amniotic fluid were further processed by passage through a Seppak cartridge and the AA content of the eluate determined after methylation by gas liquid chromatographic separation (Appendix C.6).

6.3.2 Phospholipase A₂ (PLA₂) releasable Arachidonic Acid content of the Homogenates of the Fetal Membranes

Portions of the lipid extract of the homogenates of amnion and chorion were incubated with IU Phospholipase A₂ (Boehringer Mannheim, specific activity 3000U/mg protein) under standard conditions, as described in Appendix 8.2. On completion of the incubations, the contents were extracted, methylated and AA content quantitated by gas liquid chromatography (Appendix C.6). PLA₂ releasable AA was expressed as the difference between the AA content of the incubates and the free AA content of the whole tissue homogenate extract.

6.3.3 Measurement of the Glycerophospholipid composition of the Fetal Membranes

Homogenized amnion and choriodecidua obtained from the patients in the 3 groups were extracted with a chloroform:methanol solvent mixture as described in Appendix C.5.4. Portions of the extract were then subjected to two dimensional TLC. (Appendix C.5.6) The separated phospholipids were visualized in iodine vapour, scraped off the plates and analysed for phosphate content. (Appendix C.5.7).

6.3.4 Phospholipase A₂ releasable Arachidonic Acid content of the Individual Glycerophospholipids of the Fetal Membrane

Homogenates of amnion and choriodecidua were extracted with a chloroform : methanol solvent mixture as described in Appendix

C.5.4. Portions of the extract were then subjected to two dimensional TLC (Appendix C.5.6). The separated phospholipids were visualized with dichlorofluorescein (0,02% in 100% ethanol). Each fraction was scraped off the plate into a funnel plugged with glass wool and the phospholipid eluted off the silica scrapings with methanol and dried under N_2 . The dried extract was reconstituted in Tris HCl buffer (pH8) and incubated with PLA_2 under standard conditions (Appendix C.8.2). The AA content of the sample was determined by gas liquid chromatography following extraction and methylation of the free acid (Appendix C.6).

6.4 STATISTICAL ANALYSES

Details of the statistical analyses are presented in Appendix B. When the recorded data had a parametric distribution the significance of differences was tested by the Students' t test. Non-parametric distributions were analysed by applying the Mann Whitney U-test. Differences in mean values were regarded as significant when the p value was less than 0,05.

6.5 RESULTS

6.5.1 Free Arachidonic Acid concentration in Amniotic Fluid

The AA concentrations in amniotic fluid were 2 - 3 fold higher in samples obtained after the onset of labour as compared to prelabour samples (Caesarian section patients) ($p < 0,001$; Fig 6.1). A similar increase in AA concentration in amniotic fluid obtained from

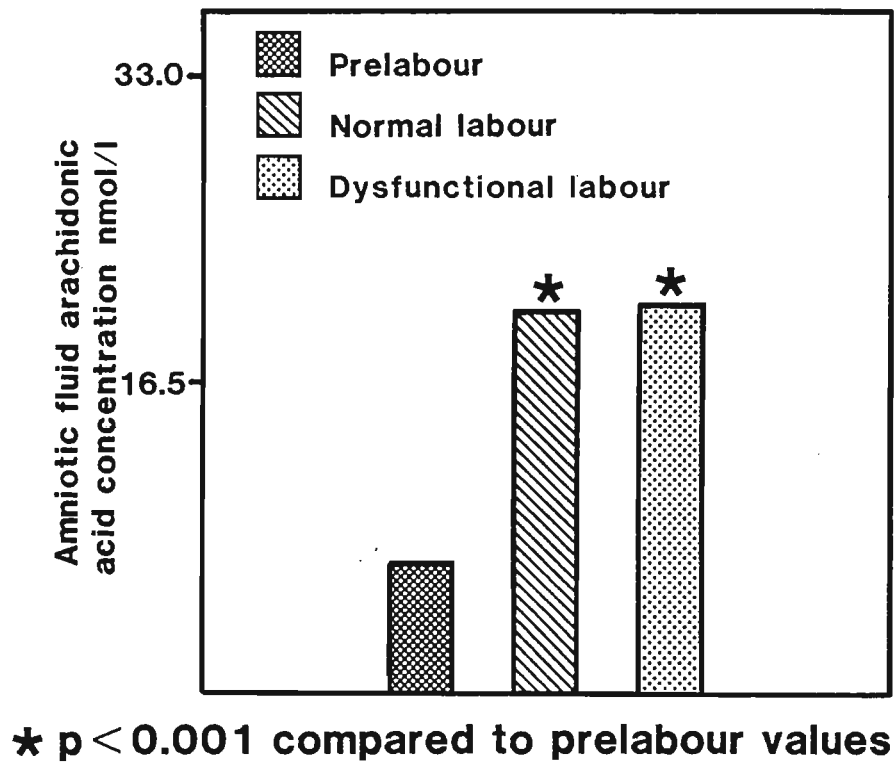


Figure 6.1 Arachidonic acid concentration in amniotic fluid obtained from patients before the onset of labour and at the end of the first stage of normal and dysfunctional labour (cervix 10 cm dilated).

patients presenting with dysfunctional labour was observed (Fig 6.1). There were no significant differences between the amniotic fluid AA concentrations in patients in normal and dysfunctional labour.

6.5.2 The Arachidonic Acid content of Homogenates of the Fetal Membranes

Although the free AA acid content of the homogenates of the fetal amnion tended to be lower in tissues obtained after normal and dysfunctional labour as compared to prelabour samples, there were no statistical differences between the 3 groups of patients tested (Table 6.1).

The free AA content of homogenates of the choriodecidua was also not different between the 3 groups of patients (Table 6.1).

6.5.3 Phospholipase-A₂ releasable Arachidonic Acid content of Homogenates of the Fetal Membranes

The PLA₂ - releasable AA content of the homogenates of the amnion obtained following normal labour was not statistically different from that obtained before the onset of labour (Table 6.1). However, the PLA₂-releasable AA content of the homogenates of amnion from patients presenting with dysfunctional labour was significantly less than that of prelabour tissue ($p < 0.001$).

The PLA₂ - releasable AA content of homogenates of the choriodecidua was not different between the 3 groups of patients (Table 6.1).

TABLE 6.1

The Free and Phospholipase A₂ releasable Arachidonic Acid content of Homogenates of the Fetal Membranes obtained from the 3 groups of patients studied.

	<u>Normal</u>	<u>Dysfunctional</u>	<u>Prelabour</u>
	<u>Labour</u>	<u>Labour</u>	
	n = 7	n = 6	n = 7
Free AA in Homogenates of:			
Amnion (nmol/g of tissue)	229 (74-248)	165 (72-172)	258 (95-598)
Choriodecidua (nmol/g of tissue)	529 (220-645)	836 (186-955)	732 (285-1116)
PLA ₂ -Releasable AA in Homogenates of:			
Amnion (nmol/g of tissue)	183 (100-251)	89*(75-210)	288 (159-409)
Choriodecidua (nmol/g of tissue)	610 (405-1220)	542 (424-665)	707 (239-1154)

Results expressed as median and range (nmol/g of tissue)

* p < 0,001 compared to prelabour.

6.5.4 Glycerophospholipid Content of the Fetal Membranes

The glycerophospholipid content of the fetal membranes is expressed as nmol of phospholipid phosphate per g of tissue.

Amnion obtained from patients after normal delivery and patients with dysfunctional labour contained significantly less phosphatidylserine (PS), phosphatidylethanolamine (PE) and phosphatidylinositol (PI) than that of amnion obtained before the onset of labour (prelabour, $p < 0.002$; Fig 6.2). Although the lecithin content of the amnion from patients with dysfunctional labour was found to be significantly less than that obtained from patients in the prelabour group (Fig 6.2), no statistical difference in the phospholipid composition of the amnion was found between patients with normal and dysfunctional labour.

There was no difference in the glycerophospholipid composition of the choriondecidua between the 3 groups of patients (Fig 6.3).

6.5.5 Phospholipase A_2 releasable Arachidonic Acid content of Specific Glycerophospholipids of the Fetal Membranes

The amount of AA released from specific glycerophospholipids following incubation of the glycerophospholipid fractions with PLA_2 is termed the PLA_2 - releasable AA content of each phospholipid. This is expressed as the percentage of AA in nmol per nmol of phospholipid phosphate. The PLA_2 - releasable AA content of each phospholipid class extracted from the amnion of the 3 groups of patients is illustrated in Fig 6.4.

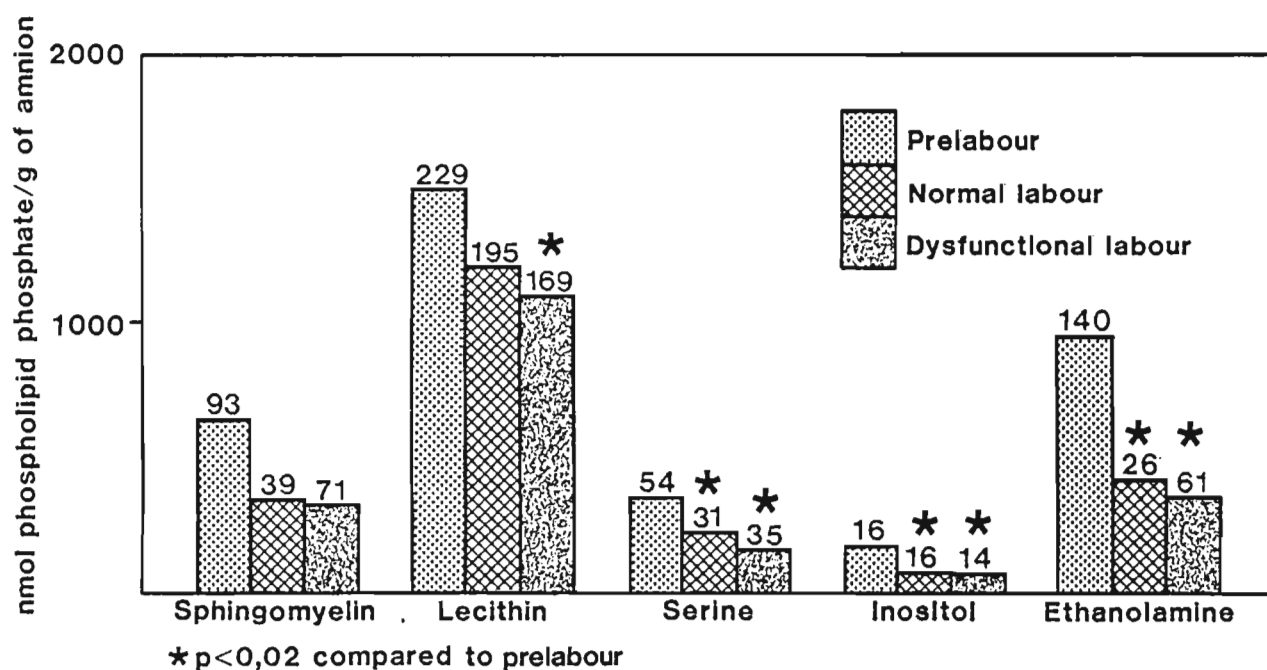


Figure 6.2 Phospholipid content of the amnion of tissues obtained before labour and following normal and dysfunctional labour (expressed as nmol phospholipid phosphate/g of tissue - wet weight). Numbers above bars represent standard error of mean (SEM).

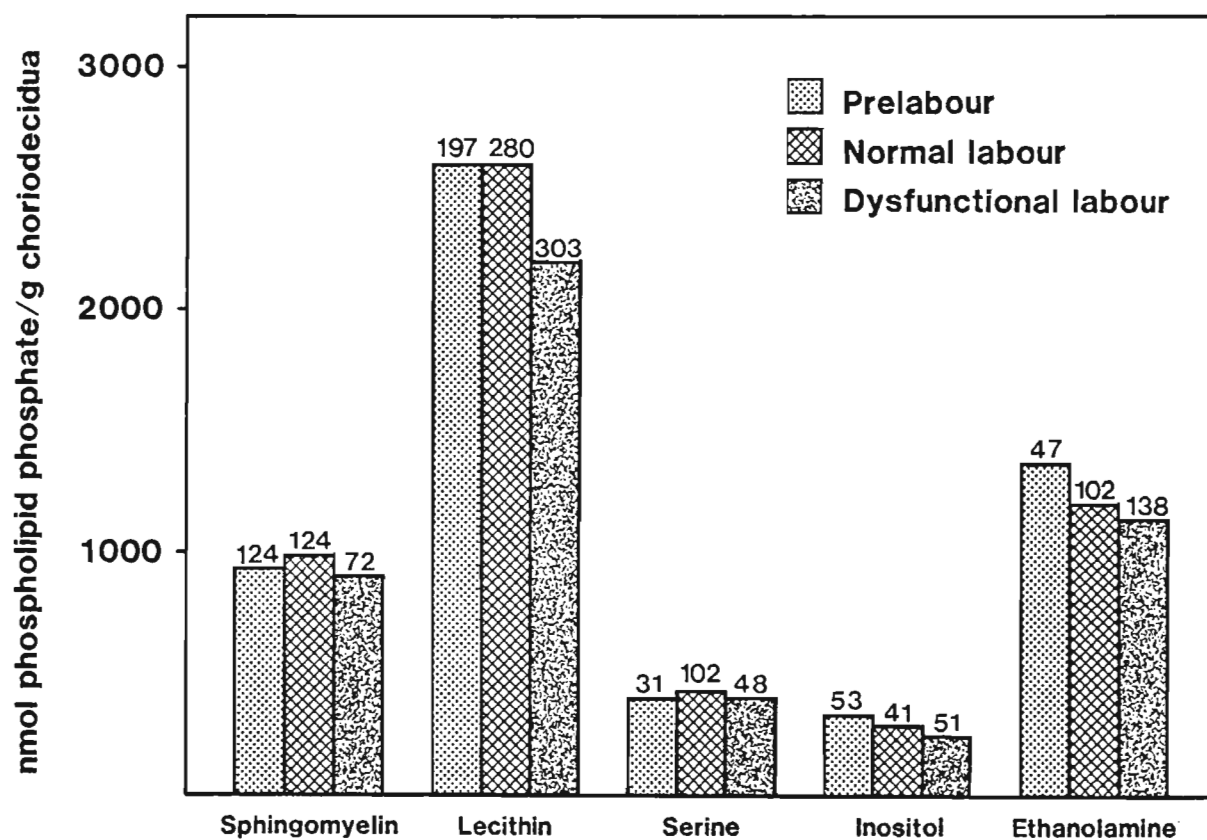


Figure 6.3 Phospholipid content of the choriodecidua of tissues obtained before labour and following normal and dysfunctional labour (expressed as nmol phospholipid phosphate/g of tissue wet weight). Numbers above bars represent standard error of mean (SEM).

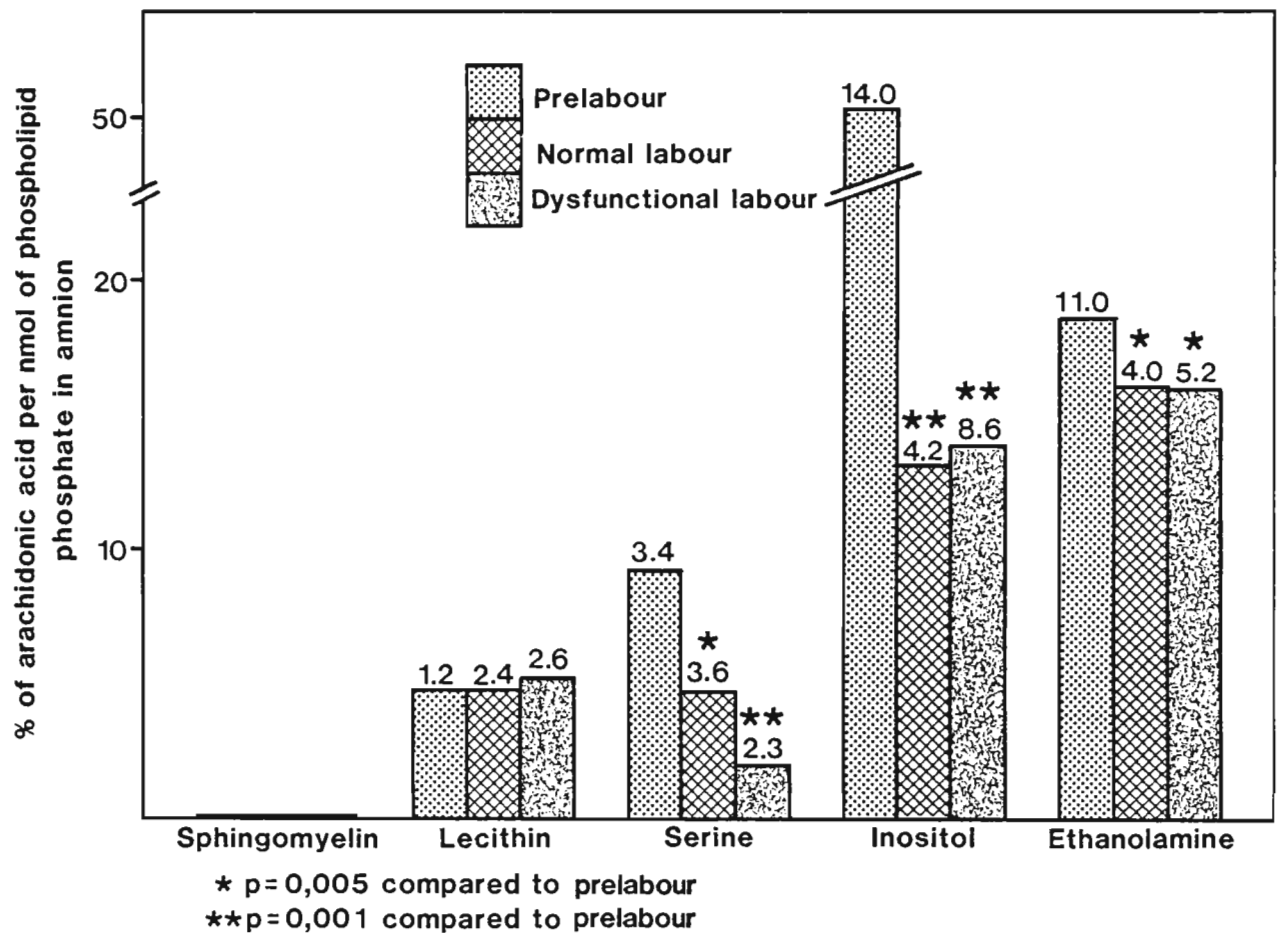


Figure 6.4 The phospholipase A_2 releasable arachidonic acid content of specific glycerophospholipids of the amnion in tissues obtained before the onset of labour and following normal and dysfunctional labour (expressed as a percentage of arachidonic acid released in nmol/nmol of phospholipid phosphate). Numbers above bars represent standard error of mean (SEM).

Sphingomyelin contained no detectable PLA₂ - releasable AA. PE and PI in the amnion obtained before the onset of labour had the highest percentage of PLA₂ - releasable AA (PI 50%, PE 19% of AA nmol /nmol of phospholipid phosphate Fig 6.4).

Normal labour resulted in a decrease in the PLA₂ - releasable AA content of PS and PI ($p < 0,001$) and PE ($p < 0,005$) extracted from the amnion (Fig 6.3). In patients presenting with dysfunctional labour, the changes in PLA₂ - releasable AA content of the amnion were similar to those described for normal labour with the exception of PS (Fig 6.4) which contained even less PLA₂ - releasable AA than that extracted from the amnion following normal labour ($p < 0,05$; Fig 6.4).

No differences in the PLA₂ - releasable AA content of the individual glycerophospholipids extracted from the choriodecidua was found between the 3 groups of patients (Fig 6.5).

6.6 DISCUSSION

The results of the present study clearly demonstrate that various structural phospholipids, specifically of the amnion, are involved in a catabolic process making available free AA as substrate for prostaglandin synthesis after the initiation of labour. These findings confirm that the amnion is an important site for the metabolic events associated with increased prostaglandin synthesis and the onset of labour. By contrast the choriodecidua, although rich in both phospholipid and AA content, does not apparently play any role in substrate provision, findings which

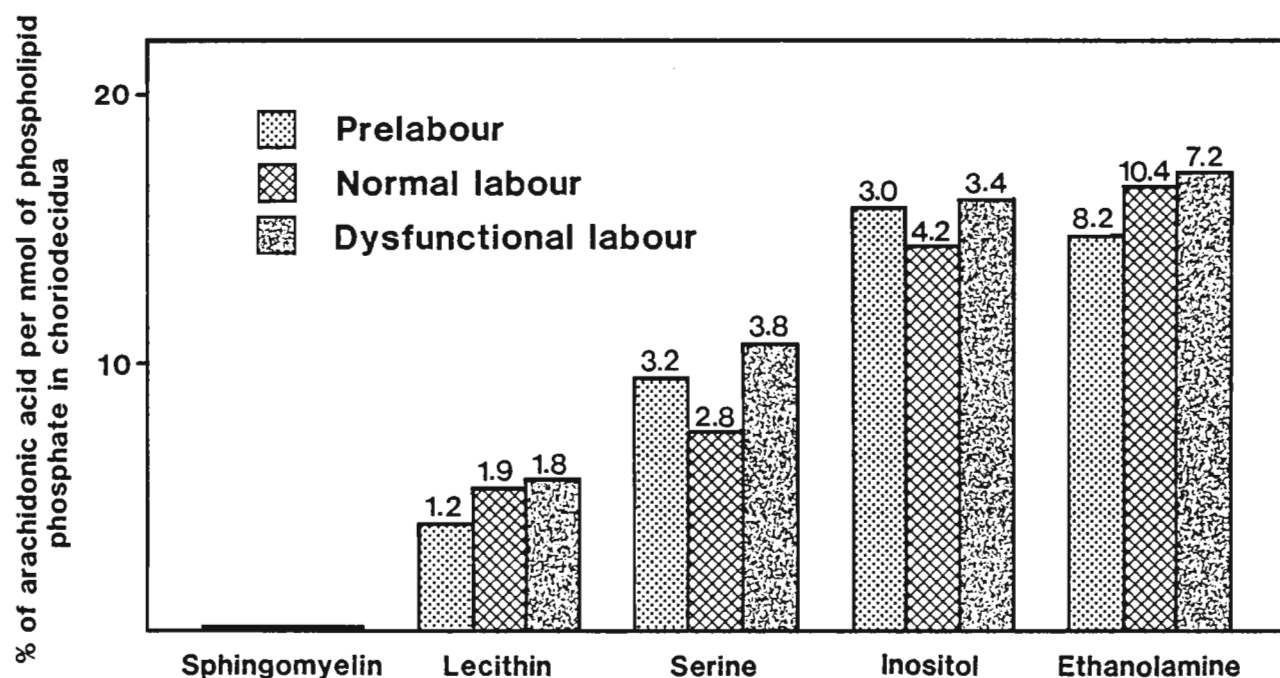


Figure 6.5 The phospholipase A_2 releasable arachidonic acid content of specific glycerophospholipids of the choriodecidua in tissues obtained before the onset of labour and following normal and dysfunctional labour (expressed as a percentage of arachidonic acid in nmol/nmol of phospholipid phosphate). Numbers above bars represent standard error of mean (SEM).

are consistent with those reported in the previous chapter on prostaglandin production by the fetal membranes (Section 5.6).

Since there is no doubt that free AA is an essential pre-requisite for prostaglandin synthesis (van Dorp 1964), the question arises whether substrate limitation might be responsible for the deficient PGF production by the amnion in dysfunctional labour (Section 5.6). Although there was a tendency towards decreased free and PLA₂-releasable AA measured in homogenates of the amnion obtained following the onset of labour, no statistical differences were demonstrated between the 3 groups of patients. More importantly, no difference in either the PLA₂-releasable or free AA content of the homogenates of the amnion was found between patients in normal and dysfunctional labour. It may be argued that with larger numbers in each group, statistical differences may be achieved between these patients and that substrate limitation may indeed be responsible for the lower PGF synthesis in dysfunctional labour. However, this argument does not seem valid. Firstly, the synthesis of PGE was not limited in dysfunctional labour. More over, amniotic fluid concentrations of AA in dysfunctional labour were not different from those in normal labour. Finally the absolute concentrations of AA in amniotic fluid and homogenates of the amnion are in vast excess of the amount of prostaglandin produced in the amnion. Thus in quantitative terms it seems unlikely that the deficiency of PGF production observed in dysfunctional labour is related to substrate deficiency.

Admittedly, as pointed out in Section 2.6.2, measurement of free AA in tissue homogenates may not necessarily reflect the amount of free AA that is available in vivo. Quite obviously the extraction procedures employed

would necessarily strip AA of proteins which in vivo would not be available as the free acid for prostaglandin synthesis. Perhaps a more accurate estimation of AA availability is the assessment of the mobilization of AA from specific glycerophospholipids during normal and dysfunctional labour.

There is no doubt from the results presented in this study that mobilization of AA from glycerophospholipids of the amnion following the onset of labour occurred primarily from PE, PI and to a lesser extent from PS. These findings are consistent with previous reports (Curbelo et al 1981, Okita et al 1982). Of importance, is that mobilization of AA from glycerophospholipids of the amnion obtained from patients with dysfunctional labour was not different from that of normal labour. The suggestion therefore, is that the activity of endogenous PLA₂ in the amnion obtained following dysfunctional labour is not less than that of the amnion obtained following normal labour. Arachidonate availability via the PLA₂ pathway is not limited in dysfunctional labour.

Alternate sources of free AA such as production by PI specific phospholipase C (PLC), which has been demonstrated in fetal membranes (Di Renzo et al 1981), were not investigated in the present study. It is possible that this may be an important source of free AA for PGF synthesis. This would be true if PLC activity in vivo is coupled to an enzyme system necessary for the production of PGF. This, as observed in Section 2.4, may constitute a regulatory mechanism in the prostaglandin biosynthetic pathway.

A more likely explanation however, for the decreased PGF synthesis in the presence of adequate substrate in dysfunctional labour would be inhibition

of PGF synthesis at a site distant to the release of AA in the prostaglandin biosynthetic pathway. Differential regulation of prostaglandin synthetase by factors present in amniotic fluid have been described (Cohen et al 1985). It seems reasonable therefore to examine the effect of such factors on prostaglandin synthetase in samples of amniotic fluid obtained from patients presenting with dysfunctional labour.

CHAPTER 7

REGULATION OF PROSTAGLANDIN SYNTHETASE BY AMNIOTIC FLUID FACTORS DURING NORMAL AND DYSFUNCTIONAL LABOUR

7.1 PURPOSE OF THE STUDY

The studies reported in the previous chapter leave no doubt that decreased PGF production during dysfunctional labour cannot be related to arachidonate deficiency. Indeed the evidence points to normal metabolic activity in the amnion of the fetal membrane in making available free arachidonate as substrate for the cyclo-oxygenase cascade.

As discussed in Section 2.6.3. factors have in fact been found in the amniotic fluid which may act as regulatory mechanisms through stimulatory or inhibitory actions on the biosynthetic train leading to prostaglandin synthesis.

It seems self evident therefore to examine the role of such factors in dysfunctional labour. In the present chapter these aspects will be studied using a crude source of prostaglandin synthetase as the target for amniotic fluid stimulators or inhibitors.

7.2 PATIENTS INCLUDED IN THE STUDY

Patients included in the present study have been described before (Section 3.2). Amniotic fluid was obtained via an indwelling intrauterine catheter during normal and dysfunctional labour as described

in Section 3.4.2 and by amniocentesis in 16 patients before the onset of labour. Amniotic fluid was obtained during early labour (cervix 3 - 5 cm dilated) and late labour (cervix 8 - 10 cm dilated) from 10 patients in normal labour and 10 patients presenting with dysfunctional labour.

7.3 METHODS

The system used for measuring inhibitory or stimulatory activity was based on the activity of a crude preparation of prostaglandin synthetase prepared from sheep seminal vesicles as described in Appendix C.8. As substrate, free AA purchased from Sigma Co (St Louis USA) was used; the more soluble sodium salt was prepared before use as described in Appendix C.8.4.

The assay of inhibitory and stimulatory activity on prostaglandin synthetase is described in detail in Appendix C.8. Results are expressed in terms of the amount of PGE and PGF produced after incubation of substrate, enzyme and amniotic fluid preparations in comparison to the prostaglandins produced in control assays in which the amniotic fluid samples were replaced by an equal volume of assay buffer (Section C.8.5).

Amniotic fluid used in the present study was either unprocessed or fractionated by passage through a microfilter (Amikon YM 10) into molecular weight fractions of greater than or less than 10 000 daltons.

7.4 CALCULATIONS

All results obtained with sample dilutions were expressed as a ratio to the control values for each assay performed. Thus ratios greater than 1 indicate a net stimulatory effect whilst those less than 1 indicate net inhibitory effect.

7.5 STATISTICAL ANALYSES

The data proved to be non-parametric in distribution and the Mann Whitney U test was therefore used in analysing results. The Wilcoxin matched pairs signed rank test was used in assessing the data obtained from individual patients during early and late labour.

7.6 RESULTS

7.6.1 *Effect of unprocessed amniotic fluid on PGE production by Prostaglandin synthetase*

Amniotic fluid obtained from patients before the onset of labour (Prelabour group) did not significantly alter PGE production by prostaglandin synthetase. In only 4 of 10 patients could some inhibitory activity be demonstrated (Fig 7.1). By contrast, amniotic fluid obtained during early normal labour (cervix 3 - 5 cm dilated), although variable between patients, resulted in a net mean stimulation of PGE production of 16% over control samples. (Fig 7.1; $p < 0,05$ compared to prelabour group). In all but 1 patient, this stimulatory effect decreased in samples of amniotic fluid obtained late in normal labour (cervix 8 - 10 cm dilated) (Fig. 7.1; $p < 0,05$ compared to early labour).

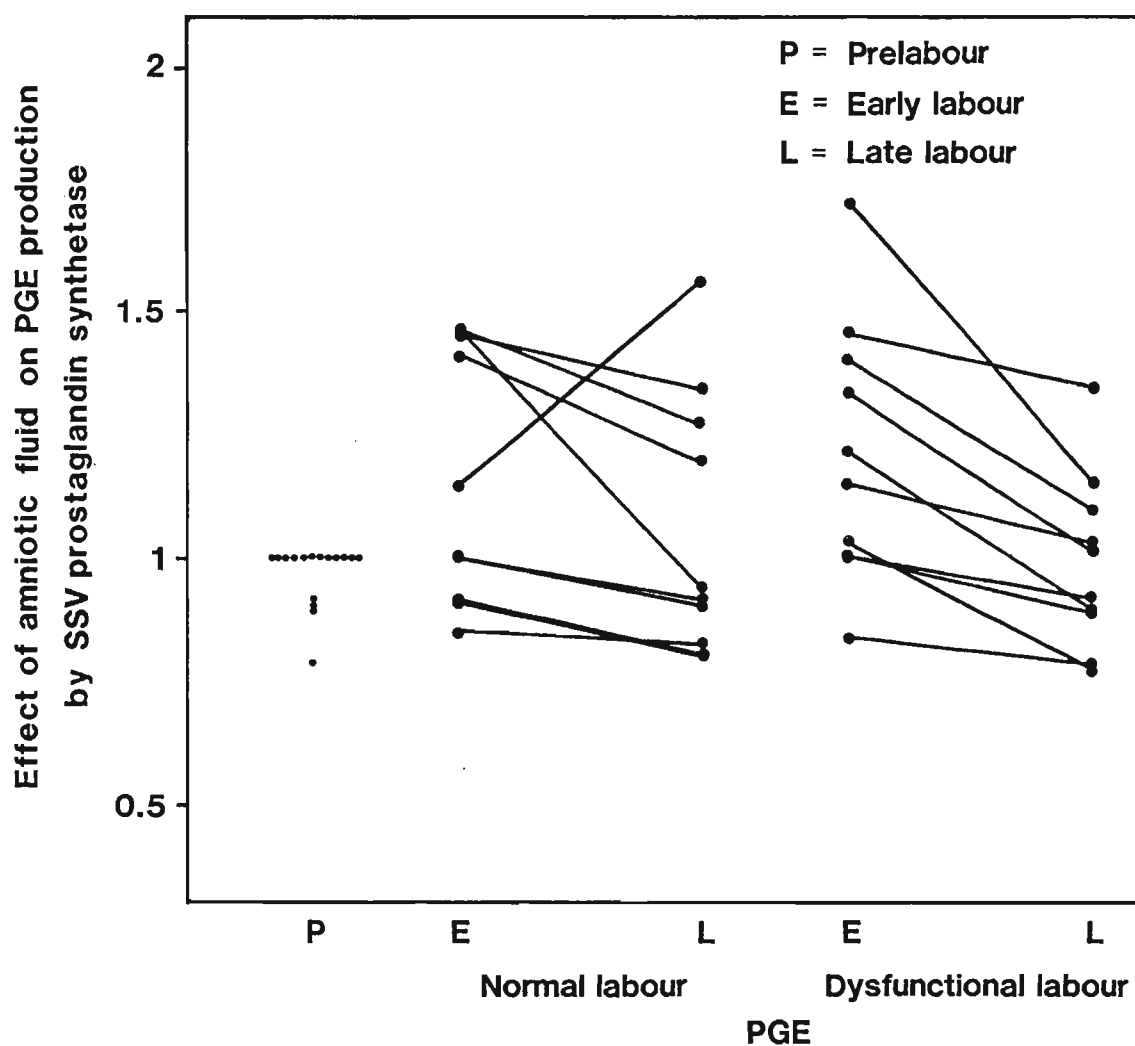


Figure 7.1 Effect of unprocessed amniotic fluid on PGE production by sheep seminal vesicle (SSV) prostaglandin synthetase. Results are expressed as a ratio over control samples. The changing effects of amniotic fluid from individual patients during normal and dysfunctional labour are also shown.

Samples of amniotic fluid obtained from patients presenting with dysfunctional labour also had a stimulatory effect on PGE production at a cervical dilatation of 3 - 5 cm; late in labour this stimulatory effect decreased in all patients as labour progressed under oxytocin stimulation. There were no significant differences in the effect of amniotic fluid factors on PGE synthesis between normal and dysfunctional labour.

7.6.2 Effect of unprocessed Amniotic Fluid in PGF production by Prostaglandin Synthetase

Amniotic fluid obtained from patients before the onset of labour did not significantly alter PGF production by prostaglandin synthetase : in 6 of the 16 samples slight inhibitory activity was noted (Fig 7.2). Amniotic fluid obtained during early normal labour had no net effect on PGF production by prostaglandin synthetase (Fig 7.2). However samples obtained in late normal labour all had significant stimulatory effects on PGF production in the assay system. ($p < 0,02$ compared to samples obtained in early labour).

Amniotic fluid from patients with dysfunctional labour had similar effects on PGF production in the assay system as found in normal labour at all stages of cervical dilatation (Fig 7.2). The effects of amniotic fluid on PGF production in normal and dysfunctional labour were not statistically different.

7.6.3 Effect of Fractionated Amniotic Fluid on PGE production by Prostaglandin Synthetase

Amniotic fluid obtained before the onset of labour (Prelabour group) exhibited both stimulatory and inhibitory effects on PGE

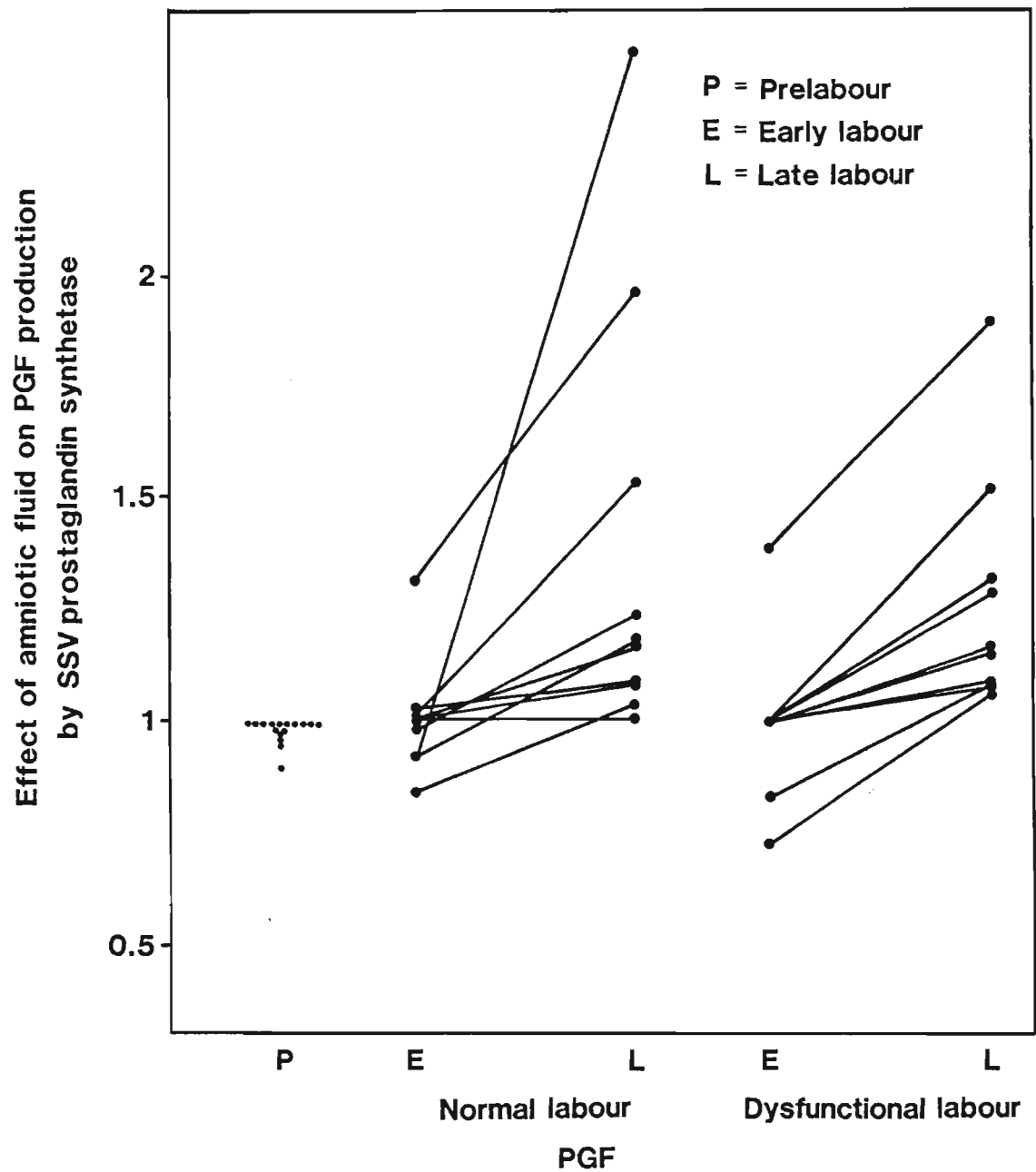


Figure 7.2 Effect of unprocessed amniotic fluid on PGF production by sheep seminal vesicle (SSV) prostaglandin synthetase. Results are expressed as a ratio over control samples. The changing effect of amniotic fluid in individual patients during normal and dysfunctional labour are also shown.

production depending on the molecular weight fraction tested : fractions of molecular weight less than 10 000 stimulated PGE production while those greater than 10 000 inhibited PGE production (Fig 7.3).

During the progress of normal active labour there was a decrease in the stimulatory activity of the low molecular weight fraction on PGE production in 4 of 5 patients in whom amniotic fluid during early and late labour was obtained. At the same time the effect of the high molecular weight inhibitory fractions increased as labour progressed (Fig 7.3; $p < 0,05$ compared to early labour).

In patients presenting with dysfunctional labour, the activity of the low and high molecular weight amniotic fluid fractions on PGE production were similar to that found in normal labour (Fig 7.3). Statistically the differences between these two groups of patients were not significant.

7.6.4 Effect of fractionated amniotic fluid on PGF production by Prostaglandin synthetase

Low molecular weight amniotic fluid fractions prepared from prelabour specimens (stimulatory fraction) did not significantly alter PGF production in the assay system (Fig 7.4). The high molecular weight fractions prepared from prelabour samples of amniotic fluid did however inhibit PGF production in all 5 samples tested (Fig 7.4).

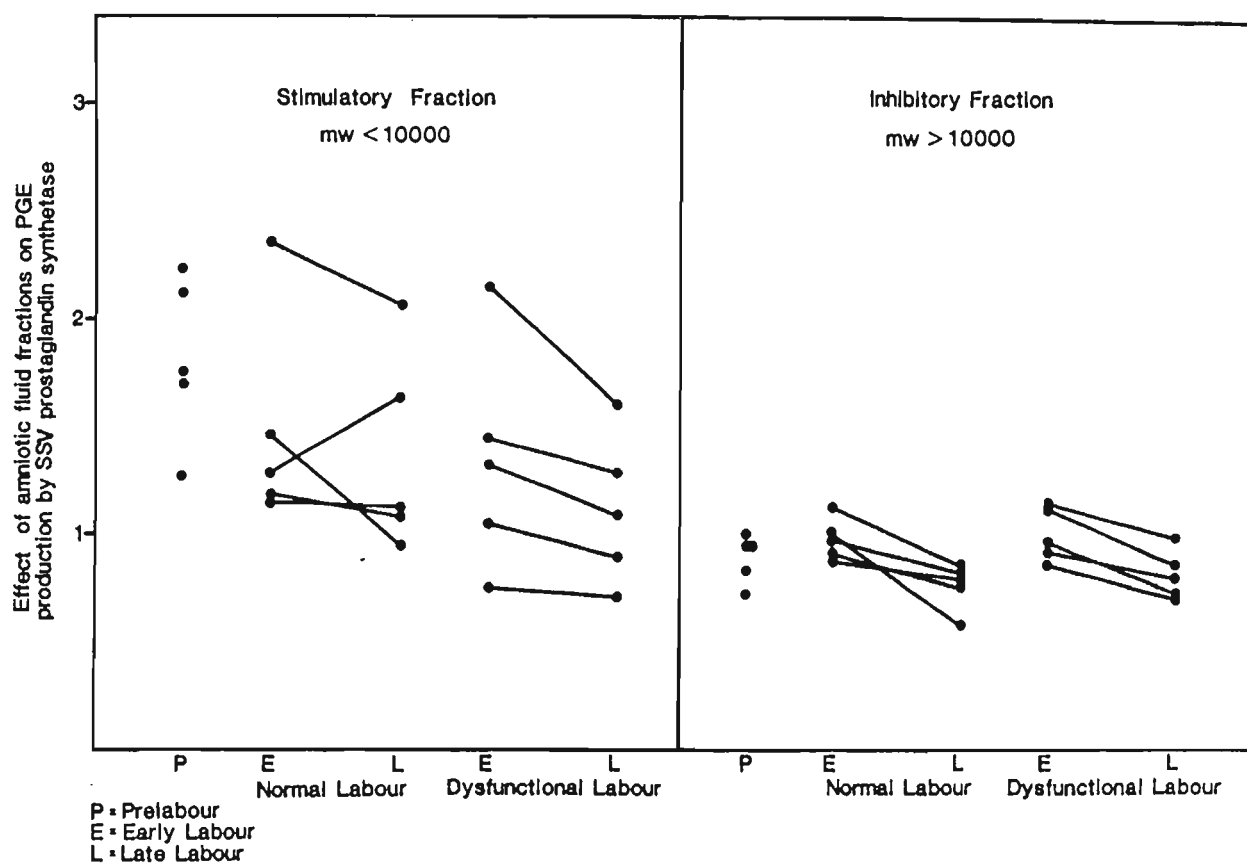


Figure 7.3 Effect of fractionated amniotic fluid on PGE production by sheep seminal vesicle (SSV) prostaglandin synthetase. Results are expressed as a ratio over control samples. The changing effects of amniotic fluid from 5 patients each during normal and dysfunctional labour are also shown.

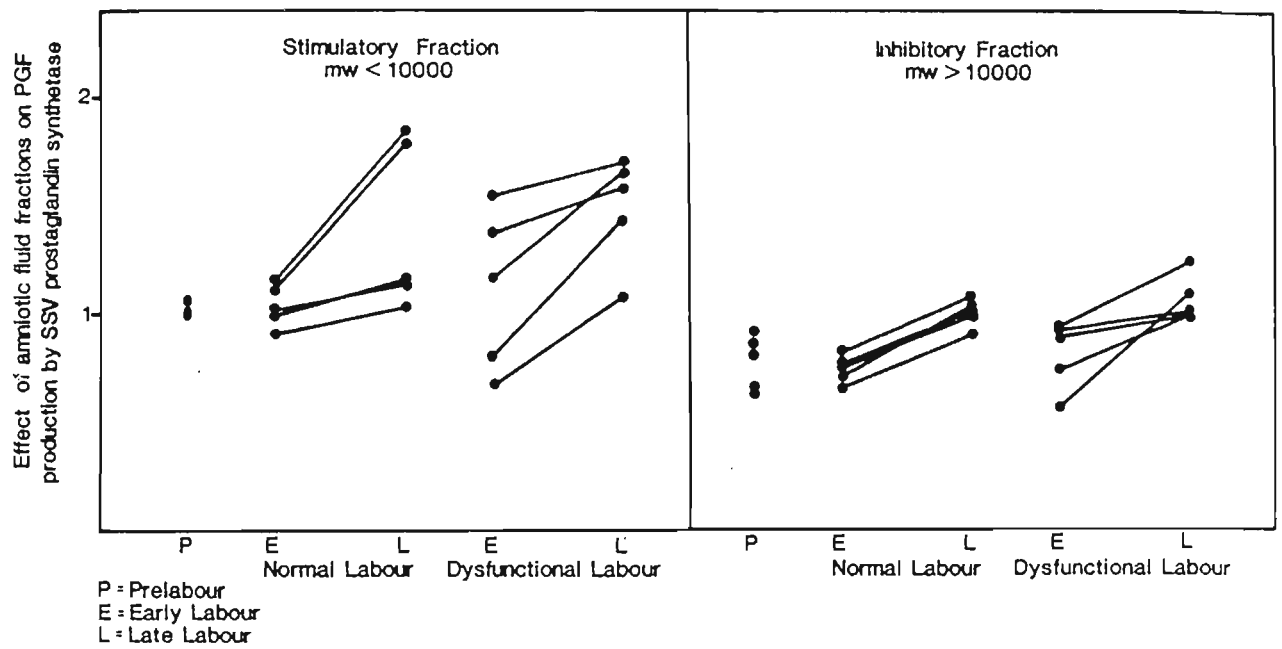


Figure 7.4 Effect of fractionated amniotic fluid on PGF production by sheep seminal vesicle (SSV) prostaglandin synthetase. Results are expressed as a ratio over control samples. The changing effects of amniotic fluid from 5 patients each during normal and dysfunctional labour are also shown.

Similar effects of both amniotic fluid fractions on PGF production were found during early normal labour as in the prelabour group (Fig 7.4). In all patients in normal labour, the stimulatory effect of the low molecular weight fraction on PGF synthesis increased while the activity of the inhibitory fraction decreased as labour progressed (Fig 7.4).

In patients presenting with dysfunctional labour, the effect of the stimulatory fraction of amniotic fluid was widely scattered (Fig 7.4). However, as described for normal labour there was a net increase in the stimulatory effect of this fraction as labour progressed under oxytocin stimulation. At the same time the activity of the inhibitory fraction decreased as labour progressed (Fig 7.4). No differences were observed in the effect of either the stimulatory or inhibitory fractions on PGF production in the assay system between patients in normal and dysfunctional labour.

7.7 DISCUSSION

The present study clearly confirms the presence of amniotic fluid factors which influence the activity of prostaglandin synthetase (Cohen et al 1985). Fractionation of the amniotic fluid has also confirmed both stimulatory (low molecular weight fraction) and inhibitory (high molecular weight fraction) effects on prostaglandin production by prostaglandin synthetase.

Of interest is the difference in activity of these fractions on PGE and PGF synthesis during labour. It is quite obvious from the results presented that the maximum stimulatory effect of amniotic fluid on PGE

production in the assay system occurred during early labour whilst that of PGF occurred only in late labour. These findings are entirely consistent with the previous hypothesis of a switch in the prostaglandin biosynthetic pathway from mainly PGE biosynthesis to that of PGF biosynthesis during normal active labour (Section 4.6).

With respect to dysfunctional labour, the findings of significantly decreased production of PGF both in vivo and in vitro (Sections 4.5 and 5.6 respectively) in the presence of adequate substrate availability (Section 6.5) certainly suggests specific inhibition of PGF synthesis. However, no differences in the regulatory effect of amniotic fluid on prostaglandin synthetase could be demonstrated between samples obtained from patients in normal and dysfunctional labour. Overtly, it would thus seem that the information presented in this chapter is inconsistent with the findings of decreased PGF concentration in amniotic fluid and the limitation of PGF production by the amnion layer of fetal membranes obtained from patients with dysfunctional labour.

It should however be recognized that amniotic fluid contents merely reflect what has been secreted into it over a period of time. When, as in the case of PGE and PGF, amniotic fluid concentrations can be related to studies which measure production by the amnion layer of the intact membranes (Sections 4.5 and 5.6 respectively), the results argue strongly for a cause and effect relationship between amniotic fluid concentrations and production by the membranes.

However, as far as the stimulatory and inhibitory activities in amniotic fluid are concerned the evidence is less explicit and interpretation

is inferential. The prostaglandin synthetase stimulatory factor(s) in amniotic fluid apparently have a molecular weight less than 1 000 daltons (Cohen et al 1985). Stimulation of prostaglandin synthetase by fetal urine has been demonstrated in vitro (Strickland et al 1983). It is thus quite feasible that the stimulatory activity demonstrated in amniotic fluid may be of fetal origin. Certainly, the molecular size described for the stimulatory fraction could easily be cleared by the fetal kidney and thus reach the amniotic fluid by excretion into fetal urine.

The activity of the stimulatory fraction with respect of PGE production in early labour is again entirely consistent with previous observations that this prostanoid is primarily involved in the events leading up to the onset of labour. This was also true of patients presenting with dysfunctional labour, and it should be borne in mind that all patients presenting with dysfunctional labour had undergone spontaneous onset of labour.

When the inhibitory factor(s) in amniotic fluid are considered, it is quite possible that the origin of this factor(s) may be different to that of the stimulatory factor(s). Manzai and Liggins (1984) have demonstrated that the amnion secretes an inhibitor of PGE and PGF synthesis by cultured endometrial cells. Hence, a reasonable expectation is that the inhibitor of prostaglandin synthesis in amniotic fluid is derived from the amnion. The inhibitory factor(s) present in amniotic fluid has been found to have a molecular weight more than 30 000 daltons (Cohen et al 1985). Even if this substance does not readily diffuse out of the amnion cells, it may still reach the amniotic fluid with the regular shedding off of amnion cells into the amniotic cavity.

In addition, in the presence of unequivocal evidence that PGF synthesis is limited in the amnion in dysfunctional labour (Section 5.5.5), it may be assumed that transfer of inhibitory factor(s) from the amnion to the amniotic cavity does not parallel in vivo activity of these factors. Thus amniotic fluid may not be the most suitable monitor of in vivo activity of prostaglandin synthetase inhibitors operative in the amnion.

Quite obviously, although not pursued within the scope of this thesis, further investigation into the presence and/or activity of these inhibitors in the amnion in dysfunctional labour would provide additional support for the role of stimulatory and inhibitory factors in the regulation of prostaglandin synthesis in the amnion during labour.

SUMMARY AND CONCLUSIONS

Investigators, for many years, have designed studies to prove that in pregnancy the fetus may be in control of its own destiny in determining the timely onset of labour and thence birth. While pioneering research in the fetal lamb has unravelled the control of parturition in this species, subsequent investigations in humans have demonstrated important points of difference, indicating that the lamb is not an adequate model for the study of human parturition.

Norman, from this laboratory, used human twin pregnancy as a model for the study of human parturition. He demonstrated clearly that PGE played a central role in the final events which initiated and maintained labour. In his elegant studies on adrenal gland function in twins he also showed that this increase in PGE biosynthesis, unlike the position in the fetal lamb, did not appear to be mediated via the fetal hypothalamus-pituitary-adrenal axis.

Further investigation into the factor(s) which may initiate PGE biosynthesis in parturition is obvious and the occurrence of dysfunctional labour appeared to present a model which could give insight into the initiation and control of prostaglandin biosynthesis during labour. A first requirement was therefore to select patients who met specific criteria indicating dysfunction in uterine contractions. This was achieved by studying patients presenting with "delayed" labour and retrospectively selecting those with no evidence of mechanical obstruction to labour and who in fact progressed to normal vaginal delivery under the stimulus of intravenous oxytocin. Having defined the

patient material, the study of prostaglandin biosynthesis before and during labour could proceed on the basis of in vivo and in vitro studies.

For the in vivo studies the concentrations of PGE, PGF, its metabolite PGFM, PGIM and TXB_2 were monitored in amniotic fluid during labour. In both normal and dysfunctional labour the PGE levels of amniotic fluid increased progressively during labour; the levels were not significantly different between the two groups although in a small subgroup of patients in dysfunctional labour levels remained low. Levels of PGF also increased significantly in amniotic fluid during normal labour and actually overtook that of PGE levels. In dysfunctional labour however both PGF and PGFM levels failed to increase during the course of labour. Oxytocin administration in these patients, although restoring functional uterine contractions, did not affect amniotic fluid PGF levels. The amniotic fluid levels of the non contractile prostanoids were not different between patients in normal and dysfunctional labour.

The obvious conclusion therefore was that since all patients were admitted into the study in active labour, in patients presenting with dysfunctional labour there was no abnormality in the initiation of labour in which PGE plays a central role. However during normal labour there appeared to be a switch from PGE to PGF biosynthesis. This did not occur in dysfunctional labour. The model of dysfunctional labour thus appeared to be a condition in which the biosynthesis of PGF was limited and was not diverted to non contractile prostaglandin biosynthesis. The next step clearly was to attempt to identify the level at which PGF biosynthesis was limited and the factor(s) responsible for the limitation.

In the review of prostaglandin biosynthesis (Section 2) it has been noted that in the biosynthetic sequence leading to prostaglandin synthesis from free AA, there are no specific rate limited steps in the classical biochemical sense.

Hence control of the prostaglandin biosynthetic pathway can only be achieved via substrate availability at any level in the biosynthetic sequence, via biological compartmentalization, via specific enzyme inhibitors or activators or a combination of these factors. The practical implication for the investigation of the prostaglandin biosynthetic pathway is that there is no alternative but to study the pathway step by step and thus by a process of elimination to attempt to pinpoint the possible cause of limitation of PGF synthesis in dysfunctional labour. To study the biosynthesis of prostaglandins it was therefore necessary to examine fetal membrane activity during dysfunctional labour. This however is only possible by in vitro techniques.

As a first step it had to be shown that the suggested limitation in PGF biosynthesis was indeed manifest in the fetal membranes in dysfunctional labour. A major criticism of past work on fetal membranes was the fact that these tissues were traumatized variously in the preparation for study and that the amnion and chorion layers were not functionally intact, a factor which in itself is known to stimulate prostaglandin production. Hence a superfusion cell was designed in which intact, untraumatized membrane could be superfused. This method also allowed simultaneous but separate superfusion of the amnion and choriodecidual surfaces, thus maintaining their functional roles without disturbance of

their anatomical relationships.

Using this in vitro preparation it was confirmed that PGE and PGF release occurred essentially from the amnion layer and that PGF release from the amnion obtained from patients with dysfunctional labour was severely limited. It seemed reasonable to conclude that the limitation of PGF production and dysfunctional labour were related as cause and effect.

The next step was the investigation of the possible factors which could limit PGF production, starting with the availability of free AA in the amnion layer of fetal membranes. Initially, free AA was measured in amniotic fluid obtained before labour and after normal and dysfunctional labour. The significant increase in amniotic fluid free AA after the onset of labour (both normal and dysfunctional labour) suggested that there was mobilization of AA from the membranes following the onset of labour.

Measurement of free AA content of homogenates of the amnion and choriodecidua revealed a wide range of results in tissue obtained before and after labour. Although levels tended to be lower in the homogenates of the amnion obtained following normal and dysfunctional labour as compared to prelabour tissue, no statistical differences were observed between the three groups of patients. Admittedly, as discussed in Section 6.6 the methodology employed in assessing free AA in tissue homogenates would necessarily also include protein bound arachidonate which in vivo would not be available as the free acid for substrate. Thus measurement of the AA content of individual phospholipids of the amnion and choriodecidua phospholipids of the amnion and choriodecidua

was performed as a more accurate assessment of arachidonate mobilization, since the source of free AA is undoubtedly the structural phospholipids, which as a result of phospholipase activity, release free AA.

These studies clearly demonstrated release of AA from PE, PI and PS from the amnion in association with labour. Failure to demonstrate any differences in arachidonate mobilization from these phospholipids of the amnion between normal and dysfunctional labour certainly suggested that the activity of endogenous PLA₂ was similar in both groups of patients and that arachidonate availability via the PLA₂ pathway was not limited in dysfunctional labour. It seems reasonable to conclude that the deficient PGF production in dysfunctional labour, in the light of adequate substrate availability, must be related to factors operative at a site distant to AA in the prostaglandin biosynthetic pathway.

The next step therefore was to examine the effect of various factors, present in amniotic fluid, that are known to regulate prostaglandin synthetase activity in patients presenting with dysfunctional labour. The findings of concomitant alterations in the effects of stimulatory and inhibitory substances in amniotic fluid on prostaglandin synthetase activity was confirmed. During normal labour these occurred in such a manner that PGE production was favoured during early labour while that of PGF during late labour, findings which are entirely consistent with the pattern of prostaglandin concentrations observed in amniotic fluid. The source of these stimulatory and inhibitory substances in amniotic fluid is inferential at this stage. The source of the stimulatory fractions could quite feasibly be fetal urine while evidence points to the amnion as the most likely source of the inhibitory substance present in amniotic

fluid. Failure to demonstrate increased inhibitory activity in amniotic fluid in dysfunctional labour as compared to normal labour with respect to PGF production by the amnion, indicates that amniotic fluid itself is not the most suitable monitor of in vivo activity of inhibitors of prostaglandin synthetase operative in the amnion. That this inhibitory activity is indeed functional in the amnion is supported by the finding that limited PGF production by the amnion in dysfunctional labour persisted during the in vitro experiments in which tissues were removed from exogenous stimuli.

It seems reasonable therefore to conclude that while amniotic fluid factors, both stimulatory and inhibitory, may have a role to play in the pattern of prostaglandin production during labour, the defect in primary dysfunctional labour is most likely due to the presence of inhibitor(s) of PGF production by prostaglandin synthetase active in the amnion. Thus, even though these inhibitor(s) have not been positively identified within the scope of this thesis, patients presenting with dysfunctional labour have proved suitable models in highlighting some aspects of the control of prostaglandin biosynthesis during human labour. Undoubtedly, this model could be further studied with respect to identification of these regulatory factors of prostaglandin synthesis and their activity in relation to human parturition.

APPENDICES

APPENDIX A

PATIENT CONSENT

All patients gave written consent to their participation in the study after full explanation of all procedures involved. The study was approved by the Ethics Committee of the University of Natal.

APPENDIX B

STATISTICAL METHODS

Patient clinical details and the results obtained for the intrauterine pressure recordings were entered into a specially designed dataform and submitted to the Institute for Medical Biostatistics for analysis

All data obtained from the various studies used were entered into an Oxstat programme. Frequency distribution of the relevant variables was then determined by examination of the degree of skewness and kurtosis of the relevant variables.

If the frequency was shown to be normal in distribution parametric statistics (unpaired Students t test) was used. If the frequency was non parametric in distribution the data was analysed using either the Mann Whitney U test, the Wilcoxin ranked sum test or Pearsons correlation co-efficient performed on the log transformed data.

Statistical significance was adjudged at the 5 percent level.

APPENDIX CLABORATORY PROCEDURESC.1 REAGENTS

C.1.1 Reagents employed were in all instances of a high grade of purity (at least Merck GR). For high performance liquid chromatography (HPLC) solvents of spectroscopic grade (Waters Associates, USA) were used. The sources of other reagents and materials such as enzymes, standards and isotopes have been indicated in the appropriate sections.

C.1.2 Phosphate Assay Buffer (A) (0,01 mol/l)

Phosphate assay buffer was used in many procedures (designated buffer A). The following were dissolved in 1 litre of distilled water at 40° C.

- | | | |
|----|--|--------|
| 1. | Potassium dihydrogen phosphate (anhydrous) | 0,140g |
| 2. | Disodium hydrogen phosphate (anhydrous) | 0,426g |
| 3. | Sodium chloride | 8,776g |
| 4. | Gelatine | 1,000g |
| 5. | Sodium azide | 1,000g |

Final pH after adjustment was 7,4. The buffer was stored at 4°C.

C.1.3 Phosphate Assay Buffer (B) (0,05 mol/l)

The following were dissolved in 1 litre of distilled water.

- | | |
|---|----------|
| 1. Potassium dihydrogen phosphate (anhydrous) | 0,7009g |
| 2. Disodium hydrogen phosphate (anhydrous) | 2,1295g |
| 3. Sodium chloride | 43,8300g |
| 4. Sodium azide | 1,000g |

Final pH was adjusted to 7,4. The buffer was stored at 4° C.

C.1.4 Dextran Charcoal Mixture (DCM)

Dextran charcoal mixture was used in the prostaglandin and thromboxane assays. Norit A, 500 mg and Dextran T70, 25mg were added to phosphate buffer A at 4° C as described in the appropriate sections.

C.2 EQUIPMENT

The equipment used in the procedures described in Appendix C were:

1. Liquid scintillation spectrometer : Packard Tricarb Model 544.
2. Refrigerated centrifuges : MSE Mistral 4L for separation of amniotic fluid samples (1000 - 2000g at 4° C).
: Hettich Rotixa for R1A separations (2500g at 4° C).

3. High performance liquid chromatograph : Waters Associates (Milford, USA) model 6000 A pumps, UK6 injector and model 490 programmable multiwave-length detector.
4. Gas liquid chromatograph : Hewlett Packard 5890A with a Hewlett Packard integrator 3392 A.
5. Spectrophotometer : Beckman DU-5.

C.3 MEASUREMENT OF PROSTAGLANDIN E_2 (PGE); $F_{2\alpha}$ (PGF), 13, 14 DIHYDRO-15-KETO PROSTAGLANDIN $F_{2\alpha}$ (PGFM); PROSTAGLANDIN-6KETO $F_{1\alpha}$ (PGIM) AND THROMBOXAME B_2 (TXB₂).

C.3.1 *Principles of the Method*

The prostaglandins in amniotic fluid and fetal membrane perfusates (Section 5) were concentrated by solvent extraction. The concentrations of prostaglandins were then measured by specific radioimmunoassay in which antigen in sample or standard competes with labelled antigen for a restricted number of binding sites on specific antibodies. The antigen antibody complexes formed after incubation were separated from unbound antigen by the addition of DCM (C.1.4) and the radioactivity counted in a liquid scintillation spectrometer.

C.3.2 Reagents for Prostaglandin Assays

C.3.2.1 The following labelled prostaglandins were purchased from Amersham International, Bucks, UK.

1. (5, 6, 8, 11, 12, 14, 15 (n) - ^3H) Prostaglandin E_2 (TRK 431); specific activity 5,92 TBq/mmol.
2. (5, 6, 8, 9, 11, 12, 14, 15 (n) - ^3H) Prostaglandin $\text{F}_{2\alpha}$ (TRK 464); specific activity 6,66 TBq/mmol.
3. 13, 14 dihydro-15-keto (5, 6, 8, 9, 11, 12, 14 (n) - ^3H) prostaglandin $\text{F}_{2\alpha}$ (TRK 517); specific activity 2,96 TBq/mmol.
4. 6 Keto (5, 8, 9, 11, 12, 14, 15 (n) - ^3H) prostaglandin $\text{F}_{1\alpha}$ (TRK 618); specific activity 5,55 TBq/mmol.
5. (5, 6, 8, 9, 11, 12, 14, 15 (n) - ^3H) Thromboxane B_2 (TRK 620); specific activity 6,71 TBq/mmol.

The contents of each vial were evaporated to dryness under a stream of nitrogen and reconstituted in absolute methanol. This stock solution was stored in a siliconized glass vial at -20°C . Prior to use an appropriate aliquot was removed, dried and the prostaglandin redissolved in phosphate buffer A. Radiochemical purity was checked regularly by thin layer chromatography employing the solvent mixture, benzene: dioxane: acetic acid 20:20:1 (v/v).

C.3.2.2 Prostaglandin Standards

Prostaglandin standards were purchased from UpJohn Co. (Kalamazoo, USA) and purity checked by thin layer chromatography as described for the radiochemicals (C.3.2.1). PGF was supplied as the Tromethamine salt. All prostaglandins (except 6 keto PGF_{1 α}) were prepared in methanol at a concentration of 1 mg/ml and stored at -20° C. Six keto prostaglandin F_{1 α} was dissolved in a minimum of acetone (0,1ml) and diluted to 1 mg/ml in distilled water containing sodium carbonate (0,5 mmol Na₂CO₃ per mmol of prostaglandin). This was divided into portions and stored at -20° C.

C.3.2.3 Prostaglandin Antisera

Prostaglandin antisera were raised in rabbits against prostaglandin-bovine serum albumin conjugate. Details of characteristics of anti-PGE₂, anti-PGF₂ and anti-PGFM were described in detail by Norman (1983). Anti-6-keto-PGF₁ (anti-PGIM) and anti-Thromboxane B₂ (anti-TXB₂) were prepared as follows:

a. Preparation of Immunogen.

PGIM and TXB₂ (5mg) were separately evaporated to dryness in a 20 ml siliconized glass scintillation vial (Beckman). While maintaining a constant pH of 5,5 and mixing, the following were added successively : an aqueous solution of

sodium carbonate (0,02 mol in 10% ethanol), bovine serum albumin (10mg) and 1-ethyl-3(-3 diethylamine propyl carbodiimide, HCl (5mg)). After 24 hours in the dark at room temperature, the mixture was dialysed for 4 days against phosphate buffered saline (0,15 mol/l sodium chloride, 0,01 mol/l phosphate, pH 7,4).

b. Immunization.

A volume of dialysate equivalent to 0,2mg of conjugate per animal was emulsified with incomplete Freund's adjuvant and injected intradermally by multiple puncture into 3 rabbits. The antibody titre was followed weekly from 4 weeks and the animals boosted monthly (x3). A study of the high titre antibody was examined for specificity and the best animal bled. Half the serum obtained was stored in 0,5ml portions at - 20° C and the rest freeze dried (0,5 ml portions) and also stored at - 20° C. Final antibody titres for PGIM and TXB₂ were 1 : 600 and 1 : 5000 respectively.

C.3.2.4 Phosphate Buffer A (Section C.1.2)

C.3.2.5 Dextran Charcoal (Section C.1.4)

C.3.3. Characteristics of the Antibodies

The specificity of the antibodies to PGIM and TXB₂ are shown in Table C.1. The percentage cross reactions were calculated from the relative amounts of prostaglandins required to reduce the initial binding of the respective ³H prostaglandin by 50%.

The sensitivity of the assay as defined by the mean of the least detectable mass resulting in a response of 2 standard deviations from the zero concentration was 28 pmol/l (PGIM) and 25 pmol/l (TXB₂).

C.3.4 Assay Procedure

The assay procedure consisted of 2 major aspects :
Sample preparation and radioimmunoassay.

C.3.4.1 Sample Preparation

Amniotic fluid samples were stored at - 20° C for up to 2 weeks prior to assay. Fetal membrane superfusates were stored for up to 7 days at - 20° C.

Extractions of samples were performed in polypropylene extraction tubes (DPC NML tubes). Samples of amniotic fluid (1ml) and tissue superfusate (2,5ml) were acidified to pH 4 using citric acid (0,1mol). Approximately 1500 cpm ³H PGFM was

TABLE C.1

Antibody cross reactions (anti PGIM and anti TXB₂) to a variety of prostaglandins.

<u>Prostaglandin</u>	<u>6-keto-F_{1α}</u>	<u>Thromboxane B₂</u>
E ₁	4,3%	< 0,1%
E ₂	0,8%	3,1%
15-keto E ₂	< 0,1%	< 0,1%
13,14-dihydro E ₂	< 0,1%	< 0,1%
13,14-dihydro-15-keto-E ₂	< 0,1%	< 0,1%
F _{2α}	< 1,0%	2,1%
15-keto F _{2α}	< 0,1%	< 0,1%
FM	< 0,1%	< 0,1%
F _{1α}	1,0%	< 0,1%
6-keto-F _{1α}	100%	< 0,1%
A ₁	0,29%	< 0,1%
A ₂	0,13%	< 0,1%
2,3,4,5,20 pentanol 11 _α 9 _α - dihydroxy-15-prostanoic acid		< 0,1%
2,3,4,5,20 11 _α -hydroxy-9,15-dioxo- 19-carboxy prostanoic acid		< 0,1%
2,3, dinor prostanoic acid		100%
B ₁	0,2%	< 0,1%
B ₂	< 0,1%	< 0,1%
D ₂	< 0,1%	
Thromboxane B ₂		100%

added to the extraction tubes for PGE, PGF and PGFM assays and 1500 cpm ^3H 6 keto PGF_1 to those tubes for assay of PGIM and TXB_2 to monitor recoveries after the extraction procedure. The prostaglandins were extracted (x2 for PGE, PGF, PGFM; x 3 for PGIM and TXB_2) with 2 volumes of ethyl acetate with vigorous shaking for 15 minutes at 4°C . After low speed centrifugation at 4°C the organic phase was separated, dried under vacuum at room temperature and reconstituted in phosphate assay buffer A. Portions were taken for assessment of radioactive recovery and for individual radio-immunoassay (RIA) of PGE, PGF, PGFM, PGIM and TXB_2 .

C.3.4.2. Radio-immunoassay

The following were placed in polypropylene assay tubes in duplicate for each assay of individual prostaglandins.

1. Sample (after extraction), standard or control (0,1 ml).
2. ^3H prostaglandin approximately 15 000 cpm (0,1ml).
3. Prostaglandin antiserum (0,1ml).
4. Phosphate Buffer A (C.1.2) 0,2ml to total and NSB tubes.

After mixing the tubes were incubated at 0°C for 18 hours. Incubation was terminated with ice cold DCM 1 ml (C.1.4) and

vortexed. After a standing time of 10 minutes the tubes were centrifuged at 2500g at 4° C for 10 minutes. The supernatant was decanted into scintillation vials and 10ml Instagel (Packard) or Readysovlv (Beckman) was added to all vials and the radioactivity counted in a liquid scintillation spectrometer.

C.3.5 Calculations

A standard curve was drawn by plotting the ratio B/B_0 for each standard value (where B is the count rate for individual standards, and B_0 is the count rate for the zero standard) against concentration of standard. Sample concentrations were found by interpolation after converting to B/B_0 ratios. Correction was made for the percent recovery of the radioactive prostaglandin following extraction of the sample. Typical standard curve graphs for PGE, PGF, PGFM, PGIM and TXB_2 are shown in Figures C1,2,3,4 and 5 respectively.

C.3.6. Comments

1. Assay blank values.
Assays were accepted only if blank values were less than the lowest standard for each assay. Therefore blanks were not subtracted from the results obtained from the standard curve.
2. Samples were only accepted if extraction recoveries were greater than 75%.

Figure C.1

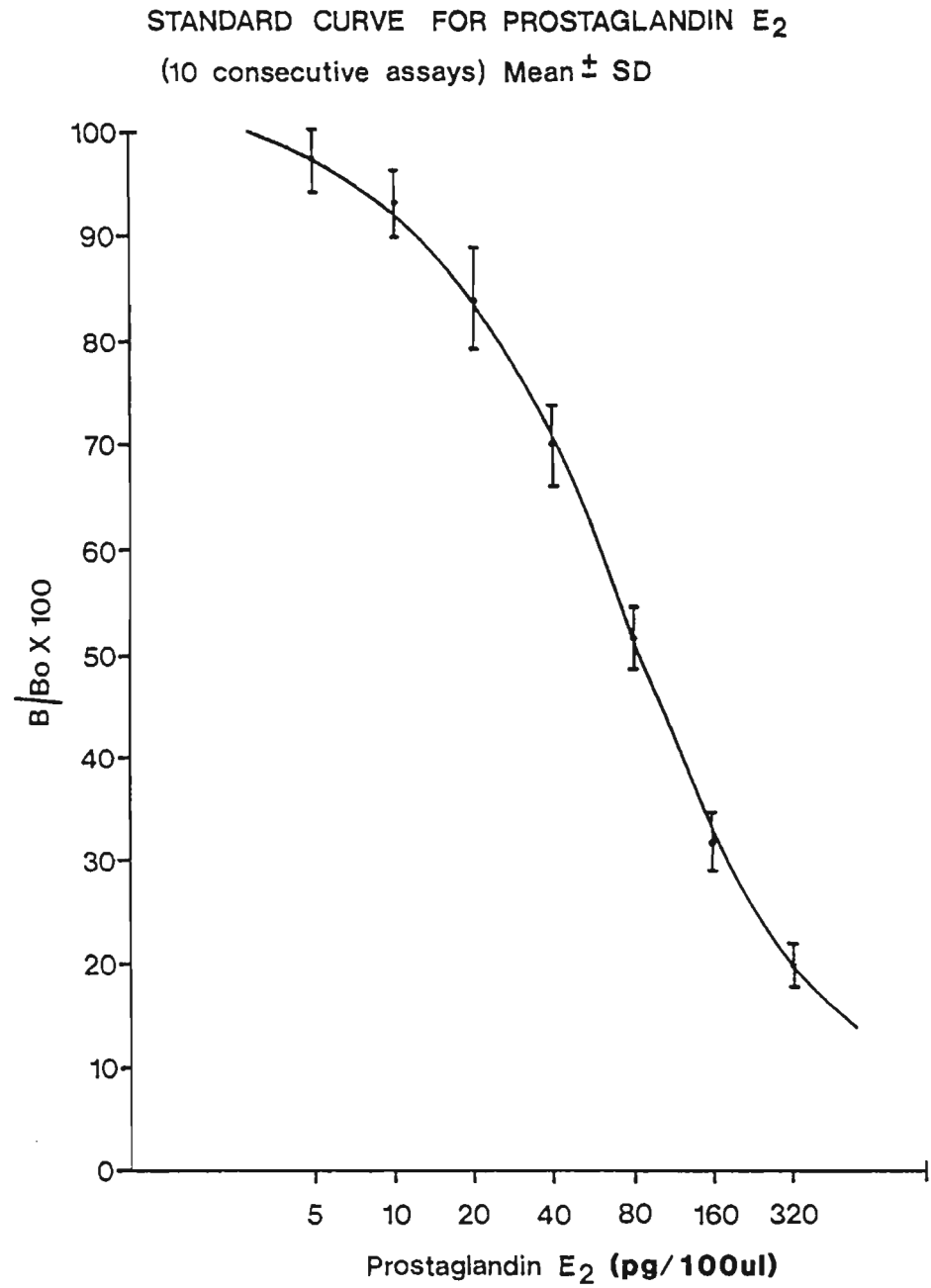


Figure C.2

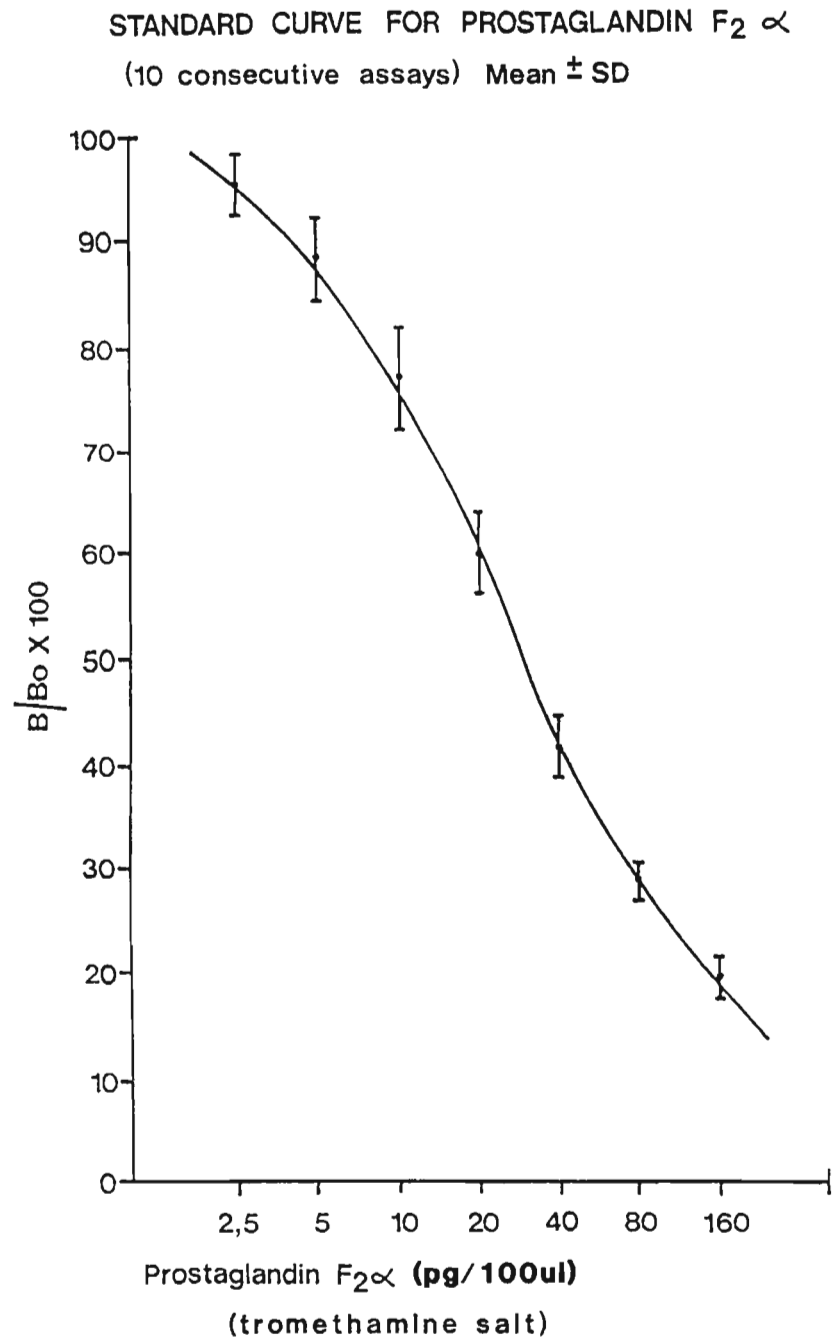


Figure C.3

STANDARD CURVE FOR PROSTAGLANDIN 13,14 DIHYDRO
15 KETO F₂ ∝ (PGFM) (10 consecutive assays) Mean ± SD

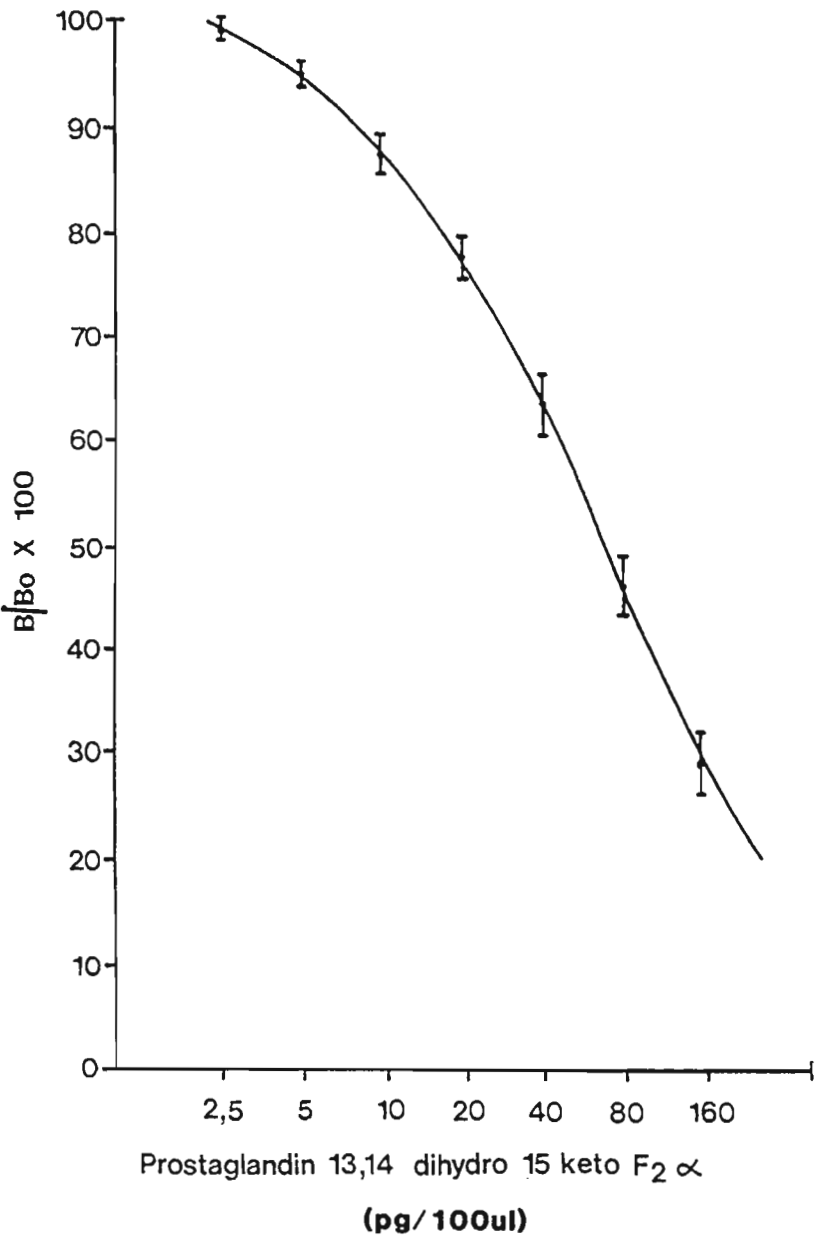


Figure C.4 **Standard curve for 6 keto prostaglandin $\text{Fl}\alpha$**
(10 consecutive assays) $M \pm \text{SD}$

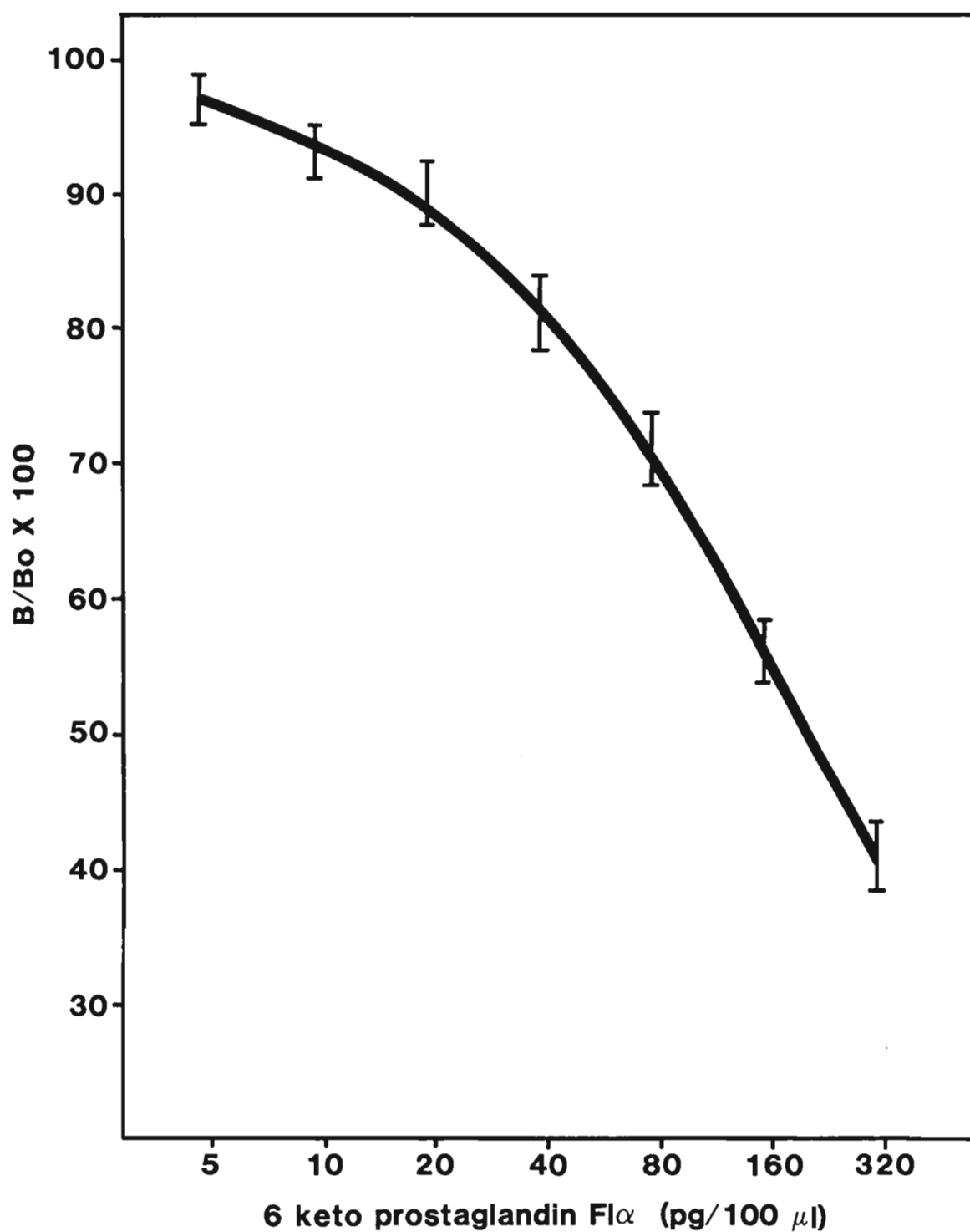
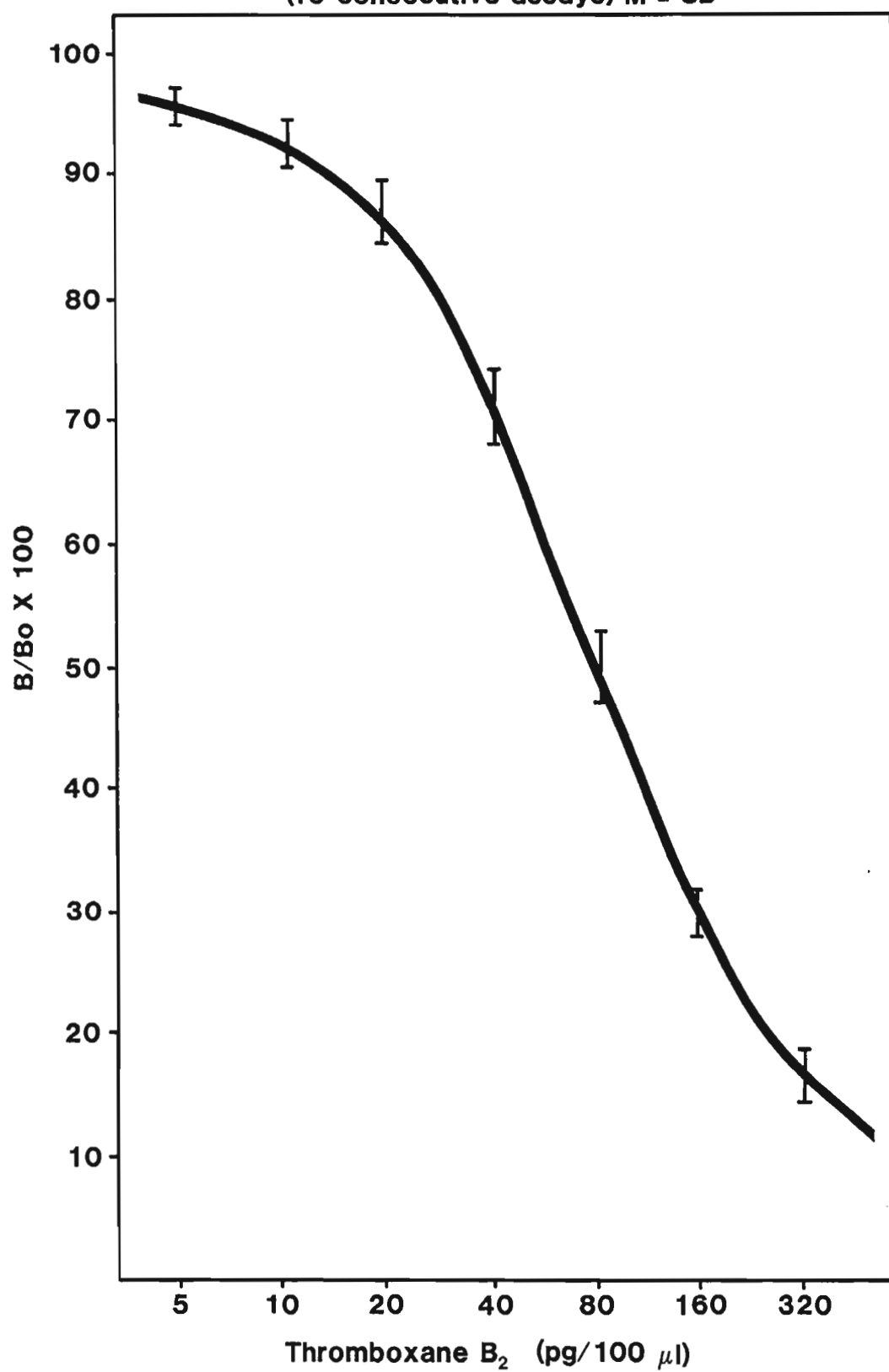


Figure C.5

Standard curve for Thromboxane B₂
(10 consecutive assays) $M \pm SD$



3. Assay tubes.

All glassware used in the assay procedure was siliconized. Polypropylene tubes (LKB Bartinger Mannheim) were used in the assay incubation procedures.

4. The non specific binding (NSB) for all assays was less than 4% and the maximum binding (Bo) between 40 - 60%.

C.4. PRODUCTION OF PROSTAGLANDINS BY FETAL MEMBRANES

Details of this method have been presented elsewhere (Section 5.3).

Superfusates of the fetal membrane were assayed for PGE, PGF and PGFM by specific RIA (Section C.3).

C.4.1 Validation of Radio-immunoassay of Superfusates of the Fetal Membranes

C.4.1.1 High Performance Liquid Chromatography of Prostaglandins

The suferfusates from either side of the fetal membranes in 4 tissue samples were extracted into ethyl acetate (x2) as described in Section C.3.4.1, dried under vacuum and resuspended in ethanol (50ml) in preparation for high performance liquid chromatography (HPLC). The column employed was a Merck Licrosorb RP-18,(5um), 250mm long x 4mm in diameter. The prostaglandins were eluted isocratically with a solvent system containing 35% acetonitrile in

distilled water adjusted to pH 3,5 with acetic acid. Fractions were collected at timed intervals for 18 minutes.

The chromatographic profile of a mixture of prostaglandin standards following HPLC is shown in Figure C.6. The RIA results for PGE and PGF on the eluted fractions following HPLC of the sample superfusates have been presented in Table 5.1 (Section 5.5.1).

C.4.1.2 Histological Examination of the Fetal Membranes

The fetal membranes were examined by light and electron microscopy before and after the superfusion experiments.

Light microscopy confirmed the presence of all 3 tissue types, amnion, chorion and decidua in the fetal membrane preparation. The electron micrographs of the amnion and choriodecidua before and after superfusion are shown in Figs C7, 8, 9 and 10 respectively.

Although there was an increase in vacuolations in both the amnion and choriodecidua following superfusion the nuclei, nucleoli and plasma membranes were intact demonstrating the viability of the tissue after the 4 h superfusion.

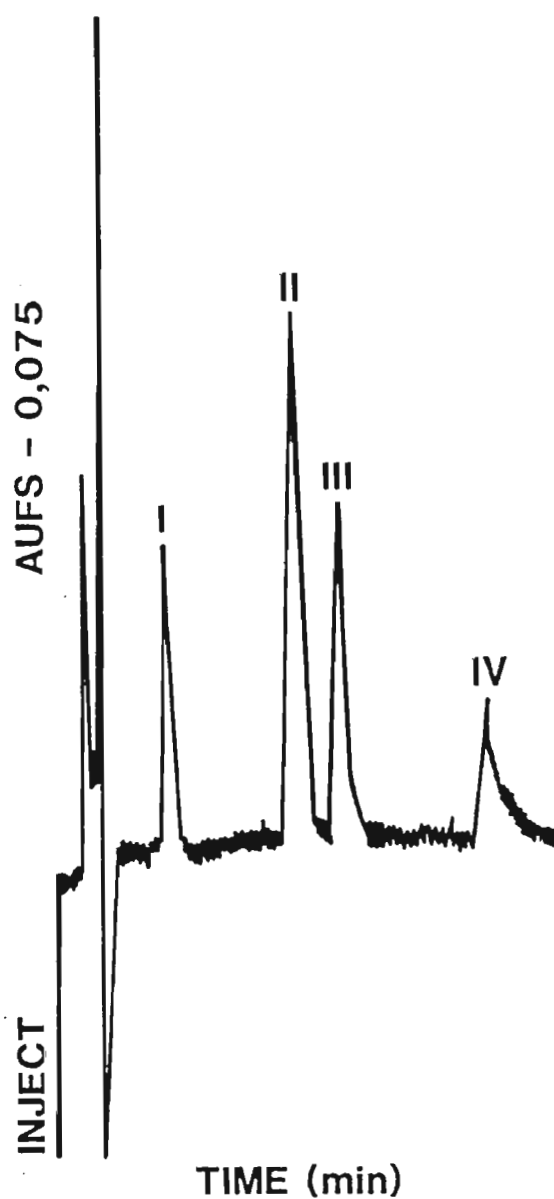


Fig C6. Chromatographic profile of a mixture of authentic prostaglandin standards following high performance liquid chromatography

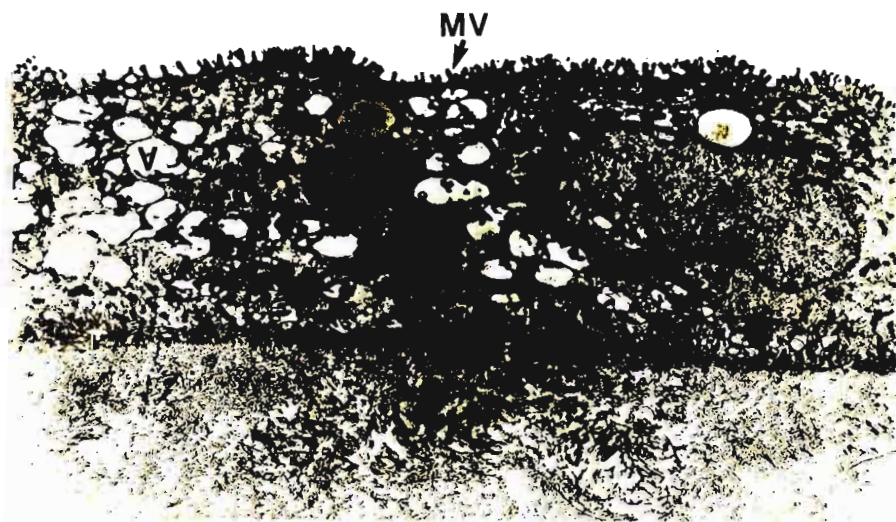
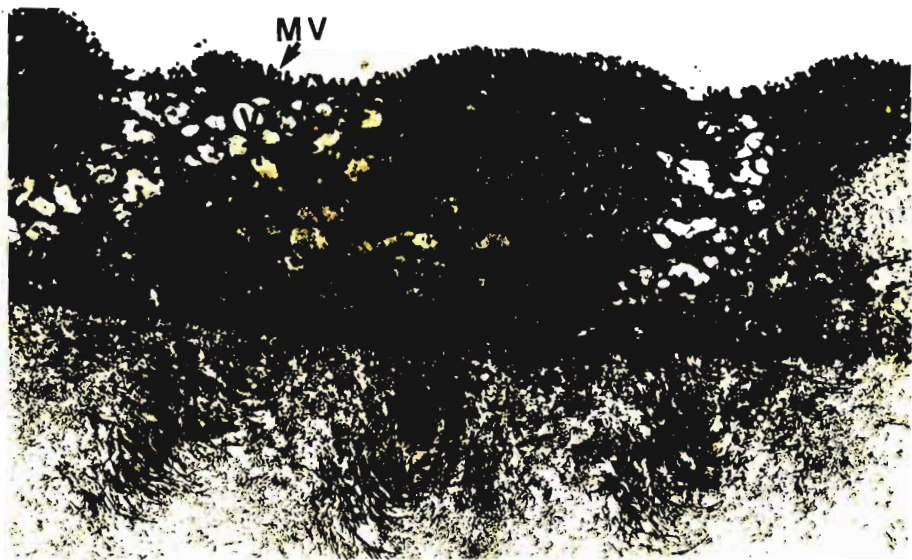
AUFS : Absorbance Units Full Scale

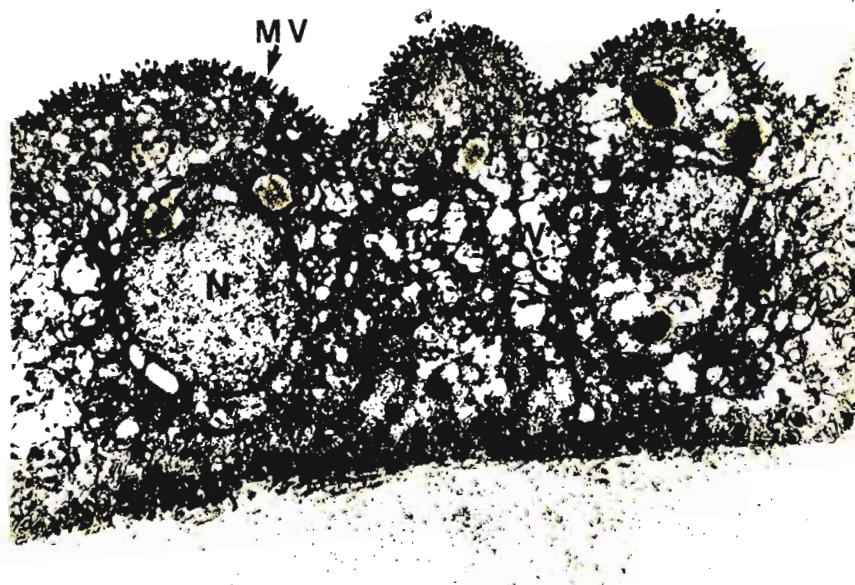
I : 6 keto prostaglandin $F_{1\alpha}$

II : prostaglandin E_2

III : prostaglandin $F_{2\alpha}$

IV : 13, 14 dihydro-15-keto $PGF_{2\alpha}$





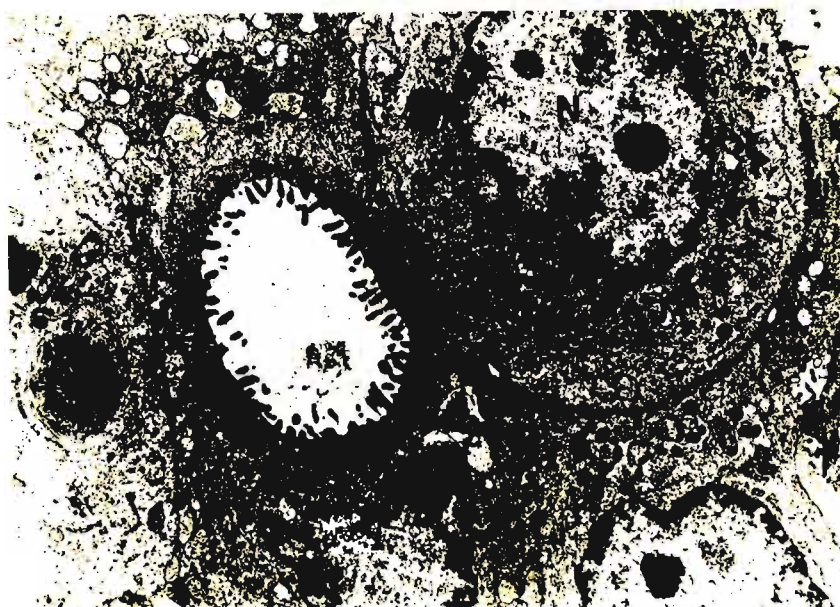


Fig C9. Electron micrograph of a section of the choriodecidua before superfusion of the tissue

N - nucleus

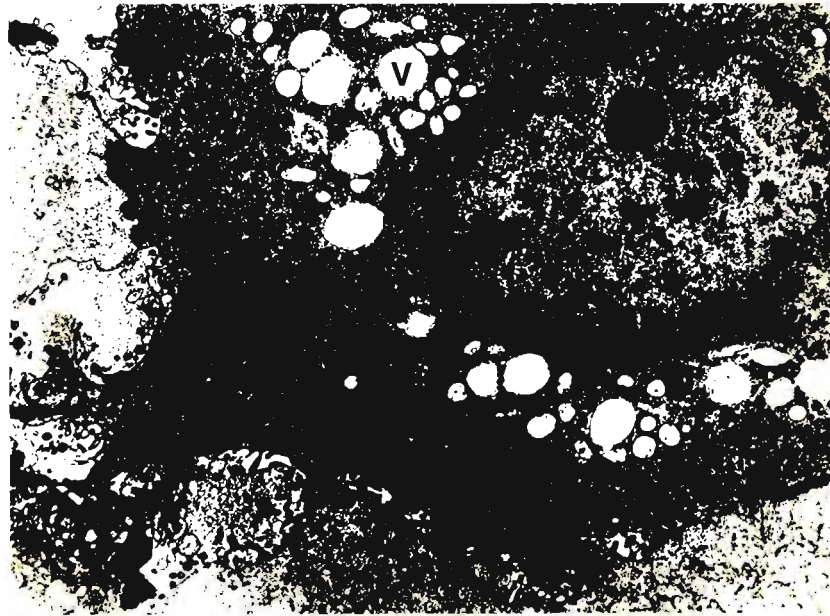


Fig C10. Electron micrograph of a section of the choriodecidua after superfusion of the tissue. An increase in the number of vacuoles in choriodecidual cells was also noted after the superfusion experiments

N - nucleus
V - vacuoles

C.5. PHOSPHOLIPID CONTENT OF FETAL MEMBRANES

C.5.1. Reagents

1. All reagents used were Merck G.R analytic grade. The various solvent mixtures used in the extraction of lipids and thin layer chromatography were:
 - a) Extraction solvents:
 - i) Chloroform : methanol 2.1 (v/v)
 - ii) Chloroform : methanol : water 3 : 48 : 47 (v/v).
 - b) Thin layer chromatography:
 - i) Solvent I Chloroform : methanol : acetic acid : water - 50 : 25 : 7 : 3 (v/v).
 - ii) Solvent II Tetrahydrofuran : methanol : ammonium hydroxide (2mmol/l) 60 : 40 : 17,5 : 5 (v/v).
2. Iodine : heated at 70° C to form iodine vapour for staining of phospholipid fractions.
3. Ammonium molybdate (Merck) : 2,5% in 3% sulphuric acid.
4. Ascorbic acid : 5% solution in distilled water.

5. Phospholipid standards : Authentic phospholipid standards were purchased from Sigma Chemical Co, St Louis, USA.

C.5.2 Principle of Method

The total lipid extract of the amnion and chorion was obtained by an extensive extraction and washing procedure commencing on the day of the tissue collection. Portions of the lipid extract were then subjected to 2 dimensional thin layer chromatography. Phospholipid fractions were identified following staining with Iodine vapour. Each phospholipid fraction was then assayed for phosphorus content.

C.5.3 Sample Collection

Placentae were collected immediately following delivery and transported on ice to the laboratory where the membranes were separated from the rest of the placenta. The amnion was separated from the choriodecidua and the tissues were washed in ice cold saline to remove blood and loose debris.

C.5.4 Preparation of Total Lipid Extract of the Fetal Membranes

Approximately 1g each of the amnion and chorio-decidua (wet weight) was homogenized in 2ml sodium chloride (0,15 mol/l) using a microdismembrator. The homogenates were then extracted with 20ml chloroform : methanol 2 : 1 v/v overnight at 4° C.

The extract was subsequently filtered through prerinsed Whatmans filter paper (No 451) to remove protein containing tissue residue. This residue was then washed with chloroform : methanol (2:1 v/v) 2 x 5 ml, and water (5ml) was added to each extract. After centrifugation at low speed, the upper aqueous phase was removed and re-extracted with chloroform (3 x 5ml). The lower chloroform layer was washed with a mixture of chloroform, methanol and water (3 : 48 : 47), the upper aqueous layer being discarded. Finally, all chloroform extracts were combined and 10 ml methanol was added. The extract was then dried under a stream of nitrogen and the residue dissolved in 0,25ml chloroform : methanol (2 : 1 v/v) and stored overnight under N₂ at - 20° C.

C.5.6 Separation of Lipid Classes by Thin Layer Chromatography

Portions of the total lipid extract of the amnion and choriodecidua were subjected to 2 dimensional thin layer chromatography using silica gel 60 HR plates (Merck Darmstadt, W. Germany). The plates were heated at 110° C for 30 minutes and subsequently cooled in a desiccator for 30 minutes prior to use. The chromatogram was developed to a height of 15 cm in solvent I (section C.6.5.1), then dried at 60° C for 15 min, cooled and redeveloped in the 2nd dimension to a height of 15 cm in solvent II (C.6.5.1). The phospholipids were visualized after exposure to Iodine vapour. Lipid classes were identified by chromatography of authentic phospholipid standards.

Figure C.11 illustrates the phospholipid fractions identified following 2 dimensional chromatography.

C.5.7 Analysis of Phosphorous Content of Phospholipids

Before analysis of phosphorous, the phospholipid fractions identified following chromatography were digested using perchloric acid (70%) at 190° C for 3 hours. The contents of the tubes were vortexed with distilled water (3,2ml) and centrifuged at 600g for 10 minutes. A portion (3ml) of supernatant was mixed with ammonium molybdate (0,3ml) and ascorbic acid (0,3ml) incubated at 100° C for 7 min and the absorbance read at 830 nm in a spectrophotometer.

C.5.7.1 Calculations

Concentration of phosphate was calculated by interpolation off a standard curve obtained from phosphate standards treated in the same way as the unknown samples.

C.6 MEASUREMENT OF ARACHIDONIC ACID

C.6.1 Principle of the Method

Free AA in amniotic fluid and homogenates of the amnion and choriodecidua were extracted into an organic solvent using

TLC PLATE SEPARATING PHOSPHOLIPIDS

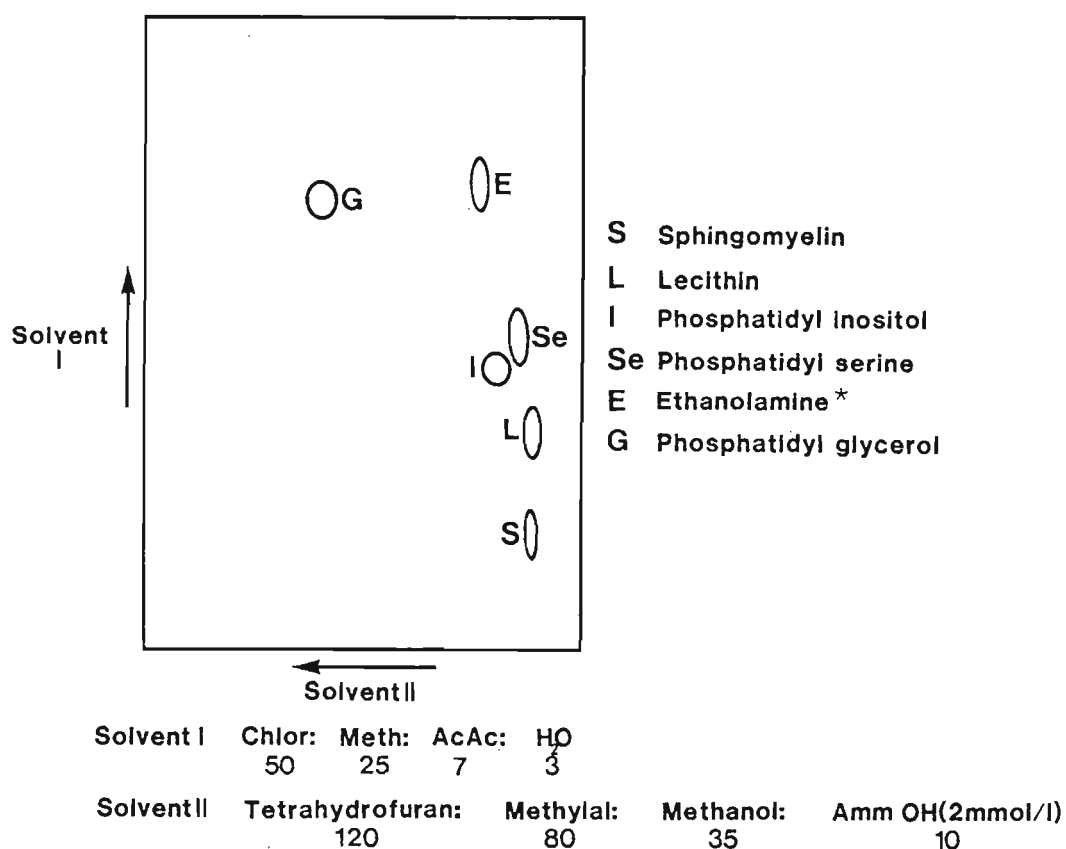


Fig C11. Separation of authentic phospholipid standards after 2-dimensional thin layer chromatography (TLC)

*Ethanolamine includes ethanolamine plasmalogen

Seppak cartridges. The free acid was methylated using benzene and boron trifluoride and quantitated by gas liquid chromatography (GLC).

C.6.2 Reagents Used

1. Stock Arachidonic Acid (Sigma St. Louis Missouri USA) 0,5 mg/ml in chloroform : methanol 1 : 12 (v/v).
2. Heptadecanoic acid (HDA; Sigma St. Louis Missouri USA) 0,5 mg/ml in ethanol.
3. Glycine buffer pH3.
 - (a) 0,1 mol/l glycine in 0,1 mol/l NaCl
 - (b) 0,1 mol/l HClMix 80 ml (a) with 20 ml (b).
4. Boron trifluoride in methanol(20%).
5. Organic Solvents. Benzene, methanol, isooctane and hexane.
6. NaCl 9g/l.

C.6.3 Sample Preparation

Amniotic fluid samples were collected from patients as described in Section 3.2. Specimens contaminated with blood and meconium were rejected. Samples were centrifuged at 230g at 4°C for 5 min and 1 ml of the supernatant used in the extraction procedure.

The total lipid extracts of the amnion and choriodecidua were prepared as described in Section C.5.4. Portions of this lipid extract were assayed for AA to determine the free AA in whole tissue homogenates. To 0,1 ml of tissue homogenates 0,9 ml of Tris HCl buffer was added and the sample then treated in the same way as amniotic fluid samples.

C.6.4 Extraction Method

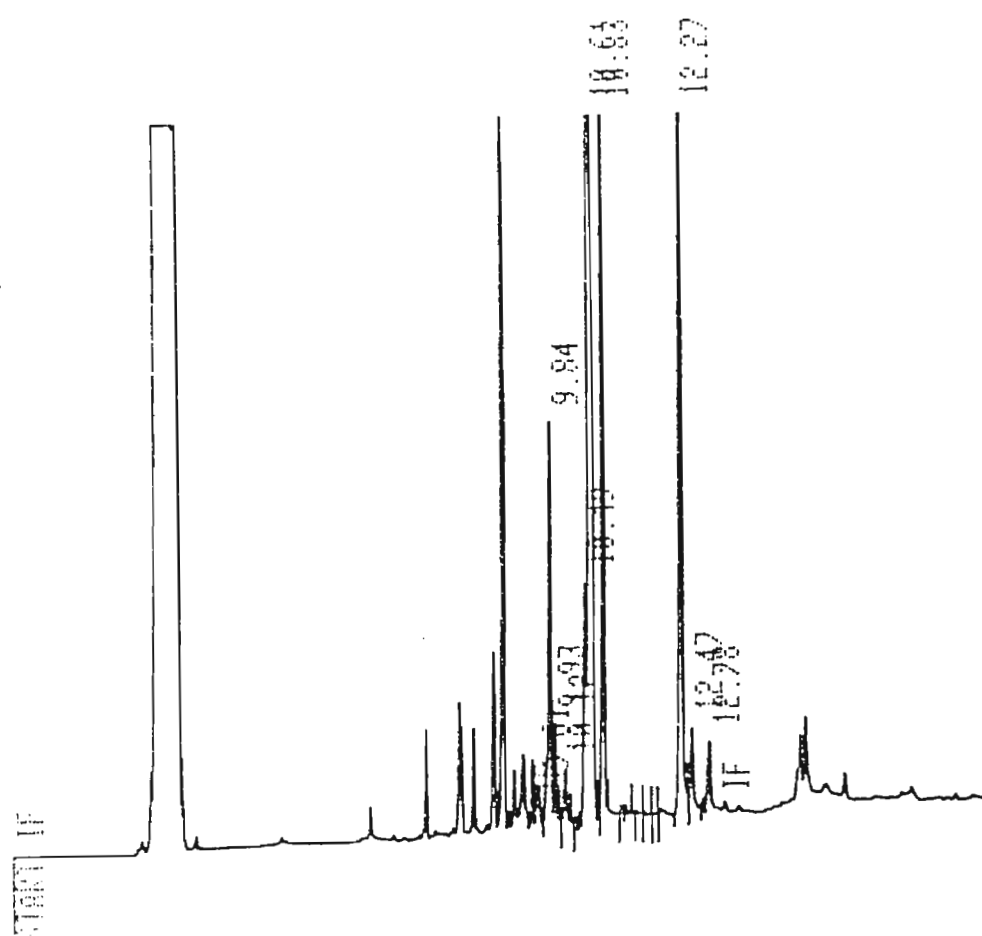
To 1 ml of amniotic fluid and samples of tissue homogenates 10 ug of heptadecanoic acid (C.6.2), to serve as internal standard, and 5 ml glycine buffer was added immediately before extraction. Seppak C₁₈ cartridges (Waters Associates, Milford, Massachusetts) pre equilibrated with methanol and water were used to extract AA and other free fatty acids. The samples were applied to the cartridges under partial vacuum followed by a 10 ml water wash. The fatty acids were eluted with 3,5 ml benzene : methanol (3:20 v/v) solvent mixture.

C.6.5 Methylation of Arachidonic Acid

Methylation of the eluted fatty acids following Seppak extraction was achieved by the addition of benzene (0,5 ml) and boron trifluoride in methanol (2 ml). The mixture was left overnight in the dark at room temperature. Hexane (4 ml) and NaCl (7 ml) were then added to each sample. The samples were centrifuged at low speed and the lower aqueous phase discarded. Samples were rewashed with NaCl (7 ml) and the hexane layer transferred to a clean stoppered tube and dried under a stream of nitrogen. The residue was dissolved in isooctane (0,2 ml) for analysis by GLC.

C.6.6 Gas Chromatography of Arachidonic Acid

Chromatography was performed with flame ionisation detection on a DB-1 fused silica column (J & W Scientific, Inc; Rancho Cordova). Column dimensions were 0,32 mm internal diameter and 30m length. Samples were dissolved in iso-octane and 0,5 μ l was injected into a cold on-column injector. Three oven temperature ramps were used. After injection of sample at 90° C the temperature was raised rapidly to 220° C (50° C/min). During the second temperature ramp to 265° C (5° C/min) chromatography of the fatty acid methyl esters took place. This was followed by a final temperature ramp to 300° C (30° C/min) to clean the column.



RUN # 283
 WORKFILE ID: C
 WORKFILE NAME:

RT	HEIGHT	TYPE	AR/HT	HEIGHT%
9.61	334	PP	0.069	0.542
*9.84	4201	PV	0.053	6.815
9.93	1019	VB	0.049	1.653
10.12	541	BB	0.052	0.878
10.49	2432	VV	0.052	3.945
10.61	35730	VV	0.058	57.964
10.83	8609	BB	0.046	13.966
** 12.27	7291	FB	0.058	11.828
12.47	780	BP	0.061	1.265
12.78	705	I VP	0.060	1.144

TOTAL HGHT= 61642
 MUL FACTOR= 1.0000E+00

C.6.7 Calculations

Arachidonic acid was calculated using the formula

$$\frac{\text{Peak height (AA)} \times 10}{\text{Peak height (HDA)} \times 0,71}$$

where

AA = Arachidonic Acid

HDA = Heptadecanoic acid

0,71 = Relative AA peak height correction factor.

This was expressed as nmol/l for amniotic fluid and nmol/g wet weight for tissue homogenates.

C.7 PHOSPHOLIPASE A₂ RELEASABLE ARACHIDONIC ACID CONTENT OF TISSUE
HOMOGENATES AND PHOSPHOLIPID FRACTIONS OF THE MEMBRANES

C.7.1 Principle of the Method

Tissue homogenates and phospholipid fractions isolated from the fetal amnion and choriodecidua were incubated with PLA₂ to hydrolyse fatty acids present in position 2 of the glycerophospholipids. The free acid was then methylated and quantitated by GLC as described in Section C.6.5 and C.6.6 respectively.

C.7.2 Reagents Used

1. Organic solvents. As described in Section C.6.2.
2. Phospholipase A₂ (PLA₂) (Boehringer Mannheim) specific activity 3000 U/mg protein.
3. Tris HCl buffer 0,05 mol/l pH8 containing 0,015 mol/l Ca²⁺ as CaCl₂.
4. Dichlorofluorescein 0,02 % in 100% ethanol.

C.7.3 Sample Preparation

The total lipid extracts of homogenates of the amnion and choriodecidua were prepared as described in Section C.5.4

Separation of the phospholipid classes of the homogenates of the amnion and choriodecidua was performed by 2 D TLC as described in Section C.5.6. These chromatographs were performed at the same time as those prepared for phosphate analysis (Section C.5.7).

The phospholipid fractions used for fatty acid analysis were visualized with dichlorofluorescein (C.7.2). Each fraction was then scraped off the silica plates into glass funnels plugged with glasswool, the phospholipids eluted with methanol (3ml) into clean glass stoppered tubes, dried under a stream of nitrogen and the residue dissolved in 0,1 ml methanol.

C.7.4 Incubation of Sample with Phospholipase A₂

Tris HCl buffer (Section C.7.2) 0,9 ml was added to portions of the lipid extract of tissue homogenates(0.1ml) and phospholipid fractions (0,1 ml). PLA₂ (1U) was added to each tube and samples were incubated at 37° C for 15 min with gentle shaking. The reaction was stopped by the addition of 5 ml glycine buffer pH3 (section C.6.2). The free AA was then extracted for analysis by gas liquid chromatography as described in Section C.6.4. For each sample a separate incubation in the absence of PLA was done to determine the endogenous (free) AA concentration.

C.7.5 Calculations

The releasable AA content of the homogenates was calculated as the difference between total AA content after incubation with PLA₂ and endogenously determined AA. This is expressed as nmol AA per nmol of phospholipid phosphate. Phospholipid phosphate analysis was performed as described in Section C.5.7.

C.7.6 Comments

1. Internal standard

Heptadecanoic acid (C17,0) is not present in significant amounts in amniotic fluid and the amnion and choriodecidua. (Schwarz 1975). This has therefore proved suitable for use as an internal standard.

2. Extraction recovery

Recovery of authentic arachidonic acid added to amniotic fluid prior to extraction was $96,6\% \pm 4,8\%$ (n = 12).

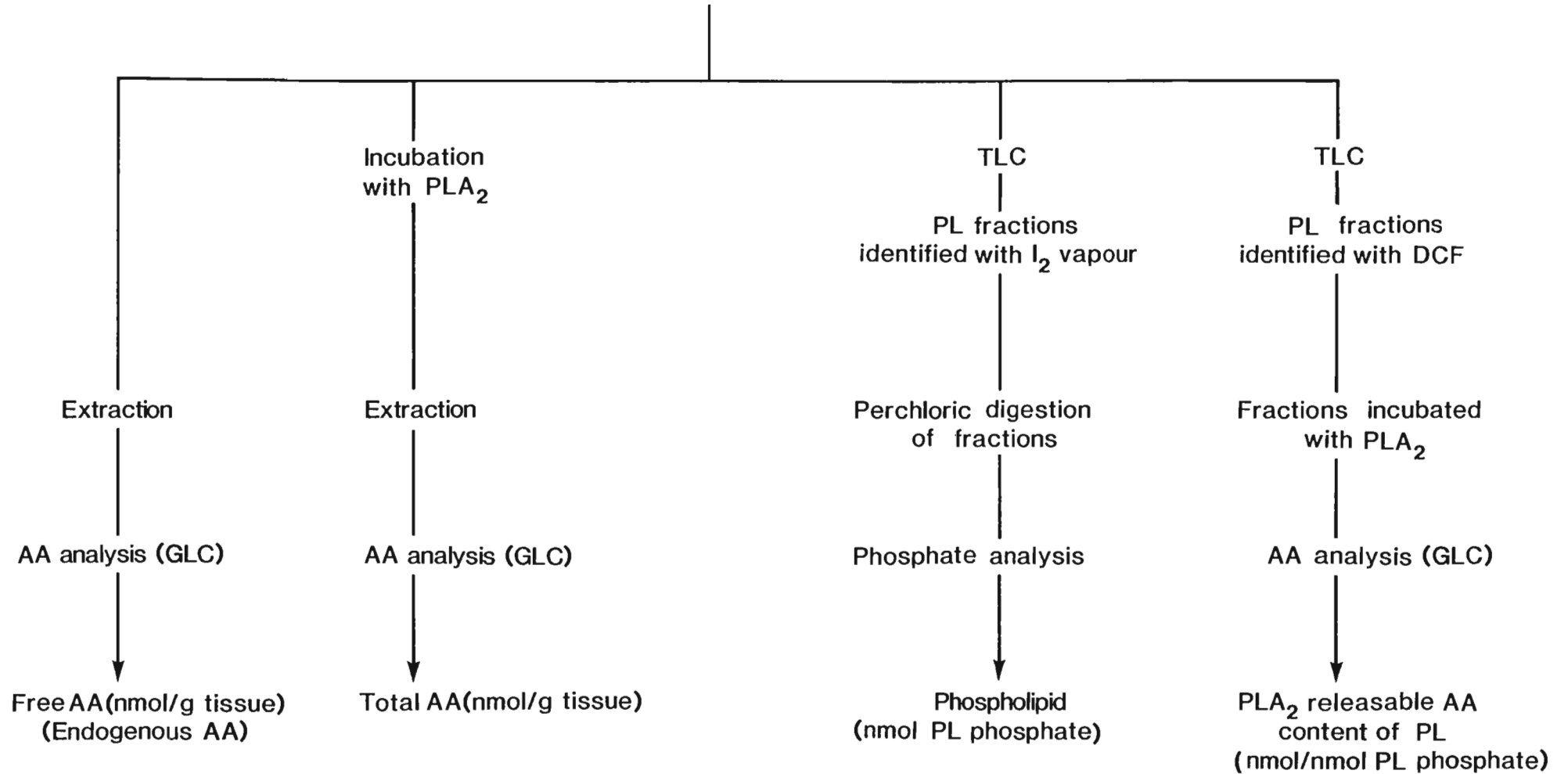
3. Assay blanks

Scrapings of silica following staining with dichlorofluorescein were processed in the same way as the identified phospholipid fractions. Arachidonic acid was always undetectable in these samples.

Fig C13. Summary of the procedures employed in phospholipid and arachidonic estimation in homogenates of the amnion and choriodecidua

TLC : thin layer chromatography
GLC : gas liquid chromatography
I₂ : iodine
DCF : dichlorofluorescein
PL : phospholipid
PLA₂ : phospholipase A₂
AA : arachidonic acid

HOMOGENATES OF AMNION AND CHORIODECIDUA



C.8 REGULATION OF PROSTAGLANDIN SYNTHETASE ACTIVITY BY AMNIOTIC FLUID

C.8.1 Principle of the Method

The ability of amniotic fluid or fractions of amniotic fluid to regulate prostaglandin synthetase activity was tested in an in vitro assay system. In the control samples prostaglandin synthetase (prepared from sheep seminal vesicles, Section C.8.3) was incubated with substrate, AA, under optimal conditions (Section C.8.6) and the products, PGE and PGF measured by specific RIA (Section C.3.4). The results obtained following the addition of amniotic fluid or fractions of amniotic fluid to the assay system were compared to those of the control samples in each assay.

C.8.2 Reagents Used

1. AA (Sigma, St Louis, Missouri, USA). Evaporated to dryness and stored under N₂ under vacuum at -20°C.
2. Prostaglandin synthetase (prepared as described in Section C.8.3), freeze dried and stored under vacuum in a desiccator at room temperature.
3. Reduced glutathione (Merck, Darmstadt, W Germany).
4. Authentic prostaglandin standards (Section C.3.2).

5. Organic solvents (Section C.1).

6. Phosphate buffer B (Section C.1.3).

C.8.3 Preparation of Prostaglandin Synthetase

This was performed as described by Wallach et al (1970).

Frozen sheep seminal vesicles were obtained from the local slaughterhouse. Approximately 300 - 400g (wet weight) was blended in a Waring Blender with 800 ml 0,154 mol/l KCl at room temperature. All further operations were performed at 0 - 3°C. The homogenate was then centrifuged for 5 min(0°C,4000g) and the resultant supernatant filtered through cheesecloth to remove fat and fibrous debris.

Acetone precipitation of prostaglandin synthetase from the supernatant of the homogenate was performed by meticulous, dropwise addition of acetone to a concentration of 30%, whilst keeping the temperature of the mixture below 3°C. The resultant beige coloured precipitate was recovered by centrifugation at 2000g for 20 min at 0°C. The precipitate was blended with approximately 15 vol acetone at -25°C - -30°C and all further operations were performed at this temperature. The mixture was filtered on a chilled Buchner funnel using Whatmans filter paper No 5. When most of the solvent had been removed the precipitate was resuspended in half the previous volume of acetone and the mixture refiltered as before.

After removal of the acetone the precipitate was blended with 5 vol ice cold pentane. The bulk of the pentane was removed by filtration. The precipitate (still wet) was then transferred to a chilled wide mouthed flask and freeze dried. The powder obtained was stored in a desiccator under vacuum at room temperature. Under these conditions the activity of the enzyme was retained for 4 - 5 months.

C.8.4 Preparation of Reagents

The following reagents were freshly prepared before each assay as described:

1. Sodium arachidonate substrate.

Before each assay the more soluble sodium salt was obtained from the parent acid. The residue of AA (Section C.8.2) was dissolved in a small volume of ethanol (final ethanol concentration in incubation tube 0,05%) to which ice cold 0,2% sodium carbonate was added (0,95 ml sodium carbonate per mg AA). This sodium salt was further diluted with ice cold phosphate buffer B to give a stock solution of sodium arachidonate of 200 mg/ml for assay use.

2. The prostaglandin synthetase powder was suspended in ice cold phosphate buffer(100mg/ml) and kept at 4°C until aliquotted into incubation tubes in each assay.

3. Reduced glutathione: reconstituted in phosphate buffer B immediately before each assay (4mg/ml).

C.8.5 Sample Preparation

Amniotic fluid was obtained from patients before labour and during labour as described in Section 3.2. Samples were centrifuged at 1000g and the supernatant stored at - 20° C.

A 2 ml aliquot of amniotic fluid was passed through a membrane with a molecular weight exclusion of 10 000 (UM 10 Amikon). Each fraction was reconstituted to 2 ml and was tested for effects on prostaglandin synthetase activity together with a sample of unprocessed amniotic fluid.

C.8.6 Method of Enzyme Incubation

This was performed as described by Brennecke et al (1982).

The standard assay mixture (total volume 1 ml) contained the following :-

- a) Prostaglandin synthetase powder (Section C.8.3) 10 mg.
- b) Sodium arachidonate 20 ug (61 nmol).

- c) Reduced glutathione (400 ug). This was added only to those tubes incubated for estimation of PGE (based on results obtained from enzyme incubation optimization studies).
- d) Amniotic fluid sample, unprocessed or fractionated (Section C.8.5). This constituted 10% of the total incubation volume.

The incubation was begun by the addition of sodium arachidonate and carried out in a water bath at 37°C for 20 min with gentle agitation. Incubation was stopped by the addition of 0,1 ml 2 mol/l citric acid. Samples were then extracted for PGE and PGF determination by RIA (Section C.3.4).

Each assay included in addition to the sample tubes the following:

- a) Active enzyme control (prostaglandin synthetase, AA, reduced glutathione (for PGE), but no amniotic fluid sample).
- b) Boiled enzyme control (as in (a) but enzyme powder boiled for 1 min immediately before assay).
- c) Blank controls (AA, reduced glutathione, no added enzyme).

- d) Prostaglandin recovery controls (1 ug of authentic prostaglandin standard (PGE or PGF), enzyme powder, reduced glutathione, but no AA) to monitor losses throughout the assay.

Where necessary the incubation volume was adjusted to 1ml using phosphate buffer B.

The enzyme incubation conditions were based on the results of optimization studies which investigated the effects of substrate concentration, enzyme concentration, reduced glutathione concentration, incubation time and incubation temperature on prostaglandin production. These results are respectively shown in Figs C.14 - C. 18 respectively.

C.8.6.1 Enzyme - incubation - radioimmunoassay reliability

The percentage conversion of arachidonate to PGE and PGF were $14,7 \pm 3,2\%$ and $8,4 \pm 2,1\%$ respectively ($M \pm SD, n=15$). Recovery of added standards were for PGE $87 \pm 6\%$ and PGF $83 \pm 5\%$ ($M \pm SD, n = 15$). Endogenous levels of prostaglandins in amniotic fluid samples were always less than 3% of the control samples.

C.8.7 Calculations

The results obtained after incubation of amniotic fluid samples to the assay system were expressed as a ratio over the control

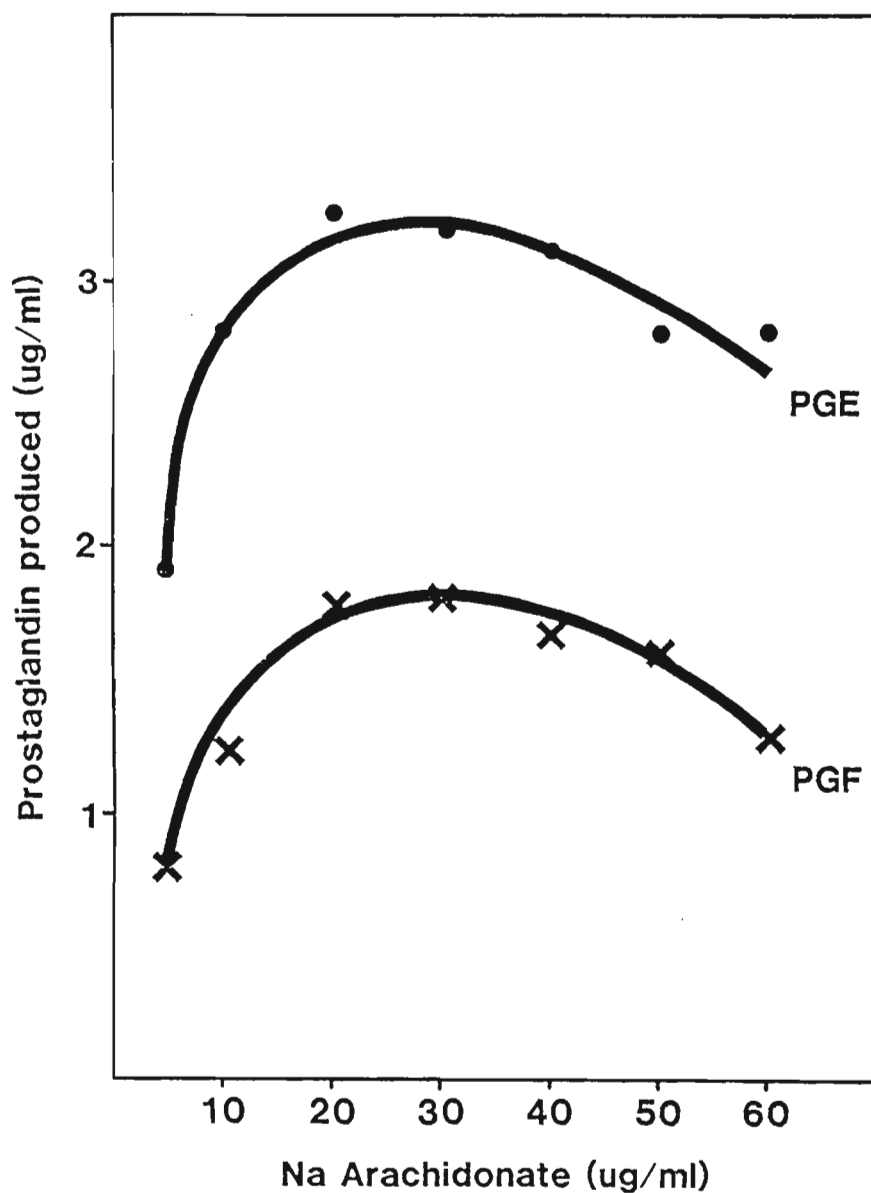


Fig C14. Relationship between sodium arachidonate concentration and prostaglandin production by sheep seminal vesicle prostaglandin synthetase. Substrate concentration of 20 $\mu\text{g/ml}$ was chosen for further experiments. Each point represents a mean of 4 readings from 3 experiments

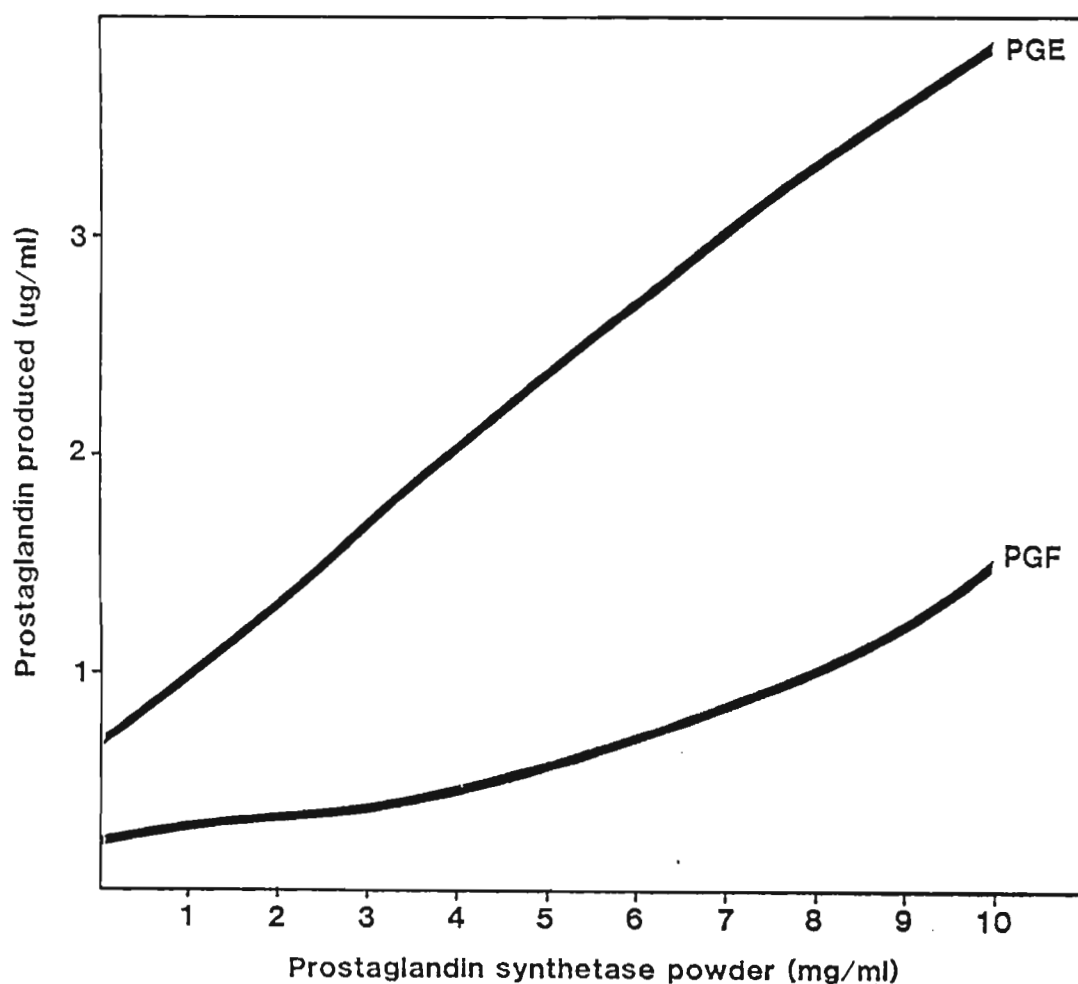


Fig C15. Relationship between enzyme powder concentration and prostaglandin production by sheep seminal vesicle prostaglandin synthetase (arachidonate concentration of 20 μ g/ml, pH 7.4 at 37°C for 30 min). Enzyme concentration used in all further assays was 10 mg/ml

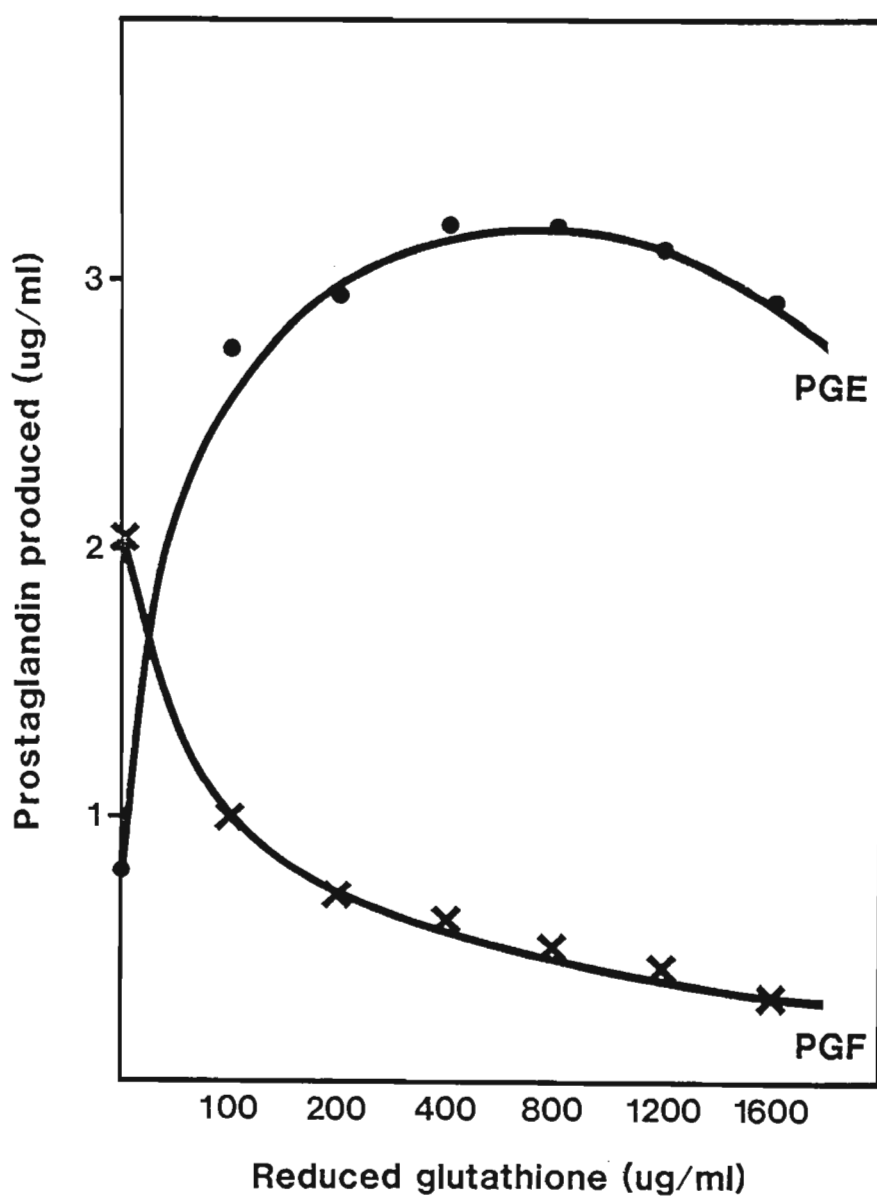


Fig C16. Relationship between reduced glutathione concentration and prostaglandin production by sheep seminal vesicle prostaglandin synthetase. The presence of reduced glutathione clearly inhibited PGF production and was therefore omitted in tubes incubated for PGF estimation. Concentration used for PGE assay was 400 $\mu\text{g/ml}$

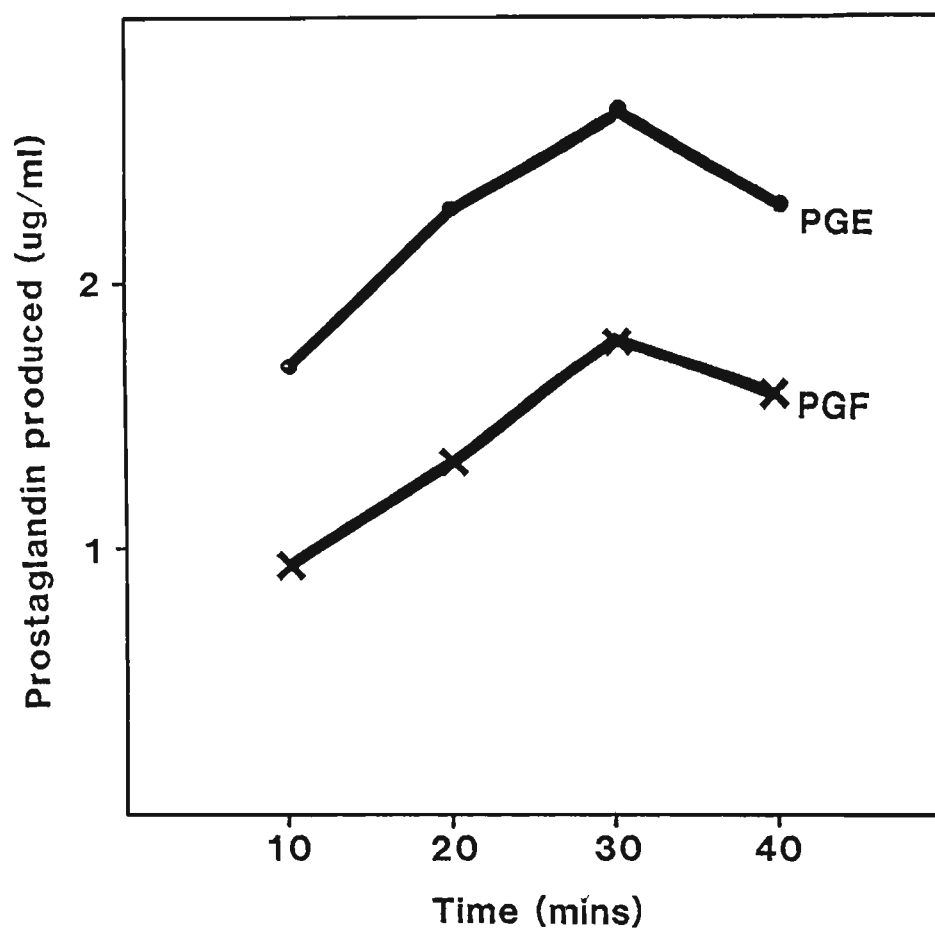


Fig C17. Relationship between time (min) and prostaglandin production by sheep seminal vesicle prostaglandin synthetase (substrate $20 \mu\text{g/ml}$, enzyme powder 10 mg/ml , incubated at 37°C). Time chosen for routine assay was 30 min

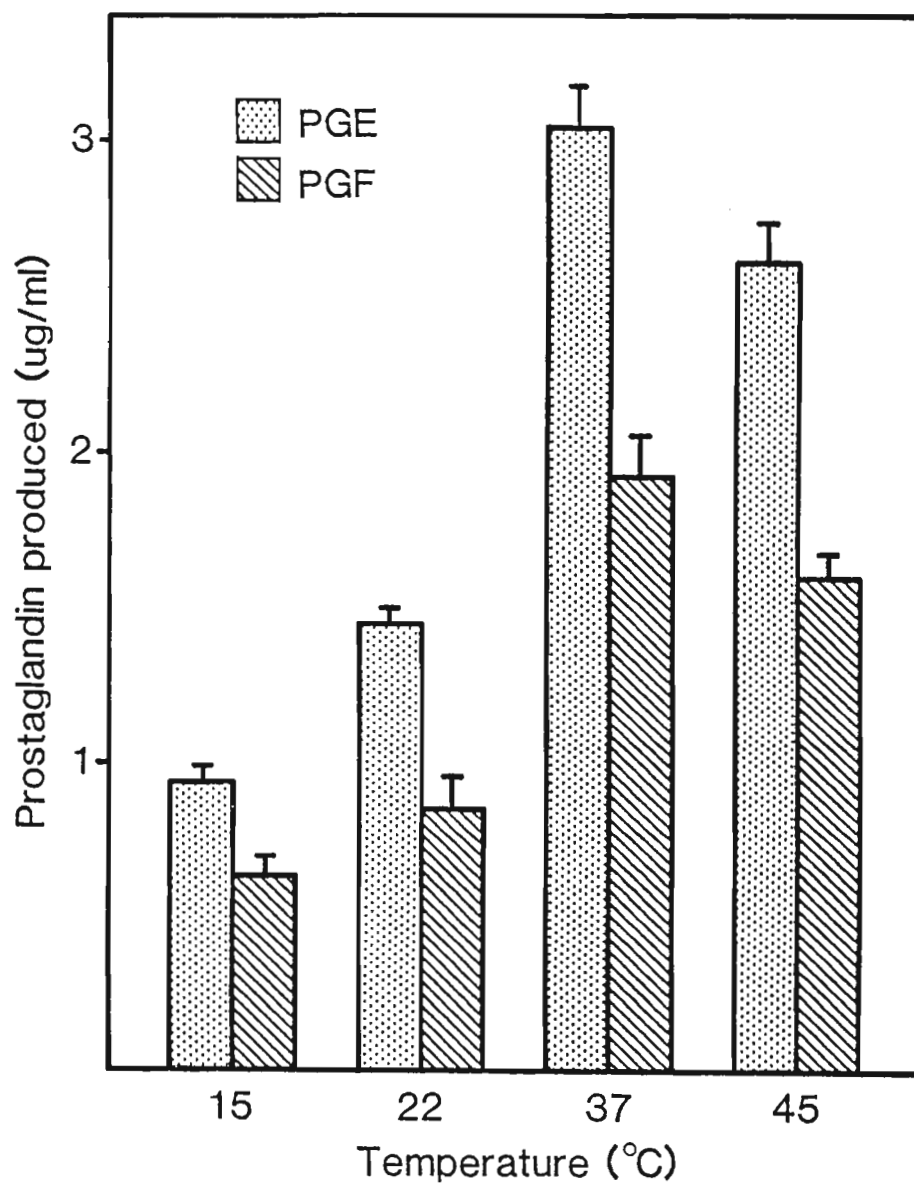


Fig C18. Relationship between temperature and prostaglandin production by sheep seminal vesicle prostaglandin synthetase (substrate 20 μ g/ml, enzyme powder 10 mg/ml and incubated for 30 min. Temperature chosen for routine assay was 37°C

samples in each assay. Thus values less than 1 indicated net inhibition of prostaglandin synthetase whilst those greater than 1 indicated net stimulation of prostaglandin synthetase activity.

C.8.8 Comments

Enzyme incubations for PGE and PGF were performed separately but simultaneously. This was necessary because reduced glutathione had to be omitted from those tubes incubated for PGF estimation since the presence of reduced glutathione inhibited PGF production.

REFERENCES

1. Brennecke et al (1982)
2. Curbelo et al (1981)
3. Norman et al (1981)
4. Norman et al (1982)
5. Okita et al (1981)
6. Wallach et al (1970)

REFERENCES

REFERENCES

- ABEL, MH ; KELLY, RW. (1979)
Differential production of prostaglandins within the human uterus. Prostaglandins 18 : 821 - 828.
- ABEL, MH ; KELLY, RW. (1983)
Metabolism of prostaglandins by the non pregnant uterus.
J Clin Endocrinol Metab 56 : 678 - 685.
- ALLAN, H; DODDS, EC.
Investigations into the cause of the onset of labour. Part I. Internal secretions in Pregnancy. J Obstet Gynaecol : 447 - 475
- AMY, JJ; THIERY, M. (1979)
Labour - spontaneous and induced. In Clinical Perinatology ed Aladjeu, S ; Brown, A K , Mosby St. Louis.
- ARULKUMARAN, S ; GIBB, DMF ; LUN, KC ; HENG, SH ; RATNAM, SS. (1984)
The effect of parity on uterine activity in labour. Br J Obstet Gynaecol 91 : 843 - 848.
- BAKHLE, YS. (1983)
Synthesis and catabolism of cyclo-oxygenase products. Br Med Bulletin 39 : 214 - 218.
- BERGSTROM, S; SJÖVALL, J. (1957)
The isolation of prostaglandins. Acta Chem Scand 11 : 1086.
- BIBBY, JG; BRUNT, JD; HODGSON, H; MITCHELL, MD; ANDERSON, ABM; TURNBULL, AC. (1979)
Prostaglandins in umbilical plasma at elective caesarian section. Br J Obstet Gynaecol 86 : 282 - 284.
- BLACKWELL, GJ; FLOWER, RJ. (1983)
Inhibition of Phospholipase. Br. Med Bulletin 39 : 260 - 264.

- BRENNECKE, SP; BESSELL, CK; WOODS, JB; TURNBULL, AC. (1983)
A proteinaceous inhibitor of Prostaglandin Synthase in human amniotic fluid. J Obstet Gynaecol 4 : 73 - 77.
- BRENNECKE, SP; BRYCE, RL; TURNBULL, AC; MITCHELL, MD. (1982)
The Prostaglandin Synthase inhibiting ability of maternal plasma and the onset of human labour. Eur J Obstet and Gynaecol Reprod Biol 14 : 81 - 88.
- BRENNECKE, SP; CASTLE, BM; DEMERS, LM; TURNBULL, AC. (1985)
Maternal plasma prostaglandin E₂ metabolite levels during human pregnancy and parturition. Br J Obstet Gynaecol 92 : 345 - 349.
- BRENNECKE, SP; COLLIER, HOJ; DENNING - KENDALL, PA. (1981)
Inhibition of prostaglandin biosynthesis by ovine and human blood plasmas in relation to reproductive function. J Physiol 315 : 39P - 40P.
- BRENNECKE, SP; COLLIER, HOJ; DENNING KENDALL, PA; McDONALD - GIBSON, WJ; SAEED SA; MITCHELL, MD. (1981)
Prostaglandin synthesis inhibition by maternal and fetal sheep plasma and its relations to haptoglobin and albumin levels. Prostaglandin and Med. 6 : 243 - 248.
- BRENNECKE, SP; LENTON, EA; TURNBULL, AC; MITCHELL, MD. (1982)
Inhibition of Prostaglandin Synthase by maternal plasma factor(s) in early human pregnancy. Br J Obstet and Gynaecol 89 : 612 - 616.
- BRUMMER, HC. (1972)
Serum F_{2α} levels during late pregnancy, labour and the puerperium. Prostaglandins 2 : 185 - 194.
- BURR, GL; BURR, MM. (1930)
Quoted by Crawford, MA. Background to essential fatty acids and their prostanoids derivatives. Br Med Bulletin (1983) 39 : 210 - 213.

- BYGDEMAN, M. (1980)
Clinical Applications. Adv. Prostaglandin Thromboxane Res. 6 87 - 94.
- CARSTEN, ME. (1974)
Hormonal regulation of myometrial Ca^{2+} transport. Gynaecol Invest 5 : 269 - 275.
- CADWELL, BV; BURSTEIN, S; BROCK, WA; SPEROFF, L. (1971)
Radioimmunoassay of the F prostaglandin. J Clin Endocrinol Metab 33 : 171 - 175.
- CASEY, ML; CUTRER, SI; MITCHELL, MD. (1983)
Origin of prostanoids in human amniotic fluid. The fetal kidney as a source of amniotic fluid prostanoids. Am J Obstet Gynaecol 147 : 547 - 551.
- CASEY, ML; MACDONALD, PC. (1986)
The initiation of labour in women : Regulation of phospholipid and arachidonic acid metabolism and of prostaglandin production. Sem Perinatol 10 : 270 - 275.
- CASEY, ML; MACDONALD, PC; MITCHELL, MD. (1985)
Despite a massive increase in cortisol secretion in women during parturition, there is an equally massive increase in prostaglandin synthesis. A Paradox? J. Clin Invest 75 : 1852 - 1857.
- CHALLIS, JRG; MITCHELL, BF. (1981)
Hormonal control of preterm and term parturition. Sem Perinatol 5 : 192 - 202.
- CHALLIS, JRG; OTHATHANOND, R; RYAN, KI; TULCHINSKY, D. (1974)
Maternal and fetal prostaglandin levels at vaginal delivery and caesarean section. Prostaglandins 6 : 281 - 288.

- COHEN, DK; CRAIG, DA; STRICKLAND, DM; McCUBBIN, JH; MITCHELL, MD. (1985)
Prostaglandin biosynthesis : Stimulatory and inhibitory substances in human amniotic fluid during pregnancy and labour. *Prostaglandins* 30 : 13 - 20.
- COLACICCO, G; BASU, MK; RAY, AK; WITTNER, R; ROSENBAUM, RM. (1971)
Effects of Prostaglandin E_2 and $F_{2\alpha}$ on lecithin biosynthesis of cultured lung cells. *Prostaglandins* 14 : 283 - 294.
- COWAN, DB; VAN MIDDELKOOP, A; PHILPOTT, RH. (1982)
Intra-uterine pressure studies in Africa nulliparae. *Br J Obstet Gynaecol* 89 : 364 - 369.
- COWAN, DB; VAN MIDDELKOOP, A; PHILPOTT, RH. (1982)
Intrauterine pressure studies in African nulliparae: delay, delivery and disproportion. *Br. J Obstet Gynaecol* 89 : 370 - 380.
- CURBELO, V; BEJAR, R; BENIRSCHKE, K; GLUCK, L. (1981)
Premature labour. 1 Prostaglandin precursors in human placental membranes. *Obstet Gynaecol* 57 : 473 - 478.
- DAWOOD, MY. (1977)
Hormones in amniotic fluid. *Am J Obstet Gynaecol* 128 : 576 - 583.
- DAWOOD, Y; YLIKORKALA, O; TRIVEDI, D; FUCHS F. (1979)
Oxytocin in maternal circulation and amniotic fluid during pregnancy. *J Clin Endocrinol Metab* 49 : 429 - 433.
- DEMBELE-DUCHESNE, MJ; THALER-DAO, H; CHAVIS, C; CRASTES DE PAULET, A. (1981)
Some new prospects in the mechanism of control of arachidonate metabolism in human placenta and amnion. *Prostaglandins* 22 : 979 - 1002.

- DI RENZO, GC; JOHNSTON, JM; OKAZAKI, T. (1981)
Phosphatidylinositol - specific phospholipase C in fetal membranes and uterine decidua. J. Clin Invest 67 : 847 - 856.
- DI RENZO, GC; SALVIOLI, G; BARTOLAMASI, A; MAMBRINI A. (1980)
Lipid and fatty acid composition of amniotic fluid and fetal blood during labour. The Human Placenta - Serono Symposium No 35. Ed A Klover, A Genazzani; P Crosignani Academic Press.
- DRAY, F; FRYDMAN, R. (1976)
Primary prostaglandins in amniotic fluid in pregnancy and spontaneous labour. Am J Obstet Gynaecol 126 : 13 - 19.
- DUBIN, NH; JOHNSON, JWC; CALHOUN, S; GHODGAONKAR, RB; BECK, JC. (1981)
Plasma prostaglandins in pregnant women with term and preterm deliveries. Obstet Gynaecol 57 : 203 - 206.
- EKBLAD, U; ERKKOLA, R; UOTILA, P. (1982)
PGE₂ is only slightly metabolized in the fetal circulation of perfused human placenta. Prostaglandins, Leukotrienes Med 8: 481 - 488.
- FERREIRA, SH; VANE, JR. (1967)
Prostaglandins : their disappearance from and release into the circulation. Nature 215 : 1237 - 1240.
- FILSHIE, GM; ANSTEY, MD (1978)
The distribution of arachidonic acid in plasma and tissues of patients near term undergoing elective or emergency caesarean section. Br J Obstet and Gynaecol 85 : 119 - 123.
- FUCHS, A R. (1983)
The role of oxytocin in parturition. Current topics in experimental endocrinology 4. Academic Press Inc. : 231 - 265.

FUCHS, AR; FUCHS, F. (1984)

Endocrinology of Human Parturition: a review. Br J Obstet Gynaecol 91 : 948 - 967.

FUCHS, AR; HUSSLEIN, P; FUCHS, F. (1981)

Oxytocin and the initiation of human parturition.
II : Stimulation of prostaglandin production in human decidua by oxytocin. Am J Obstet Gynaecol 141 : 694 - 697.

FUCHS, F. (1983)

Endocrinology of Parturition. In Endocrinology of Pregnancy
Ed Fuchs, F. and Klopper A. Harper and Row Publishers,
Philadelphia 247 - 270.

GIBB, DMF; ARULKUMARAN, S; LUN, KC; RATNAM S.S. (1984)

Characteristics of uterine activity in nulliparous labour.
Br J Obstet Gynaecol Vol 91: 220 - 227.

GORDON, AJ. (1984)

Measurement of uterine activity - a useful clinical tool?
Br J Obstet Gynaecol 91 : 209 - 210.

GRANSTROM, E. (1979)

Aspects on measurements of prostaglandin and thromboxanes.
Ann of Clin Biochem 16: 354 - 358.

GREEN, K; BYGDEMAN, M; TOPPOZADA, M; WIKQUIST, N. (1974)

The role of $\text{PGF}_{2\alpha}$ in human parturition. Am. J Obstet Gynaecol 120: 25 - 31.

GRIEVES, SA; LIGGINS GC. (1976)

Phospholipase A activity in human and ovine uterine tissues.
Prostaglandins 12: 229 - 241.

HANSEN, SH. (1976)

15 Hydroxy-prostaglandin dehydrogenase. Prostaglandins 12 :
647 - 679.

HARPER, CML; THORBURN, GD. (1984)

Inhibition of prostaglandin synthesis. Acute reduction in inhibitory activity during late gestation. Can J Physiol Pharmacol 62 : 1152 - 1157.

HARPER, MJK; KHODR, GS; VALENZUELA, G. (1983)

Prostaglandin production by human term placentas in vitro. Prostaglandin Leukotrienes Med 11 : 121 - 129.

HEALY, DL; HODGEN, GD. (1983)

The Endocrinology of the human endometrium. Obstet Gynaecol Survey 38: 509 - 530.

HENDRICKSE, W; STAMMERS, JP; HULL, D. (1985)

The transfer of free fatty acids across the human placenta. Br J Obstet Gynaecol 92: 945 - 952.

HERTELENDY, F; WOODS, R; JAFFE, BM. (1973)

Prostaglandin E levels in peripheral blood during labour. Prostaglandins 3: 223 - 227.

HIBBARD, BM; SHARMA, SC; FITZPATRICK, RJ; HAMLET JD. (1974)

Prostaglandin $F_{2\alpha}$ concentrations in amniotic fluid in late pregnancy. J Obstet Gynaecol Br Commonw 81: 35 - 38.

HILLIER, K; CALDER, AA; EMBREY, MP. (1974)

Concentrations of $PGF_{2\alpha}$ in amniotic fluid and plasma in spontaneous and induced labours. J Obstet Gynaecol Br Commonw 81: 257 - 263.

HUSSLEIN, P; SINZINGER, H. (1984)

Concentration of 13; 14 dihydro-15-keto PGE_2 in the maternal peripheral plasma during labour of spontaneous onset. Br J Obstet Gynaecol 91: 228 - 231.

HUSZAR, G. (1981)

Biology and biochemistry of myometrial contractility and cervical maturity. Sem Perinatol 5: 216 - 235.

IRVINE, RF. (1982)

How is the level of free arachidonic acid controlled in mammalian cells. *Biochem J* 204: 3 - 16.

JARABAK, J. (1980)

Early steps in prostaglandin metabolism in the human placenta. *Am J Obstet Gynaecol* 138: 534 - 540.

KARIM, SMM. (1966)

Identification of prostaglandins in human amniotic fluid. *J Obstet Gynaecol Br Commonw* 73: 903 - 908.

KARIM, SMM. (1968)

Appearance of prostaglandin $F_{2\alpha}$ in human blood during labour. *Br Med J* 4: 618 - 621.

KARIM, SMM; DEVLIN, J. (1967)

Prostaglandin content of amniotic fluid during pregnancy and labour. *J Obstet Gynaecol Br Commonw* 74: 230 - 234.

KARIM, SMM; HILLIER, K. (1979)

Prostaglandins in the control of animal and human reproduction. *Br Med Bulletin* 35: 173 - 180.

KEIRSE, MNJC. (1979)

Endogenous prostaglandin in human parturition. In Human Parturition. Ed Keirse MNJC, Anderson ABM, Bennebroek G, Leyden University Press 101 - 142.

KEIRSE, MJNC; ERWICH, JJHM; KLOK, G. (1985)

Increase in placental 15 hydroxy-prostaglandin dehydrogenase in the first half of human pregnancy. *Prostaglandin* 30 131 - 152.

KEIRSE, MJNC; FLINT, APF; TURNBULL, AC. (1974)

F prostaglandins in amniotic fluid during pregnancy and labour. *J Obstet Gynaecol Br Commonw* 81: 131 - 135.

KEIRSE, MJNC; HICKS, BR; TURNBULL, AC. (1976)

Prostaglandin dehydrogenase in the placenta before and after the onset of labour. Br J Obstet Gynaecol 83: 152 - 155.

KEIRSE, MJNC; HICKS, BR; MITCHELL, MD; TURNBULL, AC. (1977)

Increase of the prostaglandin precursor, arachidonic acid, in amniotic fluid during spontaneous labour. Br J Obstet Gynaecol 84: 937 - 940.

KEIRSE, MJNC; MITCHELL, MD; TURNBULL, AC. (1977)

Changes in prostaglandin F and 13, 14 dihydro 15-keto PGF concentrations in amniotic fluid at the onset and during labour. Br J Obstet Gynaecol 84: 743 - 746.

KEIRSE, MJNC; TURNBULL, AC. (1973)

E Prostaglandins in amniotic fluid during late pregnancy and labour. Br J Obstet Gynaecol Br Commonw 80: 970 - 973.

KEIRSE, MJNC; TURNBULL, AC. (1975)

Metabolism of prostaglandins within the pregnant uterus. Br J Obstet Gynaecol 82: 887 - 893.

KEIRSE, MJNC; TURNBULL, AC. (1976)

The fetal membranes as a possible source of amniotic fluid prostaglandins. Br J Obstet Gynaecol 83: 146 - 151.

KEIRSE, MJNC; WILLIAMSON, JG; TURNBULL, AC. (1975)

Metabolism of prostaglandin $F_{2\alpha}$ within the human uterus in early pregnancy. Br J Obstet Gynaecol 82: 142 - 145.

KINOSHITA, K; SATOH, K; SAKAMOTO, S. (1977)

Prostaglandin $F_{2\alpha}$ and E_1 in plasma and amniotic fluid during human pregnancy and labour. Endocrinol Japon 24: 155 - 162.

KINOSHITA, K; SATOH, K; SAKAMOTO, S. (1977)

Biosynthesis of prostaglandin in human decidua, amnion, chorion and villi. Endocrinol Japon 24: 343 - 350.

- KINOSHITA, K; SATOH, K; SAKAMOTO, S. (1984)
Human amniotic membrane and prostaglandin biosynthesis.
Biol Res Preg 5: 61 - 67.
- KUNZE, H; VOGT, W. (1971)
Significance of Phospholipase A for prostaglandin formation.
Ann N Y Acad Sci 180 : 123 - 125.
- KURZOK, R; LIB, CC. (1930)
Quoted by Crawford, MA. Background to essential fatty acids
and their prostanoid derivatives. Br Med Bulletin (1983) 39
: 210 - 213.
- LANDS, WEM. (1979)
The biosynthesis and metabolism of prostaglandins. Ann Rev
Physiol 41: 633 - 652.
- LANDS, WEM. (1981)
Control of prostaglandin biosynthesis. Prog Lipid Res 20:
875 - 883.
- LANDS, WE; MERKEL, I. (1963)
Metabolism of glycerolipids III. Reactivity of various acyl
esters of co-enzyme A with Alpha 1 - acyl-
glycerophosphorylcholine, and positional specificities in
lecithin synthesis. J Biol chem 238 : 898 - 904.
- LIGGINS, GC. (1972)
Fetal participation in the physiological controlling
mechanisms of parturition. Fetal and neonatal physiology :
Proceedings of the Sir Joseph Bercroft Centenary Symposium.
Cambridge University Press.
- LIGGINS, GC. (1979)
Initiation of parturition. Br Med Bulletin 35: 145 - 150.
- LIGGINS, GC; FAIRCLOUGH, RJ; GRIEVES, SA; KENDALL, JZ; KNOX, BS. (1973)
The mechanism of initiation of parturition in the ewe.
Recent Prog Horm Res 29: 111 - 159.

- LIGGINS, GC; FORSTER, CS; GRIEVES, SA; SCHWARTZ, AL. (1977)
Control of parturition in man. Biol Reprod 16: 39 - 56.
- LUNDSTROM, V; GREEN, K; WIKVIST, M. (1976)
Prostaglandins, indomethacin and dysmenorrhoea.
Prostaglandins 11: 893 - 904.
- MACDONALD, PC; PORTER, JC; SCHWARZ, BE; JOHNSTON, JM. (1978)
Initiation of parturition in the human female. Sem Perinatol
2: 273 - 286.
- MACDONALD, PC; SCHULTZ, FM; DUENHOELTER, JJ; GANT, NF; JIMENEZ, JM;
PRITCHARD, JA; PORTER, JC; JOHNSTON, JM. (1974)
Initiation of human parturition: I : Mechanism of action of
arachidonic acid. Obstet Gynaecol. 44: 629 - 635.
- MANZAI, M; LIGGINS, GC. (1984)
Inhibitory effects of dispersed human amnion cells on
production rates of prostaglandin E and F by endometrial
cells. Prostaglandins 28: 297 - 307.
- MITCHELL MD. (1981)
Prostaglandins during pregnancy and the perinatal period.
J Reprod Fert 62: 305 - 315.
- MITCHELL, MD. (1984)
The mechanism(s) of human parturition. J Develop Physiol 6:
107 - 118.
- MITCHELL, MD. (1986)
Pathways of arachidonic acid metabolism with specific
application to the fetus and mother. Sem Perinatol 10 : 242
- 254.
- MITCHELL, MD; BIBBY, J; HICKS, BR; TURNBULL, AC. (1978)
Specific production of prostaglandin E by human amnion
in vitro. Prostaglandins 15: 377 - 382.

- MITCHELL, MD; BIBBY, J; HICKS, BR; TURNBULL, AC. (1978)
Possible role for prostacyclin in human parturition.
Prostaglandins 16: 931 - 937.
- MITCHELL, MD; BRUNT, J; BIBBY, J; FLINT, APF; ANDERSON, ABM; TURNBULL, AC. (1978)
Prostaglandins in the human umbilical circulation at birth.
J Obstet Gynaecol 85: 114 - 118.
- MITCHELL, MD; ELLWOOD, DA; ANDERSON, ABM; TURNBULL, AC. (1978)
Elevated concentrations of 6 keto PGF_{1α} in fetal and maternal plasma and in amniotic fluid during ovine parturition. Prostaglandins Med 1: 265 - 266.
- MITCHELL, MD; FLINT, APF. (1978)
Prostaglandin production by intra-uterine tissue from periparturient sheep : Use of a superfusion technique.
J Endocrinol 76 : 111 - 121.
- MITCHELL, MD; FLINT, APF; BIBBY, J; BRUNT, J; ARNOLD, JM; ANDERSON, ABM; TURNBULL, AC. (1977)
Rapid increase in plasma prostaglandin concentrations after vaginal examination and amniotomy. B Med J 2 : 1183 - 1185.
- MITCHELL, MD; FLINT, AP; BIBBY, J; BRUNT, J; ARNOLD, JM; ANDERSON, ABM; ALEXANDER, C; TURNBULL, AC. (1978)
Plasma concentrations of prostaglandins during late human pregnancy. Influence of normal and preterm labour. J Clin Endocrinol Metab 46 : 947 - 951.
- MITCHELL, MD; KEIRSE, MNJC; ANDERSON, ANNE BM; TURNBULL, AC. (1977)
Evidence for a local control of prostaglandins within the pregnant human uterus. Br J Obstet Gynaecol 84 : 35 - 38.
- MITCHELL, MD; KEIRSE, MNJC; ANDERSON, ABM; TURNBULL, AC. (1978)
Thromboxane B₂ in amniotic fluid before and during labour.
Br J Obstet Gynaecol 85 : 442 - 445.

- MITCHELL, MD; KEIRSE, MJNC; BRUNT, JD; ANDERSON, ABM; TURNBULL, AC.
(1979)
Concentrations of the prostacyclin metabolite 6 keto
prostaglandin $F_{1\alpha}$ in amniotic fluid during late pregnancy and
labour. Br J Obstet Gynaecol 86 : 350 - 353.
- MITCHELL, MD; MACDONALD, PC; CASEY, ML. (1984)
Stimulation of prostaglandin E_2 synthesis in human amnion
cells maintained in monolayer culture by a substance(s) in
amniotic fluid. Prostaglandins Leukotrienes Med 15 : 399 -
407.
- MORRISON, WR; SMITH, LM. (1964)
Preparation of fatty and methyl esters and diethyl acetals
from lipids with boron trifluoride - methanol.
J Lipid Res 5 : 600 - 608.
- MORTIMER, G; HUNTER, IC; STIMSON, WH; GOVAN, ADT. (1985)
A role for amniotic epithelium in the control of human
parturition. Lancet i 1074 - 1075.
- NATHANIELSZ, PW. (1978)
Endocrine mechanism of parturition. Ann Rev Physiol 40 : 441
- 445.
- NORMAN, RJ. (1983)
The twin pregnancy as a model for studies in the
endocrinology of parturition and development. M.D. Thesis.
University of Natal.
- NORMAN, RJ; BREDENKAMP, BLF; JOUBERT, SM; BEETAR, C. (1981)
Fetal prostaglandins levels in twin pregnancies.
Prostaglandins Med 6: 309 - 316.
- NORMAN, RJ; JOUBERT, SM. (1982)
Fetal urinary prostaglandins. Prostaglandins, Leukotrienes
Med 8 : 171 - 172.

- NORMAN, RJ; JOUBERT, SM; MARIVATE, M. (1982)
Amniotic fluid phospholipids and glucocorticoids in twin pregnancy. *Br J Obstet Gynaecol* 90 : 51 - 55.
- NORMAN, RJ; MARTIN, R; MARIVATE, M. (1982)
Maternal prostaglandin levels after vaginal examination in twins. *S A Med J* 61 : 833 - 834.
- NUGTEREN, DH; HAZELHOF, E. (1973)
Isolation and properties of intermediates in prostaglandin biosynthesis. *Biochem Biophys Acta* 326 : 446 - 481.
- OGBURN, PL; JOHNSON, SB; WILLIAMS, PP; HOLMAN, RT. (1980)
Levels of free fatty acids and arachidonic acid in pregnancy and labour. *J Lab Clin Med* 95 : 943 - 949.
- OGINO, N; MIYAMOTO, T; YAMAMOTO, S; HAYAISHI, O. (1977)
Prostaglandin endoperoxide E isomerase from bovine vesicular gland microsomes, a glutathione requiring enzyme. *J Biolog Chem* 252 : 890 - 895.
- OKAZAKI, T; CASEY, ML; OKITA, JR; MACDONALD, PC; JOHNSTON, JM. (1981)
Initiation of human parturition. XII. Biosynthesis and metabolism of prostaglandins in human fetal membranes and uterine decidua. *Am J Obstet Gynaecol* 139 : 373 - 381.
- OKAZAKI, T; OKITA, JR; MACDONALD, PC; JOHNSTON, MJ. (1978)
Initiation of human parturition. X : Substrate specificity of phospholipase A₂ in human fetal membranes. *Am J Obstet Gynaecol* 130 : 432 - 438.
- OKAZAKI, T; SAGAWA, N; OKITA, JR; BLEASDALE, JE; MACDONALD, PC; JOHNSTON, JM. (1981)
Diacylglycerol metabolism and arachidonic acid release in human fetal membranes and decidua vera. *J Biolog Chem* 256 : 7316 - 7321.

OKITA, JR; JOHNSTON, JM; MACDONALD, PC. (1983)

Source of prostaglandin precursor in human fetal membranes. Arachidonic acid content of amnion and chorion laeve in diamnionic dichorionic twin placentas. Am J Obstet Gynaecol 147 : 477 - 482.

OKITA, JR; MACDONALD, PC; JOHNSTON, JM. (1982)

Initiation of human parturition. XIV : Increase in the diacylglycerol content of amnion during parturition. Am J Obstet Gynaecol 142 : 432 - 435.

OKITA, JR; MACDONALD, PC; JOHNSTON, JM. (1982)

Mobilization of AA from specific glycerophospholipids of human fetal membranes during early labour. J Biol Chem 257 : 14029 - 14034.

OLSON, DM; OPAVSKY, MA; CHALLIS, JRG. (1983)

Prostaglandin synthesis by human amnion is dependent on extracellular calcium. Can J Physiol Pharmacol 61 : 1089 - 1092.

OLSON, DM; SKINNER, K; CHALLIS JRG. (1983)

Prostaglandin output in relation to parturition by cells dispersed from human intrauterine tissues. J Clin Endocrinol Metab 57 : 694 - 699.

PEARSON MURPHY, BE. (1981)

Demonstration of novel compounds in human fetal tissues and a consideration of their possible roles in parturition. Am J Obstet Gynaecol 139 : 353 - 358.

PEEK, MJ; FRASER, JS; PHILLIPS, CA; RESTA, TM; BLACKWELL, PM; MARKHAM, R. (1985)

The measurement of human endometrial prostaglandin production. A comparison of two in vitro methods. Prostaglandins 29 : 3 - 18.

- PHILPOTT, RH. (1972)
Graphic records in labour. Br Med J IV : 163 - 165.
- PIPER, PJ; VANE, JK. (1969)
Release of additional factors in anaphylaxis and its
antagonism by anti inflammatory drugs. Nature 223 : 29 - 35.
- RAMWELL, PW; FOEGH, M; LOEB, R; LEONEY, EMK. (1980)
Synthesis and metabolism of prostaglandins, prostacyclin and
thromboxanes. The arachidonic acid cascade. Sem Perinatol
4 : 3 - 13.
- SAEED, SA; MACDONALD-GIBSON, WJ; GUTHBERT, J; COPAS, JL; SCHNEIDER, C;
GARDINER, PG; BUTT, NM; COLLIER, HOJ. (1977)
Endogenous inhibitor of prostaglandin synthetase. Nature
270 : 32 - 36.
- SAEED, SA; MITCHELL, MD. (1982)
Inhibition of prostaglandin biosynthesis by human low density
and high density lipoproteins. Prostaglandins Leukotrienes
Med 8 : 185 - 189.
- SAEED, SA; MITCHELL, MD. (1982)
Formation of arachidonate lipoxygenase metabolites by human
fetal membranes, uterine decidua vera and placenta.
Prostaglandins Leukotrienes Med 8 : 635 - 640.
- SAEED, SA; MITCHELL, MD. (1982)
Stimulants of prostaglandin biosynthesis in the human fetal
membranes, uterine decidua vera and placenta. Prostaglandins
24 : 475 - 585.
- SAEED, SA; STRICKLAND, DM; YOUNG, DC; DANG, A; MITCHELL, MD. (1982)
Inhibition of prostaglandin synthesis by human amniotic
fluid. Acute reduction in inhibitory activity of amniotic
fluid obtained during labour. J Clin Endocrinol Metab 55 :
801 - 803.

- SAGAWA, N; OKAZAKI, T; MACDONALD, PC. (1982)
Regulation of diacylglycerol metabolism and arachidonic acid release in human amniotic tissue. J Biol Chem 257 : 8158.
- SALMON, JA; AMY, JJ. (1973)
Levels of prostaglandin $F_{2\alpha}$ in amniotic fluid during pregnancy and labour. Prostaglandins 4 : 523 - 533.
- SAMUELSSON, B; GOLDYNE, M; GRANSTROM, E; HAMBERG, M; HAMMARSTROM, S; MALMSTEN, C. (1978)
Prostaglandins and Thromboxanes. Ann Rev Biochem 47 : 997 - 1029.
- SAMUELSON, B; GRANSTROM, E; GREEN, K. (1975)
Prostaglandins. Ann Rev Biochem 44 : 669.
- SATOH, H; HIRATO, K; TAHARA, R; OGAWA, K; NOGUCHI, Y; YANAIHARA, T; NAKAYAMA, T. (1984)
Enhancement of human amniotic phospholipase A_2 activity by steroid - sulphate derived from the feto placental unit. Acta. Endocrinol 107 : 420 - 424.
- SATOH, K; YASUMIZU, T; FUKUOKA, H; KINOSHITA, K; KANEKO, Y; TSUCHIYA, M; SAKAMOTO, S. (1979)
Prostaglandin $F_{2\alpha}$ metabolite levels in plasma, amniotic fluid, and urine during pregnancy and labour. Am J Obstet Gynaecol 133 : 886 - 890.
- SATOH, K; YASUMIZU, T; KAWAI, Y; OKAZI, A; WU, T; KINOSHITA, K; SAKAMOTO, S. (1981)
In vitro production of prostaglandins E, F and 6 keto prostaglandin $F_{1\alpha}$ by human pregnant uterus, decidua and amnion. Prostaglandins Med 6 : 359 - 368.
- SCHULTZ, MF; SCHWARZ, BE; MACDONALD, PC, JOHNSTON, JM. (1975)
Initiation of human parturition. II. Identification of phospholipase A_2 in fetal chorioamnion and uterine decidua. Am J Obstet Gynaecol 123 : 650 - 653.

- SCHWARZ, BE, SCHULTZ, MF; MACDONALD, PC; JOHNSTON, JM. (1975)
Initiation of human parturition. III. Fetal membrane content of prostaglandin E_2 and $F_{2\alpha}$ precursor. *Obstet Gynaecol* **46** : 564 - 568.
- SCHWARZ, BE, SCHULTZ, MF; MACDONALD, PC; JOHNSTON, JM. (1976)
Initiation of human parturition IV. Demonstration of phospholipase in the lysosome of human fetal membranes. *Am J Obstet Gynaecol* **125** : 1089 - 1092.
- SELLERS, SM; HODGSON, HT; MITCHELL, MD; ANDERSON, ABM; TURNBULL, AC. (1982)
Raised prostaglandin levels in the 3rd trimester of pregnancy. *Am J Obstet Gynaecol* **144** : 209 - 212.
- SELLERS, SM; MITCHELL, MD; BIBBY, JG; ANDERSON, ABM; TURNBULL, AC. (1981)
A comparison of plasma prostaglandin levels in term and preterm labour. *Br J Obstet Gynaecol* **88** : 362 - 366.
- SELLERS, SM; MITCHELL, MD; ANDERSON, ABM; TURNBULL, AC. (1984)
The influence of spontaneous and induced labour on the rise in prostaglandins at amniotomy. *Br J Obstet Gynaecol* **91** : 849 - 852.
- SKINNER, SJM; LIGGINS, GC; WILSON, T; NEALE, G. (1984)
Synthesis of prostaglandin F by cultured human endometrial cells. *Prostaglandins* **27** : 821 - 839.
- SKINNER, KA; CHALLIS, JRG. (1985)
Changes in the synthesis and metabolism of prostaglandins by human fetal membranes and decidua at labour. *Am J Obstet Gynaecol* **151** : 519 - 523.
- STEER, PJ; CARTER, MC; BEARD, RW. (1984)
Normal levels of active contraction area in spontaneous labour. *Br J Obstet Gynaecol* **91** : 211 - 219.

STEER, PJ; CARTER, MC; BEARD, RW. (1985)

The effect of oxytocin infusion on uterine activity levels in slow labour. Br J Obstet Gynaecol 92 : 1120 - 1126.

STEER, PJ; CARTER, MC; CHOONG, K; HANSON, M; GORDAN, AJ; PRADHAN, P. (1985)

A multicentre prospective randomized controlled trial of induction of labour with an automatic closed loop feedback controlled oxytocin infusion system. Br J Obstet Gynaecol 92 : 1127 - 1133.

STEER, PJ; CARTER, MC; GORDAN, AJ; BEARD, RW. (1978)

The use of the catheter tip pressure transducers for measurement of intrauterine pressure in labour. Br J Obstet Gynaecol 85 : 561 - 566.

STRICKLAND, DM; BRENNECKE, SP; MITCHELL, MD. (1982)

Measurement of 13, 14-dehydro-15 keto prostaglandin $F_{2\alpha}$ and 6 keto prostaglandin $F_{1\alpha}$ in plasma by radioimmunoassay without prior extraction or chromatography.

Prostaglandin, Leukotrienes Med 9 : 491 - 493.

THORBURN, GD; CHALLIS, JRG. (1979)

Endocrine Control of parturition. Physiol Rev 59 : 863 - 916.

THORBURN, GD; CHALLIS, JRG; ROBINSON, JS. (1977)

The endocrinology of parturition. In Cellular Biology of the Uterus. Ed: Wynn R.M. Plenum Press New York 653.

TURNBULL, AC. (1957)

Uterine contractions in normal and abnormal labour. J Obstet Gynaecol Br Emp 64 : 321 - 333.

TURNBULL, AC; LUCAS, A; MITCHELL, MD. (1987)

Prostaglandins in the perinatal period. Rev Perinatal Med 4 : Ed: Scarpelli E V Cosmi. Raven Press New York.

- TURNBULL, AC; MITCHELL, MD; ANDERSON, ABM. (1980)
Endocrine status at the end of pregnancy and the mechanism involved in the initiation of parturition. Serono Symp 35. "The Human Placenta" Ed : A Klopper; A Genazzani; P G Crosignani. Academic Press London and New York.
- VAN DORP, DA; BEERTHUIS, RK; NUGTEREN, DH; VONKEMAN, H. (1964)
The biosynthesis of prostaglandins. Biochim Biophys Acta 90 : 204 - 207.
- VANE, JR. (1969)
The release and fate of vasoactive hormones in the circulation. Br J Pharmacol 35 : 209 - 242.
- VON EULER U.S. (1936)
On the specific vasodilating and plain muscle stimulating substances from accessory genital glands in man and certain animals. J Physiol 88 : 213.
- WILLIAM, EA; COLLINS, WP. (1976)
Distribution of prostaglandin E_2 and $F_{2\alpha}$ within the feto placental unit throughout human pregnancy. J Endocrinol 69 : 413 - 419.
- WIKVIST, N. (1979)
The use of inhibitors of prostaglandin synthesis in obstetrics. In Human Parturition Ed: Keirse, MNJC; Anderson, ABM, Bennebroek, JG. Boerhauve Series, Leyden Universal Press 189 - 200.