The Involvement of Prostaglandins in Parturition and Implantation in the Rat

by

Christine Anne Phillips B.Sc.Hons

Thesis Presented for the Degree of Doctor of Philosophy

University of Edinburgh

December 1979

I declare that the work performed, as described and presented in this thesis is entirely my own.

To

Helen and Frank

ABSTRACT

The involvement of prostaglandins in the processes of parturition and implantation in the rat has been studied.

The release of prostaglandins and thromboxane from the term (day 22) pregnant rat uterus *in vitro* has been measured using radio-immuno-assay, gas chromatography combined with mass spectrometry and a platelet aggregation method. Prostaglandin I_2 and thromboxane A_2 (measured as their metabolites 6-oxo-prostaglandin $F_{1\alpha}$ and thromboxane B_2 respectively), were found to be released in large amounts, with prostaglandin $F_{2\alpha}$ and prostaglandin E_2 being released in smaller amounts. The compound released in by far the largest amounts was found to be prostaglandin D_2. Possible roles for these prostaglandins at parturition in the rat, are discussed.

The treatment of the day 22 pregnant rat uterus *in vitro*, with the prostaglandin I_2 synthesis inhibitors, 15-hydroperoxy arachidonic acid and tranylcypromine has been investigated. Both compounds caused a spasm of the tissue when added to the bathing fluid. 15-hydroperoxy arachidonic acid caused a dose-dependent increase in the release of prostaglandins, while tranylcypromine caused a fall in the release of prostaglandin E_2, but did not affect the release of other prostaglandins. Possible mechanisms for the effect of 15-hydroperoxy arachidonic acid on prostaglandin release from the term pregnant rat uterus, are discussed.

The production of prostaglandins by the early pseudopregnant and early pregnant rat uterus *in vitro* has been studied. 6-oxo-prostaglandin $F_{1\alpha}$ was the major product in both early pseudo-pregnancy and early pregnancy. Production of prostaglandin E_2, prostaglandin $F_{2\alpha}$ and 6-oxo-prostaglandin $F_{1\alpha}$ showed a peak on day 5 of pseudopregnancy. Prostaglandin E_2 and 6-oxo-prostaglandin $F_{1\alpha}$ only showed a peak on day 5 of pregnancy. Total prostaglandin production was higher during pregnancy than during pseudopregnancy.

Indomethacin treatment of rats during early pregnancy caused a delay in implantation. The indomethacin treatment also caused a significant reduction in uterine weight and in the number of implants per uterine horn, on day 9 of pregnancy compared to controls. Plasma progesterone levels were significantly lower on day 6 to 9 of pregnancy in indomethacin treated animals compared to controls. The possible roles of prostaglandins and the involvement of other factors, in implantation is discussed.

CONTENTS

		Page
ABSTRACT		
List of Abbreviations		
List of Prostaglandins and Thromboxanes and Metabolic Pathways of Arachidonic Acid		1
General Introduction		9
SECTION I:	Basic Methods	
	Composition of Solutions	29
	1. Solvent Extraction of Prostaglandins	30
	2. Recovery of Prostaglandins by Solvent Extraction from Krebs' Solution	30
	3. Extraction of Progesterone from Plasma Samples	31
	4. Radio-Immuno-Assays for the Measurement of Prostaglandins and Progesterone	33
	a. Prostaglandin $F_{2\alpha}$	33
	b. Prostaglandin E_2	34
	c. 6-oxo-Prostaglandin $F_{1\alpha}$	34
	d. Preparation of Samples for Assay of Prostaglandin by Radio-Immuno-Assay	37
	e. Radio-Immuno-Assay for Progesterone	37
	5. Purification of Tritiated Prostaglandin F_2 by Column Chromatography	43
	6. Derivitisation and Assay of Prostaglandins by Gas Chromatography-Mass Spectrometry using Multiple Ion Detection	45
	a. Instrument Details	45
	b. Assays for Prostaglandin D_2 and Thromboxane B_2	45
	i. Derivitisation	45
	ii. Construction of Standard Curves for Prostaglandin D_2 and Thromboxane B_2	45
	c. Assay for 6-oxo-Prostaglandin $F_{1\alpha}$ using the 3,3,4,4-tetradeuterated Compound as Internal Standard	46
	d. Preparation and Assay of Samples for Gas Chromatography-Mass Spectrometry	46
	7. Assay for Prostaglandin D_2 using a Method of Platelet Aggregation	50
SECTION II:	Prostaglandins, Thromboxanes and the Pregnant Rat Uterus at Term	
	Introduction	54
	Methods	
	1. The Release of Prostaglandins from the Day 22 Pregnant Rat Uterus <u>in vitro</u>	54

Page

2. The Effects of Prostaglandins on the Day 22 Pregnant Rat Uterus *in vitro* 55

3. The Effects of 15-hydroperoxy Arachidonic Acid and Tranylcypromine on the Day 22 Pregnant Rat Uterus *in vitro* 56

a. Preparation of 15-hydroperoxy Arachidonic Acid 56

b. Derivitisation of 15-hydroxy Arachidonic Acid for Gas Chromatography-Mass Spectrometry 57

c. The Effects of 15-hydroperoxy Arachidonic Acid and Tranylcypromine on the Day 22 Pregnant Rat Uterus *in vitro* 60

d. The Removal of 15-hydroperoxy Arachidonic Acid from Extracted Samples 60

e. To Determine the Elution of Prostaglandins by Fractions used in the Separation of 15-hydroperoxy Arachidonic Acid from Extracted Samples 61

Results

1. The Release of Prostaglandins from the Term Pregnant Rat Uterus *in vitro* 64

2. The Effects of Prostaglandins and Thromboxane on the Day 22 Pregnant Rat Uterus *in vitro* 64

3. The Effects of 15-hydroperoxy Arachidonic Acid and Tranylcypromine on the Day 22 Pregnant Rat Uterus *in vitro* 67

4. The Effects of 15-hydroperoxy Arachidonic Acid and Tranylcypromine on Prostaglandin Release 67

5. Release of Prostaglandins from the Day 22 Pregnant Rat Uterus *in vitro*, over Four Consecutive Periods of Fifteen Minutes 72

6. The Effects of Indomethacin on Prostaglandin Release from the Day 22 Pregnant Rat Uterus *in vitro* 74

Discussion 74

SECTION III: The Involvement of Prostaglandins in Implantation in the Rat

Introduction 84

Methods

1. Prostaglandin Synthesis by the Early Pseudopregnant and Early Pregnant Rat Uterus *in vitro* 84

a. Early Pseudopregnancy 84

b. Early Pregnancy 85

2. Measurement of Basal Uterine Tissue Levels of Prostaglandins in the Pregnant Rat 86

Page

3. Studies on the Metabolism of Prostaglandin $F_{2\alpha}$ by the Early Pregnant Rat Uterus *in vitro* 86

4. The Effects of Indomethacin Treatment on Early Pregnancy in the Rat 87

a. Effects on Implantation 87

b. Effects on Development of Early Pregnancy 88

Results

1. Uterine Prostaglandin Synthesis and Peripheral Plasma Progesterone Levels in the Early Pseudopregnant and Pregnant Rat *in vitro* 88

a. Early Pseudopregnancy 88

b. Early Pregnancy 91

c. A Comparison of the Values Obtained for Measurement of 6-oxo-Prostaglandin $F_{1\alpha}$ by Radio-Immuno-Assay and Gas Chromatography-Mass Spectrometry 91

2. Basal Tissue Levels of Prostaglandins During Early Pregnancy 92

3. Metabolism of Prostaglandin $F_{2\alpha}$ by Uterine Homogenates from Pregnant Rats *in vitro* 94

4. The Effects of Indomethacin on Early Pregnancy in the Rat 95

a. Effects on Implantation 95

b. Effects on the Development of Early Pregnancy 95

Discussion 96

CONCLUDING REMARKS 102

REFERENCES 107

SOURCES OF DRUGS AND CHEMICALS 115

ACKNOWLEDGEMENTS 116

List of Abbreviations

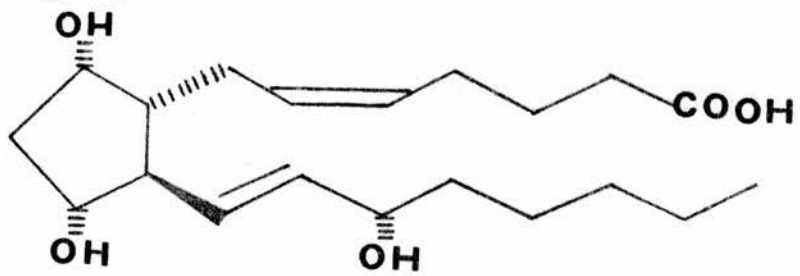
pg	picogramme
ng	nanogramme
mg	milligramme
g	gramme
kg	kilogramme
µl	microlitre
ml	millilitre
l	litre
µCi	microcurie
Ci	curie
min	minute(s)
hr	hour(s)
psi	pounds per square inch
µM	micromolar
M	molar
N	normal
xg	gravitational force
nm	nanometre
UV	ultra violet

List of Prostaglandins and Thromboxanes and Metabolic Pathways of Arachidonic Acid.

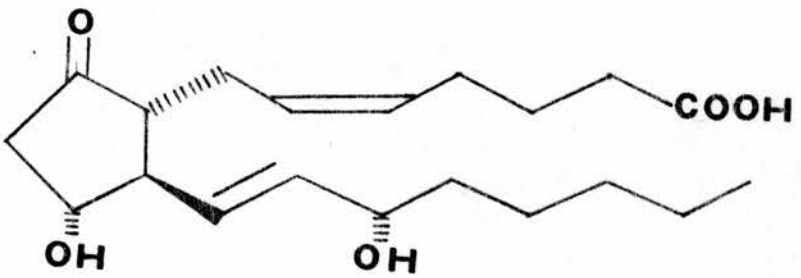
The following is a list of the prostaglandins and thromboxanes mentioned in the text of this thesis together with their structures. Structures of arachidonic acid, 15-hydroxyarachidonic acid and 15-hydroperoxyarachidonic acid, 8, 11, 14 eicosatrienoic acid and its corresponding 15-hydroxy and 15-hydroperoxy compounds are also given. Hatched lines indicate bond orientations below the plane of the paper, thickened lines, orientation above the plane of the paper. A wavy line indicates that the bond orientation is not known or can be in either plane.

The list is followed by Figs.1-4 representing the metabolic pathways of arachidonic acid. Fig.4 represents the metabolic pathway proposed recently by Jones, Kerry, Poyser, Walker and Wilson (1979) and Walker, Jones and Wilson (1979) for arachidonic acid by blood platelets.

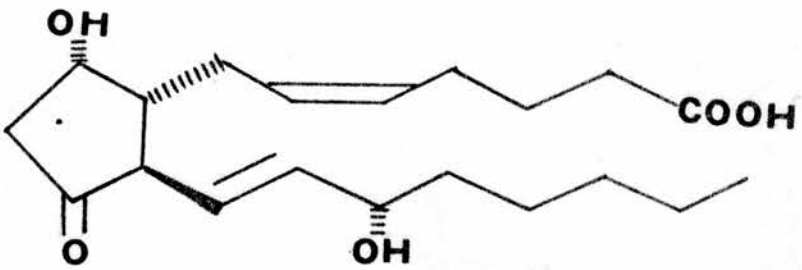
Prostaglandin $F_{2\alpha}$
$PGF_{2\alpha}$



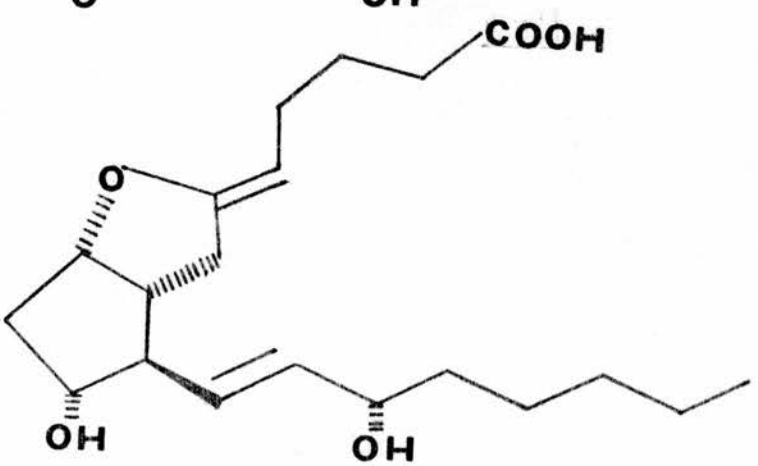
Prostaglandin E_2
PGE_2



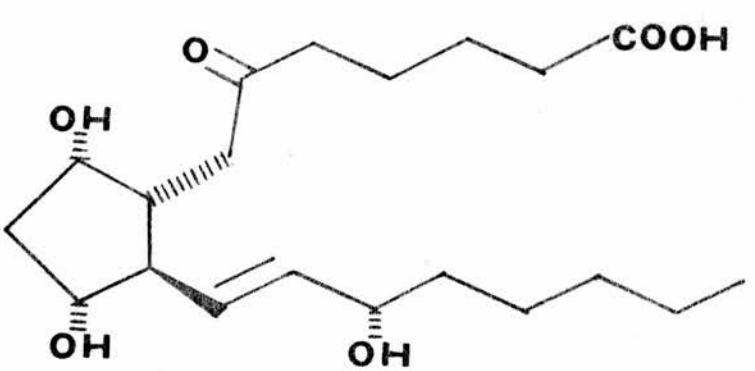
Prostaglandin D_2
PGD_2



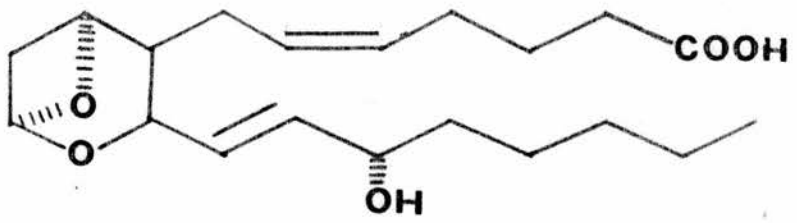
Prostacyclin
PGI_2



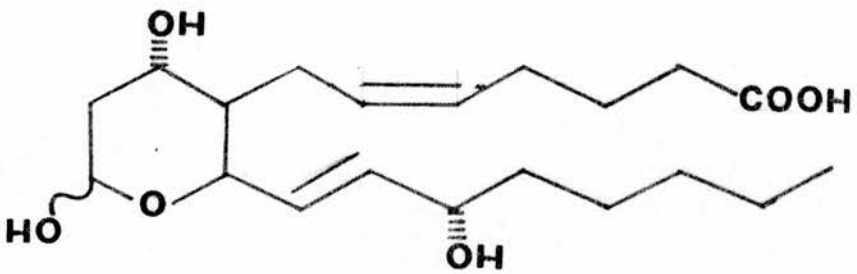
6-oxo-prostaglandin $F_{1\alpha}$
6-oxo-$PGF_{1\alpha}$



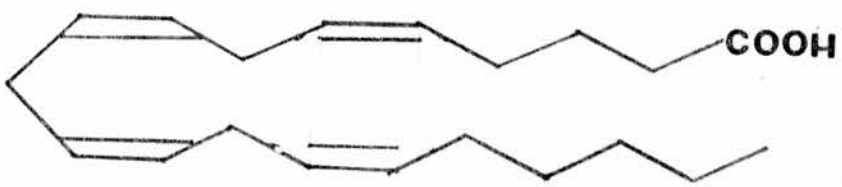
Thromboxane A_2
TXA_2



Thromboxane B_2
TXB_2

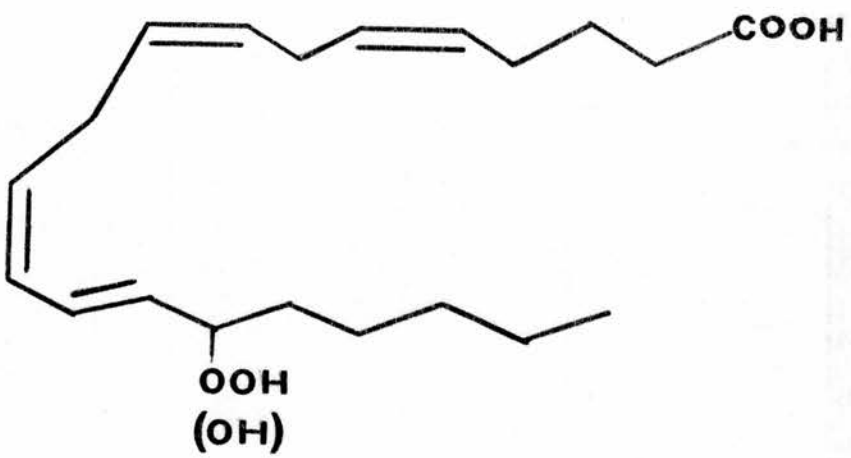


Arachidonic Acid



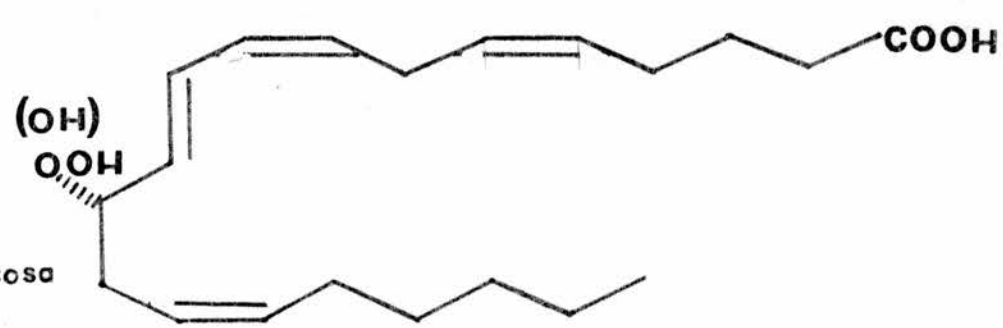
15-hydroperoxy-
(hydroxy)
arachidonic acid

15-OOH AA
(15-OH AA)

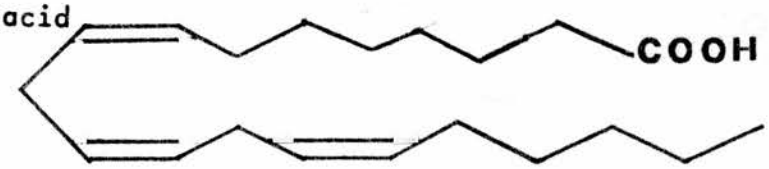


12-hydroperoxy-
(hydroxy)
5, 8, 10, 14 eicosa
tetranoic acid

HPETE
(HETE)

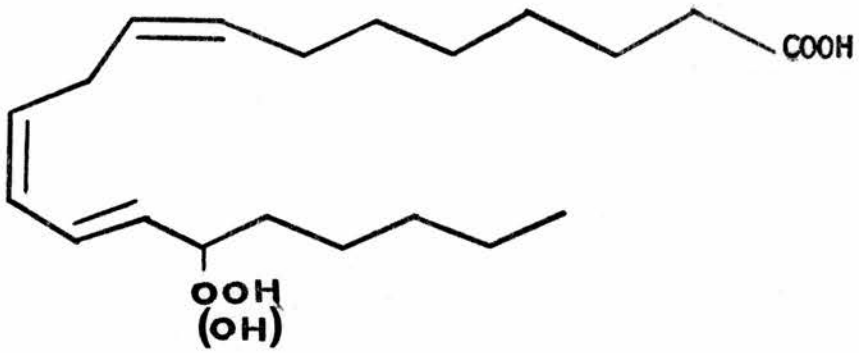


8,11,14 eicosatrienoic acid

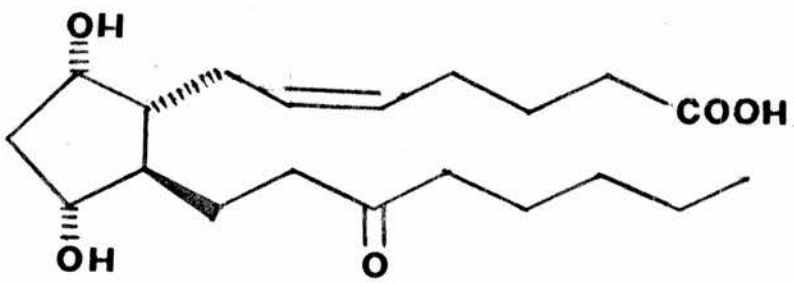


15-hydroperoxy-
(hydroxy)

8,11,13 eicosa-
trienoic acid

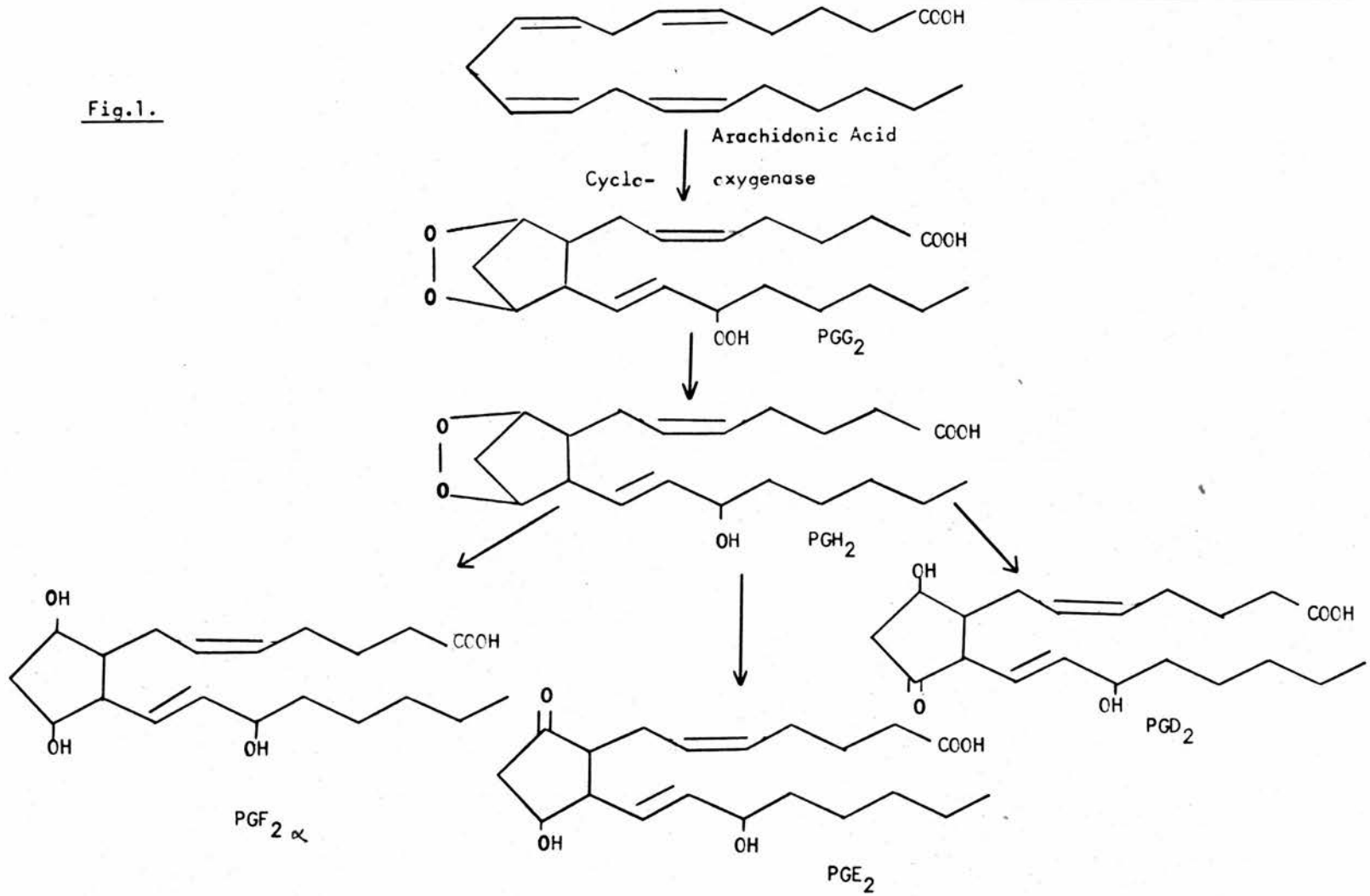


15-keto -13,14-dihydro
prostaglandin $F_{2\alpha}$



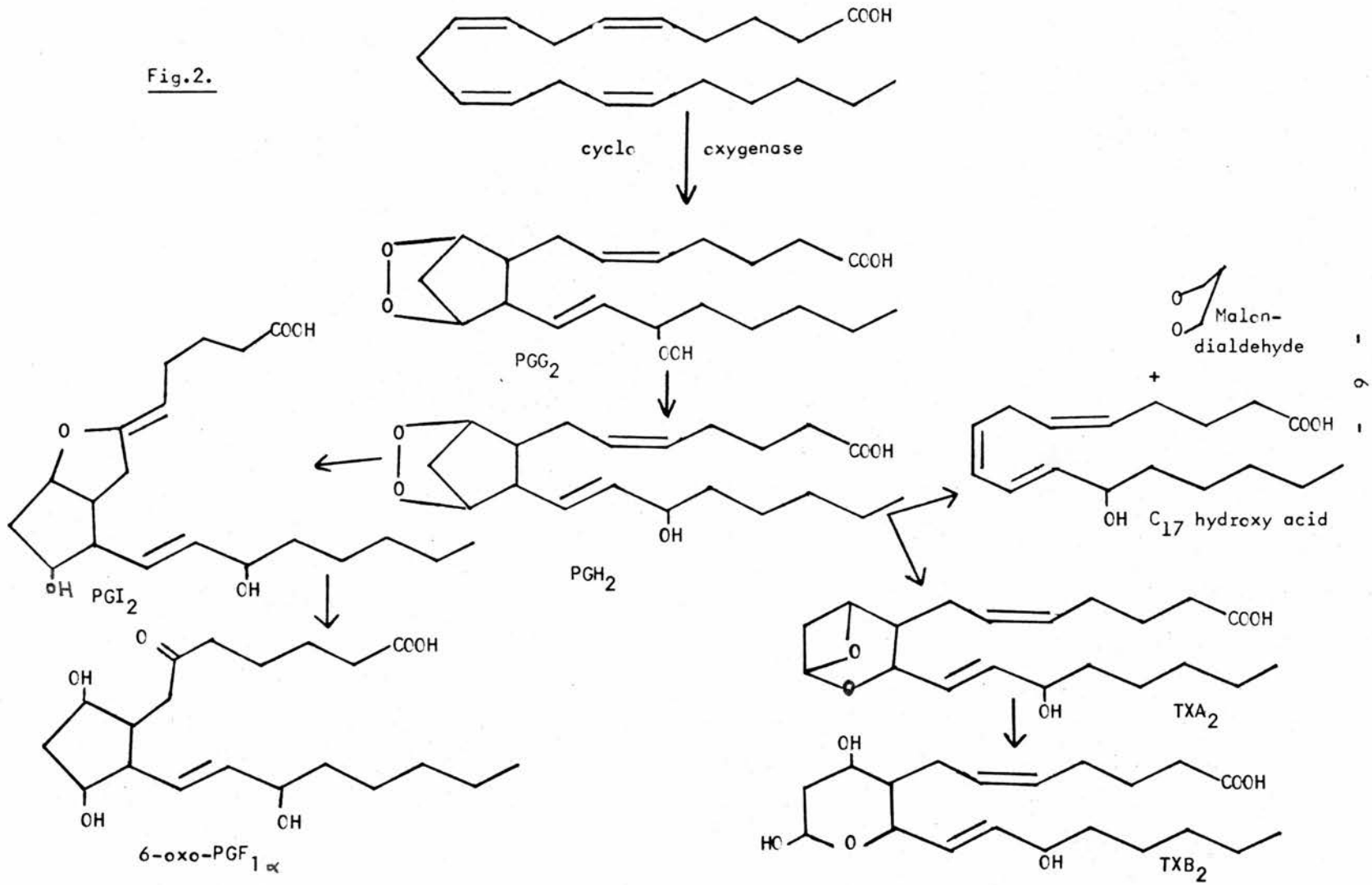
Metabolism of Arachidonic Acid into the "Classical" Prostaglandins i.e. $PGF_{2\alpha}$, PGE_2 and PGD_2

Fig.1.



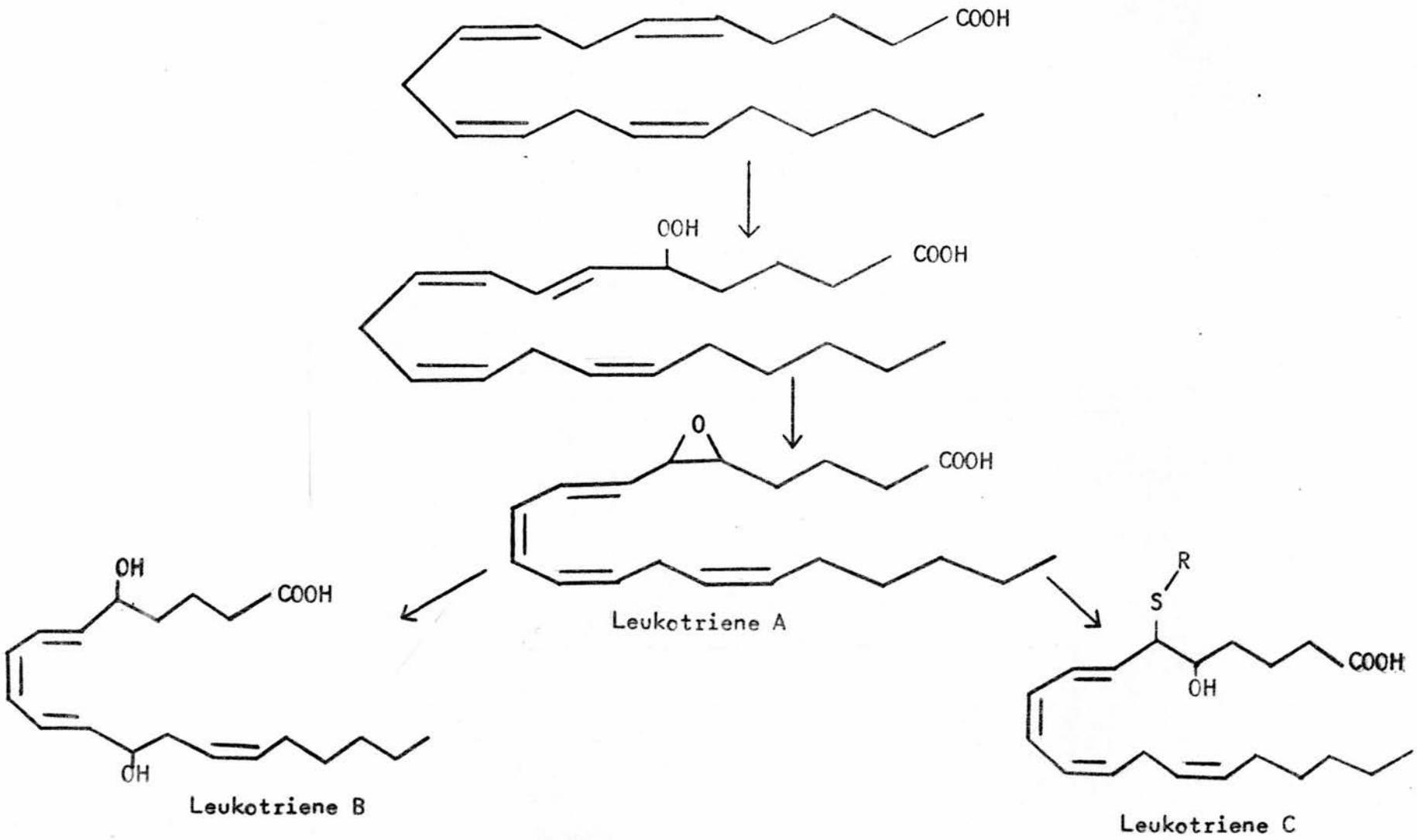
Metabolism of Arachidonic Acid into Prostacyclin and Thromboxane and their respective Major Metabolites

Fig.2.



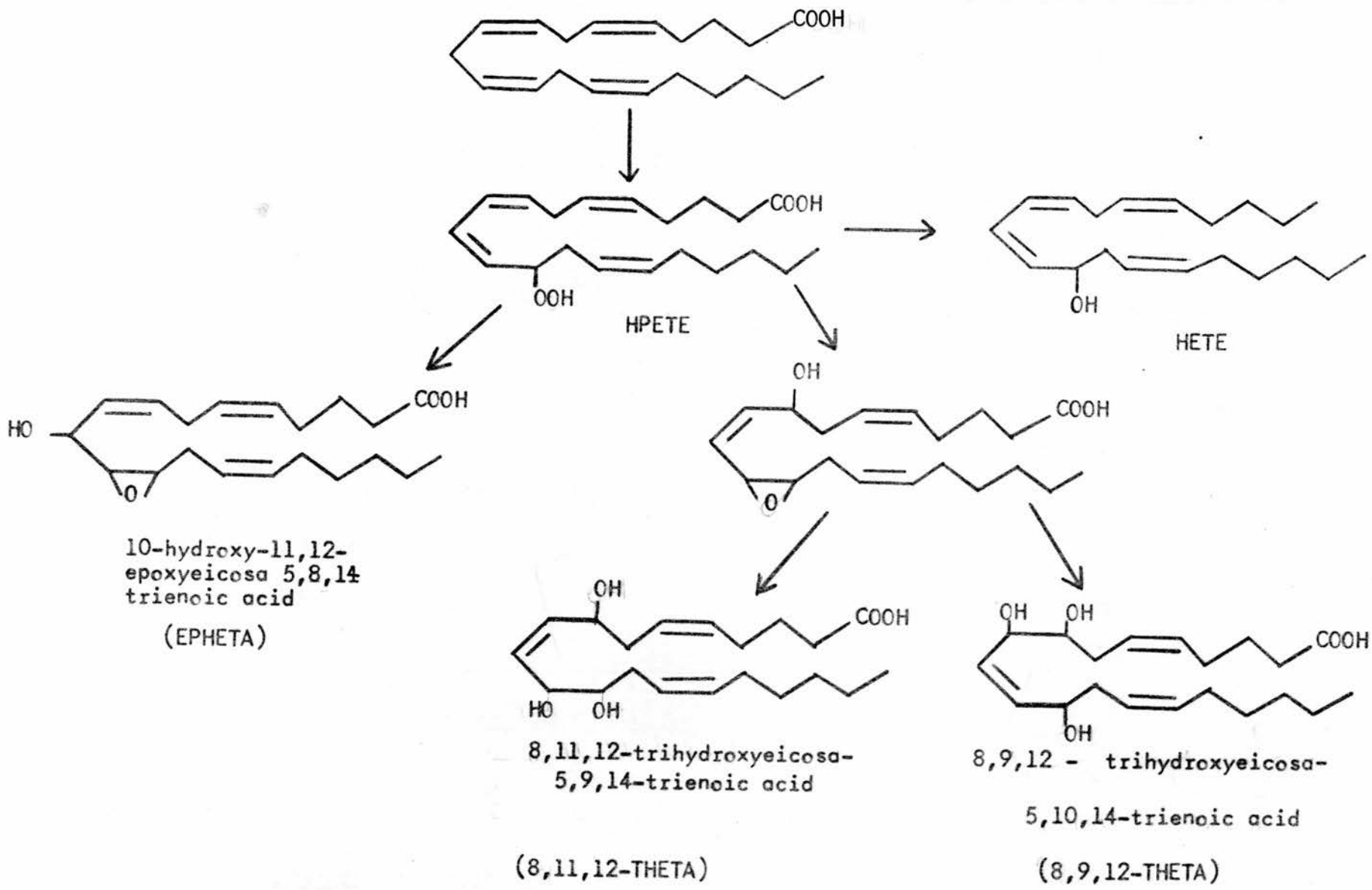
Metabolism of Arachidonic Acid into the Leukotrienes as proposed by Samuelsson (1980)

Fig.3.



Pathway of Arachidonic Acid Metabolism as proposed by Jones, Kerry, Poyser, Walker and Wilson (1979) and Walker Jones and Wilson (1979)

Fig.4



General Introduction

The involvement of prostaglandins in various reproductive processes, has been the subject of much discussion over recent years. The work reported in this thesis deals with their particular role(s) in the processes of parturition and implantation in the rat.

Karim (1966) was the first to observe increased levels of a substance, with properties similar to those of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) in the peripheral venous blood of women during labour. He also claimed that the highest concentrations were found one minute prior to and during a uterine contraction (Karim 1968), although subsequently Sharma, Hibbard, Hamlett and Fitzpatrick (1973) reported that the maximum concentrations occur after a contraction. Brummer (1972) has also reported that the concentration of F type prostaglandins in peripheral venous serum, increase during labour. These latter results must be treated with caution however, as the contribution of $PGF_{2\alpha}$ formed by platelets and blood cells during isolation of serum and also any possible non-enzymic cyclization of polyunsaturated fatty acids, is difficult to estimate. Salmon and Amy (1973) have found that $PGF_{2\alpha}$ levels, measured by radio-immunoassay (RIA) increase during labour in women, but Jouvenaz, Nugteren and Van Dorp (1973) were unable to detect $PGF_{2\alpha}$ by an electron capture method. The plasma levels of $PGF_{2\alpha}$ and its major metabolite 15-keto, 13,14-dihydro-$PGF_{2\alpha}$ (PGFM), have been measured in women by Green, Bygdeman, Toppozada and Wiquist (1974) prior to and during normal and oxytocin-induced labour, using the technique of gas chromatography combined with mass-spectrometry (GC-MS). They found that levels of PGFM were low during the last month of pregnancy but increased significantly over the 15 hr prior to parturition and showed a 10-30 times increase during active labour. The plasma levels did not vary significantly in relation to contractions, but were related to the degree of cervical dilation. Levels were found to fall after parturition. During oxytocin-induced labour, the PGFM levels were also high, but showed no relationship to the progress of labour. The authors did not confirm that peripheral levels of $PGF_{2\alpha}$ increased during labour in women, but suggest that the increase in the PGF metabolite levels reflect an increase in $PGF_{2\alpha}$ output from the uterus and that the $PGF_{2\alpha}$ is metabolised by the lungs before reaching the peripheral circulation. They further suggest that $PGF_{2\alpha}$ may

be important for the maintenance and acceleration of uterine activity during the active stage of labour. Karim and Devlin (1967) have previously proposed the decidua as the main site of prostaglandin production, but recent studies (Thorburn: In Press) have indicated that the amnion and decidua are the major sites of prostaglandin synthesis in women.

Prostaglandin F output from the uterus has been found to increase prior to and/or during parturition in the goat (Currie, Wong and Thorburn 1973), cow (Fairclough, Hunter and Welsh 1975) and sheep (Liggins and Grieves 1971). Peripheral plasma levels of $PGF_{2\alpha}$ in the rabbit tend to increase during the last week of gestation (Challis, Davies and Ryan 1973). Peripheral plasma levels of $PGF_{2\alpha}$ in the pregnant rhesus monkey are low and do not show any significant change near term (Challis, Robinson and Thorburn 1977). However, during the last 10-20 days before delivery there is a progressive increase in the concentration of $PGF_{2\alpha}$ and 15-keto,13,14-dihydro $PGF_{2\alpha}$ in samples of amniotic fluid (Challis, Hartley, Johnson, Patrick, Robinson and Thorburn 1977). Production of $PGF_{2\alpha}$ is thought to be by the decidua and amnion (Thorburn: In Press). Labhsetwar and Watson (1974) have shown that the level of $PGF_{2\alpha}$ in uterine venous blood from pregnant rats on days 21 and 22 are higher, compared to levels on days 18 and 20 (day 22 being the day of parturition). Shaikh, Naqvi, Saksena, (1977), observed a dramatic increase in uterine venous levels of $PGF_{2\alpha}$ on day 20 in rats compared to previous days of pregnancy. This was followed by a fall on day 21 and another large increase on day 22.

Less work has been performed to measure levels of prostaglandin E_2 (PGE_2) at term, although Heterlendy, Woods and Jaffe (1973) did report an increase in peripheral venous blood levels of PGE_2 during labour in women. Plasma levels of PGE_2 in the rhesus monkey did not show any changes near term, as was found for $PGF_{2\alpha}$ (Challis, Robinson and Thorburn (1977). Shaikh and Saksena (1977) found that in the rat PGE_2 showed the same pattern as $PGF_{2\alpha}$ in uterine venous blood i.e a sharp increase on day 20 followed by a fall on day 21 and a second rise on day 22 of pregnancy.

It is possible to delay the onset of parturition and to prolong its duration by the administration of drugs which inhibit prostaglandin synthesis. Indomethacin, administered to pregnant rabbits from day 20, prolonged the gestation period by two days (Challis, Davies and Ryan 1975). Gestation length is also increased in the hamster with indomethacin, given twice daily on days 14-16 of pregnancy. Injection of $PGF_{2\alpha}$ into these animals on day 15 was found to advance the onset of parturition (Lau, Saksena and Chang 1975). Neither indomethacin nor $PGF_{2\alpha}$ had any effect on the duration of parturition. In retrospective studies of pregnant women, aspirin-like drugs have been shown to produce increases in gestation length and the length of parturition (Lewis and Schulman 1973; Collins and Turner 1973).

In 1972 Aiken demonstrated that the oral administration of indomethacin and aspirin, to rats, over the last few days of gestation would lead to a prolonged parturition. Chester, Dukes, Slater and Walpole (1972), using a similar dose schedule of indomethacin to that used by Aiken, showed a delay in the onset of parturition. This also occurred with fenclozic acid, another prostaglandin (PG) synthetase inhibitor (Sykes and Maddox 1972), and naproxen also was found to prolong pregnancy and delay labour in the rat (Csapo, Csapo, Fay, Henzl and Salau 1973). Dunn, Humphries, Judkins, Kendall and Knight (1973), found that injection of an antibody to $PGF_{2\alpha}$ on day 17 of pregnancy in the rat, prolonged gestation by more than 24 hrs. All treated animals eventually delivered, but the excessive loss of blood and foetal mortality observed were similar to that found in animals treated with aspirin or indomethacin. It is clear, therefore, that $PGF_{2\alpha}$ and possibly PGE_2 are released from the pregnant uterus at term in several species and are necessary for the normal occurrence of parturition.

It would be useful at this point to consider the control (hormonal or otherwise) of prostaglandin production in the different species, at or near term.

In most mammalian species, the maintenance of pregnancy is dependent on progesterone production. In a number of species, e.g goat, cow, pig, rat, the maintenance of adequate progesterone levels is dependent on a functioning corpus luteum throught pregnancy,

and therefore delivery is dependent on luteal regression, since progesterone levels have to fall in these species if parturition is to take place. In the sheep, however, progesterone from the corpus luteum is only vital up to day 50 of pregnancy and from then on, placental production is adequate to maintain pregnancy. At term, the foetus modifies placental steroidogenesis to effect its own delivery. The guinea pig is only dependent on the corpus luteum for progesterone up to day 25 of pregnancy and progesterone levels do not fall prior to parturition in the guinea pig, which is similar to women in this feature and unlike other species (see Horton and Poyser 1976).

In the sheep, as in the goat, cow and pig, the onset of parturition depends on the presence of a foetal pituitary and an active foetal pituitary-adrenal axis (see Thorburn, Challis and Currie 1977 for review of evidence). During the last 10-15 days pre-partum in the sheep, there is a gradual rise in foetal plasma cortisol levels followed by a more rapid rise over the last 2-3 days of gestation (Thorburn, Nicol, Bassett, Shutt and Cox 1972). This is accompanied by a fall in maternal progesterone levels over the last 4 days of gestation (Bassett, Oxborrow, Smith and Thorburn 1969) and an increase in unconjugated oestrogen levels during the last 24 hr of pregnancy (Thorburn et al 1972; Challis 1971). The increase in foetal cortisol levels at term is apparently dependent on a trophic drive from the foetal pituitary and maturational changes in the foetal adrenals (Liggins 1973). Prolactin levels and foetal cortisol levels show a direct correlation during the last 30 days of gestation, suggesting prolactin may be important in promoting growth of the foetal adrenals and the stimulation of steroidogenesis, but the involvement of other pituitary hormones cannot be excluded. The PGE concentration in foetal plasma increases near term and there is evidence to suggest that PGE acts to augment the effects of ACTH on steroidogenesis in the foetal adrenal. PGE_2 may also stimulate release of pituitary hormones in the foetus (Lewis, Challis, Robinson and Thorburn 1976). The evidence suggests that the increase seen in foetal cortisol levels in sheep, is responsible for the fall in progesterone and rise in oestrogen seen in the maternal plasma. The rise in cortisol

induces the 17α -hydroxylase and C17-20 lyase enzymes in the placenta and thereby stimulates the formation of androgens from progesterone and pregnenolone. The androstenedione formed is converted into oestrogens by aromatase enzymes (Thorburn, Challis and Currie 1977). This mechanism explains the fall in maternal plasma progesterone before delivery and the increasing concentrations of 17α, 20 α - dihydroxy-pregn-4-en-3-one and androstenedione in the utero-ovarian vein (Liggins, Fairclough, Grieves, Forster and Knox 1977). These changes result in an increase in "free" oestradiol 17β in maternal plasma during the 24 hr prior to delivery. This elevation in oestrogen levels is associated with the increase in PGF levels in the utero-ovarian venous blood and the onset of active labour (Challis, Harrison, Heap, Horton and Poyser 1972; Thorburn et al 1972). It has been suggested that oestrogen acts on uterine oestrogen receptors to stimulate $PGF_{2\alpha}$ release as measured in the uterine vein (Thorburn and Challis 1978). After this initial release of $PGF_{2\alpha}$, there is a much larger release of $PGF_{2\alpha}$ during active labour. This is due to stretching of the cervix and vagina, which, via a nervous reflex releases oxytocin, which then acts back on the uterus to cause the second release of $PGF_{2\alpha}$ (Flint, Forsling and Mitchell 1978; Liggins et al 1977).

In the goat, cow and pig, the corpus luteum is the main site of progesterone synthesis throughout pregnancy (see Horton and Poyser 1976; Thorburn et al 1977). Regression of the corpus luteum and a consequent decline in plasma progesterone levels precedes the development of uterine activity and normal delivery, although in the pig, progesterone withdrawal is not complete at delivery (Robertson and King 1974). Any "message" from the foetus in these species must initially act to cause luteal regression. In the goat, there seems to be trophic support of the corpus luteum from the maternal pituitary and from the placenta, possibly by a placental lactogen, as this hormone appears in the maternal circulation around day 60 and increases up to day 125 of pregnancy (Currie and Thorburn 1977). Levels tend to fall progressively over the last 15 days of pregnancy. It may be that the fall in lactogen leads to a decrease in trophic support of the corpus luteum and, therefore, a decline in progesterone production which may facilitate the later release of $PGF_{2\alpha}$ which

ensures irreversible luteolysis. PGF is found in the uterine vein of the goat, up to 24 hr prior to the final abrupt fall in progesterone levels, in concentrations which are luteolytic (Currie and Thorburn 1977). The stimulation for PGF appears to be oestrogen (oestrone and oestradiol 17α) which show a rapid rise in the maternal plasma during the last 4-5 days prior to delivery (Thorburn et al 1972). The oestrogen rise could be due to an increase in precursors from the foetal adrenal. Increases in PGF production may involve oestrogen induced changes in the stability of lysosomal membranes in the maternal placenta, its action releasing hydrolases, consistent with the idea of oestrogen causing separation of the placenta and membranes (Currie and Thorburn 1977). However, like the sheep, there appears to be two surges of $PGF_{2\alpha}$. The smaller initial one is concerned with luteolysis, while the second, large peak is probably due to a nervous reflex and oxytocin release, as in the sheep. The second, but not the first peak can be blocked by continuous progesterone administration (see Horton and Poyser 1976).

In the cow, there is a gradual fall in plasma progesterone levels over the last 20 days and a more rapid fall 2-3 days before parturition (Donaldson, Bassett and Thorburn 1970). PGF levels in the utero-ovarian vein, increase 24-48 hr before delivery and are closely related to the sharp fall in plasma progesterone (Thorburn et al 1977). Urinary oestrogens rise progressively during the last month of pregnancy and plasma oestradiol levels show a similar rise during the last 30 days and fall post-partum (Smith, Egerton, Hafs and Convey 1973). The likely major site of oestrogen production in the cow, is the placenta (see Thorburn et al 1977). PGF may be the luteolytic factor in the cow, as its administration during early pregnancy or near term, causes luteolysis and abortion (Zerobin, Jöchle and Steingruber 1973; Lamond, Tomlinson, Drost, Henricks and Jöchle 1973).

In the pig, there is a gradual fall in progesterone levels for 20-15 days before parturition and a more rapid fall over the last 2 days (Killian, Garverick and Day 1973). Urinary oestrogens rise during pregnancy and unconjugated oestrogens and oestrone sulphate in maternal plasma rise during pregnancy, but urinary levels do not decline

post partum (Robertson and King 1974). PGF is luteolytic in the pig and parturition can be induced with $PGF_{2\alpha}$ (Diehl, Godke, Killian and Day 1974) and its analogue I.C.I. 79939 (Ash and Heap 1973). It therefore seems reasonable to suppose that luteolysis at term and parturition in the pig is dependent on increased uterine $PGF_{2\alpha}$ output.

Parturition in the pig, like the sheep, goat and cow is dependent on an intact foetal pituitary-adrenal cortex axis and the hormonal changes initiated in the foetus eventually leads to increases in uterine $PGF_{2\alpha}$ output. However, parturition in the rat is not apparently dependent on hormonal changes initiated in the foetus since embryectomy between days 9 and 13 leads to delivery of the placenta at the correct time (Selye, Collip and Thompson 1935). In the rat, as in the goat, cow and pig, the corpus luteum is the source of progesterone throughout pregnancy. In the pregnant rat, the corpus luteum receives trophic support from the maternal pituitary up to day 11 and then from a placental luteotrophin, up to day 15. There is a small decline in progesterone levels from day 15 to 19, due to decreased production of placental luteotrophin after day 15. This is followed by a more rapid fall in progesterone up to day 22, the day of parturition (Csapo and Wiest 1969; Pepe and Rothchild 1972). There is no decrease in the size of corpora lutea, but the fall in progesterone is accompanied by a rise in 20α-dihydroprogesterone, thus implying a functional luteolysis, involving a redirection of synthesis. Hence progesterone would be converted to 20α-dihydroprogesterone by the enzyme, 20α-hydroxysteroid dehydrogenase, the activity of which increases before parturition (Wiest 1970). Kuhn and Briley (1970) however, have proposed an alternative pathway of pregnenolone metabolism in the rat ovary before term, which contributes to the observed fall in plasma progesterone and rise in 20α-dihydroprogesterone. They showed a marked increase in ovarian 20α-hydroxysteroid dehydrogenase activity one to two days before parturition and proposed that this enzyme converts pregnenolone to pregnenediol, which then competes with progesterone for 3 β-hydroxysteroid dehydrogenase, to form 20α-dihydroprogesterone. Oestrogen output from the maternal ovaries is low until a few days prior to parturition (Shaikh 1971). As oestrogen levels rise, the level of $PGF_{2\alpha}$ in the utero-ovarion vein increases (Shaikh, Naqvi and Saksena 1977) as does the prostaglandin synthesising capacity of the uterus in vitro (Vane and Williams 1974). The evidence suggests that the rise in oestrogen is the stimulus for the increase in

$PGF_{2\alpha}$ synthesis and release, as ovariectomy on day 16 followed by oestrogen treatment, induces premature delivery, an effect blocked by naproxen (a prostaglandin synthesis inhibitor). The action of prostaglandins in this instance is clearly a direct one on the pregnant uterus as no corpora lutea are present (Csapo, Csapo, Fay, Henzl and Salau 1973). The stimulus for the rise in

oestrogen which occurs prior to parturition is from the pituitary, as hypophysectomy in late pregnancy prevents the rise (Wayneforth and Robertson 1972). The increase in $PGF_{2\alpha}$ release subsequent to oestrogen increasing, may cause the second, rapid fall in progesterone levels seen from day 19, due to a luteolytic action on the corpus luteum. The rapid decline in progesterone can be blocked by indomethacin treatment (Strauss, Sokolski, Caploe, Duffy, Mintz and Stanbough 1975). Experiments performed by Louis, Lawrence, Becker and Borden (1978) suggested that the pelvic nerves participate in the events leading to parturition in the rat. Pelvic neurectomy on day 8 prevented the more rapid fall in plasma progesterone which occurs on day 19 of pregnancy, although levels did gradually fall later. Parturition was also prevented on day 22. Pelvic neurectomy also prevented the rising uterine plasma PGF levels which normally occurs on the day of parturition. The increases in levels of 20 α -hydroxyprogesterone seen at parturition were not blocked. These results suggest that the pelvic nerves have some control on the increased release of $PGF_{2\alpha}$ at parturition.

The foetus of the Rhesus monkey may play an active role in initiating its own delivery, but the mechanism is different to those discussed previously for other species (for evidence see Thorburn: In Press). Before delivery, only slight increases in maternal plasma oestradiol and progesterone are observed. However, amniotic fluid levels of oestrone, oestrone sulphate, $PGF_{2\alpha}$ and 13,14-dihydro-15-keto $PGF_{2\alpha}$ rise, along with progesterone and androstenedione, during the last 10-20 days of pregnancy (Challis, Hartley, Johnson, Patrick, Robinson and Thorburn 1977). The increases in progesterone and androstenedione are considered as an expression of an increase in foetal adrenal activity which leads to a rise in amniotic oestrogen, which in turn stimulates PG production by the decidua. Oestrogen is thought to act by labilising lysosomal membranes, releasing phospholipase A_2 (PLA_2) which acts on phospholipids to release the prostaglandin precursor, arachidonic acid (Challis, Robinson and Thorburn 1977).

It is thought that prostaglandin synthesis is actively suppressed during pregnancy by the foetus and that parturition occurs as a removal of this supression, although the mechanism is uncertain (Robinson, Challis, Chapman and Thorburn 1977). The trophoblast and membranes

have been implicated in the production of a number of materials, e.g. chorionic gonadotrophin, renin, prolactin, and one or more substances may be involved in prostaglandin supression. The increase in oestrogens may also increase oxytocin receptors in the membranes and decidua, and oxytocin then stimulates prostaglandin synthesis by the amnion and decidua, which triggers parturition (Thorburn: In Press).

As in the case of non-human primates, the human shows no abrupt changes in maternal oestrogens or progesterone prior to the start of labour. Evidence for the human has been reviewed by Liggins, Forster, Grieves and Schwartz (1977). The maintenance of the pregnancy is dependent on placental hormones and the corpus luteum has no function after the second month of pregnancy (Csapo, Pulkkinen, Ruttner, Sauvage and Weist 1972; Csapo, Pulkkinen and Weist 1973). The foetus appears to play a relatively minor role in the initiation of parturition. There is no strong evidence for a rise in foetal cortisol levels before the onset of labour and no evidence for an action of cortisol on the placenta, as is the case in the sheep. Cortisol is unlikely to feature as a physiological triggering mechanism in human labour but it may share in a more complex endocrine trigger.

There is no evidence in women for thewithdrawal of a uterine inhibitory factor (the function of which would be to prevent uterine activity), for the initiation of labour, and although betamimetics such as salbutamol are very effective in inhibiting labour (Liggins and Vaughn 1973), there is no evidence that the uterus is normally strongly inhibited by catecholamines. Maximum levels of oxytocin are found during the second stage of labour and ethanol is thought to inhibit labour by inhibiting oxytocin release from posterior pituitary (Fuchs, Fuchs, Poblete and Risk 1967; Mantell and Liggins 1970). The evidence suggests that oxytocin is not involved in the initiation of human parturition, but has a role in the maintenance of labour once it has been established. There may be an interaction of oxytocin and prostaglandins, as intravenous infusion of subthreshold doses of prostaglandins enhance the oxytocin response (see Liggins et al 1977).

Prostaglandins are released into the maternal circulation (Green, Bygdeman, Toppozada and Wiquist 1974) and amniotic fluid during labour (Salmon and Amy 1973; Kierse, Flint and Turnbull 1974)

but there is no unequivocal evidence of their involvement in the initiation of labour. It has yet to be shown that there is an increased release of, or enhanced uterine response to, prostaglandins immediately prior to the onset of labour. However, significantly higher levels of $PGF_{2\alpha}$ have been found in the amniotic fluid during early spontaneous labour than in early induced labour, although uterine activity was higher in the latter group (Hillier, Calder and Embrey 1974).

The decidua and foetal membranes are the sites favoured for prostaglandin production; prostaglandin release from these tissues would not be due to contractile activity, as may be the case for the uterus. The arachidonic acid content of decidua and foetal membranes is high and it is incorporated into foetal membranes in preference to other unsaturated fatty acids (see Liggins et al 1977). PLA_2 activity of the reproductive tissues during pregnancy is highest in the deciduum and amnion (Grieves and Liggins 1976). There is little evidence in women that $PGF_{2\alpha}$ synthesis is stimulated by changes in plasma levels of oestrogens and progesterone.

Evidence is accumulating in support of the "lysosomal theory" of Gustavii (1975). In this hypothesis, a key role in labour initiation is attributed to lysomomes. The deciduum becomes rich in lysosomes which are maintained in a stable state during pregnancy by the presence of stabilizers, particularly progesterone. The onset of labour (or abortion) may be precipated by a labilising influence causing "leak" of lysosomal enzymes, including PLA_2 into the cyctoplasm. The PLA_2 acts on phospholipid, releasing arachidonic acid which in turn leads to increased prostaglandin production. Prostaglandins diffuse partly to the myometrium which they activate and into amniotic fluid where a rise in concentration is observed.

Liggins, Forster, Grieves and Schwartz (1977) suggest that the foetal membranes and deciduum may function as a unit, the membranes serving as a substantial source of arachidonic acid for prostaglandin synthesis in the deciduum and possibly the chorion. The factors which promote the selective incorporation of arachidonic acid into phospholipids and stimulate its release are unknown. It has been proposed that a specific progesterone-binding protein found

in the cytosol of human foetal membranes, in low concentrations before the 38th week of pregnancy and in much higher concentrations at term, binds progesterone, thus removing its stabilizing influence on lysosomes. Therefore, there is a local withdrawal of progesterone which is independent of maternal plasma levels, (Schwartz, Vanatta, Siiteri and MacDonald, 1974). This hypothesis of a local removal seems more acceptable than that proposed by Gustavii (1975), who attributes the initial lysosomal labilization to a rise in oestrogen and fall in progesterone levels. There is no evidence to support such abrupt changes in maternal levels of these steroids at the start of labour. The causes for the accumulation of the progesterone-binding protein proposed by Schwartz and his co-workers is not known. It seems that labour, in the human therefore, is initiated by a maturational signal arising from the foetal membranes and interacts with the deciduum. The foetal and maternal hormones play only a minor part, adding accuracy to the exact timing of parturition.

It is clear that one or more prostaglandins are released at term from the pregnant uterus and/or its contents, under the control of specific hormonal changes. These may be well defined as in the sheep and goat or less clear as in the primates. Since prostaglandin levels are elevated at term, it is assumed that they have a function in the parturition process and several roles have been postulated. A luteolytic action at term has been proposed, which leads to a fall in plasma progesterone levels followed by parturition. Administration of $PGF_{2\alpha}$ to goats during the final period of gestation, leads to a premature decline in progesterone levels and the induction of parturition (Currie and Thorburn 1973). Administration of $PGF_{2\alpha}$ to pigs in late pregnancy produces similar results (Diehl, Godke, Killian and Day 1974), and premature parturition can be induced with the $PGF_{2\alpha}$ analogue I.C.I.79939, it is presumed by a luteolytic action (Ash and Heap 1973). In the cow, increases in the ratio of plasma levels of oestrogen to progesterone over the last period

of gestation (Fairclough, Hunter and Welsh 1975; Henricks, Dickey, Hill and Johnson 1972; Robertson 1974), havebeen suggested as being responsible for an increased output of $PGF_{2\alpha}$ which in turn causes a decline in progesterone levels by causing luteal regression (Fairclough et al 1975). Treatment of rats on day 18 of pregnancy with $PGF_{2\alpha}$ leads to a decline in progesterone and premature parturition (Buckle and Nathanielsz 1973).

There is much evidence which suggests that prostaglandins contribute to the expulsion of the foetus by acting as uterine stimultants. In the goat there is a small rise in $PGF_{2\alpha}$ in the uterine vein during the 24 hr prior to normal parturition which is associated with luteolysis, but this small increase is followed by a larger increase immediately prior to birth and this second release may be involved in uterine contractions (Currie,Wong, Cox and Thorburn 1973; Thorburn, Nicholl, Bassett, Shutt and Cox 1972). Similarly in the rat, there is a rise in utero-ovarian vein levels of PGF and PGE on day 20 of pregnancy followed by a fall on day 21 and a second rise on day 22. Plasma progesterone levels fall on day 20 and parturition occurs on day 22 (Shaikh, Naqvi and Saksena 1977). Premature parturition can be induced in guinea pigs with $PGF_{2\alpha}$, probably by a direct stimulant action on the uterus (Ilingworth, Challis, Ackland, Burton, Heap and Perry 1974). In pregnant women, E and F prostaglandins have a direct oxytocic effect on the uterus (Karim 1972; Wiquist, Bygdeman and Toppozada 1973) and Karim and Hillier(1970) proposed that prostaglandins may be involved in spontaneous abortion, although whether prostaglandin production and release induced contractions, or was a result of contractions, was unclear. Intravenous PGE_2 or $PGF_{2\alpha}$ during first or second trimester of pregnancy, causes abortion (Karim and Filshie 1970a;1970b; Roth-Brandell, Bygdeman, Wiquist and Bergstrom 1970). Extra amniotic injection of hypertonic saline to women, 18-22 weeks pregnant causes the appearance of high concentrations of $PGF_{2\alpha}$ in the amniotic fluid, prior to abortion (Gustavii and Gréen 1972).

Interest has been focused recently on the actions of prostaglandins on the uterine cervix. Najak, Hillier and Karim(1970) demonstrated that the non-pregnant human cervix exhibits spontaneous contractility <u>in vitro</u> and that PGE_2 caused marked relaxation, while the effects of $PGF_{2\alpha}$ were more variable. Gréen et al (1974)

noted that the plasma levels of the $PGF_{2\alpha}$ metabolite, 15-keto-13,14-dihydro-$PGF_{2\alpha}$, were directly related to the degree of cervical dilation in women during labour. Before the process of parturition can proceed, it is necessary for the cervix to elongate and soften, thus allowing the foetus to pass through. In cases where artificial induction of labour is necessary, enforced mechanical dilation can cause a subsequent increase in the rate of cervical incompetence, spontaneous abortion and still birth. Cervical dilation has been successfully achieved prior to labour induction using oral PGE_2 (Pearce 1977; Golbus and Creasy 1977). Cervical dilation is also a pre-requisite of abortion and the same problems arise as have been discussed for labour induction. Dilation has been achieved by oral PGE_2 (Van den Bergh, Szabo, Szantagh 1976), vaginal $PGF_{2\alpha}$ (Brenner, Dingfelder, Staurovsky and Hendricks 1973), extra-amniotic $PGF_{2\alpha}$ and PGE_2 (Wiquist, Bequin, Bygdeman, Ferström and Toppozada 1972) and also by vaginal administration of $PGF_{2\alpha}$ analogues (e.g 15(s) - 15 methyl $PGF_{2\alpha}$ methylester; Zormethangi, Agarwal, Puni, Laumas, Soraya and Hingorani 1977). PGE_2 seems to be more potent than $PGF_{2\alpha}$ as a cervical relaxant. In the late pregnant rat, the extensibility of the cervix in vitro, was found to be increased by prior administration of $PGF_{2\alpha}$ or PGE_2 to the intact animal (Hollingsworth, Isherwood and Gallincie 1979). The authors suggest $PGF_{2\alpha}$ exerts its action indirectly by reducing progesterone levels and/or increasing the secretion of relaxin, but that PGE_2 has other mechanism (s) of action.

The role (s) of prostaglandins released from the rat uterus at term, has been the subject of controversy. The capacity of the uterus to synthesis prostaglandins E_2 and $F_{2\alpha}$ increases near term (Vane and Williams 1973; Harvey, Sneddon and Williams 1974; Carminanti, Luzzani, Soffientini and Lerner 1976) and there is an increase in the uterine vein levels of PGF prior to parturition (Labhsetwar and Watson 1974; Shaikh and Saksena 1977). Flower (1977) has reported that the activity of the enzyme metabolising $PGF_{2\alpha}$ in the lungs of the rat, decreases before parturition, as a result of a decrease in progesterone and an increase in oestrogen levels. This may result in an increased circulating level of $PGF_{2\alpha}$ and thus allow it to exert a spasmogenic effect on the uterus over a longer period.

Prostaglandin synthesis inhibitors will inhibit spontaneous contractions of the pregnant rat uterus *in vitro* (Vane and Williams 1973) but contractions induced by oxytocin were not inhibited, although the dose response curve was shifted to the right in the presence of the inhibitors. Contractions could be restored by addition of $PGF_{2\alpha}$ or PGE_2 to the bath fluid. Aspirin, indomethacin and naproxen, will prolong parturition in the rat *in vivo* (Aiken 1972; Csapo, Csapo, Fay, Henzl and Salau 1973; Chester, Dukes, Slater and Walpole 1972) and abortion (Deis 1969) and premature labour (Chatterjee 1976) can be induced in the rat with $PGF_{2\alpha}$.

Fuchs (1972) however, could not induce parturition on day 22 of pregnancy (the day of delivery) with infusions of PGE_2 and $PGF_{2\alpha}$. Infusions of oxytocin on day 22, did produce parturition. The contractions caused by oxytocin were indistinguishable from spontaneous contractions of labour (Fuchs and Poblete 1970). Infusion of $PGF_{2\alpha}$ on days 18-20 induces premature delivery 36-44 hr later (Fuchs and Mok 1973) and the authors suggest that this is due to a fall in plasma progesterone levels brought about by the luteolytic actions of $PGF_{2\alpha}$, as the levels of $PGF_{2\alpha}$ used were lower than those necessary for uterine stimulation. Other results have been presented by Fuchs, Smitasiri and Chantharaksri (1976) as evidence that $PGF_{2\alpha}$ released from the uterus at term, has a luteolytic function but is not involved in uterine contractions leading to expulsion of uterine contents. Indomethacin treatment of rats beginning on day 20 of pregnancy, delayed the onset of spontaneous parturition by14 hr. *In vivo* recordings of uterine contractions however, were not inhibited by indomethacin. The delay in the onset of parturition was accompanied by a similar delay in the decline in plasma progesterone levels and the appearance of 20α hydroxy-dehydrogenase in the corpus luteum, indications of retarded luteolysis, in indomethacin treated animals compared to controls. Infusion of $PGF_{2\alpha}$ on day 21 reversed the effects of indomethacin.

Drugs which have been used to inhibit uterine prostaglandin synthesis are not specific cyclo-oxygenase inhibitors (Flower 1974). Other actions include phosphodiesterase inhibition as this type of compound exerts unspecific inhibitory effects on muscle contractions (Smith, Temple and Shearman 1975) and compounds such as indomethacin and aspirin may affect the uterus by such a process.

It is possible that oxytocin exerts part of its spasmogenic action on the uterus by releasing prostaglandins, which in turn cause contractions. Isolated non-pregnant rat uterine horns have been shown to release prostaglandin-like activity on incubation with oxytocin. (Chan 1974) and oxytocin-induced parturition is inhibited in pregnant rabbits by administration of indomethacin (Heterlendy 1973). However, Ishikawa and Fuchs (1978) could not show any effects of oxytocin on the accumulation of PGF from isolated non-pregnant uteri from the rat and Roberts and McCracken (1976) concluded that increased synthesis of $PGF_{2\alpha}$ was not an essential intermediate in the activation of the myometrium by oxytocin, in sheep.

Hence, much evidence has been accumulated which suggests a role for the "classical" prostaglandins E_2 and $F_{2\alpha}$, in luteolysis, uterine contractility and cervical ripening at term, in many species. However, many of these studies were performed before it was realised that PGI_2 is a major substance produced by the uterus, Fenwick, Jones, Naylor, Poyser and Wilson (1977) showed PGI_2 detected as 6-oxo-$PGF_{1\alpha}$, to be produced by homogenates of pseudopregnant rat uteri. Many studies have also overlooked the possible production of thromboxanes during parturition. Section II of this thesis deals with PGI_2 and thromboxane production, as well as PGE_2 and $PGF_{2\alpha}$ by the pregnant rat uterus, at term.

Prostaglandins have recently been implicated as having a function in the process of implantation, although studies have so far been restricted to a few species only i.e. mice, rats, rabbits and hamsters. It is more difficult to prove an involvement of prostaglandins in implantation because the site of production is also the site of action, unlike processes such as luteolysis, for example, where the prostaglandins have to be transported to the site of action and the opportunities for experimental manipulation are therefore greater.

There is evidence in the rat and rabbit that prostaglandins are involved in decidualization of the uterine endometrium. PGE levels were found to be significantly higher in the artificially induced, decidualized horns of rat uteri, compared to control horns (Anteby, Bauminger, Zor and Lindner 1975). Prostaglandin synthetase levels were also higher in the decidualized horn. PGF levels on the other hand, were not significantly different from control levels and

were lower than PGE levels. However, $PGF_{2\alpha}$ has been found to induce decidualization when instilled into the uterine lumen of prepubertal rats maintained on progesterone (Sananes, Baulieu and Le Gascogne 1976). Scratching the endometrium in prepubertal rats maintained under the same hormonal conditions elicits the decidual response and this can be prevented by indomethacin treatment. Tobert (1976) also found that the olive oil-induced decidual cell reaction was significantly reduced by indomethacin, when given before or after the oil stimulus. Hoffman, Strong, Davenport and Frolich (1977) have found PGE_2, $PGF_{2\alpha}$ and arachidonic acid will cause decidualization when instilled into the uterine lumen of pseudopregnant rabbits. PGE_2 was the most potent.

Prostaglandin production by the pseudopregnant rat uterus in vitro, has been shown by Fenwick et al to reach a peak on day 5 (the day of implantation in the pregnant rat). PGE and PGF levels in the uterine venous plasma of pregnant rats also rise from days 1 to 5 and fall on day 6 (Shaikh, Naqvi and Saksena 1977). The levels of PGF in the peripheral plasma of pregnant and pseudopregnant hamsters show a peak late on day 4 (day 5is the implantation day)(Shaikh, Birchall and Saksena 1975). Kennedy (1977) showed in the rat and Evans and Kennedy (1978) in the hamster, that the concentration of PGE is elevated, approximately two-fold in areas showing the "uterine dye reaction" (which indicates the increase in vascular permeability at the implantation sites) compared to non-dye sites, following intravenous injection of blue dye on the day of implantation.

Treatment of rats, mice and hamsters with indomethacin, during early pregnancy, leads to inhibition or delay of implantation. Treatment of pregnant mice on day 1, 2, 3 or 4 inhibits pregnancy as determined by the absence of implantation sites on day 8. (Lau, Saksena and Chang 1973). Chatterjee (1973) found that treatment of rats on day 2 or 3 prevented implantation and this effect could be reversed by giving human chorionic gonadotrophin on day 3 or oestradiol cyclopentylpropionate on day 4. Indomethacin, given on the day of implantation was found to cause delay (as determined by the appearance of the uterine dye sites) in rats (Kennedy (1977) and hamsters (Evans and Kennedy 1978). When given to rabbits on days 4-6 of pregnancy, indomethacin was found to inhibit the uterine dye reaction normally seen on day $6\frac{1}{2}$-7 (Hoffman, Dipietro and McKenna 1978).

Prostaglandins may also be important after implantation, in the subsequent development of the foetus and maintenance of placental function. Treatment of rabbits in early pregnancy with indomethacin has been shown to produce inhibition of foetal development (Hoffman 1978) and resorption (El-Banna, Sacher and Schilling 1976; Saksena and Harper 1974). Active immunisation of rabbits against PGE_2 and $PGF_{2\alpha}$ during early pregnancy was found to cause retardation of embryonic growth, placental insufficiency and ultimately, foetal death (Elzayat and Stylos 1974). These effects on pregnancy development must be carefully separated from effects on implantation when studying the importance of prostaglandins, especially if animals are examined several days after the expected time of implantation. Studies which involve the examination of the uterus for "dye reaction sites" are particularly useful for this reason.

Aspirin-type drugs will block phosphodiesterase in concentrations slightly higher than those required for inhibition of prostaglandin synthesis (Flower 1974) and therefore, studies involving these compounds should take into account a possible effect on cyclic-3' 5'-adenosine menophosphate (cyclic AMP) levels. Any inhibition of phosphodiesterase will lead to an increase in cyclic AMP levels. Increasing the level of cyclic AMP is reputed to prevent histamine release from mast cells by inhibiting calcium uptake (Ishizaka, Ishizaka, Orange and Austen 1971). A histamine involvement in implantation in mice, rats and rabbits has been implicated. Block of implantation in the mouse, obtained with indomethacin, could be reversed in 60% of animals treated with PGE_2 and/or $PGF_{2\alpha}$ (Saksena, Lau and Chang 1976). Oestrogen-induced implantation in the rat, could not be obtained in uteri which had been pre-frozen but treatment of such uteri with a histamine infusion did induce implantation, (Ferrando and Nalbander 1968). Disodium chromoglycate (Intal) when instilled into the uterine lumen of pregnant rabbits decreased the number of implants and this action was reversed by the simultaneous instillation of histamine (Dey, Villanenva, Chien and Crist 1978). Intal is reputed to prevent histamine release from mast cells by inhibiting calcium uptake (Brogden, Speight and Avery 1974). Hence indomethacin may increase cyclic AMP levels (by inhibiting phosphodiesterase) and thereby prevent histamine release, in addition to inhibiting prostaglandin synthesis. Consequently

replacement therapy with both histamine and prostaglandins would be required to achieve implantation in indomethacin treated animals, as has been shown in the mouse (Saksena, Lau and Chang 1976).

Contrary to the above discussion, which implies that an increase in cyclic AMP.is detrimental, there is evidence that cyclic AMP may be involved in implantation. Implantation can be induced in pregnant ovariectomised mice, maintained on progesterone by cyclic AMP, (Holmes and Bergstrom 1975) and its dibutyryl analogue (Webb 1975). In pregnant rats, treatment with the adenyl cyclase inhibitor, alloxan, significantly reduces uterine PGE and PGF levels on days 5 and 6 compared to controls and this effect is reversed by concomitant treatment with cyclic AMP, (Garg, DeSouza and Chaudhury 1979). This suggests a stimulatory role for cyclic AMP on prostaglandin synthesis. Conversely, however, Vesin, DoKhac and Harbon (1978) found that treatment with the prostaglandin precursor, arachidonic acid, increased cyclic AMP accumulation in oestrogen treated rats. It may be that the time course of events during implantation is critical and that histamine is released before a rise in cyclic AMP, which then acts as a negative feedback, regulating histamine release.

When studying the effects of exogenous prostaglandins, for example, as replacement therapy after indomethacin administration, care should be taken in the interpretation of results. Effects may be observed which are a consequence of actions of prostaglandins such as luteolysis or effects on egg transport (Labhsetwar 1972). Administration of PGE_2 or $PGF_{2\alpha}$ to pregnant rats has been shown to prevent implantation (Batta and Martini 1975), probably by activation of uterine contractility.

From experimental evidence, it seems that oestrogen is responsible for the initiation of implantation. In the pregnant mouse, there is a rise in circulating oestrogen on day 4 (McCormack and Greenwald 1974), which is followed by increased uterine sensitivity to formation of decidual tissue (Hetherington 1968) essential for implantation (Humphrey 1967). In pregnant rats there is a surge of oestrone and oestradiol in peripheral plasma detectable early on day 4 (Shaikh 1971) preceding implantation on day 5. A small rise in plasma oestradiol up to day 4 is also seen in the pseudopregnant rat and so is independent of the blastocyst (Welschen, Osman, Dullaart, De Greef, Uilenbroek and De Jong 1975). Similarly, in pregnancy and pseudo-pregnancy in the hamster, oestrone and oestradiol levels in ovarian venous plasma increase from day 1 to 4 and fall on day 5 (the day of implantation), (Shaikh et al 1973). Implantation can be initiated

in pregnant ovariectomised mice (Saksena et al 1976), and rats (Ferrando and Nalbander 1968), maintained on progesterone by administration of single dose of oestrogen. In pregnant, ovariectomised rabbits, maintained on progesterone, implantation proceeds without oestrogen administration, but can be prevented by the anti-oestrogen drug Cl-628 (Dey, Dickmann and Sen Gupta 1976). It has been suggested that oestrogen, produced by the rabbit blastocyst is necessary for implantation and indeed the rabbit blastocyst has been shown capable of producing oestrogenic steroids and also to contain oestrogen (Dickman,Dey and Sen Gupta 1975). It has also been shown in the ovariectomised hamster, that oestrogen is not required for implantation (Harper, Dowd and Elliott 1969). The blastocyst of the pig has been shown to synthesize oestrogen (Perry, Heap, Burton and Gadsby 1976; Flint, Burton, Gadsby, Saunders and Heap 1978) and this has also been suggested for the hamster, mouse and rat (Dickman and Sen Gupta 1974; Dey and Dickman 1974; Dickman 1976). So, although progesterone is required for the maintenance of pregnancy, it seems that oestrogen of maternal and/or foetal origin, is needed for the initiation of implantation. The anti-oestrogen drug ,Tamoxifen, inhibits implantation in the rat, when given in early pregnancy (Harper and Walpole 1967). A significant reduction in prostaglandin synthesis by the uterus on day 5 of pseudopregnancy in the rat, has also been shown when Tamoxifen is given on day 2 (Fenwick et al 1977). This infers that the oestrogen surge on day 4 is responsible for the peak in prostaglandin production on day 5 of pseudopregnancy. Oestradiol is known to stimulate the $PGF_{2\alpha}$ synthesising capacity of the uterus by increasing the amount of synthetase present (Ham, Cirillo, Zanetti and Kuehl 1975) and oestradiol treatment of ovariectomised guinea pigs, produces similar results in vitro (Naylor and Poyser 1975). Oestrogen has also been found to stimulate uterine prostaglandin levels in hamsters (Saksena and Harper 1972) and mice (Saksena and Lau 1973). In ovariectomised rats, mice, guinea pigs and ewes, however, the optimum hormonal treatment for uterine prostaglandin production was found to be a dose of oestrogen given after a period of progesterone priming (Castracane and Graig-Jordan 1975; Saksena and Lau 1973; Caldwell,Tillson,Brock and Speroff 1972; Blatchley and Poyser 1974). These are the hormonal conditions which exist in early pregancy in the mouse, rat and hamster and hence it would seem logical to assume that

the increased prostaglandin levels observed around the time of implantation, are stimulated by an oestrogen surge of maternal and/or foetal origin following a period of progesterone priming. In support of this suggestion, it has been reported that the peak in PGF in peripheral plasma on day 4 of pregnancy in the hamster is associated with high levels of oestrone and oestradiol (Shaikh, Birchall and Saksena 1973). Also, oestrogen-induced implantation can be inhibited in mice by indomethacin and this effect can be reversed by administration of prostaglandins and histamine, as has been discussed previously. Therefore, it seems that oestrogen stimulates increased production of prostaglandins by the uterus and is involved in the events leading to implantation of the blastocyst.

Oestrogen exerts other actions on the blastocyst and uterus around the time of implantation, e.g. stimulation of RNA synthesis, initiation of changes in the surface morphology of the preimplantation blastocyst, promotion of cell division in the uterine stroma and sensitisation for decidualization. These effects can be mimicked by cyclic AMP (Mahla and Prasad 1970; Holmes and Bergstrom 1976; Webb 1977). Oestrogen has been found to increase cyclic AMP levels in the uterus (Szego and Davies 1967) and therefore, cyclic AMP may act as a "second messenger" for these oestrogen effects, as has been shown for the stimulation of prostaglandin synthesis (Garg, De Souza and Chaudhury 1979).

Experiments performed as described in Section III, have investigated changes in the synthesising capacity of the rat uterus for PGE_2, $PGF_{2\alpha}$ and PGI_2 around the time implantation. These changes have been compared with those which occur in the pseudopregnant animal. The effect of indomethacin on implantation and subsequent pregnancy development has also been studied.

SECTION I

BASIC METHODS

Composition of Solutions

Krebs' Solution 51

Glucose 10g
Sodium Chloride 34.5g
Sodium Bicarbonate 10.5g
Potassium Chloride 17.5ml
Potassium Dihydrogen Phosphate 8ml
Magnesium Sulphate. 7 H_2O 7 ml
} 10% Solution
1M Calcium Chloride 12.5 ml

Acid Citrated Dextrose

Glucose (Dextrose) 3g
Disodium Hydrogen Citrate 2g
} In 120 ml water

Tris Buffer pH 8.0

12g/l trihydroxymethylmethylamine phosphate adjusted to pH 8.0 with 1N hydrochloric acid.

For Radio-Immuno-Assay (RIA)

Diluent I

56 ml of 0.1M solution Sodium Dihydrogen Phosphate plus 34.5 Disodium Hydrogen Phosphate in 5l distilled water pH 7.5. Plus 0.1g/l Sodium Azide (antibacterial) + 1g/l Gelatin.

Diluent II

0.05M solution Trihydroxymethylmethylamine (Tris) Hydrochloride titrated to pH 6.8 using concentrated Hydrochloric Acid (HCl).

Diluent III

0.05M Tris titrated to pH 8.0 using concentrated HCl.

Scintillation Fluid A

10.5g 2,5, diphenyloxazole (PPO) in a mixture of 1.5l Toluene and 0.9l 2-ethoxyethanol.

Scintillation Fluid B

4g/l 2-(4-tertbutylphenyl)-5-(4-biphenyl)-1, 3, 4, oxadiazole (Butyl PBD) in Toluene.

15-hydroperoxy arachidonic acid, the reported prostaglandin I_2 synthesis inhibitor (Moncada, Gryglewski, Bunting and Vane 1976b), was prepared by the action of soya bean lipoxygenase on arachidonic acid (see Section I).

Synopsis of Experiments

The experiments described in the following three sections were carried out using tissue obtained from female Wistar rats. Section I describes the basic techniques used for the extraction, purification and assay of all samples obtained. Section II described the experiments performed to investigate the role(s) of prostaglandins in parturition in the rat. The release of prostaglandins from the term pregnant rat uterus *in vitro* and the effects of prostaglandins and prostaglandin synthesis inhibitors on the uterus, have been investigated. The female rats were mated with male rats of the same breed, as described in Section II. Methods.1. The experiments involved extraction and measurement of prostaglandins released from the isolated, term pregnant rat uterus, into the Kreb's bathing solution. The effects of adding exogenous prostaglandins to the bathing fluid was investigated and also the effects of prostaglandin synthesis inhibitors on the uterus and on prostaglandin release from the uterus, at term.

Section III deals with the involvement of prostaglandins in implantation in the rat. The capacity of the rat uterus to synthesise prostaglandins *in vitro* around the time of implantation, during pregnancy and also during pseudopregnancy has been investigated and the two reproductive states compared. The extraction and assay of prostaglandins from tissue homogenates has been performed in these cases. The effects of indomethacin (a prostaglandin cyclo-oxygenase inhibitor) on implantation and on the subsequent development of the pregnancy up to day 9 in the rat, has also been studied. Peripheral plasma progesterone has been measured during pregnancy and pseudo-pregnancy and also during pregnancy in indomethacin treated rats.

Female rats were mated with vasectomised or normal male rats of the same breed, in order to achieve pseudopregnancy or pregnancy, respectively, as described in Section III, Methods Ia and Ib.

1. Solvent Extraction of Prostaglandins

The acidity of samples, from which prostaglandins were to be extraced, was lowered to pH 4.0 with 1N hydrochloric acid (HCl). Each sample was then partitioned three times with two volumes of redistilled ethyl acetate. The ethyl acetate fractions were combined, evaporated to dryness in a rotary evaporator at 45°C and placed in a dessicator under vacuum for 15 min. This extraction procedure is summarised in Fig.5. The residues were redissolved in 10ml redistilled ethyl acetate and stored, until assayed, at -20°C.

2. Recovery of Prostaglandins by Solvent Extraction from Krebs' Solution

Three radioactive "counting standards" were prepared by adding 0.05μCi of radioactive prostaglandin to 10ml liquid scintillation fluid A. At the same time 0.125μCi was added to 20ml Krebs' solution containing 2μg/ml of non-radioactive "cold" prostaglandin. The pH of Krebs' solution was then lowered to 4.0 with 1N HCl and the solution was shaken three times with two volumes of redistilled ethyl acetate. The ethyl acetate fractions were pooled, evaporated to dryness at 45°C using a rotary evaporator and then placed in a dessicator under vacuum for 15 min. The residue was redissolved in 0.6ml of methanol (MeOH) and added to a vial containing 13ml liquid scintillation fluid A. The three "counting standards" and the vials containing extracted prostaglandins were then counted on a liquid scintillation counter (Nuclear Chicago) for 2 min using an external standard chemical ratios count. The number of disintegrations per minute (DPM) of the solvent extracted radioactive prostaglandin was compared to the DPM from the "counting standards". From these values the % recovery from Krebs' solution of the radioactive prostaglandin can be calculated.

$$\% \text{ Recovery} = \frac{\text{Number DPM}}{\text{Average DPM Counting Standards}} \times 100$$

This procedure was carried out for prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$), prostaglandin E_2 (PGE_2), prostaglandin D_2 (PGD_2), 6-oxo-prostaglandin $F_{1\alpha}$ (6-oxo-$PGF_{1\alpha}$) and thromboxane B_2 (TXB_2).

The efficiency of the extraction procedure for progesterone was determined by extracting a known amount of radioactive progesterone together with "cold" progesterone carrier by the methods described subsequently and counting the extracted samples as described for prostaglandin recovery from Krebs' solution, using 10ml scintillation fluid B.

Results

Prostaglandin	Number of Determinations	% Recovery Mean ± Standard Error of the Mean (S.E.M.)
$PGF_{2\alpha}$	10	92 ± 4.5
PGE_2	12	94 ± 2.5
6-oxo-$PGF_{1\alpha}$	3	65 ± 1.2
PGD_2	12	91 ± 2.5
TXB_2	10	94 ± 5.5
Progesterone	12	93 ± 1.6

As recovery of progesterone and all prostaglandins, with the exception of 6-oxo-$PGF_{1\alpha}$, was over 90%, results were not corrected for recoveries. A series of extractions were performed for 6-oxo-$PGF_{1\alpha}$ from pH 1.5 to pH 5.0, but 65% recovery was the maximum which could be obtained and was recovered in the range pH 1.5 up to 4.5.

The radio-immuno-assays for PGE_2, $PGF_{2\alpha}$ and progesterone are well established and therefore their accuracy is known to be satisfactory. The radio-immuno-assay (RIA) for 6-oxo-$PGF_{1\alpha}$ however has only recently been established and therefore it was thought necessary to test the accuracy of this assay by extracting known quantities of 6-oxo-$PGF_{1\alpha}$ from 20ml Krebs' solution and measuring the recoveries by RIA. The resultsare given below:-

Amount of 6-oxo-$PGF_{1\alpha}$	Number of Determinations	Amount measured by RIA Corrected to 100% Recovery
200ng	4	229.6 ± 13.6
500ng	4	496.5 ± 27.8
1000ng	4	976.4 ± 38.4

These results show that the RIA gives an accurate measure of the amounts of 6-oxo-$PGF_{1\alpha}$ present.

3. Extractions of Progesterone from Plasma Samples

Blood samples (2.0ml) were obtained from rats by cardiac puncture. Blood was added to centrifuge tubes containing 10i.u/ml heparin and was centrifuged at 600 x g for 10 min using a bench centrifuge (MSE).

Fig.5. Solvent Extraction of Prostaglandins

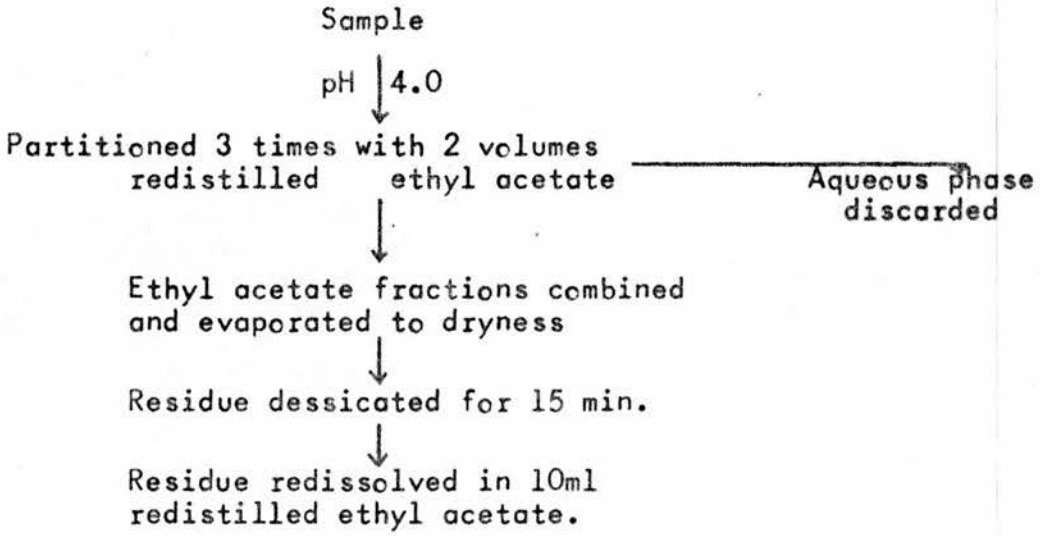
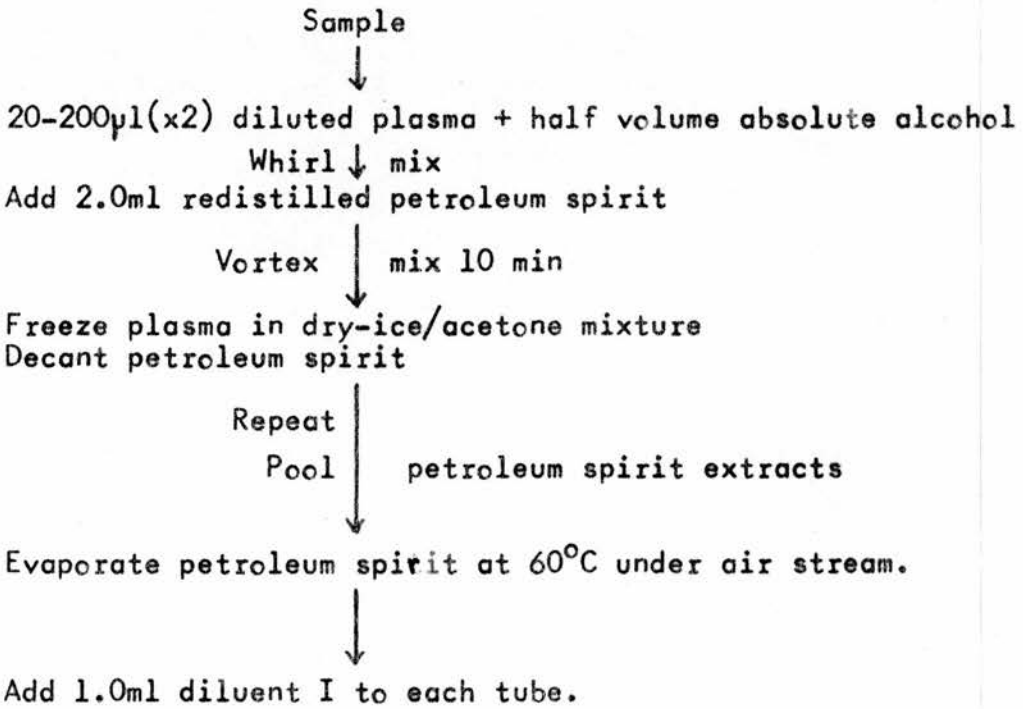


Fig.6. The Extraction of Progesterone from Plasma



After centrifugation, the supernatent plasma was removed and stored at -20°C.

For extraction, plasma was diluted ten times with distilled water and 20-200µl was taken in duplicate for each sample. Distilled water (100µl) was added to samples of less than 50µl and half the volume of redistilled alcohol was added to each sample to prevent hydrogen bonding of proteins in plasma to the antibody. Progesterone was extracted by adding 2.0ml redistilled petroleum spirit (boiling point 40-60°C) and vortex mixing for 10 min. The plasma was then frozen using a freezing mixture consisting of dry-ice in acetone and the petroleum spirit was decanted off. This process was repeated and the petroleum spirit fractions were pooled and evaporated to dryness in a stream of air at 60°C. 1.0ml of diluent I for progesterone RIA was then added to each of the tubes. The extraction procedure for progesterone from plasma is summarised in Fig.6.

4. Radio-Immuno-Assays for the Measurement of Prostaglandins and Progesterone

a. Prostaglandin $F_{2\alpha}$

Samples were assayed using an antibody raised and tested in the laboratory (Dighe, Emslie, Henderson, Rutherford and Simon 1975) using the double antibody method of separating bound from unbound radioactive prostaglandin. A list of cross reactivities of the antibody is given in table 1.

One vial was prepared as a background count for radiation by adding 0.7ml diluent III to 13ml scintillation fluid A. Four "counting standards" containing 0.65ml diluent III and 50µl (30pg) tritiated $PGF_{2\alpha}$ in 13ml scintillation fluid A were prepared. Four "zero standards" ("0") were then prepared, consisting of 0.6ml diluent III per assay tube and four "non-specific binding standards" (NSB), consisting of an excess of "cold" $PGF_{2\alpha}$ (10ng/ml) in 0.65ml diluent III. A further nine "standards" of $PGF_{2\alpha}$ were prepared dissolved in diluent III. Each standard solution of $PGF_{2\alpha}$ was dispensed out into assay tubes in triplicate (0.5ml per tube). To each tube was added 50µl (30pg) of tritiated $PGF_{2\alpha}$ ($^{3}HPGF_{2\alpha}$, "tracer") and 50µl antibody (at a dilution of 1:1500 in diluent III) and the solutions were mixed using a "whirlimix". 30pg tracer was also added to each "0" standard and each NSB standard. Antibody (50µl) was also added to the "0" standards. After an incubation

period of 1 hr at room temperature, normal rabbit serum (NRS - 50µl of 1:140 dilution in diluent III) and donkey anti-rabbit serum, (DARS-50µl of 1: 15 dilution in diluent III) were added to the nine standards and also the "0" and NSB standards. The solutions were then mixed on the "whirlimix".

Following incubation for 16 hr at 4°C, the tubes were centrifuged at 4°C and 1720 x g for 30 mins (Fisons MSE centrifuge). The supernatent liquid was then decanted off into vials containing 13ml scintillation fluid A and after shaking, the vials were counted for 4 min in a liquid scintillation counter. A standard curve (Fig.7) was plotted of % binding of the $^{3}HPGF_{2\alpha}$ against the amount of non-radioactive "cold" $PGF_{2\alpha}$.

The sensitivity of all radio-immuno assays was defined as the amount of "cold" prostaglandin or progesterone giving a 10% fall in binding, measured from the "0" standards. In all assays, the upper limit of the working range was determined as the sensitivity of the assay and the lower limit was determined as the concentration of "cold" prostaglandin or progesterone at which there was a 2% decrease in the slope of the curve.

Examples of the standard curves obtained for each assay are given in Figs. 7, 8, 9 and 10. The dotted curves represent the limits of the range of curves obtained for all assays. Standard curves for all assays fell between the upper and lower dotted curves. The values for the working range and sensitivity of each assay, in table 4, are the values obtained for the middle curve in Figs. 7, 8, 9 and 10. The highest value for sensitivity obtained during the course of the work described in this thesis for each of the assays were: $PGF_{2\alpha}$ = 80pg, PGE_2 = 30pg, 6-oxo-$PGF_{I\alpha}$ = 60pg and progesterone = 55pg. The sensitivity value of all assays were equal to, or lower than these values.

Details of all radio-immuno assays used are given in tables 3 and 4. Samples were assayed in three volumes in duplicate and the coefficient of variation between duplicates was calculated. If this value exceeded 15%, samples were re-assayed. Therefore, the maximum intra-assay coefficient of variation was 15%, however for most samples the value was less than 10%. The inter-assay coefficient of variation was calculated from two tubes containing

in duplicate, a known amount of prostaglandin or progesterone, which were added to the end of each assay. The between assay (inter-assay) coefficient of variation was calculated from these duplicated standards.

b. Prostaglandin E_2

An antibody PGE_2 was purchased from the Pasteur Institute, Paris, and a list of cross-reactivities is given in table.1. Tritiated PGE_2 ($^3HPGE_2$ - 10pg in 50µl diluent I) was used at 1:100 ampoule dilution (in 50µl), of antibody. A series of eight "standards" in duplicate (in 0.5ml diluent I) were prepared and "0" and NSB standards as for PGF_2 RIA. After a 2 hr incubation at room temperature NRS (50µl of 1:140 dilution in diluent I) and DARS (50µl of 1: 15 dilution in diluent I) were added to each tube and the contents were mixed and left for 16 hr at 4°C. The procedure was then as for $PGF_{2\alpha}$ RIA, one background and four "counting standards" for PGE_2 having been prepared as for $PGF_{2\alpha}$.

c. 6-oxo-Prostaglandin $F_{1\alpha}$

For this assay diluent I was used initially, but was then replaced by diluent II.

The antibody for this assay was raised in the laboratory and the tritiated 6-oxo-$PGF_{1\alpha}$ (^3H 6-oxo- $PGF1\alpha$) was also made in the laboratory (Dighe, Jones, Poyser 1978). The cross-reactivities

Fig.7.

Example of a standard curve for radio-immuno-assay of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$).

NSB = Non Specific Binding standard.

"0" = Zero standard N.S.B and "0" refer to middle curve. Curves for all assays fell between the upper and lower dotted curves.

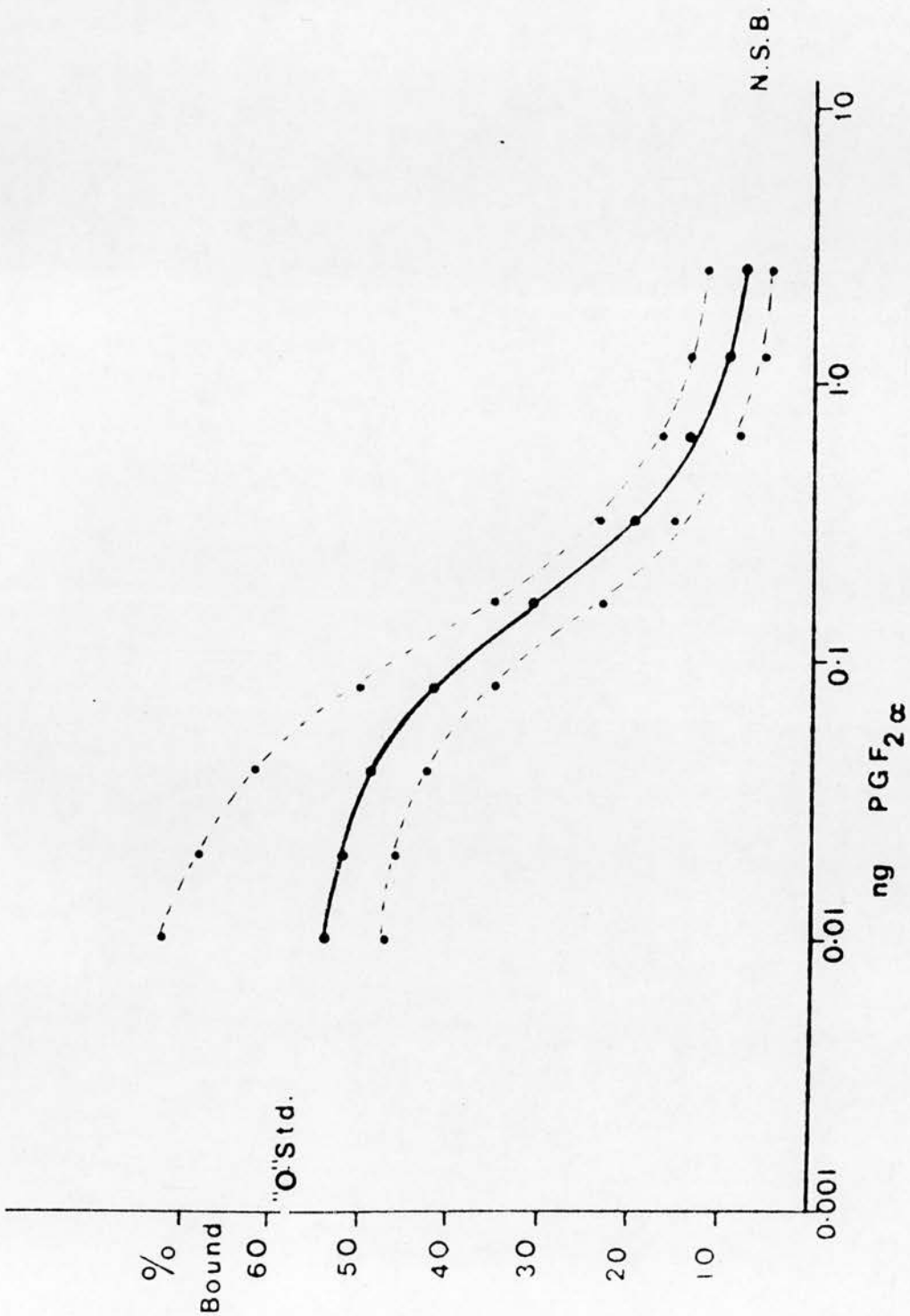
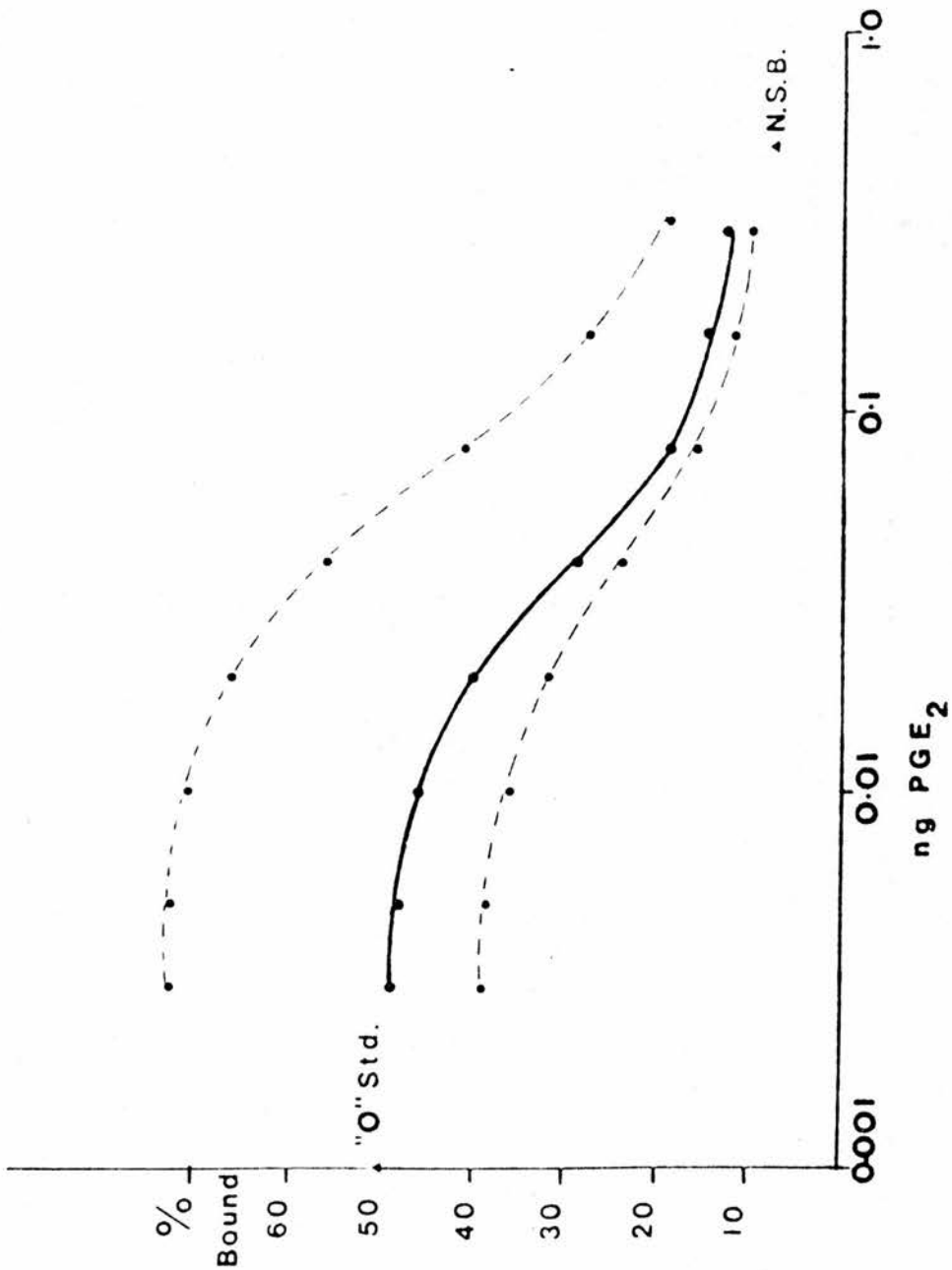

% Bound
60
50
40
30
20
10
"O" Std.
N.S.B.
0·001
0·01
0·1
1·0
10
ng PGF2α

Fig.8.

Example of a standard curve for radio-immuno-assay of prostaglandin E_2 (PGE_2).
NSB = Non Specific Binding standard.

"0" = Zero standard. N.S.B. and "0" refer to middle curve. Curves for all assays fell between the upper and lower dotted curves.


% Bound
60
50
40
30
20
10
"0" Std.
N.S.B.
0·0001
0·001
0·01
0·1
1·0
ng PGE2

for the antibody are given in table 1. Four "0" standard, four NSB standards and ten standards in triplicate were used. The antibody was used in a dilution of 1:650 in diluent II and 50µl was added to each tube. 30pg ^{3}H6-oxo-$PGF_{1\alpha}$ was used in 50µl diluent II. Incubation time was 2 hr at room temperature. DARS (50µl of 1:8 dilution of diluent II) was then added prior to incubations at 4°C for 16 hr. The procedure was then as for $PGF_{2\alpha}$ RIA.

d. Preparation of Samples for Assay of Prostaglandins by RIA

Samples to be assayed for prostaglandins were extracted into ethyl acetate (see 1. previously). Three volumes of 20-500µl were dispensed in duplicate, to check for parallelism between volumes, and the ethyl acetate was blown off under a stream of air at 45°C. A volume of 0.5ml of appropriate diluent was added to each tube prior to RIA. Two "blanks" were added to the end of each assay consisting of 20-500µl ethyl acetate evaporated to dryness and 0.5ml diluent added. Two tubes containing a known quantity of prostaglandins in 0.5ml diluent were also added to check the interassay coefficient of variation.

e. Radio-Immuno-Assay for Progesterone

Progesterone, extracted with petroleum spirit (see 3. previously) from peripheral plasma samples, was measured by an RIA developed by Dighe and Hunter (1974) and described by Poyser and Horton (1975). The cross reactivities are given in table 2. The dilution of antibody was 1:2000 in diluent I (50µl/tube) and 30pg ^{3}H Progesterone (in 50µl diluent I). Incubation time was 2 hr at room temperature. NRS (50µl of 1:140 dilution in diluent I) and DARS (50µl of 1:15 dilution in diluent I) were used. Ten triplicate "standards" in a volume of 1.0ml diluent I were used and four "0" and four NSB standards were prepared. The procedure was as for RIA for prostaglandins but with the addition of a further 1.0ml diluent I to each tube prior to centrifugation. The supernatent liquid was decanted into vials containing 10ml scintillation fluid B and the vials were shaken for 3 min before counting for 4 min on a liquid scintillation counter.

Fig.9.

Example of a standard curve for radio-immuno-assay of 6-oxo-prostaglandin $F_{1\alpha}$ (6-oxo-$PGF_{1\alpha}$).

NSB = Non Specific Binding standard.

"0" = Zero standard N.S.B. and "0" refer to middle curve. Curves for all assays fell between the upper and lower dotted curves.

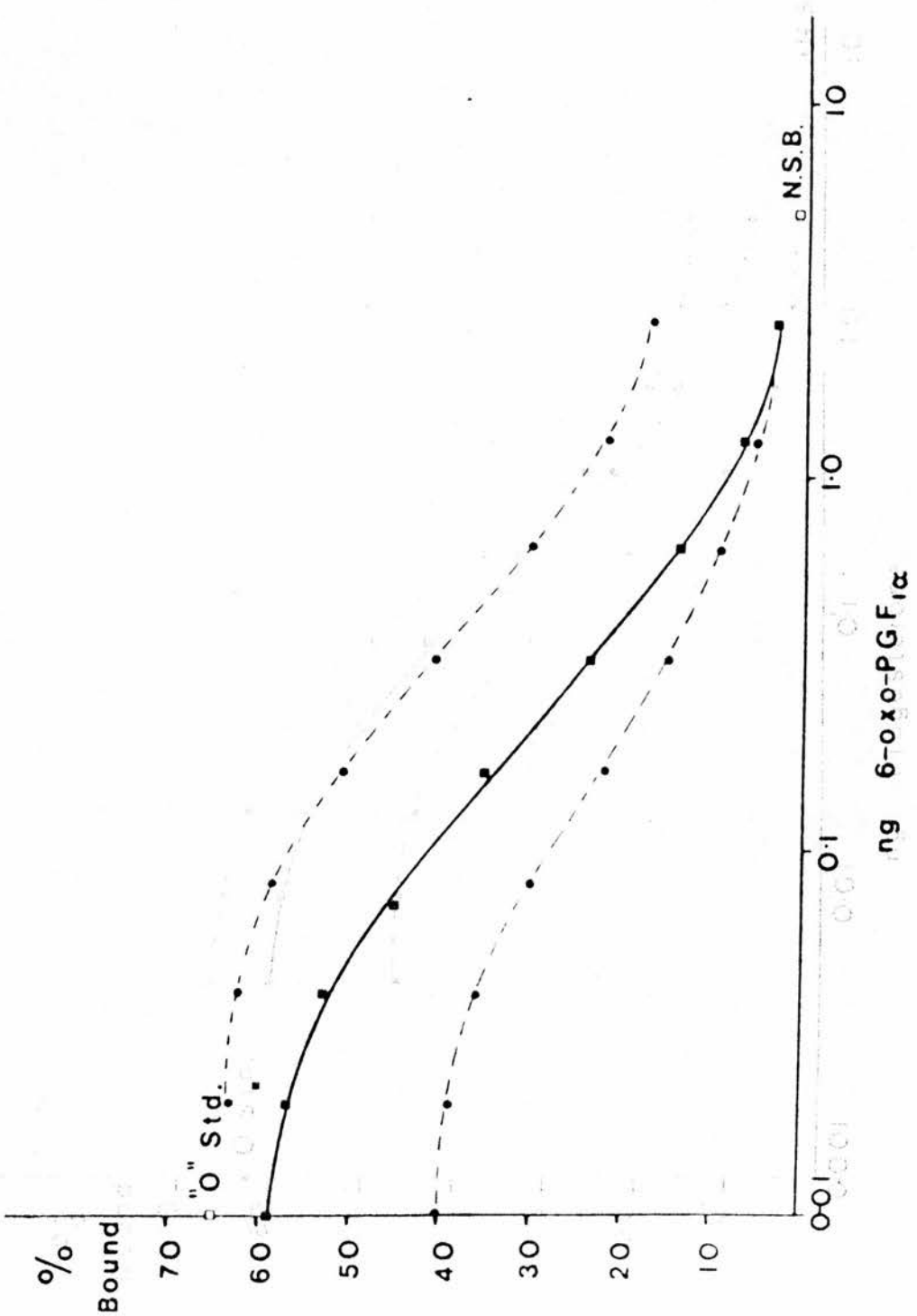

%
Bound
70
60
50
40
30
20
10
"0" Std.
N.S.B.
0·01
0·1
1·0
10
ng 6-oxo-PGF1α

Table 1. Cross Reactivities of Prostaglandin Antibodies used for Radio-Immuno-Assay.

Prostaglandin	$PGF_{2\alpha}$ 1:1500 30pg/tube "0" Std. 72%	PGE_2 1:100 10pg/tube "0" Std. 51%	6-oxo-$PGF_{1\alpha}$ 1:650 30pg/tube "0" Std. 55%
E_2	0.8%	100%	6.8%
E_1	0.65%	15.1%	2.0%
A_2	0.04%	0.14%	0.036%
B_2	0.145%	0.05%	0.031%
$F_{2\alpha}$	100%	0.17%	5.8%
$F_{2\beta}$	0.833%	-	-
15-oxo-$F_{2\alpha}$	2.8%	-	-
15-oxo-E_2	-	0.58%	-
$F_{1\alpha}$	100*%	-	0.12%
D_2	3.4%	0.008%	0.013%
13,14,dihydro 15-oxo-$F_{2\alpha}$	0.67%	-	0.105%
13,14,dihydro-15-oxo-E_2	-	0.056%	0.018%
6-oxo-$F_{1\alpha}$	1.2%	0.002%	100%

*Cross reactivity for the $PGF_{2\alpha}$ Antibody with $PGF_{1\alpha}$ is 100%, however, the rat uterus contains only trace amounts of $PGF_{1\alpha}$ (Fenwick, Jones, Naylor, Poyser, Wilson 1977) and so interference with the measurement of $PGF_{2\alpha}$ is negligible and the same applies for the cross reactivity of PGE_1 with the PGE_2 antibody.

Cross reactivities were determined by using the method of Dighe et al (1975).

Fig.10.

Example of a standard curve for radio-immuno-assay of progesterone.

NSB = Non Specific Binding standard.

"0" = Zero standard. N.S.B. and "0" refer to middle curve. Curves for all assays fell between the upper and lower dotted curves.

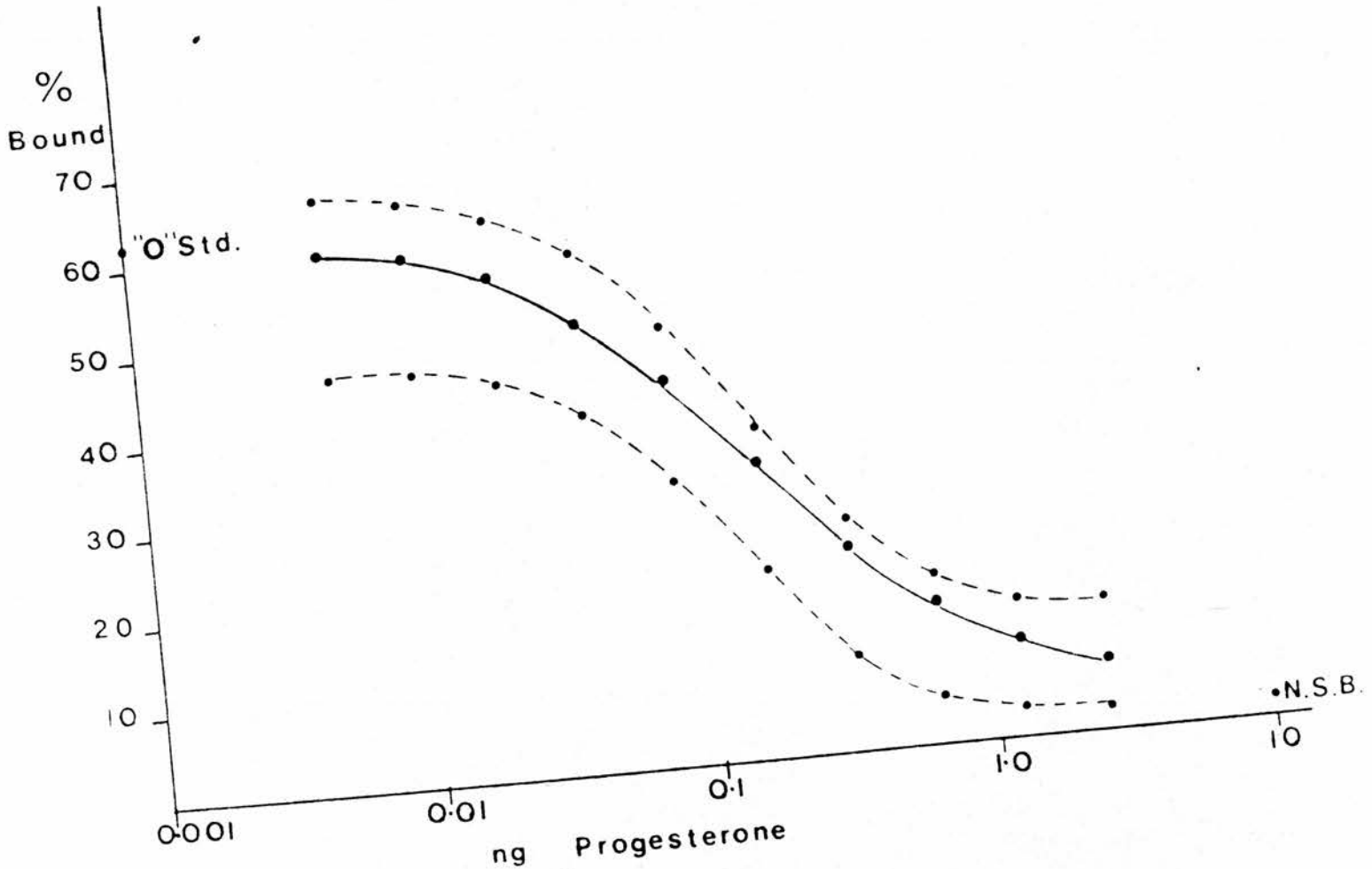

%
Bound
70
60
50
40
30
20
10
"O" Std.
N.S.B.
0·0001
0·001
0·01
0·1
1·0
10
ng Progesterone

Table 2. Cross Reactivities of Progesterone Antibody used for Radio-Immuno-Assay.

	% Cross Reactivity Progesterone Antibody *
Progesterone	100%
11∝hydroxy progesterone	31%
11β hydroxy progesterone	12%
11-oxo-progesterone	15%
6β hydroxy progesterone	0.8%
20∝hydroxy-pregn-4-ene-3-one	1.0%
20β hydroxy-pregn-4-ene-3-one	0.005%
5∝ pregnane,3,20,dione	13%
5β pregnane,3,20,dione	7%
17∝hydroxy progesterone	1.2%
3∝hydroxy5β-pregnan-20-one	0.2%
5∝pregnane,3∝ 20∝,diol	0.0001%
5∝pregnane 3β,20∝,diol	0.003%
5β pregnane, 3∝20∝,diol (pregnanediol)	0.008%
3-hydroxypregn5-en-20-one	0.13%
3β,17-OH-pregn-5-ene-20-one	0.02%
11-decxycorticcsterone	0.9%
11-deoxycortisol	0.15%
cortisol	0.02%
corticcsterone	0.43%
cortisone	0.02%
Androst-4-ene,3,17,dione	0.02%
3βhydroxyandrost-5-ene-17-one	0.004%
Cholesterol	0.0004%

*As determined by Dighe and Hunter(1974).

Tables 3 and 4 Details of Radio-Immuno-Assays for Prostaglandins and Progesterone

Table 3.

Substance	Specific Activity of Tracer ng/μCi	Amount of Tracer pg	Scintillation Fluid	No.of Stds. for assay	Vol.in tube ml	Dilution NRS	Dilution DARS
$PGF_{2\alpha}$	2.27	30	A	9(x3)	0.7	1:140	1:15
PGE_2	2.30	10	A	8(x2)	0.7	1:140	1:15
6-oxo-$PGF_{1\alpha}$	3.30	30	A	10(x3)	0.65	-	1:8
Progesterone	2.86	30	B	10(x3)	2.2	1:140	1:15

Table 4.

Substance	Working Range of assay pg	Sensitivity of assay pg	Highest value for Intra Assay Coefficient of Variation	Inter Assay Coefficient of Variation
$PGF_{2\alpha}$	50-600	50	15%	8.4%
PGE_2	20-100	20	15%	4.2%
6-oxo-$PGF_{1\alpha}$	30-1000	30	15%	6.1%
Progesterone	35-600	35	15%	7.1%

5. Purification of Tritiated Prostaglandin $F_{2\alpha}$ by Column Chromatography

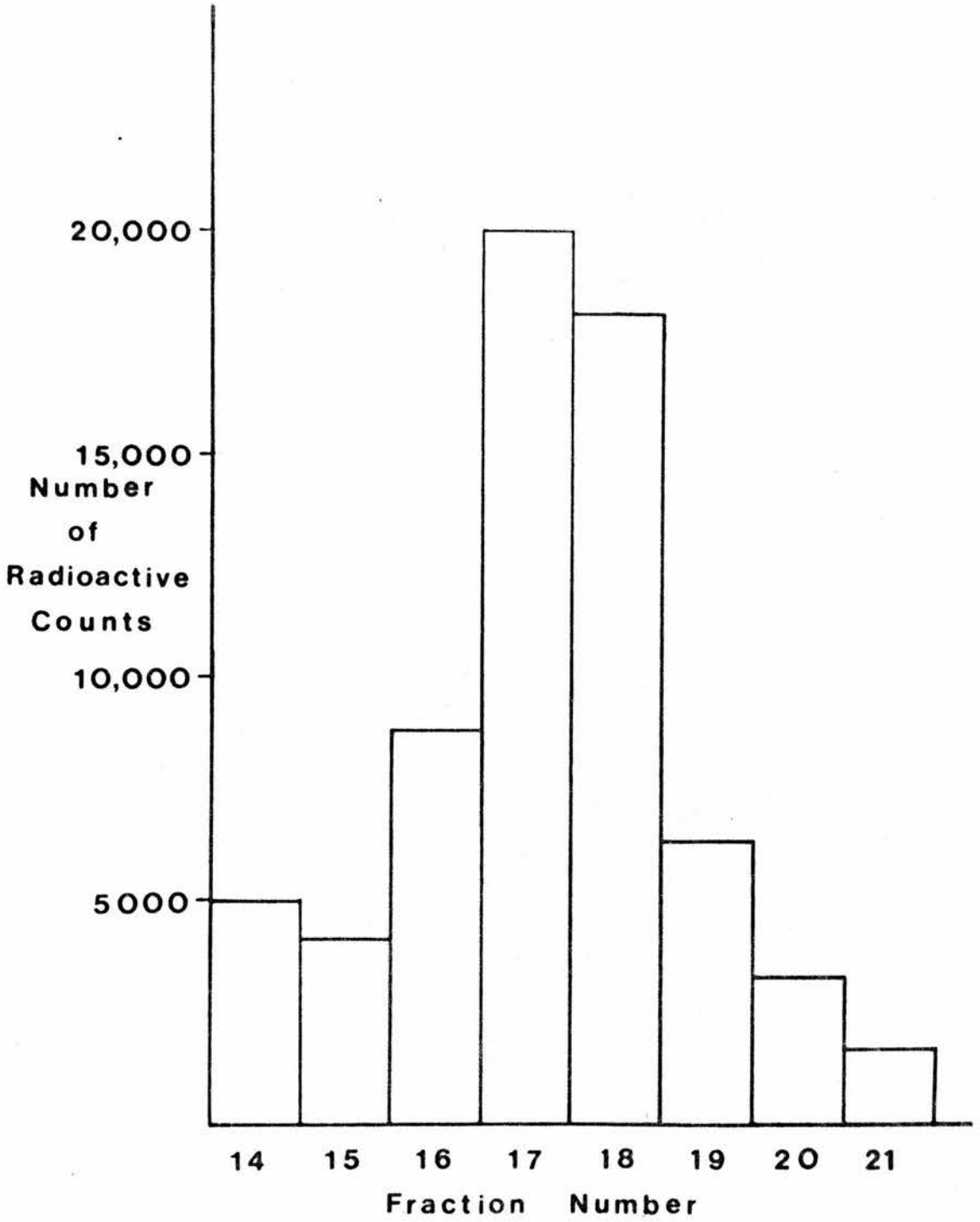
The column used for purification of tritiated $PGF_{2\alpha}$ ($^3HPGF_{2\alpha}$) for RIA, was packed with Lipidex 1000 (31cm x 3cm) and a straight phase solvent system was used to elute the prostaglandins (Brash and Jones 1974).. The column was washed and left to equilibrate overnight in the following solvent mixture: hexane (100): dichloroethane (100): ethanol (15): glacial acetic acid (0.1%). Before using the column, ten drops were taken for liquid scintillation counting to determine the level of "background" radioactivity(this was acceptable at 5% of the fraction containing the highest number of counts).

The radioactive $PGF_{2\alpha}$ was taken to dryness in a pear-shaped flask by rotary evaporation at 40°C. The residue was redissolved in 0.5ml of the solvent mixture and loaded onto the top of the column using a Pasteur pipette. The flask was then rinsed three times with 0.5ml of solvent mixture and each rinse was loaded onto the column. The columm was then run at a flow rate of 10ml/hr and thirty fractions, each of 3.0ml, were collected. A sample of 0.02ml of each fraction was taken and added to a vial containing 5ml of scintillation fluid I and the vials were counted on the scintillation counter for 1 min. A graph of fraction number against number of counts was then plotted (Fig.11). From the graph, the fractions containing $^3HPGF_{2\alpha}$ were determined i.e in this case fraction 16-19. These fractions were pooled and the test tubes were rinsed with methanol which was added to the flask. The pooled fractions were taken to dryness in a rotary evaporator at 40°C and the residue was redissolved in 7.0ml methanol. Three 0.02ml aliquots were taken and added to vials containing 5ml scintillation fluid which were counted using an external standard chemical ratios count for 4 min so the DPM could be calculated. From this value, the amount of radioactivity recovered from the column was determined and more methanol was added to give a final concentration of 5μCi/ml.

Radio-labelled PGE_2 was purified in a similar manner, but using a solvent mixture of: hexane (100): dichloroethane (100): ethanol (10): glacial acetic acid (0.1%). The $^3HPGE_2$ was found to run in fractions 18-21 in this system.

Fig.11.

Purification of radioactive prostaglandin $F_{2\alpha}$ for radio-immuno-assay, by column chromatography.


20,000
15,000
Number
of
Radioactive
Counts
10,000
5000
14
15
16
17
18
19
20
21
Fraction Number

6. Derivitisation and Assay of Prostaglandins by Gas Chromatography-Mass Spectrometry using Multiple Ion Detection.

a. Instrument Details.

The gas chromatograph - mass spectrometer used was a 7070F dual focusing model from VG Micromass Ltd. The gas chromatograph column was 3% OV1 on a diatomaceous skeleton, 100-120 mesh. Helium carrier gas pressure was 15psi. The multiple ion detection (MID) traces were recorded on a six channel Rikadenki (Mitsui Electronics U.K. Ltd.) pen recorder.

b. Assays for Prostaglandin D_2 and Thromboxane B_2

i. Derivitisation

A series of five "standards" of PGD_2 and TXB_2 in methanol were prepared i.e 0, 75, 150, 300 and 600ng/tube and the methyl ester of each was formed by adding 1.0ml diazomethane solution (9 parts diethyl ether: 1 part methanol) and allowing to react for 5 min at room temperature. The solution was then removed under a stream of nitrogen at 45°C. 300ng of the ethyl ester of PGD_2 and 300ng of the ethyl ester of TXB_2 were then added to each tube and the solvent was again removed under a stream of nitrogen at 45°C. The standards were then dessicated under vacuum for 5 min. The n-butyl oximes were formed by adding 3 drops of O-butylhydroxyhydroxylamine hydrochloride (5mg/ml in pyridine) to each tube. The tubes were left overnight at room temperature and heated for 30 min at 60°C the following morning, or alternatively, were heated at 60°C for 3 hr on the same day. Pyridine was then removed under an air jet at 60°C and the standards were placed in a dessicator under vacuum for 15 min. Finally, the trimethyl-silyl ether derivative was formed by adding 25µl bis (trimethylsilyl) -trifluoroacetamide (BSTFA) to each tube and heating at 60°C for 20 min. Standards were injected into the gas chromatograph - mass spectrometer (GC-MS) in volumes of 8µl, each"standard" of PGD_2 and TXB_2, therefore containing 100ng of the ethyl esters of PGD_2 and TXB_2 respectively, as internal standards.

ii. Construction of Standard Curves for PGD_2 and TXB_2

The retention times for the standards on the GC-MS column were recorded . Their carbon values were then determined from a plot of

retention time against the carbon number, of a series of fatty acids injected at the same column temperature. The ion at $^{m}/e$ 420 ($^{+}$M-90,-71) of the methyl ester, butyl oxime, trimethylsilyl ether derivative of PGD_2 (Me.BuO.TMS), carbon value 25.7 was monitored continuously by multiple ion detection (MID). The corresponding ion at $^{m}/e$ 434 of the ethyl ester, butyl oxime, trimethylsilyl ether of PGD_2 (Et.BuO.TMS) carbon value 26.1 was monitored as internal standard. The ion at $^{m}/e$ 301 of TXB_2 (Me.BuO.TMS) carbon value 26.4 was monitored, together with the same ion produced by the Et.BuO.TMS derivative of TXB_2, carbon value 26.9 which was used as internal standard. (See Figs. 12 and 13 for breakdown of molecules). The height of the peaks produced by continuously monitoring these ions was measured and the graph of the ratio of peak heights of standard PGD_2 or TXB_2, to the peak height of internal standard, was plotted against the amount of standard PGD_2 or TXB_2, The standard error of the mean (S.E.M.) for six injections at the mid point of each graph was less than 5% (Figs. 12 and 13).

c. Assay for 6-oxo-Prostaglandin $F_{1\alpha}$ using the 3,3,4,4-tetradeuterated Compound as Internal Standard

A series of "standards" i.e 0, 50, 100, 200, 400 and 800ng/tube of 6-oxo-$PGF_{1\alpha}$ in methanol, were prepared and 400ng of tetradueterated 6-oxo-$PGF_{1\alpha}$ was added to each tube as internal standard. The Me.BuO.TMS derivatives of each "standard" plus deuterated internal standard were prepared as described previously (6bi). Finally, 20µl BSTFA was added to each tube and 5µl were used for injection into the GC-MS. The carbon value for the second isomer of 6-oxo-$PGF_{1\alpha}$ was determined (26.2). The ion at m/e 491 ($^{+}$M-2x90) of 6-oxo-$PGF_{1\alpha}$ Me.BuO.TMS was monitored and the corresponding ion at $^{m}/e$ 495 of the tetradueterated compound was monitored as internal standard. A standard curve was then constructed for 6-oxo-$PGF_{1\alpha}$ (Fig.14) as previously described for PGD_2 and TXB_2.

d. Preparation and Assay of Samples for Gas Chromatography-Mass Spectrometry

A known proportion of each extracted sample (in 10ml ethyl acetate) was taken and evaporated to dryness in an Eppendorf tube at 45°C under a stream of air and then dessicated under vacuum for 5 min. Redistilled methanol (100µl) was added to each tube. Samples to be assayed for PGD_2 and TXB_2 were methylated, using diazomethane, as before. The corresponding ethyl ester (300ng) was added as the internal standard

Fig.12.

Standard curve for assay of prostaglandin D_2 (PGD_2), by gas chromatography - mass spectrometry. Molecular weight of methyl ester, trimethylsilyl ether, butyl oxime derivative = 581. Carbon value = 25.7. Multiple ion detection unit focused on the exact mass of the ion at $^{m}/_{e}$ 420 (^{+}M - 90, - 71) and the corresponding ion at $^{m}/_{e}$ 434 of the ethyl ester, trimethylsilyl ether, butyl oxime used as internal standard, carbon value = 26.1.

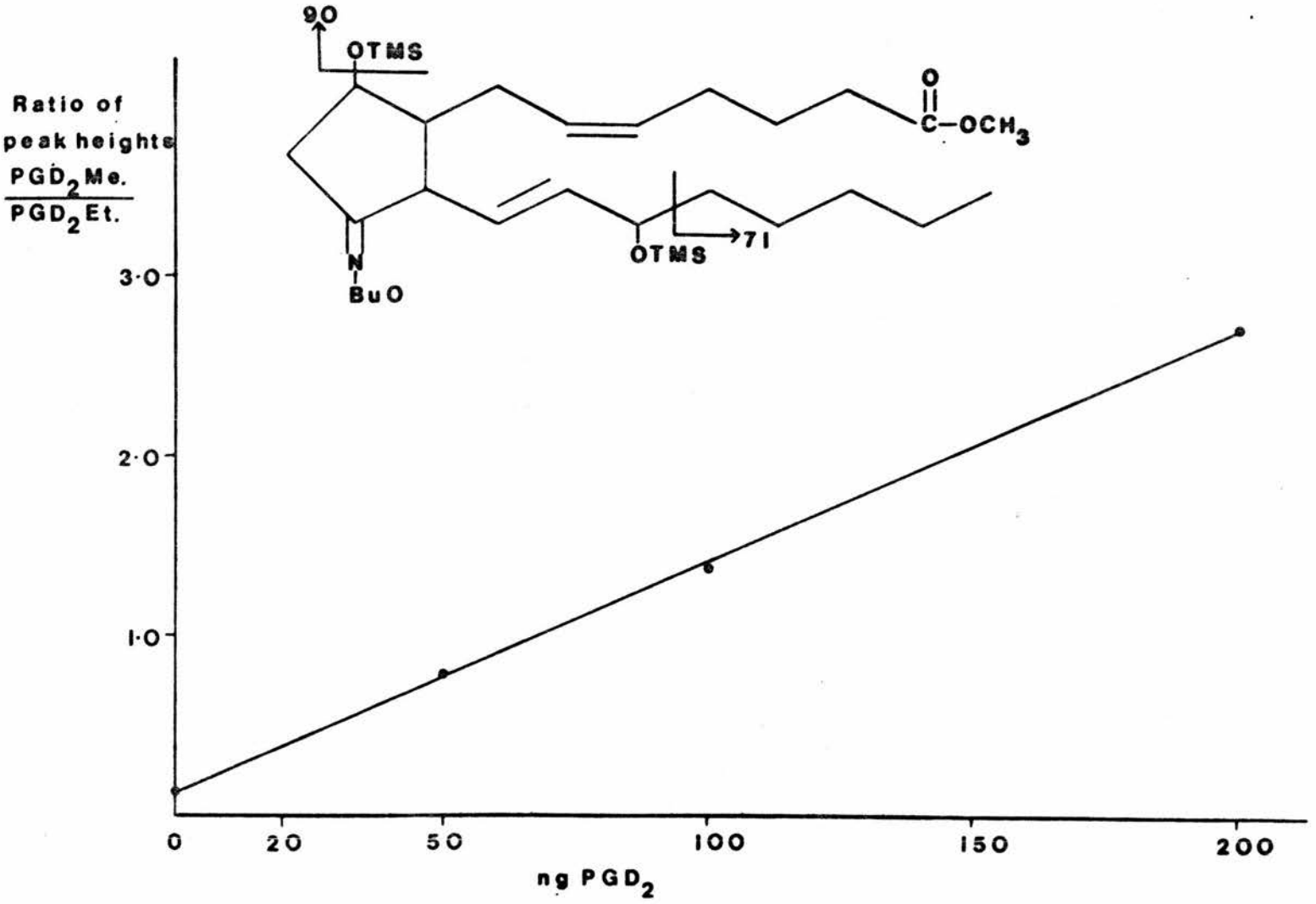

90
OTMS
O
C–OCH3
N
BuO
OTMS
71
Ratio of
peak heights
PGD2 Me.
PGD2 Et.
3·0
2·0
1·0
0
20
50
100
150
200
ng PGD2

Fig.13.

Standard curve for assay of thromboxane B_2 (TXB_2), by gas chromatography-mass spectrometry. Molecular weight of methyl ester, trimethylsilyl ether, butyl oxime derivative = 671. Carbon value = 26.4. Multiple ion detection unit focused on the exact mass of the ion at $^m/_e$ 301 of the methyl ester and also of the ethyl ester used as internal standard, carbon value = 26.9.

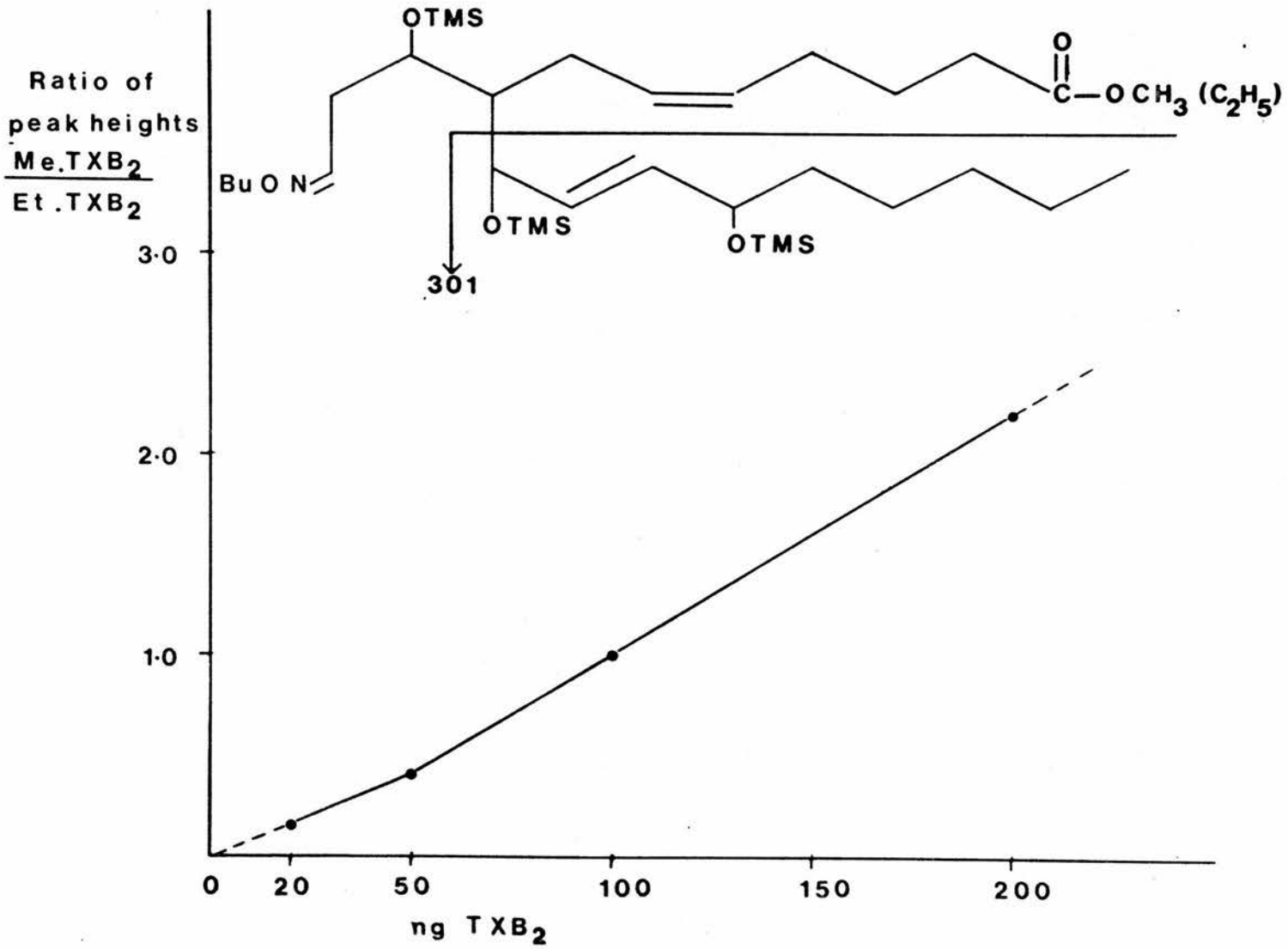
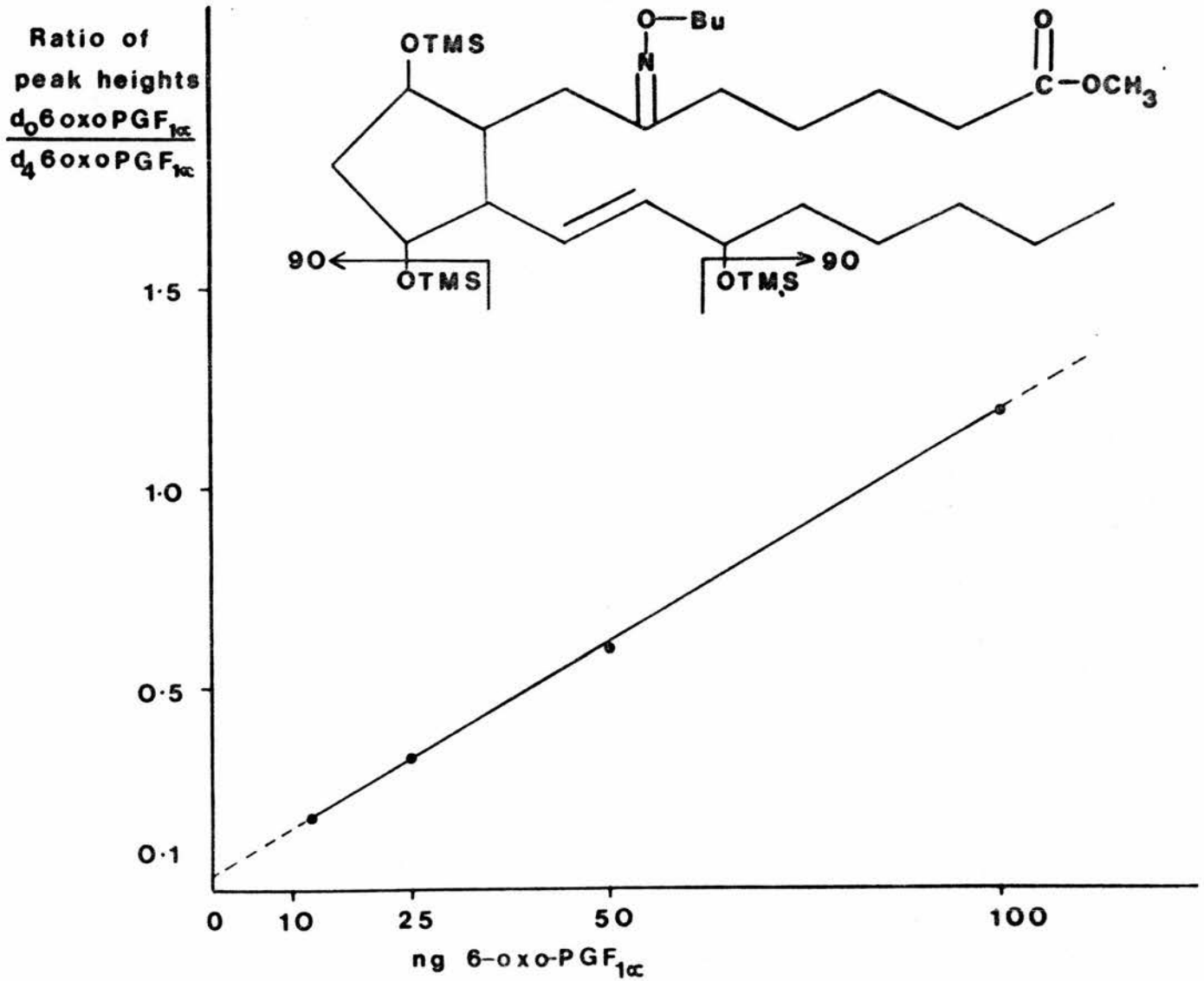

OTMS
O
C—O CH3 (C2H5)
Bu O N
OTMS
OTMS
301
Ratio of
peak heights
Me.TXB2
Et .TXB2
3·0
2·0
1·0
0
20
50
100
150
200
ng TXB2

Fig.14.

Standard curve for the assay of 6-oxo-prostaglandin $F_{1\alpha}$ (6-oxo-$PGF_{1\alpha}$) by gas chromatography-mass spectrometry. Molecular weight of the methyl ester, trimethylsilyl ether, butyl oxime derivative = 671. Carbon value = 26.2. Multiple ion detection unit focused on the exact mass of the ion at $^{m}/e$ 491 ($^{+}M - 2 \times 90$) and the corresponding ion at $^{m}/e$ 495 of the 3, 3, 4, 4-tetradeuterated compound used as internal standard, carbon value 26.2.


Ratio of
peak heights
$\frac{d_0\ 6oxoPGF_{1\alpha}}{d_4\ 6oxoPGF_{1\alpha}}$
OTMS
O—Bu
N
O
C—OCH3
90
OTMS
OTMS
90
1·5
1·0
0·5
0·1
0
10
25
50
100
ng 6-oxo-PGF1α

and the n-butyl oxime trimethylsilyl ether derivatives were formed as described for the "standards". Samples to be assayed for 6-oxo-$PGF_{1\alpha}$ were derivitised after the addition of 400ng of the deuterated compound. Samples for PGD_2 and TXB_2 assay were dissolved in a final volume of 25µl BSTFA and 8µl was used per injection into the GC-MS. Samples for 6-oxo-PGF_1 assay were dissolved in a final volume of 20µl BSTFA and 5µl/injection was used.

A standard curve was constructed for each assay as described previously (6bii). The peak height of each ion monitored was measured and the ratio of this, to the peak height of the monitored ion of the known amount of internal standard, was calculated. By reading this ratio off from the standard curve which had been constructed, the amount of prostaglandin in the tube could be determined and hence, the total amount in the original sample could be calculated.

Due to small changes in the sensitivity of the GC-MS from day to day, standard lines obtained differed slightly between assays. These small variations are compensated for however, by the construction of a new standard line for each assay, using the same range of standard concentrations and by adding the same amount of internal standard to each of the "standard" tubes and each of the assay tubes. The standard error of the mean, calculated from six injections at the mid-point of each of the standard lines (Figs. 12, 13 and 14) was less than 5% for each assay.

7. Assay for Prostaglandin D_2 using a method of Platelet Aggregation

Prostaglandin D_2 (PGD_2) released from the pregnant rat uterus *in vitro* (see section II), was also measured, after solvent extraction, by a method of platelet aggregation.

Blood was obtained from human volunteers and collected into citrated dextrose (1ml/5ml blood). The blood was then centrifuged at 600 x g for 15 min at room temperature using a Multex MSE centrifuge. After centrifugation, the supernatent platelet rich plasma (PRP) was siphoned off.

0.5ml PRP was placed in a cuvette and diluted with 0.45ml of 0.9% saline. Maximal aggregation was obtained by addition of 0.05ml adenosine diphosphate (ADP) to give a final concentration in the cuvette of 5×10^{-6}M (Fig.15). Aggregation was measured by the turbidometric method of Born (1962) using an Upchurch B1037346 aggregometer coupled to a Vitatron pen recorder.

Fig.15.

Aggregation of platelets induced by adenosine diphosphate (ADP at 5×10^{-6} M) added at arrows and inhibited by 2 min pre-incubation with PGD_2. a = 5×10^{-6}M ADP, b = 5ng PGD_2 + 5×10^{-6}M ADP, c = 10ng PGD_2 + 5×10^{-6}M ADP, d = 20ng PGD_2 + 5×10^{-6} M ADP.

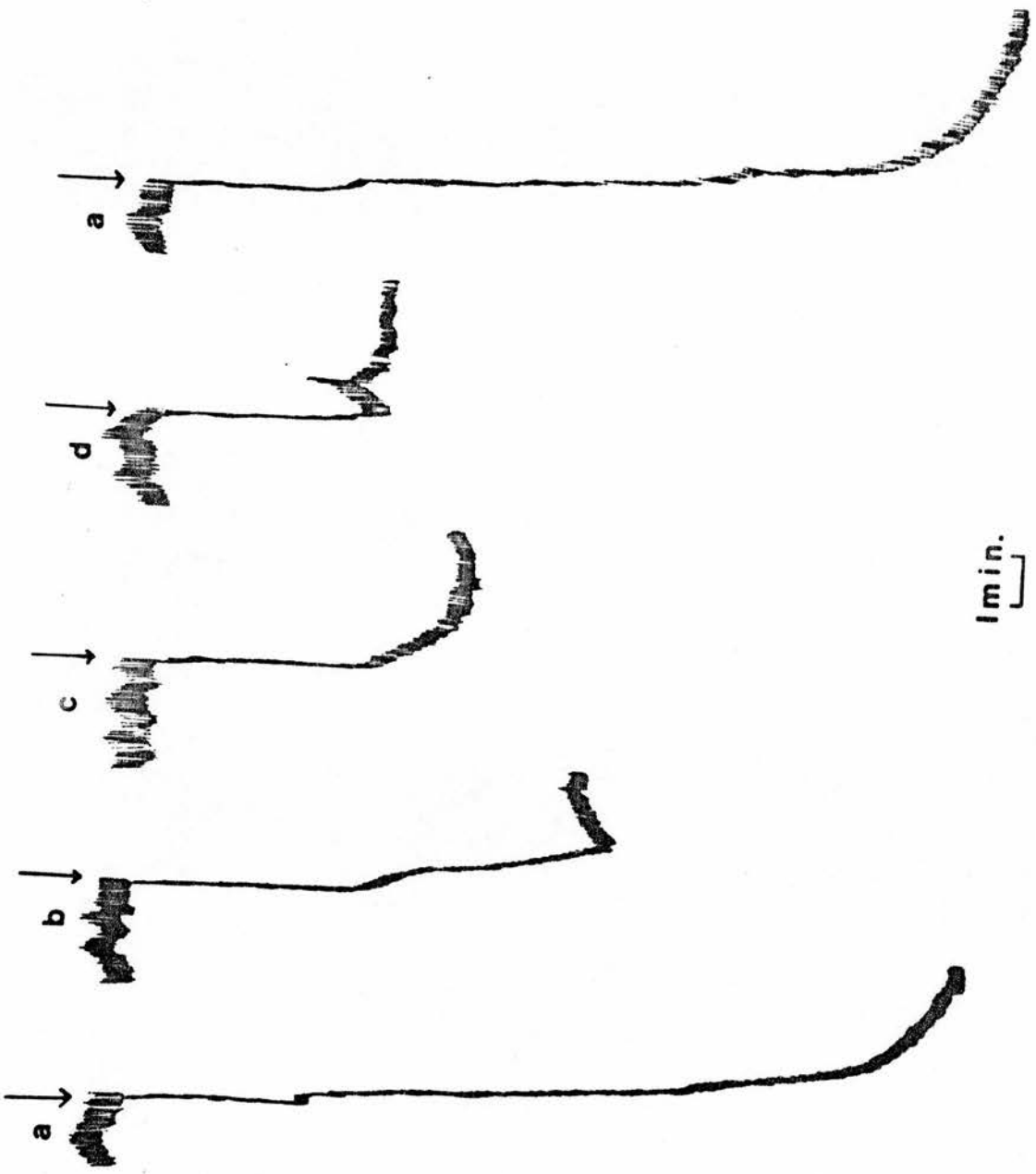
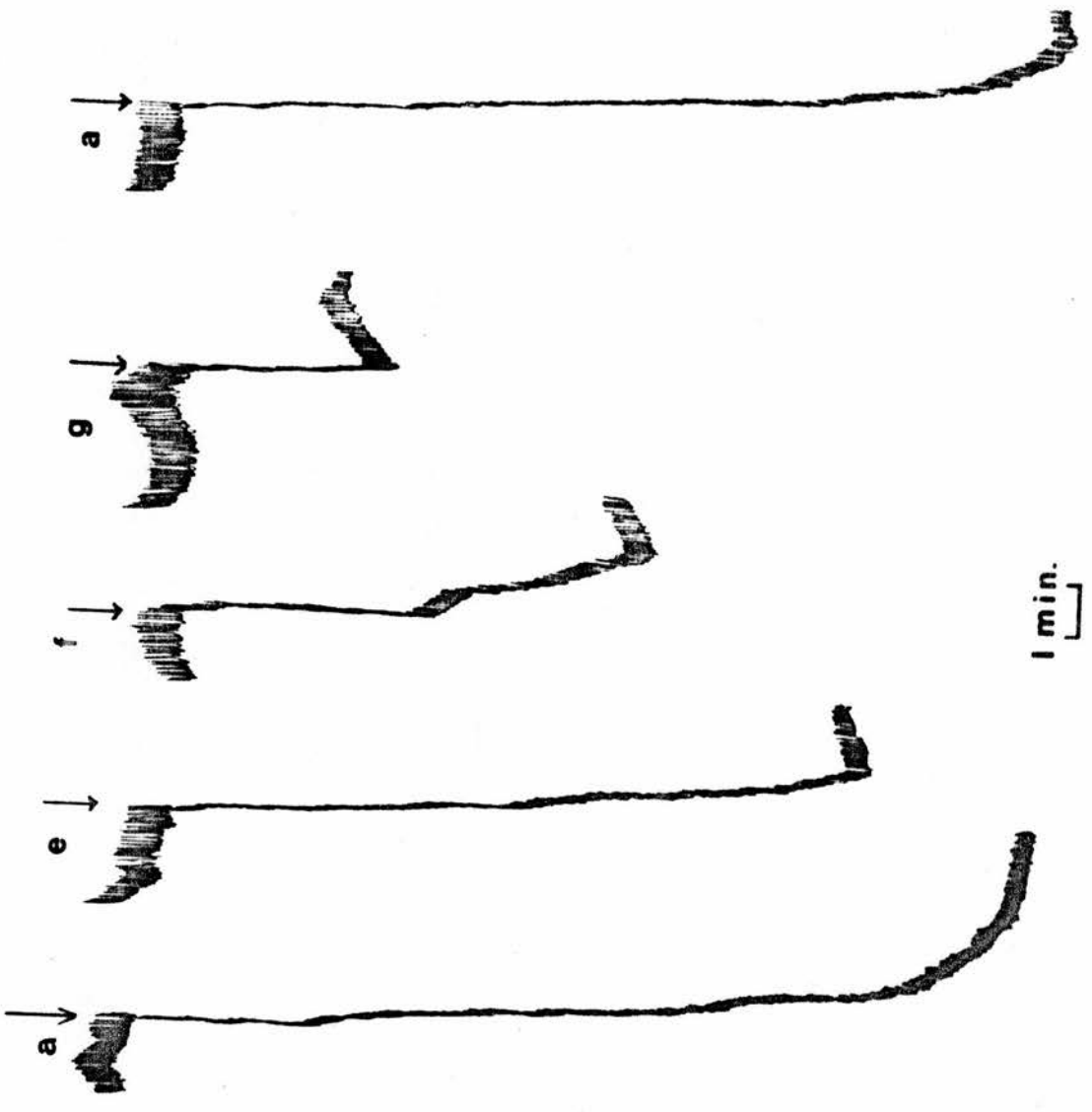


Fig.16.

Platelet aggregation induced by ADP (5×10^{-6}M) added at arrows and inhibited with proportions of sample extraced from bath fluid from a day 22 pregnant uterine horn *in vitro*. a = 5×10^{-6}M ADP, e = 20µl sample + 5×10^{-6}M ADP, f = 50µl sample + 5×10^{-6}M ADP, g = 100µl sample + 5×10^{-6}M ADP. Samples of extracted bath fluid added 2 min prior to ADP.


a
g
f
e
a
1 min.

ADP-induced platelet aggregation is inhibited by PGD_2 in a dose-dependent manner (Mills and Macfarlane 1977). A dose-response curve of the percentage inhibition of aggregation caused by $5x10^{-6}$M ADP, against the dose of PGD_2 added, was set up. PGD_2, dissolved in 0.9% saline, was added to the cuvette in a volume of 0.05ml and incubated with stirring at 37°C for 2 min prior to addition of ADP (Fig.15). A known amount of the solvent extracted sample, to be assayed for PGD_2 was taken and the ethyl acetate removed under an air stream at 45°C. The sample was then diluted with 0.9% saline and 0.05ml of each dilution was incubated with the platelets (as for standard PGD_2) until two dilutions were found which corresponded to two doses of PGD_2. (Fig.16). The total amount of PGD_2 contained in each sample could then be calculated.

The results obtained by this method are given in Section II, table 5, and are compared to those obtained by GC-MS. Values estimated by platelet aggregation are in the same range as those obtained by GC-MS and there is no significant difference between the two sets of results (Student's "t" test).

SECTION II

PROSTAGLANDINS, THROMBOXANES AND THE PREGNANT RAT UTERUS AT TERM

Introduction

The uterus from a day 22 pregnant rat, when removed and freed of conceptuses, exhibits spontaneous activity when suspended in an organ bath filled with Krebs' solution. $PGF_{2\alpha}$ and PGE_2 are released into the bathing fluid and the amounts of $PGF_{2\alpha}$ released have been measured (Vane and Williams 1973). The spontaneous contractions of the uterus and the release of prostaglandins are inhibited by the addition of prostaglandin synthetase (cyclo-oxygenase) inhibitors such as indomethacin and meclofenamate, to the organ bath. Contractions can be restored by the addition of PGE_2 and $PGF_{2\alpha}$, with PGE_2 being the more potent. Since the output of PGE_2 and $PGF_{2\alpha}$ and the spontaneous contractions in this in vitro system are greater on day 22 (the day of parturition) than on earlier days of pregnancy, a role for these prostaglandins in parturition in the rat has been suggested. However, these studies were performed before thromboxanes and prostacyclin (PGI_2) were discovered. Consequently, the following experiments were performed to measure the release of prostacyclin and thromboxane, in addition to the classical prostaglandins, from the pregnant rat uterus at term.

The more accurate methods of radio-immuno-assay (RIA) and gas chromatography-mass spectrometry (GC-MS) rather than bioassay were used for analysis. The effect of the cyclo-oxygenase inhibitor, indomethacin and the prostacyclin synthetase inhibitors, 15-hydroperoxy arachidonic acid (15-OOHAA) and tranylcypromine, on contractions and prostaglandin release has also been examined.

Methods

1. The Release of Prostaglandins from the day 22 Pregnant Rat Uterus in vitro

Mature female Wister rats were caged with a light cycle of 12 hr day and 12 hr night, light changes occurring at 08.00 hr and 20.00 hr. They received food and water ad libitum. Vaginal smears were taken daily and examined microscopically. Day 1 of the oestrous cycle was taken as the day of maximum cornification, preceding the day of leucccytic infiltration. All rats used exhibited regular 4 day cycles. The female rats were mated singly by placing them with male rats of proven fertility on the afternoon of day 4 of the oestrous cycle. Mating was assumed to have taken place during the dark period

between day 4 and day 1. This latter day became day 1 of the pregnancy and was confirmed by the appearance of spermatozoa in the vaginal smear.

Three rats were allowed to go to term and were found to deliver during the dark period between day 22 and day 23. Twelve rats for experimental purposes were used on the morning of day 22 of pregnancy. The animal was killed by cervical dislocation and the uterus quickly removed and placed in Kreb's solution. The uterus was divided into two horns which were cut longitudinally and freed of foetuses and placentae. Each horn was dried, weighed and suspended in 80ml organ bath containing Kreb's solution at 37°C bubbled with a mixture of oxygen (95%) and carbon dioxide (5%). A tension of 2g was applied to each uterine horn. Isotonic contractions were recorded using a Vitatron pen recorder for four consecutive periods of 15 min. After each 15 min period, the bath fluid surrounding each uterine horn was removed and collected and the bath refilled. The four fractions from each horn were pooled and the prostaglandins extracted (see Basic Methods Section I). The amounts of prostaglandins in the extract were then measured either by RIA (PGE_2, $PGF_{2\alpha}$ and 6-oxo-$PGF_{1\alpha}$) or by GC-MS (PGD_2, TXB_2) and PGD_2 was also measured by the platelet aggregation method (see Basic Methods Section I). The presence of PGE_2 and $PGF_{2\alpha}$ were confirmed by GC-MS by monitoring the ions at $^m/_e$ 295 and 423 respectively at the carbon values obtained for standards of PGE_2 (25.9) and $PGF_{2\alpha}$ (23.9).

In one experiment, the effect of indomethacin on the release of PGF_2 and PGE_2 was investigated. Fluid from a 15 min control period was collected and then fluid from 2 x 15 min periods in the presence of 0.6 and 1.2µg/ml indomethacin, respectively. Each sample was solvent extracted and prostaglandins measured by RIA.

In three experiments the release of prostaglandins from the term pregnant uterus (PGE_2, $PGF_{2\alpha}$ and 6-oxo-$PGF_{1\alpha}$ measured by RIA), over four consecutive 15 min periods was determined, in order to ascertain whether or not the rate of release of prostaglandins altered over the four periods of time, as this may have affected the interpretation of results of experiments performed using prostaglandin synthesis inhibitors.

2. <u>The Effects of Prostaglandins on the Day 22 Pregnant Rat Uterus in vitro</u>

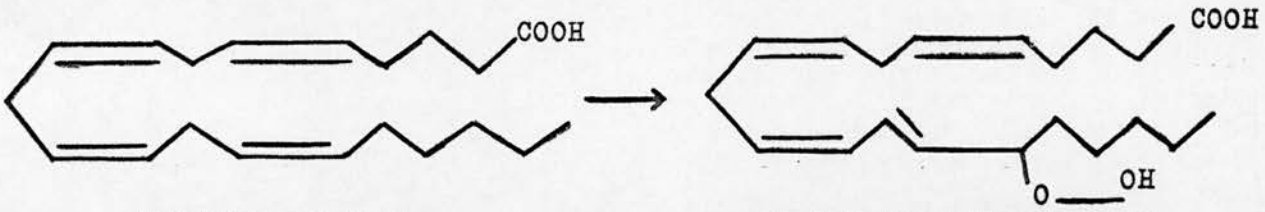
In some experiments, one uterine horn was kept as a control, while indomethacin (5-10µg/ml) was added to the bath containing the second uterine horn to render it quiescent (Vane and Williams 1973).

The effects of prostaglandins added to the bathing fluid of the quiescent horn were then recorded. Prostaglandins were dissolved 0.9% saline at pH 7.5 and added to the bath fluid in volumes up to 1.0ml. Each dose of prostaglandin was tested two or three times on a minimum of three preparations.

3. The Effects of 15-hydroperoxy Arachidonic Acid and Tranylcypromine on the Day 22 Pregnant Rat Uterus in vitro

a. Preparation of 15-hydroperoxy Arachidonic Acid (15-OOHAA)

The product is prepared by the action of the soya bean lipoxygenase enzyme on arachidonic acid:-



Arachidonic Acid 15-OOH Arachidonic Acid

The generation of a conjugated diene system allows the reaction to be followed by UV light absorption at 234nm, and similary the product can be quantified.

The compound is a rather unstable hydroperoxy acid which can be reduced to the hydroxy compound with stannous chloride in water.

A solution (0.5mg/ml) of soya bean lipoxygenase was prepared in 0.1M triphosphate buffer (Tris) pH 8.0 and 0.5ml of the enzyme solution was added to 150ml Tris buffer containing 5-10mg arachidonic acid. The mixture was stirred at room temperature for 15 min until the reaction was completed (monitored by UV light at 234 nm). The pH was reduced to 4.0 with $^{N}/_{10}$ hydrochloric acid (HCl) and the reaction mixture was shaken twice with an equal volume of ether. The ether extracts were pooled. The ether was then washed with 0.2 x the volume of water, the ether was then separated and dried with the minimum quantity of anhydrous sodium sulphate, which was removed by filtration. The ether was evaporated at room temperature on a rotary evaporator.

and the residue was redissolved in 1.0ml hexane for further purification by high pressure liquid chromatography (HPLC). The separation of 15-OOHAA from any unconverted arachidonic acid and any of the hydroxy compound which may have formed was thus achieved.

The solvent system used for HPLC was hexane and isopropanol in the ratio of 10:1 with 0.1% glacial acetic acid added. The column was packed with Partisil PAC and the HPLC was performed on an 848 Pump Model from Du Pont Instruments. The column flow rate was 2ml/min. A maximum of 50µl of the extract was injected onto the column each time. The compounds were detected by UV absorption at 234nm (Fig 17a). The first peak to appear was the hydroxy compound ($3\frac{1}{2}$ min) followed by the hydroperoxy 2 min later. The second peak was collected and quantified by UV absorption. (The extinction coefficient E for a 1M solution of 15-OOHAA is 25000)

$$1M = 334g/l = 334mg/ml = 25000$$

$$\text{therefore } 334\mu g/ml = 25$$

where α = Extinction value obtained

$$\text{therefore } \frac{\alpha}{25} \times 334 = \text{Concentration being measured } \mu g/ml$$

The 15-OOHAA was stored in methanol at-20°C before use. It was found to remain stable under these conditions for at least 2 weeks.

In order to verify that the product collected was authentic, a known volume was removed after HPLC separation and shaken with a saturated solution of stannous chloride in water for 5-10 min. The hexane fraction was then separated and subjected to HPLC to observe the change in retention time from that of the hydroperoxy to that of the hydroxy compound (Figs. 17a and 17b). The 15-OHAA was then collected and derivitised for GC-MS.

b. Derivitisation of 15-OH Arachidonic Acid for GC-MS

The methyl ester, trimethylsilyl ether derivative of 15-OHAA was formed, as previously described for prostaglandins and the derivative was then subjected to GC-MS using a 3% OVI column. The Me.TMS derivative of 15-OHAA was found to have a carbon value of 21.4. The structural analysis is shown in Fig.18, along with the mass spectra obtained. The mass ion (M^+) is at $^m/_e$ 406 and the major (base) peak was found at $^m/_e$ 225 resulting from cleavage of the omega chain. Peaks were also obtained at $^m/_e$173 (M-233) and $^m/_e$ 335 (M-71). Other fragmentations are due to loss of TMSOH (M-90)

Fig. 17 a and b.

Arrows indicate injection points.

Fig.17a (bottom of page). Separation of (a) 15-hydroxy arachidonic acid (15-OHAA) from (b) 15-hydroperoxy arachidonic acid (15-OOHAA) by high pressure liquid chromatography (HPLC) and detection by UV light absorption at wavelength 234 nm.

Fig.17b (top of page). HPLC trace obtained after reduction of 15-OOHAA to 15-OHAA, (peak a), with stannous chloride.

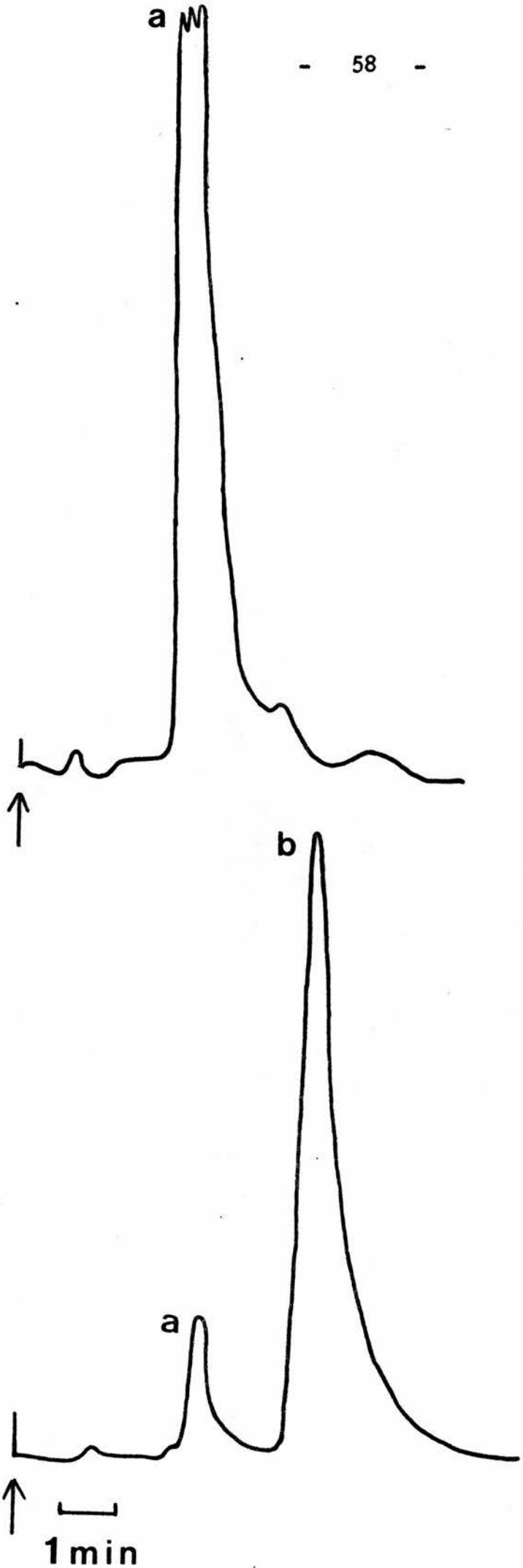
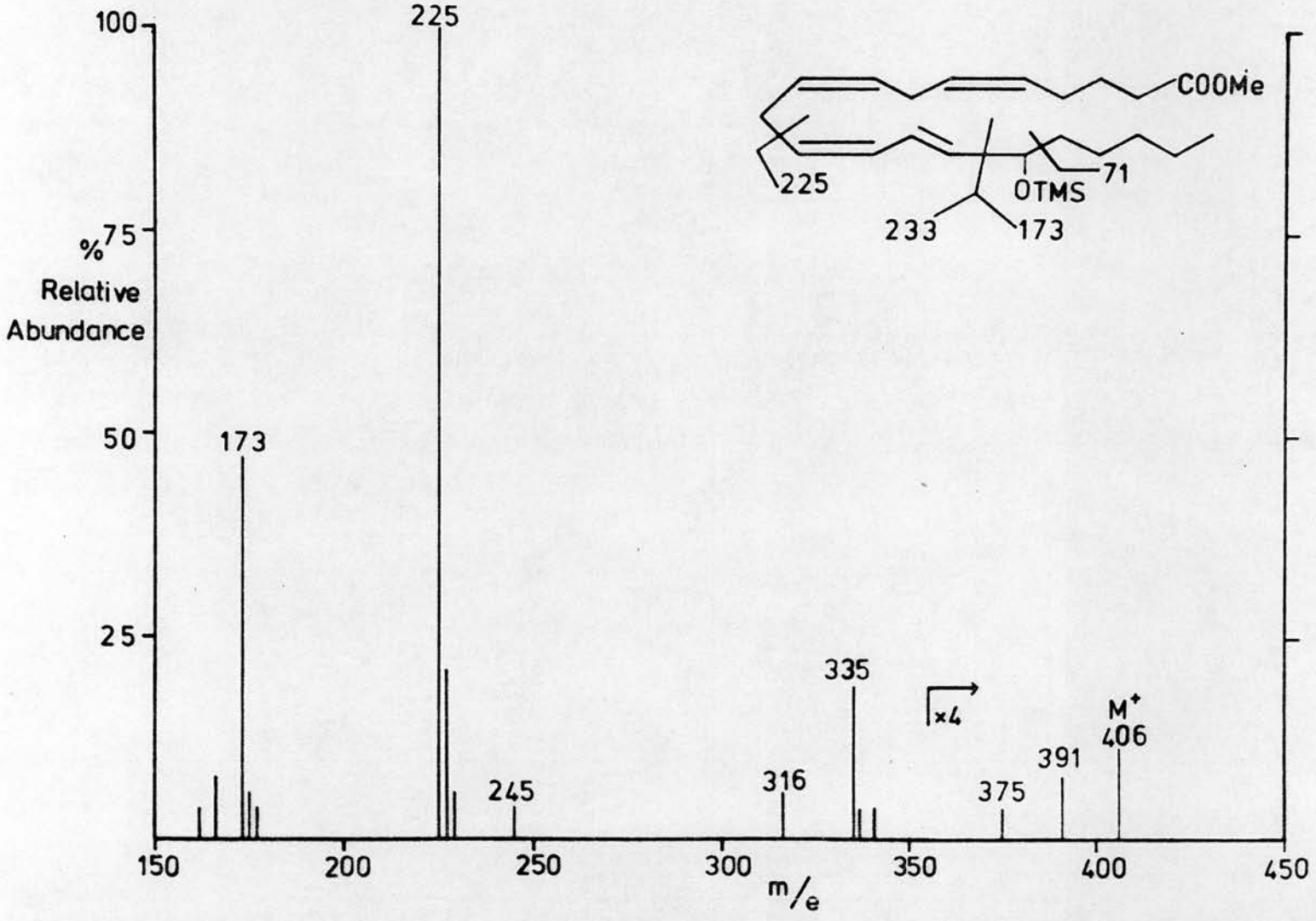

a
b
a
1 min

Fig.18.

Mass-spectrum of the methyl ester, trimethylsilyl ether of 15-hydroxy arachidonic acid, carbon value = 21.4. Molecular ion at $^{m}/_{e}$ 406. Ordinate scale: relative abundance of ions as % of base peak at $^{m}/_{e}$ 225; abscissa scale: ratio of mass (m) to charge (e), where e = 1.


100
75
50
25
% Relative Abundance
225
173
245
316
335
×4
375
391
M+
406
150
200
250
300
350
400
450
m/e
COOMe
225
OTMS
71
233
173

at $^{m}/_{e}$ 316 and (M-90-71) at $^{m}/_{e}$ 245.

Thus, the compound obtained from the action of soya bean lipoxygenase on arachidonic acid was shown to be 15-OOHAA by: reduction to the hydroxy compound with stannous chloride, shown by a change in retention time to that of the hydroxy using HPLC and detected by UV absorption. The structure of the substance thereby obtained was proved to be that of 15-OHAA by GC-MS.

c. The Effects of 15-Hydroperoxy Arachidonic Acid and Tranlcypromine on the Day 22 Pregnant Rat Uterus in vitro

The bath fluid from a 15 min control period of contractions, was collected and the bath refilled. 15-OOHAA dissolved in 0.2 - 0.6ml polyethylene glycol (PEG) or tranylcypromine dissolved in 0.9% saline pH 7.5, was then added to the bath and the effects recorded over a 15 min period. The bath fluid surrounding the uterus was collected for extraction of prostaglandins at the end of this 15 min period. Three doses of 15-OOHAA were used, 25, 30 and 40µg/ml. Tranylcypromine was used at 500 µg/ml. It was found that up to 0.6ml PEG could be added to the bath without any effect on the tissue. Saline alone, also did not effect the tissue.The number of uterine horns used was 3 or 4 per group.

d. The Removal of 15-Hydroperoxy Arachidonic Acid from Extracted Samples

The samples of extracted bath fluid were to be assayed for prostaglandins using RIA. It was therefore necessary to establish the cross-reactivity of 15-OOHAA with the antibodies used for RIA, as any of this compound which was extracted along with the prostaglandins, may have interfered with the assays.

The cross reactivity of 15-OOHAA with the $PGF_{2\alpha}$ and PGE_2 antibodies was found to be very low at 10% drop from the "0" standard (0.09% and 0.005% respectively). However, at the concentrations used for experiments, it may still have interfered with the RIA. It was therefore thought necessary to remove the 15-OOHAA from the solvent-extracted samples, by column chromatography, before performing the RIA. It is not necessary to remove the 15-OOHAA before GC-MS as the compounds are separated on the GC column. Tranylcypromine does not cross react with the RIA antibodies.

A silicic acid column was used to separate the 15-OOHAA from prostaglandins. The following three solvent fractions were used for elution.

Fraction	Toluene	Ethyl Acetate	Methanol	Total Volume(ml)
FI	80	20	-	150
FII	50	48	2.0	150
FIII	50	48	2.0	50

4g of silicic acid were weighed out and allowed to soak in a small volume of FI. The column was then packed and left to equilibrate overnight in FI.

In order to determine if the 15-OOHAA could be removed in FI, 1μCi of C^{14} arachidonic acid was added to the incubation mixture for the preparation of 15-OOHAA (see this Section, 3a). After separation and quantitation, the 15-OOHAA ("cold" plus radio labelled) was dissolved in 1.0ml FI and loaded onto the column using a Pasteur pipette. The flask was washed three times with 0.5ml FI which was also loaded onto the column. The column was then run under gravity at a flow rate of 1 drop/second and the three fractions were collected. After column elution, three 100μl aliquots of each fraction were added to vials containing 10ml scintillation fluid (PCS cocktail/toluene: 2/1) and counted for 10 min on a liquid scintillation counter. The results are given below:-

Fraction	Total Counts Per Minute (CPM)	% of Total Radioactivity Recovered
FI	456720	81
FII	8400	15
FIII	2200	3

Therefore, 81% of 15-OOHAA recovered from the column was found in FI.

e. To Determine the Elution of Prostaglandins by Fractions used in the Separation of 15-OOHAA from Extracted Samples

The column was again allowed to equilibrate overnight in FI. The following morning, 0.5ml of FI containing 0.66 μCi of tritium labelled (^3H) PGE_2 and 10ug "cold" PGE_2, was loaded onto the column. The column was run as previously described and the three fractions were collected. Each fraction was evaporated to dryness using a rotary evaporator at 40°C and the residue was redissolved in 1ml methanol and 100μl of each fraction added to vials containing 10ml PCS/toluene scintillation fluid. Three counting standards containing

0.026μCi of $^{3}HPGE_2$ in 20μl methanol were set up and the vials were counted using an external standards chemical ratios channel for 4 min. The results were as follows:-

	Number of Disintegrations Per Minute (DPM)	Efficiency of Counting	Total Radioactivity DPM	% Recovery of Radioactivity Added to Column
Counting Standards	64272.5	32.194%		
	68955.9	32.824%	1685375	
	69016.6	32.753%		
FI	3419.41	32.411%		
	3922.07	32.432%	37972.1	2.25%
	4050.14	32.604%		
FII	86413.1	32.334%		
	102198	31.904%	983400	58%
	106409	32.446%		
FIII	2515.18	32.205%		
	2683.51	32.318%	28951.7	2%
	3487.45	24.724%		

Therefore, 58% of radio-labelled PGE_2 was recovered in FII and 2% in FIII. It was assumed that 2% methanol was sufficient to elute all prostaglandins from the column. In all experiments, FII and FIII were pooled before measurement of prostaglandins.

In order to determine the recovery of prostaglandins from the columns, samples of extracted bath fluid from the day 22 pregnant uteri were assayed for prostaglandins before and after chromatography. Prostaglandins E_2, $F_{2\alpha}$ and 6-oxo-$F_{1\alpha}$ were measured by RIA and PGD_2 and TXB_2 were measured by GC-MS. Results for these recoveries are given below:-

Prostaglandin	% Recovery from Silicic Acid Column
$PGF_{2\alpha}$	30
6-oxo-$PGF_{1\alpha}$	30
PGE_2	85
PGD_2	80
TXB_2	36

The low recoveries obtained from $PGF_{2\alpha}$, 6-oxo-$PGF_{1\alpha}$ and TXB_2 suggest that not all of the prostaglandins were eluted by the solvent mixtures from the columns. A more polar mixture may have improved recoveries. In experiments to investigate the effects of 15-OOHAA and tranylcypromine, all control and treated samples were subjected to column chromatography. The results have not been corrected for recoveries since I was chiefly concerned with comparing the amounts of prostaglandins and TXB_2 produced by treated compared with untreated uterine horns and not with absolute amounts released, as these latter parameters have already been measured. Obviously, an estimate of the gross amounts can be obtained by multiplying the uncorrected value by the mean recovery. It was not possible to calculate the recoveries of all prostaglandins by using internal radioactive compounds, since only tritiated and radioactive carbon labelled prostaglandins were available, which is an insufficient number to correct for all the prostaglandins and thromboxane.

Prostaglandins in extracted samples were measured by RIA ($PGF_{2\alpha}$, PGE_2, 6-oxo-$PGF_{1\alpha}$) or by GC-MS (PGD_2). TXB_2 was also detected by GC-MS but was below the limits of sensitivity of the assay.

In three experiments, the effects of 15-OOHAA on the release of 6-oxo-$PGF_{1\alpha}$ as measured by GC-MS, was investigated. These were performed to compare the results with those obtained when the 6-oxo-$PGF_{1\alpha}$ was measured by RIA. Bath fluid was collected from one control uterine horn and one treated with 30µg/ml 15-OOHAA, after a 30 min period. Bath fluid samples were then extracted and release of PGI_2 from the control and treated horns was compared, the prostaglandins being measured as its metabolite 6-oxo-$PGF_{1\alpha}$, by GC-MS.

Results

1. The Release of Prostaglandins from the Term Pregnant Rat Uterus in vitro

The amounts of prostaglandins and thromboxane released from the day 22 pregnant rat uterus are shown in Fig.19. Prostaglandin D_2 was found to be released in the greatest quantities with a mean value ($\pm$ standard error) of 655 ± 141ng/g/hr. Thromboxane B_2 (313 ± 35ng/g/hr) and the PGI_2 metabolite, 6-oxo-$PGF_{1\alpha}$ (229 ± 28ng/g/hr) were also released in large quantities and $PGF_{2\alpha}$ (129 ± 59ng/g/hr) and PGE_2 (30 ± 11ng/g/hr) were released in smaller amounts. An example of the trace obtained after subjecting the bath fluid sample to GC-MS using MID, is given in Fig.20.

The value quoted above for PGD_2 is from results obtained by GC-MS. Some samples were assayed for PGD_2 by the method platelet aggregation as described in Section I, in addition to GC-MS assay. The two sets of results are given in the following table :-

Table 5 Release of PGD_2 from the Term Pregnant Rat Uterus in vitro

Sample	PGD_2 Release ng/g/hr	
	GC-MS	Platelet Aggregation
1	1200	1333
2	329	388
3	463	500
4	684	842
5	597	323
Mean $\pm$ S.E.M.	655 ± 141	677 ± 187

Therewas no significant difference between the values obtained by either assay method.

2. The Effects of Prostaglandins and Thromboxane on the Day 22 Pregnant Rat Uterus in vitro

The spontaneous contractions of the uterus were inhibited by adding 5-10µg/ml indomethacin to the bathing fluid. Contractions could be restored by addition of prostaglandins to the organ bath. Prostaglandin E_2 was the most potent spasmogen, restoring contractions at 2ng/ml. $PGF_{2\alpha}$ was effective at 8ng/ml and PGI_2 at 24ng/ml. It was found that the uterus could be sensitised by addition of 8ng/ml

Fig.19.

The release of prostaglandins and thromboxane from the day 22 pregnant rat uterus *in vitro*. PGE_2, $PGF_{2\alpha}$ and 6-oxo-$PGF_{1\alpha}$, were measured by radio-immuno-assay. PGD_2 and TXB_2 were measured by gas chromatography-mass spectrometry. Number of animals = 12. Mean ± S.E.M.

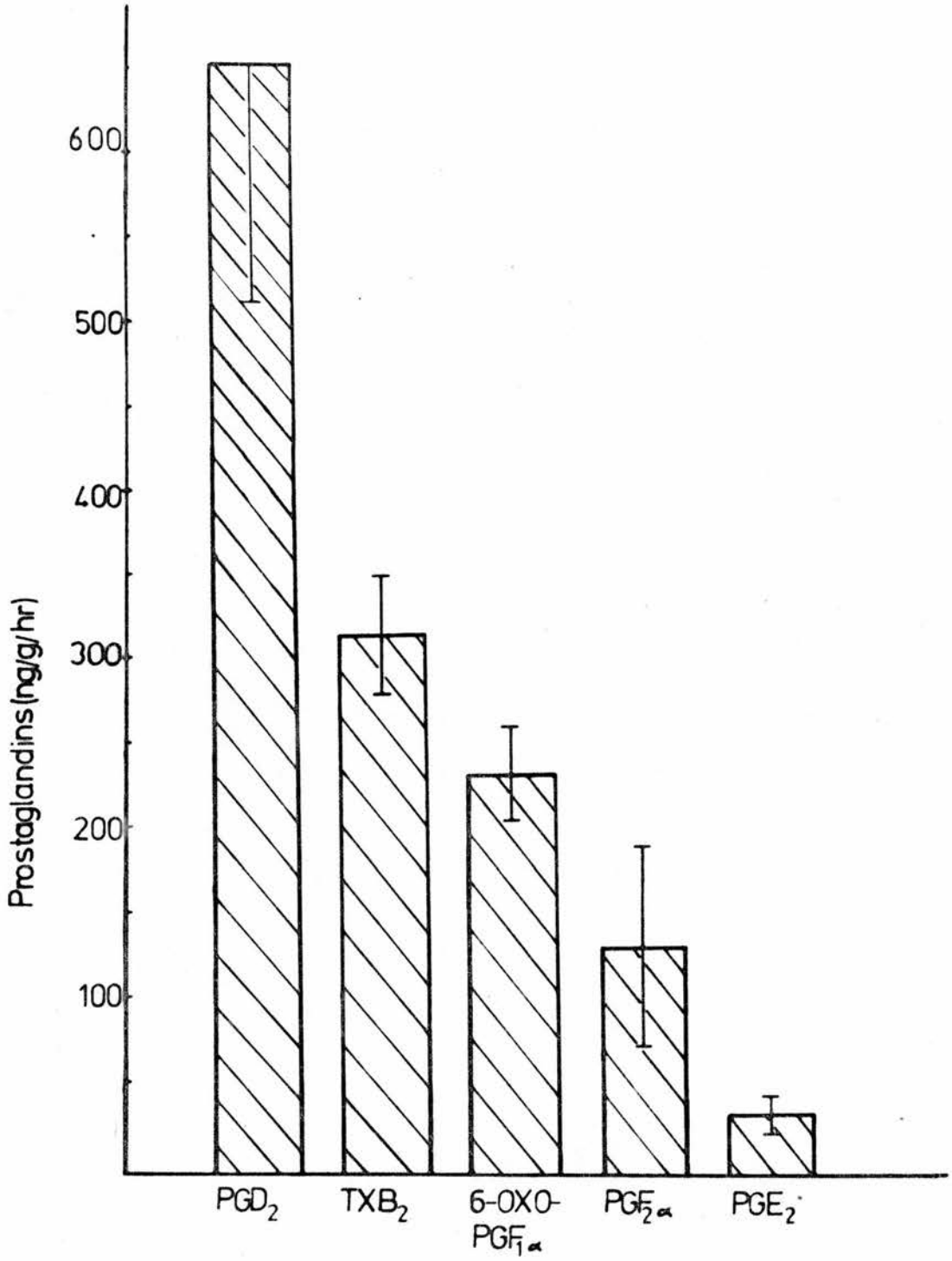
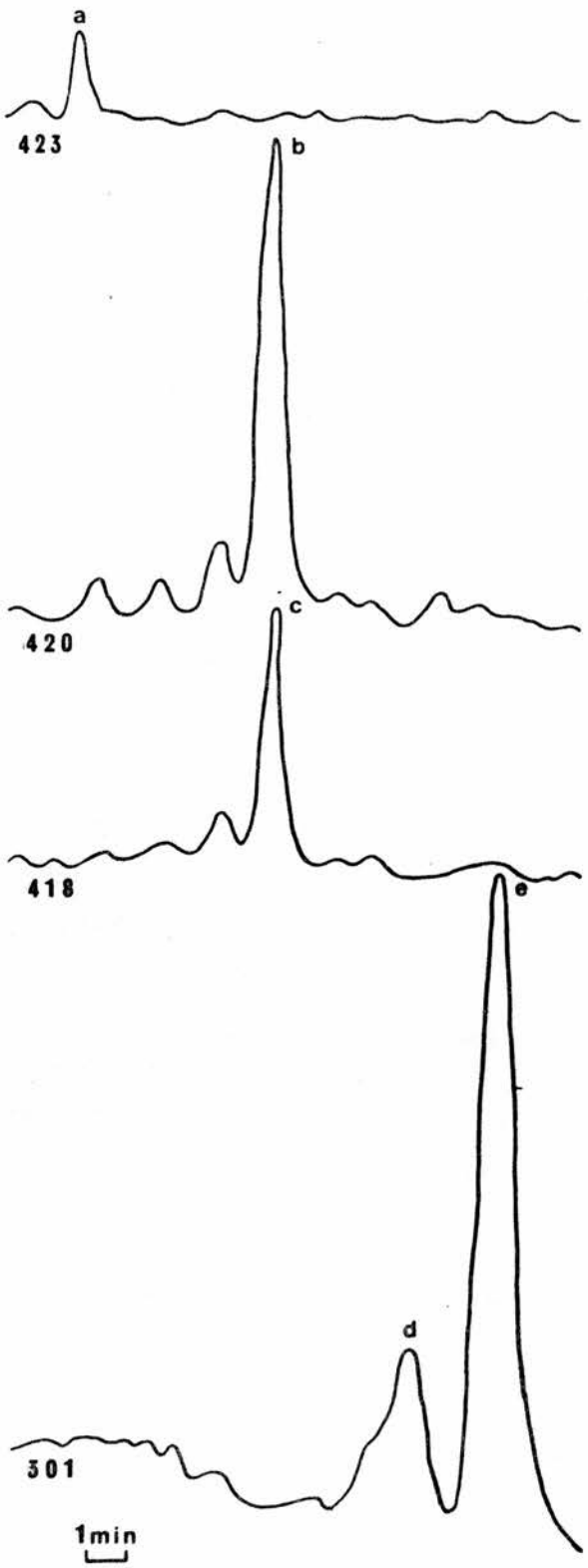

Prostaglandins (ng/g/hr)
600
500
400
300
200
100
PGD2
TXB2
6-OXO-PGF1α
PGF2α
PGE2

Fig.20.

Multiple ion detection trace of a sample of extracted bath fluid from the day 22 pregnant rat uterus *in vitro*, showing (a) $PGF_{2\alpha}$, carbon value 23.9 monitored by the ion at $^{m}/e$ 423, (b) PGD_2 carbon value of the second isomer 25.7 monitored by the ion at $^{m}/e$ 420 and 418 (c), (d) TXB_2 carbon value 26.4 monitored by the ion at $^{m}/e$ 301, the methyl ester trimethylsilyl ether, butyl oxime of each prostaglandin was used. Peak (e) is the ethyl ester, trimethylsilyl ether, butyl oxime of TXB_2 used as internal standard for assay of TXB_2.


a
423
b
420
c
418
e
d
301
1min

PGI_2 to the bath fluid and would then contract on addition of a further 16ng/ml. Contractions could not be obtained by adding 8 or 16ng/ml of PGL_2 as a single dose (Fig.21).

PGD_2 was also found to be spasmogenic, but a larger dose (60-120ng/ml) was required. The PGI_2 metabolite, 6-oxo-$PGF_{1\alpha}$ and TXA_2 metabolite, TXB_2 were found to cause contractions at a dose of 500 ng/ml (Fig.22).

Contractions obtained with PGI_2 and PGD_2 were qualitatively different from those obtained by PGE_2 or $PGF_{2\alpha}$. The contractions caused by PGI_2 and PGD_2 were regular and showed little change in basal tone of the tissues, (see Figs.21. 22). Contractions caused by PGE_2 or $PGF_{2\alpha}$ were more frequent and there was an increase in basal tone of the tissues (Fig.21).

3. <u>The Effects of 15-OOH Arachidonic Acid and Tranycypromine on the Day 22 Pregnant Rat Uterus in vitro</u>

When the 15-OOHAA was added to the bath, it was found to produce a spasm of the tissue and a large increase in tone was observed (Fig.23). This effect was produced by all three doses of the compound i.e. 25, 30 and 40µg/ml. When the vehicle (0.2-0.6ml polyethylene glycol) alone was added to the bath, this effect was not seen. A similar effect to that obtained with 15-OOHAA was seen with 500µg/ml tranylcypromine, (Fig.23). The spasmogenic effects of these two compounds was observed both in the absence and presence of indomethacin (5 - 10µg/ml).

4. <u>The Effect of 15-OOH Arachidonic Acid and Tranylcypromine on the Day 22 Pregnant Rat Uterus in vitro</u>

The results for the effects of the "PGI_2 synthesis inhibitors" on prostaglandin release from the term pregnant rat uterus are given in Fig.24. The results are expressed as the range of values obtained, n = 3 or 4 per group. 15-OOHAA increased the release of PGE_2, $PGF_{2\alpha}$, PGD_2 and also 6-oxo-$PGFI_{\alpha}$. The most dramatic increase was seen in the release of PGD_2 in the presence of the highest concentration of 15-OOHAA, where release increased from the control value of 22 ± 6 up to 116 ± 41 ng/g/min. Thromboxane B_2 was detected in these samples (by GC-MS), but the levels were below the sensitivity of the assay for reliable measurement.

Fig.21.

The effects of $PGF_{2\alpha}$, PGE_2 and PGI_2 on the uterus from a day 22 pregnant rat, rendered quiescent with 5µg/ml indomethacin in vitro.

Figure to be read from right to left. Top trace first, then bottom trace.

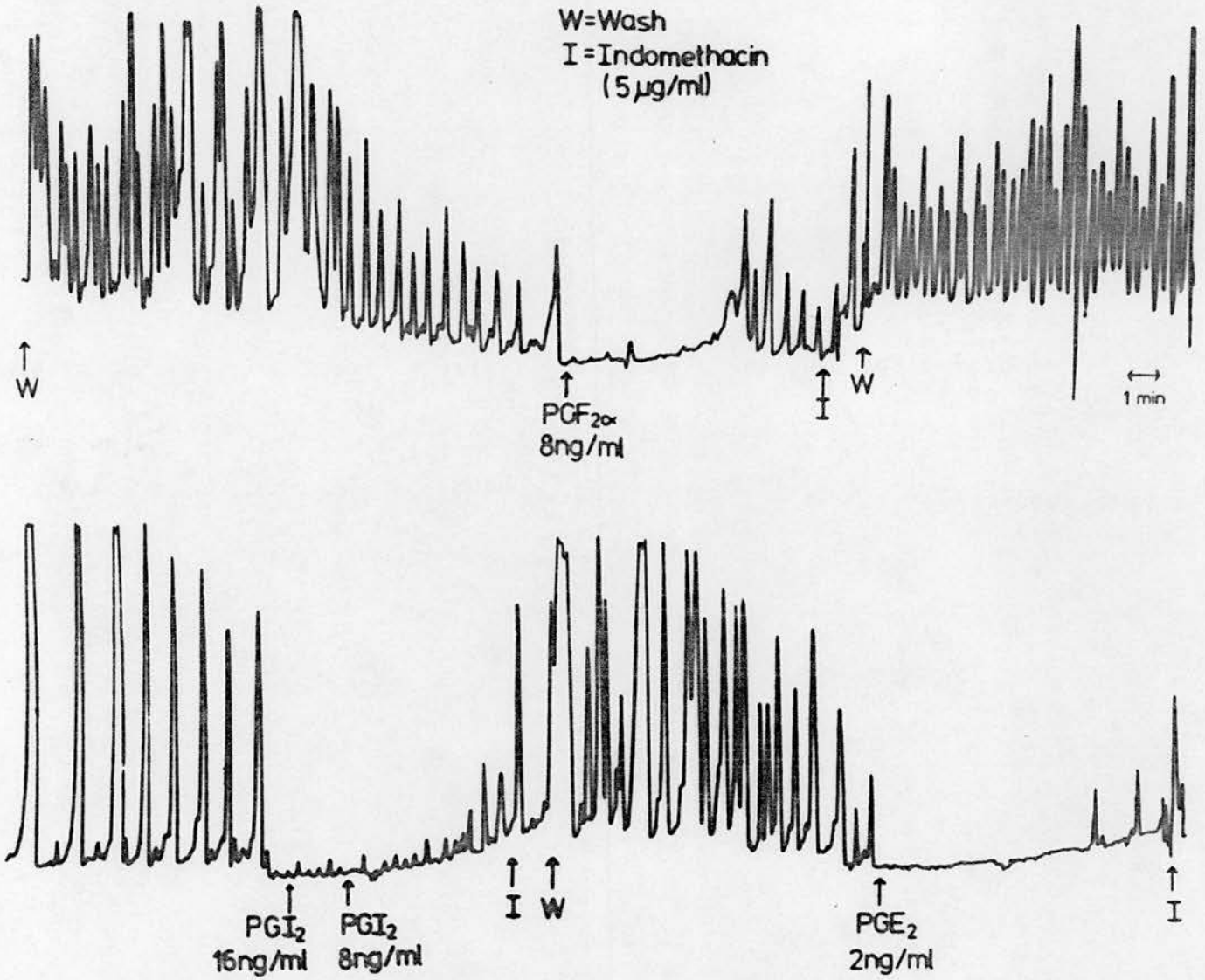

W=Wash
I=Indomethacin
(5 µg/ml)
W
PGF2α
8ng/ml
I
W
1 min
PGI2
16ng/ml
PGI2
8ng/ml
I
W
PGE2
2ng/ml
I

Fig.22.

The effects of PGD_2, 6-oxo-$PGF_{1\alpha}$ and TXB_2 on the day 22 pregnant rat uterus *in vitro*, in the presence of 10μg/ml indomethacin (I).

Figure to be read from right to left. Top trace first, then bottom trace.

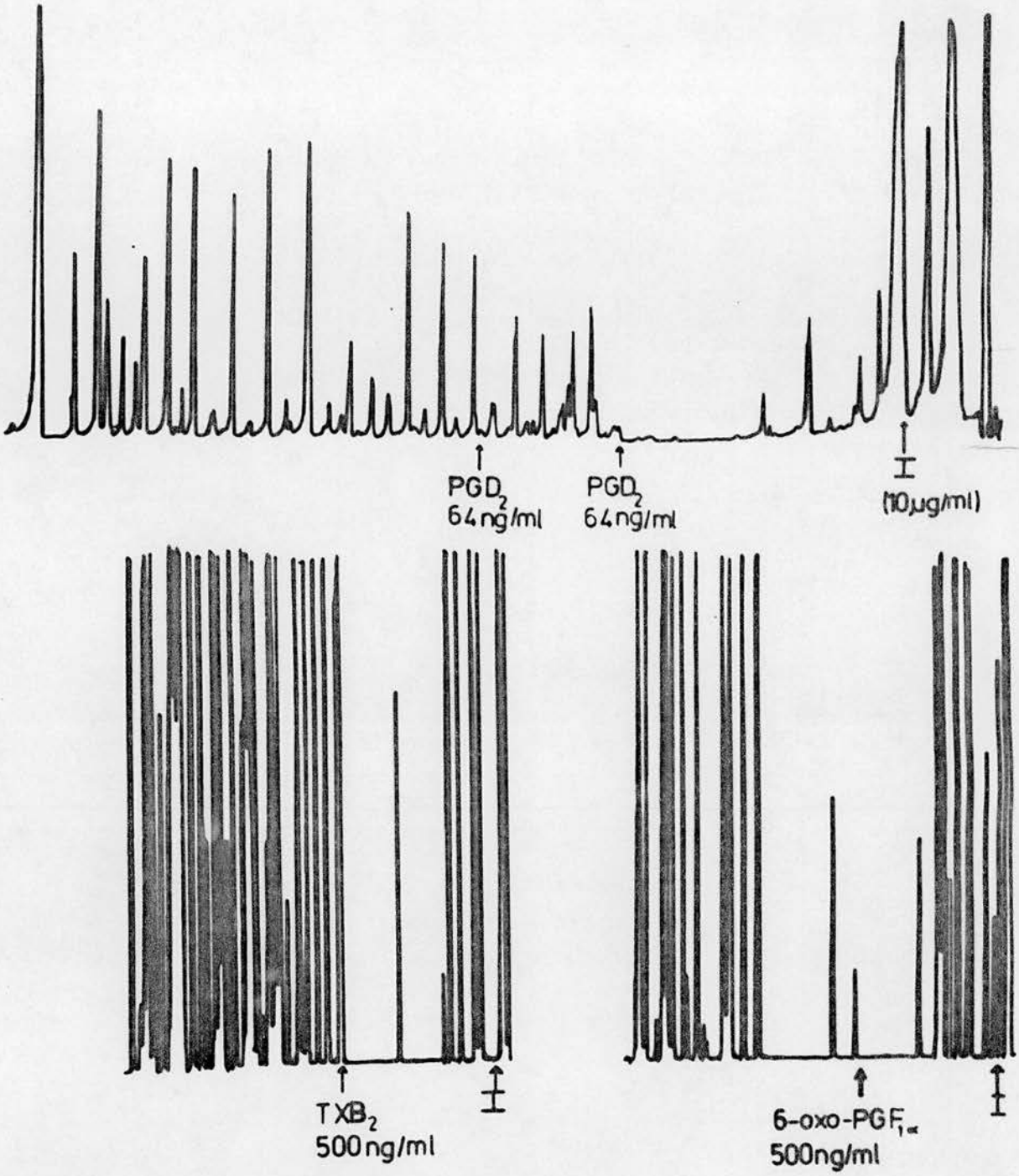

PGD2
64 ng/ml
PGD2
64 ng/ml
(10 µg/ml)
TXB2
500 ng/ml
6-oxo-PGF1α
500 ng/ml

Fig.23.

The effect of 15-hydroperoxy arachidonic acid (15-OOHAA) and tranylcypromine on the day 22 pregnant rat uterus *in vitro*.

Figure to be read from right to left. Top trace first, then bottom trace.

Effects of 15-00H-Arachidonic Acid and Tranylcypromine on the Day 22 Pregnant Rat Uterus, in vitro.

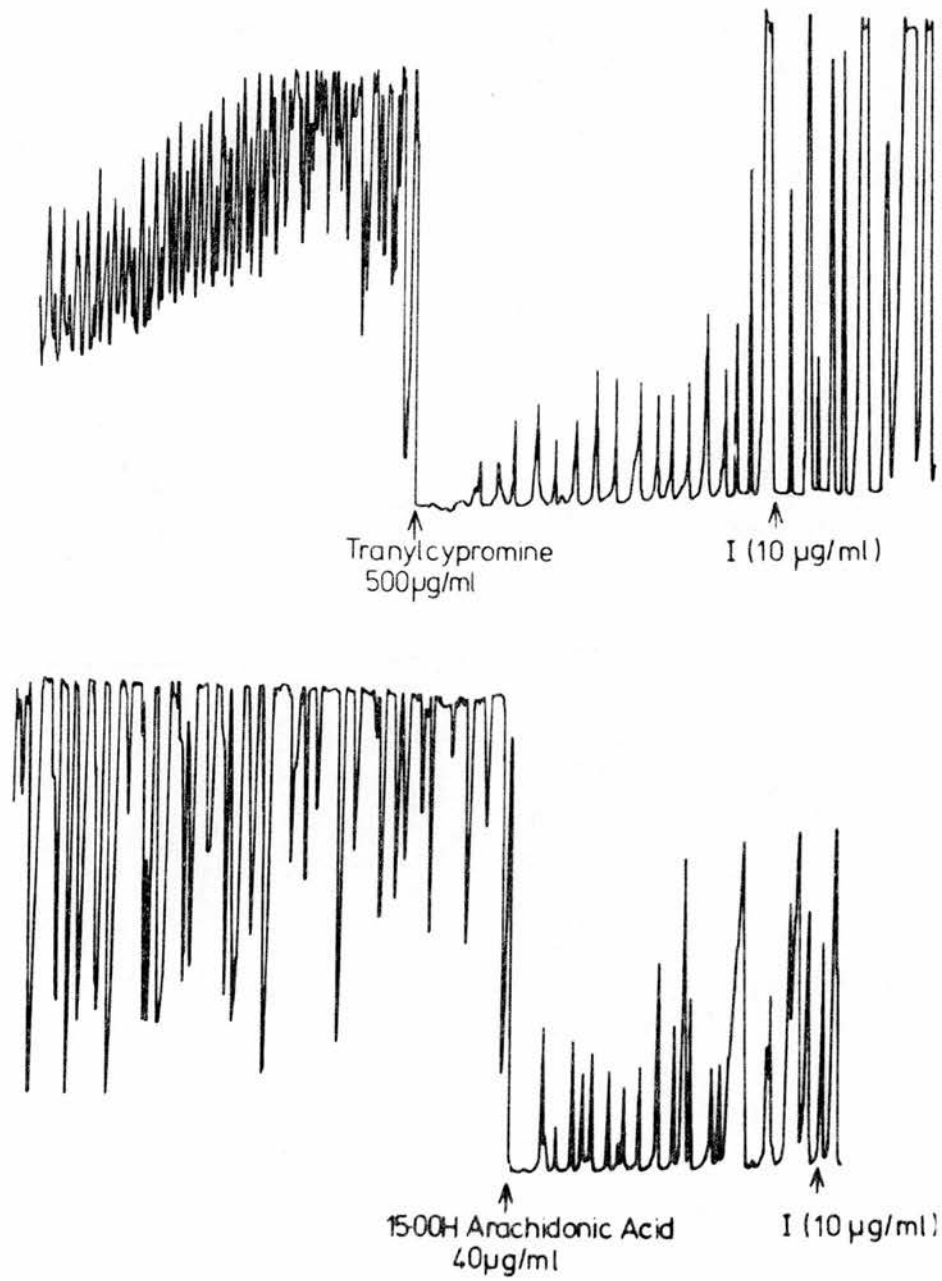
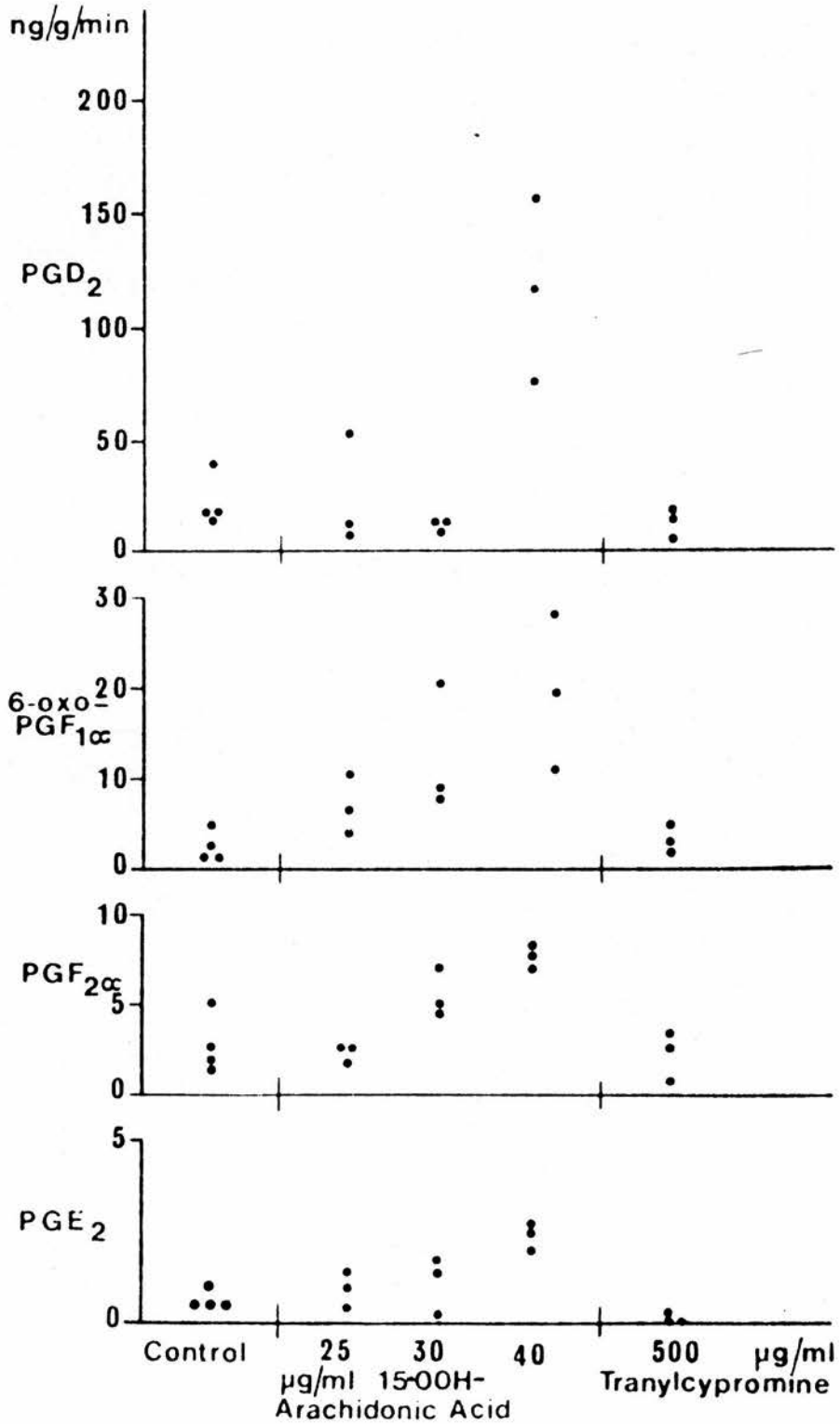


Fig. 24. The effects of 15-hydroperoxy arachidonic acid (15-OOHAA) and tranylcypromine on prostaglandin release from the day 22 pregnant rat uterus *in vitro*. Figure shows range of values obtained n=4/control group and n=3/treated group. PGE_2, $PGF_{2\alpha}$ and 6-oxo-$PGF_{I\alpha}$ measured by R.I.A PGD_2 measured by GC-MS.


ng/g/min
200
150
PGD2
100
50
0
30
6-oxo-PGF1α
20
10
0
10
PGF2α
5
0
5
PGE2
0
Control
25
30
40
500
μg/ml
μg/ml 15-OOH-
Arachidonic Acid
Tranylcypromine

(However, levels did seem to increase in the presence of 15-OOHAA).

The release of prostaglandins from the uterus was not affected by tranylcypromine (500µg/ml) except for PGE_2 which showed a significant fall ($p < 0.01$) from the control value.

The results obtained by RIA, for the release of PGI_2, reflected by the levels of its metabolite 6-oxo-$PGF_{1\alpha}$, were unexpected. The experiment was therefore repeated on uteri obtained from three rats and the 6-oxo-$PGF_{1\alpha}$ was measured by GC-MS. These experiments have been described previously (see Methods, this section) and the results are given below in table 6. An example of the trace obtained for the measurement of 6-oxo-$PGF_{1\alpha}$ by GC-MS is given in Fig.25.

Table 6.

Effect of 15-OOHAA on PGI_2 Release from the Term Pregnant Rat Uterus (measured as 6-oxo-$PGF_{1\alpha}$ by GC-MS) in vitro

Control Horn ng/g/min.	Horn Treated with 30ug/ml 15-OOH Arachidonic Acid ng/g/min.
4.2	3.7
3.8	4.2
0.5	13.5

In two experiments the release from the treated horn was not significantly different to control. In the third experiment however, there was a large increase in the amount of 6-oxo-$PGF_{1\alpha}$ obtained from bath fluid surrounding the treated horn.

5. Release of Prostaglandins from the Day 22 Pregnant Rat Uterus in vitro, over Four Consecutive Periods of Fifteen Minutes.

The increase in prostaglandins release from the uterus in the presence of 15-OOHAA was not due merely to an increased release from untreated tissue over consecutive periods of 15 min. This is illustrated by the following table 7.

The release of PGE_2, $PGF_{2\alpha}$ and 6-oxo-$PGF_{1\alpha}$ is the same or shows a slight decrease from one collection period to the next. There are no large increases in prostaglandin release as was observed in the presence of 15-OOHAA.

Fig.25.

Multiple ion detection trace of the assay for 6-oxo-$PGF_{1\alpha}$ in the extracted bath fluid from the day 22 pregnant rat uterus in vitro, monitored by the ion at $^{m}/_{e}$ 491, using the tetradeuterated compound as internal standard, monitored by the ion at $^{m}/_{e}$ 494. Carbon value of 6-oxo-$PGF_{1\alpha}$ second isomer 26.1.

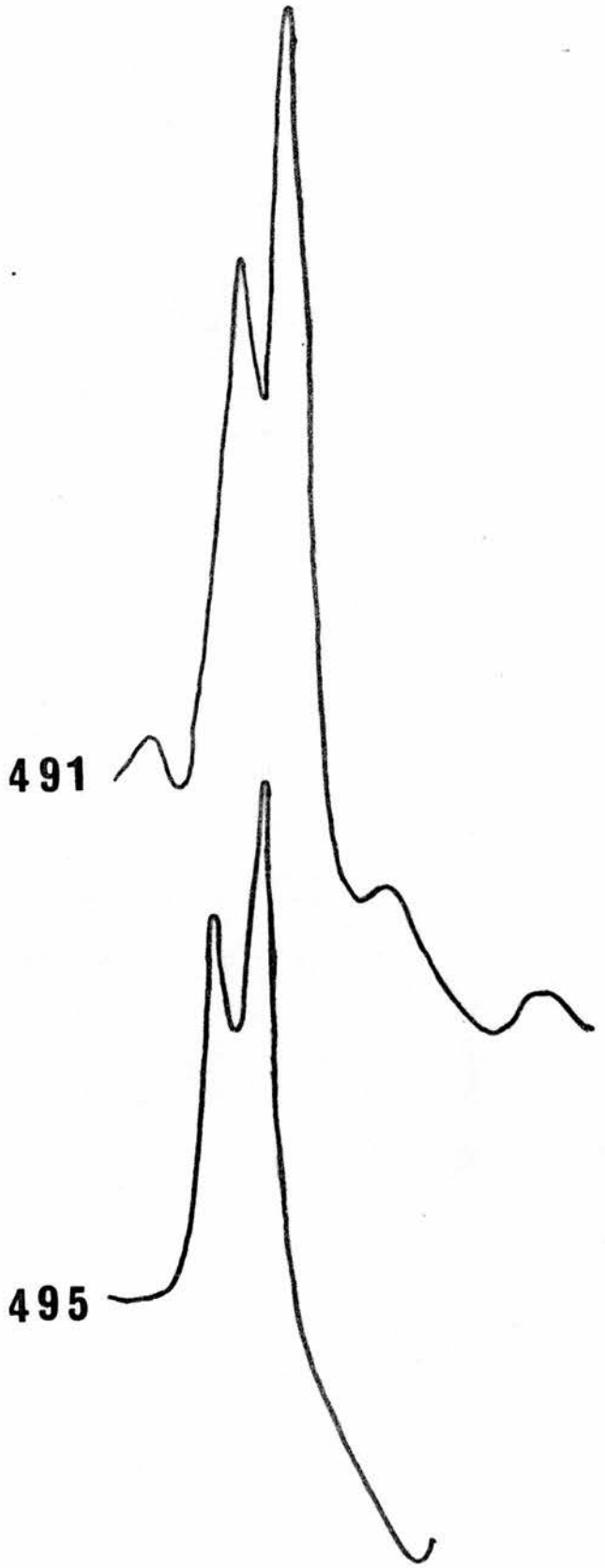

491
495

Table 7. **Release of Prostaglandins from the Term Pregnant Rat Uterus in vitro over Four Consecutive Fifteen Minute Periods.**

Prostaglandin	Release, 4 x 15 min Periods ng/g/min(n=3)			
	1	2	3	4
$PGF_{2\alpha}$	3.1	2.7	1.9	1.9
PGE_2	1.2	1.0	1.2	0.9
6-oxo-$PGF_{1\alpha}$	3.5	3.1	2.1	2.0

6. The Effect of Indomethacin on Prostaglandin Release from the Day 22 Pregnant Rat Uterus, in vitro

One experiment was performed to investigate the effect of addition of indomethacin to the bath fluid, on prostaglandin release (see Methods this section). Indomethacin treatment was found to reduce the release of PGE_2 and $PGF_{2\alpha}$ in a dose dependent manner, as shown in the table 8 below:-

Table 8 **Effect of Indomethacin on Prostaglandin Release from the Term Pregnant Rat Uterus in vitro (n = 1).**

Prostaglandin	Release of Prostaglandins ng/g/min		
	Control	Indomethacin	
		0.6µg/ml	1.2µg/ml
$PGF_{2\alpha}$	2.2	0.73	0.22
PGE_2	0.5	0.11	0.02

Discussion

These experiments have shown that prostaglandins and thromboxane are released from the rat uterus on day 22 of pregnancy in vitro. The amounts of $PGF_{2\alpha}$ released, measured by RIA were found to be in the same order, but slightly lower, than those reported by Vane and Williams (1973) who used bioassay. PGE_2 was released in smaller amounts than $PGF_{2\alpha}$. Since these initial studies of Vane and Williams, prostacyclin (Fenwick et al 1977) and thromboxane (Williams and Downing 1977), have been shown to be produced by the rat uterus. Results obtained in this section have shown the release of large amounts of PGI_2 and TXA_2 (measured as their more stable metabolites, 6-oxo-$PGF_{1\alpha}$ and TXB_2, respectively) from the day 22 pregnant uterus, in vitro, in quantities greater than $PGF_{2\alpha}$ and PGE_2. Williams, Dembinska, Kiec, Zmuda and Gryglewski (1978) have

shown that myometrial and decidual cell fractions from the day 22 pregnant rat uterus, synthesise PGI_2, as estimated by inhibition of platelet aggregation. In addition, they claimed that the rat placenta, at this time, does not produce PGI_2, due to the presence of a placental prostaglandin synthetase inhibitor (Williams, Harrowing and Downing 1979) which produced an inhibition of PGI_2 synthesis by uterine microsomes. Zamecnik and Kennedy (1979) however, found that the placental tissue from pregnant rats does produce PGI_2 when measured as 6-oxo-$PGF_{1\alpha}$ by GC-MS. Also there is an increase in the tissue level on day 20 compared to day 15, with further increases occurring on day 21, day 22 and during labour on day 23. Zamecnik and Kennedy (1979) also report increasing levels of TXB_2 in decidual and placental tissue and amniotic fluid from day 15 to day 23 of pregnancy in rats, which is in keeping with the findings of Downing and Williams (1977). In consequence therefore, it seems that any measurement of PGI_2 production and its inhibition, from tissue incubates or microsomes, by platelet aggregation methods, as used by Williams et al (1978), should be treated with caution owing to the possible antagonism of the actions of PGI_2 by TXA_2 produced simultaneously.

As has been stated previously, 6-oxo-$PGF_{1\alpha}$ and TXB_2 were found to be released from the term pregnant rat uterus <u>in vitro</u>, in amounts greater than $PGF_{2\alpha}$ and PGE_2. However, the prostaglandin found to be released in by far the largest quantities was PGD_2(Fig.19). Katori, Harada, Yamashita, Ishibushi and Miyazaki (1978) have also reported PGD_2 to be a major product released from the pregnant rat uterus, although no measurements were made. In 1973 Vane and Williams reported the appearance of a spot of unknown identity following thin layer chromatography (TLC) of an extract of fluid which had bathed a term pregnant rat uterus. The position of the spot on the TLC plate however, was not that which might have been expected for PGD_2, in the solvent system used. It may have been due to 6-oxo-$PGF_{1\alpha}$. The question arises as to a physiological role for prostaglandins and thromboxanes in parturition in the rat. In the present study, the spasmogenic potencies of PGE_2 and $PGF_{2\alpha}$ were similar to those reported by Vane and Williams (1973), with PGE_2 being more potent than $PGF_{2\alpha}$.

PGI_2 was found to be spasmogenic,

but was less potent than $PGF_{2\alpha}$. 6-oxo-$PGF_{1\alpha}$ produced contractions only at a very high dose level (500 ng/ml) and lower doses were ineffective in producing contractions. The effects of PGI_2 on the isolated, human uterus have been reported as stimulating an increase in tone and frequency of contractions (Whilhelmsson, Lindblom, Hamberger, Samsoie, Hammarstrom, Wiquist and Samuelsson 1979). PGI_2 has also been reported to relax the human uterus (Omini, Pasagaklian, Folco, Fano and Berti 1978; Omini, Pasagaklian, Burnetti, Falco and Berti 1979), although PGI_2 was found only to contract the pregnant rat uterus in the present studies. The type of contractions obtained on the pregnant rat uterus were visibly different from those obtained with PGE_2 or $PGF_{2\alpha}$. Those obtained with PGI_2 were more regular, but less frequent, with no change in basal tone of the tissue, while PGE_2 and $PGF_{2\alpha}$ produced frequent, irregular contractions and an increase in tissue tone (see Fig.21). The contractions produced by PGI_2, appear, in my opinion, to be much more related to the synchronous, regular but intermittent type of contraction necessary for expulsion of the foetus during parturition than contractions produced by PGE_2 or $PGF_{2\alpha}$ .Mitchell, Ellwood, Anderson and Turnbull (1978) have reported a marked increase in the levels of 6-oxo-$PGF_{1\alpha}$ in the maternal utero-ovarian vein, foetal plasma and amniotic fluid of sheep during active labour. 6-oxo-$PGF_{1\alpha}$ has also been found in maternal plasma and amniotic fluid of the Rhesus monkey in late pregnancy (Robinson, Natale, Clover and Mitchell 1979) and uterine tissues (particularly decidua and placenta) taken from the late pregnant Rhesus monkey produce PGI_2 <u>in vitro</u> (Mitchell, Hicks,Thorburn and Robinson 1979). It may be that the proposal of Fuchs et al (1976) discussed in the introduction is true i.e. that the role of $PGF_{2\alpha}$ produced by the endometrium (Williams, Harney and Sneddon 1974) at term, is only to cause luteolysis and a fall in plasma progesterone levels and that the myometrium synthesises another prostaglandin,PGI_2 (Williams et al 1978),which contributes to the uterine contractions of parturition. Williams, El-Tahir and Marcin-Kiewicz (1979) report a synergistic spasmogenic action of PGI_2 with oxytocin (and also of PGI_2 with $PGF_{2\alpha}$) when the two compounds are added together to the fluid surrounding the term pregnant rat uterus, in doses which are subthreshold for production of contractions when added singly. This synergism between

between these compounds may also contribute to foetal expulsion.

PGI_2 has been found to be capable of releasing renin from the kidney (Wharton, Lazar, Smigel and Oates 1979). Renin is the enzyme necessary for the formation of the vasoactive peptide, angiotensin II. Angiotensin II also has a potent spasmogenic effect on the uterus (Dubin and Ghodgoankar 1979), and the uterus of various species is known to contain renin (Gross, Schaetelin, Zeigler and Berger, 1964; Ferris, Gordon and Mulrow 1967; Hodari, Carretero and Hodgekinson 1969; Skinner, Lumbers and Symonds 1968), although to my knowledge the rat has not been investigated. When PGI_2 was added to the bath fluid, there was a short delay before contractions were seen (Fig.21). It could be that PGI_2 releases renin which causes the formation of angiotensin II, which then causes the uterine contractions. This would explain the delay observed after the addition of PGI_2 to the bath. This suggestion is purely speculative since it does depend upon the tissue containing precursor of angiotensin II. However, it would be interesting to test the hypothesis by using an angiotensin antagonist such as saralasin, or an angiotensin converting enzyme inhibitor e.g. Squib 19225.

Besides stimulating the uterus to contract, PGI_2 is also a potent vasodilator (Moncada, Gryglewski, Bunting and Vane (1976a). Its production by the uterus may be to increase the blood supply through the uterus and placenta during pregnancy. However, the effect of PGI_2 on blood flow through pregnant uterus and maternal and foetal placenta is not known. Terragno, Terragno, Pacho, Iczyk and McGiff (1974) have reported that uterine blood flow in the pregnant dog is reduced by indomethacin, and this effect can be reversed by infusion of arachidonic acid. However, it could not be reversed by PGE_2 infusion. Hence it is possible that PGI_2 and not PGE_2 was mediating the increase in uterine blood flow in this case.

It has been recently suggested that PGI_2 is involved in cervical ripening (Ellwood, Mitchell, Anderson and Turnbull 1979). There is a significant increase in production of PGE_2 and 6-oxo-$PGF_{1\alpha}$ by the sheep cervix <u>in vitro</u> at delivery compared to late pregnancy. The cervical venous effluent also shows a dramatic increase in PGE_2 and 6-oxo-$PGF_{1\alpha}$ levels during the 12 hr preceding delivery. It has been suggested that PGE_2 and PGI_2 act synergistically to soften cervical connective tissue in the sheep.

Thromboxane B_2, measured as described in Section II, presumably reflects the release of its unstable precursor TXA_2 (Hamberg, Svensson and Samuelsson 1975; Svensson 1978) from the uterus. TXB_2 levels have been found to rise in the placenta, decidua and amniotic fluid of the rat during late pregnancy (Zamecnik and Kennedy 1979). Decidual microsomes from the day 22 pregnant rat have been shown to produce TXB_2 (Downing and Williams 1977). TXA_2 is known to constrict a number of blood vessels e.g. rabbit aorta (Hamberg, Svensson, Wakabayaski and Samuelsson 1975), swine coronary artery (Svensson and Hamberg 1976) and human umbilical artery (Turemo, Strandberg, Hamberg and Samuelsson 1976). Its release from the uterus may be therefore, in order to constrict placental blood vessels and reduce blood loss after birth.

Recently TXA_2 (150ng/ml) has been found to increase the tone and frequency of contraction of strips of human uterus (Whilhelmsson, Lindblohm, Hamberg, Sansoie, Hammarstrom, Wiquist and Samuelsson 1979) and it is possible that TXA_2 may also contribute to expulsion of the foetus at term. TXB_2 was found to be a weaker spasmogen on the rat uterus in the present study, though it is possible that it could contribute to the increased contractions of the uterus at term. However, although TXB_2 has been found in the amniotic fluid and maternal plasma of sheep and human during parturition (Mitchell, Kierse, Anderson and Turnbull 1978; Mitchell, Hicks, and Robinson 1979), no distinct trends could be found and a role for thromboxane in parturition in these species was therefore not apparent. Mitchell, Hicks, Thorburn and Robertson (1978) on the other hand, have found a small increase in TXB_2 in the amniotic fluid of the Rhesus monkey at term, compared to late pregnancy, but the authors suggest that other prostaglandins rather than thromboxanes are involved in the initiation of parturition.

The finding that PGD_2 was the prostaglandin released in the largest amounts from the isolated, term pregnant uterus, was unexpected and raised the question as to why this particular pathway of arachidonic acid metabolism is so active in this situation. PGD_2 was found to have a spasmogenic effect on the isolated, pregnant uterus, but the potency was much less than that of other prostaglandins released (PGE_2 $PGF_{2\alpha}$ and PGI_2). It has been suggested, that in some instances, PGD_2 may produce its effect

by acting at $PGF_{2\alpha}$ receptors (Horton and Jones 1974). This action would explain its high equipotent molar ratio, due to lower affinity and/or efficacy when compared to $PGF_{2\alpha}$. Possibly, PGD_2 is acting via this mechanism to contract the rat uterus, which would explain its lower potency. On the other hand, Jones (1978) has reported that the rat may possess a PGD_2 sensitive vasoconstrictor system. Therefore, it is possible that PGD_2 released from the uterus brings about a vasoconstriction of placental blood vessels just after parturition and possibly acting in this way along with TXA_2.

A prostaglandin-endoperoxide isomerase, which gives a high yield of PGD, has been isolated from the rat spleen (Van Dorp, Buytenhoek, Christ-Hazlehof, Nugteren and Van Der Ouderaa 1978; Christ-Hazlehof and Nugteren 1978) and a similar enzyme has been found in rat brain tissue (Yamamoto 1979). Rat lung, stomach and intestine also give a high conversion of endoperoxide (PGH_2) to PGD (Van Dorp et al 1978)and it seems likely in view of this and of results reported here, that the rat uterus also contains such a system. The PGH_2 to PGD_2 isomerase in the rat spleen is a soluble, cytoplasmic enzyme. This is in contrast to enzymes which convert PGH_2 into PGE_2, $PGF_{2\alpha}$, PGI_2 and TXB_2, as these are membrane-bound (Ogino, Miyamoto, Yamamoto and Hayaishi 1977; Needleman, Moncada,, Bunting, Vane, Hamberg and Samuelsson 1976; Johnston, Morton, Kinner, Gorman, McQuine, Sun, Whittaker, Bunting, Salmon, Moncada and Vane 1976). Fatty acid cyclo-oxygenase, which converts arachidonic acid to PG endoperoxide is also membrane bound. Since the two enzymes needed for the conversion of arachidonic acid into PGD_2 via the reaction: arachidonic acid $\rightarrow$ PG endoperoxide $\rightarrow PGD_2$, differ in sub-cellular localisation, Christ-Hazlehof and Nugteren (1978) have suggested that any PG endoperoxide which escapes conversion into the other prostaglandin or thromboxane in the membranes, is converted by the PGH_2 to PGD_2 isomerase within the cytoplasm. This transformation protects the tissue from the possible harmful effects due to overproduction of the more potent prostaglandins and thromboxanes, The much lower potency of PGD_2 and its abundant release from the term pregnant rat uterus, compared to other prostaglandins may be explained by this theory.

Hence, one or more physiological roles can be attributed to each of the prostaglandins and thromboxane released from the term pregnant rat uterus. Although the studies presented in this thesis have been performed on the isolated rat uterus only, a function *in vivo* is implied by their actions:

1. A luteolytic action for $PGF_{2\alpha}$, followed possibly by a contribution in uterine contractions.
2. An increase in blood flow through the uterine and placental vessels for PGE_2 and PGI_2.
3. A spasmogenic effect of PGI_2 on the myometrium resulting in the synchronous, regular type of contractions associated with parturition.
4. A spasmogenic action of other prostaglandins cannot be ruled out (PGE_2, TXA_2, PGD_2).
5. A potentiating effect of PGI_2 on oxytocin-induced contractions. (Williams et al 1979).
6. A vasodilatory effect of PGI_2 is possibly overcome after parturition by a vasoconstrictor effect of TXA_2 and PGD_2, the release of which may take over the release of PGI_2 during the latter stages of parturition.
7. An action of PGI_2 and PGE_2 to induce cervical ripening at term.
8. The main function of the PGH_2 to PGD_2 pathway may be one of "mopping up" of excess precursor.

Further experimentation is needed to see whether the ratio of the prostaglandins and thromboxane released *in vitro* is reflected *in vivo*, or which prostaglandin(s) is released preferentially.

To further test the possible role of PGI_2 in causing contractions of the rat uterus at term, the reported PGI_2 synthesis inhibitors 15-hydroperoxy arachidonic (15-OOHAA) acid and tranylcypromine were used. However, both compounds were found to produce spasm of the day 22 pregnant rat uterus when added to the bath fluid. Consequently, these drugs were of little use for investigating the significance of PGI_2 in the production of uterine contractions. 15-OOHAA and tranylcypromine produced contractions of the uterus both in the presence and absence of indomethacin. Furthermore, in the absence of indomethacin, 15-OOHAA caused a dose related increase in prostaglandin from the uterus which was surprising and unexpected. This is especially true for 6-oxo-$PGF_{1\alpha}$, since 15-OOHAA has been reported to specifically inhibit PGI_2 synthesis from PGH_2 by aortic microsomes (Moncada,

Gryglewski, Bunting and Vane 1976b).

The high values for prostaglandin production obtained by RIA were not due to high cross-reactivity of 15-OOHAA with the antisera used, because the high values were obtained after removal of the 15-OOHAA by column chromatography. Another possibility, was that the 15-OOHAA may have "fed into" the prostaglandin pathways producing prostaglandin-like compounds which may then have interfered with the RIA. However, PGD_2 and 6-oxo-$PGF_{1\alpha}$ were measured by the much more sensitive method of GC-MS, so it is thought unlikely that such interference could explain the increased release of these unless any compounds so formed had identical retention times and produced the same ions as the prostaglandins being measured. In addition, the corresponding compound, 15-hydroperoxy-8,11,13-eicosatrienoic acid does not "feed into" the "1 series" prostaglandin pathway (Hamberg and Samuelsson 1967) so it is unlikely that 15-OOHAA would be converted into prostaglandins of the "2-series". It would appear, therefore, that 15-OOHAA does stimulate prostaglandin production by the pregnant rat uterus in some way. However, it was possible that the increases in prostaglandin release observed were due to a non-linear release of prostaglandins from the uterus between consecutive period of time, since prostaglandin release from the uterus in the presence of 15-OOHAA over a 15 min period, was compared with prostaglandin release over the preceding 15 min, when 15-OOHAA was absent from the bath fluid. However, this possibility was found not to be the case (see table 7). Prostaglandin release showed no increase between 4 consecutive 15 min periods of collection in the absence of 15-OOHAA. In fact, some decreases were seen over the 4 periods tested.

It has been reported that 15-OOHAA will inhibit the lipoxygenase pathway of arachidonic acid metabolism (see Fig.4 in General Introduction), in the same concentration as it inhibits PGI_2 synthase (Burka and Flower 1979). Lipoxygenase is a soluble enzyme found in the supernatent liquid fraction after ultra centrifugation of a smashed cell preparation (Nugteren 1975), whereas the PG endoperoxide PGI_2 converting enzyme is membrane bound (Johnston et al 1976). The 15-OOHAA may, therefore, have easier access to the lipoxygenase enzyme, when it is added to the bath fluid surrounding the whole uterine tissue. If this is the case, the resultant inhibition of the

lipoxygenase pathway may cause more substrate to be available for the prostaglandin pathways, thus producing an increased release of prostaglandins. 12-hydroxy-5,8,10,14-eicosatetranoic acid (HETE) the product of the lipoxygenase pathway has been found to be produced by the sheep uterus (Jones and Wilson: unpublished results) and the rat uterus may contain this lipoxygenase pathway. In addition, Hamberg and Samuelsson (1974) have demonstrated that inhibition of the cyclo-oxygenase pathway in human platelets with aspirin and indomethecin gives rise to an increase in the products of the lipoxygenase pathway suggesting that blockade of one pathway potentiates the other. Thus it is possible that the increase in prostaglandin release in the presence of 15-OOHAA is not the result of direct stimulation, but rather indicrectly, as a consequence of blocking the lipoxygenase pathway.

Tranylcypromine is, supposedly, another specific inhibitor of PGI_2 synthesis (Gryglewski, Bunting, Moncada, Flower and Vane 1976) but treatment of the isolated uterus with the recommended dose for 100% inhibition in blood vessels (500µg/ml) did not produce any reduction in PGI_2 release. However, a fall in PGE_2 release was observed. Rajtar and de-Gaetano (1979) have reported that in the rat, tranylcypromine is not selective for PGI_2 inhibition, as they found that it can also inhibit production of TXA_2 in veins and arteries.

In the case of the isolated uterus, theremay have been a problem of access of both inhibitors of PGI_2 production to the sub-cellular site of action. This was not the case with indomethacin, since treatment with this drug caused a dose-related decrease in release of PGE_2 and $PGF_{2\alpha}$ (see table 8) from the uterus, as expected. From the results obtained in these experiments it appears that 15-OOHAA and tranylcypromine are not selective inhibitors of PGI_2 synthesis in the term pregnant rat uterus. The role of PGI_2 in the production of spontaneous activity in the uterus merits further investigation.

In conclusion, this experimental section has shown the order of release of prostaglandins in the isolated spontaneously contracting rat uterus on day 22 of pregnancy is $PGD_2 > TXA_2 > PGI_2 > PGF_{2\alpha} \geq PGE_2$

This order is the reverse of their spasmogenic potency on the uterus i.e. $PGE_2 > PGF_{2\alpha} \geq PGI_2 > PGD_2$. Possible roles for these prostaglandins have been suggested, but their release in vivo needs examining. It is known that PGE_2 and $PGF_{2\alpha}$ are released into the uterine venous blood of the rat at term, but other prostaglandins and thromboxane have not been identified or measured.

SECTION III

THE INVOLVEMENT OF PROSTAGLANDINS IN IMPLANTATION IN THE RAT

Introduction

The rat uterus has been shown to synthesise prostaglandins *in vitro*, during pseudopregnancy (Fenwick et al 1977). PGE and PGF measured by RIA, showed a peak on day 5; the amounts of PGF were higher than PGE on each day studied. The major prostaglandin produced was found to be 6-oxo-$PGF_{1\alpha}$, which was detected by GC-MS, but not measured due to a lack in the availability of standard 6-oxo-$PGF_{1\alpha}$ at that time. Tamoxifen administered on day 2 of pseudopregnancy, inhibited the increase in prostaglandin production on day 5. Tamoxifen administered to pregnant rats on day 2, prevents implantation. (Harper and Walpole 1967). These results suggest that prostaglandins may be involved in implantation in the rat. It was decided, therefore, that the amounts of PGE_2, $PGF_{2\alpha}$ and 6-oxo-$PGF_{1\alpha}$ produced by the uterus during early pregnancy should be investigated and compared with the amounts produced during pseudopregnancy, using RIA and also using GC-MS for 6-oxo-$PGF_{1\alpha}$ measurements.

One of the first indications of the implantation process is an increase in vascular permeability at the implantation site. This can be detected by intravenous injection of Evans or Pontamine, Blue dye, followed by examination of the uterus 1 hr later, for dye reaction sites. The time of appearance of these sites in our rats was determined and the effects of indomethacin on this time of appearance was investigated. The effects of the indomethacin treatment on the subsequent development of pregnancy, up to day 9, has also been investigated.

Methods

1. Prostaglandin Synthesis by the Early Pseudopregnant and Pregnant Rat Uterus in vitro

a. Early Pseudopregnancy

Mature female rats undergoing regular four day oestrous cycles, were caged individually with a vasectomised male on the afternoon of day 4 of the oestrous cycle and were removed at mid-day on the following day. Mating was assumed to have taken place during the dark period between day 4 and day 1 of the oestrous cycle, this latter day becoming day 1 of pseudopregnancy. Pseudopregnancy was confirmed by the lack of subsequent oestrus and/or by the determination of peripheral plasma progesterone levels. Three rats were allowed to continue their pseudopregnancy and vaginal smears were taken every

morning to determine the length of pseudopregnancy. This was found to be thirteen days i.e. before the next oestrous cycle.

Rats (4 per day) were taken on the mornings of days 4 to 6 of pseudopregnancy and cardiac punctures were performed in order to collect blood to use for determination of peripheral plasma progesterone levels. The rats were killed by cervical dislocation and the uterus quickly removed, freed of fat, dried and weighed. The uterus was then homogenised in 15 ml Krebs' solution (pH 7.5) using a Fisal's glass homogeniser. The homogenate was transferred to a conical flask and the homogeniser was washed with 5ml Krebs' solution which was then added to the flask. Homogenates were incubated at 37°C for 1 hr in a shaking water bath, during which time they were bubbled with 95% oxygen and 5% carbon dioxide. After 1 hr, the pH of the incubate was reduced to 4.0 with 1N HCl and the incubate was shaken three times with two volumes of redistilled ethyl acetate, to extract the prostaglandins. The ethyl acetate extracts were pooled and evaporated to dryness at 45°C on a rotary evaporator and the residue was dessicated under vacuum for 5 min. Dry residues were redissolved in 10ml redistilled ethyl acetate and stored at -20°C before assay.

Blood taken from each rat, into heparinised tubes (10 i.u./ml) was spun at 600 x g for 5 min using a bench centrifuge and the supernatent plasma was removed and stored at -20°C before progesterone was extracted and measured by RIA.

b. Early Pregnancy

Mature female rats exhibiting regular 4 day oestrous cycles were mated individually with males of proven fertility on the afternoon of day 4 of the oestrous cycle. Mating was assumed to have taken place during the dark period between day 4 and day 1. The rats were removed from the male cages at mid-day on day 1 and this became day 1 of pregnancy.

Rats (5 per day) were taken on the mornings of days 3 to 7 of pregnancy and a blood sample was taken by cardiac puncture from each rat for determination of peripheral plasma progesterone levels. The rats were killed by cervical dislocation, the uterus was removed and subjected to exactly the same procedures as the uteri from pseudopregnant rats.

Samples from both early pseudopregnancy and pregnancy were assayed for $PGF_{2\alpha}$, PGE_2 and 6-oxo-$PGF_{1\alpha}$ by RIA. 6-oxo-$PGF_{1\alpha}$ was also measured by GC-MS.

2. Measurement of Basal Uterine Tissue Levels of Prostaglandins in the Pregnant Rat

The uteri from rats from days 3 to 7 of pregnancy (3 animals per day) were removed, dried and weighed and immediately placed in 15ml absolute alcohol and homogenised. The glass homogeniser was washed with 5ml absolute alcohol which was then added to the homogenate. The alcoholic homogenate was then diluted with water, the pH reduced to 4.0 with 1N HCl and shaken with three times two volume redistilled ethyl acetate and the ethyl acetate extracts were pooled and evaporated to dryness as previously described. The residue was redissolved in 10ml redistilled ethyl acetate and stored at -20°C before RIA for $PGF_{2\alpha}$, PGE_2 and 6-oxo-$PGF_{1\alpha}$.

3. Studies on the Metabolism of Prostaglandin $F_{2\alpha}$ by the Early Pregnant Rat Uterus in vitro

Uteri from rats from days 3 to 7 of pregnancy (3 animals per day) were taken, divided into the right and left horns and the two horns were weighed. One horn (a) was homogenised in 8ml Krebs' solution containing 0.25μCi $^3HPGF_{2\alpha}$. The homogeniser was washed with 2.0ml Krebs' solution which was added to the homogenate. The second horn (b) was homogenised in 8.0ml Krebs' solution containing 0.25μCi $^3HPGF_{2\alpha}$, 20μg (2μg/ml) "cold" $PGF_{2\alpha}$ and 2.0mM nicotineamide adenine dinucleotide (NAD^+). Horn (a) was to determine metabolism of $PGF_{2\alpha}$ during the incubation procedure described in (1) with endogenous co-factors and horn (b) was to determine the maximum metabolic capacity of the tissue by using saturating levels of precursor i.e. non-radioactive "cold" $PGF_{2\alpha}$ and the co-factor, NAD^+. Each homogenate was bubbled with 95% oxygen and 5% carbon dioxide and incubated for 1 hr at 37°C in a shaking water bath. After 1 hr. the pH was reduced to 4.0 with 1N HCl and the homogenates subjected to solvent extraction as described in (1).

The dried residue of each extract was taken up in 0.5ml methanol and spotted onto silica gel thin layer chromatography plates at a distance of 3cm from the end of the plate. The plates were then

run first in the FV1 solvent system (Anderson 1969) to a distance from the origin of the spot to 1cm from the top of the plate and then in the GCM solvent system (Millar 1974). The plates were then dried and scanned under a Panax radioactive plate scanner to determine the position of any radioactive peaks.

Three silica gel plates were spotted with 10µg standard $PGF_{2\alpha}$, 15-oxo-$PGF_{2\alpha}$, 13,14,dihydro-15-oxo-$PGF_{2\alpha}$ respectively and were run in theFV1 and GCM solvent systems. The plates were then dried and sprayed with phosphomolybdic acid in methanol and heated in an oven at 115°C for 10 min in order to visualize the spots. The Rf values of $PGF_{2\alpha}$ and the two metabolites for these solvent systems were then calculated and compared to those obtained for each metabolsm study. The Rf value of the standards are given below:-

$PGF_{2\alpha}$	= 0.35
15-oxo-$PGF_{2\alpha}$	= 0.54
13,14,dihydro-15-oxo-$PGF_{2\alpha}$	= 0.59

The Rf values for the peaks detected by the radioactive scanner were determined. It was found that the two peaks of prostaglandin metabolites did not separate completely and so the plates were divided into two zones only, for scraping i.e the zone corresponding to the $PGF_{2\alpha}$ peak and the zone corresponding to the metabolites. Each scraping was washed twice with 5.0ml methanol to elute the prostaglandins and the methanol washings were pooled and taken to dryness on a rotary evaporator at 45°C. The dry residues were redissolved in 0.6ml methanol of which 0.5ml was added to vials containing 10ml scintillation fluid A (see Basic Methods Section) and the vials were counted for 4 min on a liquid scintillation counter.

4. The Effects of Indomethacin Treatment on Early Pregnancy in the Rat

a. Effects on Implantation

Pregnant rats were taken on the evening of day 5 of pregnancy in order to determine the time of the appearance of the "uterine dye reaction site" indicative of the start of the implantation process.

Rats were anaesthetised with 7ml/kg 25% urethane solution given intraperitoneally (i.p.). The jugular vein was cannulated and 0.5ml of a 0.5% solution of "Evans blue" dye was injected. One hr later

blood samples for progesterone measurement were taken by cardiac puncture (Evans blue dye did not cross react with the progesterone antibody). The rats were killed and the uterus examined for dye reaction sites. The first appearance of the dye reaction sites in the rats used for these experiments was 02.00 hr on day 6 of pregnancy (6 animals).

One group of rats was treated with indomethacin, in order to investigate the effects of inhibition of prostaglandin synthesis on implantation. Indomethacin was given sub-cutaneously in a dose of 3mg/Kg, dissolved in 0.7ml of polyethylene glycol on the morning (09.00) and evening (17.00) of days 3 and 4 of pregnancy. Plasma half life of indomethacin in the rat is 4 hr and indomethacin is cleared from the plasma at 24 hr (Hucker, Zacchei, Cox, Brodie, Cantrell 1966).

Five of the rats treated with indomethacin were examined at 02.00 hr on day 6 of pregnancy and five at 10.00 hr on day 6 of pregnancy. Peripheral plasma progesterone levels were measured in control (vehicle treated) and indomethacin treated rats at 02.00 hr on day 6 and at 10.00 hr on days 6 to 9 of pregnancy (5 animals/group).

b. Effects on Development of Early Pregnancy

One group of animals (n=11) treated with indomethacin on days 3 and 4 of pregnancy were killed on day 9 and the uteri removed. Uterine weight was taken and the appearance and number of implantation sites per horn was noted. These parameters were compared to those obtained in control animals (n=12) examined on day 9 of pregnancy.

Results

1. Uterine Prostaglandin Synthesis and Peripheral Plasma Progesterone levels in the Early, Pseudopregnant and Pregnant Rat in vitro

a. Early Pseudopregnancy

The amounts of prostaglandins synthesis by homogenates of pseudopregnant rat uteri, during a 60 min incubation period are shown in Fig.26. The production of all prostaglandins showed a peak on day 5 of pseudopregnancy. The amounts of PGE_2, $PGF_{2\alpha}$ and 6-oxo-$PGF_{1\alpha}$ formed were significantly higher on day 5 than on day 4 ($p<0.05$, $p<0.005$, $p<0.005$ respectively), or day 6 ($p<0.05$, $p<0.005$, $p<0.05$ respectively). The ratio of the amounts of 6-oxo-$PGF_{1\alpha}$, $PGF_{2\alpha}$ PGE_2 formed was 4:2:1, for all three days.

Fig.26.

Prostaglandin production by the pseudopregnant rat uterus *in vitro*. n = 4/group. Mean ± S.E.M. Measurements by R.I.A

PGE_2 ▲—▲—▲

$PGF_{2\alpha}$ •——•——•

6-oxo-$PGF_{1\alpha}$ ■—■—■

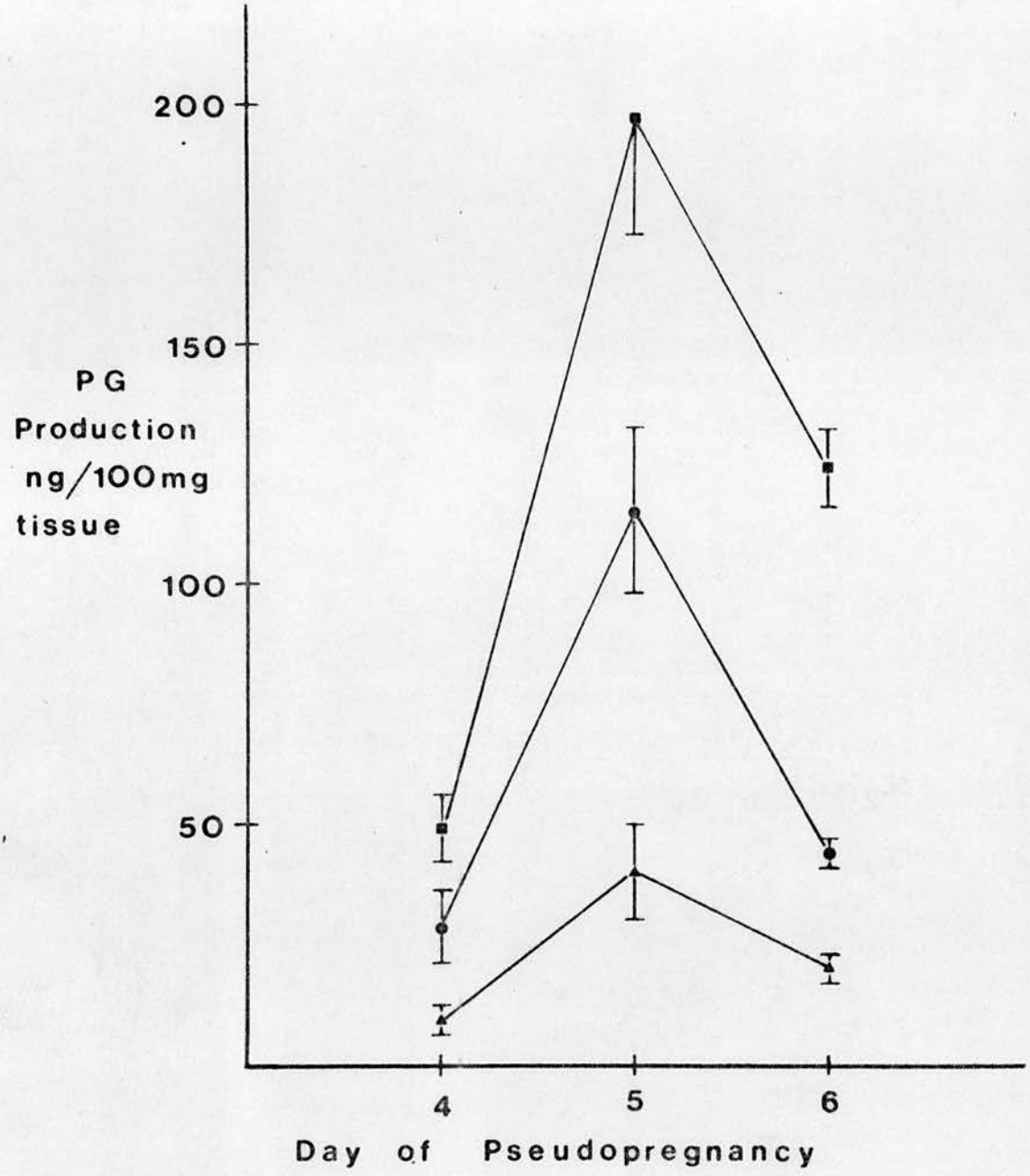

200
150
100
50
PG
Production
ng/100mg
tissue
4
5
6
Day of Pseudopregnancy

Fig.27.

Prostaglandin production by the pregnant rat uterus in vitro. n = 5/group. Mean ± S.E.M. Measurements by R.I.A.

PGE_2 ▲----▲----▲

$PGF_{2\alpha}$ • — • — •

6-oxo-$PGF_{1\alpha}$ ■ — ■ — ■

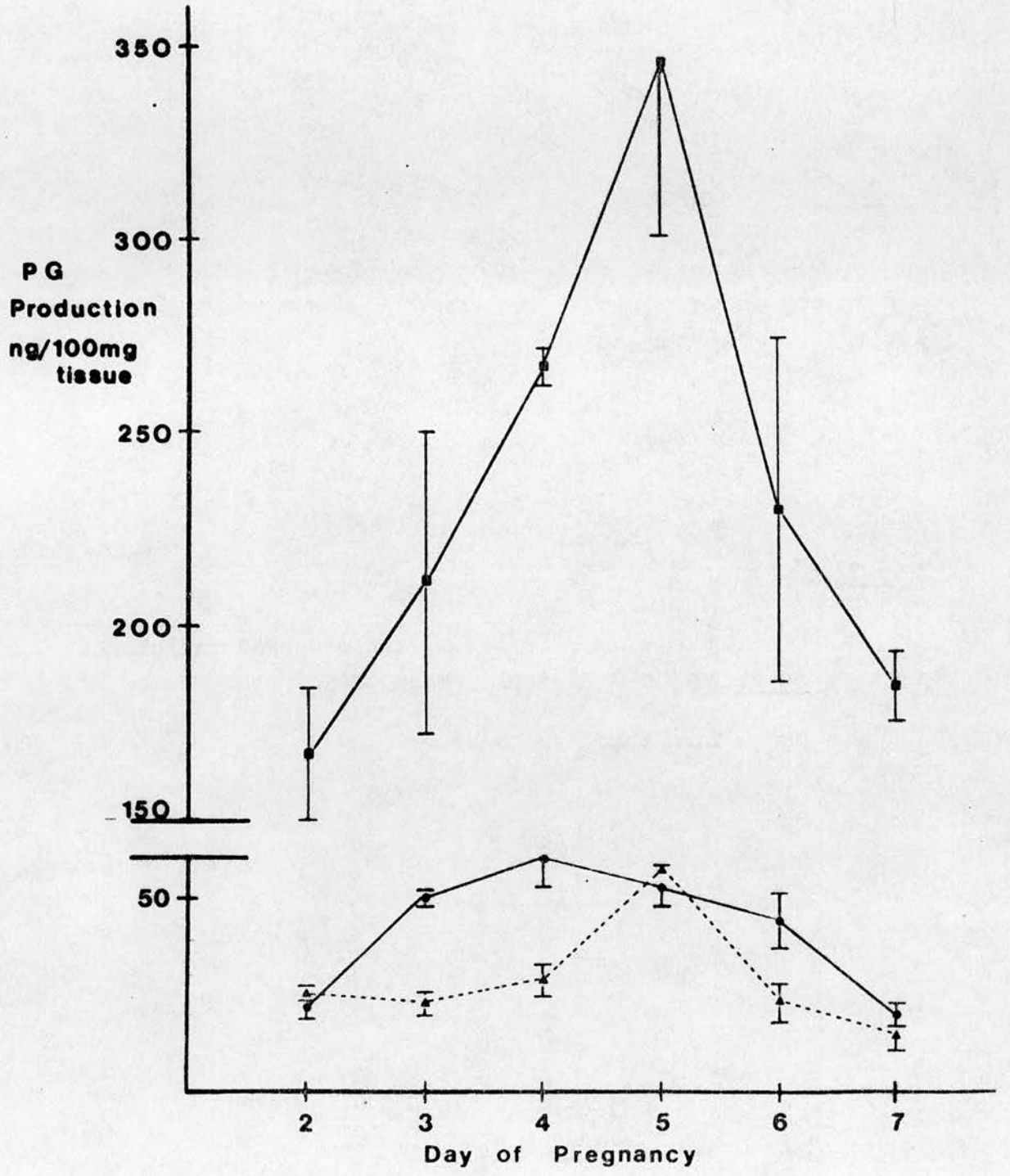

350
300
PG
Production
ng/100mg
tissue
250
200
150
50
2
3
4
5
6
7
Day of Pregnancy

Table 9a Prostaglandin Production by Uterine Homogenates from Pregnant and Pseudopregnant Rats. Mean ± S.E.M.

Day	Pregnancy ng/100 mg tissue n=5/group			Pseudopregnancy ng/100mgtissue n = 4/group		
	$PGF_{2\alpha}$	PGE_2	6-oxo-$PGF_{1\alpha}$	$PGF_{2\alpha}$	PGE_2	6-oxo$PGF_{1\alpha}$
4	60±8	29±4	267±5	28±9	9±3	49±7
5	53±5	57±6	346±46	115±17	40±10	198±25
6	44±6	24±5	230±47	44±3	18±3	124±8

b. Early Pregnancy

The amounts of prostaglandins synthesised by homogenates of pregnant rat uterus during a 60 min incubation period are given in Fig. 27. The production of PGE_2 showed a peak on day 5 of pregnancy, as was seen with the pseudopregnant uterus. Production on day 5 was significantly higher than on day 4 or day 6 ($p < 0.005$). The amounts produced by the pregnant and pseudopregnant uterus were comparable.

The production of $PGF_{2\alpha}$ by the pregnant uterus, did not show a peak on day 5. This is in contrast to the pseudopregnant uterus (compare Figs.26and 27). Production of $PGF_{2\alpha}$ by the uterus did show a small but significant increase from day 2 to day 3 of pregnancy and the levels plateaued between day 3 and day 6 and fell(again not significantly) on day 7. $PGF_{2\alpha}$ production was significantly higher than PGE_2 on days 3 and 4 of pregnancy ($p < 0.0005$ and $p < 0.005$ respectively) but there were no significant differences on days 2, 5, 6 and 7.

The major product formed by the pregnant rat uterus was 6-oxo-$PGF_{1\alpha}$ as was the case for the pseudopregnant uterus. Levels of 6-oxo-$PGF_{1\alpha}$ were five or six times higher than those of $PGF_{2\alpha}$ or PGE_2. The production of 6-oxo-$PGF_{1\alpha}$ was found to increase from day 2, reach a peak on day 5 and then fall on days 6 and 7. Production of 6-oxo-$PGF_{1\alpha}$ tended to be higher in the pregnant than in the pseudopregnant uterus (day 4 $p < 0.0005$, day 5 $p < 0.01$ and day 6 $p < 0.05$).

c. A Comparison of the Values obtained for Measurement of 6-oxo-$PGF_{1\alpha}$ by RIA and GC-MS

The amounts of 6-oxo-$PGF_{1\alpha}$ produced by the pseudopregnant and pregnant rat uterus were measured by GC-MS, in addition to RIA, in order to compare the two methods. The results are given in table 9. There was no significant difference between the results obtained by either method (Student's t test). The trend in prostaglandin production by the pregnant and pseudopregnant rat uterus from day to day (Fig. 26 and 27) measured by RIA, was also obtained in measurements by GC-MS.

Table 9.

Production of PGI_2 by the Pregnant and Pseudopregnant Rat Uterus in vitro. Measured as 6-oxo-$PGF_{1\alpha}$ by RIA AND GC-MS. Mean ± S.E.M. n= 4 or 5/Group.

		ng/100mg tissue	
Day		RIA	GC-MS
3	Pregnancy	211±41	226±32
4	Pregnancy	267±5	245±10
	Pseudopregnancy	49±7	79±17
5	Pregnancy	346±46	309±12
	Pseudopregnancy	198±25	260±77
6	Pregnancy	230±47	169±40
	Pseudopregnancy	124±8	161±13

d. Peripheral Plasma Progesterone Levels

The results for peripheral plasma progesterone levels in the rat during pregnancy are given in Fig. 28. Levels increased from 33 ng/ml on day 3 to reach a peak of 94 ng/ml on day 5 and the levels then plateaued. These results agree with previously reported findings (Wiest, Kidwell and Balogh 1968; Pepe and Rothchild 1974). The peripheral progesterone levels during pregnancy were not significantly different from those measured over a similar period of pseudopregnancy (see table 10). The levels found in pseudopregnancy also agree with previously reported results (Fenwick et al 1977).

Table 10 Peripheral Plasma Progesterone Levels in Pregnant and Pseudopregnant Rats. Mean ± S.E.M n = 4/5 Group.

Day	Peripheral Plasma Progesterone ng/ml	
	Pregnancy	Pseudopregnancy
4	53±4	63±7
5	94±20	99±13
6	70±9	92±7

2. Basal Tissue Levels of Prostaglandins During Early Pregnancy

The tissue levels of PGE_2, $PGF_{2\alpha}$ and 6-oxo-$PGF_{1\alpha}$ from day 3 to day 7 of pregnancy are given in table 11 below. Tissue levels of all three prostaglandins are low and there was no significant difference

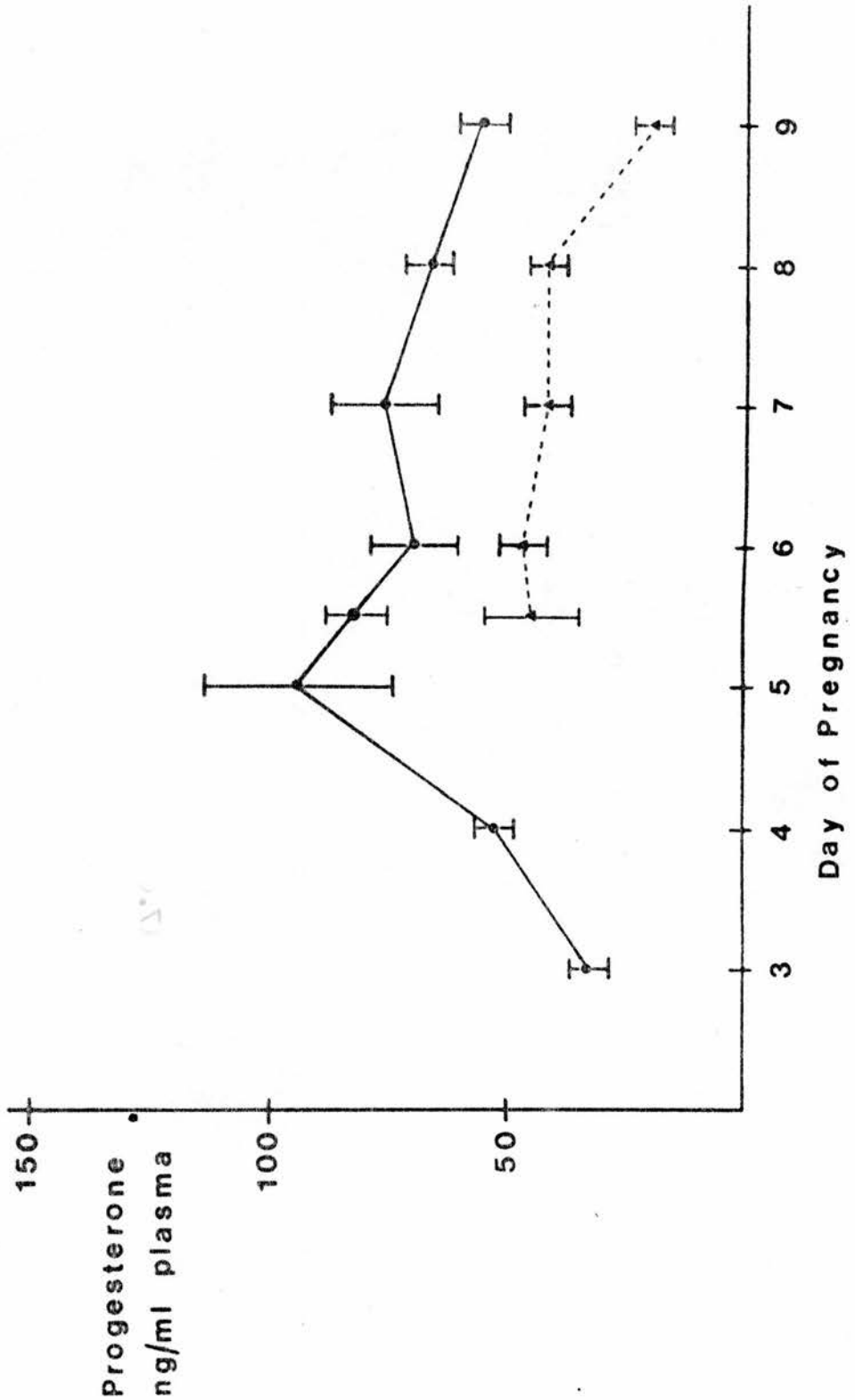
Fig.28.

Peripheral plasma progesterone levels in pregnant rats. n = 5 or 6/group. Mean ± S.E.M.

Control • —— • —— •

Indomethacin treated days 3 and 4 ▲ ---- ▲ ----- ▲
of pregnancy 3mg/Kg 09.00 and 17.00 hr.

Measurements by R.I.A.


150
100
50
Progesterone
ng/ml plasma
3
4
5
6
7
8
9
Day of Pregnancy

in the levels between any two days. Levels tended to be slightly lower on day 6 than on the other days, although not significantly.

Table 11 Basal Uterine Tissue Prostaglandin Levels During Early Pregnancy. Mean ± S.E.M. n = 3/group.

Day of Pregnancy	PG levels ng/100mg tissue		
	E_2	$F_{2\alpha}$	6-oxo-$F_{1\alpha}$
3	1.1±0.5	2.2±0.5	1.8±0.6
4	1.8±0.8	3.0±1.0	3.1±1.3
5	1.5±0.4	3.0±0.6	3.3±0.6
6	0.9±0.2	0.9±0.2	1.6±0.4
7	1.0±0.1	2.2±0.3	3.6±0.5

3. Metabolism of Prostaglandin $F_{2\alpha}$ by Uterine Homogenates from Pregnant Rats in vitro

The results, expressed as % metabolism of $PGF_{2\alpha}$ per unit weight of uterine tissue, obtained from rats from day 3 to day 7 of pregnancy. are shown below in table 12.

Table 12 Metabolism of $PGF_{2\alpha}$ by Uterine Homogenates from Pregnant Rats in vitro. Mean ± S.E.M. n = 3/group.

Day of Pregnancy	% Total Metabolism $PGF_{2\alpha}$/100mg Tissue	
	Horn a	Horn b with cofactors added
3	2.8±0.2	3.5±1.5
4	3.0±1.1	4.4±1.1
5	2.4±0.8	4.8±0.6
6	6.9±2.1	7.8±0.5
7	9.4±1.0	11.6±0.9

Metabolism tended to be slightly higher (though not significantly) in the horn (b) with added co-factor and an excess of substrate, on each of the days of pregnancy. Metabolism of $PGF_{2\alpha}$ did show a significant increase ($p < 0.05$) however, on day 6 and day 7 of pregnancy in the horn with added co-factor (b).

4. The Effects of Indomethacin on Early Pregnancy in the Rat

a. Effects on Implantation

The sites for implantation in the uterus were clearly visible at 02.00 hr on day 6 of pregnancy, due to the increased permeability of the blood vessels at these sites and consequently, penetration of the Evans blue dye.

In animals treated with indomethacin on days 3 and 4 of pregnancy and examined at 02.00 hr on day 6, there were no blue dye sites visible. Some animals showed small, dark spots in the uterus which may have been blood clots. Animals treated with indomethacin and examined at 10.00 hr on day 6 did show dye sites and the number was not significantly different from vehicle-treated controls, (table 13).

Table 13.
Effects of Indomethecin on the Appearance of Uterine "Dye Sites" in the Early Pregnant Rat. Mean ± S.E.M.

Group (n=5/group)	Mean No. "Dye Sites"/Horn
Control Day 6 02.00 hr	5.6±0.6
Treated Day 6 02.00 hr	0
Control Day 6 10.00 hr	5.5±0.4
Treated Day 6 10.00 hr	5.8 ±0.4

The peripheral plasma progesterone levels of pregnant rats treated with indomethacin were significantly lower than controls at 02.00 hr on day 6 and at 10.00 hr of subsequent days of pregnancy (see Fig. 28).

b. Effects on the Development of Early Pregnancy

The number of implantation sites per horn and the uterine weight of a vehicle-treated control group of rats and on group treated with indomethacin, all examined on day 9 of pregnancy, are given in table 14 below.

Table 14.
Effects of Indomethacin on the Development of Early Pregnancy in the Rat. Mean ± S.E.M.

Group (Day 9 of Pregnancy)	Mean Number of Implants per Uterine Horn	Uterine Weight (mg)
Control (n=12)	5.5±1.2	912±104
Treated:-		
i(n=7)	0	374±31
ii(n=4)	2.6±1.1	349±65

Seven of the animals treated with indomethacin showed no implantation sites and four showed a significant reduction in the number of implants per uterine horn compared with control rats ($p<0.01$). The uterine weight of both groups of indomethacin treated animals was significantly lower than controls ($p<0.01$). The implanted blastocysts in the indomethacin treated group of rats, were visibly smaller than those of controls. Two of the rats in the indomethacin treated group showed clumping of the blastocysts at the cervical end of the uterine horns. This is in contrast to control animals which exhibited even spacing of implants throughout both uterine horns.

Discussion

The major product formed by both the early pseudopregnant and early pregnant rat uterus when homogenised and incubated *in vitro* was found to be 6-oxo-$PGF_{1\alpha}$. The amounts produced by the pregnant uterus were higher than those produced by the pseudopregnant uterus and showed a peak on day 5 of both pregnancy and pseudopregnancy. The amounts of PGE_2 formed during pregnancy and pseudopregnancy were comparable and again showed a peak on day 5 in each case. However, $PGF_{2\alpha}$ production did not show a peak in production on day 5 of pregnancy in contrast to day 5 of pseudopregnancy.

Fenwick et al (1977) have also shown peak production of PGE_2 and $PGF_{2\alpha}$ by homogenates of rat uterus on day 5 of pseudopregnancy. However, the levels they report are slightly higher than those reported here. 6-oxo-$PGF_{1\alpha}$ was also reported to be

the major product formed but no measurements were made in that study.

The work of Kennedy (1976) and Evans and Kennedy (1978) reported an increase in the concentration of PGE_2 at the site of implantations compared with other uterine areas in the rat and hamster. Kennedy and Zamecnik, (1978) later reported similar results when measuring the concentration of 6-oxo-$PGF_{1\alpha}$ in the rat. The increase in synthesising capacity of PGE_2 and 6-oxo-$PGF_{1\alpha}$ by the pregnant rat uterus obtained in this section may correspond to the increase in levels of PGE_2 and 6-oxo-$PGF_{1\alpha}$ at the sites of implantation reported by other workers. In the rabbit, Sharma (1979) has recently reported that PGE levels in the endometrium and in the uterine venous plasma, increase during the preimplantation period, reaching a maximum value on day 6 (implantation in the rabbit occurs between day $6\frac{1}{2}$ and 7). He found a change in the ratio of E to F over the period studied, this change being more marked at the implantation sites. The results obtained in the present studies in the rat show a similar change in the ratio of PGE to PGF production by the pregnant uterus over the period studied with a peak in PGE_2 production on day 5, the day of implantation. The results obtained in this section and by other workers strongly suggest a role for PGE_2 and PGI_2 in implantation. The increased production of prostaglandins appears to be due to an increase in the synthesising capacity of the tissue for two reasons. Table 11 shows that the basal tissue levels of prostaglandins are very low, indicating that fresh synthesis of prostaglandins must have taken place during incubation of the homogenised uterus. The basal levels of prostaglandins in the pregnant rat uterus showed no significant variation, in contrast to uterine production from day to day. The metabolism of $PGF_{2\alpha}$ by the pregnant rat uterus was also low (table 12), but did show small increases on day 6 and 7 of pregnancy in the horns with added co-factor. This is in accordance with the observation that uterine prostaglandin metabolising capacity increases during pregnancy in the rat (Flower 1976). However, the small increase in metabolism of $PGF_{2\alpha}$ on day 7 could not account for the decrease in production observed on this day, especially as NAD was not added to incubates

in which prostaglandin production was measured. However, metabolism of $PGF_{2\alpha}$ only, has been studied in these experiments and it would be appropriate to perform such studies with PGE_2 and 6-oxo-$PGF_{1\alpha}$, which show larger changes in production than $PGF_{2\alpha}$ over the period of early pregnancy in the rat. Unfortunately, due to lack of time, such experiments were not possible in the present study.

Changes at the nidation site have been likened to an inflammatory process (Horan 1971). These include vasodilation and increased vascular permeability. PGE_2 and PGI_2 are known vasodilators (Jones 1978; Moncada, Gryglewski, Bunting and Vane 1976) and recently Kennedy (1979) reported that PGE_2, when instilled into the uterine lumen of indomethacin treated rats, increased vascular permeability at decidual sites. Similar results were not obtained with PGI_2, suggesting that it may not increase vascular permeability in the uterus. However, the negative results may have been due to the inherant instability of PGI_2, which may not have had sufficient time to act before being hydrolysed to 6-oxo-$PGF_{1\alpha}$.

Treatment with indomethacin on day 3 and 4 of pregnancy, delayed the appearance of dye reaction sites, which has normally occurred by 02.00 hr on day 6. Dye sites were visible however at 10.00 hr on day 6. Similar results have subsequently been reported by Kennedy (1977), who injected indomethacin on day 5 only. These findings further suggest a role for prostaglandins in implantation. However, other possible actions of indomethacin such as delaying the tubal transport of ova, cannot be excluded.

The indomethacin treatment also interfered with the subsequent development of pregnancy. Animals treated on day 3 and 4 and examined on day 9 showed an absence, or a reduction in number of implanted blastocysts. The implants were visibly smaller in treated animals, compared with controls on day 9 and the uterine weight was significantly lower in treated animals. A reduction in the weight of uterine areas containing implants on day 8, in indomethacin treated rats was reported by Kennedy (1976).

Horan (1971) also observed reductions in uterine weight on day 7 in animals treated with sodium salicylate during early pregnancy. In the rabbit, treatment with indomethacin or antibodies to PGE_2 and $PGF_{2\alpha}$ produces retardation of embryonic growth and resorption (El-Banna, Sacher and Schilling 1976; Elzayat and Stylos 1974; Hoffman 1978). It would be of interest to examine the effects of treatment of rats with antibodies to prostaglandins, on implantation and pregnancy development. The effects of treatment with indomethacin *in vivo*, on the production of prostaglandins by the rat uterus *in vitro* during early pregnancy, is another obvious experiment to carry out. There was a significant reduction in plasma progesterone levels from days 6 to 9 of pregnancy, in treated animals compared to controls. This was unexpected, and probably indicates that progesterone production by the ovary was inhibited either by a direct effect on the ovary, or indirectly by a reduction in trophic support. It is not known if the fall in progesterone occurred as a result of the failure of normal pregnancy development, or whether in fact the fall in progesterone leads to foetal resorption. Further investigations into the mechanism of action of indomethacin would be interesting to carry out. This result was not shown by Kennedy (1976; 1977) who used a lower dose of indomethacin and who measured progesterone levels on day 5 only.

Some animals in the group treated with indomethacin and examined on day 9 of pregnancy, showed a clumping of implants at the cervical end of the uterine horn. This was in contrast to controls which showed an even distribution of implantation sites throughout both horns. This observation was also made by Kennedy (1977). It is interesting that indomethacin should adversely affect the even distribution of implants, in view of the findings that the concentrations of PGE_2 and 6-oxo-$PGF_{1\alpha}$ are higher at implantation sites compared to non-implantation sites in the uterus (Kennedy 1977; 1978; Evans and Kennedy 1978). It may be that a local increase in uterine production of prostaglandins is responsible for the correct spacing of the blastocyst in the uterus prior to implantation, though this is only speculation.

The increase in prostaglandin synthesis may be stimulated by the surge in plasma oestrogen observed early on day 4 of

pregnancy (Shaikh 1971). However, some workers (Dickman and Dey 1974; Dey and Dickman 1974) have measured changes in Δ^5-3β-hydroxysteroid dehydrogenase and oestradiol-17β-hydroxysteroid dehydrogenase activity, which is first detectable in the blastocyst on day 4, peaks on day 5 and declines on day 6. If these changes in enzyme activity reflect changes in steroid synthesis, then oestradiol may be synthesised by the blastocyst. Hence the stimulus for local uterine prostaglandin production may come from oestrogen secreted by the blastocyst and taken up at local sites by the uterus. Alternatively, synthesis of oestrogen by the blastocyst may stimulate its own prostaglandin synthesis which could be released and taken up by the endometrium and contribute to vasodilatation and increased vasular permeability at nidation sites. Sharma (1979) has reported a rise in oestrogen in the blastocyst preceding a rise in PGE in the blastocyst on day 6 of pregnancy in the rabbit. It would be appropriate to measure prostaglandin production by the rat blastocyst.

The profile of prostaglandin production during pregnancy compared to that during pseudopregnancy, infers that the presence of the blastocyst is affecting uterine prostaglandin production. It is possible that factor (s) from the blastocyst may cause an inhibition of $PGF_{2\alpha}$ synthesis or a redirection of synthesis from precursor, into other pathways e.g. 6-oxo-$PGF_{1\alpha}$. Production of 6-oxo-$PGF_{1\alpha}$ during pregnancy was higher than that during pseudopregnancy, while PGE_2 levels were comparable. Overall prostaglandin production during pregnancy was greater than that during pseudopregnancy, and this may be due to stimulation by factor(s) released from the blastocyst.

No measurement of uterine cyclic AMP levels has been attempted during this study. It would be interesting, in the light of evidence presented in the introduction for a role of cyclic AMP in implantation, to investigate whether there is a rise in cyclic AMP on day 5 of pregnancy which coincides with the rise in prostaglandin production. Garg, De Souza and Chaudhury (1979) have reported that alloxan (an adenyl cyclase inhibitor) reduces uterine production of PGE and PGF on day 5 and 6 of pregnancy. Conversely, Vesin, Do-Khac and Harbon (1979) report that PGI_2 stimulates a rise in cyclic AMP in the endo-

metrium and myometrium of oestrogen-treated rats. Other studies which could be performed would be to investigate the effect of alloxan on implantation and on uterine 6-oxo-$PGF_{1\alpha}$ production around the time of implantation.

In conclusion, the experiments performed in this section support a role for prostaglandins in implantation in the rat. Since there is no difference in the number of uterine dye sites in indomethacin treated rats at 10.00 hr of day 6, but there is a reduced number or absence of implanted blastocysts on day 9, compared with the control group of animals, it would appear that prostaglandins may also be involved in the development of the foetus and placenta after the initial increase in uterine capillary permeability. However, the fact that prostaglandins cannot reverse completely the inhibition of implantation caused by indomethacin suggests that prostaglandins are not the only local hormones involved in implantation. They probably act together with other local hormones such as histamine which has also been implicated in the implantation process (see General Introduction).

Concluding Remarks

It seems that prostaglandins are involved in both parturition and implantation in the rat, from evidence of an increase in release from and/or synthesis by the uterus during each process. In both cases, more than one prostaglandin is implicated. PGE_2 and PGI_2 appear to be the main prostaglandins involved in implantation, while roles are implied for PGE_2, $PGF_{2\alpha}$, PGI_2, PGD_2 and TXA_2, in parturition. Prostaglandin synthesis inhibitors have been found to produce detrimental effects on both processes. Treatment with aspirin, indomethacin, fendozic acid or naproxen causes increased gestation length and prolonged parturition (Aiken 1972; Chester, Dukes, Slater and Walpole 1972; Sykes and Maddox 1972; Csapo, Csapo, Fay, Henzl and Salau 1973) and injection of $PGF_{2\alpha}$ antibody prolongs gestation in the rat (Dunn, Humphries, Judkins, Kendall and Knight 1973). Injection of $PGF_{2\alpha}$ into pregnant rats leads to a premature decline in plasma progesterone levels (Strauss, Sokolski, Caploe, Duffy, Mintz and Stanbough 1975), premature labour (Chatterjee 1976) and abortion (Deis 1969). Fuchs, Smitisari and Chantharaksri (1976) found that indomethacin treatment of pregnant rats near term causes a delayed decline in progesterone and increase in 20α-hydroxyprogesterone and also delayed parturition. These effects could be reversed by infusion of $PGF_{2\alpha}$. Indomethacin treatment has been shown to delay implantation in the rat in the present studies, and also by Kennedy (1977).

Although the evidence strongly suggests a role or roles for prostaglandins in parturition and implantation, it appears that in each case, other factors are involved. There is evidence that oxytocin plays an important role in parturition in the rat. The uterine contractions of labour are indistinguishable from those induced by oxytocin and there is a marked increase in uterine sensitivity to oxytocin during the 12 hr pre-partum (Fuchs 1978). Oxytocin has not been measured in the plasma of rats during parturition, but there is a decline in pituitary content of oxytocin during labour (Fuchs and Saito 1971). In addition, stimulation of the neurohypophysis will induce delivery (Buer, Lincoln and Swaab 1975). It is possible that oxytocin causes release of prostaglandins during parturition. Oxytocin has been shown to stimulate

prostaglandin release from the non-pregnant rat uterus *in vitro* (Chan 1974), although Ishikawa and Fuchs (1978) could not demonstrate any stimulation of PGF release under the same conditions. Pelvic neurectomy before mating abolished labour completely (Carlson and De Feo 1965). The mechanism of this effect was thought to be a removal of the afferent pathway which causes reflex activation of the neurohypophysis and oxytocin release, in response to cervical and uterine stretching. Thus parturition was prevented due to an absence of oxytocin. Louis, Lawrence, Becker and Borden (1978) have found that pelvic neurectomy performed on day 8, also prevents the increase in plasma PGF levels which normally occurs on the day of parturition, although the rise in plasma 20α hydroxyprogesterone levels which occurs prior to parturition is not affected. These results suggest that the pelvic nerves may be necessary for the second surge of prostaglandins, seen on the day of parturition, but possibly not for the first surge which initiates functional luteolysis, and that the second increase in prostaglandin output from the uterus occurs as a result of oxytocin release from the pituitary.

As regards other factors being involved in implantation, the delay in implantation in the mouse, due to indomethacin treatment can only be partially reversed by replacement therapy with PGE_2 or $PGF_{2\alpha}$(Lau, Saksena and Chang 1973). Complete reversal can be achieved however by using a combination of $PGF_{2\alpha}$ and histamine (Saksena, Lau and Chang 1976). The results presented in this thesis suggest the involvement of both PGI_2 and PGE_2 in implantation in the rat. It would be interesting therefore to investigate the effects of treatment with PGI_2 alone and in combination with PGE_2 and/or histamine, on the indomethacin induced delay of implantation in the rat.

There may be important roles for PGE_2 and PGI_2 in both parturition and implantation, as vasodilatory substances. PGE_2 seems also to be involved in the increase in vessel permeability seen at implantation sites (Kennedy 1979b). At parturition, the vasodilatory action of PGI_2 is possibly balanced against the vasoconstrictor effect of TXA_2 and possibly PGD_2. The suggested function of PGI_2 to maintain placental and uterine blood flow

before and during parturition, becomes unnecessary after parturition. Consequently, in the immediate post-partum period, this vasodilatory action of PGI_2 may be over-ridden by an increased release of TXA_2 and PGD_2, in order to reduce blood loss after birth. The spasmogenic effects of PGI_2 on the pregnant rat uterus appear to be like those one would associate with the uterine contractions of parturition. PGI_2 may therefore have a role in initiating and maintaining uterine contractility, though it is possible that PGE_2, $PGF_{2\alpha}$ and also TXA_2 also have a contributory role in the expulsion of the foetus. PGI_2 also potentiates the spasmogenic effect of oxytocin on the uterus (Williams, El-Tahir and Marcin-Kiewicz 1979) and this action may also aid in foetal expulsion.

The extensibility of the cervix of the late pegnant rat can be increased by prior administration of PGE_2 or $PGF_{2\alpha}$ to the animal (Hollingsworth, Isherwood and Gallincie 1979). Ellwood, Mitchell, Anderson and Turnbull (1979) have shown dramatic increases in the production and release from the cervix of PGE_2 and 6-oxo-$PGF_{1\alpha}$ at term compared to late pregnancy in sheep. In view of the high levels of 6-oxo-$PGF_{1\alpha}$ released from the rat uterus at term and the effects of other prostaglandins in the cervix, a possible role for PGI_2 in cervical ripening is implied in the rat.

The increased prostaglandin synthesis at both parturition and implantation appears to be under the control of oestrogen as in each case there is a surge of oestrogen prior to the rise in prostaglandins. Oestrogen is known to stimulate PGF production by uterine microsomes from oestrogen-treated, ovariectomised rats, by an effect on the synthesising enzymes involved (Ham,Cirillo, Zanetti and Kuehl 1975; Kuehl, Cirillo, Zanetti, Beveridge and Ham 1976). The rise in PGF production occurs, to some extent, at the expense of PGE, although PGE production is not completely abolished. However, in pseudopregnancy, increases occurred in the production of PGE_2, $PGF_{2\alpha}$ and 6-oxo-$PGF_{1\alpha}$ by the uterus, reaching a peak on day 5. The increase in the production of any one prostaglandin did not occur at the expense of another. Since Tamoxifen treatment on day 2 largely prevented the increases in prostaglandin production seen on day 5 of pseudopregnancy,

Fenwick et al (1977), suggested that the increase in uterine prostaglandin production occurred as a result of the surge of oestradiol in the early hours of day 4. Since the oestradiol would then have to stimulate synthesis of all prostaglandins, this hypothesis does not agree with that of Ham et al(1975). However, the oestrogen surge which occurs early on day 4 of pregnancy (Shaikh 1971), may be responsible for the rise in PGE_2 and 6-oxo-$PGF_{1\alpha}$ production by the uterus on day 5. The increase in 6-oxo-$PGF_{1\alpha}$ production by the pregnant uterus was higher on day 5 of pregnancy than day 5 of pseudopregnancy and occurred partly at the expense of $PGF_{2\alpha}$ production. Consequently, the peak in $PGF_{2\alpha}$ production seen on day 5 of pseudopregnancy was absent on day 5 of pregnancy. However, overall prostaglandin production on day 5 of pregnancy was greater than on day 5 of pseudopregnancy. It may be that oestrogen from the ovary stimulates the peak in prostaglandin production seen on day 5 of pseudopregnancy and pregnancy, though oestrogen and possibly other factor(s) released from the blastocyst, stimulates a redirection of synthesis and also a further increase in prostaglandin production during pregnancy.

It is known that PGE_2 will stimulate a rise in cyclic AMP levels in the rat ovary (Kuehl, Humes, Tarnoff, Cirillo and Ham 1970) and uterus (Bhalla, Sanborn and Korenman 1972) and more recently, PGI_2 has been shown to stimulate cyclic AMP in uterine tissues (Vesin, Do-Khac and Harbon 1979). It is also known that cyclic AMP is involved in the implantation process (see General Introduction) and hence it may be that there is a directing of prostaglandin synthesis towards PGE_2 and PGI_2 by factor(s) released from the blastocyst, in order to cause the increase in cyclic AMP required for implantation. Alternatively, theremay be release of prostaglandins from the blastocyst, in addition to those produced by the uterus, to stimulate a rise in uterine cyclic AMP levels.

On day 5 of pregnancy, the increase in prostaglandin E_2 and I_2 synthesis appears to be stimulated by an oestrogen surge, possibly together with factors from the blastocyst, against a background of high progesterone levels. In contrast, prostaglandin release at

term seems to be stimulated initially by an oestrogen surge on a falling background of progesterone. Zamecnik and Kennedy (1979) observed an increase in the levels of 6-oxo-$PGF_{1\alpha}$ and TXB_2 in the placenta, decidua and amniotic fluid of rats on day 20 of pregnancy compared to day 15. Levels continued to increase from day 20 and reached a maximum at parturition on day 23. Hence, at some time between day 15 and day 20, the stimulus for the rise in PGI_2 and TXA_2 production occurs. There is a surge in plasma oestrogen levels from day 20 - 21 of pregnancy (Shaikh 1971; Labhsetwar and Watson 1974) which is superimposed on a rapidly declining background of plasma progesterone, beginning on day 18 - 19 (Shaikh, Naqvi and Saksena 1977; Labhsetwar and Watson 1974). Consequently, this changing hormonal environment may be the stimulus for the initial increased prostaglandin and thromboxane synthesis and release from the pregnant uterus at term in the rat. A possible role for oxytocin in the subsequent release of prostaglandins from the uterus on the day of parturition has been discussed previously.

In conclusion, I should like to suggest that there are important roles for prostaglandins in parturition and implantation in the rat and that they have more than one function in each process. In parturition they are involved in luteolysis, maintenance of adequate uterine and placental blood flow during pregnancy and parturition (by vasodilatation) and then reducing blood loss after birth (by vasoconstriction) and I believe contributing to foetal expulsion, particularly in the case of PGI_2. Prostaglandins seem also to be involved in cervical ripening in the rat. Finally, earlier in pregnancy prostaglandins appear to be involved in the timing of implantation, in the even spacing of implants in the uterus, vasodilatation and increased permeability at the implantation sites and the subsequent development of placental function and normal pregnancy.

References

Aiken, J.W. (1972). Nature (Lond.), 240: 21-25.

Anderson, N.H. (1969). J.Lipid Res., 10: 316-319.

Anteby, S.O., Bauminger, S., Zor,U. and Lindner, H.R. (1975) Prostaglandins, 10: 991-1000.

Ash, R.W., and Heap, R.B. (1973). J.Agric.Sci., 81: 365-368.

Basset, J.M., Oxborrow, T.A., Smith,I.D. and Thorburn, G.D.(1969). J.Endocr., 45: 449-457.

Batta, S.K. and Martini, L. (1975). Prostaglandins, 10: 1075-1083.

Behrman, H.R., Yoshinaga, K., Wyman, H. and Greep, R. (1971). Am.J. Physiol., 221: 189-193.

Bernie, B., Kupresanin, M. and Kepor-Stanulovic, N. (1973).Am.J.Obstet. Gynec., 116: 813-821.

Bhatta, R.C., Sanborn, B.M and Korenman, S.G. (1972).Proc.Nat.Acad.Sci. U.S.A., 69: 3761-3764.

Blatchley, F.R. and Poyser, N.L. (1974).J.Reprod.Fertil., 40:205-209.

Born, G.V.R. (1962). J.Physiol.Lond., 162: 67P.

Brash, A.R. and Jones, R.L. (1974). Prostaglandins, 5: 441-454.

Brogden, R.N., Speight, T.M. and Austen, G.S. (1974). Drugs, 7: 164-282.

Brenner, W.E., Dingfelder, J.K., Staurovsky,L.G. and Hendricks, M.D. (1973). Prostaglandins, 4: 819-834.

Brummer, H.C. (1972). Prostaglandins, 2: 185-194.

Buckle, J.W. and Nathanielz, P.W. (1973). Prostaglandins, 4: 437-443.

Buer, K., Lincoln,D.W. and Swaab, D.F. (1975). J.Endocr., 65: 163-176.

Burka, J.F. and Flower, R.J. (1979). Br.J.Pharmac., 65: 35-41.

Caldwell, B.V., Tillson, S.A., Brock,W.A. and Speroff, L. (1972). Prostaglandins, 1: 217-228.

Carlson, R.R. and De Feo, V.J. (1965). Endocrinol., 77: 1014-1022.

Carminanti, P., Luzzani, F., Soffientini, A. and Lerner, L.J.(1975). Endocrinol., 97: 1071-1079.

Challis, J.R.G. (1971). Nature Lond.), 229: 208.

Challis, J.R.G., Harrison, F.A., Heap, R.B., Horton E.W. and Poyser,N.L. (1972). J. Reprod.Fertil., 30: 485-488.

Challis, J.R.G., Davies, I.J. and Ryan, K.J. (1973).Prostaglandins, 4: 509-516.

Challis, J.R.G., Davies, I.J. and Ryan, K.J. (1975).J.Endocr.,64: 363-370

Challis, J.R.G., Hartley,Phillida., Johnson,P., Patrick, J.E., Robinson, J.S. and Thorburn, G.D. (1977).J.Endocr., 73: 355-363.

Challis, J.R.G., Kendall, I.Z., Robinson,J.S. and Thorburn,G.D.(1977). Biol.Reprod., 16: 57-69.

Christ-Hazlehof, E. and Nugteren, D.H. (1978).Pop.Kerf.Ges.Biol.Chem 25th: 201 - 206. ed. Seis/Wendel. Springer, Berlin.

Challis, J.R.G., Robinson, J.S. and Thorburn.G.D (1977). In: "The Fetus and Birth": 211-227. Ciba Fdn. Symp.No.47. (ed.J.Knight and M.O'Connor). Elsevier.Amsterdam.Oxford.New York.

Chan, Y.W. (1974). Life Sci., 14: 2385-2392.

Chatterjee, A. (1973). Proc. India.Nat.Sci.Acad., 39: 408-419.

Chatterjee, A. (1976). Prostaglandins, 12: 1053-1059.

Collins, E., and Turner, G. (1973). Lancet, ii:1494.

Cross, F. (1964). Lancet, i: 914-916.

Csapo, A.I. (1955). In: "Modern Trends in Obstetrics and Gynaecology": 20-49 (ed. K. Bowes). London. Butterworth.

Csapo, A.I., Pulkinnen, M.D., Ruttner, B., Sauvage, J.P. and Weist,W.G. (1972). Am.J. Obstet. Gynec., 112: 1061-1067.

Csapo, A.I., Csapo, E.F., Fay,E., Henzl,M.K. and Salau, G. (1973). Prostaglandins. 6: 839-846.

Csapo, A.I.,Pulkinnen,M.D. and Weist,W.G. (1973). Am.J.Obstet.Gynec., 115: 759-765.

Currie, W.B. and Thorburn, G.D. (1973). Prostaglandins, 4: 201-214.

Currie, W.B., Wong, M.S.F., Cox,R.I. and Thorburn,G.D. (1973). Mem.Soc. Endocr., 20: 95-118.

Currie, W.B. and Thorburn G.D. (1977). In: "The Fetus and Birth":49-66. Ciba Fdn. Symp. No.47.(ed.J.Knight and M.O'Connor) Elsevier. Amsterdam. Oxford. New York.

Deis, R.P. (1969). Nature (Lond.), 229:568.

Dey, S.K. and Dickman, Z. (1974). Steroids, 24: 57-62.

Dey, S.K., Dickman, Z. and Sen Gupta, J. (1976). Steroids, 28: 481-486.

Dey, S.K., Villanenva, C., Chien, S.M. and Christ,R.D. (1978). J.Reprod. Fertil., 53: 23-26.

Dickman, Z. and Dey, S.K. (1974). J. Reprod.Fertil., 37: 91-93.

Dickman, Z. and Sen Gupta, J. (1974). Dev. Biol., 40: 196-198.

Dickman, Z. (1975). Res. Reprod., 7:3.

Dickman, Z. Dey, S.K. and Sen Gupta, J. (1975).Proc.Nat.Acad.Sci.U.S.A., 72: 298-301.

Dickman, Z. and Spilman, C.M. (1975) Science, 190: 997-998.

Dickman, Z. (1976). Vitam. and Horm., 34: 215-242.

Dickman, Z., Sen Gupta, J. and Dey, S.K. (1977) Scientc, 195: 687-688.

Diehl, J.R., Godke, R.A., Killian, D.B and Day,B.N. (1974).J.Anim.Sci., 38: 1234-1239.

Dighe, K.K. and Hunter, W.M. (1974). Biochem.J., 143: 219-231.

Dighe, K.K., Emslie, H.A., Henderson, L.K., Rutherford, F.and Simon, L. (1975). Br.J.Pharmac., 55: 503-514.

Dighe, K.K., Jones, R.L. and Poyser, N.L. (1978). Br.J.Pharmac.,63:406P.

Donaldson, L.G., Bassett,J.M. and Thorburn, G.D. (1970). J. Endocr., 48: 599-614.

Downing, I. and Williams, K.I. (1977). Br.J.Pharmac.,61: 158P

Dubin, N. and Ghodgaonkar, R. (1979). 4th International Prostaglandin Conference: In Press.

Dukes, M., Russel,W. and Walpole, A.L. (1974). Nature, 250: 330-331

Dunn, M.V., Humphries, N.G., Judkin, G.R., Kendall,J.Z. and Knight G.W. (1973). Prostaglandins, 3: 509-514.

El-Banna, A.A., Sach, B. and Schilling, E. (1976). J.Reprod. Fertil., 46: 375-378.

Ellwood, D., Mitchell, M., Anderson, M. and Turnbull, A. (1979). 4th International Prostaglandin Conference: In Press.

Elzayat, S. and Stylos, W.A. (1974). Endocrinl., 95: 1642-1648.

Eto, T.H., Masuda, M., Suzuki, Y. and Hosi, R. (1962).Jap.J.Anim. Reprod. 8: 34-40.

Evans, C.A. and Kennedy, T.G. (1978). J.Reprod.Fertil., 54: 255-261.

Fairclough, R.J., Hunter, J.T. and Welsh, R.A.S. (1975). Prostaglandins, 9: 901-916.

Fenwick, L., Jones, R.L., Naylor, B., Poyser, N.L. and Wilson N.H.(1977). Br.J.Pharmac., 59: 191-199.

Ferrando, G. and Nalbander, A.V. (1968). Endocrinol., 83: 933-937.

Ferris, T.F. (1967). Am.J.Physiol., 212: 698-702.

Flint, A.P.F., Burton,R.D., Gadsby, J.E., Saunders, P.T.K. and Heap,R.B. (1978). In:"Maternal Recognition of Pregnancy",209:-228. Ciba Fdn. Symp.No.64.Elsevier, Amsterdam.Oxford.New York.

Flint, A.P.F., Farsling, M.L. and Mitchell, M.D. (1978).Horm.and Metab.Res., 10: 545-548.

Flower, R.J. (1974). Pharmacol.Rev., 26: 23-67.

Flower, R.J. (1977)."The Fetus and Birth" :297-309 (ed.J.Knight and M.O'Connor). Ciba Fdn.Symp.No.47.Elsevier.Amsterdam.Oxford.

Ferris, T.F., Gorden, P., and Mulrow, P.J. (1967).Am.J.Physiol. 212.698-702.

Frank,M., Bazer, F.W., Thatcher, W.W. and Wilcox,C.J. (1977). Prostaglandins. 14: 1183-1196.

Fuchs, A.R. and Poblete, V.F. (1970). Biol.Reprod., 2: 387-400.

Fuchs, A.R. and Saito, S. (1971). Endocrinol., 88: 574-578.

Fuchs, A.R. (1972). Fertil.Steril,23: 410-416.

Fuchs, A.R. and Mok, E. (1973). Fertil. Steril., 24: 275-283.

Fuchs, A.R., Smitisari, Y. and Chantharaksri, H. (1976). J.Reprod. Fertil., 48: 331-340.

Fuchs, F., Fuchs, A.R., Poblete,V.F. and Risk,A. (1967).Am.J.Obstet. Gynec. 99: 627-637.

Garg, S., De-Zouza,A. and Chaudhury, R. (1979). 4th International Prostaglandins Conference: In Press.

Gross, F. Schaetelin, G., Zeigler M. and Berger,M.(1964). Lancet,i. 914-916.

Gréen, K., Bygdeman, M., Toppozada, M. and Wiquist, N. (1974). N.Amer. J.Obstet. Gynaec. 120: 25-31.

Grieves, S.A. and Liggins, G.C. (1976). Prostaglandins, 12: 229-241.

Gryglewski, R., Bunting, S., Moncada, S., Flower, R.J. and Vane, J.R. (1976). Prostaglandins, 12: 685-713.

Gustavii, B., and Gréen, K. (1972). Am.J. Obstet.Gynaec. 114:1099-1100.

Gustavvi, B. (1975). Br.J.Obstet.Gynaec., 82: 177-181.

Ham, E.A., Cirillo, V.J., Zanetti, M.E. and Kuehl, F.A. Jnr. (1975). Proc.Nat.Acad.Sci. U.S.A., 72: 1420-1424.

Hamberg, M. and Samuelsson,B. (1967). J.Biol.Chem., 242: 5336-5343.

Hamberg, M. and Samuelsson,B. (1974). Proc.Nat.Acad.Sci.U.S.A., 71: 3400-3404.

Hamberg, M., Svensson, J. and Samuelsson, B. (1975).Proc.Nat.Acad.Sci. U.S.A., 72: 2994-2998.

Hamberg, M. and Samuelsson, B. (1976). Nature, 261: 558-560.

Harney, P.J., Sneddon, J.M. and Williams, K.I. (1974). J.Endocr. 60: 343-351.

Harper, M.J.K. and Walpole, A.L. (1967). J.Endocr., 37: 83-92.

Harper, M.J.K., Dowd, D. and Elliott, A.S.W. (1969). Biol.Reprod.1:253-257

Heap, R.B. and Perry, J.S. (1974). J.Endocr., 63: 570-577.

Henricks, D.M., Dickey,J.R., Hill, J.R. and Johnston, W.E.(1972). Endocrinol. 90: 1336-1342

Heterlendy, F. (1973). Life Sci., 13: 1581-1589.

Heterlendy, F., Wood, R. and Jaffe, B.M. (1973). Prostaglandins,3:223-227.

Hetherington, C.M. (1968). J.Reprod. Fertil., 17: 391-393.

Hillier, K., Calder, A.A. and Embrey, M.P. (1974). J.Obstet.Gynaec. Br.Commonw., 81: 251-263.

Hodari., A.A., Carretero, D.A. and Symonds,E.M. (1968).Am.J.Obstet.Gynaec.. 34: 358 - 362.

Hoffman, L.H., Strong, G.B., Davenport, G.R. and Frolich, J.C. (1977). J. Reprod. Fertil., 50: 231-237.

Hoffman, L.H. (1978). Biol.Reprod., 18:148-153.

Hoffman, L.H, DiPietro,D.L. and McKenna, T.S. (1978).Prostaglandins, 15: 823-838.

Hollingsworth, M., Isherwood,C.and Gallincie,S.(1979).4th International Prostaglandin Conference: In Press.

Holmes, B.V. and Bergstrom,S. (1975).J.Reprod.Fertil.43: 329-332

Holmes, B.V. and Bergstrom,S. (1976).Am.J.Obstet.Gynaec., 124:301-306.

Horan, A.H. (1971). Fertil.Steril., 22: 392-397.

Horton, E.W.and Jones, R.L. (1974).Br.J.Pharmac., 52: 110-111P.

Horton, E.W.and Poyser,N.L. (1976).Physiological Rev., 56: No.4.

Ilingworth,D.V., Challis,J.K.G., Ackland,N., Burton,A.M., Heap, R.B., and Perry, J.S. (1974). J.Endocr., 63: 557-570.

Hucker, H.B., Zacchei, A.G., Cox, S.V., Brodie, D.A. and Cartwell,N.H.R. (1966) J.Pharmacol. Exp.Ther., 153: 237 - 249.

Ishikawa, M. and Fuchs,A.R.(1978). Prostaglandins, 15:89-101.

Ishizaka, T., Ishizaka, K., Orange, R.P. and Austen,K.F.(1971). J.Immunol., 106: 267-273.

Johnson, R.A., Morton,D.R., Kinner, J.H., Gorman,R.R., McQuire,J.C., Sun, F.F.,Whittaker,N., Bunting,S., Salmon,J.A.,Moncada,S. and Vane, J.R. (1976). Prostaglandins, 12: 915-928.

Jones, R.L. (1978). Acta.Biol.Med.Germ., 37 : 837-844.

Jones, R.L., Kerry,P.J., Poyser, N.L.,Walker, I.C.W. and Wilson,N.H. (1979). In: "Chemistry, Biochemistry and Pharmacological Activity of Prostanoids", :138-148. (ed.S.M.Roberts and F. Scheinman).Pergamon Press, Oxford and New York.

Jouvenaz, G.H.,Nugteren, D.H. and Van Dorp,D.A.(1973).Prostaglandins, 3: 175-187.

Karim, S.M.M. (1966). J.Pharm.Pharmac., 18: 519-530.

Karim, S.M. and Devlin, J. (1967). J.Obstet.Gynaec.Br.Commonw., 74: 230-234.

Karim, S.M.M. (1968). Br.Med.J., 4: 618-621.

Karim, S.M.M. and Hillier, K. (1970). J.Obstet.Gyneac.Br.Commonw., 77: 837-839.

Karim, S.M.M. and Filshie, G.M. (1970a). Br.Med.J., 3: 198-200.

Karim, S.M.M. and Filshie, G.M. (1970b). Lancet, i :157-159.

Katori, M., Horada, Y., Yamashita, Y., Ishbushi, M. and Miyazaki, M. (1978). Prostaglandins, 15:697P.

Kennedy, T.G. (1976). Vth.Int.Cong.Endocrinol.,Abst., 83.

Kennedy, T.G. (1977). Biol.Reprod., 16: 286-291.

Kennedy,T.G. and Zamecnik, J. (1978). Prostaglandins,16: 599 - 605.

Kennedy, T.G. (1979). Biol.Reprod., 20, Suppl.1 :99A.

Kierse, M.J., Flint, A.P.F., and Turnbull. A.C. (1974).Am.J.Obstet. Gynaec., 81: 131-135.

Killian, D.B., Garverick, H.A. and Day, B.A.(1973).J.Anim.Sci., 37: 1371-1375.

Kuehl, F.A.Jnr., Humes, J.L.,Tarnoff, J., Cirillo,V.J.and Ham,E.A. (1970). Science, 169:883-886.

Kuehl, F.A.Jnr., Cirillo, V.J. Ham,E.A. and Humes,J.L. (1973). In: "Advances in the Biosciences",9:155-172.(ed.S.Bergstrom and S. Bernhard). Pergamon Press. Oxford.

Labhsetwar, A.P. (1972).J.Endocr., 53: 201-213.

Labhsetwar, A.P. and Watson, B.J. (1974). Biol.Reprod., 10:103-110.

Lamond, D.R., Tomlinson, R.V., Drost,M., Henricks,D.M. and Jöchle,W. (1973). Prostaglandins, 4: 209-284.

Lau, I.F., Saksena,S.K. and Chang, M.C. (1973).Prostaglandins,4:795-803.

Lau, I.F., Saksena,S.K. and Chang, M.C. (1975).Prostaglandins,10:1011-1018

Lewis,T.M., Challis, J.R.G.,Robinson,J.S.and Thorburn,G.D. (1976). Nature, 264:797-799.

Kuhn, N.J. and Briley, M.S. (1970). Biochem. J., 117: 193 - 201.

Liggins,G.C.(1973).Rec.Prog.Horm,Res., 29: 111-159.

Liggins,G.C. and Vaughn,G.S. (1973).J.Obstet.Gyneac.Br.Commonw., 80:29-33.

Liggins,G.C., Fairclough,R.J., Grieves,S.A., Forster,C.S. and Knox.B.S. (1977). In: "The Fetus and Birth" : 5-25. Ciba Fdn.Symp.No.47. Elsevier, Amsterdam. Oxford. New York.

Louis, T.M., Lawrence, I.E.Jnr.,Becker,R.F. and Borden, H.W. (1978). Proc.Soc. Exp.Biol.Med., 158: 631-636.

McCormack, J.T. and Greenwald,G.S. (1974).J.Reprod.Fertil., 41:297-301.

McQuire, J.C., Sun, F.F., Whittaker, N.,Bunting,S., Salmon,J.A., Moncada,S. and Vane, J.R. (1976).Prostaglandins, 12:915-928.

Mahla, S. and Prasad, M.R.N. (1970).J.Reprod.Fertil., 23:327-329.

Millar, G.C. (1974). Prostaglandins, 10: 207-208.

Mills,D.C.B. and FacFarlane, D.E. (1977). In: "Prostaglandins in Haematology; 219-233. Spectrum Publications Ltd.

Mitchell, M.D., Hicks, B.R., Thorburn,G.D. and Robinson,J.S.(1978). J.Endocr.,79: 103-106.

Mitchell, M.D., Kierse, M.J.N.C., Anderson,A.B.M.and Turnball. A.C. (1978). Br.J.Obstet.Gyneac., 85: 442-445.

Mitchell, M.D., Hicks,B.R. and Robinson, J.S. (1979).J.Reprod.Fertil., 55:147-151.

Mitchell, M.D., Hicks. B.R., Thorburn, G.D.and Robinson. J.S. (1979).J. Endocr., 81: 339-343.

Mitchell, S., Golbus, M.D. and Creasy, R.K. (1977).Prostaglandins, 14: 577-581.

Moncada, S. and Vane, J.R. (1976). Prostaglandins, 12: 915-928.

Moncada, S., Gryglewski, R., Bunting, S. and Vane,J.R.(1976a). Nature, 263: 663. -665

Moncada, S., Gryglewski, R., Bunting, S. and Vane,J.R.(1976b). Prostaglandins, 12: 715-737.

Najak, Z., Hillier, K. and Karim.S.M.M. (1970).J.Obstet.Gyneac.Br. Commonw., 77: 701-709.

Naylor, B. and Poyser, N.L. (1975). Br.J.Pharmac.,55: 229-232.

Needleman, P., Moncada,S., Bunting, S.Vane,J.R., Hamberg,M. and Samuelsson. S (1976). Nature, 261: 558-560.

Nugteren,D.H. (1975). Biochem.Biophys.Acta., 380:299-307.

Ogino, N., Miyamoto, T., Yamamoto, S. and Hayaishi,O. (1977).J.Biol. Chem. 252:890-895.

Omini, C., Pasagaklian, R., Folco, G. Fano, M. and Berti,F. (1978). Prostaglandins, 15: 1045-1054.

Ominic, C., Pasagaklian,R.Burnelli,G., Folco, G. and Berti, F.(1979). 4th International Prostaglandins Conference: In Press.

Pearce, D.J. (1977). Prostaglandins,14: 571 - 576.

Pepe, J.G. and Rothchild,J. (1974) Endocrinol.,95: 275-279.

Perry, J.S., Heap,R.B., Burton, R.D. and Gadsby, J.E.(1976).J.Reprod. Fertil. Suppl. 25: 85-96.

Poyser, N.L. (1977). Experientia, 33: 1561.

Poyser, N.L. and Horton, E.W. (1975). J. Endocr. 67: 81 - 88.

Rajtar, G. and de Gaetano, G. (1979).Thrombosis Res., 14:245-248.

Roberts, J.S. and McCracken, J.A. (1976). Biol.Reprod.15: 457-463.

Robertson,H.A., (1974). Reprod.Fertil., 30: 1-7.

Robertson,H.A. and King, G.S. (1974). J.Reprod.Fertil., 40:133-141.

Robinson., J.S. Natale,R.Clover,L. and Mitchell,M.D. (1979).J.Endocr., 81: 345-349.

Roth-Brandel, U., Bygdeman, M. Wiquist,N. and Bergstrom,S. (1970). Lancet,i: 190-191.

Saksena, S.K. and Harper, M.J.K. (1972).Prostaglandins, 2: 405-411.

Saksena, S.K. and Lau, I.F. (1973). Prostaglandins, 3: 317-322.

Saksena, S.K. and Harper, M.J.K. (1974). Fertil. Steril., 25: 121-126.

Salmon, J.A. and Amy, J.J. (1973). Prostaglandins, 4: 523-533.

Salmon, J.A., Moncada,S. and Vane, J.R. (1976).Prostaglandins,12: 915-928.

Samuelsson, B. (1976). Acta.Physiol. Scand., 96: 145-149.

Sananes, S.N., Baulieu, E.C. and Le Gascogne, C. (1976).Mol.Cell. Endocrinol., 6: 153-158.

Shaikh, A.A. (1971). Biol.Reprod., 5: 297-307.

Shaikh, A.A., Birchall, Kathleen and Saksena, J.K. (1977).Prostaglandins, 4: 17-30.

Shaikh, A.A. Naqvi, R.H. and Saksena, S.K. (1977).Prostaglandins. 13: 311-320.

Sharma, S.L., Hibbard, B.M., Hamlett, J.D. and Fitzpatrick,R.J.(1973). Br.Med.J.,1: 709-711.

Sharma, S.L. (1979). Colloque International.Montpelier.France.: In Press.

Skinner, S.L., Lumbers, M.B. and Symonds, E.M. (1968).Am.J.Obstet.Gynaec., 101: 529 - 533.

Schwartz, B.E., Vanatta, P., Siiteri, P.K. and MacDonald,K.C.(1974). Gyneac.Invest., 5: 21.

Smith, V.G.,Edgerton, L.A., Hafs,H.D. and Convey,E.M. (1973).J.Anim. Sci., 36: 391-396.

Soraya, L.K. and Hingorani, V. (1977).Contraception, 15: 285-295.

Strauss,J.F. Sokolski,J., Caploe, P., Duffy,P. and Mintz, G. and Stanbough, R.L. (1975). Endocrinol., 96: 1040-1043.

Svensson, J. and Hamberg,M. (1976). Prostaglandins, 12: 943-950.

Szego,C.M. and Davies, J.S. (1967).Proc.Nat.Acad.Sci.U.S.A.,58:1711-1718.

Thorburn, G.D., Nicoll, D.H., Bassett, H.J.A., Shutt, D. and Cox,R.I. (1972). J. Reprod.Fertil. Suppl., 16: 61-84.

Thorburn, G.D., Challis,J.R.G. and Currie, W.B. (1977).Biol.Reprod., 16: 18-27.

Thorburn, G.D. and Challis.J.R.G. (1978).Physiological Rev.: In Press.

Tobert,J.A(1976).J.Reprod.Fertil., 47: 391-393.

Turemo, T.,Strandberg, K.,Hamberg,M. and Samuelsson. B.(1976). Acta. Physiol.Scand., 96: 145-149.

Terragno, N.A., Terragno,D.A, Pacho,D., Iczyk, D. and McGiff,J.C.(1974). Nature, 249: 57 - 58.

Samuelsson, B. (1980).Advances in Prostaglandin and Thromboxane Research, Vols. 6, 7 and 8: In Press.

Vane, J.R., and Williams,K.I. (1973).Br.J.Pharmac., 43:629-639.

Vane, J.R. (1976). Prostaglandins, 12: 715-737.

Van den Bergh, A.S., Szabo, E. and Szontagh, F.E. (1976).Contraception, 14: 631-637.

Van Dorp, D.A., Buytenhoek.,M. Christ-Hazlehof, E., Nugteren, D.H. and Van der Ouderaa, E.J. (1978). Acta.Biol.Med.Germ., 37:691-697.

Vesin, M.F., Do-Khac, L. and Harbon, S. (1978). Mol.Pharmacol., 14: 24-37.

Vesin, M.F., Do-Khac,L. and Harbon. S. (1979). 4th International Prostaglandins Conference: In Press.

Walker, I.C.W., Jones, R.L. and Wilson, N.H. (1979). Prostaglandins, 18: 173-178.

Waltham, R. Tricani, V., Shabanah, E.H. and Arenas, R. (1973). Prostaglandins, 4: 93-106.

Wayneforth, H.B. and Robertson. D.M. (1972). J.Endocr., 54: 79-85.

Weist, W.G., Kidwell, W.R. and Balogh, K. (1968). Endocrinol, 82: 844-85.

Weist, W.G. (1970). Endocrinol. 87: 43-48.

Welschen, R. Osman, P., Bullaart, J., De Greef, W.J.,Uilenbroek, J. and De Jong, F.H. (1975). J. Endocr., 64: 37-47.

Whilhelmsson, L., Lindblom, B., Hamberger,L., Sausoie, S.,Hammarstrom,S. Wiquist, N. and Samuelsson, B. (1979).4th International Prostaglandin Conference.: In Press.

Whorton, A.R. Lazar, J., Smigel,M., and Oates, J.A. (1979).4th International Prostaglandins Conference.: In Press.

Wiquist, N., Bequin,F., Bygdeman, M. Fernström, I. and Toppozada, M. (1972). Prostaglandins, 1: 37-53.

Williams, K.I. and Downing. I. (1977).Prostaglandins, 14: 813-817.

Williams, K.I.,Dembinsca-Kiec, D.,Zmuda, S. and Gryglewski, R. (1978). Prostaglandins, 15: 343-350.

Williams, K.I., El-Tahir, K.E.H, and Marcin-Kiewicz,E. (1979). Prostaglandins, 17: 667-672.

Yamamoto, S. (1979). 4th International Prostaglandins Conference:In Press

Zamecnik, J. and Kennedy. T.G. (1979).4th International Prostaglandins Conference: In Press.

Zerobin, K., Jöchle, W. and Steingruber, C. (1973).Prostaglandins, 4: 891-901.

Zormethangi, B., Agdrwal, N., Puin, C.P., Laumas, K.R., Soraya,K.K. and Hingorani, V. (1977). Contraception, 15: 285-293.

Sources of Drugs and Reagents

Heparin - Weddel Pharmaceuticals Ltd.
Indomethacin - Merck, Sharp and Dohme Ltd.
Soyabean Lipoxidase - Sigma.
Tranylcypromine - Smith, Kline and French Ltd.
Silicic Acid - Unisil.
Arachidonic Acid (99% pure) - Sigma.
PCS Scintillation fluid cocktail - Amersham/Searle Corporation.

Tritiated Prostaglandin $F_{2\alpha}$ and E_2 - Amersham Co. Ltd.
Tritiated Prostaglandin D_2 and Thromboxane B_2 - New England Nuclear Laboratories.

Deuterated 6-oxo-prostaglandin $F_{1\alpha}$
Prostaglandins $F_{2\alpha}$, E_2, D_2, 6-oxo-$F_{1\alpha}$ and Thromboxane B_2.
} Gifts from the Upjohn Company, Kalamazoo, Michigan, U.S.A.

6-oxo-prostaglandin $F_{1\alpha}$ - Gift from I.C.I. Ltd.
Prostaglandin I_2 - Gifts from I.C.I. Ltd., and The Welcome Foundation.

All other solvents and reagents used, were from B.D.H. Ltd. (Analar Grade), which were redistilled before use.

ACKNOWLEDGEMENTS

I should like to thank Dr. Norman Poyser for his guidance and encouragement, during his supervision of this work at the Department of Pharmacology of the University of Edinburgh.

I should also like to thank Professor. E.W. Horton of the Department of Pharmacology, for the facilities extended to me and also for his moral support and kindness.

I am grateful to Dr. R.L. Jones and Mr. C. Marr, for their help and advice.

My thanks are further extended to Mr. K. Bell for his assistance in the operation of the gas chromatograph-mass specrometer and also for the reproduction of illustrations. I am also grateful to Mrs. J. Hunter and the technical staff of the Animal House for their care of and attention to the animals used.

This work was carried out during the tenure of a Medical Research Council Studentship, which I gratefully acknowledge receipt of, and a Medical Research Council Project Grant to Professor Horton.

Finally, I am gratefully indebted to my mother, Helen, for her conscientious work and patience in the typing of this thesis.

Advances in Prostaglandin and Thromboxane Research, Vol. 8, edited by B. Samuelsson, P. W. Ramwell, and R. Paoletti. Raven Press, New York © 1980.

Prostaglandins and Implantation in the Rat

Christine A. Phillips and N. L. Poyser

Department of Pharmacology, University of Edinburgh, Edinburgh EH8 9JZ, Scotland

There is evidence to suggest an involvement of prostaglandins (PG) in implantation and subsequent fetal development in rabbits (1,3), mice (6), and rats (4). Fenwick et al. (3) reported a significant increase in the uterine production of PGE and PGF on day 5 of pseudopregnancy *in vitro*. 6-oxo-$PGF_{1\alpha}$ was reported as the major product formed by the pseudopregnant rat uterus. Prostaglandin production by the pregnant rat uterus around the time of implantation has not been measured *in vitro*. Production of 6-oxo-$PFG_{1\alpha}$ by the pseudopregnant rat uterus was also measured. The effect of indomethacin on implantation and subsequent fetal development has also been investigated.

METHODS AND RESULTS

PGE_2 and $PGF_{2\alpha}$ were measured by radioimmunoassay (RIA) while 6-oxo-$PGF_{1\alpha}$ was measured by gas chromatography-mass spectrometry (GC-MS).

PGE_2 production by uterine homogenates was found to increase significantly on day 5 of pregnancy, the reported day of implantation in the rat. $PGF_{2\alpha}$ production was found to plateau from day 3 to day 6 of pregnancy. $PGF_{2\alpha}$ production was significantly higher than PGE_2 on all days measured except day 5 when there was no significant difference. 6-oxo-$PGF_{1\alpha}$ (reflecting PGI_2 production) showed a significant increase on day 5 of both pregnancy and pseudopregnancy. Production was maximum on day 5 of pregnancy and was five times higher than production of PGE_2 or $PGF_{2\alpha}$. 6-oxo-$PGF_{1\alpha}$ was significantly higher on day 4 of pregnancy compared with pseudopregnancy but was not significantly different on days 5 and 6.

The increase in vascular permeability at the implantation site can be demonstrated by intravenous injection of a 0.5% solution of Evans Blue dye. Pregnant rats were injected with the dye and the uterus examined for "dye reaction sites" 1 hr later. Our control rats were found to show the dye reaction by 2:00 A.M. on day 6 of pregnancy. However, pregnant rats treated with indomethacin (3 mg/kg at 9:00 A.M. and 5:00 P.M. on days 3 and 4) and examined at 2:00 A.M. on day 6 did not show any dye reaction sites in the uterus. If indomethacin-treated rats were examined at 10:00 A.M. on day 6, however, dye reaction

sites were present, their number not being significantly different from control rats injected with vehicle, 0.7 ml polyethylene glycol.

The effect of the indomethacin treatment by day 9 of pregnancy was also investigated. In the treated group of animals, seven did not show any implantation sites and four showed a significant reduction in the number of implants per uterine horn compared to controls. There was a significant reduction in the mean uterine weight in animals treated with indomethacin (with and without implants) compared to controls. Plasma progesterone levels measured by RIA were significantly lower from day 6 to day 9 of pregnancy in animals treated with indomethacin on days 3 and 4, compared with controls. Some of the animals treated with indomethacin and examined on day 6 and some of those examined on day 9 showed a clumping of the developing embryos around the cervical end of the uterine horns. This was in contrast to control animals which showed an even distribution throughout both horns.

DISCUSSION

The increase in synthesizing capacity of the uterus for PGE_2 and 6-oxo-$PGF_{1\alpha}$ on day 5 of pregnancy suggests a role for these PGs in the process of implantation in the rat. Further evidence for a role for PGs in implantation is the observation that implantation is delayed by treatment with indomethacin. This drug also interferes with subsequent development of the blastocyst, again suggesting that PGs are important during early pregnancy in the rat. The reduction in plasma progesterone seen from day 6 to day 9 of pregnancy may be due to a disturbance of the pregnancy due to the action of indomethacin on the developing embryos. Alternatively, the normal development of the pregnancy may be interrupted by a fall in plasma progesterone levels, possibly due to an action of indomethacin on the corpus luteum. The disturbance in distribution of the implants within the uterus in animals treated with indomethacin may indicate that PGs are also necessary for even spacing of implants. Kennedy and Zamecnik (5) have reported that the concentration of 6-oxo-$PGF_{1\alpha}$ is elevated at the site of implantation compared with adjacent uterine areas in the rat. This observation, together with our findings that 6-oxo-$PGF_{1\alpha}$ is the major PG produced by the uterus around the time of implantation, supports a role for PGI_2 in the implantation process.

ACKNOWLEDGMENT

This work was supported by a long-term support grant to Professor E. W. Horton from the Medical Research Council.

REFERENCES

1. Al Barna, A. A., Sacher, B., and Schilling, E. (1976): *J. Reprod. Fert.*, 46:375–378.
2. Fenwick, L., Jones, R. L., Maylor, B., Poyser, N. L., and Wilson, N. H. (1977): *Br. J. Pharmacol.*, 59:191–199.

3. Hoffman, L. H. (1978): *Biol. Reprod.*, 18:148–153.
4. Kennedy, T. G. (1977): *Biol. Reprod.*, 16:286–291.
5. Kennedy, T. G., and Zamecnik, J. (1978): *Prostaglandins*, 16:599–605.
6. Lau, I. F., Saskena, S. K., and Chang, M. C. (1973): *Prostaglandins*, 4:795–803.